CHANGES IN SOIL PH, POLYPHENOL CONTENT AND MICROBIAL COMMUNITY MEDIATED BY *EUCALYPTUS CAMALDULENSIS*

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Abstract. *Eucalyptus camaldulensis* has been the main exotic species planted in reforestation programs in the tropics due to its fast growth and adaptability to climate variations. Based on the premise that the conversion from natural grazed pastures to commercial *Eucalyptus* plantations generates significant changes in soil properties, we assessed the impact of this exotic plantation on soil chemical and biological indicators. The study was conducted in 6 plantations across Senegal following a decreasing rainfall gradient from south to north. The plantations were divided in three lots according to their age: young plantations (established in 2003, 6 years old); intermediate plantations (established in 1998, 11 years old) and old plantations (established in 1982 and 1983, 26 years old). Our results clearly showed that *E. camaldulensis* plants significantly modified soil pH and soil bacterial community at all sites regardless of the age of the plantation. Microbial biomass (assessed by substrate-induced respiration), community structure (assessed by denaturing gradient gel electrophoresis profiles) and function (assessed by Catabolic Response Profile using different substrates) were all significantly decreased. The acidifying effect of *E. camaldulensis*, the effect of high level of polyphenols and their impact on microbial communities and ecosystem functioning were discussed.

Keywords: *eucalyptus, allelopathy, microbial diversity, catabolic diversity, enzyme activities*

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Introduction

Plantations of fast-growing trees have been extended in Sahelian zones because of their ecological plasticity (Bernhard-Reversat and Loumeto, 2002). Expansion of *Eucalyptus* plantations has been the focus of several studies throughout the world, usually related to their economic importance. *Eucalyptus* is commonly used in reforestation because of its high cellulose production, high fiber quality, and resistance to environmental stresses and diseases (Turnbull, 1999; Berthelot et al., 2000; Cossalter and Pye-Smith, 2003). Several environmental impact studies on carbon flux, water balance and soil fertility have been conducted in industrial plantations in Congo and Brazil, in response to criticisms by opponents of *Eucalyptus* plantations (Bouillet et al., 1997; Bouillet and Bernhard-Reversat, 2001; Laclau et al., 2004). It has been shown that, in addition to their acidifying affects (Cannell, 1999; Rhoades and Binkley, 1996; Farley et al., 2008), exotic species have mineral and water needs significantly higher than native species (Benhard-Reversat, 1987; Laclau, et al., 2005). It is noteworthy that, despite being very informative, these studies did not take into account the microbiological characteristics and more specifically, microbial genetic and functional diversities in soils with *Eucalyptus* plantations.

In Senegal, many *Eucalyptus* plantations have been planted in different regions by the Senegalese Institute of Agricultural Research (DRFP/ISRA), in its tree improvement program. The extent of studies conducted in the past encouraged researchers to select *E. camaldulensis* as the species which is best adapted to soil and climatic conditions of the experimental stations throughout Senegal (Giffard, 1969; Hamel, 1981). But little attention has been paid to diversity and functionalities of microbial communities under *Eucalyptus* soil. Yet, microbial communities are the most sensitive and most affected by the replacement of native vegetation by exogenous plant cover (Yu, 2005).

In fact, soil microorganisms are extremely diverse and play an important role in ecosystems. In soils, they mediate nutrient cycling, organic matter decomposition, soil aggregate formation, soil carbon storage (Zinn et al., 2002), and have an impact on composition of plant communities (Hooper et al., 2000; Wardle, 2002) and plant disease prevention and bio-control (Kennedy, 1998; Biró et al., 2000; Artursonn, et al., 2006). The microbial community composition and functioning can be influenced by exudates from roots and litter quality and quantity. According to Powlson et al. (1987), soil microbial biomass measurement can give an early indication of soil health before changes in total organic C and N can be reliably detected. In order to draw meaningful conclusions we assessed a set of parameters (microbial biomass, enzyme activities, functional diversity, etc.) as early indicators of stress and disturbance (Dick et al., 1996; Nannipieri and Eldor, 2009).

So far in Senegal, studies on impact of *Eucalyptus* plantations on soil health were limited to greenhouse or site scale. This is the first large scale study conducted in *Eucalyptus* plantations in Senegal (across several sites and different ages of plantations).

The aim of this study was to determine the impact of *E. camaldulensis* plantations on microbial genetic and functional diversities, including microbial biomass. Importantly, studies have indicated that *Eucalyptus* leaves contain toxic organic compounds (high quantities of lignin, polyphenol compounds, allelochemicals, etc.) which may have a deleterious impact on soil microorganisms. We hypothesized that *E. camaldulensis* will lead to changes in soil chemical and microbiological properties that will ultimately limit the catabolic capabilities of native microbial communities.

Materials and Methods

Study sites and soil sampling

The sampling sites were selected in 6-, 11- and 26-year-old *Eucalyptus* plantations considered respectively as young, intermediate and old. Young plantations correspond to Sinthiou, Kolda and Nioro. As shown on the map (*Fig 1*), Nioro is located in south-west of Senegal (15°19N; 04°17W), Sinthiou is in the North (17° 15N; 06° 82W while Kolda is in South Senegal (14° 27 N; 05°77W). Intermediate plantation corresponds to Matam (North) collection (17°30N; 06° 87W) and old plantations correspond to Lompoul (17°07 N; 03°69 W) and Tamba sites (15°23 N; 06°40W) respectively in the center and in the North of Senegal. The rainfall distribution patterns across Senegal are presented in *Fig 1*. From each site, five soil samples were collected under five *Eucalyptus* trees from 0 to 30 cm soil depth and mixed to form one composite sample (noted SC). And three composite samples were collected per plantation. This soil was considered to be influenced by *Eucalyptus*. At each site, three other composite soil sample was formed with soil collected in areas free of *Eucalyptus* and located at a distance of 30 m from *Eucalyptus* plantings (control sample or HC). These soil samples were supposedly uninfluenced by *Eucalyptus.*

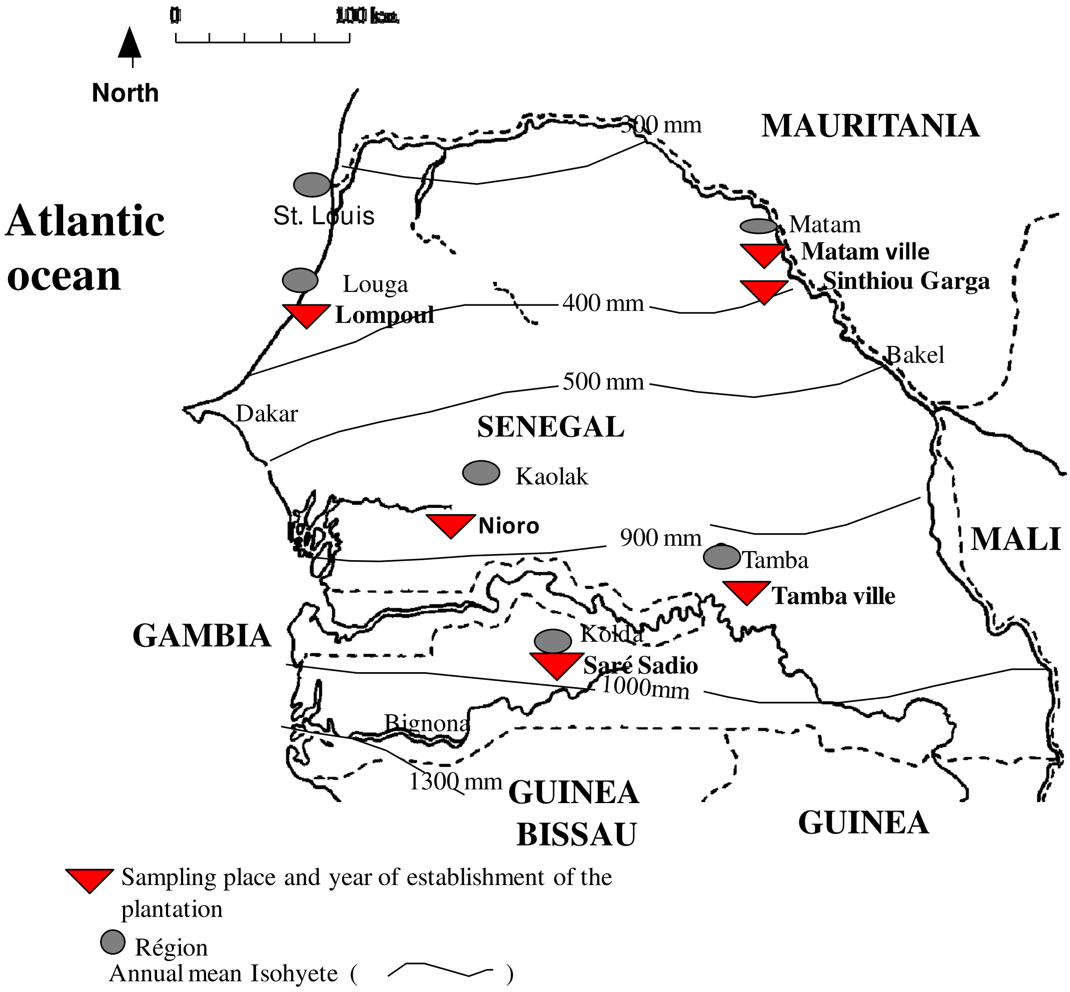

Figure 1. *Study site and the exact locations of sampling*

Soil analyses

Chemical analysis of soil

All soil samples were characterized by measuring pH, total soil C and N after dry combustion in Elemental Analyzer (*LECO Corporation, St. Joseph, MI, USA*). Total and available P were analyzed by Olsen-Dabin method (Olsen et al., 1954; Aubert, 1978). Analyses were performed at LAMA (Laboratoire des Moyens Analytiques IRD/ISRA, laboratory certified by International Organization for Standardization ISO 9001 version 2008).

Total polyphenol content

The method described by Macheix et al. (1990) was adapted to determine total polyphenol content. One gram of each soil sample was shaken in 20 ml cold methanol (80% v/v) during 15 minutes and the mixture was centrifuged for 3 minutes at 5000 rpm at 4°C. This step was repeated three times before the supernatants were evaporated to remove methanol. A solution of ammonium sulphate (40% v/v) was added to the aqueous extract in order to increase the ionic strength of the solution and metaphosphoric acid solution 20% (1/10 v/v) was also added to prevent oxidation of phenolic compounds.

This phase was followed by depigmentation and defatting of aqueous extract with petroleum ether (v / 2). The extract was purified by ethylene acetate (v/v) and evaporated to dryness at 35°C with a rotary evaporator and the residue was recovered in 2 ml of pure methanol. Extraction was repeated 3 times for each soil sample.

Total polyphenol content was determined by spectrophotometry, using (+/-)-catechin as standard range (Singleton and Rossi, 1965). Briefly, 50 µl of the diluted sample extract was mixed with 1.35 ml of distilled water and 200 µl of Folin-Ciocalteu's reagent. Then, 400 µl of sodium carbonate solution (20% w/v) was added. The test tubes were placed in a water bath for 20 minutes at 40 ° C before absorbance was measured at 760 nm. The concentration of polyphenols in samples was derived from a standard curve (+/-) - catechin ranging from 5 to 30 µg ml^{-1}.

Total microbial biomass

Microbial biomass (MB) was estimated using substrate induced respiration method (Anderson and Domsch, 1978). The soil microbial content was estimated from the maximum rate of glucose-induced respiration by applying the formula below:

$$x = 40.04y + 0.37 \tag{Eq. 1}$$

where y = the maximum initial rate of respiration (in ml CO_2 100 g $soil^{-1}$ h^{-1}), and x = mg microbial-C 100 g $soil^{-1}$. Microbial CO_2 respiration was determined by direct injection into a micro GC Analytical Instruments SRA (MTI P200, Microsensor Technology Inc., Fremont, CA.) equipped with a TCD detector using helium as the carrier gas.

Soil enzyme activity

Acid and alkaline phosphatases and dehydogenase activity were determined on *Eucalyptus* rhizospheric soils and compared to control soil samples.

These phosphatase activities (alkaline and acid) were determined on 0.5 g of dry soil according to the method of Eivazi and Tabatabai (1977).

For each soil sample, soil were mixed with 400 µL of modified universal sterile buffer (at pH 5.8 for acid phosphatase and pH 11 for alkaline phosphatase) and 100 µl of p-nitrophenyl phosphate solution (pNPP) before being incubated for 1 hour at 37°C on a rotary shaker. The reaction was stopped by adding 100 µl of $CaCl_2$ and 400 µl of NaOH, and the soil suspensions were centrifuged at 12000 rpm for 10 minutes. Then, the absorbance readings were taken at 400 nm. Analyses were conducted in triplicate and one non-substrate control and results are expressed as µg p-nitrophenol release.g^{-1} h^{-1} at 37°C.

Dehydrogenase activity was assayed by a method from Casida et al. (1964) with few modifications. One g soil sample was mixed with 1 ml of tris buffer, 1 ml 4% (w/v) of 2, 3, 5-triphenyltetrazolium chloride (TTC) and incubated for 24 h at 37 °C. Dehydrogenase enzymes convert TTC to 2, 3, 5-triphenylformazan (TPF). 1 ml of 4 % TTC and 2.5 ml of H_2O were added to each tube before they were inverted a few times and incubated at 37°C for 24 hours. A control sample contained all the chemicals mentioned above except the TTC. Each soil sample was extracted with 5 ml of acetone after incubation. The extract was centrifuged and the optical density (O.D.) of the supernatant was read at 546 nm in a spectrophotometer. Results were expressed as µg TPF g^{-1} h^{-1}.

Catabolic Response Profiles

The functional diversity of heterotrophic microbial communities was determined by measuring the patterns of *in situ* catabolic potential (ISCP, CRP). The ISCP is a physiological approach developed by Degens and Harris (1997). This method is based in a serial measure of CO_2 production in a short term after adding different organic substrates. Thirty three substrates (33) belonging to various chemical groups: eleven amino acids (arginine, aspargine, L-cysteine, L-glutamic acid, L-hisdidine, L-serine, L-tyrosine, L-lysine, L-leucine, L-proline, L-glutamine), six carbohydrates (amidon, D-mannose, D-glucose, sucrose, threalose, maltose), twelve organic acids (gallic acid, ascorbic acid, citric acid, fumaric acid, malic acid, quinic acid, succinic acid, tartaric acid, malonic acid, α-ketoglutaric acid, oxalic acid, panthothenic acid), one amine (D-glucosamine) and three alcohols (meso inositol, sorbitol, mannitol) were used on three replicate soil samples to evaluate soil microflora capacity to catabolize different C sources. Each substrate was added at 0.5 mg C.g $soil^{-1}$ concentration in 70% water-holding capacity of each soil in vacutainers bottles. Solutions were adjusted to pH 5.8-6.2 before addition to soil, using NaOH or HCl to increase or decrease the pH at the time of preparation in order to avoid any substrate-pH effects on microbial communities. A control without substrate, with only water added was included. Bottles were incubated for 2 h at ambient temperature (25°C) and CO_2 fluxes from the soils were measured by µCPG (Analytical Instruments SRA (MTI P200, Microsensor Technology Inc., Fremont, CA.) equipped with a TCD detector using Helium as the carrier gas). Results were expressed as µgCO_2 g^{-1} soil h^{-1}.

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Catabolic evenness (E), defined as the variability in substrate utilization, was evaluated by $E=1/\Sigma p_i^2$ where pi = ratio of respiration response on the i^{th} substrate to the sum of the respiration responses on all substrates.

Genetic diversity

Whole-community DNA was directly extracted from 0.5 g of sample using a bead-beating method (Fast DNA SPIN Kit for soil, Bio 101 Inc., USA) following manufacturer's instructions.

The 16S rDNA (V3 sequence) fragments of the bacterial soil community were amplified with 338f-GC and 518r primers (Muyzer et al., 1993). The PCR and DGGE were performed as described by Fall et al. (2004). The number of bands and their positions on the DGGE gel were analyzed using the Bio-Profil Biogene program (Vilber Lourmat) and dendrograms were created based on Dice coefficient of similarity.

The species richness on DGGE gels (R) was calculated as the mean number of bands present (Vivas et al., 2008, 2009). The structural diversity of the microbial community was examined by the Shannon index of general diversity and Simpson index of dominance D (Simpson, 1949). For these analyses, each band was presumed to represent the ability of that bacterial species to be amplified (Vivas et al., 2008, 2009). The intensity of the bands was reflected as peak heights in the densitometric curve. The Shannon H' and Simpson D indexes were calculated from the following equations: $H' = -\Sigma\ (P_i \log P_i^2)$ $D = \Sigma P_i^2$; $P_i = ni/N$; ni = height of peak and N = sum of all peak heights in the curve.

Statistical analyses

Co-inertia analysis (CIA) was used to analyze the relationship between chemical and microbial properties and sample type in different sites. Additionally, Student-Newman Keuls test was done to separate the means when the ANOVA revealed significant differences at 5% level. The relationship of Karl Pearson is used to correlate the different variables measured in this experiment. Between-group analysis (BGA) was used for the SIR responses because number of cases is lower than the number of variables (12 soil samples and 33 SIR substrates). The free ADE4 software (Thioulouse et al., 1997) was used to perform BGA computations.

Results

ACP analysis showed clear separation between plantations according to age. Young and old plantations (young SC and old SC) correspond to axis 1 (38.44% of the total contribution rate) and were associated with low pH, low MB, and low diversity index (*Fig. 2, Table 1*). While intermediate plantation (med SC) was associated to axis 2 (31.41% of the total contribution rate) and is mainly characterized by very high levels of phenols and phosphatase (*Fig. 2; Table 1*).

Table 1. *Change in chemical and biological variables according to plantation age*

	Desy	Ac. phos	Al. Phos	H	E	MB	Total poly	pH_2O
med	8,48 a	764,12a	364,28a	1,47ab	28,81ab	4276,14a	18,1a	6,67a
old	6,91 b	382,93b	95,99b	1,45b	30,83a	1015,40b	6,44b	5,49b

young	3,07 c	300,71c	51,93c	1,49a	26,49b	737,79b	6,06b	5,47b
	med> old> young			med= old< young	med= old> young	med> old= young		

Data in the same column followed by the same letter are not significantly different according to one-way ANOVA ($P < 0.05$). Abbreviations: Shannon (H), Simpson (D), Species richness (R), total microbial biomass (MB); alkaline phosphatase (Al. phos), acid phosphatase (Ac. Phos), dehydrogenase (Desy), total polyphenol content (Total poly)

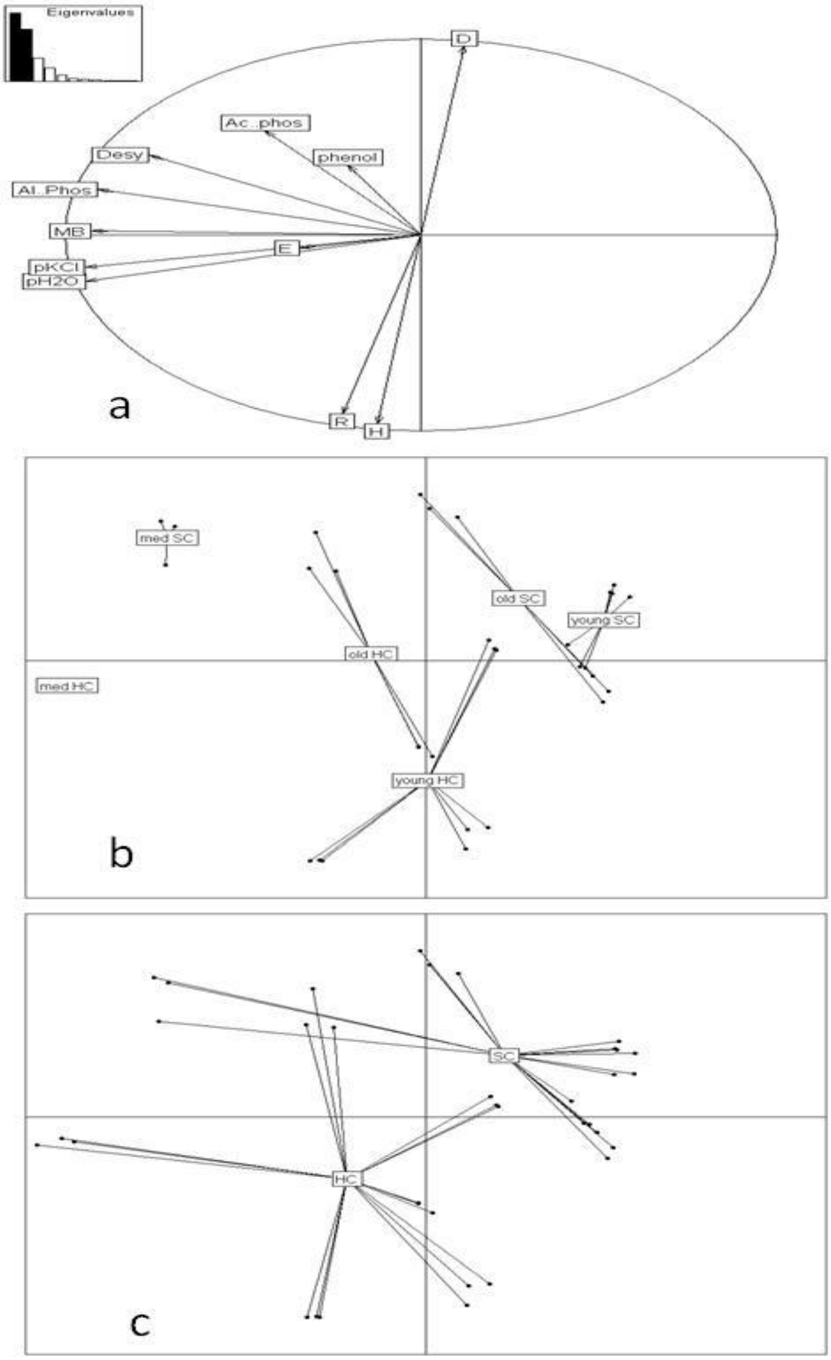

Figure 2. *Principal Component Analysis showing the relationship between the chemical and microbiological properties(a) of Eucalyptus plantations based on age (b) and sample type (HCvsSC) (c); SC: under Eucalyptus samples and HC : control samples*

Canonical correlation analysis (*Fig. 2c*) showed a sharp contrast between HC and SC samples on basis of different variables measured. Irrespective of the age, the control samples (HC) were associated with low total polyphenol, high pH, high microbial biomass, high alkaline phosphatase, high dehydogenase and greater diversity index (H' and R). ANOVA between separate means is presented in *Table 2* and *Table 3*.

Differences in pH between HC and SC were significantly lower in soils under *Eucalyptus* compared to soils without *Eucalyptus* in all age groups (*Table 2*). However, this acidity was not associated with age of plantation. For example, old plantation of Lompoul and young plantation of Nioro showed the same Δ_{pH} (HC-SC) (1.2 and 1.3 respectively). Also, acidification effect on microbial activities was different. In the old plantation of Lompoul, microbial biomass was not changed but its activity and diversity were strongly modified (dehydogenase, alkaline phosphatase, E and H'). In contrast, in the old planting of Tamba, BM was significantly reduced as well microbial activities although, catabolic diversity remained intact. Within the group of young plantations, the effect of planting *Eucalyptus* on biological variables appeared similar through reduced BM, enzymatic activity and microbial diversity. ANOVA analysis showed that the difference in polyphenol content between HC and SC were significantly higher in SC irrespective of the age group (*Fig. 2*).

Functional diversity

In terms of functional diversity in the different age groups, Acid phosphatase activity did not present any significant difference between HC under SC (except in the intermediate plantation at Matam). While alkaline phosphatase activity was significantly highest in *Eucalyptus*-free soil (HC) (*Table 2*, *Fig. 2a* and *2c*). In most of the case, microbial biomass and dehydrogenase activity showed the same trend in different age groups.

The ISCP patterns highlight a strong site effect mainly due to the opposition of Matam/Lompoul, Sinthiou and Kolda. The soil of Matam was characterized by high levels of P, N, and high pH compared to other samples (*Table 4* and *Fig. 3a*).

If the site effect is removed, the difference between HC and SC is very clear (*Fig. 3a*). All groups of substrates induced significantly higher CO_2 emissions in control soils (HC) compared to SC samples except for the old plantation of Lompoul (*Fig. 3a*). The higher catabolic evenness E found in HC samples (*Table 2*) confirmed the functional difference. This difference in ISCP response between HC and SC samples was more marked in younger plantations (Sinthiou Garba, Nioro and Kolda sites) (data not shown) than old and intermediate plantations. However the permutation test of BGA between HC/SC was not significant (*Fig. 3b*).

Genetic diversity

Table 3 showed the effect of *Eucalyptus* plantations on bacterial communities. Importantly, the Shannon index showed that the microbial community diversity, characterized by the number and intensity of DGGE bands, was significantly higher in sites free of *Eucalyptus* (HC) compared to *Eucalyptus*-covered sites (SC) for each couple of soil samples. Specific richness (R) showed the same trend although the differences were not significant in all site. Contrarily, Simpson's dominance (D) was higher in SC samples compared to the controls for all groups (old, young and med). Dominance measures the relative abundances and decreases progressively as the relative abundance of microbes become more equitable. The maximum dominance (value 1) corresponds to a site that contains one dominant species.

Table 2. *pH and biological characteristic of soils (means of three replicates)*

	old				med		young					
	Lompoul		Tamba		Matam		Sinthiou		Kolda		Nioro	
	HC	SC	HC	SC	HC	SC	HC	SC	HC	SC	HC	SC
pH_{H2O}	6.34 a	5.12 c	6.41 b	5.87 a	7.44 a	6.87 b	6.17 b	5.35 e	5.28 c	5.55 d	6.83 a	5.53 d
pH_{KCl}	5.37 b	4.46 c	5.62 b	5.30 a	6.86 a	6.00 b	5.04 c	4.28 e	5.28 b	4.95 c	6.32 a	4.66 d
Total poly. *mg. g soil*$^{-1}$	4.81 bc	10.58 a	2.37 c	6.52 b	7.78 b	28.41 a	2.78 c	17.09 a	0.617 c	10.40 b	3.10 c	4.67 c
MB (µg*C-CO2 .100g soil*$^{-1}$*. h*$^{-1}$*)*	49,16 c	49,07 c	187,36 a	120,57 b	541,25 a	313,98 b	58,67 b	21,51 d	45,28 b	30,13 c	242,86 a	44,23 b
Simpson-Yule index (*E*)	30.10 b	28.73 c	32.2 a	32.3 a	30.20 a	27 ,0 b	31.70 a	30 ,0 b	24.1 d	18.2 e	29.6 b	25.2 c
Ac. phos. (*µg* pNPP g^{-1} .h^{-1})	206.1 c	346.7 cd	415.7 ab	509.2 a	333.6 b	1194.6 a	262.5 b	264.5 b	370.7 a	429.0 a	221.2 b	256.1 b
Al. phos (*µg* pNPP g^{-1}.h^{-1})	89.0 b	25.0 c	188.0 a	81.7 b	388.8 a	339.6 b	73.8 b	6.4 e	119.0 a	33.9 d	54.9 c	23.4 d
Desy (µg TPF g^{-1}. h^{-1})	6.51 b	1.22 c	11.02 a	8.56 b	8.765 a	8.194 a	2.11 b	1.24 c	5.09 a	2.978 b	4.64 a	2.392 b

Data in the same line followed by the same letter in each age range are not significantly different according to one-way ANOVA ($P < 0.05$). HC and SC as in *Figure 2.* MB: total microbial biomass, Al. phos: alkaline phosphatase, Ac. Phos: acid phosphatase, Desy: Dehydrogenase.

Table 3. *Shannon (H), Simpson (D), Species richness (R) diversity indexes values for DGGE profiles under and out cover of E. camaldulensis*

Age	old				med		young					
	LHC	LSC	THC	TSC	MHC	MSC	SHC	SSC	KHC	KSC	NHC	NSC
H	1,523 a	1,480 b	1,426 c	1,397 d	1.484 a	1.460 a	1.574 b	1.452 e	1,470 d	1,486 c	1,586 a	1,426 f
D	0,031 e	0,034 d	0,03 a	0,04 a	0,034 b	0,04 a	0,03 d	0,04 a	0,035 b	0,038 c	0,04 d	0,03 a
R	37 b	36 b	31 a	27 c	35,6 a	34 ,6 a	40 a	40 a	33 b	32,6 b	41 a	29 c

Data in the same line followed by the same letter in each age range are not significantly different according to one-way ANOVA ($P < 0.05$). Abbreviations for sampling site L: Lompoul, M: Matam, S: Sinthiou, T: Tamba, K: Kolda, and N: Nioro; HC : control samples and SC : Under *Eucalyptus* samples.

Table 4. *Soil chemical characteristics*

age	old				med		young					
site	Lompoul		Tamba		Matam		Sinthiou		Kolda		Nioro	
sample	HC	SC	HC	SC	HC	SC	HC	SC	HC	SC	HC	SC
N total (%)	0.028	0.056	0.069	0.056	0.093	0.062	0.024	0.022	0.045	0.049	0.050	0.044
Carbone total (%)	0,295	0,688	0.992	0.807	0,95	0,699	0,24	0,224	0.599	0.671	0,629	0,54
P. total (mg/kg)	28	44	82	58	351	237	70	48	49	53	98	56
P. available (mg/kg)	3.49	11.35	6.11	8.73	141.40	47.13	4.36	6.11	3.93	4.80	20.08	9.16

HC: control samples, SC: Under *Eucalyptus* samples

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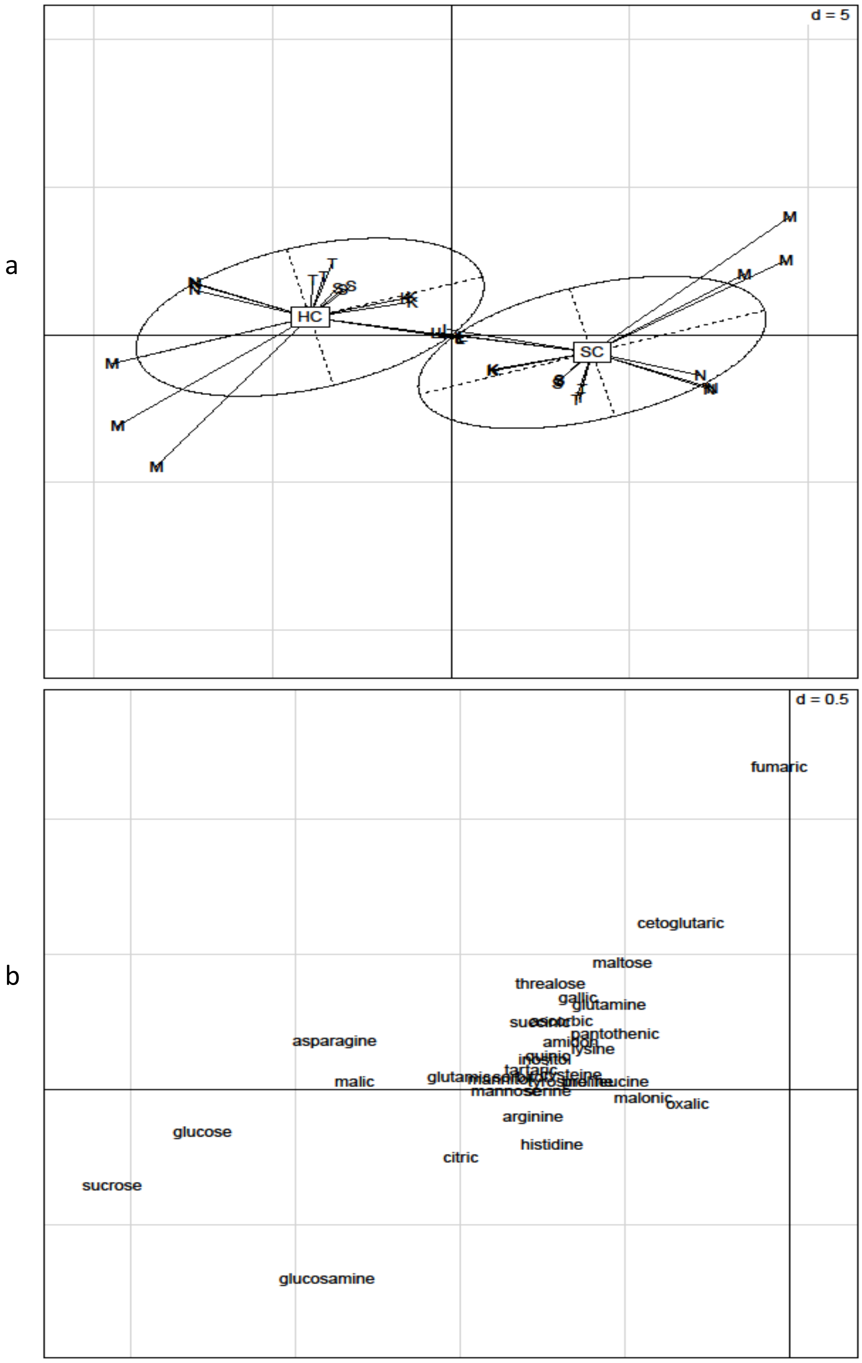

Figure 3. *Between-group analysis (BGA) of in situ catabolic potential (ISCP) responses of soils under Eucalyptus compared with control soils. M: Matam, N: Nioro, L: Lompoul, S: Sinthiou Garba, K: Kolda , T: Tamba, HC : control samples and SC: Under Eucalyptus samples. a) Factor map of subtracts b) Factor map of SIR responses of soils.*

Comparison of the bacterial community from *Eucalyptus* sites and control displayed distinct profiles on the DGGE gel (*Fig. 4*). The UPGMA dendrograms revealed that the structures of the bacterial communities from the young plantations were similar (similarity coefficient between 70 and 85%) to that from the old plantations (similarity coefficient 60%) (*Fig. 4a* and *4b*).

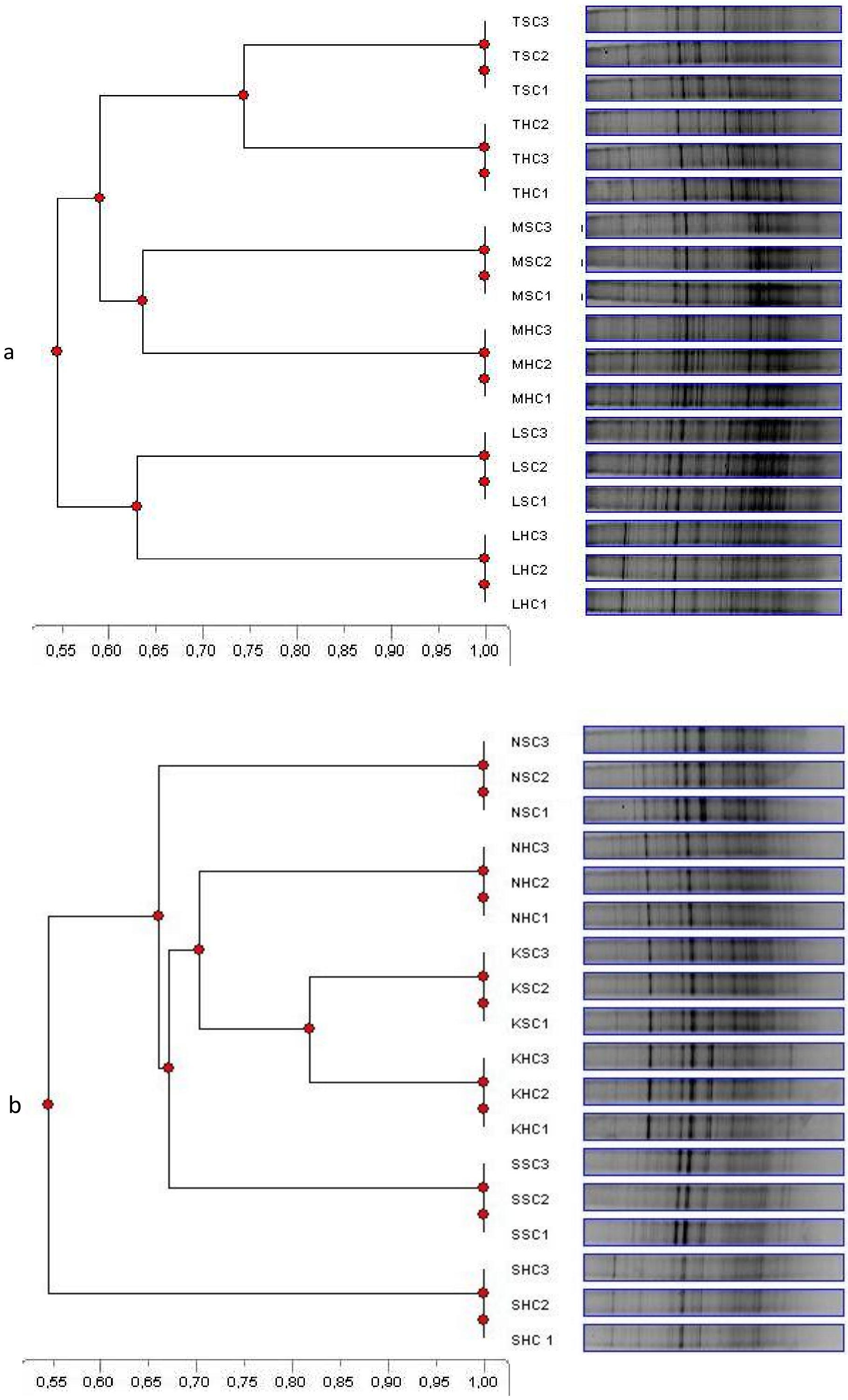

Figure 4. *Dendrograms comparing the DGGE profiles of bacterial communities of soil sampling in HC and SC of E. camaldulensis plantations. L, M, S, T, K, N; HC: and SC are the same as in figure 3. Number 1, 2 and 3 correspond to three replications of each sample*

There were positive and significant correlations between microbial biomass and soil pH (R^2=0.87, $p<0.05$) and between MB and alkaline phosphatase (R^2=0.88, $p<0.05$). Also, positive correlations were found between soil total polyphenols content and acid phosphatase (R^2=0.65, $p<0.05$). In contrast, significant negative correlation existed between catabolic evenness (E) and total polyphenols content (R^2 = - 0.618, $p<0.05$) (*Table 5*).

Table5. *Correlation analysis between different variables measured in soils*

Variables	Desy	Ac. phos.	Al. phos.	BM	pH	Total poly	E	H
Déshy	**1**							
Ac. phos.	**0,477**	**1**						
Al. phos.	**0,710**	0,563	**1**					
BM	**0,616**	0,329	**0,881**	**1**				
pH	0,393	0,202	**0,792**	**0,868**	**1**			
poly	0,208	**0,654**	0,377	0,342	0,054	**1**		
E	-0,391	**-0,824**	**0,697**	-0,517	-0,351	**-0,618**	**1**	
H	-0,301	-0,171	-0,278	-0,083	0,139	0,387	-0,061	**1**

Data values are different from 0 at significance level alpha = 0.05. Abbreviations: Al. phos: alkaline phosphatase, Ac. phos: acid phosphatase, Desy: dehydrogenase, Total poly: total polyphenol content.

Discussion

Ours results showed that *Eucalyptus* modified soil properties by acidification and /or accumulation of antimicrobial compounds such as phenols. In intermediate plantations, high levels of polyphenol in soils resulted from litter accumulation because plants were at the shorter spacing (observed during the sampling). In old and young plantations acidification seemed most obvious. This acidification of soils under *Eucalyptus* could be caused by an accelerated extraction of basic cations by this fast-growing tree species, especially when extraction was not offset by fertilization. Indeed, a recent study reported an acidification of surface water due to soil depletion of cations when watershed and pasture were replaced by *Eucalyptus* plantations (Farley et al., 2008). *Eucalyptus* litter can also release many acidic compounds. According to Swift et al. (1979), decreasing pH in decaying litter was mainly due to the leaching of acidic material from the vacuoles. This acidifying effect was also reported by a set of studies conducted in Uruguayon an experimental site, ten years after the original vegetation was replaced by *Eucalyptus* plantation (Durán et al., 2001; Pérez Bidegain et al., 2001; Sicardi et al., 2004).

The consequences of these soil chemical modifications were the decline in microbial biomass, activity and diversity. In fact, the results obtained from the current study showed low microbial biomass and low catabolic capacity and low diversity (Shannon index and Evenness) under *Eucalyptus* soil. Similar results were obtained on *Gmelina arborea* Roxb and *E. camaldulensis*, two exotic species, by Sanon et al. (2006)

and Kisa et al. (2007) respectively. These authors have shown that the exotic plants considerably alter soil properties by modifying both microbial community structure and functional diversity.

Ours results showed that acidification was not proportional to plantation age and its effect on microbial activity and biomass could be different even within the same age plantation. This difference could be related to soil texture which unfortunately was not measured in this study. But according to early studies, the soil of Matam (intermediate plantation) was a loam clay soil (Maymard, 1962) and was closed to a river, thus releasing few cations so little subject to acidification. Thus soil pH was highest (pH 6.87) in Matam. Whereas Nioro is a ferruginous soil (Pieri, 1969), Sinthiou and Lompoul are sandy (Maymard, 1962). These soils were more susceptible to acidification. Highest pH in Matam site compared to other plantations could explain highest enzymatic activities and microbial biomass.

Low microbial biomass and activity found under *Eucalyptus* plantation could be due to the toxic impact of harmful allelochemical compounds (including polyphenols) released from the *Eucalyptus* leaf litter. Negative correlation between catabolic Evenness (E) and total polyphenol contents suggests that phenols reduce microbial activity. Previously, Dellacassa et al. (1989) have reported antimicrobial activity of *Eucalyptus* leaf extract toward pure culture of soil bacteria. Consistently, our study also reported high rates of total polyphenols under *Eucalyptus* soil samples compared to adjacent soils. Polyphenols are known for their negative role in the mineralization process of organic matter either by complexing the protein nitrogen which would make it inaccessible to microorganisms or by the inhibition of microorganisms or enzymes responsible for the transformation of ammonium nitrate (Mangelot and Toutet, 1980; Duponnois et al., 2001; Diallo et al., 2006). The results of Blum and Shafer (1988) and Diallo et al. (2006) showed that the effect of phenols depend on the phenolic acid composition rather than concentration. According to these authors, acidic phenolic compounds have more negative effects alkaline than phenolic. The determination of soil phosphatase activities indicated less bacteria activity under *Eucalyptus* (evidenced by alkaline phosphatase) than in adjacent samplings. Furthermore, high fungal activity (evidenced by acid phosphatase) was found in samples under *Eucalyptus*. This change in soil microorganism activities could be related to changes in pH induced by *E. camaldulensis*. Similar results have previously been documented by Bradget et al. (1993) who showed that acidification increased abundance of fungal communities in the Boreal forest, whereas bacteria increase in alkaline soils. Our findings thus support previous observations suggesting that soil microbial communities composition is greatly controlled by pH as shown by the strong and positive correlation between pH and MB.

Changes (acidifying effects and/or high polyphenol content) mediated by *E. camaldulensis* in soils can reduce catabolic groups and/or soil catabolic ability. Indeed, *E. camaldulensis*, by its root exudates and litter fall select microorganisms able to use its carbon residues and support its acidifying effect. Meyer (1994) and Kourtev et al. (2003) demonstrated that exotic species promote changes in functional groups within the microbiota. Similar results were reported by Saetre and Bååth (2000) and Yao et al. (2000) which showed that changes in the ratio of Gram-negative to Gram-positive bacteria were related to quality of organic matter in the soil under exotic species. Recent studies that have used BIOLOG (Yu et al., 2005), noted great modifications in catabolic activity forms of soil bacterial community with 31 carbon resources in soils treated by *Eupatorium adenophorum* (an exotic invasive species) root water extract. In our study, the combined effect of low pH and litter accumulation could explain the lack of herbaceous layer noted under *E. camaldulensis*.

Conclusion

The results showed that *Eucalyptus* cultivation negatively impacted on soils at all sites regardless of the age of the plantation. Intermediate plantation appeared to be characterized by high levels of phenols, while in young and old plantations pH effect was dominant. In both situations, this had a negative effect on microbial biomass, activity and diversity.

Among all the measured indicators, acidification, phosphatases activities and the accumulation of phenols were the most common observations in all sites. The effect of *E. camadulensis* would start with changes in these parameters, which will in turn affect microbial communities by changing their biomass, structure and/or catabolic diversity. The synergistic effect of these changes in soil might resulted in a novel ecological niche in soil which is less favorable to herbaceous plants growth and might probably induce the lack of herbaceous layer under *Eucalyptus* plants. Our study thus supports the existence of environmental hazards that may result from the use of this exotic plant for reforestation purposes. The present findings should therefore be of great importance for reforestation programs challenging plant biodiversity preservation.

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REFERENCES

[1] Anderson, J.P.E., Domsch, K.H. (1978): A physiological method for measurement of microbial biomass in soils. - Soil Biology and Biochemistry 10: 215-221

[2] Aubert, G. (1978) : Méthodes d'Analyse des sols. - Edition CRDP, Marseille, p. 360.

[3] Artursson, V., Finlay, R.D., Jansson, J.K. (2006): Interactions between arbuscular mycorrhizal fungi and bacteria and their potential for stimulating plant growth. - Environmental Microbiology 8: 1-10. doi:10.1111/j.1462-2920.2005.00942.x

[4] Bardgett, R.D., Frankland, J.C., Whittaker, J.B. (1993): The effects of agricultural practices on the soil biota of some upland grassland. - Agriculture, Ecosystems & Environment 45: 25-45.

[5] Bernhard-Reversat, F., Loumeto, J.J. (2002): The litter system in African forest tree plantations. - In : Vikram Reddy M, Management of tropical plantation forests and their soil litter system. -Science Publishers, Inc. Enfield (NH, USA) 11-39.

[6] Bernhard-Revesat, F. (1987) : Les cycles des éléments minéraux dans un peuplement de *Acacia seyal* et leur modification en plantation de *Eucalypus* au Sénégal. - Acta oecologica, Oecalogia generalis 8 : 3-16.

[7] Berthelot, A., ranger, J., Gelhave, D. (2000): Nutrient uptake and immobilization in short-rotation coppice stand of hybride poplars in North-West France. - Forest Ecology and Management 128: 167-179.

DOI: http://dx.doi.org/10.15666/aeer/1403_001019

[8] Biró, B., Köves-Péchy, K., Vörös, I., Takács, T., Eggenberger, P., Strasser, R.J. (2000): Interrelations between *Azospirillum* and *Rhizobium* nitrogen fixers and arbuscular mycorrhizal fungi in the rhizosphere of alfalfa in sterile AMF free or normal conditions. - Applied Soil Ecology 15: 159-168.

[9] Blum, U., Shafer, S.R. (1988): Microbial populations and phenolic acids in soil. - Soil Biology and Biochemistry 20: 793-800.

[10] Bouillet, J.P., Nizinski, G., Nzila, J.D., Ranger, J. (1997): The sustainability of Eucalyptus commercial plantation : The Congolese approach. - In: Proceedings IUFRO Meetings 4: 232-237.

[11] Bouillet, J.P., Bernhard-Reversat, F. (2001): General objectives and sites. In: Bernhard-Reversat, F(Ed), Effect of Exotic tree plantation on Plant Diversity and Biological Soil Fertility in the Congo Savanna: A Reference to Eucalyptus. - CIFOR, Bogor, Indonesia, pp.13-21.

[12] Cannell, M.G. (1999): Environmental impacts of forest monocultures: water use, acidification, wildlife conservation, and carbon storage. - New Forests 17(1): 239-262.

[13] Casida, L.E., Klein, D.A., Santoro, T. (1964) : Soil dehydrogenase activity. - Soil Science 98: 371-376.

[14] Cossalter, C., Pye-Smith, C. (2003): Fast-wood Forestry: Myths and Realities. - Center for international Forest Research, Indonesia.

[15] Degens, B.P., Harris, J.A. (1997): Development of a physiological approach to measuring the catabolic diversity of soil microbial communities. - Soil Biology and Biochemistry 9:1309-1320.

[16] Degens, B.P., Schipper, L.A., Sparling, G.P., Duncan, L.C. (2001): Is the microbial community in a soil with reduced catabolic diversity less resistant to stress or disturbance. - Soil Biology and Biochemistry 33: 1143-1153.

[17] Diallo, M.D., Duponnois, R., Guisse, A., Sall, S., Chotte, J.L., Thioulouse, J. (2006): Biological effects of native and exotic plant residues on plant growth, microbial biomass and N availability under controlled conditions. - European Journal of Soil Science 42: 238-246.

[18] Dellacassa, E., Mendez, P., Cerdeiras, P., Moyna, P. (1989): Antimicrobial activity in *Eucalyptus* essential oils. - Fitoterpia 60: 544-546.

[19] Dick, R.P., Breakwill, D.,Turco, R. (1996): Soil enzyme activities and biodiversity measurements as integrating biological indicators. - In: Doran, J.W., Jones, A.J. (Eds.), Handbook of Methods for Assessment of Soil Quality. Soil Sci Society of America Specific Publications, Madison, WI: 242-272.

[20] Duponnois, R., Chotte, J.L., Sall, S.N., Cadet, P. (2001): The effect of organic amendments on the interaction between a nematophagous fungus *Arthrobotrys oligospora* and the roots knot nematodes *meloidogyne mayaguensis* parasitizing tomato plants. - Biology and fertility of soils 34: 1-16.

[21] Durán, A., García-Préchac, F., Pérez Bidegain, M., Frioni, L., Sicardi, M., Molteni, C., Bozzo, A. (2001): Propiedades físicas, químicas y biológicas. Cap. 2.5. Suelos y Vegetación. - In: Informe Final, Proyecto Monitoreo ambiental de plantaciones forestales en Uruguay, Convenio UDELAR-División Forestal MGAP-Banco Mundial.

[22] Eivazi, F., Tabatabai, M.A. (1977): Phosphatases in soil. - Soil Biology and Biochemistry 9: 67-172.

[23] Fall, S., Nazaret, S., Chotte, J.L., Brauman, A. (2004): Bacterial density and community structure associated with aggregate size fractions of soil-feeding termite mounds. - Microbial Ecology 48: 191-199.

[24] Farley, K.A., Pineiro, G., Palmer, S., Jobbagy, E., Jackson, R. (2008): Stream acidification and base cation losses with grassland afforestation. - Water Research 44

[25] Giffard, P.L. (1969):Recherches effectuées sur les Eucalyptus. - Rapport d'activité du CTFT au Sénégal.

[26] Hamel, O., Bailly, C.R. (1981) : Premières observations sur les potentialités de l'Eucalyptus au Sénégal. - Contribution volontaire au Congrès IUFRO de Kyoto – Japon

[27] Hooper, D.U., Bignel, D.E., Brown, V.K., Brussaard, L., Dangerfield, J.M., Wall, D.H., Wardle, D.A., Coleman, D.C., Gille, K.E., Lavelle, P., van der Putten, W.H., de Ruiter, P.C., Rusek, J., Silver, W.L., Tiedje, J.M., Wolters, V. (2000): Interactions between aboveground and belowground biodiversity in terrestrial ecosystems: patterns, mechanisms, and feedbacks. - Bioscience 50: 1049-106.

[28] Kisa, M., Sanon, A., Thioulouse, J., Assigbetse, K., Sylla, S., Spichiger, R., Dien, L., Berthelin, J., Prin, Y., Galiana, A., Lepage, M., Duponnois, R. (2007): Arbuscularmycorrhizal symbiosis can counterbalance the negative in£uence ofthe exotic tree species *Eucalyptus camaldulensis* on the structure and functioning of soil microbial communities in a sahelian soil. - FEMS Microbiology Ecology 62: 32-44.

[29] Kennedy, A.C. (1998): The rhizosphere and spermosphere - In: Sylvia, D.M., Fuhrmann, J.J., Hartel, P.G., Zuberer, D.A. (Eds.) Principles and Applications of Soil Microbiol., Prentice-Hall, Englewood Cliff, NJ, pp. 389-407.

[30] Kourtev, P.S., Ehrenfeld, J.G., Haeggblom, M. (2003): Experimental analysis of the effect of exotic and native plant species on the structure and function of soil microbial communities. - Soil Biology and Biochemistry 35: 895-905.

[31] Laclau, J.P., Ranger, J., Deleporte Nouvellon, Y., Saint-André, L., Marlet, S., Bouillet, J.P. (2005): Nutrient cycling in a clonal stand of Eucalyptus and an adjacent savanna ecosystem in Congo 3. Input–output budgets and consequences for the sustainability of the plantations. - Forest Ecology and Management 210: 375-391.

[32] Laclau, J.P., Toutain, F., Thongo, M'bou, A., Arnaud, M., Joffre, R., Ranger, J. (2004): The function of the superficial root mat in the biogeochemical cycles of nutrients in Congolese Eucalyptus plantations. - Annals of Botany 93: 249-261.

[33] Macheix, J.J., Fleuriet, A., Billot, J. (1990): Fruit phenolics. - CRC Press: Boca Raton, Florida.

[34] Mangelot, F., Toutet, F. (1980) : Les litières. - In : Pesson, P. (Ed.) Actualités d'Ecologie Forestière, Gauthier-Villars, Paris, pp. 3-59.

[35] Maymard, J. (1962) : Les sols de la région de Matam. - SOGETHA, Paris (France), Vol.2. 166p.

[36] Muyzer, G., de Wall, E., Uitterlinden, A. (1993): Profiling of complex microbial populations by DGGE of PCR-amplified genes coding for 16S rRNA. - Applied Environmental Microbiology 59: 695-700

[37] Meyer, O. (1994): Functional groups of microorganisms. - In: Schulze, E.D., Mooney, H. (Eds.) Biodiversity and Ecosystem Function, Springer-Verlag, Berlin, pp. 67-97.

[38] Nannipieri, P., Eldor, P. (2009): The chemical and functional characterization of soil N and its biotic components. - Soil Biology and Biochemistry 41: 2357-2369.

[39] Olsen, S.R., Cole, C.V., Watanabe, F.S., Dean, L.A. (1954) : Estimation of available phosphorus in soils by extraction with sodium bicarbonate. U.S. Department of Agriculture circular, vol. 939. U.S. Department of Agriculture,Washington, DC. p. 19.

[40] Pérez Bidegain, M., Garcìa-Préchac, F., Durán, A. (2001): Soil use change effect from pastures to *Eucalyptus* spp., on some soil physical and chemical properties in Uruguay. - In: Proceedings of the 3rd International Conference on Land Degradation, Rio de Janeiro, in CD-ROM.

[41] Pieri, C. (1969) : Etude pédologique de la région de Nioro du Rip au 1/50.000. - IRAT, Dakar, Sénégal.

[42] Powlson, D.S., Brookes, P.C., Christensen, B.T. (1987): Measurement of soil microbial biomass provides and early indication of chances in total soil organic matter due to straw incorporation. - Soil Biology and Biochemistry 19: 159-164.

[43] Rhoades, C., Binkley, D. (1996): Factors influencing decline in soil pH in Hawaiian *Eucalyptus* and *Albizia* plantations. - Forest Ecology and Management 80: 47-56.

[44] Saetre, P., Bååth, E. (2000): Spatial variation and patterns of soil microbial community structure in a mixed spruce-birch stand. - Soil Biology and Biochemistry 32: 909-917.

[45] Sanon, A., Martin, P., Thioulouse, J., Plenchette, C., Spichiger, R., Lepage, M., Duponnois, R. (2006): Displacement of an herbaceous plant species community by mycorrhizal and non-mycorrhizal *Gmelina arborea*, an exotic tree, grown in a microcosm experiment. - Mycorrhiza 16: 125-132.

[46] Sicardi, M., García-Préchac, F., Frioni, L. (2004): Soil microbial indicators sensitive to land use conversion from pastures to commercial *Eucalyptus grandis* (Hill ex Maiden) plantations in Uruguay. - Applied Soil Ecology 27: 125-133.

[47] Simpson, E.H. (1949): Measurement of diversity. - Nature 163: 688.

[48] Singleton, V.L., Rossi, J.A. (1965): Colorimetry of total phenolics with phosphomolybdic–phosphotungstic acid reagents. - American Journal of Enology and Viticulture 16: 144-158.

[49] Swith, M.J., Heal, D.W., Anderson, J.M. (1979): Decomposition in Terrestrial Ecosystems. - Blackwell Scientific publications, Oxford.

[50] Thioulouse, J., Chessel, D., Dolédec, S., Olivier, J.M. (1997): ADE-4: a multivariate analysis and graphical display software. - Statistics Computing 7: 75-83.

[51] Turnbull, J.W. (1999): Eucalyptus plantation. -New Forest 17: 37-52.

[52] Vivas, A., Moreno, B., del Val, C., Macc, C., Masciandaro, G., Benítez, E. (2008): Metabolic and bacterial diversity in soils historically contaminated by heavy metals and hydrocarbons. - Journal of Environmental Monitoring 10: 1287-1296.

[53] Vivas, A., Moreno, B., Garcia-Rodriguez, S., Benitez, E. (2009): Assessing the impact of composting and vermicomposting on bacterial community size and structure, and microbial functional diversity of an olive-mill waste. - Bioresource Technology 100: 1319-1326.

[54] Wardle, D.A. (2002): Communities and Ecosystems: Linking the aboveground and belowground components. Monographs in Population Biology. - Princeton University Press, Princeton, USA. 34-392.

[55] Yao, H., He, Z., Wilson, M.J., Campbell, C.D. (2000): Microbial biomass and community structure in a sequence of soils with increasing fertility and changing land use. - Microbial Ecology 40: 223-237.

[56] Yu, X., Yu, D., Lu, Z., MA, K. (2005): A new mechanism of invader success: Exotic plant inhibits natural vegetation restoration by changing soil microbe community. - Chinese Science Bulletin 50: 1105-1112.

[57] Zinn, Y.L., Resck, D.V.S., Da Silva, J.E. (2002): Soil organic carbon as affected by afforestation with *Eucalyptus* and *Pinus* in Cerrado region of brazil. - Forest Ecology and Management 166: 287-294.

SOIL CARBON DIOXIDE EFFLUX RESPONDS TO LAND-USE CHANGES IN THE SUB-URBAN REGIONS OF NANJING, CHINA

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Abstract. The aim of this study was to assess the influence of urbanization on soil carbon dioxide efflux. We investigated how the soil environment affects the soil carbon dioxide efflux in native grassland, residential lawns and agricultural land. Continuous measurements of the soil carbon dioxide efflux were made once a week for a 10-week period during July-September 2013 in Nanjing China. Our results demonstrated that the soil carbon dioxide efflux significantly decreased during the10-week period in the three land-use types. Soil carbon dioxide efflux in urban lawns was the highest among the three land-use types in the following order: residential land > agricultural land > native grassland. The soil water-soluble organic carbon (WSOC), the labile organic carbon (LOC), and the basal respiration, as covariates, did not significantly influence the soil carbon dioxide efflux. The soil temperature and land-use type had significant contributions to the carbon efflux. A higher soil temperature in the lawns was attributed to the urban heat island effect. Our data suggest that human-induced land-use changes, such as urbanization and agricultural practice, could enhance the local soil carbon dioxide emissions, especially when urban development increased the local soil temperature.

Keywords: *backward elimination stepwise linear regression, soil carbon dioxide efflux, soil moisture, soil temperature, urbanization*

Introduction

Soil carbon dioxide efflux is a major contributor to the carbon emission from the terrestrial ecological system into the atmosphere and is an important source of atmospheric carbon dioxide (Hou, 2011) that is associated closely with the global terrestrial-atmospheric carbon exchanges. Approximately 25% of global carbon emissions is derived from land-use change and is the second largest source of human-induced greenhouse gas emissions (Houghton, 2003; Zheng et al., 2011). To date, urbanization is increasing in developing countries, especially in China, and is now one of the burgeoning types of land-use change that is altering ecosystem processes (Pickett et al., 2011; Siciliano, 2012). The urbanization process can change the natural and agricultural landscapes into urban commercial, industrial, greenbelt, and residential uses, thereby altering the carbon stocks and carbon fluxes through the destruction of habitat, climate change, and urban pollutant deposition (Pouyat et al., 2002; Fenn et al., 2003; Trusilova and Churkina, 2008; Duan et al., 2013). Thus, a question of increasing interest is how urban land-use impacts ecosystem processes, such as soil carbon cycling.

In fact, the influences of urbanization on soil carbon dioxide efflux depend on climatic conditions, the urban land-use and vegetation types. Koerner and Klopatek (2010) found that the soil respiration rates along an urban–rural gradient in a desert landscape in Phoenix, Arizona. The research on carbon dynamics in urban and rural forests in humid Baltimore demonstrated that soil respiration rates were higher at the

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urban sites (Groffman et al., 2006). Kaye et al. (2005) reported that urban ecosystems had dramatically altered the soil respiration compared with native grasslands and agricultural ecosystems. Similarly, Bowne and Johnson (2013) observed that the carbon dioxide efflux of the residential lawns was significantly higher than that of the corn fields in Elizabethtown, PA. Obviously, the spatial–temporal variability of the soil carbon is controlled by the soil temperature, soil moisture, photosynthetic nutrition supply to roots, and substrate availability (Balogh et al., 2011). Sheng et al. (2010) found that soil respiration rates were significantly reduced following the conversion of natural forest to agricultural land in subtropical China, which was explained by the decrease of the topsoil organic carbon and the light-fraction organic carbon storages. For two decades, however, most of studies on the effects of urbanization on carbon dynamics have mainly focused on temperate urban ecosystems (Carlisle et al., 2006; Kellman et al., 2007; Raciti et al., 2011); little is known regarding the soil carbon dioxide efflux response to land-use changes in tropical–subtropical urban ecosystems.

Therefore, we investigated: the potential influence of urbanization on carbon dynamics by monitoring the soil carbon dioxide efflux in subtropical urban ecosystems, such as the residential lawns, native grasslands and agricultural fields in Nanjing, China. More specifically, the objective of this study was to investigate: a) the soil carbon dioxide efflux differences among three land-use types in the suburban environment and b) the main factors influencing the soil carbon dioxide efflux. Based on previous research on the variations of the soil carbon dioxide efflux in urban ecosystems, we hypothesized that the soil carbon dioxide efflux in residential lawns would be higher than that in the adjacent agricultural land and native grassland due to the urban heat effect.

Materials and methods

Study area and field experiment

The field experiment was conducted from July to September 2013 in the Xianlin Campus of the Nanjing Sport Institute (32°6′45.3″ - 32°7′35.6″N, 118°57′51.5″ - 118°58′34.8″E), which is located in the northeast of Nanjing, China *(Fig. 1)*. Nanjing, with a total land area of 6,598 square kilometers, is situated in one of the largest economic zones of China, the Yangtze River Delta, which is part of the downstream Yangtze River drainage basin. With a total population of 8.16 million and an urban population of 6.55 million, Nanjing is the second-largest commercial center in the East China region, after Shanghai. A maritime subtropical monsoon climate covers this region, which has a mean annual air temperature of 18.7 °C and a mean annual rainfall of 1106 mm. A typical urban and agricultural land-use change sequence in this region was chosen, including residential lawns, agricultural land, and native grassland, which allows us to compare the undisturbed soil and the disturbed soil. Three fields of each land-use type were studied, and the distance between these fields ranged from 250 to 1400 meters. The native grassland has had an absence of human mediated disturbance for at least 30 years. The native grassland was covered mainly by native herbaceous species, such as *Humulus scandens* (Lour.) Merr (45%), *Conyza canadensis* L. (25%), *Juncus effuses* L. (18%) and *Solidago altissima* (12%). Agricultural land was in rotation with wheat (*Triticum aestivum* L.) and rape (*Brassica campestris* L.). Approximately 225-300 kg ha^{-1} of nitrogenous fertilizer was applied to the rape field during the cultivation, and the wheat field was fertilized using approximately 180-215 kg ha^{-1} of nitrogenous fertilizer. Our experiment began approximately two weeks after the harvest of the wheat. During the study, the agricultural fields were no-till. The agricultural fields have been in cultivation for over 30 years since conversion from native grassland. To study the impact of urban land-use, three 10-year old residential lawn fields were selected for the study, which were transformed from agricultural fields. These lawns were treated with nitrogenous fertilizer at a rate of approximately 150-225 kg ha^{-1} each March. Moreover, manila grass（*Zoysia japonica*）and tall fescue grass（*Festuca arundinacea*）were planted in the lawns that were mowed once a month, after which the residue was removed from the lawns. These land-uses are similar in topography and soil type, with elevations varying from 19.16 to 24.88 m. The loam soil at the experimental site is a Haptic Luvisol derived from the Xiashu loess. None of the study sites were irrigated because the study occurred during the rainy season in Nanjing.

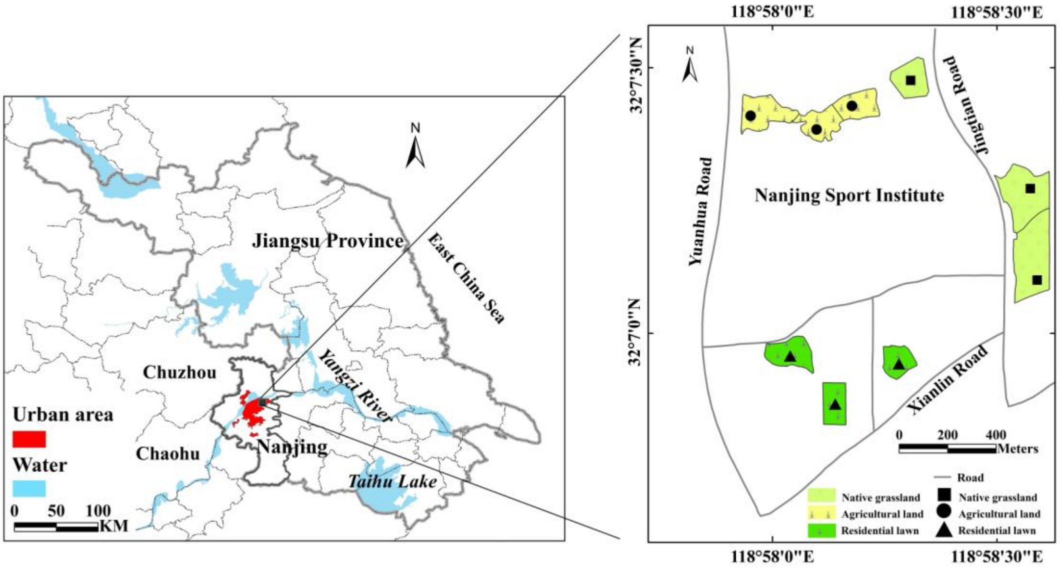

Figure 1. *Geographic location and land-use situation of the study site*

Soil carbon dioxide efflux, temperature, and moisture measurements

Three polyvinyl chloride (PVC) collars (11-cm inner diameter and 20-cm height) were randomly inserted into the soil at each location for continuous soil carbon dioxide efflux measurement throughout the entire study period. The collars were inserted in the soil of a 10-cm depth to monitor the soil carbon dioxide efflux. Sampling areas in the agricultural fields were of soil between the wheat rows. The vegetation inside the collar was clipped to the soil surface to avoid the interference of aboveground plant respiration during measurements. Meanwhile, we attempted to not disturb the soil to minimize root disturbance and soil porosity. We measured the soil carbon dioxide efflux at three locations per field, for a total of 27 measurements once a week on a sampling day from 11 July to 12 September 2013. The measuring time per field was over a 7 min period. Measurements in three land-use types were performed in random visits to each site between 8:00 and 11:30 (Beijing Time) in the absence of rain. For determining the daily variation of soil respiration, three fields per land-use type were measured continuously every 15 min between 8:00-20:00 (Beijing Time) on 22 September 2013. The soil carbon dioxide efflux and temperature were measured using a Leaf Chamber & Soil Respiration Analysis System (LCpro$^+$, ADC Bioscientific Ltd.) with a 10 -cm temperature probe. Soil moisture was measured using a Time Domain Reflectometry unit (Model TRIME-PICO, Germany) at a 0-10 cm depth at each location (Zhu et al., 2012).

Soil property determinations

Three randomized topsoil samples (0-10 cm) in each land-use field were collected using a sampling ring knife for soil property determinations on August 27, 2013. The soil bulk density (0-10 cm) was measured using the cutting ring method (100 cm^3) according to Sheng et al. (2010). Approximately 500-g subsamples were air-dried at room temperature, homogenized, and then ground in an agate mortar and sieved to 2 mm, 0.25 mm and 0.149 mm for further analysis. Total carbon and nitrogen in each of the 27 samples were determined by the elemental combustion of 25.0 mg of dried 0.149-mm sieved soil using a CHN elemental analyzer (Heraeus Elementar Vario EL, Germany) in Modern Analysis Center of Nanjing University. Labile organic carbon (LOC) was determined using the modified method described by Blair et al,. (1995). Approximately 15 g of the 0.25-mm sieved soil was added into 30-mL plastic centrifuge tubes with 25 mL of 333 mmol L^{-1} $KMnO_4$. The centrifuge tubes were tumbled for 1 h at 25 °C, centrifuged for 5 min at 2000 rpm. The supernatants were diluted with deionized water (v/v, 1:250). The absorbance of the diluted samples was read on a split beam spectrophotometer at 565 nm. Water-soluble organic carbon (WSOC) and basal respiration were analyzed according to Wei et al. (2014), with a minor modification. The soil sample (15 g of 2 mm sieved) was mixed with 30 mL of distilled water, shaken for 2 h at 25°C, and then centrifuged at 5000 rpm at 4°C for 15 min. The supernatant was filtered through a 0.45-µm carbon-free membrane for water-soluble organic carbon (WSOC). Regarding basal respiration, approximately 50 g of soil (2 mm sieved) were added into plastic bottles (500 mL). The soil water content was adjusted to 60% of full water holding capacity and then pre-incubated in the dark for 7 days at 25 ˚C to restore the microbial activity for soils. Next, in each jar, a beaker with 10 mL of 0.6 mol L^{-1} NaOH was placed to absorb the evolved carbon dioxide during the incubation, and the solution of NaOH was renewed after 1, 2, 3, 4, 5 and 6 d. The carbon dioxide trapped in the NaOH was determined by back-titration of excess NaOH with 1.5 mol L^{-1} after precipitation with 1 mol L^{-1} $BaCl_2$. Blank samples containing no soil and samples of a standard soil were analyzed in each run. The calculation of the basal respiration was determined using the day average of the 7-day period cumulative CO_2 efflux (mg CO_2-C kg^{-1} d^{-1}). To determine the influence of the surrounding land-use on soil temperature, we extracted the information urban land-use within 175 m of each experimental site from the Second National Land Survey Dataset of China to calculate the area of developed land-use in a geographic information system (ArcGIS 10.0, ESRI) according to Gunawardhana and Kazama (2012).

Data analysis

The temporal-variability data of the soil carbon dioxide efflux were analyzed using a repeated measures general linear model (GLM). The weekly differences of the mean soil carbon dioxide efflux, soil temperature and soil moisture for three land-use types soil were analyzed using the respective GLM. The soil carbon content, nitrogen content, water-soluble organic carbon (WSOC), labile organic carbon (LOC), and basal respiration acted as covariates and were added into the GLM. The influence of the soil temperature, soil moisture and land-use types on the soil carbon dioxide efflux, independent of time, was analyzed using backward elimination stepwise linear regressions. The soil carbon dioxide efflux was $\log_{10}$ transformed because the soil carbon dioxide efflux did not correlate linearly with the soil temperature. The differences of the soil carbon content, nitrogen content, water-soluble organic carbon (WSOC), labile organic carbon (LOC), and basal respiration across three land-use types were compared by factorial ANOVA with $\alpha = 0.05$ (Duncan's multiple range test). All of the statistical analyses were performed in SPSS 19.0 (IBM SPSS, New York) and SigmaPlot 12.0 (Systat Software Inc., San Jose, CA).

Results

Soil Properties

As presented in *Table 1*, the soil carbon and nitrogen contents did not significantly differ ($F_{2,24} = 2.432$, $p = 0.109$; $F_{2,24} = 1.449$, $p = 0.255$) in the samples from the lawns, agricultural lands and native grasslands. The soil labile organic carbon (LOC), water-soluble organic carbon (WSOC) and basal respiration ($F_{2,24} = 3.911$, $p = 0.034$; $F_{2,24} = 6.670$, $p = 0.005$; $F_{2,24} = 6.954$, $p=0.004$, respectively) in agricultural lands and urban lawns were significantly lower than those in native grasslands. The soil carbon contents and nitrogen contents as covariates were incorporated into the GLM. The results suggested that the soil carbon contents ($F_{1,4} = 0.522$, $p = 0.510$) and nitrogen contents ($F_{1,4} = 6.270$, $p = 0.066$) had no significant influence on the soil carbon dioxide efflux, but they decreased the significance of land-use on efflux over time ($F_{2,4} = 41.986$, $p = 0.002$). LOC, WSOC and basal respiration as covariates were also added into the GLM. The results exhibited that LOC ($F_{1,3} = 6.439$, $p = 0.085$), WSOC ($F_{1,3} = 0.321$, $p = 0.610$) and basal respiration ($F_{1,3} = 8.364$, $p = 0.063$) did not significantly influence the soil carbon dioxide efflux, but it also reduced the effect of land-use on the efflux over time ($F_{2,3} = 5.841$, $p = 0.092$).

Table 1. *Selected soil (0–20 cm) and surrounding development characteristics of three land-use types (native grassland, agricultural land and residential lawns) in the study site of Nanjing, China. Values are means ± standard errors (n = 9), and the significant differences ($p < 0.05$) between the land-use types are noted using superscripts a, b, c. C = carbon; N = nitrogen; LOC = labile organic carbon; WSOC = water-soluble organic carbon.*

Experimental site description	**Land-use**		
	Native grassland	**Agricultural land**	**Residential lawns**
Bulk density (g cm^{-3})	1.34±0.07a	1.18±0.00b	1.40±0.01a
Carbon content (g kg^{-1})	11.50±0.40a	12.73±0.53a	11.40±0.19a
Nitrogen content (g kg^{-1})	1.13±0.02a	1.30±0.03a	1.13±0.05a
LOC (g kg^{-1})	2.56±0.11a	2.10±0.06b	2.16±0.17b
WSOC (mg kg^{-1})	144.91±4.57a	131.35±2.70b	124.64±4.44b
Basal respiration(mg CO_2-C kg^{-1} d^{-1})	15.50±0.48a	12.95±0.55b	13.71±0.43b
Impervious surface area（%）	4.46±0.72c	16.47±1.15b	30.49±1.27a

Soil carbon dioxide efflux

The soil carbon dioxide efflux of three land-use types significantly decreased over 10 weeks ($F_{9,\,54} = 5.453$, $p < 0.001$; *Fig. 2a*). The mean soil carbon dioxide efflux was significantly different among the three land-use types ($F_{2,\,6} = 25.745$, $p = 0.001$; *Fig. 2a*) in the following order: residential lawn (4.37±0.317 µmol co_2 m^{-2} s^{-1}) > agricultural land (2.9±0.354 µmol co_2 m^{-2} s^{-1}) > native grassland (1.21±0.164 µmol co_2 m^{-2} s^{-1}). The mean soil carbon dioxide efflux of residential lawns was 1.51 and 3.61 times higher than those of agriculture land and native grassland, respectively.

The diurnal carbon dioxide efflux of soil for three land-use types exhibited a unimodal pattern peaking at 13:00 (*Fig. 2b*). Compared to the native grasslands, the diurnal variation of soil respiration in lawns and agricultural were greater over the 12-hour measurement. In addition, the mean diurnal soil carbon dioxide efflux of lawns and agricultural fields was higher than that of native grasslands ($F_{2,\,6} = 33.395$, $p = 0.001$), as was also observed in the weekly measurements of the soil carbon dioxide efflux.

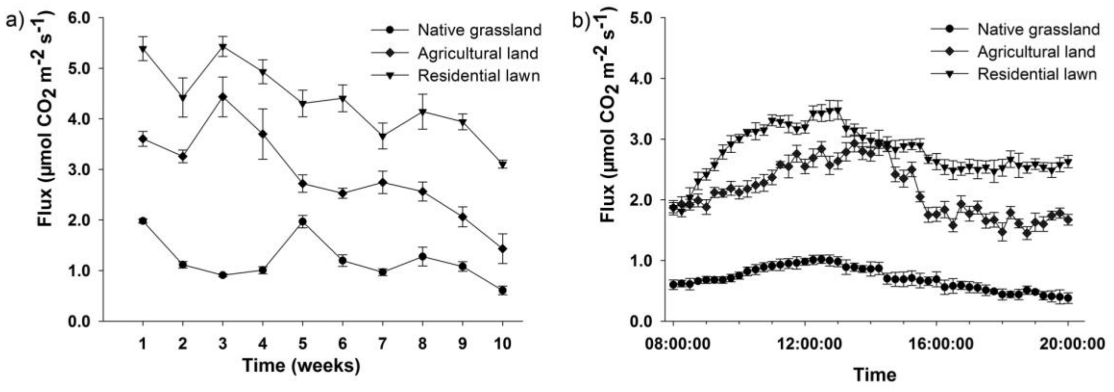

Figure 2. *Soil carbon dioxide efflux from native grassland, agricultural land, and residential lawn in Nanjing, China. Points are the means ± s.e. a) Means from each site type from 11 July to 12 September, 2013. b) Diurnal variation from 8:00 to 20:00 on 22 September 2013.*

Soil temperature and moisture for the three land-use types

The mean soil temperature of all land-use types decreased significantly over time ($F_{9,\,54} = 284.187$, $p < 0.001$; *Fig. 3a*), whereas the mean soil moisture generally increased during the duration of the experiment ($F_{9,\,54} = 35.773$, $p < 0.001$; *Fig. 3b*). Both the soil temperature and moisture for each of the three land-use types differed significantly from each other over the 10 weeks ($F_{2,\,6} = 218.374$, $p < 0.001$; $F_{2,\,6} = 27.229$, $p < 0.001$; *Fig. 3a, b*). Residential lawns had significantly higher soil temperatures compared to wheat and native grasslands over the duration of the study, while the soil moisture ranged between 20 % and 45 % among all land-use types, following the order: native grassland > residential lawn > agricultural land.

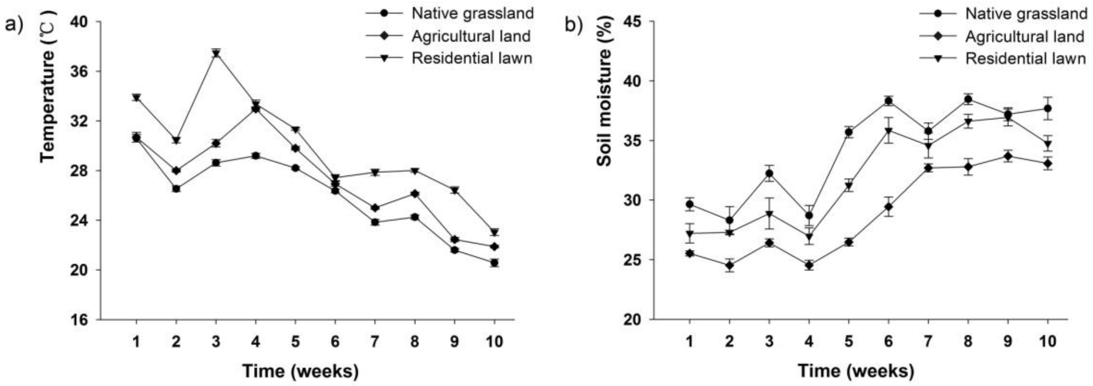

Figure 3. *Mean ± s.e. of (a) soil temperature and (b) soil moisture from the sites of native grassland, agricultural land, and residential lawns from July 11 to September 12, 2013 in Nanjing, China.*

Soil carbon dioxide efflux responses to land-use change

To investigate the multiple factors influencing soil respiration, a backward elimination stepwise linear regression model was used to analyze the influence of soil temperature, soil moisture and the land-use types on soil carbon dioxide efflux. Our results indicated that both land-use type and soil temperature had a significant influence on soil carbon dioxide ($R^2 = 0.783$, $F_{2,87} = 161.410$, $p < 0.001$). Based on the model, the soil carbon dioxide efflux was significantly influenced by the land-use type ($R^2 = 0.686$, $F_{1,88} = 192.101$, $p < 0.001$; *Fig. 4a*). The soil temperature significantly affected the soil carbon dioxide efflux ($R^2 = 0.396$, $F_{1,88} = 57.802$, $p < 0.001$; *Fig. 4b*). The soil moisture also significantly influenced the soil carbon dioxide efflux ($R^2 = 0.143$, $F_{1,88} = 14.815$, $p < 0.001$; *Fig. 4c*), but it reduced the significance of the multiple-factor regression model ($t= -0.884$, $p = 0.042$) after it was added into the model.

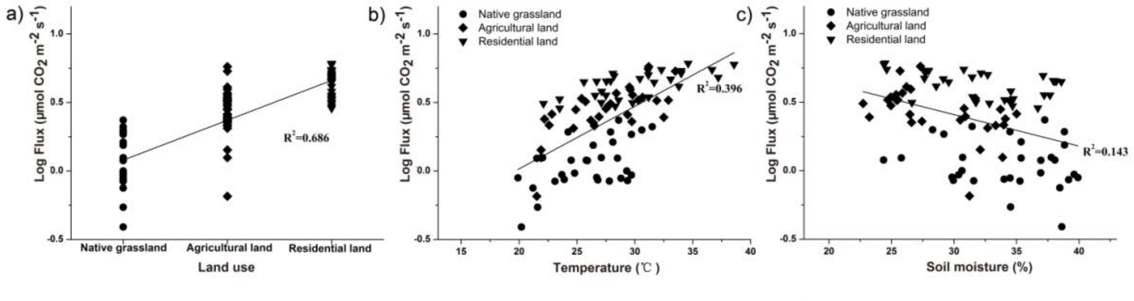

Figure 4. *Influence on soil carbon dioxide efflux of (a) land-use ($R^2 = 0.686$, $F_{1,88} = 192.101$, $p < 0.001$), (b) soil temperature ($R^2 = 0.396$, $F_{1,88} = 57.802$, $p < 0.001$), and (c) soil moisture ($R^2 = 0.144$, $F_{1,88} = 14.815$, $p = 0.010$). There is no significant influence from soil moisture to soil carbon dioxide; after soil moisture was added to the model, it reduced the significance of the model.*

Discussions

Anthropogenic land-use changes, such as urbanization, agricultural cultivation, and afforestation, often not only alter carbon stocks but also possibly alter carbon efflux

(Schimel et al., 1997). Most of the literature suggests that human-induced land-use change could reduce the carbon content in the soil (Davidson and Ackerman, 1993; Houghton, 1994; Murty et al., 2002; Zhao et al., 2012). However, in the present study, the results suggested that the soil carbon and nitrogen content were not significantly affected by urban or agricultural land-use and did not significantly influence the soil carbon dioxide efflux, according to the GLM (*Table 1*). The similarity in carbon and nitrogen concentrations among the three land-use types is likely because current management practices in lawns and wheat field includes fertilizer applications, and carbon is returned to agricultural soils through wheat straw residues (Tian et al., 2010). In the study by Bowne and Johnson (2013), the carbon concentrations between the lawns and corn fields did not significantly differ due to the increasing carbon input into the soil under no-till management (Powlson et al., 2011). The reason why the carbon and nitrogen content as covariates had no significant effect on soil carbon dioxide efflux after analysis with repeated measures was because the two covariates reduced the power from 0.999 to 0.987, resulting in an increase in the probability of committing a type III error. LOC, WSOC and basal respiration were significantly lower for residential lawns and native grassland, and these covariates also did not significantly influence the soil carbon dioxide efflux, according to the GLM (*Table 1*). The phenomena may be explained by the fact that higher soil microbe activity and available carbon fractions in non-till field increase the soil microbe respiration, but not enough to affect the total carbon dioxide efflux (Chu et al., 2006).

Meanwhile, we found that the mean weekly soil respiration rates of anthropogenic land-use changes (residential lawns and agricultural fields) in the subtropical city were significantly higher than those of native grasslands, which supports our hypothesis that soil carbon dioxide efflux in urban lawns would be highest (*Fig. 1*). The soils from urban lawns also had an increased soil carbon content relative to soil from corn fields in the temperate northeast United States, according to Bowne and Johnson (2013). Groffman et al., (2009) found an equal or higher efflux from managed lawns compared to forest soils in Baltimore, MD. Similarly, in arid and semiarid ecosystems, Koerner and Klopatek (2002) reported a greater carbon dioxide efflux in human-controlled ecosystems, such as lawns, golf courses, and agriculture, than in native ones in the Phoenix, AZ, metropolitan area.

Generally, the spatial-temporal variations of the soil carbon dioxide efflux results from a combined effect of root growth and soil environment characteristics, such as microorganisms, temperature, and moisture (Norman et al., 1997; Davidson et al., 1998). The soil temperature and soil moisture are the most important factors in the soil environment controlling the soil carbon effluxes (Raich and Potter, 1995; Koerner and Klopatek, 2002; Ni et al., 2012). In our study, residential lawn sites had the highest average temperature (*Fig. 4a*), which can explain why the soil carbon dioxide efflux was greater in residential lawns than in agricultural land and native grassland during the study period. In addition, the change of diurnal carbon dioxide efflux was also significantly affected by the diurnal variation of the soil temperature (*Fig. 2b*). A similar study demonstrated that the soil temperature had a strongly exponential positive correlation with the soil respiration rates for all land-use sites (Sheng et al., 2010). A higher soil temperature can stimulate the turf grass roots and enhance biological decomposition by microbes, thereby releasing carbon dioxide (McCulley et al., 2007; Kaur et al., 2010). An urban heat island effect contributes to the higher soil temperatures in residential lawns as a consequence of more intensive urban development (Gunawardhana and Kazama, 2012; Bowne and Johnson, 2013). In our study, the average area of the developed land was 30.5% within a 175-m radius of the lawn sites, which was significantly higher than that of the wheat (16.5%) and native grasslands (4.5%) (*Table 1*). The area of the developed land significantly affected the mean soil temperature ($F_{1,7} = 6.69$, $p = 0.036$; $r^2 = 0.489$). In addition to the temperature response, the soil carbon dioxide efflux was demonstrated to be significantly limited by the soil water content and was a crucial limiting factor, especially in the arid and semi-arid region (Shen et al., 2008; Makhado and Scholes, 2011). In this study, the soil water content varied significantly among the three land-use types (*Fig. 4b*), and therefore, the contributions of moisture to carbon dioxide efflux is significantly different in lawns, wheat fields and native grasslands. One probable cause of the discrepancy was the limited soil microbial diversity and activity and root respiration because of the low soil water content; in contrast, too much water in the soil pore space restricted or slowed diffusion of carbon dioxide efflux out of the soil profile (Cook and Orchard, 2008; Nusier and Rousan, 2008). However, our soil water content did not significantly influence the soil carbon dioxide efflux when incorporated into the multiple-factor regression model; the influence of soil water on soil respiration was probably offset by soil other factors (e.g., soil temperature and organic fractions). Furthermore, the soil active organic carbon fractions have been suggested as potential drivers for contrasting the microbial activities associated with the soil carbon influx between the land-use types (Wei et al., 2012). However, our results demonstrate that the soil temperature associated with land-use changes was the greatest sensitivity factor of soil respiration under the urbanization background. This result may be related with the effect of the study scales of the soil environment, including abiotic and biotic factors.

In China and at a global scale, urbanization processes, such as the conversion of agricultural fields to urban lawns, is a major component of environmental change (Siciliano, 2012). In our study, urban lawns had a higher soil carbon dioxide efflux during the growing season. Meanwhile, there is widespread concern that urbanization causes a net increase in the atmospheric carbon dioxide. Thus, the issue of the sequestration of carbon in agricultural and residential soils must be clarified to more fully understand the dynamics of local carbon cycling in an urban ecosystem. Several studies have evaluated the carbon sequestration potential of agricultural and urban soils at a local scale (Pouyat et al., 2002; Pataki et al., 2006; Lal, 2008). Zirkle et al. (2011) reported that home lawns in the USA can be a net sink for atmospheric carbon dioxide under all three evaluated levels of management practices (mowing, irrigating, and fertilizing). In addition, a study indicated that the soil nitrous oxide and carbon dioxide emissions in ornamental lawns during management were offset by the soil carbon sequestration according to Townsend-Small and Czimczik (2010). However, the impacts of urbanization on regional ecosystem functions and the global carbon cycle are not entirely clear (Pataki et al., 2006; Hutyra et al., 2011). Thus, future studies on the impacts of urbanization on soil carbon cycling explicitly including how land-use change impacts soil temperature and subsequently influences soil respiration should be investigated at the regional and global scales.

Conclusions

In conclusion, our study demonstrated that the anthropological land-use changes, especially urban lawn development, increased both the weekly and the daily soil carbon dioxide efflux in summer. Although soil microbes and active organic carbon are important factors controlling soil respiration，the soil carbon dioxide efflux at our site was predominantly influenced by the soil temperature and land-use type. The variation in soil moisture among all of the sites was less significant than that of the soil temperature and land-use type. The soil temperature change in three land-use fields is positively correlated with the degree of impervious areas, suggesting that an urban heat island would increase soil carbon emission due to urban development. Thus, how urban land conversion affects the soil carbon dioxide efflux by altering the soil temperature at different scales requires further investigation.

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REFERENCES

[1] Balogh, J., Pintér, K., Fóti, S., Cserhalmi, D., Papp, M., Nagy, Z. (2011): Dependence of soil respiration on soil moisture, clay content, soil organic matter, and CO_2 uptake in dry grasslands. – Soil Biology and Biochemistry 43(5): 1006-1013.

[2] Blair, G.J., Lefroy, R.D.B., Lisle, L. (1995): Soil carbon fractions based on their degree of oxidation, and the development of a carbon management index for agricultural systems. – Australian Journal of Agricultural Research 46(7): 1459-1466.

[3] Bowne, D.R., Johnson, E.R. (2013): Comparison of soil carbon dioxide efflux between residential lawns and corn fields. –Soil Science Society of America Journal 77(3): 856-859.

[4] Carlisle, E.A., Steenwerth, K.L., Smart, D.R. (2006): Effects of Land Use on Soil Respiration. – Journal of environmental quality 35(4): 1396-1404.

[5] Chu, J.X., Zhang, X.Q. (2006): Dynamic and fractionalization of soil respiration under three diferent land use/covers in the subalpine region of western Sichuan Province, China. – Acta Ecologica Sinica 26(6): 1693-1700. (in Chinese)

[6] Cook, F.J., Orchard, V.A. (2008): Relationships between soil respiration and soil moisture. – Soil Biology and Biochemistry 40(5): 1013-1018.

[7] Davidson, E., Belk, E., Boone, R.D. (1998): Soil water content and temperature as independent or confounded factors controlling soil respiration in a temperate mixed hardwood forest. –Global change biology 4(2): 217-227.

[8] Davidson, E.A., Ackerman, I.L. (1993): Changes in soil carbon inventories following cultivation of previously untilled soils. – Biogeochemistry 20(3): 161-193.

[9] Duan, Y.P., Meng, X.Z., Wen, Z.H., Chen, L. (2013): Acidic pharmaceuticals in domestic wastewater and receiving water from hyper-urbanization city of China (Shanghai): environmental release and ecological risk. Environ. – Environmental Science and Pollution Research 20(1): 108-116.

[10] Fenn, M.E., Haeuber, R., Tonnesen, G.S., Baron, J.S., Grossman-Clarke, S., Hope, D., Jaffe, D.A., Copeland, S., Geiser, L., Rueth, H.M. (2003): Nitrogen emissions, deposition, and monitoring in the western United States. – Bioscience 53(4): 391-403.

[11] Groffman, P.M., Pouyat, R.V., Cadenasso, M.L., Zipperer, W.C., Szlavecz, K., Yesilonis, I.D., Band, L.E., Brush, G.S. (2006): Land use context and natural soil controls on plant community composition and soil nitrogen and carbon dynamics in urban and rural forests. – Forest Ecology and Management 236(2):177-192.

[12] Groffman, P.M., Williams, C.O., Pouyat, R.V., Band, L.E., Yesilonis, I.D. (2009): Nitrate leaching and nitrous oxide flux in urban forests and grasslands. – Journal of

environmental quality 38(5): 1848-1860.

[13] Gunawardhana, L.N., Kazama, S. (2012): Using subsurface temperatures to derive the spatial extent of the land use change effect. – Journal of Hydrology 460-461: 40-51.

[14] Hou, Y. (2011): Review of effects of urbanization on soil respiration. – Ecology and Environmental Sciences 20(12):1945-1949. (in Chinese)

[15] Houghton, R. (1994): The worldwide extent of land-use change. – Bioscience(44): 305-313.

[16] Houghton, R.A. (2003): Revised estimates of the annual net flux of carbon to the atmosphere from changes in land use and land management 1850–2000. – Tellus 55(2): 378-390.

[17] Hutyra, L.R., Yoon, B., Alberti, M. (2011): Terrestrial carbon stocks across a gradient of urbanization: a study of the Seattle, WA region. – Global Change Biology 17(2): 783-797.

[18] Kaur, K., Jalota, R.K., Midmore, D.J. (2010): Soil respiration rate and its sensitivity to temperature in pasture systems of dry-tropics. – Acta Agriculturae Scandinavica Section B 60(5): 407-419.

[19] Kaye, J., McCulley, R., Burke, I. (2005): Carbon fluxes, nitrogen cycling, and soil microbial communities in adjacent urban, native and agricultural ecosystems. – Global Change Biology 11(4): 575-587.

[20] Kellman, L., Beltrami, H., Risk, D. (2007): Changes in seasonal soil respiration with pasture conversion to forest in Atlantic Canada. – Biogeochemistry 82(1): 101-109.

[21] Koerner, B., Klopatek, J. (2002): Anthropogenic and natural CO_2 emission sources in an arid urban environment. – Environmental Pollution 116: S45-S51.

[22] Koerner, B.A., Klopatek, J.M. (2010): Carbon fluxes and nitrogen availability along an urban–rural gradient in a desert landscape. – Urban Ecosystems 13(1): 1-21.

[23] Lal, R. (2008): Promise and limitations of soils to minimize climate change. – Journal of Soil and Water Conservation 63(4): 113A-118A.

[24] Makhado, R.A., Scholes, R.J. (2011): Determinants of soil respiration in a semi-arid savanna ecosystem, Kruger National Park, South Africa. – Koedoe-African Protected Area Conservation and Science 53(1): 1-8.

[25] McCulley, R.L., Boutton, T.W., Archer, S.R. (2007): Soil respiration in a subtropical savanna parkland: Response to water additions. – Soil Science Society of America Journal 71(3): 820-828.

[26] Murty, D., Kirschbaum, M.U., Mcmurtrie, R.E., Mcgilvray, H. (2002): Does conversion of forest to agricultural land change soil carbon and nitrogen? A review of the literature. – Global Change Biology 8(2): 105-123.

[27] Ni, K., Ding, W.X., Cai, Z.C., Wang, Y.F., Zhang, X.L., Zhou, B.K. (2012): Soil carbon dioxide emission from intensively cultivated black soil in Northeast China: nitrogen fertilization effect. – Journal of Soils and Sediments 12(7): 1007-1018.

[28] Norman, J., Kucharik, C., Gower, S., Baldocchi, D., Crill, P., Rayment, M., Savage, K., Striegl, R. (1997): A comparison of six methods for measuring soil-surface carbon dioxide fluxes. – Journal of Geophysical Research 102(D24): 28771-28777.

[29] Nusier, O.K., Rousan, L.M. (2008): Soil air permeability and carbon dioxide flux measurements from the soil surface. – Soil Science and Plant Analysis 39 (9-10): 1406-1415.

[30] Pataki, D., Alig, R., Fung, A., Golubiewski, N., Kennedy, C., McPherson, E., Nowak, D., Pouyat, R., Romero, L.P. (2006): Urban ecosystems and the North American carbon cycle. – Global Change Biology 12(11): 2092-2102.

[31] Pickett, S., Cadenasso, M., Grove, J.M., Boone, C.G, Groffman, P.M., Irwin, E., Kaushal, S.S., Marshall, V., McGrath, B.P., Nilon, C.H. (2011): Urban ecological systems: Scientific foundations and a decade of progress. – Journal of Environmental Management 92(3): 331-362.

[32] Pouyat, R., Groffman, P., Yesilonis, I., Hernandez, L. (2002): Soil carbon pools and fluxes in urban ecosystems. – Environmental pollution 116(S1): S107-S118.

[33] Powlson, D., Whitmore, A., Goulding, K. (2011): Soil carbon sequestration to mitigate climate change: a critical re-examination to identify the true and the false. – European Journal of Soil Science 62(1): 42-55.

[34] Raciti, S.M., Groffman, P.M., Jenkins, J.C, Pouyat, R.V, Fahey,T.J., Pickett, S.T., Cadenasso, M.L. (2011): Accumulation of carbon and nitrogen in residential soils with different land-use histories. – Ecosystems 14(2): 287-297.

[35] Raich, J.W., Potter, C.S. (1995): Global patterns of carbon dioxide emissions from soils. – Global Biogeochemical Cycles 9(1): 23-36.

[36] Schimel, D.S., Participants, V., Braswell, B. (1997): Continental scale variability in ecosystem processes: models, data, and the role of disturbance. – Ecological Monographs 67(2): 251-271.

[37] Shen, W.J., Wu, J.G., Grimm, N.B., Hope, D. (2008): Effects of urbanization-induced environmental changes on ecosystem functioning in the Phoenix metropolitan region, USA. – Ecosystems 11(1): 138-155.

[38] Sheng, H., Yang, Y.S., Yang, Z.J., Chen, G.S., Xie, J.S., Guo, J.F., Zou, S.Q. (2010): The dynamic response of soil respiration to land–use changes in subtropical China. – Global Change Biology16(3): 1107-1121.

[39] Siciliano, G. (2012): Urbanization strategies, rural development and land use changes in China: A multiple-level integrated assessment. – Land use policy 29(1): 165-178.

[40] Tian, S.Z., Ning, T.Y., Wang, Y., Li, H.J., Zhong, W.L., Li, Z.J. (2010): Effects of different tillage methods and straw-returning on soil organic carbon content in a winter wheat field. – Chinese Journal of Applied Ecology 21(2): 373-378. (in Chinese)

[41] Townsend-Small, A., Czimczik, C.I. (2010): Carbon sequestration and greenhouse gas emissions in urban turf. –Geophysical Research Letters 37(2): 1-5.

[42] Trusilova, K., Churkina, G. (2008): The response of the terrestrial biosphere to urbanization: land cover conversion, climate, and urban pollution. – Biogeosciences 5(6): 1505-1515.

[43] Wei, Z.Q., Wu, S.H., Zhou, S.L., Li, J.T., Zhao, Q.G. (2014): Soil organic carbon transformation and related properties in urban soil under impervious surfaces. – Pedosphere 24(1): 56-64.

[44] Zhao, R.Q., Huang, X.J., Zhong, T.Y., Chuai, X.W. (2012): Carbon Storage and Fluxes of Different Land Use Types in Nanjing. – Journal of Soil and Water Conservation 26(6): 164-170. (in Chinese)

[45] Zheng, S.Q., Wang, R., Glaeser, E.L., Kahn, M.E. (2011): The greenness of China: household carbon dioxide emissions and urban development. – Journal of Economic Geography 11(2): 761-792.

[46] Zhu, Q., Liao, K. H., Xu, Y., Yang, G.S., Wu, S.H., Zhou, S.L. (2012): Monitoring and prediction of soil moisture spatial–temporal variations from a hydropedological perspective: a review. – Soil Research 50(8): 625-637.

[47] Zirkle, G., Lal, R., Augustin, B. (2011): Modeling carbon sequestration in home lawns. – HortScience 46(5): 808-814.

INFLUENCE OF SEASONAL DROUGHT ON ECOSYSTEM WATER USE EFFICIENCY IN A SUBTROPICAL EVERGREEN CONIFEROUS PLANTATION

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Abstract. Seasonal drought (low precipitation combined with high atmospheric temperature) typically occurs during the active vegetative growth season in subtropical regions of southeast China. We examined the responses of water use efficiency (WUE) and its components, gross primary productivity (GPP) and evapotranspiration (ET), to water limitations and high atmospheric temperature (T_a) in a subtropical evergreen coniferous plantation. The effect of high temperature and low water availability on the water use efficiency (WUE) at the site was analyzed using six years (2003–2008) of eddy covariance flux observations. During the active growing season (June–August), GPP and ET did not decrease remarkably and successively unless extreme drought occurred, i.e. mean soil relative extractable water decreased below 0.4. Low precipitation and high T_a (26–32°C) at the peak of the active growing season decreased GPP. Furthermore, the response of water consumption (i.e. ET) to high T_a and drought lagged behind that of carbon fixation (i.e. GPP). Whether daily WUE increases or decreases in response to drought depends on temperature. For the present study site, at daily time scale, variations of WUE during the active growing season were determined mostly by air temperature while at longer (annual) time scales the influence of solar radiation may exert greater control over interannual variation in WUE than air temperature.

Keywords: *evergreen coniferous plantation, evapotranspiration, gross primary production, seasonal drought, water use efficiency*

Introduction

When considering the relationship between average climate and forest productivity among sites – or inter-annual variations within one site – water and carbon (C) fluxes and, therefore, productivity of forest ecosystems are strongly influenced by drought (Granier et al., 2007). Since water and C fluxes are strongly linked by their passage through the stomata, water use efficiency (WUE), the ratio of carbon dioxide (CO_2) assimilation to water losses, has been identified as an effective integral trait for assessment of ecosystem response to drought (Reichstein et al., 2007; Linares and Camarero, 2012). Therefore, understanding ecosystem WUE and its key controlling processes in response to drought by using long-term observations is helpful to project drought–terrestrial C feedbacks.

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Drought stress occurs whenever soil water drops below a threshold that induces restrictions to growth and transpiration (Bréda et al., 2006). Transpiration, gross photosynthesis and respiration decrease sharply when the soil relative extractable water (REW) drops below 0.4 (Granier et al., 2007). Repeated drought induces a reduction in leaf area index (LAI) (Battaglia et al., 1998; Zhang et al., 2005) that subsequently decreases gross primary production (GPP) (Law et al., 2002; Hoff and Rambal, 2003). Drought in European forests during an extremely dry year (2003) reduced annual tree growth and had consequences in subsequent years (Granier et al., 2007).

High atmospheric temperatures (T_a) have been shown to adversely affect GPP in many forest ecosystems. For example, Griffis et al. (2003) found that boreal conifers experienced a pronounced mid-season decline in GPP. Grant et al. (2001) showed that the GPP of boreal black spruce declined under higher T_a. The sensitivity of GPP to high T_a in forests may be due to biochemical or stomatal factors (Grant et al., 2005). Méthy et al. (1997) found degradation of photosystem II photochemical efficiency in *Pinus halapensis* only at temperatures > 35°C, which is well above those at which GPP becomes constrained. However stomatal conductance (g_l) of most vegetation (e.g. boreal conifers and deciduous trees) has been frequently observed to decline under the higher atmospheric vapor pressure deficits (VPDs) (Grelle et al., 1999; Ohta et al., 2001; Wang et al., 2009, 2012) that can occur at temperatures > 25°C. Reduced g_l caused by higher VPD limits a plant's ability to use light for photosynthesis because of reduced CO_2 concentrations inside leaves (Schulze et al., 1987). Transpiration is also reduced by stomatal control and affects leaf energy balance (Baldocchi, 1997). Consequently, there is an increase in leaf temperature, which promotes photorespiration and affects electron transport and carboxylation capacity, thereby potentially reducing C gain (Harley and Tenhunen, 1991; Baldocchi, 1997).

Empirical evidence has shown strong coupling between transpiration and photosynthesis, and the effect that drought can have on both processes (Bernier et al., 2006). Moreover, temperate and subtropical forest ecosystems have been shown to have different relationships between GPP and evapotranspiration (ET) (Yu et al., 2008). For temperate forest ecosystems, GPP was significantly correlated with ET at different temporal scales, showing a strong linear relationship between C gain and water loss. For subtropical forest ecosystems, the relation between GPP and ET was nonlinear indicating that the coupling between GPP and ET was weak under the changing environmental variables (Yu et al., 2008). Ruehr et al. (2012) also reported that C and water fluxes were tightly coupled, but became less so during drought for a young ponderosa pine forest. The ratio of GPP to ET, expressed as WUE, was found to decrease slightly during severe drought in a European forest in the summer of 2003 (Reichstein et al., 2007). As described by Reichstein et al. (2002), this pattern challenges many current ecosystem model formulations in which pure stomatal control implies increasing WUE under soil drying conditions (Schulze et al., 2005). Indeed there are also observations that WUE increases during the seasonal drought (Vickers et al., 2012).

Due to the effect of the subtropical continental monsoon climate, the mid-subtropical region in southeast China is rich in water and heat resources. However, the lower atmosphere layer of this region is controlled by a West Pacific subtropical high pressure system and a descending airflow prevails in summer, which often leads to difficulty in forming precipitation (Sun et al., 2006). Hence, seasonal drought (less precipitation combined with high air temperature) usually occurs during the active vegetative growth season, which is typical in subtropical regions of southeast China (Sun et al., 2006; Yu et al., 2008; Wen et al., 2010). The changing precipitation regime (such as rainfall deficit) accompanied by increased air temperature has a profound impact on ecosystem C exchanges (e.g. Ciais et al., 2005; Niu et al., 2011). Some studies have also reported that during dry seasons in wet–dry regions, many tree species do not suffer from significant water stress as a result of one or several adaptive mechanisms (Eamus and Prior, 2001). To date, the behavior of whole ecosystems in the humid climate zone in response to seasonal drought has not been thoroughly investigated.

The objectives of this study were: (i) to describe the seasonal and inter-annual patterns of WUE for a subtropical plantation forest; (ii) to investigate the coupling relationship between GPP and ET and their dependence on T_a and explain the reason for decreasing WUE during the peak of the active growing season; (iii) to explore whether decreased soil water content led to decreased C fixation during the active growing season.

Materials and methods

Study site

The study site in ChinaFLUX is located at the Qianyanzhou Experimental Station of the Chinese Ecosystem Research Network in southeast China (26°44′N, 115°03′E and 102 m a.s.l.). The vegetation is mainly evergreen coniferous trees planted after 1985 and the dominant species include slash pine (*P. elliottii*), Masson pine (*P. massoniana*) and Chinese fir (*Cunninghamia lanceolata*) with an average canopy height of 13 m (Wen et al., 2010). The summer maximum LAI is 5.6 m^2 m^{-2} and tree density is 1463 stems ha^{-1}. The study site is in a typical subtropical monsoon climate region with predominant south-southeast winds in summer and north-northwest winds in winter. With the aid of FSAM (flux source area model), Mi et al. (2006) indicated that the flux footprint was 120 m under unstable conditions, while it was 1655 m under stable conditions. The red soil is weathered from red sand rock, and the soil texture is divided into 2.0–0.05 (17%), 0.05–0.002 (68%) and < 0.002 mm (15%), with pH 4.7 and organic matter content of 2.14% (Wang et al., 2004). The average annual air temperature over 20 years (1985–2007) was approximately 17.9°C and mean annual precipitation was 1475 mm (Wen et al., 2010). Further detailed descriptions of the region are available in the literature (Wen et al., 2006, 2010; Zhang et al., 2011a, 2011b).

Flux measurements and data processing

Ecosystem ET and C exchange were measured continuously at 39.6 m using the eddy covariance (EC) technique from late August 2002. Briefly, the EC measurement setup consisted of a three-dimensional sonic anemometer (Model CSAT3, Campbell Scientific Inc., Logan, UT, USA) for wind speed and temperature measurement and an open-path infrared absorption gas analyzer (Model LI-7500, Licor Inc., Lincoln, NE, USA) for measurement of CO_2 and water vapor concentration. The signals from the EC instruments were sampled at 10 Hz and recorded on a data logger (Model CR5000, Campbell Scientific Inc.) for subsequent processing.

Meteorological parameters included atmospheric temperature and relative humidity (Model HMP45C, Campbell Scientific Inc.), photosynthetically active radiation (Model LI190SB, Licor Inc.), net radiation (Model CNR-1, Kipp & Zonnen, Delft, The Netherlands) and precipitation (Model 52203, RM Young Inc., Michigan, USA) that were measured at 39.6 m above the ground. Soil water content was measured at depths of 5, 20, and 50 cm using automated time domain reflectometry probes (Model CS615-L, Campbell Scientific Inc.). Soil temperatures were measured at five depths (2, 5, 20, 50 and 100 cm) using thermocouples (105T and 107L, Campbell Scientific Inc.). Meteorological variables were sampled at 1 Hz with 30-min averages calculated by CR10X-TD data loggers (Campbell Scientific Inc.).

REW is defined as:

$$\mathrm{REW} = \frac{\theta_m - \theta_{wp}}{\theta_{fc} - \theta_{wp}} \qquad \text{(Eq. 1)}$$

where θ_m is the soil water content at a soil depth of 0.5 m, θ_{fc} is field capacity and θ_{wp} is wilting point (all in $cm^3\ cm^{-3}$). For values of θ_{fc} and θ_{wp} refer to Mi et al. (2009). REW was calculated for the single soil depth of 50 cm, as this is approximately the average depth from which the majority of water extraction occurs (Mi et al., 2009).

The fluxes of net ecosystem exchange (NEE, mg $CO_2\ m^{-2}\ s^{-1}$) and ET (g $H_2O\ m^{-2}\ s^{-1}$) were obtained from covariance computed at 30-min intervals.

$$F_{\mathrm{NEE}} = \overline{w'\rho_c'(z_r)} + \int_0^{z_r} \frac{\partial \overline{\rho}_c}{\partial t} dz \qquad \text{(Eq. 2)}$$

where w and ρ_c represent vertical velocity and CO_2 density, respectively. The overbars denote time averaging and primes represent fluctuations from the mean. The first term on the right-hand side is the eddy flux for CO_2 or water vapor, the second term is the storage of CO_2 or water vapor below the height of observation (z_r), and all advective terms in the mass conservation equation were ignored (Baldocchi, 2003).

Spurious data were removed from the dataset if the instrument performance and experimental conditions were abnormal. Such problems were largely related to rainfall, water condensation, system failure or weak turbulent mixing during the night (Wen et al., 2006). Three-dimensional rotation of the coordinates was applied to the wind components to avoid the effects of instrument tilt or irregular airflow (Aubinet et al., 2000). Corrections were made for the effects of fluctuations of air density on the fluxes of CO_2 and water vapor (Webb et al., 1980). Storage below the EC height was also corrected (Hollinger et al., 1994). When the value of friction velocity (u_*) was $< 0.19\ m\ s^{-1}$, the values observed of

NEE and ET in the night (global radiation<1 W m^{-2}) were excluded. The energy balance closure was 77% (Li et al., 2005) and Wen et al. (2005) indicated that the co-spectral and power-spectral distributions both followed theoretical laws, and there was no obvious loss of high-frequency flux.

The gap-filling of the half-hourly ET was performed based on the methods described by Falge et al. (2001), but modified according to the gap-filling methodology of Reichstein et al. (2005), which considers the temporal auto-correlation of ET as well as the covariance of ET with environmental variables. The data gaps of NEE were primarily filled by the nonlinear regression method (Falge et al., 2001). For small gaps (< 2 h), the missing data were linearly interpolated. For large gaps, the missing daytime NEE was estimated using the Michaelis–Menten equation with an independent 10-d window (Wen et al., 2010). The nighttime NEE was defined as nighttime ecosystem respiration. The missing nighttime ecosystem respiration was estimated as a function of soil temperature and soil moisture with a yearly interval (Reichstein et al., 2002).

Unfortunately, direct measurements of GPP are usually not available in most ecosystem studies. Following micrometeorological conventions, C uptake by an ecosystem is denoted by negative values of CO_2 flux (NEE), while C release through ecosystem respiration (RE) is reported as positive values. Thus, GPP (mg CO_2 m^{-2} s^{-1}) can be estimated by subtracting CO_2 flux from ecosystem respiration: GPP = RE – NEE. To estimate GPP, the daytime RE was estimated by extrapolation of the functional relationship between nighttime RE and soil temperature and water content (Wen et al. 2010). Similarly to Ju et al. (2010), we considered the GPP derived from measured NEE, soil temperature and soil water content as measured GPP.

WUE at the ecosystem level, measured as the ratio between daily-integrated GPP and daily-integrated ET, is a useful indicator of the relationship between terrestrial water and C cycles (Jongen et al., 2011). Daily ecosystem WUE (g C kg^{-1} H_2O) is calculated as:

$$\mathrm{WUE} = \mathrm{GPP}/\mathrm{ET} \qquad \text{(Eq. 3)}$$

WUE expresses the relationship between C fixation and water consumption during a day. The reciprocal of WUE reflects the water cost per unit C assimilation during a day. Additionally, annual WUE is defined as the ratio of annual sums of GPP to ET. Daily WUE during the active growing season (June-August) and annual WUE and its control environments were analyzed in this study.

Statistical analysis

The relationships between GPP, ET and air temperature were fitted with linear ($y = ax + b$) and quadratic ($y = ax^2 + bx + c$) equations using the Origin software package. The air temperature when achieving the maximum GPP and ET was obtained by calculating $-b/2a$. Pearson's correlation analysis was used to determine the relationship between temperature when GPP achieved the maximum value and the average of REW during

June–August. The difference of GPP and ET under different soil water conditions was performed by *t*-test using the SPSS software package.

Results

Environmental conditions

Figure 1 illustrates the seasonal variation of daily precipitation, REW, daily air temperature and VPD during 2003–2008. Overall, 46.6% (in 2007) to 59.1% (in 2003) of the annual rainfall was received during March–June (*Fig. 1a–f*). Rainfall in July was only 3.9 mm in 2003 and 2.7 mm in 2007, respectively, accounting for 0.5 and 0.2% of the annual rainfall. Moreover, July had mean daily temperatures of 31.9 and 30.4°C in 2003 and 2007, respectively (about 2°C more than other years), with the high temperature period corresponding to the summer less-rainy season. Rainfall in July 2004 and 2005 was also relatively less compared to 2006 and 2008, accounting for 7.8 and 4.0% of annual rainfall, respectively (for 2006 and 2008 the value was about 11%).

During the active growing season, there was temporarily low REW (≤ 0.5) except in 2006 (*Fig. 1a–f*). REW < 0.5 occurred for the longest period in 2003 (36 d), during which time the mean value of REW was 0.33, which was almost the same as in 2007 (0.34 for 25 d). Additionally, REW < 0.5 occurred for 18, 29 and 6 d in 2004, 2005 and 2008, respectively, during which time average REW (0.44) was greater than in 2003 and 2007. VPD showed a similar pattern in 2003 and 2007, achieving maximum values of 3.9 kPa in summer of 2003 (mid-July) and 3.8 kPa in summer of 2007 (late July) (*Fig. 1g–l*). The average VPDs during the active growing seasons of 2003 and 2007 were 2.0 and 1.8 kPa, respectively, which were higher than for 2004 (1.5 kPa), 2005(1.6 kPa), 2006(1.5 kPa) and 2008 (1.6 kPa). The minimum VPD values (near 0 kPa) occurred during winter for all years.

Daily temperature progressively increased in February–August and decreased in August–February (*Fig. 1g–l*). The minimum temperature occurred in January 2003 (–2.3°C) and the maximum in July 2003 (34.0°C).

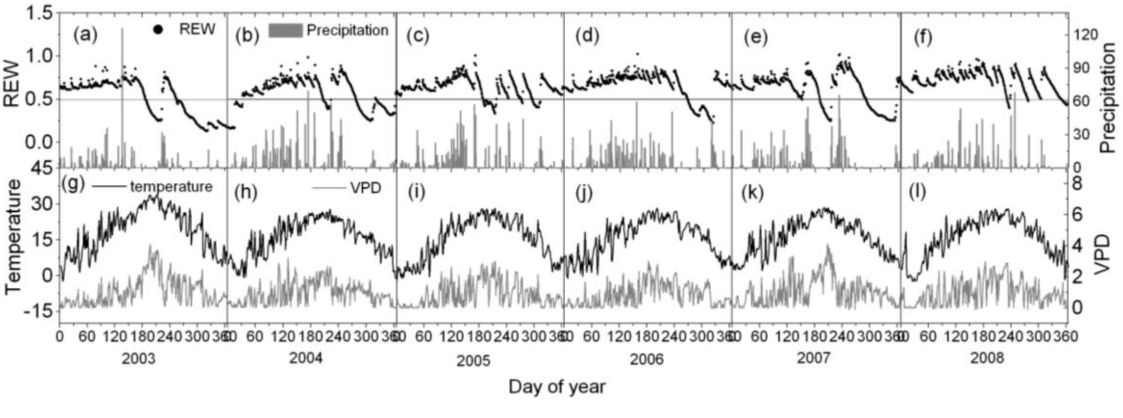

Figure 1. *Seasonal and inter-annual variations of daily precipitation (P), soil relative extractable water (REW) (a–f), daily air temperature and vapor pressure deficit (VPD, 8:00–18:00) (g–l) during 2003–2008 in a subtropical evergreen coniferous plantation.*

Seasonal variation of WUE and annual WUE

For most of the time, the variation in WUE was in the range of 1.5–4.5 g C kg^{-1} H_2O. Within any single year, during 2003–2008, a decline in WUE was always observed during June from the seasonal variation of WUE (*Fig. 2*). Accordingly, the daily WUE formed a trough during the active growing season for all years (*Fig. 2*).

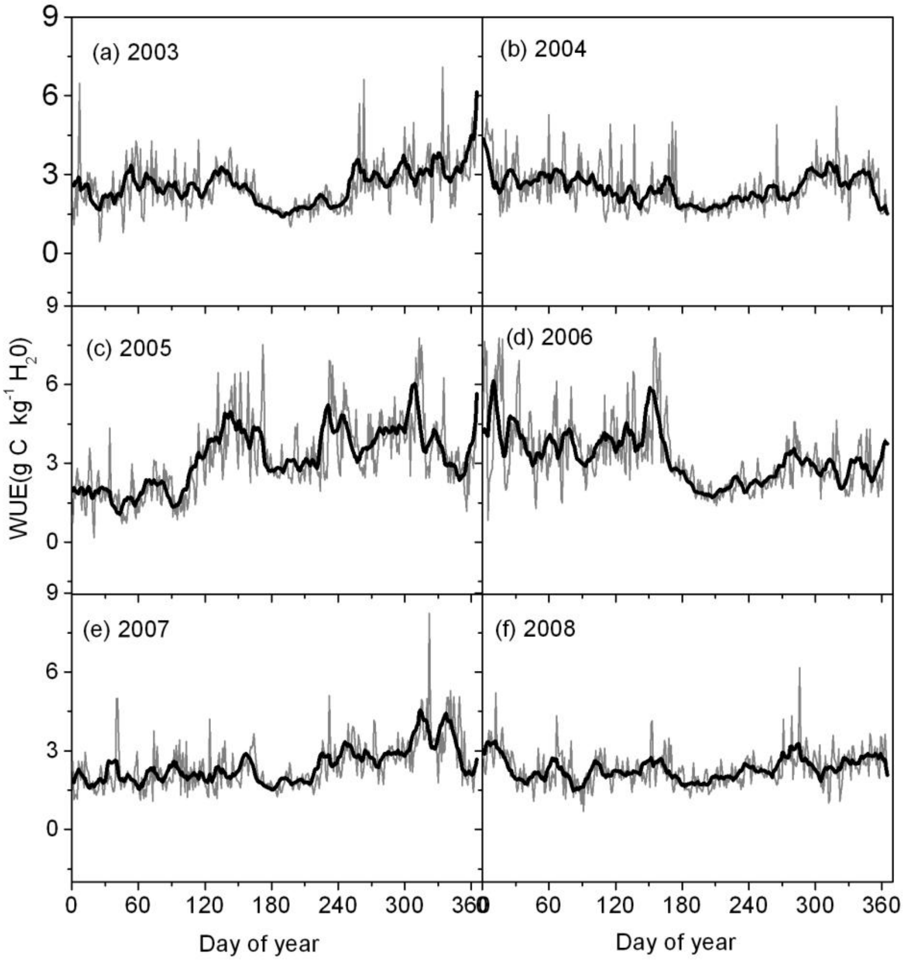

Figure 2. *Seasonal and inter-annual variations of water use efficiency (WUE, g C kg^{-1} H_2O) during 2003–2008 in a subtropical evergreen coniferous plantation. The black lines are 10-d running means.*

Annual WUE (the ratio of annual sums GPP and ET) in 2005 and 2006 (2.7–2.9 gC kg^{-1}H_2O) was higher than for the other four years (2.1–2.2 gC kg^{-1} H_2O) (*Fig. 3*). The slope of the linear fit between GPP and ET in 2005 and 2006 was also higher than for the other four years (*Fig. 3*). As far as the whole year was concerned, annual WUE in the years of poor water conditions during the active growing season (e.g. 2003 and 2007) was consistent with those of 2004 and 2008 (normal years). Annual WUE was in proportion to annual solar radiation for the year 2003 to 2008 (*Fig. 4*). Annual solar radiation explained 81% of the variations in annual WUE (*Fig. 4*).

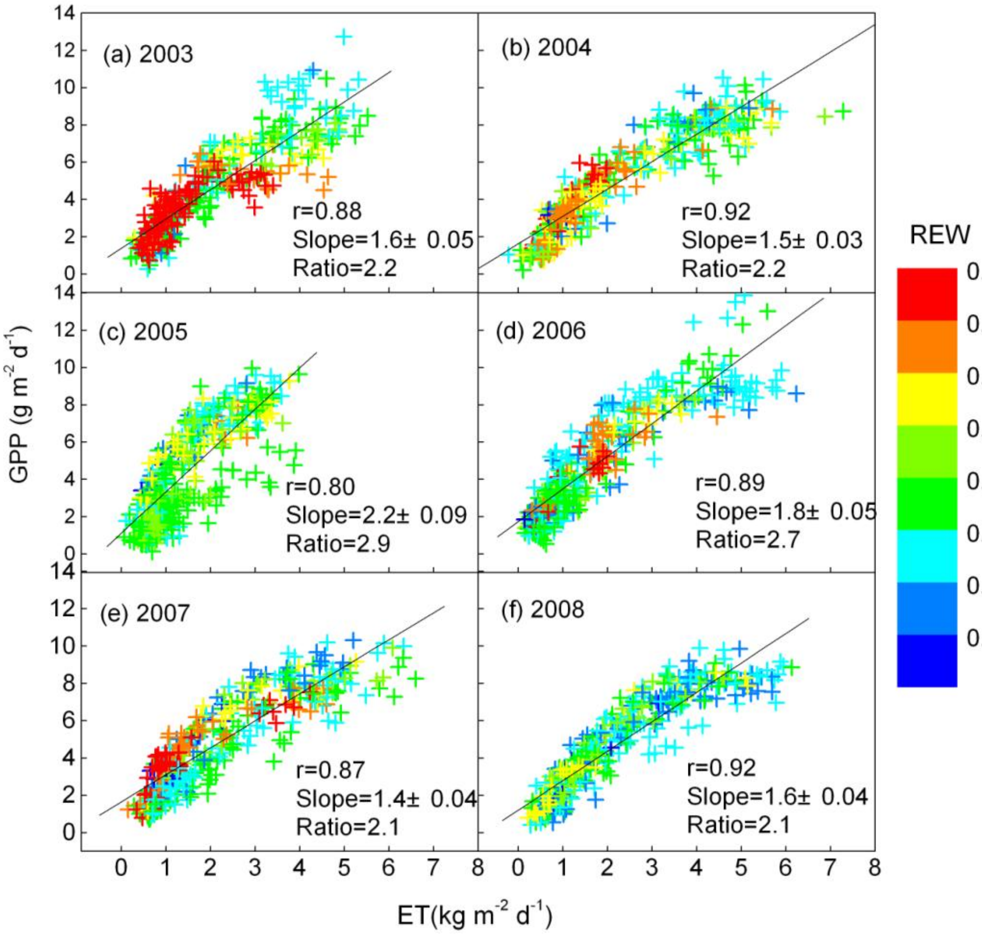

Figure 3. *Relationship between GPP and ET during 2003–2008 in a subtropical evergreen coniferous plantation. Also shown are the correlation coefficient, the slope of the linear fit, and the ratio of annual GPP to ET) and the REW on the color scale. All regressions are significant at $P < 0.001$.*

GPP, ET and WUE under different REW intervals relative to temperature

Figure 5 compares the responsive characteristics of GPP and ET to air temperature during June–August of 2003–2008. GPP response characteristics to air temperature for all years could be fitted using quadratic equations. The air temperature at which the maximum of GPP (T_{GPP}) was achieved could be calculated from the fitted site-specific parameters (see ‘Statistical analysis’ section). For 2003 and 2007, GPP was depressed when $T_a > 26$ and 28°C respectively, while in years other than 2003 and 2007, GPP began to decrease when $T_a > 30$ or 32°C (*Table 1*). T_{GPP} was proportional to the average value of REW during June–August ($\mathrm{REW}_{6\text{-}8}$) and Pearson’s correlation coefficient (r) between them was 0.90 ($P = 0.01$) (*Fig. 6*). Our results showed that high T_a (> 26–32°C) became a restriction for C assimilation during the active growing season. The temperature when GPP achieved the maximum (T_{GPP}) was significantly (P =0.015, r = 0.899) correlated with $\mathrm{REW}_{6\text{-}8}$, indicating that T_{GPP} was not a constant but a variable determined by soil water conditions and became lower if drought occurred (e.g. in 2003 and 2007).

Table 1. *Temperature when gross primary productivity (GPP) and evapotranspiration (ET) achieved a maximum value during June–August and the average of REW at that time*

	2003	2004	2005	2006	2007	2008
Temperature when GPP achieved the maximum value (T_{GPP}, °C)*	26	30	30	32	28	32
Temperature when ET achieved the maximum value (T_{ET}, °C)*	30	-	-	-	31	-
Average REW during June–August (REW_{6-8})	0.547	0.663	0.603	0.762	0.640	0.755

*This value obtained by calculating –b/2a from the quadratic equation in *Fig. 3*.

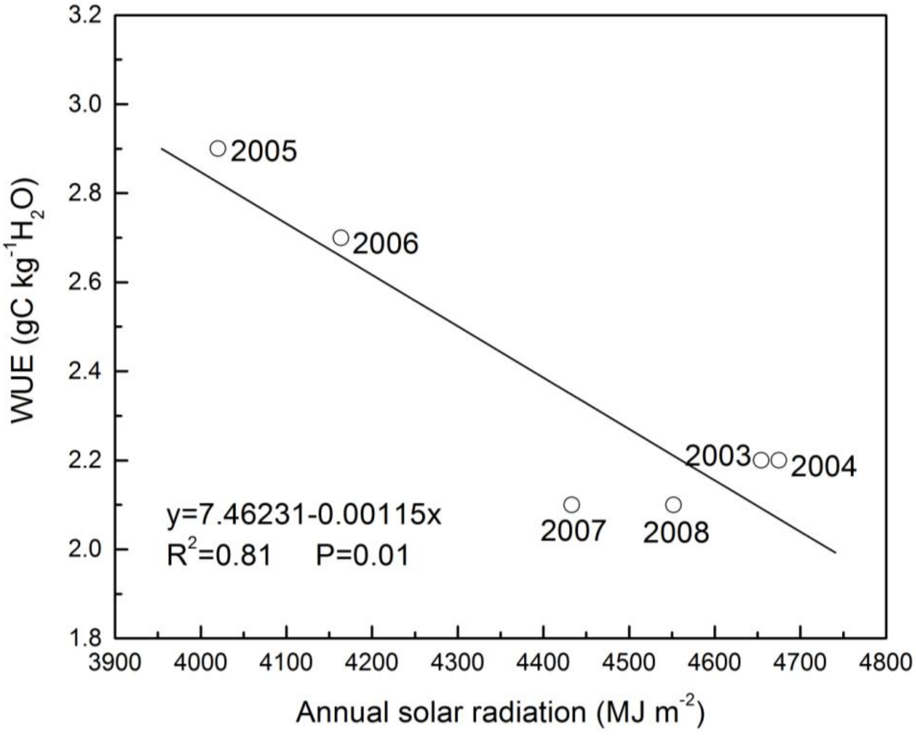

Figure 4. *The relationship between annual WUE and annual solar radiation during 2003–2008 in a subtropical evergreen coniferous plantation.*

The relationship between T_a and ET could be fitted either by a linear or a quadratic equation (*Fig. 5*), which was determined by soil water availability. The optimum temperature of ET for 2003 and 2007 was greater than T_{GPP}. For 2003 and 2007, the response characteristics of GPP and ET to T_a suggested that high T_a (> 26–28°C) induced a decrease in GPP and, at higher T_a (> 30–31°C), also induced a decrease in ET, which lagged behind GPP in the active growing season (*Table 1*). For years other than 2003 and 2007, ET showed a linear increase with temperature although GPP was stable when temperature reached around T_{GPP}. Compared to GPP, ET showed an increase (*Fig. 5d, f, h* and *l*) or a lag-decrease (*Fig. 5b* and *j*) when air temperature was higher than T_{GPP}.

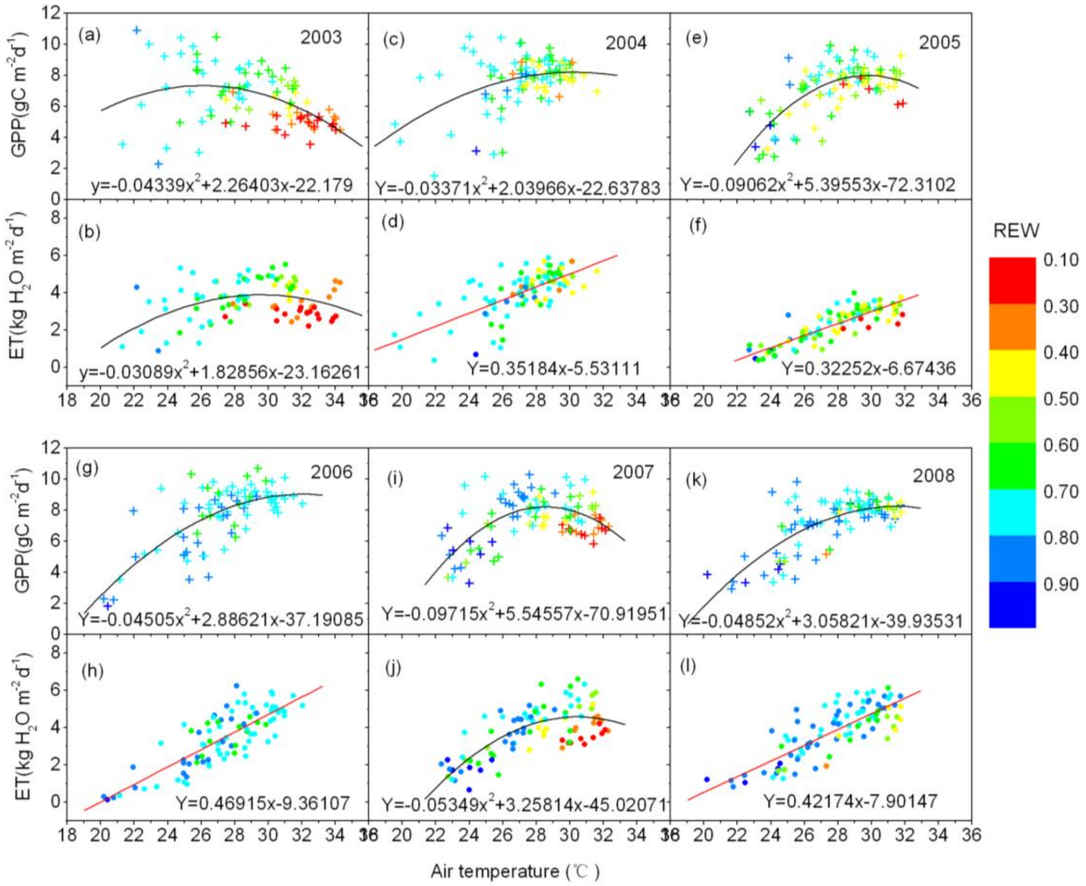

Figure 5. *The dependence of gross primary productivity (GPP, g C m^{-2} d^{-1}) and evapotranspiration (ET, kg H_2O m^{-2} d^{-1}) on air temperature on a daily basis during June–August of 2003–2008 in a subtropical evergreen coniferous plantation. Also shown are the fitting equation according to y = ax2 + bx + c and y = ax + b, and REW on the color scale. All fitted equations are P < 0.001.*

The ratio between GPP and ET, i.e. WUE, was also strongly related to T_a, decreasing by about 60–70% when T_a increased from 22 to 32°C (*Fig. 7*). The relationship between T_a and WUE can be expressed by $y = a - b\ln(T_a)$, which explained > 60% (60–78%) of the variations in daily WUE. The higher WUE in the years 2005 and 2006 was due to lower solar radiation (see ‘Discussion’).

In the active growing seasons of 2003 and 2007, differences in GPP and ET were observed among different REW intervals when $T_a > T_{GPP}$ (*Table 2*). For 2003, GPP when REW ≥ 0.5 was an average of 7.34 ± 1.15 g C m^{-2} d^{-1}. GPP was reduced to 6.26 ± 0.50 g C m^{-2} d^{-1} when 0.4 < REW < 0.5, and to 5.02 ± 0.60 g C m^{-2} d^{-1} when REW ≤ 0.4. GPP showed significant differences between REW ≥ 0.5 and 0.4 < REW < 0.5 ($P < 0.05$), and between 0.4 < REW < 0.5 and REW < 0.4 ($P < 0.05$). ET when REW ≥ 0.5 and 0.4 <

REW < 0.5 was almost the same (*Table 2*). There was a significant difference for ET between 0.4 < REW < 0.5 and REW ≤ 0.4 ($P < 0.05$). In 2007, GPP showed significant differences when REW ≤ 0.4.

Table 2. *GPP and ET under different soil water conditions when Ta > 26°C in 2003 and Ta > 28°C in 2007. Also shown is the average of REW, Ta and the number of days (N) under different REW intervals.*

Year	Soil water conditions	GPP (g C m^{-2} d^{-1})	ET(kg H_2O m^{-2} d^{-1})	N	REW	$\overline{T}_a$ (°C)
2003	REW ≥ 0.5	7.34±1.15a	4.09±0.88a	38	0.66	28.9
$T_a > 26$°C	0.4 < REW < 0.5	6.26±0.50b	4.08±0.35a	7	0.45	30.8
	REW ≤ 0.4	5.02±0.60c	3.13±0.59b	29	0.30	32.0
2007	REW ≥ 0.5	8.40±0.88d	5.22±0.83c	22	0.68	29.9
$T_a > 28$°C	0.4 < REW < 0.5	7.79±0.88d	4.20±0.57d	7	0.44	29.0
	REW ≤ 0.4	6.90±0.50e	3.79±0.53d	18	0.31	31.1

Means within columns not followed by the same letter are significantly different at P<0.05.

Discussion

The overall mean annual WUE (2.4±0.3 gC kg^{-1} H_2O, mean for six years) in the subtropical evergreen coniferous plantation in the present study was similar to that in a similar latitude (29°45′N) for evergreen needle-leaved forest (2.35 gC kg^{-1} H_2O; Clark et al., 2004), but was less than for evergreen needle-leaved forest growing at a higher latitude (2.66–5.42 gC kg^{-1} H_2O; Beer et al., 2009). High precipitation (historic mean 1475 mm) in the present study site may have caused low WUE. Generally, the Qianyanzhou site is subject to episodic summer droughts. During a study conducted by Wen et al. (2010), there were extremely dry summers in 2003 and 2007. In the present study, we also observed pronounced drought effects on ecosystem fluxes during the active growing seasons in 2003 and 2007, likely driven by a continuous decline in soil water availability and high atmospheric temperature. All of the study years (2006 excluded) had a REW < 0.5 during the active growing season, but typical seasonal drought was especially long and severe in 2003 and 2007. Drought conditions in 2003 exceeded those in 2007 in terms of both severity and longevity.

The observed threshold for water limitation was about 40–50% REW, which appears to be consistent for a wide range of ecosystems – crop (Ding et al., 2013), forest (Ruehr et al., 2012; Granier et al., 2007) – and may emerge as a general approximation to identify forests under drought stress (Ruehr et al., 2012). GPP and ET were reduced by drought when REW dropped below ca. 0.4 (Granier et al., 2007). The typical threshold of the REW (40–50%) inducing drought effects and significant reduction of GPP and ET was confirmed in the present study and whether the GPP was reduced when REW dropped below 0.5 or 0.4 seemed related to T_a (*Table 2*).

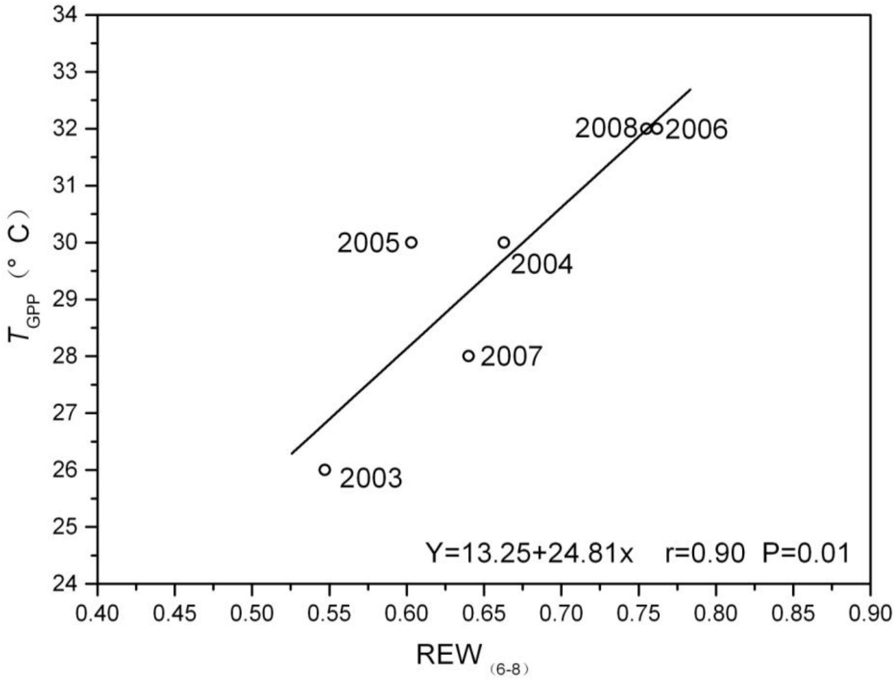

Figure 6. *The relationship between average REW during June–August (REW6-8) and air temperature when GPP achieved the maximum (TGPP).*

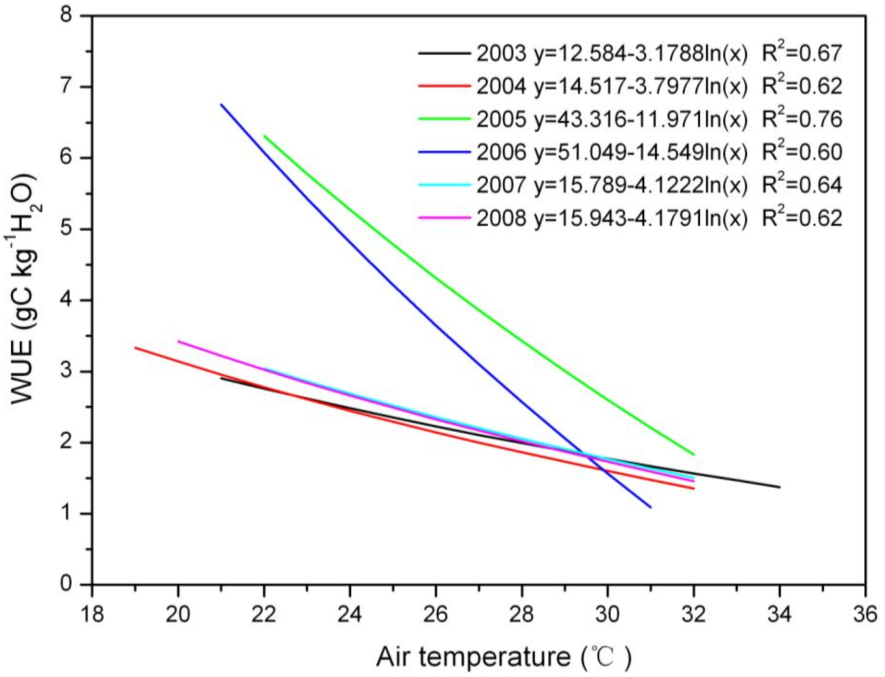

Figure 7. *The relationship between water use efficiency (WUE, g C kg^{-1} H_2O) and atmospheric temperature (T_a) on a daily basis during the active growing season (June–August) of 2003–2008 in a subtropical evergreen coniferous plantation. WUE vs. air temperature represented by curves of y =a – bln Ta. All curves are significant at $P < 0.001$.*

At the stand and ecosystem levels, the variability of WUE results from the trade-off between water loss and C gain during plant photosynthetic assimilation (Yu et al., 2008). During the studied years (2003–2008), variation of daily WUE in each year formed a trough during the active growing season (*Fig. 2*). In years other than 2003 and 2007, the linear increasing rate of ET was much larger than that of GPP when T_a was close to T_{GPP} and the ratio between them (i.e. WUE) was reduced. This phenomenon cannot be interpreted by stomatal limitations to photosynthesis because ET showed no decrease; instead non-stomatal limitations caused by higher temperature (due to either mesophyll conductance or leaf biochemistry or both) can reasonably explain this phenomenon. Proposed non-stomatal mechanisms include reduced RuBisCO activity, reduced electron transport capacity and reduced mesophyll conductance (Flexas et al., 2012; Zhou et al., 2013). The decrease in WUE in response to seasonal drought in 2003 and 2007 may have occurred because higher temperature (larger than optimum) induced reduction in electron transport and carboxylation capacity and restricted CO_2 uptake (non-stomatal limitation). Additionally drought constrained leaf photosynthesis (stomatal limitation) caused GPP to decline earlier than ET in response to the seasonal drought (*Fig. 5a, b, i, j*), which was also observed in a ponderosa pine forest (Ruehr et al., 2012). Similarly, Zhou et al. (2013) stated that stomatal and non-stomatal limitations to photosynthesis must both be considered for the short-term response to drought. To be more specific, J_{max} (rate of electron transport) is hypothesized to be more sensitive to drought than V_{cmax}(maximum carboxylation capacity) (Misson et al., 2006) supported by the observations of Wright et al. (2013) that during a drought year the J_{max}/V_{cmax} ratio was significantly lower than during a non-drought year.

There are no uniform conclusions on how ecosystem WUE responds to drought – an opinion confirmed by the report of Wright et al. (2013) that the response of WUE to drought was not uniform across sites. WUE increased during drought for two semi-arid pine forests (young and mature) (Vickers et al., 2012) and a young ponderosa pine stand (Ruehr et al., 2012) as water loss was restricted more than the inhibition of photosynthesis. The research of Reichstein et al. (2002) showed that ecosystem WUE decreased during drought at three Mediterranean evergreen sites due to the large decline (by up to 90%) in light-saturated ecosystem gross C uptake and daytime averaged canopy conductance. For the short-term response to drought, the reduction in WUE observed in some ecosystems should be ascribed to the stomatal and non-stomatal limitations to photosynthesis which decreased more than could be explained by the reduction in stomatal conductance. The results of the present study are consistent with other studies showing that both stomatal and non-stomatal processes are affected by drought (Keenan et al., 2009; Egea et al., 2011). The correlation of T_a with daily WUE (*Fig. 7*) was greater than for soil water availability (data not shown), suggesting that T_a was a main determinant of variation in daily WUE for this subtropical evergreen coniferous plantation. Reports of increase in WUE during drought have often concerned environments with maximum daily $T_a < 20°C$ (e.g. Vickers et al., 2012). Comparably, the study sites that have found decreased WUE during drought have often been for environments with maximum daily $T_a > 20°C$ and sometimes > 30°C (e.g. Reichstein et al., 2002 and *Fig. 1*). Thus we concluded that T_a may be a factor that determines how daily WUE responds to drought because T_a greatly determines the extent of non-stomatal limitation impacts on photosynthesis. Certainly this conclusion requires validation at a larger regional scale.

At an annual timescale, WUE in 2005 and 2006 was higher than for other years (*Fig. 3*), possibly due to the low annual solar radiation – 10% below normal at ~400 MJ m^{-2} (Zhang et al., 2011b) – which caused a disproportionate change in the relationship between evapotranspiration and photosynthesis (Rocha et al., 2004). On an annual time scale, WUE seemed relatively conservative at this study site and tended to increase in years of low solar radiation (*Fig. 3c, d*). This result is in accordance with the conclusion of Reichstein et al. (2007) who reported that for most sites the year-to-year changes in WUE were small (<1g kg^{-1}). As demonstrated by Yang et al. (2010), different data selection can lead to different conclusions on the relationship between WUE and its controls. For the present study site, at a daily time scale, variations in WUE during the active growing season were determined mostly by air temperature (*Fig. 7*); while at longer (annual) time scales the variability in WUE may be influenced by solar radiation (*Fig. 4*). This is attributed to high temperature and drought during the active growing season that induced a shift from optimal stomatal regulation to nonstomatal regulation at leaf scale and affected daily GPP to a large extent, while at longer (annual) time scales solar radiation mostly determined the ET and finally affected annual WUE.

Conclusions

The following conclusions can be drawn from this study:

(1) GPP and ET did not decrease remarkably and successively during the active growing season (June–August) unless extreme drought had occurred (mean REW < 0.4).

(2) During the active growing season (June–August), the WUE showed a trough, regardless of whether ET was an increasing or decreasing response to seasonal drought. The reduction of water consumption (i.e. ET) in response to high temperature and drought lagged behind the process of C sequestration (i.e. GPP).

(3) The optimum temperature for GPP to achieve a maximum was related to soil water conditions. Our results demonstrated that the threshold of temperature became lower if drought occurred.

(4) Environmental temperature was a factor that determined the daily WUE response to drought. For the present study site, on a daily time scale, variations in daily WUE during the active growing season were mostly determined by air temperature. At a longer (annual) time scale the influence of solar radiation may be more dominant in controlling the variability of WUE than air temperature.

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REFERENCES

[1] Aubinet, M., Grelle, A., Ibrom, A., Rannik, Ü., Moncrieff, J., Foken, T., (2000): Estimates of the annual net carbon and water exchange of European forests: the EUROFLUX methodology. – Adv. Ecol. Res. 30:113–174.

[2] Baldocchi, D.D. (1997): Measuring and modeling carbon dioxide and water vapour exchange over a temperate broad-leaved forest during the 1995 summer drought. - Plant Cell Environ. 20: 1108–1122.

[3] Baldocchi, D.D. (2003): Assessing the eddy covariance technique for evaluating carbon dioxide exchange rates of ecosystems: past, present and future. - Global Change Biology 9: 479–492.

[4] Battaglia, M., Cherry, M.L., Beadle. C.L., Sands, P.J., Hingston, A. (1998): Prediction of leaf area index in eucalypt plantations: effects of water stress and temperature. - Tree Physiol. 18: 521–528.

[5] Beer, C., Ciais, P., Reichstein, M., Baldocchi, D., Law, B.E., Papale, D., Soussana, J.F., Ammann, C., Buchmann, N., Frank, D., Gianelle, D., Janssens, I.A., Knohl, A., Köstner, B., Moors, E., Roupsard, O., Verbeeck, H., Vesala, T.,Williams, C.A., Wohlfahrt, G. (2009): Temporal and among-site variability of inherent water use efficiency at the ecosystem level. - Global Biogeochem. Cycles 23 : GB2018, doi: 10.1029/2008GB003233.

[6] Bernier, P.Y., Bartlett, P., Black TA (2006) Drought constraints on transpiration and canopy conductance in mature aspen and jack pine stands. – Agric. For. Meteorol. 40:64–78.

[7] Bréda, N., Huc, R., Granier A, Dreyer, E. (2006): Temperate forest trees and stands under severe drought: a review of ecophysiological responses, adaptation processes and long-term consequences. - Ann. Forest. Sci. 63: 625–644

[8] Ciais, P.H., Reichstein, M., Viovy, N. et al. (2005) Europe-wide reduction in primary productivity caused by the heat and drought in 2003 - Nature 437:529–533.

[9] Clark, K., Gholz, H., Castro, M. (2004): Carbon dynamics along a chronosequence of slash pine plantations in north Florida.- Ecol. Appl. 14:1154–1171.

[10] Ding, R.S., Kang, S.Z., Li, F.S., Zhang, Y.Q., Tong, L. (2013): Evapotranspiration measurement and estimation using modified Priestley–Taylor model in an irrigated maize field with mulching. – Agric. For. Meteorol. 168: 140–148.

[11] Eamus, D., Prior, L.D. (2001): Ecophysiology of trees of seasonally dry tropics: Comparisons among phonologies. – Adv. Ecol. Res. 32:113–197.

[12] Egea, G., Verhoef, A., Vidale, P.L. (2011): Towards an improved and more flexible representation of water stress in coupled photosynthesis–stomatal conductance models. - Agric. For. Meteorol. 151: 1370–1384.

[13] Falge, E., Baldocchi, D, Olson, R et al. (2001): Gap filling strategies for long term energy flux data sets. – Agric. For. Meteorol. 107: 71–77.

[14] Flexas, J., Barbour, M.M., Brendel, O., Cabrera, H.M., Carriquí, M., Díaz-espejo, A.,Douthe, C., Dreyer, E., Ferrio, J.P., Gago, J., Gallé, A., Galmés, J., Kodama, N., Medrano, H., Niinemets, Ü., Peguero-pina, J.J., Pou, A., Ribas-carbó, M., Tomás, M., Tosens, T., Warren, C.R.(2012) Mesophyll diffusion conductance to CO2: An unappreciated central player in photosynthesis.- Plant. Sci. 194:70–84.

[15] Granier, A., Reichstein, M., Bréda, N. (2007): Evidence for soil water control on carbon and water dynamics in European forest during the extremely dry year: 2003. - Agric. For. Meteorol. 143: 123–145.

[16] Grant, R.F., Arain, A., Arora, V., Barr, A., Black, T.A.,Chen, J., Wang, S., Yuan, F., Zhang,

Y. (2005): Intercomparison of techniques to model high temperature effects on CO2 and energy exchange in temperate and boreal coniferous forests, - Ecological Modelling 188: 217–252.

[17] Grant,R.F.,Goulden, M.L., Wofsy, S.C., Berry, J.A. (2001): Carbon and energy exchange by a black spruce – moss ecosystem under changing climate: testing the mathematical model *ecosys* with data from the BOREAS experiment. - J. Geophys. Res. 106: 33605–33621.

[18] Grelle, A., Lindroth, A., Mölder, M. (1999): Seasonal variation of boreal forest surface conductance and evaporation. – Agric. For. Meteorol. 98–99: 563–578.

[19] Griffis, T.J., Black, T.A., Morgenstern, K., Barr, A.G., Nesic, Z. Drewitt, G.B. Gaumont-Guay, G., McCaughey, J.H.(2003): Ecophysiological controls on the carbon balances of three southern boreal forests. -Agric For Meteorol 117: 53–71.

[20] Harley, P.C., Tenhunen, J.D. (1991): Modelling the photosynthetic response of C3 leaves to environmental factors. - In: Boote, K.J., Loomis, R.S. (eds.) Modelling crop photosynthesis-from biochemistry to canopy. Madison, WI, USA: American Society of Agronomy and Crop Science Society of America pp 17–39.

[21] Hoff, C., Rambal, S. (2003): An examination of the interaction between climate, soil and leaf area index in a Quercus ilex ecosystem. – Ann. For. Sci. 60:153–161.

[22] Hollinger, D.Y., Kelliher, F.M., Byers, J.N., Hunt, J.E., McSeveny, T.M., Weir, P.L. (1994): Carbon dioxide exchange between an undisturbed old-growth temperate forest and the atmosphere. - Ecology 75:134–150.

[23] Jongen, M., Pereira, J.S., Aires, L.M., Pio, C.A. (2011): The effects of drought and timing of precipitation on the inter-annual variation in ecosystem-atmosphere exchange in a Mediterranean grassland. – Agric. For. Meteorol. 151: 595–606.

[24] Ju, W., Wang, S., Yu, G., Zhou, Y., Wang, H. (2010): Modeling the impact of drought on canopy carbon and water fluxes for a subtropical evergreen coniferous plantation in southern China through parameter optimization using an ensemble Kalman filter. - Biogeosciences 7: 845–857.

[25] Keenan, T., Garcia, R., Friend, A.D.,Zaehle, S., Gracia, C., Sabatéi Jorba, S. (2009): Improved understanding of drought controls on seasonal variation in Mediterranean forest canopy CO_2 and water fluxes through combined in situ measurements and ecosystem modelling. - Biogeosciences 6: 1423–1444.

[26] Law, B., Falge, E., Gu, L. et al. (2002): Environmental controls over carbon dioxide and water vapour exchange of terrestrial vegetation. – Agric. For. Meteorol. 113: 97–120.

[27] Li, Z.Q., Yu, G.R., Wen, X.F., Zhang, L.M. Ren, C.Y., Fu, Y.L. (2005): Energy balance closure at ChinaFLUX sites. - Sci. China Ser. D 48: 51–62.

[28] Linares, J.C. and Camarero, J.J. (2012): From pattern to process: linking intrinsic water–use efficiency to drought-induced forest decline. - Global Change Biol. 18: 1000–1015.

[29] Méthy, M., Gillon, D., Houssard, C. (1997): Temperature-induced changes of photosystem II activity in Quercus ilex and Pinus halepensis. – Can. J. For. Res. 27: 31–38.

[30] Mi, N., Yu, G.R., Wang, P.X., Wen, X.F., Sun, X.M. (2006): A preliminary study for spatial representativeness of flux observation at ChinaFLUX sites. – Sci. China Ser. D. 49: 24–35.

[31] Mi, N., Yu, G.R., Wen, X.F., Sun, X.M., Wang, S.S., Zhang, L.M., Song, X. (2009): Use of ecosystem flux data and a simulation model to examine seasonal drought effects on a subtropical coniferous forest. - Asia-Pac. J. Atmos. Sci. 45: 207-220.

[32] Misson, L., Tu, K.P., Boniello, R.A., Goldstein, A.H. (2006): Seasonality of photosynthetic parameters in a muti-specific and vertically complex forest ecosystem in the Sierra Nevada of California. - Tree Physiology 26: 729-741.

[33] Niu, S.L., Xing, X.R., Zhang, Z., Xia, J.Y., Zhou, X.H., Song,B., Li, L.G., Wan, S.Q. (2011): Water-use efficiency in response to climate change: from leaf to ecosystem in a temperate steppe. - Global Change Biol. 17: 1073-1082

[34] Ohta, T., Hiyam, T., Tanaka, H., Kuwada, T., Maximov, T.C., Ohata, T., Fukushima, Y.

(2001): Seasonal variation in the energy and water exchanges above and below a larch forest in eastern Siberia. – Hydrol. Proc. 15:1459–1476.

[35] Reichstein, M., Ciais, P., Papale, D. (2007): Reduction of ecosystem productivity and respiration during the European summer 2003 climate anomaly: a joint flux tower, remote sensing and modeling analysis. - Global Change Biol. 13: 634–651

[36] Reichstein, M., Falge, E., Baldocchi, D. et al. (2005): On the separation of net ecosystem exchange into assimilation and ecosystem respiration: review and improved algorithm. - Global Change Biol. 11: 1424–1439.

[37] Reichstein, M., Tenhunen, J.D., Roupsard, O., Ourcival, J., Rambal, S., Miglietta, F., Peressotti, A., Pecchiari, M., Tirone, G., Valentini, R. (2002): Severe drought effects on ecosystem CO_2 and H_2O fluxes at three mediterranean sites: revision of current hypothesis? - Global Change Biol. 8: 999–1017.

[38] Rocha, A.V., Su, H.B., Vogel, C.S., Schmid, H.P. Curtis, P.S. (2004): photosynthetic and water use efficiency responses to diffuse radiation by an aspen-dominated northern hardwood forest. - Forest Science 50: 793-801

[39] Ruehr, N.K., Martin, J.G., Law, B.E. (2012): Effects of water availability on carbon and water exchange in a young ponderosa pine forest Above- and belowground responses. – Agric. For. Meteorol. 164: 136-148

[40] Schulze, E.D., Beck, E., Müller-Hohenstein, K. (2005): Plant Ecology. Berlin/ Heidelberg: Springer

[41] Schulze, E.D., Robichaux, R.H., Grace, J., Rundel, P.W., Ehleringer, J.R. (1987): Plant water balance. - BioScience 37: 30–37

[42] Sun, X.M., Wen, X.F., Yu, G.R., Liu, Y., Liu, Q. (2006): Seasonal drought effects on carbon sequestration of a mid-subtropical planted forest of southeastern China. – Sci. China Ser. D. 49: 110-118.

[43] Vickers, D., Thomas, C.K., Pettijohn, C., Martin, J.G., Law, B.E. (2012): Five years of crbon fluxes and inherent water-use efficiency at two semi-arid pine forests with different disturbance histories. - Tellus B 64, 17159

[44] Wang, S. (2012): Evaluation of Water Stress Impact on the Parameter Values in Stomatal Conductance Models Using Tower Flux Measurement of a Boreal Aspen Forest. - Journal of Hydrometeorology 13: 239–254.

[45] Wang, S., Yang, Y., Trishchenko, A.P., Barr, A.G., Black, T.A., Mccaughey, H. (2009): Modelling the response of canopy stomatal conductance to humidity. - Journal of Hydrometeorology 10: 521–532.

[46] Wang, S.Q., Liu, J.Y., Yu, G.R., Pan, Y.Y. (2004): Effects of land use change on the storage of soil organic carbon: A case study of the Qianyanzhou Forest Experimental Station in China - Climate Change 67: 247-255.

[47] Webb, E.K., Pearman, G.I., Leuning, R. (1980): Correction of flux measurement for density effects due to heat and water vapor transfer. – Quart. J. Roy. Meteor. Soc. 106: 85-100.

[48] Wen, X.F., Yu, G.R., Sun, X.M., Liu, R.F. (2005): Turbulence flux measurement above the overstory of a subtropical *Pinus* plantation over the hilly region in southeastern China. – Sci. China Ser. D. 48 : 63–73.

[49] Wen, X.F., Wang, H.M., Wang, J.L., Yu, G.R., Sun, X.M. (2010): Ecosystem carbon exchanges of a subtropical evergreen coniferous plantation subjected to seasonal drought, 2003-2007. - Biogeosciences 7: 357–369.

[50] Wen, X.F., Yu, G.R., Sun, X.M., LI, Q.K., Liu, R.F. (2006): Soil moisture effects on the temperature dependence of ecosystem respiration in a subtropical pinus plantation of southeastern China. – Agric. For. Meteorol. 137: 166–175.

[51] Wright, J.K., Williams, M., Starr, G., Mcgee, J., Mitchell, R.J. (2013): Measured and modelled leaf and stand-scale productivity across a soil moisture gradient and a severe drought. -Plant, Cell and Environment 36: 467–483.

[52] Yang, B., Pallardy, S.G., Meyers, T.P., Gu, L.H., Hanson, P.J., Wullschleger, S.D., Heuer, M., Hosman, K.P., Riggs, J.S., Sluss, D.W. (2010): Environmental controls on water use efficiency during severe drought in an Ozark Forest in Missouri, USA .- Global Change

Biol. 16:2252–2271.

[53] Yu, G.R., Song, X., Wang, Q.F., Liu, Y.F., Guan, D.X., Yan, J.H. Sun, X.M., Zhang, L.M., Wen, X.F. (2008): Water-use efficiency of forest ecosystems in eastern China and its relations to climatic variables. - New Phytol. 177: 927–937.

[54] Zhang, W.J., Wang, H.M., Wen, X.F.,Yang, F.T., Ma, Z.Q., Sun, X.M., Yu, G.R. (2011a): Freezing-induced loss of carbon uptake in a subtropical coniferous plantation in southern China. – Ann. Forest Sci. 68:1151–1161.

[55] Zhang, W.J., Wang, H.M., Yang, F.T., Yi, Y.H., Wen, X.F., Sun, X.M., Yu, G.R., Wang, Y.D., Ning, J.C. (2011b): Underestimated effects of low temperature during early growing season on carbon sequestration of a subtropical coniferous plantation. - Biogeosciences 8:1667–1678.

[56] Zhang, Y., Grant, R.F., Flanagan, L.B., Wang, S., Verseghy, D.L. (2005): Modelling CO_2 and energy exchange in a northern semiarid grassland using the carbon- and Nitrogen-coupled Canadian Land Surface Scheme (C-CLASS). – Ecol. Model. 181: 591–614.

[57] Zhou, S.X., Duursma, R. A., Medlyn, B. E., Kelly, W.G., Prentice, I.C. (2013): How should we model plant response to drought? An analysis of stomatal and non-stomatal responses to water stress.- Agriculture and Forest Meteorology 182-183: 204–214.

ABOVEGROUND NET PRIMARY PRODUCTIVITY AND COVER OF VEGETATION EXPOSED TO VARIOUS DISTURBANCES IN ARID ARGENTINA

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Abstract. We contrasted total herbaceous ANPP and cover between various disturbance types (Control, Burning, Shrub Control, Old Field 1, Old Field 2, Overgrazing) at a functional group (CSPG= cool-season perennial grasses; WSPG= warm-season perennial grasses; CSAG + D= cool-season annual grasses + dicots) and a species scale during 1984-1992. Results showed that (1) precipitation appeared to be more important than disturbance type in determining total ANPP (CSPG + WSPG + CSAG + Dicots); (2) CSPG made the greatest ($P<0.05$) contribution to ANPP and plant cover compared with that made by the other two functional groups in all disturbance types and years; (3) the preferred, late-seral *Nassella clarazii* and *Poa ligularis* showed a greater ($P<0.05$) ANPP than the earlier-seral, intermediate *Pappostipa speciosa* and non-preferred *Amelichloa ambigua* and *N. trichotoma* in 23 out of 27 comparisons in the Control, Burning and Shrub Control disturbance types. In the Old Field 1, Old Field 2 and Overgrazing disturbance types, however, *N. clarazii*, *P. ligularis*, *P. speciosa*, *A. ambigua* and *N. trichotoma* showed a similar ($P>0.05$) ANPP most of the times during 1984-1992; (4) Total plant cover of CSAG+D was most often greater ($P<0.05$) than that of WSPG at the Old Field 1 and Overgrazing disturbances.

Keywords: *aboveground net primary productivity, plant cover, disturbances, late- and early-seral plant species, Argentina*

Abbreviations: ANPP: Aboveground net primary productivity**; CSPG,** Cool-season perennial grasses**; WSPG:** Warm season perennial grasses**; CSAG + D:** Cool-season annual grasses + Dicots

Plant nomenclature: Instituto de Botánica Darwinion. Access date: 13 March 2015
http://www2.darwin.edu.ar/Proyectos/FloraArgentina/BuscarEspecies.asp

Introduction

Assessment of annual net primary productivity (ANPP) is important for planning range development (Le Houérou et al., 1988). Various disturbances can produce dynamic shifts on ANPP or total herbaceous plant cover: burning (Wright and Bailey,

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1982), control of woody vegetation using herbicides (Meyer and Bovey, 1985), removal of woody plants (Vallentine, 1990), and overgrazing (Vallentine, 1990). Use of these disturbances as a management tool is expected to increase the forage value of the treated area. Despite the relative impacts of these disturbances might vary in concert with climatic variations (e.g., precipitation: Le Houérou et al., 1988) and post-treatment management (Gartner, 1988), no studies have evaluated the effects of any of these disturbances on ANPP or plant cover during several years after their occurrence. Some studies have compared the effects of (1) ungrazed lands or (2) burning or (3) woody vegetation control using herbicides or (4) long-term undisturbed lands previously exposed to cultivation with those of long-term, abusive overgrazing on ANPP (Holechek, 1981; Fernández and Busso, 1999; Moreira et al., 2012). These studies have reported a lower ANPP and/or rangeland degradation on areas exposed to severe, abusive overgrazing.

Several studies have reported the effects of various disturbances on vegetation cover at a scale of individual plant species or functional groups. Fire, together with grazing and precipitation, is a major factor in shaping plant community structure in the Phytogeographical Province of the Caldenal, in semiarid, temperate, central Argentina (Distel and Bóo, 1996; Busso, 1997). Thousands of hectares of natural grassland are burnt every year at the south of the Caldenal (Bóo, 1990). However, the frequency of fire occurrence has decreased notably since the introduction of domestic livestock; the high grazing pressures altered the natural fire regimes because of the reduction of fine combustible material (Peláez et al., 2003). As a result, grasslands were transformed to either shrublands or areas of unpalatable grass species for domestic livestock (Pelaez et al., 2003). However, Distel and Bóo (1996) proposed that appropriate management of the frequency and intensity of fire could revert the processes of scrub formation and the presence of unpalatable grasses to states with less bushes and a greater cover of palatable perennial grasses. Several studies have reported an increase in the cover of palatable, rangeland forage species after the shrub stratum was controlled using various herbicides (Baker et al., 1980; Martin and Morton, 1980; Jacoby and Meadors, 1982). Cramer et al. (2008) reported that the abandonment of traditional agricultural lands in some areas can create old fields that require limited or no restoration. In Patagonia, plant communities are characterized by a low vegetation cover which may range from 10% in the most arid areas to 60%. They have been used as grazing areas since the early 90’s. As reported for other ecosystems, grazing causes an overall reduction of plant cover through the decrease in cover, or eventually the extinction, of palatable grass species (Schlesinger et al., 1990; Bertiller, 1996). Deflation and deposition processes, and organic matter and nutrient losses, are triggered by wind and precipitation after grazing, thus creating large areas of bare soil (Mazzarino et al., 1998). Fernández and Paruelo (1993) have reported that cover of the most palatable grass species (i.e., *Poa ligularis*) decreases, while that of the non-palatable grass species (i.e., *Amelichloa ambigua*) increases after grazing in the Occidental District of Patagonia. These changes are triggered by the direct effect of grazing (Sala, 1988), which may lead to plant death in extreme cases, or by indirect effects following cover reduction, such as erosion, and losses of soil organic matter, nutrients, and seeds that limit plant establishment (Bertiller, 1998). The increase of the relative or absolute shrub cover, and the decrease of the absolute cover of perennial grasses, occur as an extended process in grazed rangelands of Patagonia (Perelman et al., 1997). These studies have determined the benefits of using either fire or shrub control using herbicides or management of abandoned, previously-cultivated lands or proper grazing as management tools to improve rangeland forage production. However, no studies have compared the relative effects of these disturbance types occurring at the same time in determining the plant cover of palatable rangeland species.

A major determinant of the structure and function of terrestrial ecosystems is precipitation (Webb et al., 1983). However, the relative importance of precipitation or disturbance type in modifying the ANPP of herbaceous vegetation has not been studied to date. These studies are essential in arid zones where small precipitation events (<5 mm: Sala and Lauenroth, 1982) are common. For example, more than 60% of the rainfall events (mean=1983-2000) might be <5mm at the study site of the current research work (Páez et al., 2005).

The relative abundance of C_3 and C_4 plants can be controlled by the interaction between precipitation and temperature (Huang et al., 2001). Liu et al. (2005) demonstrated that increased C_4/C_3 ratios are positively correlated with higher temperatures. However, at sites with less than 500 g/m^2, annual precipitation is more strongly correlated than temperature to ANPP (Huxman et al., 2004).

The effects of various abiotic and biotic disturbances (e.g., drought, fire, soil tillage, pesticide applications, herbivory) on ANPP and total herbaceous plant cover have been assessed mostly at a plant species scale (Bedunah and Sosebee, 1995; Flemmer et al., 2003; Peláez et al., 2010; Köhl et al., 2014). However, research of the effects of these disturbances on those parameters at a plant functional group scale is rather scarce. Thus, this information is important to have a more generalized scenario on how various disturbance types might affect productivity and total herbaceous plant cover of the species within any of those functional groups under local climate conditions.

Poa ligularis Nees ex Steud, *Nassella clarazii* (Ball) Barkworth, *N. tenuis* (Phil.) Barkworth, *Pappostipa speciosa (Trin. & Rupr.) Romasch.*, *Amelichloa ambigua* (Speg.) Arriaga & Barkworth and *N. trichotoma* (Nees) Hack. Ex Arech. are abundant C_3 perennial, native grass species (please see *Table 1* for a complete species listing) in semiarid, temperate rangelands of central Argentina (Rúgolo de Agrasar et al., 2005). These species regrow in late summer, early fall, remain vegetative during winter, and flower and set seed in spring and early summer, when become dormant until the next growing season (Cano, 1988) . However, the abundance of any of these species at any one time depends, at least partially, upon grazing and fire management of the vegetation (Busso, 1997). Various studies have determined that the late-seral *N. clarazii* and *P. ligularis* are more competitive grass species than the earlier-seral *N. tenuis*, *N. trichotoma*, *A. ambigua* or *P. speciosa* (Moretto and Distel, 1997, 1999; Saint Pierre et al., 2004a,b,c; Cenzano et al., 2013). The WSPG [mostly the C_4 *Sporobolus cryptandrus* (Torr.) A. Gray , *Aristida spp.*and *Pappophorum vaginatum* Fries] start their growing cycle during November (mid spring), flower and produce fruits during early summer (December) until early fall (May), when plants enter into their dormant stage. Cool-season annual grasses and dicots [mostly *Bromus hordaceus* L., *Schismus barbatus* (L.) Thellung, *Medicago minima* (L) Grufberg and *Erodium cicutarium* (L.) L'Herit. et Ait] complete their life cycle within the period from fall to spring (Cano, 1988). Greater competitive ability in various C_3 or C_4 perennial grass species in central Argentina has been attributed to several traits like higher rates of nutrient uptake, root length density, root proliferation, mycorrhizal colonization of the root system, and more often greater relative growth rates of aboveground tissues (Moretto and Distel, 1999; Saint Pierre et al., 2002; Saint Pierre et al., 2004 a,b; Busso et al., 2008, Torres et al., 2013). However, no studies have yet addressed the effects of various disturbance types on the ANPP and plant cover of the study plant species.

Table 1. *Species composition and its degree of preference by livestock on each of the study functional groups (i.e., CSPG= cool-season perennial grasses; WSPG= warm-season perennial grasses; CSAG+D= cool-season annual grasses + dicots).*

Functional	Dominant herbaceous vegetation	Preference by
CSPG	*Nassella tenuis* (Phil.) Barkworth	Preferred
	Nassella longiglumis (Ball) Barkworth	Preferred
	Poa ligularis Nees ex Steud	Preferred
	Jarava plumose (Spreng.) SLW Jacobs &	Preferred
	Piptochaetium napostaense (Speg.)	Preferred
	Poa lanuginosa Poir	Preferred
	Bromus brevis Nees ex Steud	Preferred
	Koeleria permollis Nees ex Steud	Preferred
	Pappostipa speciosa (Trin. & Rupr.)	Intermediate
	Amelichloa ambigua (Speg.) Arriaga &	Not preferred
	Amelichoa brachychaeta (Godr.) Arriaga	Not preferred
	Nassella trichotoma (Nees) Hack. Ex	Not preferred
	Sporobolus rigens (Trin.) E. Desv.	Not preferred
WSPG	*Pappophorum vaginatum* Buckley	Preferred
	Sporobolus cryptandrus (Torr.) A. Gray	Preferred
	Aristida spegazzinii Arechav.	Intermediate
	Aristida subulata Henrard	Intermediate
	Aristida pallens Cav.	Intermediate
	Aristida trachianta Henrard	Intermediate
CSAG + Dicots	*Bromus hordeaceus* L.	Preferred
	Schismus barbatus (L.) Thellung	Preferred
	Vulpia megalura (Nutt.) Rydb	Preferred
	Lolium multiflorum Lam.	Preferred
	Hordeum murinum (Link) Arcang	Preferred
	Medicago minima (L.) Grufberg	Preferred
	Erodium cicutarium (L.) L´Herit. ex Ait	Preferred

Our objective was to compare the dynamics of total ANPP (ANPP), and total herbaceous plant cover, between different functional groups and plant species exposed to various disturbance types in northwestern Patagonia, Argentina. We hypothesized that (1) CSAG + D show a greater ANPP and plant cover at sites exposed to a severe overgrazing than at the other disturbance types, and (2) ANPP and/or plant cover are greater (a) in the CSPG than in the WSPG and CSAG + D, and (b) in the late-seral, more competitive *N. clarazii* or *P. ligularis* than in the earlier seral, less competitive *N. tenuis*, *N. trichotoma*, *A. ambigua*, or *P. speciosa* in all study disturbance types and years.

Materials and methods

Study site

Studies were performed in the Chacra Experimental de Patagones, Buenos Aires, Argentina (40º39'49.7"S, 62º53'6.4"W; 40 m.a.s.l.), within the Phytogeographical Province of the Monte (Cabrera, 1976) during 1984 through 1992. Long-term (1981-2011) mean monthly temperature and precipitation data are shown in *Figure 1*.

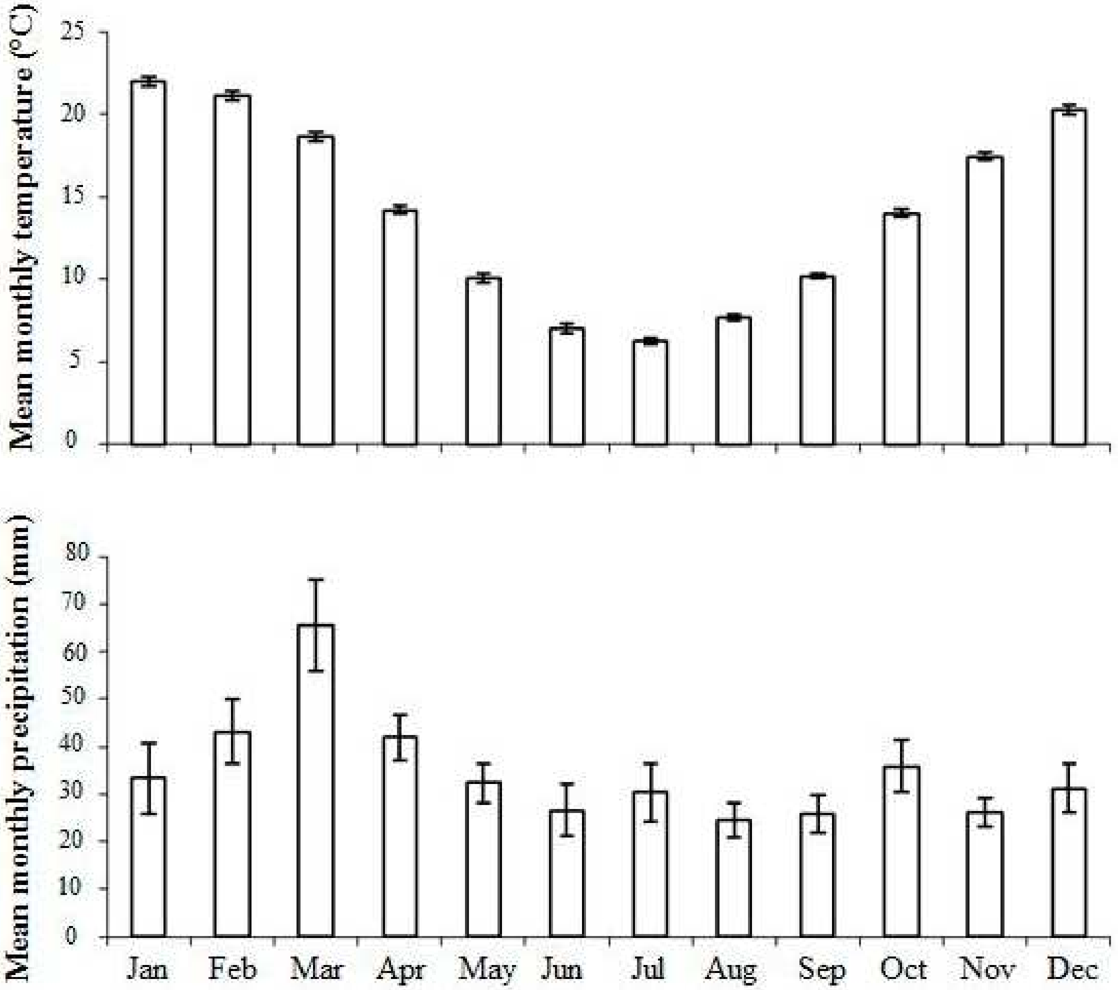

Figure 1. *Long-term (1981 to 2011) mean monthly temperatures and precipitations. Vertical bars represent ± 1 SE of the long-term mean.*

Various climatic parameters were obtained from 1984 to 1992 using a meteorological station located at 1 km away from the study area (*Figures 2 and 3*).

Climate is temperate semiarid, with higher precipitations during the spring and fall seasons (Giorgetti et al., 2000). Values for climatic variables during 1901-1950 were: mean annual temperature: 14.6^{o}C: absolute minimum temperature: -7.6^{o}C (August): absolute maximum temperature: 43 ^{o}C (January); mean annual relative humidity: 60%, and mean annual wind speed: 13 km h^{-1}.

Soil is a typical haplocalcid, with an A horizon having a loamy-clay-sandy texture; 0.20 m deep; 1.69% organic matter; 28.7 ppm available phosphorus, 0.123% total

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nitrogen. AB_w horizon was found below 0.20 m of soil depth followed by a BC_k horizon between 0.28 and 0.43 m depth. A C_k horizon existed below 0.43 m with very scarce roots. Average pH was 7.

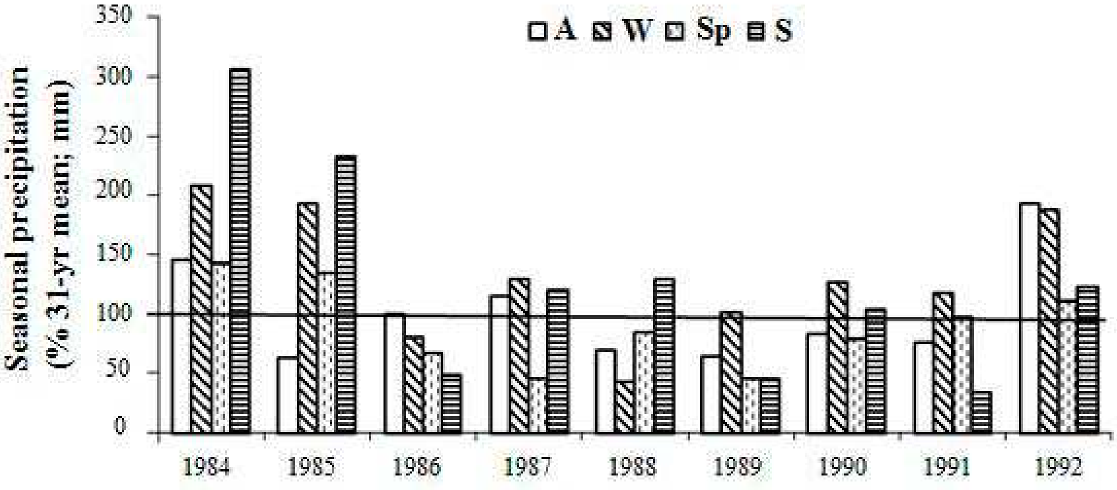

Figure 2. *Seasonal precipitation during each year of the study period (1984-1992). For any given year, each histogram represents the percentage contribution of any season to the long-term average (1981-2011) for that season. The horizontal line (417.6 mm: 100%) indicates the long-term average precipitation. A= Autumn; W= Winter; Sp= Spring; S= Summer.*

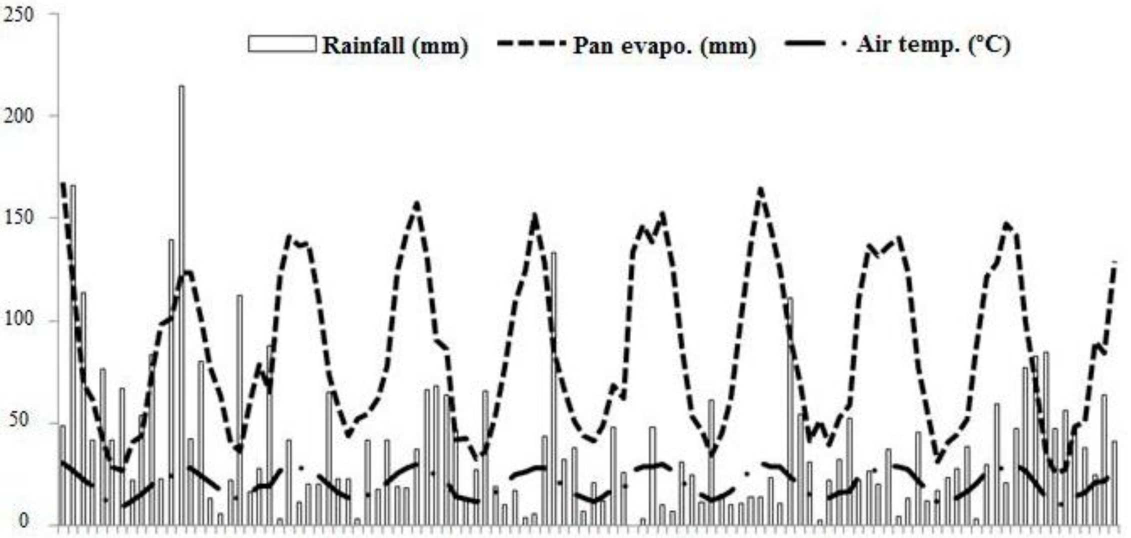

Figure 3. *Monthly precipitation, air temperature and pan evaporation during 1984 to 1992.*

The plant community is characterized by an open, shrubby stratum which includes different-quality, herbaceous species for cattle production (Busso, 1997). Dominance of a particular grass or shrubby species in the study region is partially dependent on grazing history and fire frequency and intensity (Distel and Bóo, 1996).

Labour and budget constraints made replication of this study in space and time impossible. Despite emphasis is placed on statistical differences, caution is called for extrapolating the results of this study. These should be better viewed under the specific conditions they were obtained.

Disturbances

Before disturbances were imposed at the study site, the plant community was characterized (n=20 stands) by using the abundance-dominance/sociability index of Braun Blanquet (Mueller-Dombois and Ellenberg, 1974) on 1 November 1974. *Chuquiraga erinacea* (mean = 2.3), *Bacharis ulicina* (mean = 1.2) and *Nassella tenuis* (mean = 4.4) were the species with the highest index for the shrubby, forb and grass layers, and the community was then classified as an open shrubland of *Ch. erinaea* and *Condalia microphylla* within a continuous herbaceous layer of *N. tenuis*. The study was initiated thereafter on areas which had been previously exposed to continuous grazing by cattle and sheep, and then exposed to different managements. One area (20 ha) was cleared of trees and undergrowth, and cropped from 1951 until 1975. Previous to cultivation, half of this area (Old Field 1: OF1) had been exposed to a more severe grazing than the other half (Old Field 2: OF2) because of its greater proximity to a water source for animals. During this period, an adjacent area (95 ha) which had not been cleared was grazed by cattle and sheep. Access of domestic herbivores was then excluded in both areas from 1975 to 1993. However, the 95 ha area was exposed to three different managements between December 1977 and March 1978: one site (34 ha) remained untreated (control: C), another site (37 ha) was burned (burned: B), and herbicides (shrub control: Sc) were applied on the third site (24 ha) for controlling shrubs. The last studied area was an adjacent site which had been severely overgrazed (overgrazing: O) until 1981, and then excluded from domestic herbivory until 1993.

Burning was conducted on 3 March 1978. At this time, maximum and mean air temperatures were 23.5 and 14.4 °C, respectively, mean relative humidity was 49%, and wind speed and dry weight of fine fuel load were 22 km h^{-1} and 438 kg ha^{-1}, respectively. More than or equal to 50% of plants of the shrubs *Geoffroea decorticans, Condalia microphylla*, *Lycium chilensis*, *Chuquiraga erinacea*, *Larrea divaricata* and *Schinus fasciculatus* had produced basal regrowth one year after burning. Digiuni (1983) has already reported the chemical shrub control for this study. Briefly, an aerial application of Tordon 213 (2 l ha^{-1}) and 2,4,5,-T (4 l ha^{-1}) was made on 29 December 1977, when mean air temperature and relative humidity were 18.7 °C and 58.0%, respectively, rainfall was 108 mm during December, and shrubs were at the reproductive morphological stage of development. Herbicides were very effective in producing death or total defoliation with no basal regrowth in *G. decorticans*, *C. microphylla*, *L. chilensis* and *L. divaricata*, and less than 50% defoliation in *C. erinacea* immediately after their application. Sixteen months later, however, 80-90% of *G. decorticans* and *C. microphylla* plants had not produced any regrowth, but the remaining plants and those of *L. chilensis*, *C. erinacea* and *L. divaricata* were less than 50% defoliated. Lack of enough manpower at the research station, however, prevented us to study how shrubs recovered afterwards.

Procedures

By mid-November 1978, percentage cover was determined per species within each disturbance type (n=50) by randomly distributing 20 x 20 cm quadrats following the canopy-cover method of Mueller-Dombois and Ellenberg (1974). Maximum aboveground standing crop was also estimated at the Control, Old Field 1, Old Field 2, Burned and Shrub Control disturbance type sites in 1978 (n=50), and at these and the Overgrazed site from 1984 to 1992. In late January 1984 for the CSPG and the CSAG + Dicots, and late May-early June 1984 for the WSPG, plants of the species pertaining to the various functional groups were clipped at their dormant (i.e., physiologically inactive) morphological stage of development (Rúgolo et al., 2005). In this way, this clipping did not act as a clipping treatment, and allowed that only current year's growth was included in the subsequent once-a-year harvests from 1984 to 1992 (i.e., January or May). Similar to that reported by Singh et al. (1975), aboveground standing crop [i.e., live + recent dead tissues (current growth´s production)] was taken as an approximation of ANPP. No samplings were conducted during 1979 to 1983 because of economic constraints. At harvesting time during 1984 to 1992, 30 randomly distributed, permanent plots (0.5 x 0.5 m) were clipped to 30 to 50 mm stubble height on each disturbance type. Herbage was separated by species, except in the 1978 sampling when only total herbaceous standing crop was measured, and dried in a forced draft oven at 70^{o}C until constant weight. Vegetation ANPP was then expressed on a dry weight basis. Within the desirable annual grass or forb group, a species was separated from the remaining total ANPP when its contribution to it was substantial. Previous to clipping from 1984 to 1990, plant cover of the herbaceous vegetation (i.e., cool- or warm-season perennial and annual grasses, and dicots) was also determined at a functional group scale on 20 out of the 30 randomly distributed, permanent plots (0.5 x 0.5 m) per disturbance type following the canopy-cover method of Daubenmire (1959).

Aboveground standing crop was measured by species and plant functional groups, which were comprised of cool-season, C_3 perennial grasses (CSPG); warm-season, C_4 perennial grasses (WSPG), and cool-season, C_3 annual grasses and forbs (CSAG+D). Annual forbs were not included in another functional group because they were just two plant species (i.e., *M. minima* and *E. cicutarium*), and their dry matter contribution to ANPP and plant cover were minimal. Estimates of ANPP were comprised from total standing crop harvests in all species. Total production was then calculated by summing up the individual plant functional group estimates. *Table 1* shows the species composition of each functional group and its preference by grazing livestock. Root biomass was not collected or used in estimates of production.

Statistical analysis

At first, a multivariate analysis approach was conducted using the statistic of Wilks (Wilk´s lambda) (Wilks, 1932). A repeated measures analysis was made for ANPP and plant cover between the study factors (functional groups x disturbance types) and years. Since there was an interaction between factors and years ($P<0.05$), each year was analyzed separately. Analyses were limited to annual means because seasonal analysis were not interpretable due to our inability to quantify the intra-seasonal impacts of carry-over soil water on ANPP, and herbaceous plant cover estimates. Standing crop and plant cover data were analyzed using two factorial ANOVA [functional group x disturbance types within each year]. When F tests were significant, means were always compared using the Tukey's test ($P<0.05$). The tests of Kolmogorov-Smirnov and Levene were used to evaluate normality and homoscedasticity assumptions, respectively. Because of this, data (x) of standing crop needed to be transformed to $log (x+1)$ before analysis (Zar, 1996). Untransformed values are shown in *Table 2* and *Figures 4* and *5*. Data were analyzed using the statistical software INFOSTAT (Di Rienzo et al., 2013).

Results

Precipitation

Annual precipitation across years showed a variation coefficient of 27.84% (e.g., see *Figure 2*). Average precipitation during the 1984-1992 study period (473.91 mm) was 11.9% above the long-term (1981-2011) average (417.6 mm=100%: *Figure 2*). As a percentage of the long-term average, annual precipitation ranged from 61.7 to 210.1% [257.5 (1989) to 877.3 mm (1984)]. In only 2 out of 9-study years (i.e., 1984 and 1992: *Figure 2*), each season precipitation exceeded the long-term average for that season. Means of long-term (1984-1992) seasonal precipitation were 101.2, 132, 90.4, and 127.8 mm for autumn, winter, spring and summer, respectively (*Figure 2*). Other precipitation features included (1) amounts of precipitation received in a single day ranged from 0 mm (no rainfall during November 1988) to 95.5 mm (28 Dec 1984), and (2) numbers of days with more than or equal to 5 mm rainfall fallen in a single day were 211 out of the 609 days with rainfall during the period 1984 to 1992. Monthly pan evaporation was greater than monthly precipitation most of the times during 1984 to 1992 (*Figure 3*).

Aboveground Net Primary Productivity

Total

There was an interaction between disturbance types and years ($P<0.05$). Total ANPP was higher ($P<0.05$) in the Old Field 1 and/or Field 2 than in the (1) Overgrazing and (2) Control, Burning, and/or Shrub Control disturbance types during 1984 and 1985, 1987 and 1988, and from 1990 to 1992 (*Figure 4*). Annual net primary productivity was lower ($P<0.05$) in the Overgrazing disturbance than in the Control in 1985, 1988 and 1992 (*Figure 4*). The lowest ANPP within each disturbance type was achieved in 1986 and 1989 on all disturbance types, except for the Overgrazing disturbance type in 1986 and 1991 (*Figure 4*). Also, ANPP was most often lower ($P<0.05$) in 1987, 1990 and 1991 than in 1984, 1985 and 1992 in the Control, Burning, Shrub Control and Old Field 1 disturbance types (*Figure 4*).

Functional groups

Cool-season perennial grasses showed the greatest ($P<0.05$) ANPP among functional groups in all disturbance types and study years (*Figure 5*). The greatest ($P<0.05$) ANPP of the CSPG occurred in 1984, 1985 and 1988 in the Old Field 1 disturbance type (*Figure 5*). ANPP in the cool-season perennial grass functional group was lowest ($P<0.05$) (1) in the Old Field 2 than on any other disturbance type in 1987, (2) in the Overgrazing than in the Old Field 2, Burning and Control disturbance types in 1989, and (3) in the Overgrazing than in the Old Fields 1 and 2 in 1991 (*Figure 5*). The warm-season perennial grasses showed their greatest (P<0.05) ANPP in the Old Field 2 than in any of the others disturbance types in all study years (*Figure 5*). Most of the times, the CSAG + D showed a greater (P<0.05) ANPP in the Old Field 1 and/or the Overgrazing than at the other disturbance types (e.g., OF1 and O in 1984; OF1 in 1985, 1987 and 1992; O in 1989: *Figure 5*).

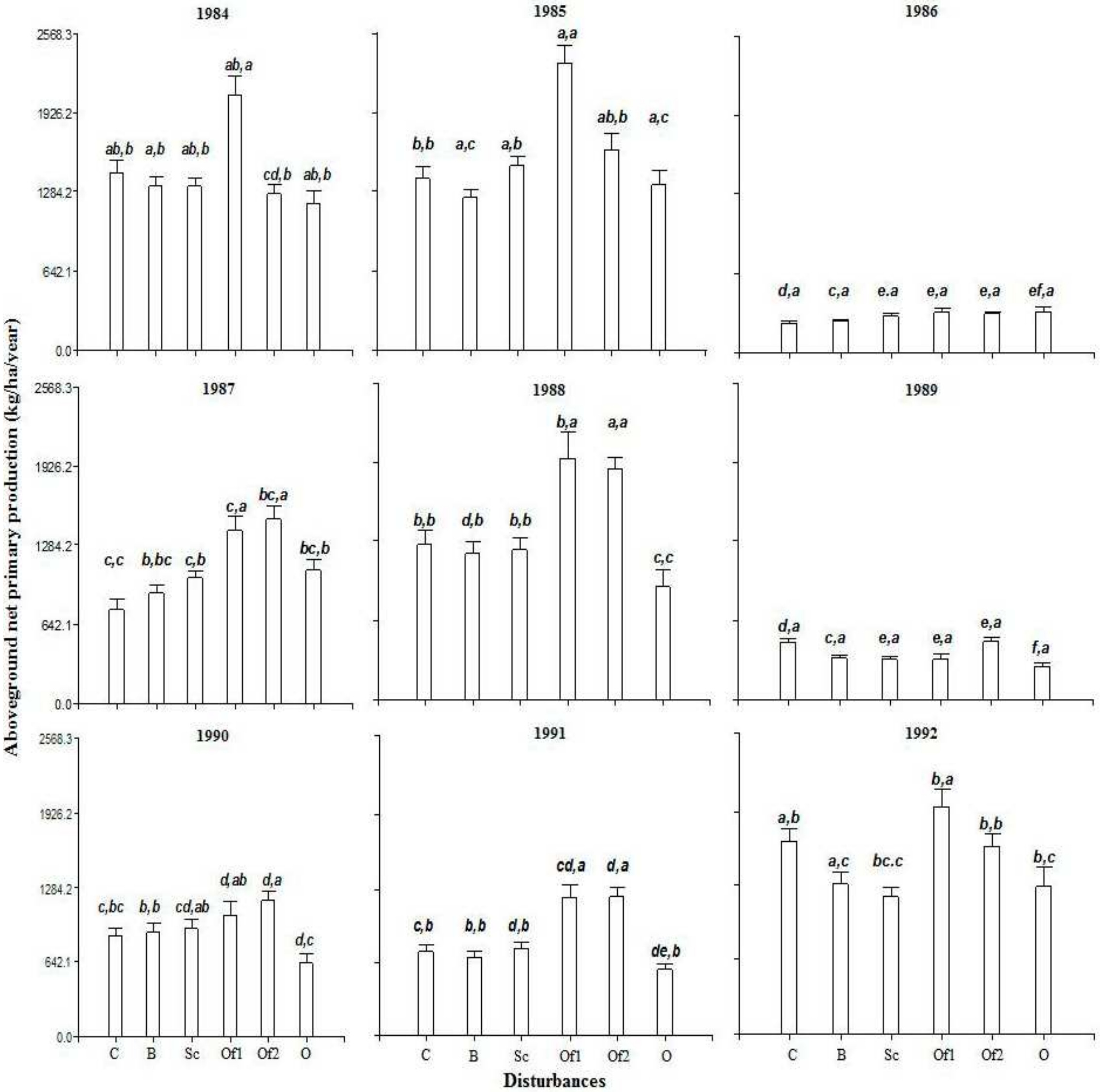

Figure 4. *Total ANPP (kg/ha/year; CSPG + WSPG + CSAG+Dicots) in the different disturbance types (C= Control, B= Burning; Sc= Shrub Control; OF1= Old Field 1; OF2= Old Field 2; O= Overgrazing) during the study years (1984 to 1992). Each histogram is the mean of n=30. Vertical bars are 1 SE of the means. Different letters to the left of the comma indicate significant differences (P<0.05) among years within each disturbance type. Different letters to the right of the comma indicate significant differences (P<0.05) among disturbance types within each year.*

Analysis of the temporal ANPP dynamics revealed that relatively, the lowest values of ANPP occurred in 1986 and 1989 in all disturbance types on the three functional groups (*Figure 5*).

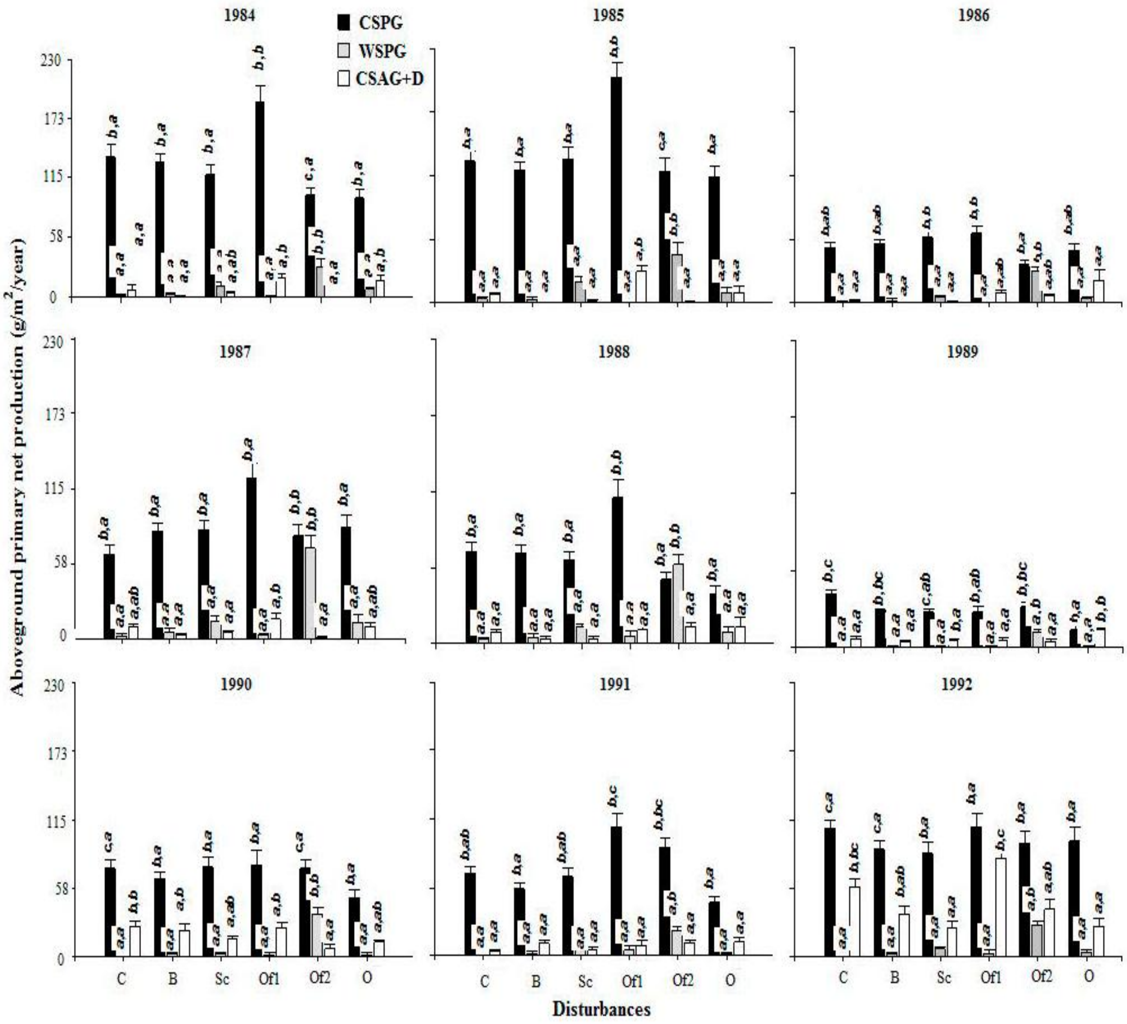

Figure 5. *Aboveground net primary productivity (gr/m²/year) of the various functional groups (CSPG= cool-season perennial grasses, WSPG= warm-season perennial grasses, CSAG+D= cool-season annual grasses + dicots) in the different disturbance types (C= Control, B= Burning; Sc= Shrub Control; OF1= Old Field 1; OF2= Old Field 2; O= Overgrazing) during the study years (1984 to 1992). Each histogram is the mean of n=30. Vertical bars are 1 SE of the means. Different letters to the left of the comma indicate significant differences (P<0.05) among functional groups within each disturbance type and study year. Different letters to the right of the comma indicate significant differences (P<0.05) within each functional group in the various disturbance types on each study year.*

Plant Species

The preferred *N. clarazii* and *P. ligularis* showed a greater (P<0.05) ANPP than the intermediate *P. speciosa* and the non-preferred *A. ambigua* and *N. trichotoma* in 23 out of 27 comparisons among these species in the Control, Burning and Shrub Control disturbance types (*Table 2*). In the Old Field 1, Old Field 2 and Overgrazing disturbance types, however, *N. clarazii*, *P. ligularis*, *P. speciosa*, *A. ambigua* and *N. trichotoma* showed a similar (P>0.05) ANPP during 1984-1992 with only a few exceptions (*Table 2*). These exceptions were (1) *A. ambigua*, Old Field 1, 1984, (2) *N. trichotoma*, Old Field 1, 1992, and (3) *N. clarazii*, Old Field 2 in 1991, and Overgrazing in 1984.

Table 2. *Annual net primary productivity (g/m2) of various CSPG on different disturbance types from 1984 to 1992. Each value represents the mean of n=30. Different letters indicate significant differences (P<0.05) among plant species within each disturbance type and study year. Plant species are Nt= Nassella tenuis; Nc= Nassella clarazii; Pl= Poa ligularis; Ps= Pappostipa speciosa; Aa= Amelichloa ambigua; Ntr= Nassella trichotoma. Disturbance types are C= Control; B= Burning; Sc= Shrub Control; OF1= Old Field 1; OF2= Old Field 2; O= Overgrazing.*

	Species	**1984**	**1985**	**1986**	**1987**	**1988**	**1989**	**1990**	**1991**	**1992**
C	Nt	25.4±2.5 b	30.6±3.2 b	17.4±2.7 c	24.9±5.4 c	14.7±3.3 b	10.5±1.8 b	20.3±3.3 b	23.1±3.5 b	25.7±4.0 b
	Nc	58.2±9.9 c	51.2±7.6 c	13.0±2.0 bc	19.1±2.9 bc	28.8±4.4 c	11.1±1.9 b	23.1±3.8 b	23.1±3.5 b	39.6±6.4 b
	Pl	31.9±5.9 b	22.0±6.0 b	9.3±1.8 b	9.0±2.2 ab	13.7±3.0 b	10.2±2.2 b	18.6±4.3 b	13.6±2.7 b	27.7±5.7 b
	Ps	1.1±0.5 a	0.2±0.2 a	0.1±0.1 a	0.0±0.0 a	0.4±0.3 a	0.0±0.0 a	0.3±0.3 a	1.5±1.5 a	0.2±0.2 a
	Aa	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0 ±0.0 a
	Ntr	0.1±0.1 a	0.8±0.8 a	0.1±0.1 a	0.3±0.3 a	0.7±0.7 a	0.7±0.7 a	0.7±0.7 a	0.9±0.9 a	0.6±0.6 a
B	Nt	25.7±3.3 b	24.8±2.4 b	12.9±1.9 b	10.0±1.9 ab	5.6±1.0 a	3.8±0.8 ab	5.3±1.3 a	5.4±1.6	7.1±2.6 a
	Nc	46.6±5.8 c	42.3±4.3 c	15.2±2.1 b	33.0±4.1 c	27.0±4.3 b	6.7±1.0 bc	15.9±2.4 b	19.2±2.8	31.9±4.6 b
	Pl	24.3±4.8 b	19.4±4.7 b	11.6±2.2 b	17.7±4.5 b	21.3±5.0 b	11.0±2.5 c	28.5±5.5 c	18.6±3.6	35.4±6.4 b
	Ps	0.00±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0	0.0±0.0 a
	Aa	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0	0.0±0.0 a
	Ntr	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0	0.0±0.0 ab
Sc	Nt	9.0±1.7 a	17.4±2.7 b	9.7±02.0 b	10.4±2.6 ab	4.9±1.6 ab	1.5±0.5 a	3.9±1.5 a	3.7±1.0	5.8±2.3 b
	Nc	36.0±6.8 b	30.2±4.7 bc	11.5±1.9 bc	19.5±3.8 bc	13.4±2.7 b	3.2±1.0 a	9.5±2.5 a	11.8±3.0	16.5±4.1 c
	Pl	41.6±5.1 b	33.0±4.8 c	16.5±2.4 c	28.6±4.6 c	28.3±5.5 c	13.0±2.1 b	46.1± 7.7 b	37.8±5.8	42.9±7.3 b
	Ps	0.00±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0	0.0±0.0 a
	Aa	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0	0.0±0.0 a
	Ntr	3.3±2.6 a	3.7±3.5 a	0.4±0.4 a	0.8±0.7 a	1.1±0.9 a	0.6±0.3 a	0.6±0.3 a	1.0±0.8	2.0±1.8 a
OF1	Nt	80.2±14.0	105.2±19.7 b	34.6±6.9 b	53.6±10.2 b	36.5±6.8 b	12.6±3.4 b	27.9±6.0 b	45.0±9.7	45.3±8.6 b
	Nc	5.5±1.7	11.4±4.9 a	5.3±1.8 a	15.3±5.4 a	13.1±4.5 a	1.5±0.6 a	6.6±2.1 ab	12.9±4.2	18.5±5.7 a
	Pl	6.4±3.7	6.6±3.3 a	4.6±2.3 a	8.2±03.6 a	9.9±4.0 a	2.7±1.3 a	9.5±4.1 ab	19.8±8.1	21.5±8.6 a
	Ps	0.0±0.0	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0	0.0±0.0 a
	Aa	44.1±8.8	28.4±8.5 a	6.9±2.1 a	17.2±6.5 a	5.0±2.6 a	2.7±2.0 a	18.7± 11.0 ab	6.9±3.9	9.7±3.8 a
	Ntr	0.0±0.0	0.0±0.0 a	0.3±0.3 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0	0.0±0.0 b

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OF2	Nt	69.4±8.4 b	57.2±8.2 b	19.3±3.0 b	36.9±6.8 b	24.0±4.7 b	22.3±3.7 b	51.3±7.2 b	49.2±7.2	35.5±5.8 b
	Nc	4.0±1.8 a	4.6±1.9 a	3.3±1.2 a	5.2±2.0 a	6.5±2.9 a	3.4±1.1 a	7.4±2.6 a	18.4±5.3	27.8±8.2 a
	Pl	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.4±0.3 a	1.4±1.0	3.0±2.1 a
	Ps	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0	0.0±0.0 a
	Aa	0.2±0.2 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0	0.0±0.0 a
	Ntr	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0	0.0±0.0 a
O	Nt	28.7±2.8 c	27.4±4.8 b	8.7±1.8 b	11.1±2.7 b	4.2±1.1 a	1.2±0.4 b	3.2±1.1 a	2.5±1.1 a	16.2±7.6 b
	Nc	8.1±1.8 b	7.7±2.7 a	2.1±0.7 a	4.1±1.3 a	3.3±1.5 a	1.0±0.5 ab	3.5±2.1 a	2.7±1.7 a	6.6±4.0 ab
	Pl	1.5±1.0 b a	1.1±1.0 a	1.3±1.3 a	1.4±1.4 a	2.1±1.9 a	0.5±0.3 ab	5.3±3.6 a	3.7±2.3 a	3.5±2.5 ab
	Ps	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a
	Aa	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a
	Ntr	3.6±2.1 a	6.9±4.5 a	0.6±0.4 a	1.4±1.4 a	0.4±0.4 a	0.0±0.0 a	0.9±0.9 a	0.00±0.0	1.7±1.2 ab

Nassella clarazii showed a similar (P>0.05) or greater (P<0.05) ANPP than *P. ligularis* during all years and disturbance types (*Table 2*). The only exceptions occurred in the Shrub Control disturbance type during 1988 to 1992, and the Burning disturbance type in 1990, where *P. ligularis* had a greater (P<0.05) ANPP than *N. clarazii* (*Table 2*). *Nassella tenuis* showed the greatest (P<0.05) contribution among all six study species in the Old Field 1, Old Field 2 and Overgrazing disturbance types except in 7 (Old Field 1: 1990; Old Field 2: 1991 and Overgrazing: 1988 to 1992) out of 27 comparisons (*Table 2*).

Plant cover

Plant Functional Groups

Except in the Old Field 2 from 1987 to 1990, the CSPG showed a greater (P<0.05) percentage cover than the other two functional groups in all disturbance types and years (*Figure 6*). In 1988, 1989 and 1990, the WSPG showed a greater (P<0.05) percentage plant cover than the CSPG at the Old Field 2 (*Figure 6*). In 1984 and from 1987 to 1990, percentage plant cover was greater (P<0.05) on the CSAG + D than on the WSPG (P<0.05) in the Old Field 1 and Overgrazing disturbance types (*Figure 6*). At these disturbance types and in the Control, the CSAG + D also showed a greater (P<0.05) percentage cover than the WSPG from 1988 to 1990 (*Figure 6*).

Disturbance types

Percentage plant cover of CSPG was lower (P<0.05) in the Old Field 2 and Overgrazing disturbance types than in Control in all study years (*Figure 6*). Percentage plant cover of the CSPG was greater (P<0.05) at the Shrub Control than at any of the other disturbance types in 1987 (*Figure 6*). Cool-season perennial grasses showed a similar (P>0.05) percentage plant cover at the Old Field 1 and Overgrazing disturbance types from 1984 to 1990 (*Figure 6*). Warm-season perennial grasses showed the greatest (P<0.05) percentage cover in the Old Field 2 that at any of the other disturbance types from 1984 to 1990 (*Figure 6*). Percentage cover of this functional group was greater (P>0.05) in the Shrub Control than in the Old Field 1 disturbance type during the whole study period (1984 to 1990) (*Figure 6*). From 1986 to 1989, percentage cover of WSPG was also lower (P<0.05) in the Overgrazing than in the Shrub Control disturbance type (*Figure 6*). Percentage cover of the CSAG + D was greater (P<0.05) at the Old Field 1 than at the (1) other disturbance types (but the Old Field 2 disturbance type) in 1989, (2) Shrub Control and Burning disturbance types in 1987 and 1989, and (3) Burning disturbance type in 1988 (*Figure 6*). Also, percentage cover of this functional group was similar (P>0.05) at the Old Field 1, Old Field 2 and Overgrazing disturbance types from 1987 to 1989, and greatest (P<0.05) at the Old Field 2 than at any other disturbance type in 1990 (*Figure 6*).

Discussion

Lowest values of ANPP were achieved at the lowest values of annual precipitation in all study disturbance types. This indicates that annual precipitation would be relatively more important than disturbance type in determining ANPP in the study functional groups.

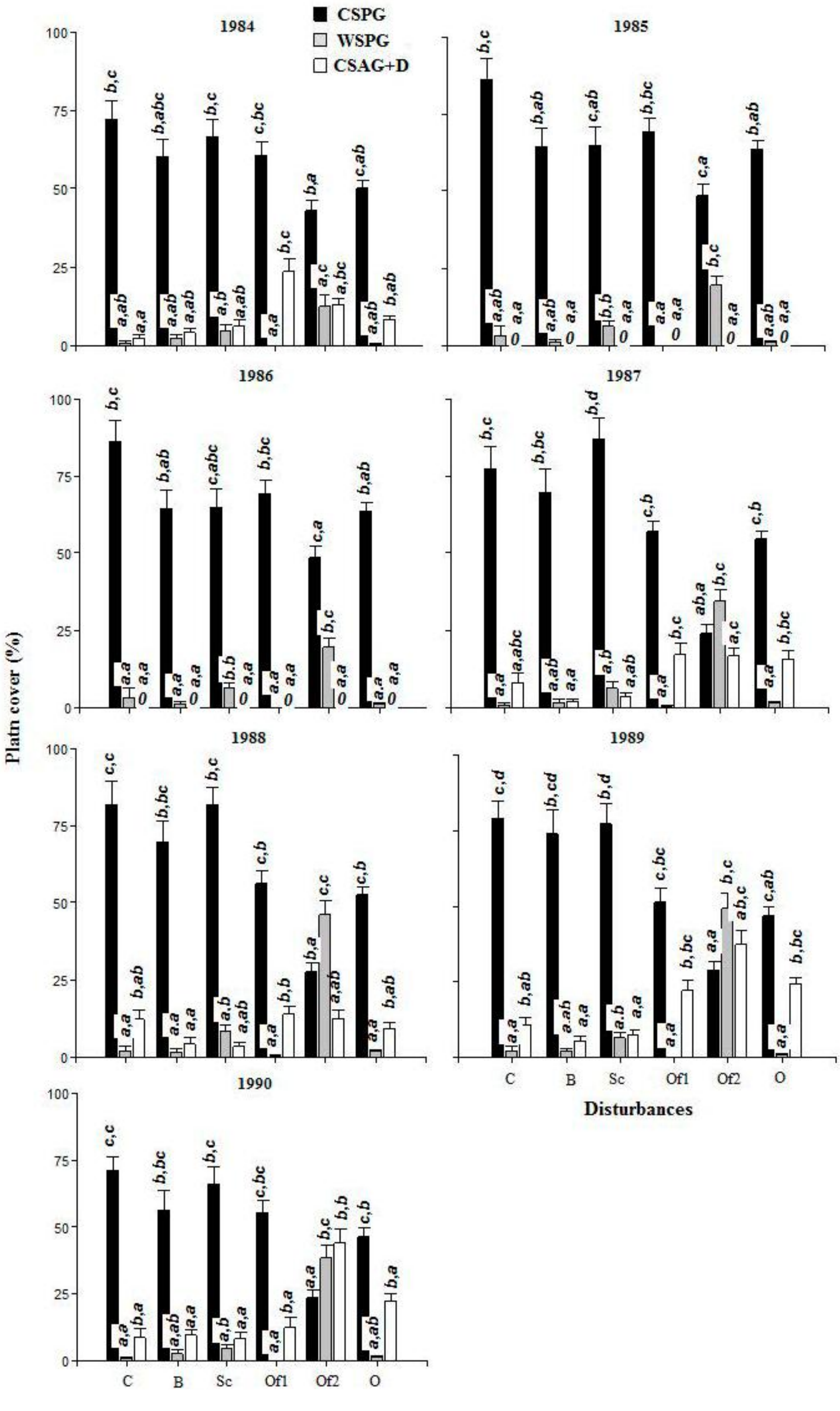

Figure 6. *Total herbaceous plant cover (%) of the various functional groups (CSPG= cool-season perennial grasses, WSPG= warm-season perennial grasses, CSAG+D= cool-season annual grasses + dicots) in the different disturbance types (C= Control, B= Burning; Sc= Shrub Control; OF1= Old Field 1; OF2= Old Field 2; O= Overgrazing) during the study years (1984 to 1992). Each histogram is the mean of n=30. Vertical bars are 1 SE of the means. Different letters to the right of the comma indicate significant differences (P<0.05) among disturbance types within each functional group and study year. Different letters to the left of the comma indicate significant differences (P<0.05) among functional groups within each disturbance type and study year.*

Total Annual Net Primary Productivity

The lowest and highest total annual herbaceous ANPP were obtained in the Burned (mean=967 kg ha^{-1} yr^{-1}, 52% of control) and Old Field 2 (mean=3804 kg ha^{-1} yr^{-1}, 203% of control) disturbances, respectively, one year after treatments (data not shown). Bartos et al. (1994) and Cook et al. (1994) reported similar results. Other studies have reported an increased forage production after mechanical disturbances in rangelands (Griffith et al., 1985), very likely because of the release of plant nutrients as a result of soil disturbance (Haferkamp et al., 1993). This might be because total ANPP was higher in the Old Field 1 and/or Old Field 2 than in the other disturbance types during most of the study years.

The only years where all disturbance types showed a similar, low ANPP were 1986 and 1987. These years were when monthly precipitation most of the months, and total annual precipitation, were below the long-term average. In the other years, which showed a similar or higher annual precipitation than the long-term average, there were significant differences among disturbance types to a greater or lower extent. This indicates that the amount of annual precipitation imposed a greater constraint than disturbance type to ANPP. A myriad of studies have shown decreases in total ANPP with decreases in total annual precipitation (Walker, 1999), emphasizing that precipitation is a major determinant of the structure and function of terrestrial ecosystems (Webb et al., 1983).

In some years (1985, 1988, 1992), total annual net primary productivity was lower in the Overgrazing than in the Control disturbance type. Other studies have also shown a decrease in ANPP on grazed than ungrazed perennial rangeland grasses (Busso and Richards, 1995).

During 1984 to 1992, total ANPP ranged from 261.2 (Control, 1986) to 2481.21 (Old Field 1, 1985) kg ha^{-1} yr^{-1} (Figure 4). These values are within the range reported by Digiuni (1983), Cano (1988) and Fresnillo Fedorenko et al. (1991) for rangelands within the Phytogeographical Province of the Espinal, Argentina. Total ANPP was lower in the Control than in the (1) Old Field 1 disturbance in 1984-1988 and 1991, and (2) Old Field 2 disturbance in 1986-1988 and 1991 (Figure 4). Once again, nutrient release as a result of soil disturbance in Old Fields 1 and 2 might have contributed to the increased ANPP in both study Old Fields than in the Control (Haferkamp et al., 1993).

The Control and Shrub Control disturbances had a similar total ANPP between 1984-1992, except in 1989 and 1992, when it was greater in the Control than in the Shrub Control disturbance. Meyer and Bovey (1985) also reported that dry weight of grasses on a rangeland pasture in east-central Texas treated with 2,4,5-T was similar to that in untreated areas 4-17 month after treatment. In 1989 and 1990, total ANPP was greater in the Control than in the Overgrazing treatment. Increased grazing intensity has already been shown to decrease herbaceous standing crop in several arid or semi-arid ecosystems of North and South America, and Australia (Archer and Smeins, 1991).

As a result of overgrazing, open, nude patches can appear between vegetation patches in arid and semiarid rangelands of central Argentina. These open patches, where colonies of the herbivore vizcacha (*Lagostomus maximus*, Chinchillidae) might establish (Bontti et al., 1999), are ideal for the germination, establishment, and substantial dry matter production of mostly *Medicago minima* and *Erodium cicutarium* in wet years (Fresnillo Fedorenko et al., 1991). This might be one of the major reasons to explain why forage production of the CSAG+D was greater in the Old Field 1 and

Overgrazing than in the remaining disturbance types during most of the years above the long-term average annual precipitation.

Functional groups

The greatest productivity among the study functional groups was that on CSPG in all disturbance types and study years. Giorgetti et al. (1999) reported that plant cover of CSPG is dominant in well-managed rangelands of arid, southwestern Buenos Aires, Argentina. The cattle production industry in 75% of continental Argentina, characterized by arid and semiarid areas, is based upon grazing of native vegetation (Fernández and Busso, 1999). Unfortunately, most of these areas have been exposed to grazing and fire mismanagement, resulting in shrub enchroachment and the local disappearance of acceptable perennial forage grasses for cattle grazing (Fernández and Busso, 1999). As a result, rangelands of central Argentina have few warm-season, native perennial grasses that are acceptable forages to grazing livestock (i.e., *Pappophorum vaginatum*; Busso et al., 2004). This helps to explain the low ANPP of the WSPG in most disturbance types and study years. The introduction of WSPG which are a food constraint for grazing cattle and sheep constitutes an unsolved, critical challenge to date in arid, northeastern rangelands of Patagonia (Torres et al., 2013). The great contribution of CSAG to total ANPP in the Old Field 1 and Overgrazing disturbance types is coincident with the state-and-transition model reported by Distel and Bóo (1996). These authors stated that annuals will take over the open, nude surface areas left between vegetated patches when range management is characterized by very high grazing intensities and fire mismanagement. LLorens (1995) also reported that annuals occur on highly disturbed sites on a variety of soil types.

Plant Species

The combined contribution of *N. clarazii and P. ligularis* to total ANPP was less or greater than 24 or 44% in the Overgrazing or Control treatment, respectively, during 1984-1992 (data not shown). Bóo et al. (1993) reported that these two grazing tolerant, species (Saint Pierre et al., 2004 a,b; Gittins, 2011) are the most highly preferred by cattle in semi-arid rangelands of central Argentina, and tend to disappear from the pastures with heavy grazing.

Non-preferred perennial grasses (*Table* 1) were mostly confined to the Old Field1 (e.g. *A. ambigua*: *Table* 2) and Overgrazing (e.g., *N. trichotoma*: *Table* 2) disturbance types. Species of this group, namely *A. ambigua*, *A. brachychaeta* and *N. trichotoma* (*Table* 1) are indicative of previous grazing mismanagement conducive to rangeland deterioration (Cano, 1988). A greater ANPP of *A. ambigua* in the Old Field 1 was probably due to the greater proximity of this site to a water source for cattle, causing a more severe and continuous grazing.

Nassella clarazzi and *P. ligularis* are among the most competitive native perennial grasses in the study region (Saint Pierre et al., 2004b; Ithurrart, 2014). It has been reported that non-preferred, native perennial grasses (e.g., *A. ambigua*), avoided by grazing animals, have a higher tissue C/N and lignin/N ratios than preferred, native perennial grasses (e.g., *Nassella clarazzi* and *P. ligularis*) in central Argentina (Moretto et al., 2001). The similar N concentration on green leaves of *P. ligularis* than on those of *N. clarazii* (Saint Pierre et al., 2004b; Moretto et al., 2013) and the lower lignin content on leaf cells of *P. ligularis* than on those of *N. clarazii* (Moretto et al., 2001)

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suggests that grazing livestock might have a relatively greater preference of *P. ligularis* than *N. clarazii*. This might help to explain the greater ANPP in *N. clarazii* than *P. ligularis* most of the times. Both species have been shown to be very competitive for soil resources (Saint Pierre et al., 2004b; Ithurrart, 2014). However, which species is more competitive than the other one has not yet been determined.

Cultivation over 25 years must have eliminated the original vegetation in the open shrubland with a continuous herbaceous layer of *N. tenuis*. After abandonment, successful seed germination and establishment from existing seeds of *N. tenuis* in areas with low interference from surrounding vegetation (Distel et al., 1992), and the very high vegetative reproduction reported for this species (Busso et al., 1993), may help to explain its persistence and high contribution to total herbaceous standing crop in both Old Field treatments.

The Burning disturbance may have been severe enough to negatively affect intermediate (i.e., *P. speciosa*, *Table 1*) and not-preferred (*A. ambigua* and *N. trichotoma*) perennial grasses which were absent during 1984-1992 (*Table 2*). Bóo et al. (1996) reported very high mortality rates in *P. speciosa* (previously *S. speciosa*) after a very severe wildfire during summer time. These authors attributed this response to the fact that *P. speciosa* is not readily consumed by cattle, and accumulates old growth which may explain the high burning temperatures. A similar response might be expected with the other grass species of these groups because they may only be grazed when better forage is not available (Cano, 1988), which would also allow for old growth accumulation. Species such as *P. ligularis*, *N. clarazii* and *N. tenuis* have had lower mortality rates than *P. speciosa* after a severe wildfire partly because they are readily grazed by cattle and do not accumulate as much old growth around the growing points (Bóo et al., 1996).

Cool-season annual grasses + Dicots were either absent or contributed up to approximately 50% of total ANPP in 1989 (Overgrazing disturbance type) (*Figure 5*). The contribution of the desirable annual grasses or dicots to total ANPP was usually higher in the Overgrazing or Old Field 1 disturbance type than in others. Annual grasses have been shown previously to increase with overgrazing in semi-arid rangelands of central Argentina (Cano, 1988), and with soil disturbance in South Dakota (Gartner et al., 1986). Our ANPP values for *M. minima* and *E. cicutarium* during 1984-1992 in all treatments (*Figure 5*) are lower than those reported by Fresnillo Fedorenko et al. (1991) on overgrazed and ungrazed sites of the Phytogeographical Province of the Espinal, Argentina. *Medicago minima*, and to a lesser extent *E. cicutarium*, however, can become major forages and an important complement of the herbivorous diet under conditions of aridity and overgrazing (Fresnillo Fedorenko et al., 1991).

Herbaceous plant cover

Functional groups

We showed that contribution to total herbaceous plant cover was greater on CSPG than on WSPG and CSAG+D in most of the disturbance types and study years. Volesky et al. (2010) reported that warm-season grasses usually have less total annual production because they do not actively grow during as much of the growing season as do cool-season grasses in Nebraska. These authors also considered that warm-season grasses cannot be made to grow at all during early spring and fall when soil and air temperatures are cool. This agrees with the shorter growing cycle on WSPG than on

CSPG in rangelands of central Argentina. The total growth cycle is from late-February (i.e., late summer) to mid-December (i.e., late spring) for CSPG, and from mid-spring (i.e., November) to late fall (i.e., May) for WSPG (Giorgetti et al., 2000).

Most of the times, total herbaceous plant cover of CSAG+D was greater than that of WSPG at the Old Field 1 and Overgrazing disturbance types. These results are similar to those reported by Digiuni (1983) who showed that desirable annual grasses [e.g., *Bromus catharticus* Vahl; *B. mollis* L.; *Hordeum murinum* L.; *Lolium multiflorum* Lam.; *S. barbatus*; *Vulpia megalura* (Nutt.) Rydb.] or forbs (e.g., *E. cicutarium*; *M. minima*) contributed up to 45% or 22%, respectively, of herbaceous standing crop on overgrazed or abandoned fields, respectively. Annual grasses and dicots have been shown previously to increase with overgrazing in semi-arid rangelands of central Argentina (Cano, 1988; Distel and Bóo, 1996), and with soil disturbance in the Great Basin (Klemmedson and Smith, 1964) and South Dakota (Gartner et al., 1986). Fresnillo Fedorenko et al. (1991) showed that the dry matter production of dicots (e.g., *M. minima*, *E. cicutarium*, and species of the genus *Plantago*, *Herniaria*, *Capsella* and *Geranium*) was greater on grazed than on ungrazed sites in rangelands of central Argentina. Species of this group (i.e., CSAG+D) are indicative of previous grazing mismanagement conducive to rangeland deterioration (Cano, 1988). However, the legume *M. minima* and the Geraniaceae *E. cicutarium* can become major forages and an important complement of the herbivorous diet under conditions of overgrazing in years when annual precipitation exceeds the long-term average (Fresnillo Fedorenko et al., 1991). This is similar to that reported by White (1985) and Whisenant (1990) that the standing crop of desirable annuals does positively correlate with annual rainfall.

Disturbance types

Plant cover of CSPG was greatest in the Shrub Control disturbance type in 1987. Similarly, plant cover of WSPG was greater at the Shrub Control than at the Old Field 1 (1984-1990) and Overgrazing (1986-1989) disturbance types. Bonan (2002) reported that control of sagebrush is a proven method to increase forage for livestock in the semi-arid intermountain west of the United States. Sturges (1993) showed that herbicide application virtually eliminated sagebrush throughout 20 years; productivity of grasses more than double compared with untreated sites for the first three years following treatment, and was still higher than untreated sites 10 to 17 years following shrub removal. Increases from 5 to 15 times in desirable perennial grasses after herbicide application to shrubs have also been reported by Baker et al. (1980).

Our results are similar to those of Tizón et al (2010). These authors reported the convenience of using controlled fires for reversing processes of scrub formation to states with more diverse and productive grassland communities. Using a fire frequency of 7 to 8 years, Tizón et al. (2010) demonstrated a proliferation of palatable perennial grasses at a study site of the Caldén Phytogeographical District in central Argentina. A characteristic palatable perennial grass in the region, *Nassella clarazii*, showed the greatest cover when the controlled-fire frequency was from 7 to 8 years. Their results showed that use of intermediate controlled-fire frequencies would favor the aerial cover of palatable perennial grasses in states that are most useful for a sustainable cattle production.

Plant cover of CSAG+D at the Old Field 1 was either greater than that at (a) any other disturbance type (but OF2) in 1989, and (b) the Shrub Control and Burning disturbance types in 1987, 1988 and 1989, or similar to that in the OF2 and Overgrazing disturbance types from 1987 to 1989. These results are similar to those of Distel and Bóo (1996), who reported the appearance of annual grasses and dicots under extreme conditions of land degradation in the Phytogeographical Province of the Espinal, namely ''The Caldenal'', in central Argentina as a result of grazing mismanagement.

Plant cover of the WSPG (1984 to 1990) and CSAG+D (1990) were the greatest at the Old Field 2 disturbance type. Du et al. (2007) demonstrated that as community biomass increased with time from farmland abandonment, various soil nutrients (e.g., P, total N, organic matter, nitrate nitrogen) also increased at the same time. Thus, it is possible that soil nutrient increases with time from farmland abandonment (i.e., Old Field 2) might have contributed to increase plant cover of the WSPG and the CSAG+D.

Conclusions

In most study years (1984 to 1992), values for total (*Figure 4*) and functional-group ANPP (*Figure 5*), and total plant cover (*Figure 6*) were disturbance-type dependent. This emphasizes the importance of the previous-land history in determining the subsequent values for those study variables. However, lowest values of total ANPP were achieved in years with the lowest annual precipitation in all disturbance types. This indicates that annual precipitation would be more important than disturbance type in determining ANPP in the study functional groups.

Cool-season perennial grasses made the greatest ($P<0.05$) contribution to ANPP and plant cover (*Figures* 5 and 6) compared with that of the other two functional groups in all disturbance groups and years.

In more than 55% of the study years, ANPP of CSAG + D was greater ($P<0.05$) than that of the WSPG at the Old Field 1 and/or Overgrazing disturbance types (*Figure 5*). This was most likely because of the creation of nude, un-vegetated patches as a result of abusive over-grazing in those disturbance types. Legume production at these excessively overgrazed locations (e.g. *Medicago minima*) has been particularly high during wet years in similar arid to semiarid zones (see Fresnillo Fedorenko et al., 1991).

The preferred, late-seral *N. clarazii* and *P. ligularis* showed a greater ($P<0.05$) ANPP than the earlier-seral, intermediate *P. speciosa* and the non-preferred *A. ambigua* and *N. trichotoma* in 23 out of 27 comparisons among these species in the Control, Burning and Shrub Control disturbance types. In the Old Field 1, Old Field 2 and Overgrazing disturbance types, however, *N. clarazii*, *P. ligularis*, *P. speciosa*, *A. ambigua* and *N. trichotoma* showed a similar ($P>0.05$) ANPP during 1984-1992 with only a few exceptions [i.e., (1) *A. ambigua*, Old Field 1, 1984, and (2) *N. clarazii*, Old Field 2 in 1991, and Overgrazing in 1984]. These results indicate that ANPP would be most likely greater in the late-seral, preferred (e.g., *N. clarazzii*, *P. ligularis*) than in the earlier-seral, non-preferred (e.g., *A. ambigua*, *P. speciosa*) perennial grasses under good management conditions.

REFERENCES

[1] Archer, S., Smeins, F. E. (1991): Ecosystem-level processes. – In: Heitschmidt, R.K., Stuth, J.W. (Eds.) Grazing Management. An ecological perspective, Timber Press, Portland, 109-139.

[2] Baker, S. L., Powell, J., Morrison, R. D., Strizke, J. F. (1980): Effects of atrazine, 2,4-D and fertilizer on crude protein content of Oklahoma Tall-Grass Prairie. – Journal of Range Management 33: 404-408.

[3] Bartos, D .L., Brown, J. F., Booth, G. D. (1994): Twelve years biomass response in aspen communities following fire. – Journal of Range Management 47: 79-83.

[4] Bedunah, D. J., Sosebee, R. E. (1995): Wildland plants. Physiological Ecology and Developmental morphology, Society for Range Management, Denver, U.S.A.

[5] Bertiller, M. B. (1996): Grazing effects on sustainable semiarid rangelands in Patagonia. The state and dynamics of the soil seed bank. – Environmental Management 20: 123-132.

[6] Bertiller, M. B. (1998): Spatial patterns of the germinable soil seed bank in northern Patagonia. – Seed Science Research 8: 39-45.

[7] Bonan, G. B. (2002): Agroecosystems. - Ecological Climatology: concepts and applications. – Cambridge University Press, Cambridge, U.S.A, 508-521.

[8] Bontti, E. E., Bóo, R. M., Lindstrom, L. I., Elía, O. R. (1999): Botanical composition of cattle and vizcacha diets in central Argentina. – Journal of Range Management 52: 370-377.

[9] Bóo, R. M. (1990): Algunos aspectos a considerer en el empleo del fuego. – Revista de la Facultad de Agrononía de la UNLPampa 5: 63-80.

[10] Bóo, R. M., Lindstrom, L. I., Elia, O. R., Mayor, M. D. (1993): Botanical composition and seasonal trend of cattle diets in central Argentina. – Journal of Range Management 46: 479-482.

[11] Bóo, R. M., Pelaez, D. V., Bunting, S. C., Elia, O. R., Mayor, M. D. (1996): Effect of fire on grasses in central semi-arid Argentina. – Journal of Arid Environments 32: 259-269.

[12] Busso, C. A. (1997): Towards an increased and sustainable production in semiarid rangelands of Central Argentina: Two decades of research. – Journal of Arid Environments 36: 197-210.

[13] Busso, C. A., Giorgetti, H. D., Montenegro O. A., Rodríguez G. D. (2004): Perennial grass species richness and diversity on Argentine rangelands recovering from disturbance. – ΦYTON, International Journal of Experimental Botany 73: 9-27.

[14] Busso, C .A., Bolletta, A. I., Flemmer, A. C., Montani, T. (2008): Influence of field soil water status on arbuscular mycorrhiza in three semi-arid perennial grasses of different successional stages in rangelands of central Argentina. – Annales Botanici Fennici 45: 435-447.

[15] Busso, C .A., Bóo, R. M., Peláez, D.V. (1993): Fire effects on bud viability and growth of *Stipa tenuis* in semiarid Argentina. – Annals of Botany 71: 377-381.

[16] Busso, C. A., Richards, J. H. (1995): Drougth and clipping effect on tiller demography and growth of two tussock grasses in Utah. – Journal of Arid Environments 29: 239-251.

[17] Cabrera, A. L. (1976): Regiones fitogeográficas Argentinas. Enciclopedia Argentina de Agricultura y Jardinería, Tomo II, Editorial ACME, S.A.C.I Buenos Aires, Argentina.

[18] Cano, E. (1988): Pastizales naturales de La Pampa. Descripción de las especies más importantes, Convenio AACREA-Provincia de La Pampa, La Pampa, Argentina.

[19] Cenzano, A. M., Varela, M. C., Bertiller, M. B., Luna, M. V. (2013): Effect of drought on morphological and functional traits of *Poa ligularis* and *Pappostipa speciosa*, native perennial grasses with wide distribution in Patagonian rangelands, Argentina. – Australian Journal of Botany 61: 383-393.

[20] Cook, J. G., Hershey, T. J, Irwin, L. L. (1994): Vegetative response to burning on Wyoming mountain-shrub big game ranges. – Journal of Range Management 47: 296-302.

[21] Cramer, V. A., Hobbs, R. J., Standish, R.J. (2008): What´s new about old fields?. Land abandonment and ecosystem assembly. – Trends in Ecology and Evolution 23: 104-112.

[22] Daubenmire, R. (1959): A canopy-coverage method for vegetation analysis. – Northwestern Scientist 33: 43-64.

[23] Digiuni, D. (1983): Métodos de desmonte y su impacto sobre el pastizal natural en el noroeste de Río Negro. – Estación Experimental IDEVI, Serie Técnica 11: 1-73.

[24] Di Rienzo, J. A, Casanoves, F., Balzarini, M. G, Gonzalez, L., Tablada, M., Robledo, C. W. (2013): InfoStat versión 2013. Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina

[25] Distel, R. A., Bóo R. M. (1996): Vegetation states and transitions in temperate semi-arid rangeland of Argentina. – In: West, N (Ed.) Proceedings of the Fifth International Rangeland Congress, Society for Range Management, Denver, U.S.A., 117-118.

[26] Distel, R. A., Peláez, D. V., Fernández, O. A. (1992): Germination of *Piptochaetium napostaense* (Speg.) Hackel and *Stipa tenuis* Phil. and seedling survival under field conditions. – Rangeland Journal 14: 49-55.

[27] Du, F., Liang, Z., Xu, X., Shan, L., Zhang, X. (2007): Community biomass of abandoned farmland and its effects on soil nutrition in the Loess hilly region of Northern Shaanxi, China. – Acta Ecologica Sinica 27: 1673−1683.

[28] Fernández, O. A., Busso, C. A. (1999): Arid and semi-arid rangelands: two thirds of Argentina. – In: Arnalds, O., Archer, S. (Eds.) Case Studies of Rangeland Desertification, Agricultural Research Institute, Reykjavik, Iceland, 41-60.

[29] Fernández, R .J., Paruelo, J. M. (1993): Estepas arbustivo-graminosas de *Stipa* spp. del centro-oeste del Chubut. – In: Paruelo, . M.., Bertiller, M. B., Schlichter, T., Coronato F. (Eds.) Secuencias de deterioro en distintos ambientes patagónicos. Su Caracterización mediante el modelo de estados y transiciones, Convenio Argentino-Alemán de Cooperación Técnica INTA-GTZ. Proyecto LUDEPA-SME. San Carlos de Bariloche, Argentina, 40-46.

[30] Flemmer, A. C., Busso, C. A., Fernández, O. A., Montani, T. (2003): Effects of defoliation under varying soil water regimes on aboveground biomass of perennial grasses. – Arid Soil Research and Management 17: 139-152.

[31] Fresnillo Fedorenko, D. E., Fernández, O. A., Busso, C. A. (1991): Forage production of the annual legume *Medicago minima* in semiarid rangelads of central Argentina. – In: International Rangeland Congress. Montpellier, France, 372-374.

[32] Gartner, F. R. (1988): Improvements practices that increase range efficiency. – In: White, R.S., Short, R.E. (Eds.) Achieving Efficient use of Rangeland Resources, Montana Agriculture Experiment Station, Montana, U.S.A., 86-91.

[33] Gartner, F. R., White, E. M. and Butterfield, R. I. (1986): Mechanical treatment and burning for high quality range forage. – In: Agriculture Experiment Station and Coop Extension Service, Brookings, 135-140.

[34] Giorgetti, H. D., Manuel, Z., Montenegro, O. A., Rodríguez, G. D., Busso, C. A. (2000): Phenology of some herbaceous and woody species in central, semiarid Argentina. – ΦYTON, International Journal of Experimental Botany 69: 91-108.

[35] Giorgetti, H. D., Montenegro, O. A., Rodríguez, G. D., Busso, C. A. (1999): Influencia de manejos previos en la Provincia Fitogeográfica del Monte: Porcentaje de cobertura. XIX Reunión de la Asociación Argentina de Ecología, Tucumán, 100.

[36] Gittins Lopez, C. G. (2011): Mecanismos de persistencia de *Poa ligularis* expuesta a varias frecuencias de defoliación en el noroeste de la Patagonia. PhD Dissertation, Universidad Nacional del Comahue, Bariloche, Río Negro, Argentina. 370 p.

[37] Griffith, L. W., Schuman, G. E., Rauzi, F., Baumgartner, R. E. (1985): Mechanical renovation of shortgrass prairie for increased herbage production. – Journal of Range Management 38: 7-10.

[38] Haferkamp, M. R., Volesky, J. D., Borman, M. M., Heithschmidt, R. K., Currie, P. O. (1993): Effects of mechanical treatments and climatic factors on the productivity of Northern Great Plains rangelands. – Journal of Range Management 46: 346-350.

[39] Holechek, J.L. (1981): Livestock Grazing Impacts on Public Lands: A Viewpoint. – Journal of Range Management 34: 251-254.

[40] Instituto de Botánica Darwinion, http://www2.darwin.edu.ar/Proyectos/FloraArgentina/BuscarEspecies.asp, Access date: 26 February 2016.

[41] Huang, Y., Street-Perrott, F. A, Metcalfe, S. E., Brenner, M., Moreland, M., Freeman, K. H. (2001): Climate change as the dominant control on glacial-interglacial variations in C_3 and C_4 plant abundance. – Science 293: 1647-1651.

[42] Huxman, T. E., Smith, M. D., Fay, P. A., Knapp, A. K., Shaw, M. R., Lolk, M. E., Smith, S. D., Tissue, D. T., Zak, J. C., Weltzin, J. F., Pockman, W. T., Sala, O. E., Haddad, B. M., Harte, J., Koch, G. W., Schwinning, S., Small, E. E., Williams, D. G. (2004)**:** Convergence across biomes to a common rain-use efficiency. – Nature 429: 651-654.

[43] Ithurrart, L. S. (2014): Efectos de la defoliación luego de la quema de gramíneas perennes nativas, palatables y no palatables, en el sudoeste Bonaerense. PhD Dissertation, Departamento de Agronomía, Universidad Nacional del Sur, Bahía Blanca, Argentina. 203 pp.

[44] Jacoby, P. W., Meadors, C. H. (1982)**:** Control of sand shinnery oak (*Quercus havardii*) with pelleted picloram and tebuthiuron. – Weed Science 30: 594-597.

[45] Klemmedson, J. O., Smith, J. G. (1964): Cheatgrass (*Bromus tectorum L.*). – The Botanical Review 30: 226–262.

[46] Köhl, L., Oehl, F., van der Heijden, M. G. A. (2014): Agricultural practices indirectly influence plant productivity and ecosystem services through effects on soil biota. – Ecological Applications 24: 1842–1853.

[47] Le Houérou, H. N., Bingham, R. L., Skerbek, W. (1988): Relationship between the variability of primary production and the variability of annual precipitation in world arid lands. – Journal of Arid Enrivonments 15: 1-18.

[48] Liu, W., Huang, Y., An, Z., Clemens, S. C., Li, L., Prell, W. L., Ning, Y. (2005)**:** Summer monsoon intensity controls C_4/C_3 plant abundance during the last 35 ka in the Chinese Loess Plateau: Carbon isotope evidence from bulk organic matter and individual leaf waxes. – Palaeogeography, Palaeoclimatology, Palaeoecology 220: 243-254.

[49] Llorens, E.M. (1995): Viewpoint: the state and transition model applied to the herbaceous layer of Argentina's calden forest. – Journal of Range Management 48: 442-447.

[50] Martin, S. C., Morton, H. L. (1980): Response of falsemesquite, native grass and forbs, and lovegrass after spraying with picloram. – Journal of Range Management 35: 219-222.

[51] Mazzarino, M. J., Bertiller, M. B., Sain, C. L., Satti, P., Coronato, F .R. (1998)**:** Soil nitrogen dynamics in northern Patagonia steppe under different precipitation regimes. – Plant and Soil 202: 125–131.

[52] Meyer, R. E., Bovey, R. W. (1985): Response of herbaceous vegetation in East-Central Texas to herbicides. – Weed Science 33: 81-90.

[53] Moreira, F., Arianoutsou, M., Corona, P., De las Heras, J. (2012): Post-Fire management and restoration of southern european forests. – Springer, Dordrecht, The Netherlands.

[54] Moretto, A. S., Distel, R. A. (1997): Competitive interactions between palatable and unpalatable grasses native to a temperate semi-arid grassland of Argentina. – Plant Ecology 130: 155-161.

[55] Moretto, A.S., Distel, R.A. (1999): Effects of selective defoliation on the competitive interaction between palatable and unpalatable grasses native to a temperate semi-arid grassland of Argentina. – Journal of Arid Environments 42: 167-175.

[56] Moretto, A. S., Distel, R. A., Didoné, N .G. (2001): Decomposition and nutrient dynamics of leaf litter and roots from palatable and unpalatable grasses in a semi-arid grassland. – Applied Soil Ecology 18: 31–37.

[57] Moretto, A. S., Didoné, N. G., Distel, R. A. (2013)**:** Reabsorción de nitrógeno en gramíneas perennes de diferente palatabilidad, nativas del Caldenal. – Ecologia Austral 23: 1-7.

[58] Mueller-Dombois, D., Ellenberg, H. (1974): Aims and Methods of Vegetation Ecology. – John Wiley & Sons, New York, U.S.A.

[59] Paez, A., Busso, C. A., Montenegro, O. A., Rodríguez, G. D., Giorgetti, H. D. (2005)**:**

Seed weight variation and its effects on germination in *Stipa* species. – ΦYTON, International Journal of Experimental Botany 74: 1-14.

[60] Pearcy, R. W., Ehleringer, J. (1984): Comparative ecophysiology of C_3 and C_4 plants. – Plant Cell and Environment 7: 1-13.

[61] Peláez, D. V, Bóo, R. M., Mayor, M. D. (2003): El Fuego y la Vegetación del Sur del Caldenal. - In: Kunst, C.R., Bravo, S., Panigatti, J.L. (Eds.) Fuego en los Ecosistemas Argentinos. Ediciones INTA, Buenos Aires, Argentina, 71-78.

[62] Peláez, D. V., Giorgetti, H. D., Montenegro, O. A., Elía, O. R., Rodríguez, G. R., Bóo, R. M., Mayor, M. D., Busso, C. A. (2010): Vegetation response to a controlled fire in the Phytogeographical Province of the Monte, Argentina. – ΦYTON, International Journal of Experimental Botany 79: 169-176.

[63] Perelman, S. B., León, R. J. C., Bussacca, J. P. (1997): Floristic changes related to grazing intensity in a Patagonian shrub steepe. – Ecography 20: 400-406.

[64] Rúgolo de Agrasar, Z. E., Steibel, P. E. and Troiani, H. O . (2005): Manual ilustrado de las gramíneas de la Provincia de La Pampa. Universidad Nacional de La Pampa, Santa Rosa, Universidad Nacional de Rio Cuarto, Córdoba, Argentina.

[65] Saint Pierre, C., Busso, C. A., Montenegro, O. A., Rodríguez, G. D., Giorgetti, H. D., Montani, T., Bravo, O. A. (2002): Root proliferation in perennial grasses of low and high palatability. – Plant Ecology 165: 161-169.

[66] Saint Pierre, C., Busso, C. A., Montenegro, O. A,, Rodríguez, G. D., Giorgetti, H. D., Montani, T., Bravo, O. A. (2004a): Defoliation tolerance and ammonium uptake rate in perennial tussock grasses. – Journal of Range Management 57: 82-88.

[67] Saint Pierre, C., Busso, C. A., Montenegro, O. A., Rodríguez, G. D., Giorgetti, H. D., Montani, T., Bravo, O. (2004b): Direct assessment of competitive ability and defoliation tolerance in perennial grasses. – Canadian Journal of Plant Science 84: 195-204.

[68] Saint Pierre, C., Busso, C. A., Montenegro, O. A., Rodríguez, G. D., Giorgetti, H. D., Montani, T., Bravo, O. A. (2004c): Soil resource acquisition mechanisms, nutrient concentrations and growth in perennial grasses. – Interciencia 29: 303-311.

[69] Sala, O. E. (1988): The effect of herbivory on vegetation structure. – In: Werger , M. J. A., van der Aart, P. J. M., During, H. J, Verhoeven, J. T. A. (Eds.) Plant form and vegetation structure. – Academic Publishing, Verboeven, 317-330.

[70] Sala, O. E., Lauenroth, W.K. (1982): Small rainfall events: an ecological role in semiarid regions. – Oecologia 53: 301-304.

[71] Schlesinger, W. H., Reynolds, J. F., Cunningham, G. L., Huenneke, L. F., Jarrell, W. M., Virginia, R. A., Whitford, W. G. (1990): Biological feedbacks in global desertification. – Science 247: 1043-1048.

[72] Singh, J. S, Lauenroth, W. K., Steinhorst, R. K. (1975): Review and assessment of various techniques for estimating net aerial primary production in grasslands from harvest data. – Botanical Review 41: 181-232.

[73] Sturges, D. L. (1993): Soil-water and vegetation dynamics through 20 years after big sagebrush control. – Journal of Range Management 46: 161-169.

[74] Tizón, F. R., Peláez, D. V., Elía, O. R. (2010): The Influence of controlled fires on a plant community in the South of the Caldenal, and its relationship with a regional state and transition model. – Phyton, International Journal of Experimental Botany 79: 141-146.

[75] Torres, Y. A., Busso, C. A., Montenegro, O. A., Ithurrart, L., Giorgetti, H., Rodríguez, G., Bentivegna, D., Brevedan, R., Fernández, O., Mujica, M. M., Baioni, S., Entío, J., Fioretti, M., Tucat, G. (2013): Plant growth and survival of five perennial grass genotypes exposed to various defoliation managements in arid Argentina. – Grass and Forage Science 69: 580-595.

[76] Torres, Y. A., Busso, C. A., Montenegro, O. A., Ithurrart, L., Giorgetti, H., Rodríguez, G., Bentivegna, D., Brevedan, R., Fernández, O., Mujica, M. M., Baioni, S., Entío, J., Fioretti, M., Tucat, G. (2014): Root proliferation in perennial grasses in arid Patagonia, Argentina. – Journal of Arid Land 6: 195-204.

[77] Vallentine, J. F. (1990): Grazing management. – Academic Press, San Diego, U.S.A.

[78] Volesky, J. D., Anderson, B. E., Nichols, J. T. (2010): Perennial Forages for Irrigated Pasture. http://www.ianrpubs.unl.edu/epublic/live/g1502/build/. Access date: 11 Feb 2015.

[79] Walker, L. R. (1999): Ecosystems of disturbed grounds. – Elsevier Science, Amsterdam, The Netherlands.

[80] Webb, W. L., Lauenroth, W. K., Szarek, S. R., Kinerson, R. S. (1983)**:** Primary production and abiotic controls in forests, grasslands, and desert ecosystems in the United States. – Ecology 64: 134-151.

[81] White, L. M. (1985)**:** Stand age, precipitation, and temperature effects on forage yield. – Journal of Range Management 38: 39-43.

[82] Wilks, S. S. (1932)**:** Certain generalizations in the analysis of variance. – Biometrika 24: 471-494.

[83] Wright, H. A., Bailey, A. W. (1982)**:** Fire Ecology. – John Wiley and Sons, New York, U.S.A.

[84] Zar, J.H. (1996): Biostatistical analysis 3rd ed. – Prentice Hall, New Jersey, U.S.A.

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CAN WATER HYACINTH (*EICHHORNIA CRASSIPES*) BE CONTROLLED BY REDUCING NITROGEN AND PHOSPHORUS POLLUTION OF WATER BODIES?

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Abstract. Increasing concentration of nutrients in water is regarded as one of the most important external factors responsible for the invasion of the common water hyacinth. In order to test whether nutrient availability limits the growth and spread of this species, we investigated the influence of water nitrogen (N) and phosphorus (P) concentrations on growth using both greenhouse and field experiments. We found that in the greenhouse experiment, only high P concentration (>1.25 mg l^{-1}) can significantly increase numbers of ramets and leaves, and N concentration exceeding 62.5 mg l^{-1} can greatly increase water hyacinth biomass in a tank. In the field experiment, the clonal growth of water hyacinth was not correlated with N and P concentrations in water bodies where the range of N and P concentrations was narrow compared with their range in the greenhouse. This suggests that controlling water hyacinth through minimizing sewage discharging is impractical, the importance of the ability of water hyacinth to grow clonally should be considered.

Keywords: *invasive species, eutrophication, aquatic plant, wetlands*

Introduction

Invasive plants are attracting increasing research attention, and the objective of most research on invasive species is how to control their spread. Compared to terrestrial invasive plants, the control of aquatic invasive plants is more difficult because flowing water can transport plants or their propagules, aiding their spread. Therefore, many control methods applied in the case of aquatic invasive plants are ineffective (Rinella et al., 2009; Kovalenko et al., 2010; Baars, 2011; Patel, 2012; Paynter et al., 2012).

Eichhornia crassipes (Mart.) Solms, commonly known as water hyacinth, is a widespread aquatic invasive plant. This plant continues to be perceived as amongst the worst of invading aquatic weeds, despite the effort and resources spent over many years to improve management strategies (Heard et al., 2014). It now occurs in at least 62 countries between 40°N and 45°S latitude, and interferes with fishing, transport, the use of water for drinking purposes, irrigation, electric power generation, and also

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affects biodiversity (Howard and Harley, 1997; de Groote et al., 2003). Much effort has been made to control it (Hill, 2014). For the management of water hyacinth, given current knowledge, the best we can hope for is to reduce its biomass and reproductive potential, and to reduce the surface area covered by the weed, thus reducing the cost and impact of infestations and slowing the rates of spread (Julien, 2008). To counter the threat of water hyacinth in ecosystems, different types of control methods, including chemical, mechanical, and biological control, have been developed. However, to date these methods either have negative side effects, or have not been effective (Cilliers, 1991).

Although intentional introductions and environmental change caused by human activities are among conditions that have facilitated water hyacinth invasions, the biological traits of water hyacinth, such as high population growth rate (Sale et al. 1985; Gopal, 1987; Santamaría, 2002), and broad tolerance to a range of pH and nutrient concentrations are regarded as the most important factors responsible for their rapid spread (Xie andYu, 2003; Xie et al., 2004). With an average annual productivity of 50 dry (ash-free) tonnes per hectare, the water hyacinth is one of the most productive plants in the world (Abbasi and Nipaney, 1986). Many studies have shown that the clonal growth of water hyacinth is correlated with the nutrient level of water bodies, especially with nitrogen (N) and phosphorus (P) (Xie and Yu, 2003; Bownes et al., 2013; Hill, 2014), and based on this methods for controlling water hyacinth through minimizing water pollution by N and P have been suggested (Zulu et al., 2000; Sinkala et al., 2002; Chamier et al., 20112). Although water hyacinth makes use of nutrients where available, it can also grow well in clear lakes with low nutrient availability. It has also been observed to grow in extremely polluted waters containing rich organic matter, minerals and heavy metals, as well as in waters with high acidity or alkalinity (Gopal, 1987). Is it possible to control water hyacinth through controlling nutrient concentrations in water? In other words, is nutrient availability in aquatic environments a factor that limits the growth of water hyacinth? If nutrient availability of an aquatic environment is not a limiting factor, any attempts to control the weed by reducing water pollution will be not effective or practical.

In this study, we conducted a greenhouse experiment in which water hyacinth was grown at experimentally manipulated levels of nutrients, and a field experiment in which water hyacinth was grown across a naturally varying range of nutrient availability. We also conducted a broad field observational study in which naturally occurring water hyacinth and associated nutrient availabilities were monitored. Our aims were to examine the relationship between water hyacinth growth and nutrient availability in water bodies, and to test whether nutrient availability in aquatic environments is a factor that limits the growth and spread of water hyacinth.

Materials and Methods

Greenhouse experiment design

The greenhouse experiment was carried out in August 2004. The temperature range during the experiment was from 29°C to 36°C. The experiment was conducted under natural light. The experimental plants were cultured in plastic tanks, which were 70 cm long, 50 cm wide and 40 cm deep, each of which contained 100 liters of culture solution with varying N and P concentrations. Water hyacinth plants used in the experiment were collected from the river located in Shanghai suburbia. In order to ensure the consistency of plant material for the experiment, the collected water hyacinth plants were cultivated in a big concrete tank in an unheated greenhouse for several months before the experiment to produce enough ramets. Only newly produced ramets of similar size (bearing 5-6 leaves) were chosen for the experiment. Each tank contained only one healthy ramet.

The formulation of the culture solution consisted of a single concentration Hoagland's solution (Torrey and Machlis, 1956), with NH_4NO_3 and KH_2PO_4 as N and P sources, respectively. Studies have shown that water hyacinth can tolerate high concentrations of N (300 mg l^{-1}) (Fox et al., 2008) and P (40 mg l^{-1}) (Haller et al., 1974) in water. It has also been shown that water hyacinth can absorb and store N and P in excess of what it requires for growth (hyper-accumulation or luxury uptake) (Alves et al., 2003; Chen et al., 2010)—a reason for its use in numerous waste water treatment systems. Based on water hyacinth's wide range of nutrient tolerance, we set up a complete factorial experiment with five levels of concentration of both N and P, i.e., N0 (Hoagland's solution but without added N, as control), N1 (0.5 mg l^{-1}), N2 (2.5 mg l^{-1}), N3 (12.5 mg l^{-1}), N4 (62.5 mg l^{-1}), P0 (Hoagland's solution but without added P, as control), P1 (0.05 mg l^{-1}), P2 (0.25 mg l^{-1}), P3 (1.25 mg l^{-1}), P4 (6.25 mg l^{-1}). With the exception of N and P, other nutrient elements were in accordance with their concentration in Hoagland's solution. This made for a total of 25 nutrient treatments (5N×5P). Each set of 25 treatments was replicated three times for a total of 75 tanks. The depth of the culture solution was approximately 30 cm, which was maintained through adding culture solution (twice) during the experimental period.

Numbers of ramets and leaves were recorded once every two days from the second day of cultivation to harvest day. In previous experiments with water hyacinth cultivation, we found that a single ramet of water hyacinth could produce approximately 25-30 ramets and cover the surface of the experimental tanks within 30 days. Thus, we ran the experiment for only 30 days following which all ramets were collected and their biomass was obtained after oven drying at 80 °C for 24 hours.

Field experiment

To test whether water hyacinth responded to nutrient concentrations similarly in the field and in controlled environments, we grew it in water bodies at several locations. The field observations were performed in Liantang town, Xiqin town, Zhujiajiao town and Qingpu town in Shanghai in August 2004. All these towns are in Qingpu district, to the west of Shanghai.

At these four towns we randomly chose one water body (for a total of three rivers and one pond), and set up five plots in each. Each plot, was a netted square (1 m×1 m) enclosed by a wooden frame. A single ramet of water hyacinth was grown in each plot. We recorded the number of ramets 5 times in each plot during the 40-day cultivation period. In addition, we sampled water using a water sampler in each plot from the surface to 30cm depth, and analyzed these samples for N and P concentrations.

Field observations

Water hyacinth occurs in almost all the water bodies of Shanghai between June and October every year. The massive invasions of water hyacinth in Shanghai might be related to the nutrient concentrations in these water bodies. To examine the relationship between the abundance of water hyacinth and nutrient availability in water, we conducted field observations in Shanghai. Water hyacinth biomass per unit area was randomly sampled, and associated samples of water for nutrient analysis were obtained from nine water bodies of Shanghai suburbia. Water hyacinth was harvested from an area of 1 m^2, and weighed to estimate the biomass (fresh weight). We sampled water to a depth of 30 cm using a water sampler. Four samples were randomly taken from each water body, which were then mixed to make a single, composite sample. All these water samples were transported in cooler boxes to the laboratory at Institute of Soil Science, Chinese Academy of Sciences in Nanjing for analysis. Water samples were filtered through Whatman GF/F filters, and filtrates were analyzed for total organic carbon (TOC), N and P concentrations. The concentration of TOC was determined by potassium dichromate - concentrated sulphuric acid method, N was tested by Kjeldahl method, and P was tested by Mo-Sb colorimetric method; all these methods were according to standard protocols (Eaton et al. 1995).

Data analysis

We analyzed the effects of the N and P treatments on numbers of ramets and leaves in the greenhouse experiment using a repeated measure two-way ANOVA with a *post hoc* Tukey test. In two-way repeated measure ANOVA, Mauchly's Test of Sphericity showed that numbers of ramets and leaves increased significantly with cultivation days ($p<0.001$), so the assumption of the Sphericity test was rejected, and Greenhouse Geisser test of within-subjects effects was conducted. Also, after harvesting all the water hyacinth in each tank, we analyzed the effects of the N and P treatments on dry mass of water hyacinth using two-way ANOVA with a *post hoc* Tukey test. Before the analysis, the data were square-root-transformed to improve normality.

For the field experiment, the concentrations of N and P sampled from water bodies and the number of ramets recorded from the same sites were analyzed by one-way ANOVA to determine the significant differences among the 4 sites.

Pearson's linear simple correlation was used to determine whether N and P concentrations of water were related to number of ramets (in the field experiment), and to test the relationship between water hyacinth biomass and water nutrient concentration (TOC, N, P) (in the observational study). All analyses were performed using SPSS 19.0 (SPSS Inc., Chicago, IL 60606).

Results

Table 1. *The effects of N and P treatments on ramet number, leaf number and biomass of water hyacinth, analyzed by two-way ANOVA, cultivation days were considered as repeated measure factors in using two-way ANOVA*

Items	Factors	df	F	p
Ramet No.	Days	5.20	1702.67	0.000
	Days*N	20.82	2.10	0.004
	Days*P	20.82	7.43	0.000
	Days*N*P	83.27	1.34	0.043
Leaf No.	Days	1.00	788.71	0.000
	Days*N	4.01	3.51	0.013
	Days*P	4.01	4.09	0.006
	Days*N*P	16.06	0.96	0.514
Biomass	N	4	5.93	0.001
	P	4	3.73	0.01
	N*P	16	2.34	0.011

The results of the greenhouse experiment showed that addition of N, P and their interaction all significantly affected ramet and leaf production over time, with a significant increase in biomass by the end of the 30-day period (*Table 1*) (*Appendix 1*). The post-hoc Tukey test showed that the number of ramets was significantly higher in P4 than in P0 and P2 treatments ($p=0.001$, 0.039), but there were no differences with other P treatments, or among N treatments (*Fig. 1*). In the case of number of leaves treatments N3 and P4 had significant higher numbers of leaves than the nutrient controls ($p=0.045$, 0.006), but there were no differences among the N- or P-addition treatments (*Fig. 2*). The dry mass of water hyacinth was significantly higher in N4 than in the other N-addition treatments ($p<0.01$); dry mass was significantly higher in P3 than in the P0, P1 and P2 treatments ($p< 0.05$) (*Fig. 3*). These results showed that within the range of N and P concentrations in this experiment, high concentrations of N (N4) or P (P3)

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increased water hyacinth biomass, and high P significantly increased the number of leaves and ramets.

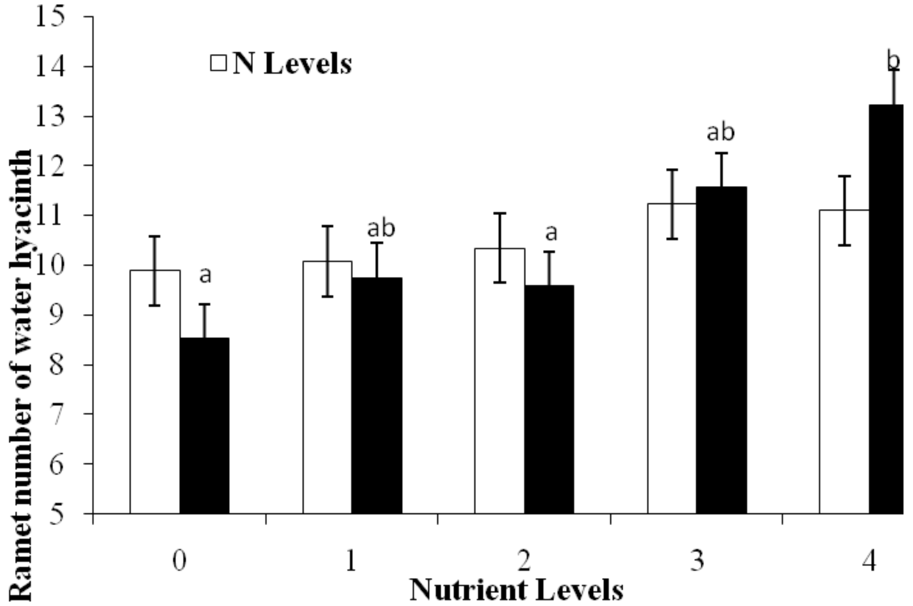

Figure 1. The number of ramets of water hyacinth grown in the greenhouse at different N and P levels show marginal Mean±S.E., and different letters in capital and lowercase indicate statistically significant differences (p < 0.05) among N or P levels.

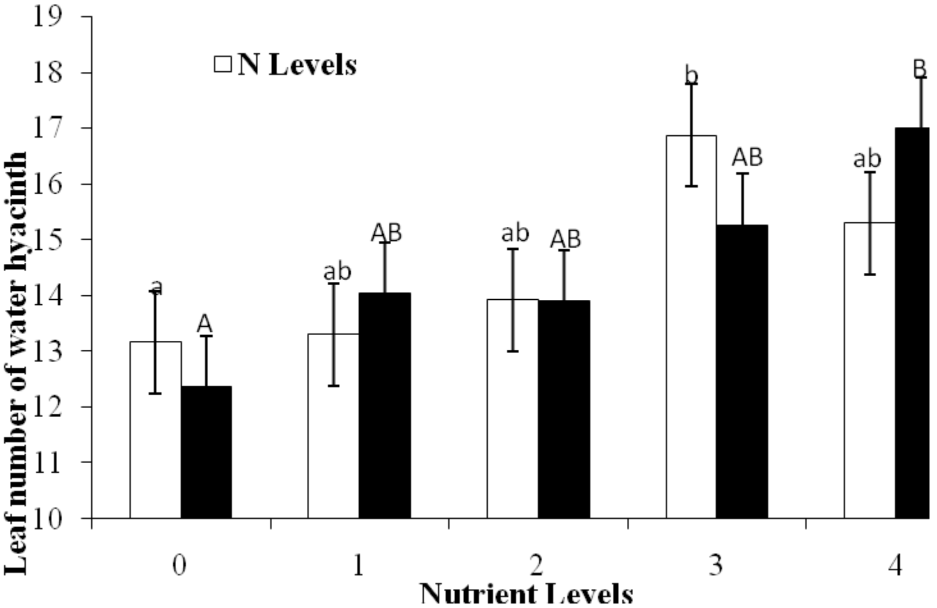

Figure 2. The number of leaves of water hyacinth grown in the greenhouse at different N and P levels, show marginal Mean±S.E., and different letters in capital and lowercase indicate statistically significant differences (p <0.05) among N or P levels.

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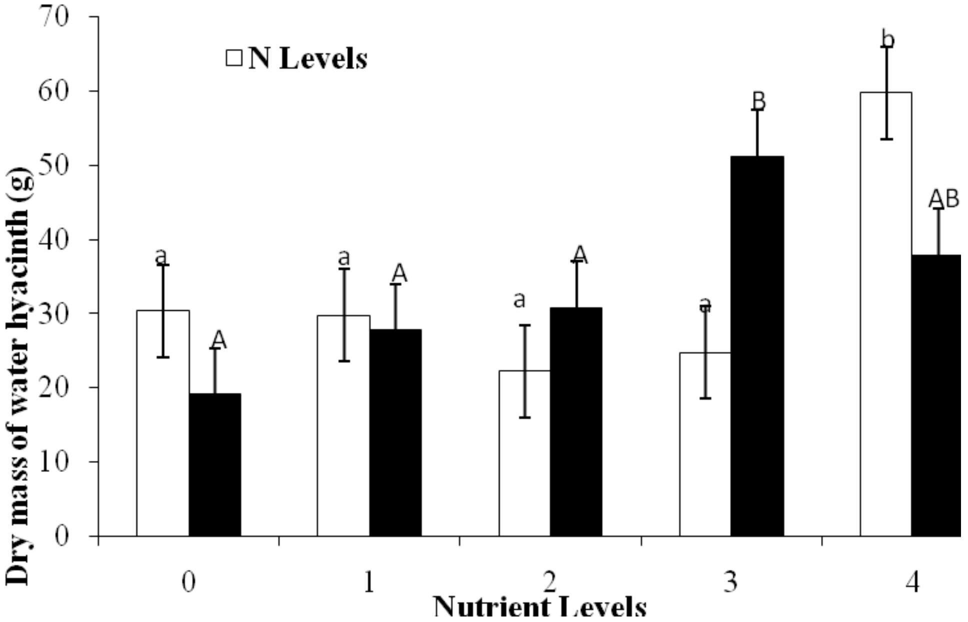

Figure 3. *The dry mass of water hyacinth grown in the greenhouse at different N and P levels, show marginal Mean±S.E., and different letters in capital and lowercase indicate statistically significant differences (p <0.05) among N or P levels.*

In the rivers and pond, water hyacinth grew very rapidly. The rate of ramet production in the field was greater than in the greenhouse. The mean ramet number of water hyacinth at the four sites all increased over time (*Fig. 4*), but there were no significant differences among the four water bodies (p=0.39 >0.05, *Table 2*), although the concentrations of both N (p< 0.001, *Table 2*) and P (p < 0.05, *Table 2*) differed significantly among the four sites. The concentration of N ranged from 1.68 ± 0.118 mg l^{-1} to 3.65 ± 0.217 mg l^{-1}, while the concentration of P ranged from 0.28 ± 0.03 mg l^{-1} to 0.43 ± 0.08 mg l^{-1}. However, there was no significant relationship between number of ramets at the time of harvest and N and P concentration of water bodies (with N: r=0.15, p=0.54, with P: r=0.19, p=0.42, *Fig. 5*).

Table 2. *The differences of N, P concentrations and ramets of water hyacinth cultivated in different water bodies, analyzed by one-way ANOVA, cultivation days were considered as repeated measure factors in using one-way ANOVA.*

Items	Factors	df	F	p
N	Site	3	29.51	0.000
P	Site	3	4.40	0.019
Ramets	days	2.28	511.75	0.000
	Days*Site	6.83	1.08	0.39

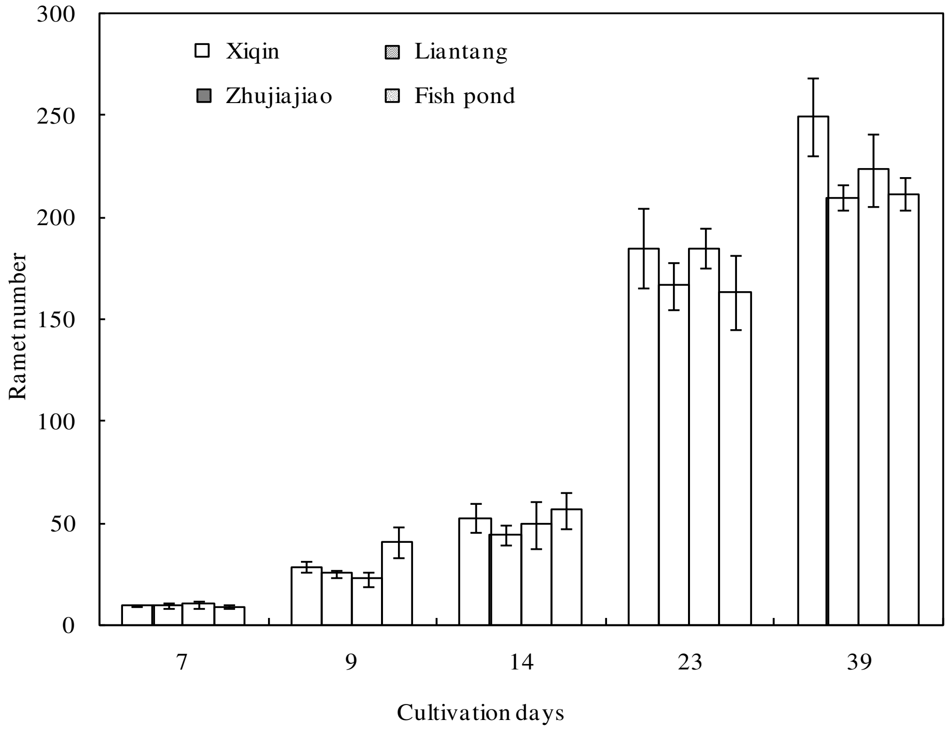

Figure 4. *The change in number of ramets (Mean± SE) produced by a single parent ramet in the field experiment conducted in four different aquatic bodies.*

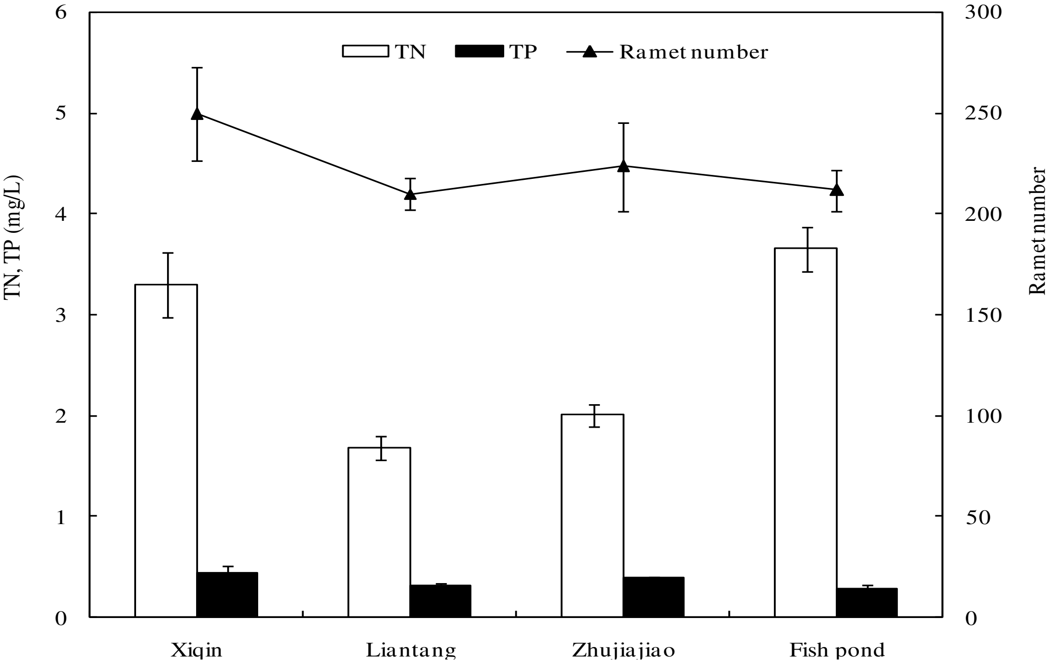

Figure 5. *The N, P concentration of different water bodies, together with the total number of ramets produced by parent ramets after 39 days of cultivation; data depicted are Mean ± SE.*

In the observational study of nine water bodies, although the samples of TOC, N and P have big coefficient of variances (CV), they showed no correlations with water hyacinth biomass sampled in the same sites ($p > 0.05$) (*Table 3*).

Table 3. *Water hyacinth biomass (fresh weight) and concentrations of TOC, N and P sampled from different water bodies of Shanghai, and the correlations between biomass and TOC, N and P, analyzed by Pearson's linear correlation method.*

Sampling site	TOC (mgl^{-1})	N (mgl^{-1})	P (mgl^{-1})	Biomass (kg m^{-2})
Sitang, SJ	11.17	13.67	0.58	14.6
Yuhu, JS	6.55	2.97	0.06	19.6
Hongguang, JS	17.51	3.69	0.11	41.4
Baiyang, JS	22.10	3.80	0.06	18.0
Dongcun, JS	15.40	3.66	0.03	25.6
Mianzhang, FX	30.26	4.01	0.05	21.5
Caoyang, FX	7.60	5.40	0.08	20.6
Donghai, NH	24.42	5.03	0.19	35.7
Junken, NH	27.13	4.81	0.18	20.1
Mean±SE	18.02±2.85	5.23±1.09	0.15±0.05	24.1±2.9
CV	47.45%	62.35%	114.98%	36.46%
r (with biomass)	0.30	-0.233	-0.142	/
p (two-tailed)	0.433	0.546	0.715	/

Discussion

Numerous studies on the response of water hyacinth to nutrient concentrations in water, have suggested that water hyacinth is highly responsive to nutrients, with increased productivity correlated with increased N (Reddy et al., 1989, 1990; Heard and Winterton, 2000; Wilson et al., 2005; Coetzee et al., 2007) and P (Xie et al., 2004; Chen et al., 2010; You et al., 2014). However, this does not explain why water hyacinth is widely distributed and has strong invasive capacity since its wide distribution would suggest a wide tolerance for environmental conditions, including nutrient availability. Our greenhouse experiment supports this proposition. The experiments performed in the greenhouse showed that there was a significant increase in the production of ramets and leaves of water hyacinth and in water hyacinth biomass only at very high N ($N > 62.5$ mg l^{-1}) and P ($P > 1.25$ mg l^{-1}) concentrations. However, water hyacinth was able to grow in water with low concentrations of N or P – at lower nutrient concentrations the growth of water hyacinth was not significantly different from the controls. Studies have also shown that the overall nutrient requirement of water hyacinth is very low (Carignan et al., 1994; DeBusk et al., 2001; Xie and Yu, 2003; Xie et al., 2004). Therefore, whether water hyacinth can grow and whether water hyacinth can produce more leaves and ramets are different. The invasion and spread of water hyacinth, and even the control to water hyacinth are mainly related to whether water hyacinth can grow in water bodies.

From the results of field study, the concentrations of N and P in the field water were lower and quite narrow compared with those set in the greenhouse. Thus, in the field experiment the clonal growth of water hyacinth was not correlated with N and P concentrations of water bodies. The results of field observation were consistent with findings from the greenhouse experiment at nutrient levels that were comparable to those encountered in the field. Thus, typical ranges of variation in the concentration of N and P in the field water are unlikely to influence the growth and clonal reproduction of water hyacinth. The studies thought that the clonal growth of water hyacinth was correlated to the concentrations of N and P, only when the concentration of N or P was in relatively high situation, the growth of water hyacinth will increase significantly. But in water bodies of field, such high concentration of N and P was not common. And also, in these water bodies of field, water hyacinth can also grow and grow out daughter ramets in clonal way in these N and P concentration ranges. Besides, due to the floating characteristic of water hyacinth, and flowing of field water, the lack of nutrients can be compensated through the moving of water hyacinth or the flow of water. Therefore, in the field water bodies, the concentration of N and P nutrients will not be a limiting factor for the growth of water hyacinth.

In addition, from the water quality data of field observation (*Table 3*), it can be seen that the concentrations of N and P of water bodies (N: 1.68 ± 0.118 mg l^{-1}-3.65 ± 0.217 mg l^{-1}, P: 0.28 ± 0.03 mg l^{-1} -0.43 ± 0.08 mg l^{-1}) were also close to the low nutrient levels we set in greenhouse experiment. From the data of greenhouse and field experiments, we can see the growth and clonal action would not be affected in these nutrient ranges. N: 1.5mgL^{-1}, P: 0.3mgL^{-1}And also, the survey of Shanghai water quality conducted in 2001 indicated that more than 88% of water bodies were classified as Grade V(those used for agriculture, N: 1.5mgL^{-1}, P: 0.3mgL^{-1}) (Wang, 2001). It further showed that all most all the water bodies of Shanghai were suitable for the growth of water hyacinth. Therefore, although N and P concentrations might not be very high, the N and P pools in the urban water are large enough to support the growth of water hyacinth. So, under the current status of N and P distribution in Shanghai water bodies, N and P availability would not be the main or only factors that determine whether or not water hyacinth thrives and flourishes. In addition, the control of aquatic invasive plants can not only stay in control of its biomass growth, the important goal should be to control the coverage area of invasive plants on the water (Gao et al., 2013). The ramet number and leaf number of water hyacinth are involved to the coverage, so the biomass growth may not be the final control purpose for aquatic invasive plants.

Many researchers have suggested that an effective method for controlling water hyacinth growth should be to reduce the discharge of sewage with high N and P to water bodies (Howard and Harley, 1997; Zulu et al., 2000; Sinkala et al., 2002). However, in the case of Shanghai, the purification of its water bodies to the standards of drinking water resources (N: 0.2 mgl^{-1}, P: 0.02 mgl^{-1}) by reducing the discharge of pollutants may be difficult. Our greenhouse experiment showed that water hyacinth can still grow and propagate well even under low N and P availability. Therefore, under the current situation it may be impractical to control water hyacinth invasion by controlling sewage discharge. As one of the world's most widespread aquatic weeds, water hyacinth has a strong invasive capacity, and it has special adaptations which allow it to grow and spread rapidly in freshwater. It is also known that water hyacinth interferes with agricultural (irrigation) and urban waterways (Kathiresan, 2000). So, the underlying mechanisms for successful invasions of water hyacinth appear to be far more complicated than pollution of waterways.

The development of effective management strategies requires an understanding of the weed's biology, ecology and how these change in response to changing conditions and to control techniques. An important role for weed scientists and weed managers is to offer plausible, information based, strategies and thus assist the decision making process (Julien, 2008). Although improving aquatic environments may play an important role in controlling water hyacinth in the long term, across a wide range of concentrations N and P do not appear to limit water hyacinth spread. For these reasons, we should consider not only the invasibility of the environment, in this case, nutrient rich water bodies (Lonsadle, 1999; Davis et al., 2005), but also the invasive capacity of the invader (Lonsadle, 1999; Davis et al., 2005) before controlling water hyacinth, therefore, those control ways against the growth characteristic of water hyacinth should be recommended, such as biological control (Ding et al., 2006): introducing natural enemies (Hill and Cilliers, 1999; Coetzee et al., 2007; Coetzee et al., 2011) and allelopathic control (Kathiresan, 2000; Kathiresan and Dhavabharathi, 2008; Kong, 2010; Khaket et al., 2012; Chai et al., 2013). In addition, the biological control of water hyacinth has also been highly successful in a number of large tropical water bodies, mainly in Africa (Marlin et al., 2013; Venter et al., 2013), but also in Australia (Dhileepan et al., 2013).

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REFERENCES

[1] Abbasi, S. A., Nipaney, P. C. (1986): Infestation by aquatic weeds of the fern genus Salvinia: its status and control. – Environ Conserv 13:235-241

[2] Alves, E., Cardoso, L. R., Scavroni, J., Ferreira, L. C., Boaro, C. S. F., Cataneo, A. C. (2003): Physiological and biochemical evaluations of water hyacinth (*Eichhornia crassipes*), cultivated with excessive nutrient levels. – Planta Daninha 21:27-35

[3] Baars, J. R. (2011): Classical Biological Control for the Management of Alien Invasive Plants in Ireland. – Biol Environ 111B:213-222

[4] Bownes, A., Hill, M. P., Byrne, M. J. (2013): The role of nutrients in the responses of water hyacinth, *Eichhornia crassipes* (Pontederiaceae) to herbivory by a grasshopper *Cornops aquaticum* Bruner (Orthoptera: Acrididae). – Biol Control 67:555-562

[5] Carignan, R., Neif, J. J., Planas, D. (1994): Limitation of water hyacinth by nitrogen in subtropical lakes of the Parana floodplain (Argentina). – Limnology and Oceanography 39:439-443

[6] Chai,T. T., Ooh, K. F., Ooi, P. W., Chue, P. S., Wong, F. C. (2013): *Leucaena leucocephala* leachate compromised membrane integrity, respiration and antioxidative deffence of water hyacinth leaf tissues. – Bot Stud 54:1-8

[7] Chamier, J., Schachtschneider, K., le Maitre, D. C., Ashton, P. J., van Wilgen, B. W. (2012): Impacts of invasive alien plants on water quality, with particular emphasis on South Africa. – Water SA 38:345-356

[8] Chen, X., Chen, X. X., Wan, XW, Weng, B. Q., Huang, Q. (2010): Water hyacinth (*Eichhornia crassipes*) waste as an adsorbent for phosphorus removal from swine wastewater. – Bioresource Tech 101:9025-9030

[9] Cilliers, C. J. (1991): Biological control of water hyacinth, *Eichhornia crassipes* (Pontederiaceae), in South Africa. – Agriculture, Ecosystem and Environment 37:207-217

[10] Coetzee, J. A., Bownes, A., Martin, G. D. (2011): Prospects for the biological control of submerged macrophytes in South Africa. – Afr Entomol 19:469-487

[11] Coetzee, J. A., Byrne, M. J., Hill, M. P. (2007): Predicting the distribution of *Eccritotarsus catarinensis*, a natural enemy released on water hyacinth in South Africa. – Entomol Exp Appl 125:237-247

[12] Davis, M. A., Thompson, K., Grime, J. P. (2005): Invasibility: the local mechanism driving community assembly and species diversity. – Ecography 28:696-704

[13] De Groote, H., Ajuonu, O., Attignon, S., Djessou, R., Neuenschwander, P. (2003): Economic impact of biological control of water hyacinth in Southern Benin. – Ecol Econ 45:105-117

[14] DeBusk, T. A., Dierber, F. E., Reddy, K. R. (2001): The use of macrophyte-based systems for phosphorus removal: an overview of 25 years of research and operational results in Florida. – Water Sci Techol 44:39-46

[15] Dhileepan, K., Taylor, D. B. J., McCarthy, J., King, A., Shabbir, A. (2013): Development of cat's claw creeper leaf-tying moth *Hypocosmia pyrochroma* (Lepidoptera: Pyralidae) at different temperatures: Implications for establishment as a biological control agent in Australia and South Africa. – Biol Control 67:194-202

[16] Ding, J. Q., Reardon, R., Wu, Y., Zheng, H., Fu, W.D. (2006): Biological control of invasive plants through collaboration between China and the United States of America: a perspective. – Biol Invasions 8:1439-1450

[17] Eaton, A. D., Clesceri, L.S., Greenberg, A. E. (1995): Standard methods for the examination of water and wastewater. 19 edn. – Am. Pub. Health Assoc., Washington

[18] Fox, L. J., Struik, P. C., Appleton, B. L., Rule, J. H. (2008): Nitrogen phytoremediation by water hyacinth (*Eichhornia crassipes* (Mart.) Solms). – Water Air Soil Poll 194:199-207

[19] Gao, L., Li, B., Liu, W. Y., Shen, Y. X., Liu, W. J. (2013): Inhibition effects of daughter ramets on parent of clonal plant *Eichhornia crassipes.* – Aquatic Botany 107:47-53

[20] Gopal, B. (1987): Aquatic Plant Studies 1: Water Hyacinth. – Elsevier Science Publishers, Oxford

[21] Haller, W. T., Sutton, D. L., Barlowe, W. C. (1974): Effects of salinity on growth of several aquatic macrophytes. – Ecology 55:891-894

[22] Heard, T. A., Zonneveld, R., Fichera, G. (2014): *Megamelus scutellaris* (Hemiptera: Delphacidae), a biocontrol agent of water hyacinth, is not sufficiently specific for release in Australia. – Biocontrol Sci Techn 24:554-560

[23] Hill, J. M. (2014): Investigations of growth metrics and delta N-15 values of water hyacinth (*Eichhornia crassipes*, (Mart.) Solms-Laub) in relation to biological control. – Aquatic Botany 114:12-20

[24] Hill, M. P., Cilliers C. J. (1999): A review of the arthropod natural enemies, and factors that influence their efficacy, in the biological control of water hyacinth, *Eichhornia crassipes* (Mart.) Solms-Laubach (Pontederiaceae), in South Africa. – Afr Entomol 1:103-112

[25] Howard, G. W., Harley, K. L. S. (1997): How do floating aquatic weeds affect wetland conservation and development? How can these effects be minimized? – Wetland Ecol Managem 5:215-225

[26] Julien, M. (2008): Plant biology and other issues that relate to the management of water hyacinth: a global perspective with focus on Europe. – Bulletin OEPP/EPPO Bulletin 38:477-486

[27] Kathiresan, R. M. (2000): Allelopathic potential of native plants against water hyacinth. – Crop Protect 19:705-708

[28] Kathiresan, R. M., Dhavabharathi, J. (2008): Rice allelopathy for weed management in aquatic ecosystems. – Allelopathy J 22:413-416

[29] Khaket, T. P., Singh, M., Dhanda, S., Singh, T., Singh, J. (2012): Biochemical characterization of consortium compost of toxic weeds *Parthenium hysterophorus* and *Eichhornia crassipes.* – Bioresource Tech 123:360-365

[30] Kobayashi, J. T., Thomaz, S. M., Pelicice, F. M. (2008): Phosphorus as a Limiting Factor for *Eichhornia Crassipes* Growth in the Upper Parana River Floodplain. – Wetlands 28:905-913

[31] Kong, C. H. (2010): Ecological pest management and control by using allelopathic weeds (*Ageratum conyzoides*, *Ambrosia trifida*, and *Lantana camara*) and their allelochemicals in China. – Weed Biol Manag 10:73-80

[32] Kovalenko, K. E., Dibble, E. D., Slade, J. G. (2010): Community effects of invasive macrophyte control: role of invasive plant abundance and habitat complexity. – J Appl Ecol 47:318-328

[33] Lonsadle, W. M. (1999): Global patterns of plant invasions and the concept of invisibility. – Ecology 80:1522-1536

[34] Marlin, D., Hill, M. P., Byrne, M. J. (2013): Interactions within pairs of biological control agents on water hyacinth, *Eichhornia crassipes.* – Biol Control 67:483-490

[35] Patel, S. (2012): Threats, management and envisaged utilizations of aquatic weed *Eichhornia crassipes*: an overview. – Rev Environ Sci Bio 11:249-259

[36] Paynter, Q., Overton, J. M., Hill, R. L., Bellgard, S. E., Dawson, M. I. (2012): Plant traits predict the success of weed biocontrol. – J Appl Ecol 49:1140-1148

[37] Reddy, K. R., Agami, M., Tucker, J. C. (1989): Influence of nitrogen supply rates on growth and nutrient storage by water hyacinth (*Eichhornia crassipes*) plants. – Aquatic Botany 36:33-43

[38] Reddy, K. R., Agami, M., Tucker, J. C. (1990): Influence of phosphorus on growth and nutrient storage by water hyacinth (*Eichhornia crassipes* (Mart) Solms) plants. – Aquatic Botany 37:355-365

[39] Rinella, M. J., Maxwell, B. D., Fay, P. K., Weaver, T., Sheley, R. L. (2009): Control effort exacerbates invasive-species problem. – Ecol Appl 19:155-162

[40] Sale, P. J. M., Orr, P. T., Shell, G. S., Erskine, D. J. C. (1985): Photosynthesis and growth rates in *Salvinia molesta* and *Eichhornia crassipes*. – J Appl Ecol 22:125-137

[41] Santamaría, L. (2002): Why are most aquatic plants widely distributed? Dispersal, clonal growth and small-scale heterogeneity in a stressful environment. – Acta Oecol 23:137-154

[42] Sinkala, T., Mwase, E. T., Mwala, M. (2002): Control of aquatic weeds through pollutant reduction and weed utilization: a weed management approach in the lower Kafue River of Zambia. – Physics and Chemistry of the Earth 27:983-991

[43] Torrey, J. G., Machlis, L. (1956): Plants in action: a laboratory manual of plant physiology. – WH. Freeman, San Francisco

[44] Venter, N., Hill, M. P., Hutchinson, S. L., Ripley, B. S. (2013): Weevil borne microbes contribute as much to the reduction of photosynthesis in water hyacinth as does herbivory. – Biol Control 64:138-142

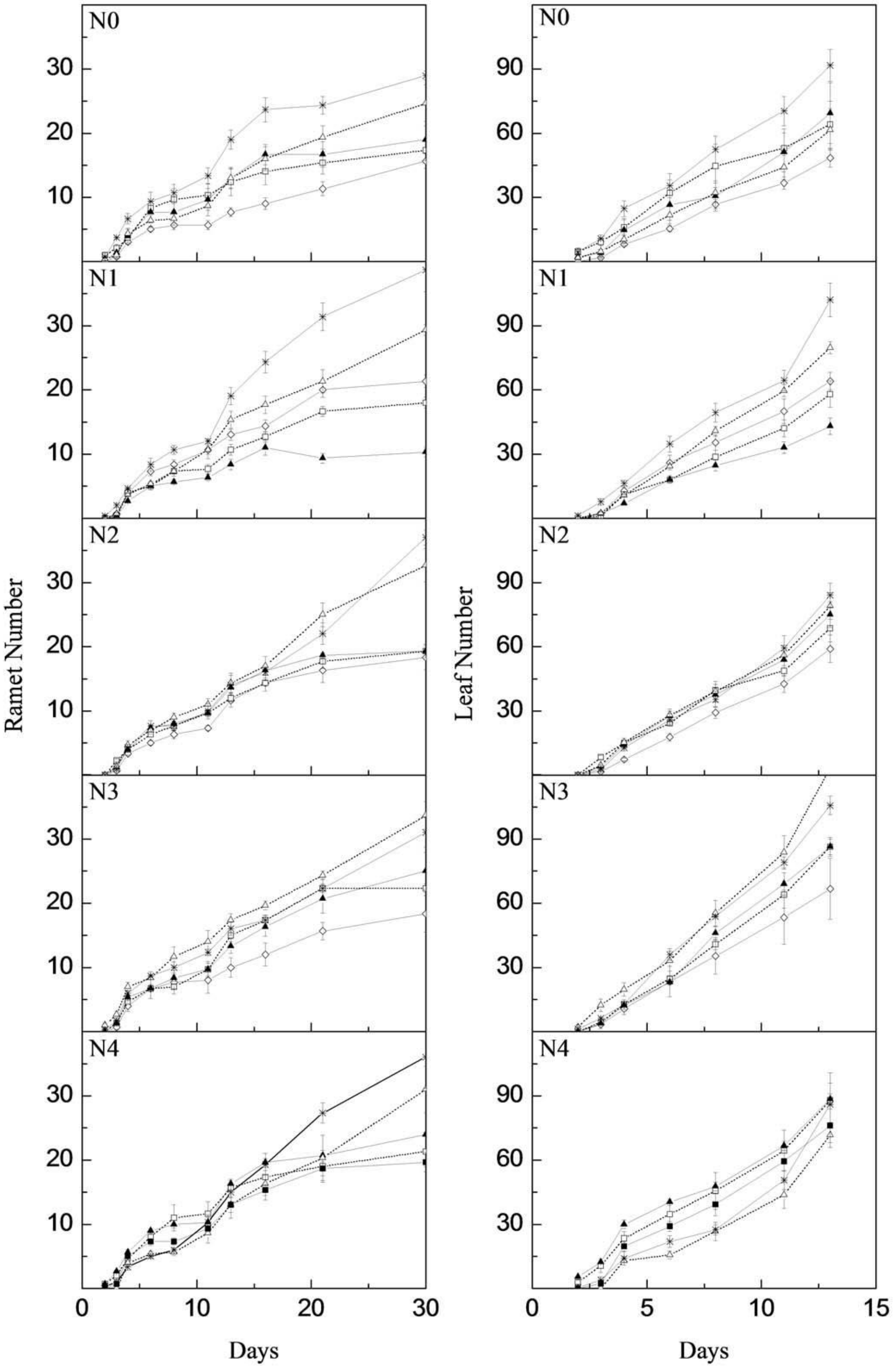
[45] Wang, S. N. (2001): Shanghai Water Resource Survey Report. – Shanghai Science and Technology Press, Shanghai

[46] Xie, Y. H., Yu, D. (2003): The significance of lateral roots in phosphorus (P) acquisition of water hyacinth (*Eichhornia crassipes*). – Aquatic Botany 75:311-321

[47] Xie, Y. H., Yu, D., Ren, B. (2004): Effects of nitrogen and phosphorus availability on the decomposition of aquatic plants. – Aquatic Botany 80:29-37

[48] Zulu, P., Sinkala, T., Mbewe, A. (2000): UNEP first cleaner roundtable for Africa: the status of cleaner production in Zambia. – University Publishing, Nairobi, Kenya

APPENDIX



Appendix 1. *At each N level in the greenhouse experiment, the changes in number of ramets (left) and number of leaves (right) in different P concentration with time. The data are mean±S.E.*

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PHYLOGENETIC RELATIONSHIPS OF INSECT-ASSOCIATED FREE-LIVING RHABDITID NEMATODES FROM EASTERN MEDITERRANEAN REGION OF TURKEY

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Abstract. Free-living nematodes play an important role in nutrient cycling. The study was designed to characterize insect-associated free-living soil nematodes in Rhabditida order from Eastern Mediterranean region of Turkey using their 28S rDNA sequences and phylogenetic analysis. A total of 105 soil samples were taken from different habitats. *Galleria* baiting technique was used to obtain free-living nematodes. The nematodes were harvested from cadavers of last instar *Galleria mellonella* larvaeafter trapping process. Free-living soil nematodes were detected in 14 soil samples (13.3%). PCR products obtained from 28S rDNA of isolates were sequenced and phylogenetic trees created. BLASTN homology searches and phylogenetic tree results constructed by Neighbour Joining and Bootstrap Tree methods. Results showed that these insect-associated isolates were determined as species belonging to Rhabditidae (*Rhabditis terricola* and *Mesorhabditis* sp.), Chambersiellidae (*Macrolaimus* sp.) and Cephalobidae (*Cephalobus* sp., *Pseudacrobeles* sp. and *Acrobeloides* sp.) families in Rhabditida. Free-living nematodes interact with many arthropods and other invertebrate species. These interactions are also important for their use in biological control program. In this study, these interactions between free-living nematode-insect,–arthropod and–invertebrate are discussed.

Keywords: *DNA sequence analysis, phylogeny, nematode-insect association, free-living soil nematode, Rhabditida*

Introduction

Nematodes (roundworms) are the most common, abundant, and genetically diverse metazoan organisms found in many habitats particularly soils and sediments, even in the most extreme environments (Baldwin et al., 1999; Derycke et al., 2008). Rhabditida order (rhabditids) is an interesting nematode group found in abundance in all kinds of soil. The free-living forms of this organisms display different feeding habits such as saprophagous or bacteriophagous, and as animal parasites (enthomopathogenic forms) (Yeates et al., 1993; Abolafia and Pena-Santiago, 2007). Because of their abundance, rapid life cycle, and strong interactions with other soil microbes and predators, free-living nematodes play an important role in nutrient cycling (Yeates and Wardle, 1996; Ferris et al., 1997; Carrascosa et al., 2014). Their functional guilds respond to food web enrichment, environmental perturbation, and recovery (Wang et al., 2004). They

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indirectly contribute to nitrogen mineralization by grazing on decomposer microbes, excreting ammonium, and immobilizing nitrogen in live biomass (Ferris et al., 1998; Neher, 2001). Due to their high occurrence, diversity, different tropic levels, and sensitivity to abiotic alterations free-living nematodes also contribute as bioindicators to environmental pollution and soil quality studies (Levi et al., 2012). Their high phenotypic plasticity among populations reduces the number of diagnostic characters in rhabditid nematode groups. Thus their classification has been a matter of long and strong discussions and diversity is far from being well known (Nadler, 2002; Abolafia and Pena-Santiago, 2007; Derycke et al., 2008). Molecular techniques and phylogenetic analyses can potentially overcome this problem and are promising tools to assess biodiversity (Derycke et al., 2008). In recent years, DNA sequence data have brought a revival in the field of systematic (Blaxter et al., 1998; De Ley and Blaxter, 2002; Subbotin et al., 2006). Rhabditids have been identified on the basis of large subunit (LSU or 28S) ribosomal DNA (rDNA) sequences. Trees inferred from LSU or 28S rDNA sequences have been used in deducing relationships among certain closely related species, primarily congeners (De Ley et al., 1999; Stock et al., 2001; Nadler et al., 2003).

The study was planned to characterize insect-associated free-living soil nematodes in Rhanditida order from East-Mediterranean Turkey using their 28S rDNA sequences and phylogenetic analysis.

Materials and methods

Nematode isolation

A total of 105 soil samples were taken from different locations and habitats in Adana, Osmaniye and Kahramanmaraş provinces at October 2010 (Table 1) (Karabörklü, 2012). Soil samples were baited with last instar *Galleria mellonella* L. (Lep.: Pyralidae) larvae (Bedding and Akhurst, 1975) for screening entomopathogenic nematodes (EPN) in our previous study (Karabörklü, 2012; Karabörklü et al., 2015a). However, many rhabditid nematodes also detected during these procedures (Duncan et al., 2003, Campos-Herrera et al., 2012). These rhabditid nematodes harvested from dead *G. mellonella* larvae were placed individually into modified White traps (Kaya and Stock 1997) at room temperature (~25 ºC). Harvested nematodes were washed in dH_2O and stored at 10ºC, 60%±5 relative humidity and full darkness until DNA extraction (Karabörklü, 2012; Karabörklü et al., 2015a).

DNA extraction and PCR amplification

DNA extraction and PCR amplification were done as described in our previous study with some modifications in method suggested by Williams et al. 1994 (Karabörklü, 2012; Karabörklü et al., 2015a,b). DNA was extracted by using 10–20 nematodes. In 20 µl of worm lysis buffer (50 mM KCl, 0.05% gelatin, 10 mM Tris pH 8.2, 10 mM DTT, 0.45% Tween 20, 2.5 mM $MgCl_2$ and 60 µg μl^{-1} proteinase K) nematodes were cut and transferred to sterile microfuge tubes (0.5 ml) on ice. Lysate were frozen at -80ºC for 20 min and then incubated at 65ºC for 1 h, followed by inactivation of proteinase K for 15 min at 95ºC (Zhang et al., 2008). The lysates were centrifuged at 6000 rpm for 30 sec after cooling. Then, 2.5 µl DNA suspension were added to a PCR reaction mixture containing 2.5 µl of 10X PCR buffer, 1.5 µl of 25 mM $MgCl_2$, 1 µl of 10 mM dNTP mixture, 1 μl of each primer pair (100 μM), 0.25 μl *Taq* polymerase (5 U μl^{-1}) and completed to 25 μl with distilled water. 28S rDNA primer pairs 5'-F'AGCGGAGGAAAAGAAACTAA-3' and 5'-R'ATCCGTGTTTCAAGACGGG-3' were used in amplification reaction (Nadler and Hudspeth, 1998). PCR was adjusted at 94 ºC for 3 min, followed by 33 cycles of 94 ºC for 30 sec, 52 ºC for 30 sec, and 72 ºC for 1 min, followed by final extension at 72 ºC for 7 min (Stock et al., 2001). Electrophoresis was performed on 1% agarose gel.

Phylogenetic analysis

PCR products were purified using of PCR purification kit (Fermentas) and sequenced by RefGen Biotechnology Laboratory (METU, Turkey). Alignments of sequences were performed using the National Centre for Biological Information's (NCBI) Basic Local Alignment Search Tool (BLAST) to compare the sequence data with known sequences submitted on the NCBI. Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 5 (Tamura et al., 2011; Yilmaz et al., 2012). Phylogenetic trees were constructed using the Neighbor Joining (NJ) and Bootstrap Tree (BT) methods (based on 1000 bootstrap replications) of the MEGA version 5 program. Alignment gaps and missing data were eliminated in pairwise sequence comparisons (Karabörklü et al., 2015a).

Results

Isolates

Free-living soil nematodes were detected in 14 out of 105 soil samples (13.3%) (*Table 1*). Nematodes were harvested from cadavers of last instar *G. mellonella* larvae after trapping process. Nematode densities on cadavers varied depending on isolates. SK-3, SK-16, SK-18 and SK-75 were the highest, SK-20 and SK-51 were medium, and SK-13, SK-29, SK-30, SK-32, SK-34, SK-76, SK-94 and SK-101 isolates were the lowest in density. Nematodes were isolated from forests, grasslands, and agricultural fields. Percentage of the nematodes isolated from forest, grassland, agronomical and horticultural areas were found to be 13.3, 13.3, 46.7 and 26.7%, respectively (*Table 1*).

Table 1. *Nematode isolation regions and their habitation properties*

Isolate	Isolation region	Habitat
SK-3	Pozantı/Adana	Forest (Pinery)
SK-13	Kozan/Adana	Horticultural area (Citrus orchard)
SK-16	Sülemişli-Feke/Adana	Grassland
SK-18	Saimbeyli/Adana	Grassland
SK-20	Pınarlar-Tufanbeyli/Adana	Agronomical area (Wheat field)
SK-29	Selimiye/Osmaniye	Agronomical area (Cornfield)
SK-30	Akyar/Osmaniye	Agronomical area (Cornfield)
SK-32	Toprakkale/Osmaniye	Agronomical area (Cornfield)
SK-34	Ceyhan/Adana	Horticultural area (Olive orchard)
SK-51	Çona/Osmaniye	Agronomical area (Peanut field)
SK-75	Süleymanlı Yolu/Kahramanmaraş	Forest (Pinery)
SK-76	Süleymanlı/Kahramanmaraş	Horticultural area (Grape orchard)
SK-94	Yeniköy/Osmaniye	Agronomical area (Cornfield)
SK-101	Buruk-Sarıçam/Adana	Horticultural area (Citrus orchard)

Molecular identification

28S rDNA PCR products of the isolates were individually purified and sequenced. Isolates were clustered on the basis of genus. Isolates compared with 20 nematode species showing highest similarity. Detailed phylogenetic analysis was given previously for each isolate. SK-3 and SK-75 isolates were indicated 99 and 98% identity with *Rhabditis terricola* (EF417152) after BLASTN homology search, respectively. Phylogenetic tree was constructed using the Neighbor Joining (NJ) and Bootstrap Tree (BT) methods for two nematode species were given in *Figure 1*. Similarly, SK-13 displayed 95% identity with *Pseudacrobeles variabilis* (AF143368) (*Fig. 2*). Similarity rates and Bootstrap Trees (BT) of other isolates were given in *Table 2* and *Figures (3−6)*.

Table 2. *Similarity rates of free-living nematode isolates with accession numbers*

Isolates (AN)	Species (AN)	Similarity (%)
SK-3 (KP027186)	*Rhabditis terricola* (EF417152)	99
SK-13 (KP027183)	*Pseudacrobeles variabilis* (AF143368)	95
SK-16 (KP027182)	*Acrobeloides maximus* strain JB-89 (DQ903097)	95
SK-18 (KP027184)	*Acrobeloides* sp. JB-68 (DQ903091)	99
SK-20 (KP027191)	*Cephalobus* sp. PS-1143(DQ903100)	99
SK-29 (KP027193)	*Macrolaimus* sp. SAN-2005 (DQ145639)	94
SK-30 (KP027181)	*Acrobeloides nanus* (EF417139)	88
SK-32 (KP027189)	*Acrobeloides* sp. JB-68 (DQ903091)	97
SK-34 (KP027190)	*Acrobeloides* sp. JB-68 (DQ903091)	99
SK-51 (KP027192)	*Cephalobus* sp. PS-1143(DQ903100)	99
SK-75 (KP027185)	*Rhabditis terricola* (EF417152)	98
SK-76 (KP027194)	*Mesorhabditis longespicula* strain DF5017 (EU195980)	92
SK-94 (KP027187)	*Acrobeloides* sp. DWF-1106 (DQ903080)	80
SK-101 (KP027188)	*Acrobeloides* sp. DWF-1106 (DQ903080)	89

AN: Accession Number

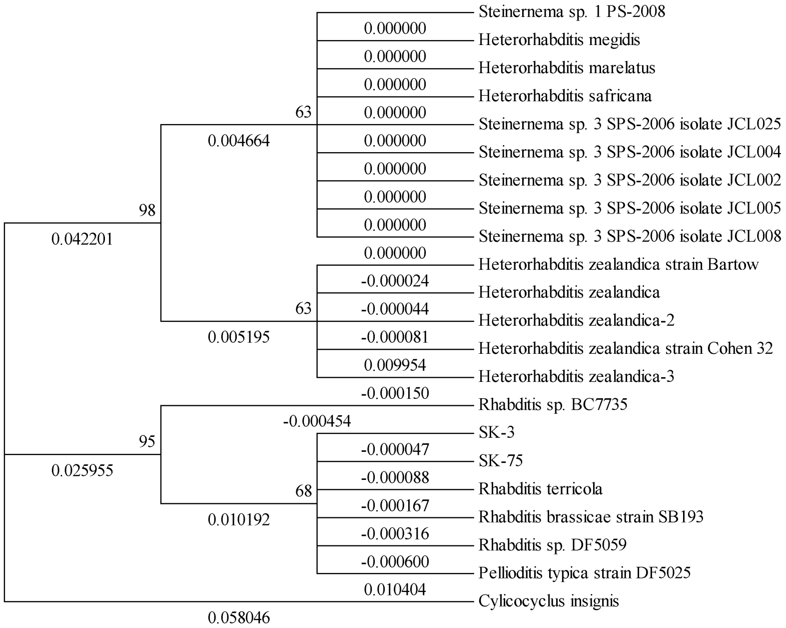

Figure 1. *Phylogenetic relationships of different nematode species with SK-3 and SK-75 isolates. Phylogeny inferred from the alignment of the 719 and 583bp of 28S rDNA region, respectively. The horizontal bar represents 0.005% differences in nucleotide identities*

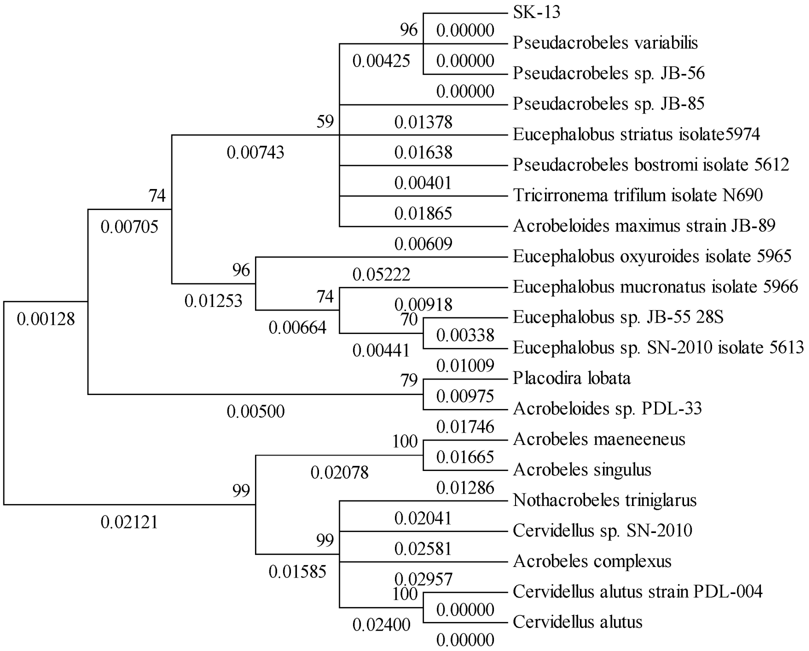

Figure 2. *Phylogenetic relationships of different nematode species with SK-13 isolate. Phylogeny inferred from the alignment of the 620bp of 28S rDNA. The horizontal bar represents 0.005% differences in nucleotide identities*

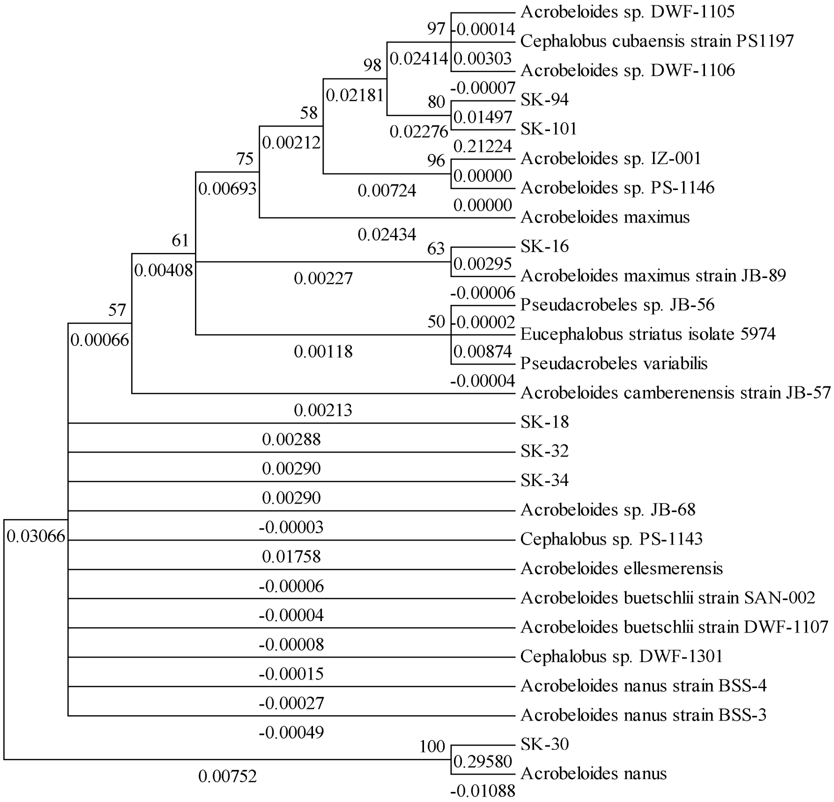

Figure 3. *Phylogenetic relationships of different nematode species with SK-16, SK-18, SK- 30, SK-32, SK-34, SK-94 and SK-101 isolates. Phylogeny inferred from the alignment of the 727, 919, 763, 752, 934, 679 and 684bp of 28S rDNA, respectively. The horizontal bar represents 0.005% differences in nucleotide identities*

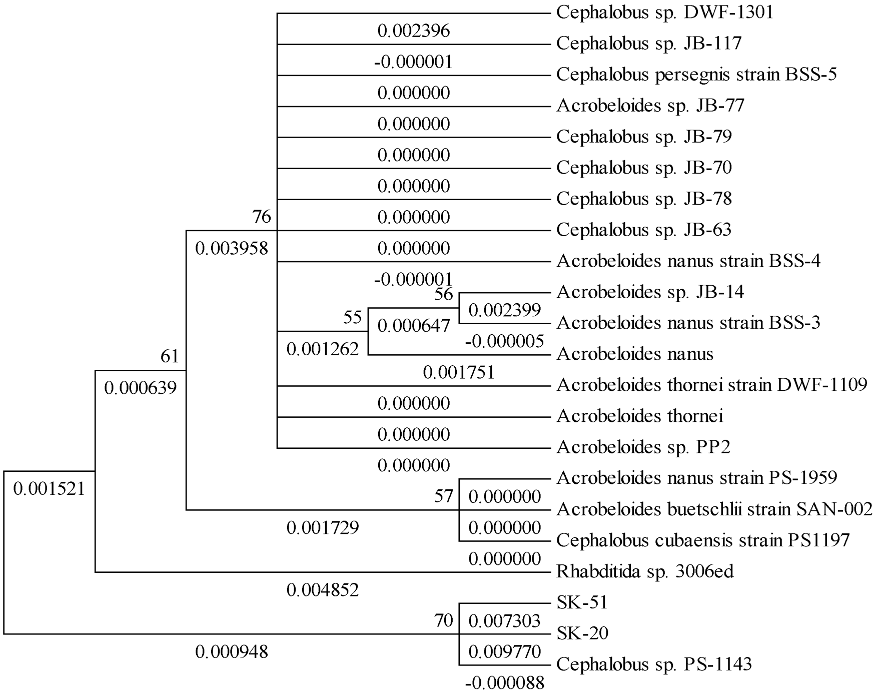

Figure 4. Phylogenetic relationships of different nematode species with SK-20 and SK-51 isolates. Phylogeny inferred from the alignment of the 746 and 927bp of 28S rDNA, respectively. The horizontal bar represents 0.005% differences in nucleotide identities

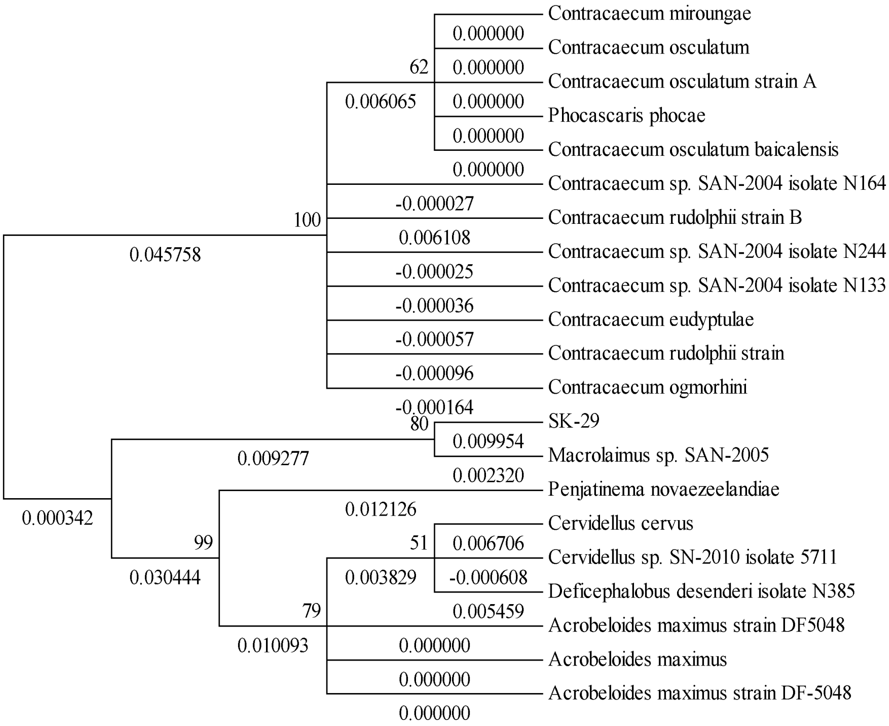

Figure 5. Phylogenetic relationships of different nematode species with SK-29 isolate. Phylogeny inferred from the alignment of the 632bp of 28S rDNA. The horizontal bar represents 0.005% differences in nucleotide identities

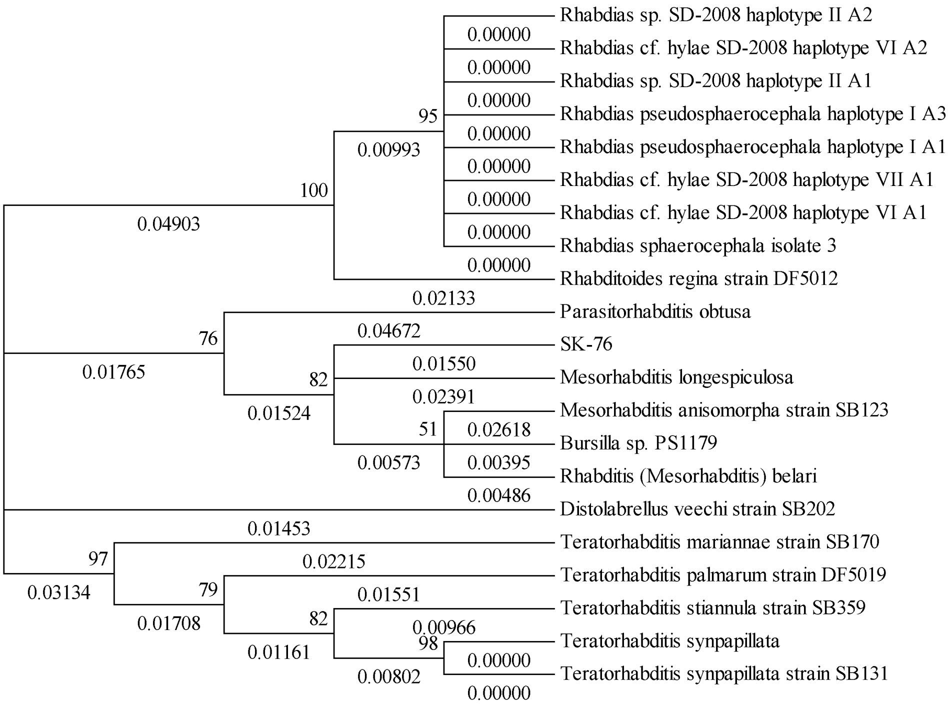

***Figure 6.** Phylogenetic relationships of different nematode species with SK-76 isolate. Phylogeny inferred from the alignment of the 688bp of 28S rDNA. The horizontal bar represents 0.005% differences in nucleotide identities*

Discussion

The free-living nematodes undertake an important role in nutrient cycling. Besides, contributions of these organisms to nutrient cycling, nitrogen mineralization and distribution have been well documented (Ferris et al., 1997, 1998; Neher, 2001). Entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) are also included in free-living rhabditid nematode groups and used as excellent biocontrol agents for many insect pests (Grewal et al., 2005).

In this study, 14 free-living nematode isolates were determined. Free-living nematodes, isolated from different habitats of East-Mediterranean region of Turkey, were characterized after BLASTN homology and phylogenetic analysis based on Neighbor Joining and Bootstrap Tree methods. The free-living nematode isolates were identified as *Rhabditis terricola* (SK-3 and SK-75), *Mesorhabditis* sp. (SK-76) (Rhabditidae), *Macrolaimus* sp. (SK-29) (Chambersiellidae), *Cephalobus* sp. (SK-20 and SK-51), *Pseudacrobeles* sp. (SK-13) and *Acrobeloides* sp. (SK-16, SK-18, SK-30, SK-32, SK-34, SK-94 and SK-101) (Cephalobidae) from order Rhabditida. Isolates were mainly obtained from agricultural fields with a rate of 73.3%. *Rhabditis terricola* strains were obtained from forest area, but *Pseudacrobeles* sp. and *Mesorhabditis* sp. strains were isolated from horticultural fields of different regions. On the other hand *Cephalobus* sp. and *Macrolaimus* sp. strains were also isolated from various agronomical fields. *Acrobeloides* sp. strains were collected from grassland, agronomical and horticultural fields. Our results indicated that the free-living nematodes can be obtained from habitats as forest, grassland and agricultural fields. Abolafia and Pena-

Santiago (2007) supports our finding in that rhabditid nematodes are very abundant in all types of soil and sediments.

In the current study, the free-living nematode strains (*Rhabditis terricola, Pseudacrobeles* sp., *Mesorhabditis* sp., *Cephalobus* sp., *Macrolaimus* sp. and *Acrobeloides* sp.) were isolated from last instar *G. mellonella* larvae cadavers. Large numbers of *Rhabditis terricola* determined in earthworm cocoons (*Lumbricus rubellus,* Hoffmeister and *Eisenia foetida,* Savigny) and earthworm cultures suffered extensive productivity loss (Taboga, 1981). The relationship of the *Rhabditis* species with arthropods could be described as phoretic, moderately pathogenic, and facultatively parasitic (Carta and Osbrink, 2005). Poinar (1971) reported that *Rhabditis adenobia* Poinar lived and reproduced within the colleterial glands and endophallic passages of the male dynastid beetle *Oryctes monoceros* L. (Col.: Scarabaeidae) without apparent harm to the host. *R. brevispina* (Claus) was found on the body surface of mole crickets, *Gryllotalpa unispina* (Ort.: Gryllotalpidae) and has phoretic relationship (Gulyamova, 1990).

Rhabditis sp. nematodes are also reported as opportunistic invader of cadavers (Garcia et al., 2011). However, *Rhabditis blumi* Sudhaus showed high pathogenicity (>78%) against major cruciferous insect pests, *Artogeia rapae* L. (Lep.: Pieridae), *Mamestra brassicae* L. (Lep.: Noctuidae) and *Plutella xylostella* L. (Lep.: Plutellidae) in laboratory experiments (Park et al., 2012). Dauer larvae of *Rhabditis rainai* Carta and Osbrink (2005) experimentally infested two subterranean termite species *Reticulitermes flavipes* Kollar and *R. hesperus* Banks (Massey, 1971). Schulte (1989) reported that *Rhabditis (Oscheius) necromena* Sudhaus and Schulte infected three millipede species, Australian *Akamptogonus novarae* (Humbert and Saussure) (Dipl.: Paradoxosomatidae), Australian *Oncocladostoma castaneum* (Attems) (Dipl.: Paradoxosomatidae) and Portuguese pest *Ommatoiulus moreletii* (Lucas) (Dipl.: Julidae) at different levels. It is also reported that dauer juveniles of *R. necromena* remain inside the haemocoel of this host until it dies and resume their development after feeding on bacteria present in the decaying carcass ('necromeny'). Moreover, soil bacteria that are attached to the surface of the invading nematode juveniles regularly cause a lethal contamination in the non-adapted *O. moreletii* (Massey, 1971). It was also reported that *Rhabditis* species use many mollusk species as host (Grewal et al., 2003).

Mesorhabditis irregularis (Körner) was found on body surface of mole crickets, *G. unispina*. *M. irregularis* was associated only with host phoretically (Poinar, 1971). *M. irregularis* and *M. quercophila* (Rühm) were observed in *Melolontha afflicta* Ball. (Col.: Scarabaeidae). *M. oschei* Körner has relationship with *Ips sexdentatus* (Börner) (Col.: Curculionidae) (Grewal et al., 2003). On the other hand *M. spiculigera* species were ectoparasite of *Scarabaeus sacer* (Col.: Scarabaeidae) (El-Kifl et al., 1971). *Macrolaimus* species are commonly recovered from bark infested with beetles and they may be carried by other insects (Massey, 1974). *M. canadensis* isolated from the frass of the bark beetle *Phloeosinus canadensis* Swaine (Col.: Curculionidae) (Sanwal, 1960). *M. crucis* was isolated from *Pissodes piniphilus* Herbst. (Col.: Curculionidae) (Poinar, 1975). The other species belong the genus *Cephalobus* was indicated to have interaction with some mollusks, annelids and insect species. *Cephalobus (Acrobeloides) buetschlii* de Man and *C. persegnis* use mollusk species *Polygyra albolabrus* Say and *Helix hortensis* Muller as host (Grewal et al., 2003). *C. persegnis* species was determined to have infestation on earthworm cocoons and act as ectoparasite on *Scarabaeus sacer* L. (Col.: Scarabaeidae) (El-Kifl et al., 1971). No study has been conducted previously concerning the relationship of *Pseudacrobeles* and *Acrobeloides* with insect and arthropod species. However, *Acrobeloides nanus* (De Man) infestation was reported in earthworm cocoons (Kraglund and Ekelund, 2002). Contribution of free-living nematodes to soil nutrient cycle and soil mineralization are evident. During these processes, free-living nematodes interact with many arthropods and other invertebrate species. Interactions of free living nematodes with arthropods and other invertebrates vary from phoretic to pathogenic relations. These interactions are also important for their use in biological control program. However, more detailed studies need to be carried out to clarify these relationships.

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REFERENCES

[1] Abolafia, J., Pena-Santiago, R. (2007): Nematodes of the order Rhabditida from Andalucía Oriental, Spain. The genera *Protorhabditis* (Osche, 1952) Dougherty, 1953 and *Diploscapter* Cobb, 1913, with description of *P. spiculocrestata* sp. n. and a species Protorhabditis key. - Journal of Nematology 39: 263-274.

[2] Baldwin, J.G., Nadler, S.A., Freckman, D.W. (1999): Nematodes–pervading the earth and linking all life. In Raven PH, Williams T, editors. Proceedings of the Second National Forum on Biodiversity, Nature and Human Society. The Quest for a Sustainable World. - Washington: National Academy Press; p. 176-191.

[3] Bedding, R.A. (1975): Akhurst RJ. A simple technique for the detection of insect parasitic Rhabditid nematodes in soil. - Nematologica 21: 109-110.

[4] Blaxter, M.L., De Ley, P., Garey Liu, J.R., Liu, L.X., Scheldeman, P., Vierstraete et al., (1998): A molecular evolutionary framework for the phylum Nematoda. - Nature 392: 71-75.

[5] Campos-Herrera, R., El-Borai, F.E., Duncan, L.W. (2012): Wide interguild relationships among entomopathogenic and free-living nematodes in soil as measured by real time qPCR .- Journal of Invertebrate Pathology 111: 126-235.

[6] Carrascosa, M., Sanchez-Moreno, S., Alonso-Prados, J.L. (2014): Relationships between nematode diversity, plant biomass, nutrient cycling and soil suppressiveness in fumigated soils. - European Journal of Soil Biology 62: 49-59.

[7] Carta, L.K., Osbrink, W. (2005): *Rhabditis rainai* n. sp. (Nematoda. Rhabditida) associated with the Formosan subterranean termite, *Coptotermes formosanus* (Isoptera. Rhinotermitidae). - Nematology 7: 863-879.

[8] De Ley, P., Felix, M.A., Frisse, L.M., Nadler, S.A., Sternberg, P.W., Thomas, W.K. (1999): Molecular and morphological characterisation of two reproductively isolated species with mirror-image anatomy (Nematoda. Cephalobidae). - Nematology 1: 591-612.

[9] De Ley, P., Blaxter, M.L. (2002): Systematic position and phylogeny. In Lee DL, editor. The Biology of Nematodes. - London: Taylor and Francis; p. 1-30.

[10] Derycke, S., Fonseca, G., Vierstraete, A., Vanfleteren, J., Vincx, M., Moens, T. (2008): Disentangling taxonomy within the *Rhabditis (Pellioditis) marina* (Nematoda, Rhabditidae) species complex using molecular and morhological tools. - Zoological Journal of The Linnean Society London 152: 1-15.

[11] Duncan, L.W., Dunn, D.C., Bague, G., Nguyen, K. (2003): Competition between entomopathogenic and free-living bactivorous nematodes in larvae of the weevil Diaprepes abbreviates. - Journal of Nematology 35: 187-193.

[12] El-Kifl, A.H., Abdel-Wahab, A.I., Ali, M.A.M. (1971): Some insects parasitized by nematodes in Egypt. - Bulletin Societe Entomologique Egypte 54: 87-99.

[13] Ferris, H., Venette, R.C., Van Der Meulen, H.R., Lau, S.S. (1998): Nitrogen mineralization by bacterial-feeding nematodes: Verification and measurement. - Plant Soil 203: 159-171.

[14] Ferris, H.R., Venette, C., Lau, S.S. (1997): Population energetics of bacterial-feeding nematodes. Carbon and nitrogen budgets. - Soil Biology and Biochemistry 29: 1183-1194.

[15] Garcia, J.M., Jenkins, D., Chavarria, J.A., Shapiro-Ilan, D.I., Goenaga, R. (2011): Interactions of a *Rhabditis* sp. on the virulence of *Heterorhabditis* and *Steinernema* in Puerto Rico. - Florida Entomologist 94: 701-702.

[16] Grewal, P.S., Ehlers, R.U., Shapiro-Ilan, D.I. (2005): Nematodes as biological control agents. - Wallingford: CAB International Publishing p: 505.

[17] Grewal, P.S., Grewal, S.K., Tan, L., Adams, B.J. (2003): Parasitism of molluscs by nematodes: Types of associations and evolutionary trends. - Journal of Nematology 35: 146-156.

[18] Gulyamova, D.B. (1990): Nematodes of mole cricket (*Gryllotalpa unispina*) in Ferghana district. – In: Sonin, M.D. (ed) Helminths of insects. New Delhi: Oxonian Press Pvt Ltd., pp. 51-52.

[19] Karabörklü, S. (2012): Isolation and identification of entomopathogenic nematodes from different regions and their use as biological control agent on pine processionary moth (*Thaumetopoea wilkinsoni* Tams). PhD Dissertation. Erciyes University, Graduate School of Natural and Applied Sciences: Kayseri.

[20] Karabörklü, S., Ayvaz, A., Yilmaz, S., Azizoglu, U., Akbulut, M. (2015a): Native entomopathogenic nematodes isolated from Turkey and their effectiveness on pine processionary moth, *Thaumetopoea wilkinsoni* Tams. - International Journal of Pest Management 61: 3-8.

[21] Karabörklü, S., Ayvaz, A., Yilmaz, S., Azizoglu, U. (2015b): Fungi associated with free-living soil nematodes in Turkey. - Archives of Biological Sciences 67: 1173-1183.

[22] Kaya, H.K., Stock, S.P. (1997): Techniques in insect nematology. In Lacey LA, editor. Manual of techniques in insect pathology. - San Diego: Academic Press p. 281-324.

[23] Kraglund, H.O., Ekelund, F. (2002): Infestation of natural populations of earthworm cocoons by rhabditid and cephalobid nematodes. - Pedobiologia 46: 125-135.

[24] Levi, T., Shermani, C., Pen-Mouratov, S., Steinberger, Y. (2012): Changes in soil free-living nematode communities and their trophic composition along a climatic gradient. - Open Journal of Ecology 2: 79-89.

[25] Massey, C.L. (1971): Two new genera of nematodes parasitic in the eastern subterranean termites *Reticulitermes flavipes*. - Journal of Invertebrate Pathology 17: 238-242.

[26] Massey, C.L. (1974): Biology and Taxonomy of nematode parasites and associates of bark beetles in the United States. Agriculture handbook no. 446. Washington: USDA forest service, p: 233.

[27] Nadler, S.A., Hudspeth, D.S.S. (1998): Ribosomal DNA and phylogeny of the Ascaridoidea (Nemata: Secernentea): implications for morphological evolution and classification. - Molecular Phylogenetics and Evolution 10: 221-236.

[28] Nadler, S.A. (2002): Species delimitation and nematode biodiversity. Phylogenies rule. - Nematology 4: 615-625.

[29] Nadler, S.A., Carreno, R.A., Adams, B.J., Kinde, H., Baldwin, J.G., Mundo-Ocampo, M. (2003): Molecular phylogenetics and diagnosis of soil and clinical isolates of *Halicephalobus gingivalis* (Nematoda. Cephalobina. Panagrolaimoidea), an opportunistic pathogen of horses. - International Journal for Parasitology 33: 1115-1125.

[30] Neher, D.A. (2001): Role of nematodes in soil health and their use as indicators. - Journal of Nematology 33: 161-168.

[31] Park, H.W., Kim, H.H., Youn, S.H., Shin, T.S., Bilgrami, A.L., Cho, M.R., Shin, C.S. (2012): Biological control potentials of insect-parasitic nematode *Rhabditis blumi* (Nematoda. Rhabditida) for major cruciferous vegetable insect pests. - Applied Entomology and Zoology 47: 389-397.

[32] Poinar, G.O. (1971): *Rhabditis adenobia* sp. n. (Nematoda. Rhabditidae) from the colleterial glands of *Oryctes monoceros* L. and other tropical dynastid beetles (Coleoptera. Scarabaeidae). - Proceedings of the Helminthological Society of Washington 38: 99-108.

[33] Poinar, G.O. (1975): Entomogenous nematodes. A manual and host list of insect-nematode associations. - Leiden: Brill p: 317.

[34] Sanwal, K.C. (1960): *Macrolaimus canadensis* n. sp. (Nematoda. Panagrolaiminae), from the frass of the bark beetle *Phloeosinus canadensis* swaine, 1917, with remarks on other species of the genus *Macrolaimus* Maupas, 1900. - Canadian Journal of Zoology 38: 1127-1131.

[35] Schulte, F. (1989): The association between *Rhabditis necromena* Sudhaus and Schulte, 1989 (Nematoda. Rhabditidae) and native and introduced millipedes in South Australia. - Nematologica 35: 82-89.

[36] Stock, S.P., Campbell, J.F., Nadler, S.A. (2001): Phylogeny of *Steinernema* Travassos, 1927 (Cephalobina. Steinernematidae) inferred from ribosomal DNA sequences and morphological characters. - Journal of Parasitology 87: 877-889.

[37] Subbotin, S.A., Sturhan, D., Chizhov, V.N., Vovlas, N., Baldwin, J.G. (2006): Phylogenetic analysis of Tylenchida Thorne, 1949 as inferred from D2 and D3 expansion fragments of the 28S rRNA gene sequences. - Nematology 8: 455-474.

[38] Taboga, L. (1981): *Rhabditis terricola*. An opportunistic nematode parasite of earthworm cocoons. - Journal of Invertebrate Pathology 38: 2-25.

[39] Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., Kumar, S. (2011): MEGA5. Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. - Molecular Biology and Evolution 28: 2731-2739.

[40] Wang, K.H., Mcsorley, R., Marshall, A.J., Gallaher, R.N. (2004): Nematode community changes associated with decomposition of *Crotalaria juncea* amendment in litterbags. - Applied Soil Ecology 27: 31-45.

[41] Williams, B.D., Schrank, B., Huynh, C., Shownkeen, R., Waterston, R.H. (1994): A genetic mapping system in *Caenorhabditis elegans* based on polymorphic sequence-tagged sites. - Genetics 131: 609-24.

[42] Yeates, G.W., Wardle, D.A. (1996): Nematodes as predators and prey: relationships to biological control and soil processes. - Pedobiologia 40: 43-50.

[43] Yeates, G.W., Bongers, T., De Goede, R.G.M., Freckman, D.W., Georgieva, S.S. (1993): Feeding habits in soil nematode families and genera- an outline for soil ecologists. - Journal of Nematology 25: 315-331.

[44] Yilmaz, S., Ayvaz, A., Akbulut, M., Azizoglu, U., Karabörklü, S. (2012): A novel *Bacillus thuringiensis* strain and its pathogenicity against three important pest insects. - Journal of Stored Products Research 51: 33-40.

[45] Zhang, C., Liu, J., Xu, M., Sun, J., Yang, S., An, X., et al. (2008): *Heterorhabditidoides chongmingensis* gen. nov., sp. Nov. (Rhabditida. Rhabditidae), a novel member of the entomopathogenic nematodes. - Journal of Invertebrate Pathology 98: 153-168.

ANALYSIS OF GENETIC SIMILARITY OF *LIMNOCHARIS FLAVA* INDIVIDUALS GROWING AROUND A GOLD MINING AREA WITH ARSENIC CONTAMINATION

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Abstract. This study aimed to determine the concentration of arsenic in the aquatic plant *Limnocharis flava* as well as this plant's genetic variability. Sediment and *L. flava* samples were collected from three studied sites at the edge of a stream near a gold mine. The arsenic concentrations in sediment and *L. flava* samples were analyzed using induction coupled plasma-mass spectrometry (ICP-MS). Genetic similarities (S) were studied using random amplified polymorphic DNA (RAPD) with dendrogram construction. The results showed that the arsenic concentrations in sediment and *L. flava* samples ranged from 4.595±0.318 to 223.434±2.118 and 0.081±0.020 to 0.291±0.106 mg/kg, respectively. To compare the samples studied to the reference site, RAPD fingerprints from 25 primers successfully produced 1,947 total bands used for dendrogram construction and S value analysis. The dendrogram construction separated *L. flava* into four clusters corresponding to their sampling sites. The S values of the studied sample sites compared to the reference site were 0.715-0.771, 0.696-0.729 and 0.757-0.836 for sites 1, 2 and 3, respectively, whereas the values of the individual samples within each site were as high as 0.981. Therefore, the genetic variation of this species is high and indicates that As is the one factor contributing to its genetic variations.

Keywords: *sediment, stream, arsenic concentration, RAPD*

Introduction

Gold mining activities have a considerable impact on the environment. Thailand has a gold mine located in the Wangsaphung district of the Loei province, resulting in affected human life. The gold mining industry has long encountered problems with heavy metals during gold extraction. Heavy metal pollutes the environment by separating and diffusing into soil and water (Henke, 2009). In addition to the local disturbance of the soil and aquatic ecosystems, a more widespread contamination of soils, vegetation and water sources by toxic concentrations of heavy metals can occur.

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The type of heavy metal contamination around gold mines mainly depends on the composition of the mined Au ore and the accompanying gangue. Several studies have been conducted to investigate the effects of heavy metals on some plant species. The effects of Cd, Cu, Ni, and Zn on *Medicago sativus* (alfafa) (Peralta-Videa et al., 2002), and the toxicity of Pb and Zn on *Avicennia marina* (gray mangrove) (MacFarlance and Murchett, 2002) have previously been reported. Arsenic (As) is an environmental toxin that is found naturally in all soils (Cullen and Reimer, 1989; Smedley and Kinniburgh, 2002). As adversely affects biological activities as a teratogen, carcinogen or mutagen and has detrimental effects on the digestive system, respiratory system and immune system (Zhou and Huang, 2000; Zhou and Song, 2004; Liao et al., 2005). Due to the past and present use of As-based pesticides, mining operations, irrigation with As-contaminated groundwater and fertilization with municipal solid wastes (Meharg et al., 2009), As has become part of the human solid food chain through contaminated crops and fodder. As contamination of the human food chain is a worldwide concern that is not restricted by economic boundaries. As is a heavy metal found in contaminated gold mining areas and can affect plant species (Boonmee et al., 2015). As is non-essential and generally toxic to plants. Roots are usually the first tissue to be exposed to As, where the metalloid inhibits root extension and proliferation. Upon translocation to the shoot, As can severely inhibit plant growth by slowing or arresting expansion and biomass accumulation, as well as compromising plant reproductive capacity through losses in fertility, yield and fruit production (Garg and Singla, 2011).

Aquatic plants are unique bioindicator species for the evaluation of water quality and subsequent risk assessment. Not only can we determine the bioavailability of contaminants, but also plant biomonitors allow for the detection of early signs of environmental disturbance before upper trophic levels are affected (Lafabrie et al., 2011). Aquatic plants have long been used for the evaluation and monitoring of metals in water (Cardwell et al., 2002). Higher plants produce varied responses to heavy metals in their environment that interfere with the genetic constitution of plants (De Wolf et al., 2004). Several plants, such as *Allium cepa*, *Hordeum vulgare*, *Arabidopsis thaliana*, *Glycine max*, *Vicia faba* and *Zea mays*, have been used as strong bioindicators of the genetic toxicity of environmental pollutants in recent years. Research on the effects of contaminant exposure on biological systems has historically focused on mechanisms of damage, and researchers have primarily studied test organisms exposed in laboratory settings (Anderson et al., 1994; Shugart and Theodorakis, 1994). Belfiore and Anderson (2001) have presented a summary of contemporary genetic assessment methods and a review of published studies of genetic effects in field-exposed aquatic organisms. Genetic techniques offer a powerful approach to assess contaminant-induced changes in populations. Direct tests of genetic adaptation are very effective in establishing a concrete and potentially deleterious population-level effect of contaminant exposure, but they are difficult to accomplish with most field-exposed organisms. Molecular genetic techniques have the potential to be very effective. As genotoxicity has been suggested because many plants are known to be injured by As contamination (Carbonell-Barrachina et al., 1997; Liu et al., 2005; Requejo and Tena, 2005), and some plant systems have been suggested as indicators of arsenic exposure (Steinkellner et al., 1998; Kovalchuk et al., 2001). Various molecular approaches, such as DNA fingerprinting based on inter-simple sequence repeat (ISSR) and random amplified polymorphic DNA (RAPD) methods, are generally used to effectively indicate genetic relationships. RAPD assays can be used to detect various types of genetic differentiation or DNA damage and mutations (point mutations, rearrangements and small deletions or insertions of DNA). This technique has been successfully applied to the study of heavy metals in plants and animals (De Wolf et al., 2004; Neeratanaphan et al., 2014; Boonmee et al., 2015). The development of this technology has provided a molecular marker for the detection of genetic alterations in response to heavy metal tolerance by looking directly at DNA sequence and structure, such as phylogenetic analyses using a highly conserved genetic marker (Héry et al., 2008).

Limnocharis flava is an aquatic plant species (family *Limnocharitaceae*) that is native to Central America and grows in wetlands. It is an important aquatic plant in the food chain ecosystem. *L. flava* is a noxious weed in irrigation channels in Indonesia and currently has invaded Sri Lanka and most of the Southeast Asian countries (Waterhouse, 2003). *L. flava* is a popular aquatic plant for consumption in a group of local Thai people. Knowledge of heavy metal accumulation and genetic similarity in plant species should clarify the bioindicator model of plants. This study aimed to determine the concentration of As in sediment and *L. flava* samples from the area around a specified gold mine. This study also explored the genetic similarity of *Limnocharis flava* growing around a gold mining area by RAPD with dendrogram construction and compared to a reference site, which was assumed to have no As contamination.

Materials and methods

Sampling sites

The three sampling sites utilized were located at the edge of a stream near the gold mine in the Wangsaphung district of the Loei province of Thailand (*Fig. 1*). The reference site was defined as the Pong River, where no gold mining activity occurs. Most of the land near the gold mine was engaged in farming and cropping plants such as rice, bananas, cassava, soybeans and rubber trees. Within the catchment area where the gold mine is situated, there are many small waterways (site 2) running from high elevation at the top of the plateau to lower areas. These waterways combine (sites 1 and 3) and eventually join the Loei River (Weerasiri et al., 2013).

Sample collections

L. flava and sediment samples were collected from three sampling sites at the edge of the stream near the gold mine (*Fig. 1*) and a reference site near the Pong River, which is located in a different province approximately 230 km from the studied sites. In each sampling site, *L. flava* samples were collected from three plants for As concentration analysis using an individual young *L. flava* leaf for DNA extraction. Three sediment samples were collected from the location of the plants and dried by air in a laboratory for analysis.

Analysis of As concentration in L. flava and sediment samples

A total of 2.5 g of each sample was predigested with 3 ml of concentrated nitric acid overnight at 40°C. After cooling, 2 ml of 30% hydrogen peroxide was added. The container was covered, placed in a high-pressure stainless steel bomb and placed in an oven at 160°C for 4 h. After cooling, each solution was diluted with Milli-Q water and transferred into a PET bottle for a total mass of 50 g. The As concentration in each sample was determined using induction coupled plasma-mass spectrometry (ICP-MS; model 7500C) (Bailey et al., 2003). The wavelength analysis of ICP-MS for As was set to 75 nm. The accuracy of the As concentration results was evaluated with certified reference material (CRM) via the 3111C method (APHA, 2005). Two aliquots of the CRM were spiked with a known amount of As spike standard. One spike was analyzed according to the 3111C method, and the other was analyzed with the 3111B method (APHA, 2005). The As recoveries were in the 96–100% range, which was determined to be acceptable (USEPA, 1994).

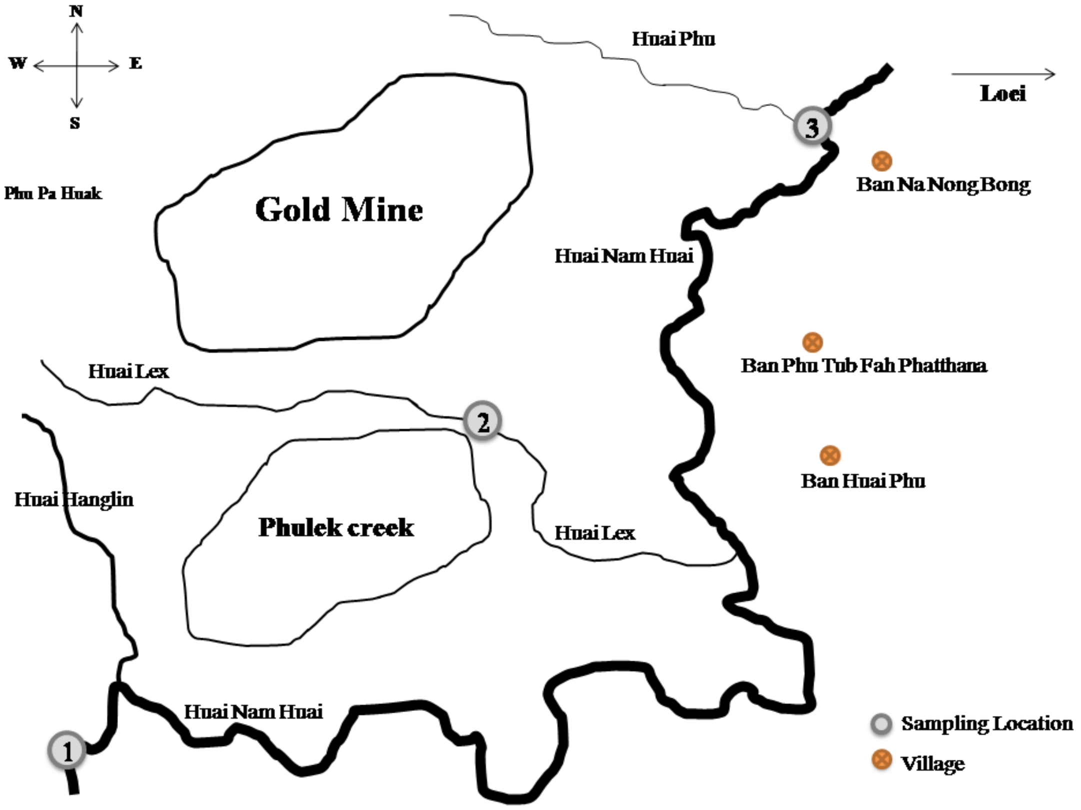

Figure 1. Overview of the gold mining area and locations of the three studied sites, indicated as sites 1 through 3

DNA extraction

Young leaves from individual *L. flava* plants at the three sampling sites and the reference site were collected and preserved in absolute ethyl alcohol for DNA extraction. The genomic DNA of plant samples was extracted using a Genomic DNA Extraction Kit (RBC Bioscience, Taiwan) following the manufacturer’s instructions. The extracted DNA was checked by 0.8% agarose gel electrophoresis. DNA samples were diluted to a final concentration of 20 ng/µl, and these dilutions were used as DNA templates in the PCR reactions.

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DNA fingerprinting and analysis using RAPD markers

DNA fingerprinting using RAPD markers was carried out on the *L. flava* samples in 25 µl reactions consisting of GoTaq Green Master Mix (Promega), 0.5 µM primer and 5 ng of DNA template. Ninety-six RAPD primers were screened; the 25 primers that successfully amplified clear bands are as follows (5′ to 3′): GGGTAACGCC, AGGTGACCGT, GTTGCGATCC, GTTTCGCTCC, TTTGCCCGGA, CCACAGCAGT, GTGAGGCGTC, GGGGGTCTTT, GATGACCGCC, GACGGATCAG, CACACTCCAG, GGACCCAACC, GAGAGCCAAC, TTATCGCCCC, CTGAGACGGA, GGTGGTCAAG, TCAGTCCGGG, AAGTCCGCTC, AGGCCCGATG, GTAGACGAGC, ACGACGTAGG, TGACCCCTCC, CAGGCGGCGT, AACGGGCAGC and CCGTCATTGG. The reaction mixture was incubated at 94°C for 3 min, and the amplification was performed using the following thermal cycles: 35 cycles of denaturation for 1 min at 94°C, annealing for 2 min at 40°C, extension for 2 min at 72°C followed by a 7 min final extension at 72°C using a thermal cycler (Swift^{TM} Maxi Thermal Cycler, Esco Micro Pte. Ltd.). The amplification products were detected using 1.2% agarose gel electrophoresis in TAE buffer and visualized using ethidium bromide. The resulting RAPD bands were used for the dendrogram construction.

The RAPD bands from all of the successful primers were discerned on an agarose gel and documented as the following diallelic characters: present = 1 and absent = 0. These resulting bands were used to construct a dendrogram using NTSYSpc 2.10p (Rohlf, 1998). The dendrogram and genetic similarities were produced and assessed.

Results and Discussion

As concentrations in sediment and L. flava

The As concentrations in the sediment and *L. flava* samples from the three studied sites and reference site are shown in *Table 1*. The average As concentration values in the sediment and *L. flava* samples at all sites ranged from 4.595±0.318 to 223.434±2.118 and 0.081±0.020 to 0.291±0.106 mg/kg, respectively. The As concentration in the sediment samples was higher than Thailand's soil quality standard level (3.9 mg/kg) (Pollution Control Department of Thailand, 2001), while the As concentration in the *L. flava* samples was lower than Thailand's food quality standard level (2.0 mg/kg) (Pollution Control Department of Thailand, 2001). The As concentrations in the sediment and *L. flava* samples at the reference site were lower than both of Thailand's standard control levels.

Table 1. *Concentration of As in sediment and L. flava samples ($\overline{X}$ ±SD)*

Samples		Sediment (mg/kg)	*L. flava* (mg/kg)
Site 1	Individual 1	4.232	0.071
	Individual 2	4.725	0.067
	Individual 3	4.829	0.105
	$\overline{X}$ ±SD	4.595±0.318	0.081±0.020
Site 2	Individual 1	231.671	0.370
	Individual 2	227.809	0.333
	Individual 3	210.823	0.170
	$\overline{X}$ ±SD	223.434±2.118	0.291±0.106

Site 3	Individual 1	18.199	0.119
	Individual 2	16.610	0.131
	Individual 3	17.543	0.129
	$\overline{X}$ ±SD	17.450±0.798	0.126±0.006
Reference site	Individual 1	3.307	0.003
	Individual 2	2.907	0.006
	Individual 3	2.728	0.007
	$\overline{X}$ ±SD	2.980±0.296	0.005±0.002
Thailand standard		3.9	2.0

This research revealed that the As concentration in sediment and the three studied plant samples correlates with genetic similarity. In addition, the Groundwater Research Center of Thailand (2010) has reported that the highest As contamination in water near the gold mine areas is 300 mg/l. The mean concentration of As in the sediment from the different sites shows values decreasing in the sequence of site 2 > site 3 > site 1. The highest As concentration in the sediment from site 2 could be due to soil erosion and water runoff from the rainy season. Alternatively, this site is nearest to the gold mine, which means that As contamination could also be caused by gold mining activities. The lower areas combine into one stream before finally joining the Loei River (*Fig. 1*). Study site 2 has a small stream near the gold mine and a lower flow rate than sites 1 and 3. Thus, the As concentration in the sediment of site 2 is higher than those of sites 3 and 1. The stream runoff from site 1 to 3 and As contamination was deposited into the sediment at site 3. The As concentration in sediment was higher than that of the plant samples at all sites. Fortunately, the As concentration of *L. flava* is lower than the standard level of Thailand. The sampling sites have running water leaching As from the gold mine into sites 1 and 3, which is then deposited into the sediments and accumulates in *L. flava*. This process likely accounts for the increased As concentration during the rainy season. As this study is a field investigation, there are other possible environmental measures of toxicity in addition to As contamination.

DNA fingerprinting of L. flava

The 25 successful RAPD primers mentioned generated clear and invaluable fingerprinting profiles; example RAPD profiles are shown in *Figure 2*. The RAPD patterns produced 1,947 total bands ranging in size from 100 bp to 2,500 bp; these bands were used for dendrogram construction. The dendrogram distinguished the studied samples into four groups based on their sites of study. The first group comprised the individuals (1, 2, and 3) of the reference site, the second group comprised the individuals (1, 2, and 3) of site 1, the third group comprised the individuals (1, 2, and 3) of site 2, and the fourth group comprised the individuals (1, 2, and 3) of site 3 (*Fig. 3*). The pairwise genetic relationships in terms of similarity (S) for all of the studied samples are shown in *Table 2*. The S values among the individual samples of each site ranged from 0.921–0.981, 0.921–0.963, 0.902–0.958, and 0.893–0.935 for the reference site, site 1, site 2 and site 3, respectively. The S values of the samples from each site compared to the reference site were 0.715–0.771, 0.696–0.729 and 0.757–0.836 for sites 1, 2 and 3, respectively. The average As concentration values in the *L. flava* samples at sites 1, 2 and 3 in addition to the reference site were 0.081±0.020, 0.291±0.106, 0.126±0.006 and 0.005±0.002 mg/kg, respectively. The aquatic plant *L. flava* and the As concentration of each studied site demonstrated a correlation according to the grouping S. The S values of the samples from site 2 compared to the reference site were lower than those of the other sites. The As concentration in *L. flava* samples at site 2 affected the genetic material compared to the reference site. The As concentration in the plant samples at site 2 was higher than those at sites 3 and 1; the lowest S value of an individual studied sample was lower than for samples at sites 1 and 3. These data indicated that the *L. flava* in the area near the gold mine adapted to their environment. Thus, plants growing in As-contaminated sites need to develop some degree of tolerance to As toxicity to survive. The aquatic plant *L. flava* in this area is affected by the gold mine but can still be of good quality. This aquatic plant can endure As contamination and survive in the environment near the gold mine, which is a highly contaminated area. Additionally, this species has very high genetic diversity, which is demonstrated by the low S value of the species. Normally, the S value of a species should be 85–100%, but *L. flava* has a wider range of S values (0.692–0.981), indicating genetic diversity. Therefore, the S value indicates that it endures a varied environment, including one with high As contamination.

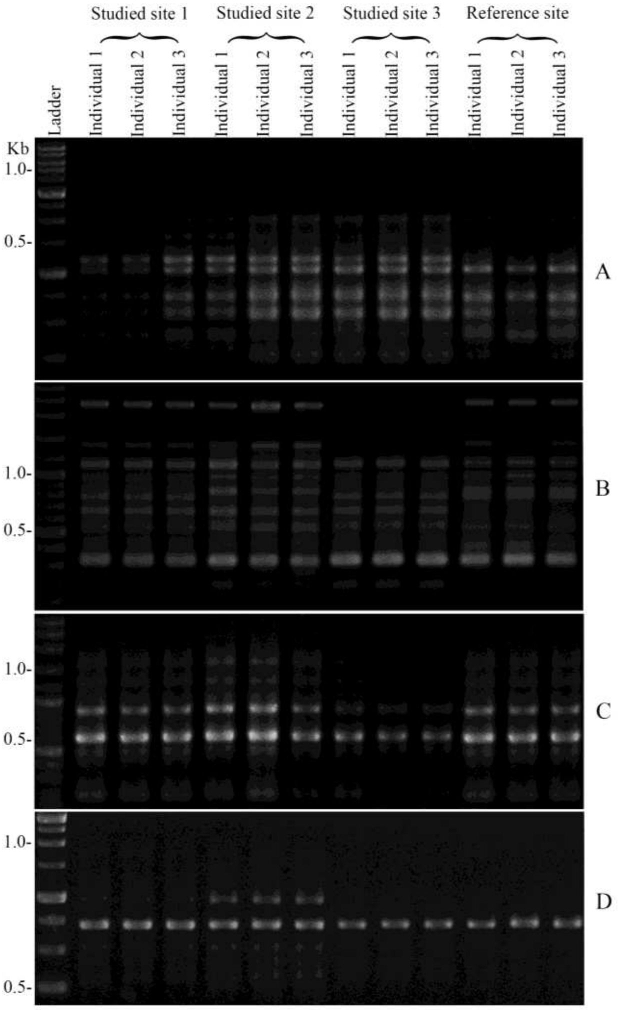

Figure 2. *Examples of RAPD fingerprints from L. flava samples in the studied sites and reference site generated using the primers GTTGCGATCC (A), CACACT CCAG (B), CTGAGACGGA (C) and AGGCCCGATG (D)*

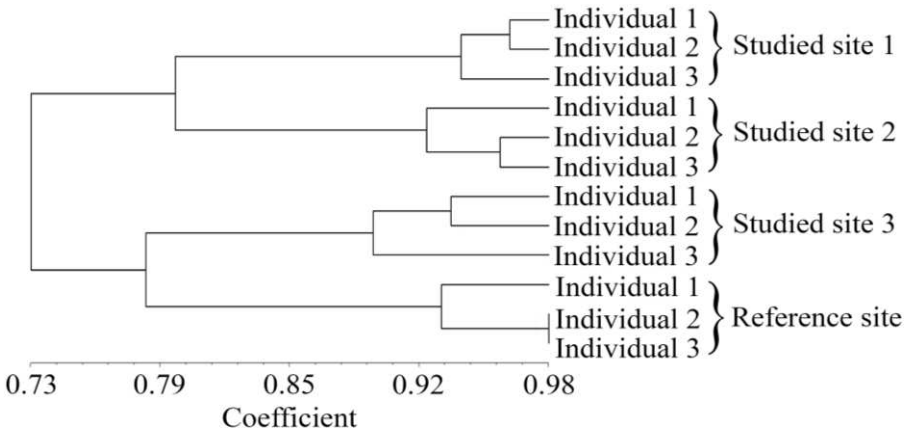

Figure 3. *The dendrogram constructed using 25 RAPD primers and the NTSYSpc 2.10p program, showing the genetic relationships among the individual samples at the three studied sites and the reference site*

Table 2. *Relationships between all pairwise individual studied samples at the three studied sites and reference site, indicating genetic similarity values*

		Studied site 1			Studied site 2			Studied site 3			Reference site		
		Individual 1	Individual 2	Individual 3	Individual 1	Individual 2	Individual 3	Individual 1	Individual 2	Individual 3	Individual 1	Individual 2	Individual 3
Studied site 1	Individual 1	1.000											
	Individual 2	0.963	1.000										
	Individual 3	0.921	0.958	1.000									
Studied site 2	Individual 1	0.780	0.818	0.860	1.000								
	Individual 2	0.762	0.799	0.841	0.944	1.000							
	Individual 3	0.748	0.785	0.827	0.902	0.958	1.000						
Studied site 3	Individual 1	0.757	0.757	0.752	0.724	0.752	0.776	1.000					
	Individual 2	0.729	0.738	0.762	0.724	0.752	0.776	0.935	1.000				
	Individual 3	0.724	0.724	0.729	0.692	0.701	0.724	0.902	0.893	1.000			
Reference site	Individual 1	0.771	0.752	0.738	0.729	0.720	0.724	0.790	0.771	0.794	1.000		
	Individual 2	0.748	0.738	0.724	0.715	0.706	0.720	0.766	0.766	0.827	0.921	1.000	
	Individual 3	0.748	0.738	0.715	0.706	0.696	0.710	0.785	0.757	0.836	0.939	0.981	1.000

Conclusions

Sediment and *L. flava* samples were taken from three sites near a gold mining area for analysis of As contamination. The concentration of As in *L. flava* did not exceed the standard level of contamination in food as determined by the Pollution Control Department of Thailand. Unfortunately, the concentration of As in the sediment was higher than the standard level. Although the As concentration in *L. flava* is low, it may still have an effect on the extent of genetic similarity of the plant based on RAPD marker analysis. In addition, this study suggests that RAPD assays are very useful tools for ecotoxicology and are useful biomarker assays for the detection of the genotoxic effects of As on plants. Lastly, the accumulation of As in aquatic plant species should be of concern, not only because As potentially affects aquatic organisms but also because it potentially affects human health. The accumulation of As in other aquatic plants, especially edible plants, should be studied. The public should be informed of the results of this research so that they can properly consider the consumption of aquatic plants grown in contaminated areas.

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REFERENCES

[1] Anderson, S., Sadinski, W., Shugart, L., Brussard, P., Depledge, M., Ford, T., Hose, J., Stegeman, J., Suk, W., Wirgin, I., Wogan, G. (1994): Genetic and molecular ecotoxicology: a research framework. – Environ. Health. Perspec. 102: 3–8.

[2] APHA. (2005): Standard methods for the examination of water and wastewater. – American Public Health Association, 21 st edition, Washington DC. 2001-3710.

[3] Bailey, R.M., Stokes, S., Bray, H. (2003): Inductively coupled plasma mass spectrometry (ICPMS) for dose rate determination: some guidelines for sample preparation and analysis. – Oxford, UK:Oxford Luminescence Research Group, School of Geography and the Environment, University of Oxford.

[4] Belfiore, N.M., Anderson, S.L. (2001): Effects of contaminants on genetic patterns in aquatic organisms: a review. – Mutat. Res. 489: 97–122.

[5] Boonmee, S., Neeratanaphan, L., Tanee, T., Khamon, P. (2015): The genetic differentiation of *Colocasia esculenta* growing in gold mining areas with arsenic contamination. – Environ. Monit. Assess. 187(5): 227–234.

[6] Carbonell-Barrachina, A.A., Burló, F., Burgos-Hernández, A., López, E., Mataix, J. (1997): The influence of arsenite concentration on arsenic accumulation in tomato and bean plants. – Sci. Hortic. 71: 167–76.

[7] Cardwell, A., Hawker, D., Greenway, M. (2002): Metal accumulation in aquatic macrophytes from Southeast Queensland, Australia. – Chemosphere. 48: 653–663.

[8] Cullen, W.R., Reimer, K.J. (1989): Arsenic speciation in the environment. – Chem. Rev. 89: 713–764.

[9] De Wolf, H., Blust, R., Backeljiau, T. (2004): The use of RAPD in ecotoxicology: A review. – Mutat. Res. 566: 249–262.

[10] Garg, N., Singla, P. (2011): Arsenic toxicity in crop plants: physiological effects and tolerance mechanisms. – Environ. Chem. Lett. 9: 303–321.

[11] Groundwater Research Center of Thailand. (2010): Performance report of prevention in environmental impact and environmental monitoring of gold mining project (Phu Thap Pha, Loei province). – Performance Report, Faculty of Technology, Khon Kaen University, Khon Kaen, Thailand.

[12] Henke, K.R. (2009): As environmental chemistry health threats and waste treatment. – 1st ed., John Wiley and Sons. Ltd., Kentucky.

[13] Héry, M., Gault, A.G., Rowland, H.A., Lear, G., Polya, D.A., Lloyd, J.R. (2008): Molecular and cultivation dependent analysis of metal-reducing bacteria implicated in arsenic mobilization in south-east asian aquifers. – Appl. Geochem. 23: 3215–3223.

[14] Kovalchuk, O., Titov, V., Hohn, B., Kovalchuk, I. (2001): A sensitive transgenic plant system to detect toxic inorganic compounds in the environment. – Nat. Biotechnol. 19: 568–72.

[15] Lafabrie, C., Major, K.M., Major, C.S., Cebrián, J. (2011): Arsenic and mercury bioaccumulation in the aquatic plant, *Vallisneria neotropicalis.* – Chemosphere. 82: 1393–1400.

[16] Liao, X.Y., Chen, T.B., Xie, H., Liu, Y.R. (2005): Soil As contamination and its risk assessment in areas near the industrial districts of Chenzhou City, southern China. – Environ. Int. 31: 791–798.

[17] Liu, X., Zhang, S., Shan, X., Zhu, Y.G. (2005): Toxicity of arsenate and arsenite on germination, seedling growth and amylolytic activity of wheat. – Chemosphere. 61: 293–301.

[18] MacFarlance, G.R., Murchett, M.D. (2002): Toxicity, growth and accumulation relationships of copper, lead and zinc in the grey mangrove *Avicennia marina* (Forsk.) Vierch. – Mar. Environ. Res. 54: 65–84.

[19] Meharg, A.A., Williams, P.N., Adomako, A., Lawgali, Y.Y., Deacon, C., Villada, A., Cambell, R.C.J., Sun, G., Zhu, Y.G., Feldmann, J., Rabb, A., Zhao, F.J., Islam, R., Hossain, S., Yanai, J. (2009): Geographical variation in total and inorganic arsenic content of polished (white) rice. – Environ. Sci. Technol. 43: 1612–1617.

[20] Neeratanaphan L., Sudmoon, R., Chaveerach, A. (2014): Genetic erosion in freshwater snail, *Filopaludina martensi* affected by lead and cadmium. – Appl. Ecol. Env. Res. 12(4): 991–1001.

[21] Peralta-Videa, J.R., Gardea-Torresdey, J.L., Gomez, E., Tiermann, K.J., Parsons, J.G., Carrillo, G. (2002): Effect of mixed cadmium, copper, nickel and zinc at different pHs upon alfafa growth and heavy metal uptake. – Environ. Pollut. 119: 291–301.

[22] Pollution Control Department of Thailand. (2001): Water quality standards. – http://www.pcd.go.th/. Accessed 10 Aug 2012.

[23] Requejo, R., Tena, M. (2005): Proteome analysis of maize roots reveals that oxidative stress is a main contributing factor to plant arsenic toxicity. – Phytochemisty. 66: 1519–28.

[24] Rohlf, F. J. (1998): NTSYS_pc: Numerical taxonomy and multivariate analysis system, version 2.1. – Applied Biostatistics, New York.

[25] Shugart, L., Theodorakis, C. (1994): Environmental genotoxicity: probing the underlying mechanisms. – Environ. Health. Perspect. 102: 13–17.

[26] Smedley, P.L., Kinniburgh, D.G. (2002): A review of the source, behaviour and distribution of arsenic in natural waters. – Appl. Geochem. 17: 517–568.

[27] Steinkellner, H., Mun-Sik, K., Helma, C., Ecker, S., Ma, T.H., Horak, O. (1998): Genotoxic effects of heavy metals: comparative investigation with plant bioassays. – Environ. Mol. Mutagen. 31: 183–91.

[28] U.S. Environmental Protection Agency. (1994): Sample preparation procedure for spectrochemical determination of total recoverable element, method 200.2. – Environmental Monitoring Systems Lab, Cincinnati, Ohio.

[29] Waterhouse, B.M. (2003): Know your enemy: records of potentially serious weeds in northern Australia, Papua New Guinea and Papua (Indonesia). – Telo-pea. 10: 477–84.

[30] Weerasiri, T., Wirojanagud, W., Srisatit, T. (2013): Localized profile of As in sediment and water in the area around gold mine. – Curr. World. Environ. 8: 231–240.

[31] Zhou, Q.X., Huang, G.H. (2000): Environmental biogeochemistry and global environmental changes. – Science Press, Beijing, China.

[32] Zhou, Q.X., Song, Y.F. (2004): Principles and methods of contaminated soil remediation. – Science Press, Beijing, China.

IMPACT OF SHEEP GRAZING ON SMALL MAMMALS DIVERSITY IN LOWER MOUNTAIN CONIFEROUS FOREST GLADES

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Abstract. Sheep grazing has once been a traditional mode of exploitation of forest as well as alpine zones in the majority of European montane regions. Nowadays, in Tatra Mts, sheep grazing is limited to a number of glades of anthropogenic origin, primarily as a method of protection against overgrowing with trees. Results of the present study demonstrate that sheep grazing causes a decrease in species diversity of small mammals Micromammalia in forest zone glades. The values of biodiversity indicators in sheep-grazed glades are lower than those in the non-managed or mown ones. On sheep-grazed glades, a lower biodiversity of Soricomorpha and a domination of common vole *Microtus arvalis* have been found.

Keywords: *glades management, sheep grazing, micromammals biodiversity, Tatra Mountains*

Introduction

There has always been a controversy over agricultural use of areas located within high nature value lands. Generally, there is a trend to exclude or reduce agricultural activities in protected ecosystems for the purpose of eliminating anthropogenic factors that may distort natural relationships. Cultivation practices in arable grounds, mowing of meadows, and animal grazing always affect the utilized land. Alterations in flora and fauna composition are the most significant effects.

Large herbivorous mammals have a great meaning when it comes to shaping the grassland ecosystems (Hobbs, 1996; Frank, 1998; Frank et al., 2002; Moore et al., 2015). In many regions of Europe, the presence of grazing animals is considered as a method of natural forest succession control, e.g., in high nature value heathland (Newton et al., 2009). Grazing is often considered to be a factor which contributes to vegetation stability. Moreover, it is regarded as a conservation method to protect a unique ecosystem, especially in regions, e.g., the Pyrenees, where animal grazing is a long-time tradition (Casasús et al., 2007; Sebastiá et al., 2008).

It is generally believed that the presence of farm animals, such as sheep or cattle, significantly affects the vegetation. Austrheim and Eriksson (2001) consider grazing as a key process stimulating biodiversity in alpine and subalpine ecosystems in Scandinavia. However, there are differences in vegetation between areas of different grazing intensity (Austrheim et al., 2014). Intensive grazing causes negative changes in the flora of alpine ecosystems (Austrheim et al., 2008; Mysterud, 2008; Lanta et al., 2014). Sheeps demonstrate large diet selectivity preferring herbs over grasslands which are eaten afterwards. Thus they significantly change the flora increasing the share of

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grasslands in the species groups (Bowns and Bagley, 1986; Hülber et al., 2005). Pasture and cutting also influences the structure of the flora. Low density of sheep has no effect on vegetation (Kausrud et al., 2006; Mayer and Huovinen, 2007; Austrheim et al., 2008) but it can affect vegetation when long-term (Mayer et al., 2009).

Changes in vegetation caused by grazing farm animals in natural environment affect wild animals that inhabit these areas. It is especially noticeable in lowland areas. Studies on small mammals population structure show unequivocally that the effect is negative (Schmidt et al., 2005; Torre et al., 2007). Nevertheless, grazed areas are important as feeding grounds for birds (Wheeler, 2008).

In montane ecosystems, studies on the effect of grazing on wild montane animals are relatively rare. In Britain, no effect of sheep grazing on insects was found (Mysterud et al., 2005). However, in central Argentina mountains, negative consequences of grazing was reported in subalpine meadows. Intensive cattle grazing causes a decrease in insect biomass and species diversity (Cagnolo et al., 2002). Extensive use of semi-natural montane meadows causes an increase in species diversity of birds (Rolando et al., 2006). An increase in grazing intensity can hinder that process (Loe, 2007). Negative effect of grazing on the populations of rodents was reported from subalpine zone in Norway (Steen et al., 2005; Austrheim et al., 2007).

The origins of grassland ecosystems in the mountain areas are usually difficult to determine unequivocally. In some cases they were created as a result of uniqe climate conditions and are maintained thanks to large herbivorous animals (Weigl and Knowls, 2014). Tatra glades are the artifacts of human activities in the forest areas. They are located among and surrounded by subalpine forests. The grounds within Tatra Mts have been utilized for pasturage for hundreds of years. The first remarks on the glades appear in written documents as early as in XVI^th^ century. For agricultural purposes, forest areas on mountain slopes were slashed-and-burned. 120 glades were created in that way (Hołub-Pacewiczowa, 1931).

In Polish Tatras, the intensive sheep grazing was continued up to the ‘80 of the 20th century. Sheep grazing was primarily taking place in mountaine forests and on alpine meadows, the areas situated above the upper range of the forest. The pressure of the shepherding on the natural ecosystems resulted in the changes in the natural reach of plant communities in many places, e.g. in the lowering of the upper range of the forest (Skawiński, 1993). Currently, sheep grazing in Polish Tatras is mainly of extensive character and takes place only on selected glades. Sheep grazing above the upper range of the forest is no longer cultivated (Kaźmierczakowa, 1990). The changes in the structure of grazing are connected with restrictions introduced by the Tatra National Park. Tatra glades, once used agriculturally, are now maintained only by a continuous human intervention. Otherwise they would be overgrown by forests in the process of natural succession. The utilization of Tatra glades has resulted in the evolution of endemic meadow association *Gladiolo-Agrostietum* as well as crocuses *Crocus scepusiensis* – on the areas with high intensity of sheep grazing (Kaźmierczakowa, 1990; Piękoś-Mirkowa and Mirek, 1996). The agricultural activities which aim at the maintenance of plant community include mowing and sheep grazing.

There is no information on the effect of various agricultural practices on small mammals populations in montane mid-forest glades. The aim of the study was to evaluate the effect of montane mid-forest glades utilization practices on small mammals communities and assess transformations in glades that were excluded from pasturage.

Material and methods

Study area

The studies were carried out in mid-forest glades located within Tatra Mts. The range of Tatra Mts is the highest massif within the Carpathians. Tatra are distinctly separated from the surrounding area although the length of the range is only 57 km and its width – 18 km. The height above sea level ranges from 900 m at the foothills up to 2499 m at the top of Rysy that is the highest summit in the Polish part of Tatra. The landscape of Tatra is of alpine type with altitudinal zonation: low mountain zone forests (to 1200 – 1250 m a.s.l.), high mountain zone forests (to 1500 m a.s.l.), subalpine zone (to 1800 m a.s.l.), alpine grasslands (to 2300 m a.s.l.), subnivean zone (from 2300 m a.s.l. up). Polish and Slovak parts of Tatra are protected as national parks and as a Biosphere Reserve of UNESCO.

The study areas were located in mid-forest glades within lower mountain zone forests. The glades were surrounded by silver fir-spruce forest and differed in the utilization mode. Three categories of glades were distinguished: (I) non-managed glades: Brzanówka (B), Dudowa (D), Gronik (G), Wyżnia Rówień Miętusia (WRM), Przysłup Miętusi (PM); (II) glades mowed annually: Huciska Niżne (HN) and Palenica Pańszczykowa (PP); (III): grazed glades: Biały Potok (BP), Huciska Wyżnie (HW), Molkówka (M), Siwa Polana (SP). All glades were situated at elevations ranging from 920 to 1174 m a.s.l. Animal traps were located in the centre of each glade to avoid ecotone effects.

The vegetation of the glades consists of semi-natural meadows (*Tab. 1*). The most common type of vegetation is mesotrophic grassland represented by *Gladiolo-Agrostietum* (association of *Gladiolus imbricatus* and *Agrostis capillaris*). There are also patches of flora belonging to the association of *Hieracio-Nardetum, Festucetum rubrae* as well as *Festuco-Cynosuretum.* Associations of *Cirsietum rivularis, Festuco-Cynosuretum*, and *Rubetum idaei,* as well as ruderal species, patches covered with *Rubus* spp. and scraps with *Deschampsia caespitosa* occur on the non-managed glades. Grazed glades feature associations of *Deschampsietum caespitosae, Festuco-Cynosuretum* as well as degraded association of *Gladiolo-Agrostietum.* Sheep-grazed glades are characterized by the predominance of *Deschampsia caespitosa* as well as ruderal flora with dominance of *Artemisia vulgaris*. *Crocus scepusiensis* occurs with various incidence on the grazed glades.

The plant cover of glades was categorized into four types, depending on vegetation density:

1. strongly grazed vegetation with *Deschampsia caespitosa*; vegetation cover very scarce (M)
2. semi-grazed vegetation with *Deschampsia caespitosa* and *Cirsium rivulare*; vegetation cover relatively scarce (SP, HW, BP),
3. *Deschampsia caespitosa* – dominated meadow with abundance of herbaceous plants; vegetation cover relatively dense (B, PP, HN, G, PM)
4. meadow with *Rubus* spp. and *Aconitum firmum*; vegetation cover very dense (D, WRM).

Sheep grazing occurred from the beginning of May until the end of October. The glades were grazed by herding sheep from one glade to another. The glades Molkówka, Siwa Polana, and Biały Potok were grazed by one flock (300 sheep in 2004, 220 sheep in 2005) and Huciska Wyżnie by another flock (190 sheep in 2004 and 170 sheep in 2005). While herded, sheep grazed along central part of the glade. Additionally, in Siwa Polana, some cattle were kept during the vegetation season (7 cattle in 2004 and 8 cattle in 2005).

Table 1. *Forms of exploitation, geographical location and botanic characteristic of the investigated Tatra glades*

Forms of exploitation of a glade	**Glade**	**Geographical location**	**Area ha**	**Altitude asl.**	**Type of flora**
Non-managed	B	N 49°17′22″ E 20°05′21″	4	932	wet tall-forb grassland
	D	N 49°14′58″ E 19°49′37″	3	1174	secondary forb communities
	G	N 49°16′28″ E 19°53′39″	3	972	grassland
	WRM	N 49°15′08″ E 19°53′43″	5	1142	wet tall-forb grassland
	PM	N 49°15′47″ E 19°53′22″	9	1147	secondary forb communities
Mown	PP	N 49°17′22″ E 20°05′21″	4	925	grassland with *Deschampsia caespitosa*
	HN	N 49°17′22″ E 20°05′21″	4	985	grassland
Grazed	M	N 49°16′44″ E 19°49′39″	18	991	intensely eaten grass with *Deschampsia caespitosa*
	SP	N 49°16′40″ E 19°50′16″	53	936	poorly eaten grass with *Deschampsia caespitosa*
	HW	N 49°17′22″ E 20°05′21″	2,5	1026	poorly eaten grass with *Deschampsia caespitosa* and *Cirsium rivulare*
	BP	N 49°16′42″ E 19°50′55″	30	920	poorly eaten grass with *Deschampsia caespitosa* and *Cirsium rivulare*

Methods

Trapping of mammals was carried out at the turn of July and August of 2004 and 2005. The research was conducted in all glades in the same time period in 2004 and repeated in 2005. Micromammalia were caught using 45 cm high cone traps. 20 traps were placed in each study area (10 traps in 2 rows). The distance between traps and rows was 10 m. All traps were located in the central part of a glade. CMR (capture/mark/recapture) method was applied (Gurnell and Flowerdew, 1994). Trapped animals were marked by cutting a small fragment of fur on their dorsal side. Trapping was conducted during 5 consecutive nights (2200 trapnights). Traps were emptied four times per day (6.00 a.m., 8.00 a.m., 5.00 p.m., and 8.00 p.m.). If it was raining, the traps were visited more frequently or the traps were blocked.

The structure of mammal communities in Tatra glades was characterized using several parameters that depict species diversity: dominance coefficient D and Shannon-Wiener diversity index *H'* (log base 2).

The numbers of caught mammals in 2004 and 2005 were compared using Pearson's χ^2 test. The differences in abundance of individual species were also analysed. The effect of sheep herding on small mammals communities was determined using Pearson's

χ^2 test with Yates' continuity correction. Chi-squared test was applied to establish the relationship between the number of small mammals and the year of study.

The areas of study were of the same size. It has been assumed that population density is the number of mammals per one study area. It has been assumed that the average density of mammals on a trapping area matches the number of trapped individuals on a 1 ha area.

Multivariate analysis of variance (MANOVA) was carried out to determine which factor (year of study, mode of utilization, density of vegetation cover, sheep herding) has the most significant effect on population density. In all analyses, the significance level was set to 0.05.

Results

The study on the small mammals populations show that there are differences in species composition and population number between the glades that differed in the mode of use. The richest in species were non-managed glades (13 species of small mammals). In mown glades, there were 11 species and in grazed glades – 7 species (*Tab. 2, Fig. 1*).

Table 2. *The effect of the exploitation of the Tatra glades on the species diversity of small mammals (Micromammalia)*

Parameter	**Non-managed glades**				**Mown glades**		**Grazed glades**				
	G	**WRM**	**PM**	**D**	**B**	**PP**	**HN**	**M**	**SP**	**HW**	**BP**
Number of species	7	7	7	7	7	9	6	2	6	3	4
Number of individuals	32	89	68	71	30	41	19	5	69	10	20
Proportion of shrews %	37.5	33.7	23.5	36.6	23.3	36.6	36.8	-	5.8	20	10
Proportion of rodents %	62.5	66.3	76.5	63.4	76.7	63.4	63.2	100	94.2	80	90
Trapping success (per 100 trap-nights)	1.6	4.45	3.4	3.55	1.5	2.05	0.95	0.25	3.45	0.5	1.0
Shannon-Wiener index *H'*	2.02	2.12	2.24	2.08	2.53	2.77	2.18	0.72	0.77	1.16	1.32

The highest number of small mammals occurred in non-managed glades (on average, 58 individuals/glade). The mean number of individuals in mown glades was 30, and in the grazed glades – 26. The relatively high mean number of mammals in grazed glades was associated with the high number of common vole *Microtus arvalis* that occurred in extensively used Siwa Polana. Significant differences in the number of mammals caught in different years occurred in mown glades (HN, PP), non-managed glades (G, PM, WRM), and grazed glades (SP). Significant decrease in the number of caught mammals occurred in 2005 in comparison to 2004 (χ^2 = 12.72, df = 1, p = 0.0003) (*Tab. 3*).

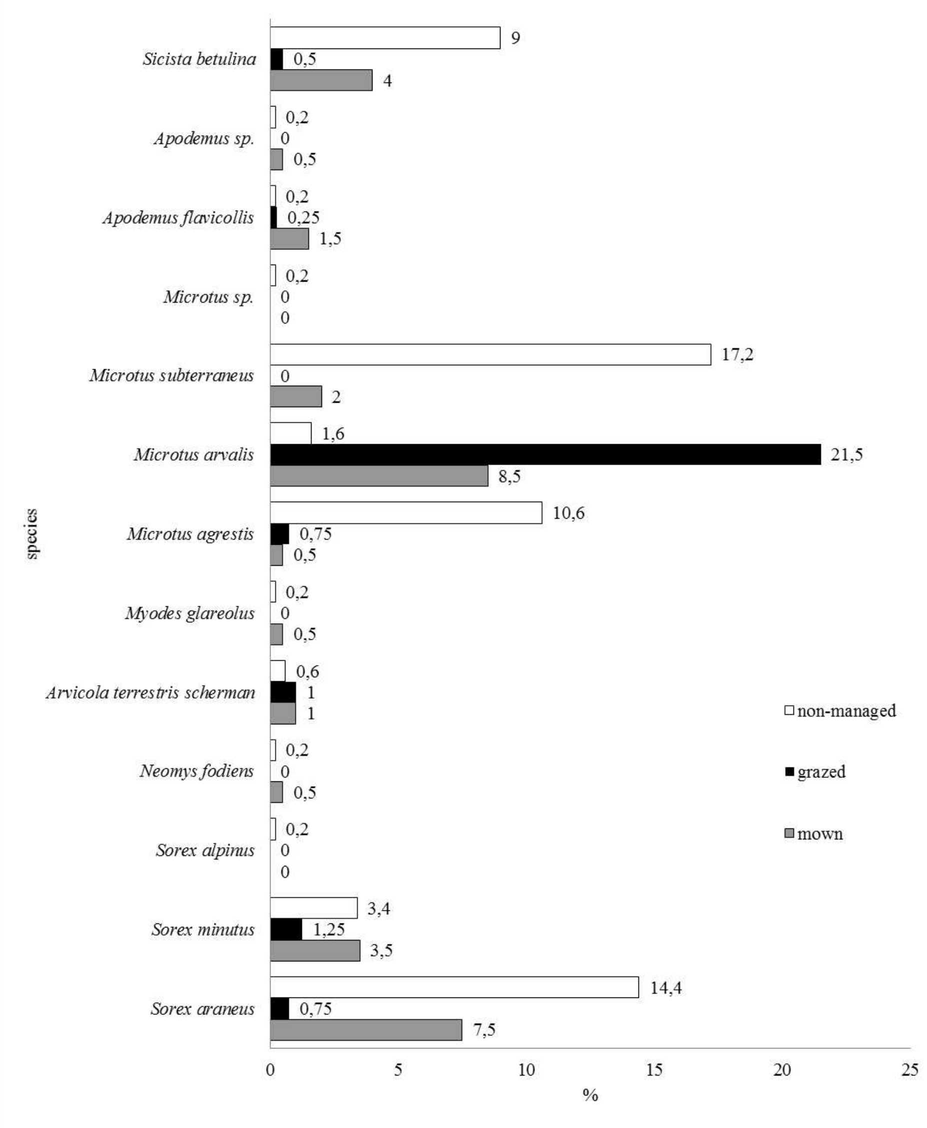

Figure 1. *Structure of small mammals communities on non-managed, mown and grazed Tatra glades in 2004-05*

Table 3. *Differences in the number of caught mammals (Micromammalia) on the Tatra glades in the years 2004 and 2005 (Pearson's χ^2 test)*

Forms of exploitation of a glade	**Glade**	**Number of individuals N**		***p***
		2004	**2005**	
Mown	HN	15	4	0.0116
	PP	27	14	0.0423
Non-managed	B	20	10	0.0679
	D	29	42	0.1229
	G	25	7	0.0015
	PM	48	20	0.0007
	WRM	62	27	0.0002
Grazed	BP	9	11	0.6547
	HW	8	2	0.0578
	M	4	1	0.1797
	SP	18	51	0.0001

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Within all small mammals that were caught in all types of glades, rodents Rodentia were the predominating group. Their number in non-managed and mown glades was comparable: 62-77% of all small mammals. In grazed glades, the number of rodents was very high and reached 80-100% of all small mammals, depending on a glade. In grazed glades, Soricomorpha were scarce: the maximum number of individuals of that group amounted to 20% of all small mammals and one species – common vole (70%) – clearly predominated (*Tab. 2*). The highest density of common vole occurred in grazed glades with a relatively low density of sheep (ca. 5-9 sheep/ha). The density of common vole is distinctly lower in glades where more sheep 15-28 sheep/ha) are grazing. This result shows that grazed glades are the worst environment for Soricomorpha. In other types of glades, no clear predominance of any of species occurred. In non-managed and mown glades, a high number of other vole species was found: field vole *Microtus agrestis,* common pine vole *Microtus subterraneus*, and of other rodents – birch mice *Sicista betulina.* In non-managed glades, common shrew *Sorex araneus* and pygmy shrew *Sorex minutus* were co-dominating (*Fig. 2*). The number of individuals of several species differed in different years of study and the difference was significant (*Tab. 4*). Differences in the value of Shannon-Wiener Index on a grazed glades, cut glades and non-managed glades were statistically significant (χ^2 = 10.2657, df = 2, p = 0.0059). The post-hoc test revealed that the differences occurred between grazed and non-managed glades (non-managed vs. mown p = 0.343; grazed vs. mown p = 0.162, grazed vs. non-managed p = 0.007).

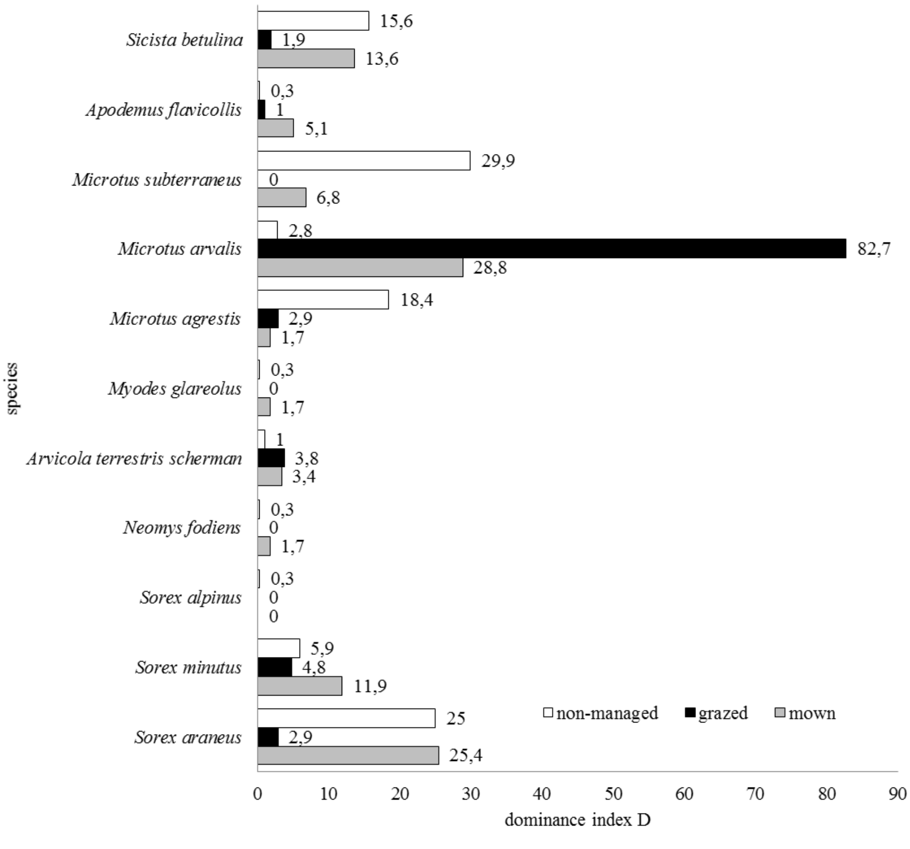

Figure 2. *The values of the dominance index (D) on non managed, mown and grazed Tatra glades in 2004-05*

Table 4. *Differences in the total number of caught mammal species (Micromammalia) on the Tatra glades in the years 2004 and 2005 (Pearson's* χ^2 *test)*

Species	Number of individuals N		*p*
	2004	2005	
Sorex araneus	68	22	0.0000
Sorex minutus	22	7	0.0053
Sorex alpinus	1	0	-
Neomys fodiens	1	1	1.0000
Arvicola terrestris scherman	8	1	0.0196
Myodes glareolus	0	2	-
Microtus agrestis	45	12	0.0000
Microtus arvalis	31	80	0.0000
Microtus subterraneus	56	34	0.0204
Microtus sp.	0	1	-
Apodemus flavicollis	5	0	-
Apodemus sp.	2	0	-
Sicista betulina	26	29	0.6858

The mode of exploitation of mid-forest glades affects the species diversity as well as the number of individuals. The most important factor is the grazing of sheep and the differences observed were mainly due to the intensity of grazing. Of the grazed glades, the lowest biodiversity occurred in the glades at Molkówka (intensive grazing: 17 sheep/ha in 2004 and 12 sheep/ha in 2005) and the highest in Siwa Polana (species spectrum comparable to non-managed glades; extensive grazing: 6 sheep/ha in 2004 and 4 sheep/ha in 2005). The limited grazing in Siwa Polana allowed higher number of species and higher number of individuals than in other grazed glades (*Tab. 2*).

The MANOVA showed that the density of vegetation and type of exploitation in glades have significant impact on the density of small mammals (p = 0.002 and p = 0.004, respectively). The remaining factors analyzed, year of study and herding of sheep did not affect the density of mammals (p = 0.7455 and p = 0.6688, respectively).

Discussion

Presence of large herbivorous mammals strongly impacts local populations of small mammals causing the increase of one species' population and decrease of other species' population (Keesing, 1998). Negative influence is demonstrated by the decrease of shelters' accessibility, reduction of nutritional base abundance and greater risk of predator's attack (Flowerdew, 2001). Diversity and number of small mammals that are particularly tied to grassland ecosystems is greater on the areas protected from livestock (Pedo et al., 2010).

In Polish Tatra, sheep grazing is of extensive character and occurs only in selected glades within forest zone. The exploitation of the forest zone glades affects the small mammals population structure, depending on the mode of use. Environmental preferences of small mammals are diversified depending on the species. Glades' use is favourable for some species while for others it may be negative (*Tab. 5*). The most important result associated with sheep grazing is the appearance of common vole. In grazed glades, this species predominates. However, it prefers meadows that are exploited extensively, that is where the density of sheep is low. Common vole is a species characteristic of agricultural land. It is likely that in Tatra, it follows the herded sheep because it does not occur in other than the glades, habitats. In Slovak parts of Tatra, the common vole was found only in the areas where sheep grazed (Kratochvíl and Pelikán, 1955; Rosický and Kratochvíl, 1955). In the 1950s, after the national park had been erected, the sheep grazing was abandoned. Twenty years later, no common voles were found in the areas where sheep grazing occurred and where common vole occurred (Zima et al., 1984). Single individuals of that species occurred only in deforested and partly urbanized areas at low altitudes (Štollmann and Dudich, 1985). At the same time, a high proportion (87-100%) of common vole in small mammals populations was reported from the neighbouring mountain range Gorce, where intensive sheep grazing occurred (Białas et al., 1989).

Table 5. *Habitat preferences of mammal species (Micromammalia) found on the Tatra glades in the years 2004-2005*

Species	**Preferred habitats**	
Sorex araneus	cool habitats with dense vegetation cover	Anděra, 1999
Sorex minutus	damp areas with dense vegetation including swamps, grasslands, heaths, sand dunes, woodland edge, rocky areas, scrubland, and montane forests	Hutterer, 1999
Sorex alpinus	cool and humid environment, densely vegetated	Spitzenberger, 1999
Neomys fodiens	wetland habitats, damp grasslands, humid woodlands	Spitzenberger, 1999
Arvicola terrestris scherman	dry or mesic grasslands, meadows pastures and occasionally in wooded areas	Saucy, 1999
Myodes glareolus	all kinds of woodlands	Spitzenberger, 1999
Microtus agrestis	moist habitats with rich grass cover, woodlands, marshes, wet meadows	Zima, 1999
Microtus arvalis	open cultivated agricultural land, grazed pastures, meadows	Zima, 1999
Microtus subterraneus	a variety of meadows habitats and pastures	Kryštufek, 1999
Apodemus flavicollis	a variety of woodland habitats, open scrublands and secondary habitats	Juškaitis, 2002
Sicista betulina	a variety of habitats including boreal and montane forests, subalpine meadows, wet meadow habitats	Pucek, 1999

In the grazed areas, the number of field vole decreases. This phenomenon was described by Steen et al. (2005) who found a negative correlation between an increase of grazing sheep and field vole population number. In the present study, the frequency of common vole in traps decreased drastically in mown and extensively grazed glades. Sheep grazing causes a decrease in the availability of food for the rodents, which may affect their population number (Austrheim et al., 2007).

Moser and Witmer (2000) showed that in the montane meadows, cattle grazing and red deer *Cervus elaphus* grazing caused a decrease in species diversity and number of small mammals and an elimination of shrews. Similar results were found in the present study. The sheep grazing caused a decrease in the number of caught shrews. It is interesting though, that mowing does not affect shrews.

In grazed glades, the decrease in number of birch mouse, field vole, common pine vole, and common shrew coincides with the increase of number of common vole. The change in species composition and species domination structure (prevalence of common vole) does not affect the overall number of small mammals in grazed glades, which was confirmed using MANOVA.

The distinctive character of sheep grazing is that the animals do not exploit the whole glade at one time. Therefore, the species spectrum and population number may be different in different parts of a glade. At the same time, species diversity of small mammals in montane deforested areas may be affected by the size of a glade. In small glades, the diversity indices are close to those of the surrounding forests (Bryja et al., 2002). In the present study, biodiversity indices of non-managed or mown glades were higher than the indices calculated for the forests in lower as well as upper mountain forest zones by Juchiewicz et al. (1986).

The high numbers of birch mouse in non-managed and mown glades is associated with the occurrence of plants that grow high that are preferred by this species. Changes in exploitation mode of glades affect the stability of these plant populations. Birch mouse is less frequent in grazed glades where the vegetation structure is changed due to grazing. Individual glades are isolated from each other by compact forest areas. Therefore, grass mowing causes a complete destruction of the birch mouse habitat. Birch mouse may occur in montane forests (Bobretsov et al., 2005), but spruce forests that surrounded Tatra glades studied do not have grass undergrowth, so they are not a good habitat for this species.

In the montane-zone glades studied, a distinct negative effect of grazing on biodiversity of small mammals occurred. Sheep grazing in relatively small glades changes habitat conditions profoundly. It is grazing as well as trotting that have an influence. The changes include also a shift in domination structure: one species (common vole) becomes a dominant one. The influence is particularly negative in the cases of common pine vole, field vole, birch mouse, and shrews. Even the extensive grazing may change vegetation structure in a negative way for mammals that occur there. The abundance of common vole may also be connected with the presence of sheep (Kratochvíl and Pelikán, 1955; Rosický and Kratochvíl, 1955). The occurrence of that species in the areas distant from agricultural lands is not likely.

Mowing or the abandonment of exploitation has a positive impact on the biodiversity of montane-forest zone glades. Nevertheless, the lack of human activity and natural succession will promote the appearance of typical forest fauna.

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REFERENCES

[1] Anděra, M. (1999): *Sorex araneus* Linnaeus, 1758. - In: Mitchell-Jones, A.J., Amori, G., Bogdanowicz, W., Kryštufek, B., Reijnders, P.J.H., Spitzenberger, F., Stubbe, M., Thissen, J.B.M., Vohralík, V., Zima, J. (eds.) The Atlas of European Mammals, Academic Press, London.

[2] Austrheim, G., Eriksson, O. (2001): Plant species diversity and grazing in the Scandinavian mountains – patterns and processes at different spatial scales. - Ecography 24: 683-695.

[3] Austrheim, G., Mysterud, A., Hassel, K., Evju, M., Økland, R.H. (2007): Interactions between sheep, rodents, graminoids, and bryophytes in an oceanic alpine ecosystem of low productivity. - Ecoscience 14: 178-187.

[4] Austrheim, G., Mysterud, A., Pedersen, B., Halvorsen, R., Hassel, K., Evju, M. (2008): Large scale experimental effects of three levels of sheep densities on an alpine ecosystem. - Oikos 117: 837-846.

[5] Austrheim, G., Speed, J.D.M., Martinsen, V., Mulder, J., Mysterud, A. (2014): Experimental effects of herbivore density on aboveground plant biomass in an alpine grassland ecosystem. - Arctic, Antarctic, and Alpine Research 46: 535-541.

[6] Białas, I., Chętnicki, W., Kupryjanowicz, J. (1989): Small mammals of Gorce National Park. - Acta Zoologica Cracoviensia 32: 631-652.

[7] Bobretsov, A.V., Luk´yanova, L.E., Poroshin, E.A. (2005): Structure of the communities of small mammals (Micromammalia) in the forests on western slopes of the Northern Urals. - Russian Journal of Ecology 36: 120-126.

[8] Bowns, J.E., Bagley, C.F. (1986): Vegetation responses to long-term sheep grazing on mountain ranges. - Journal of Range Management 39: 431-434.

[9] Bryja, J., Heroldová, M., Zejda, J. (2002): Effects of deforestation on structure and diversity of small mammals communities in the Moravskoslezské Beskydy Mts (Czech Republik). - Acta Theriologica 47: 295-306.

[10] Cagnolo, L., Molina, S.I., Valladares, G.R. (2002): Diversity and guild structure of insect assemblages under grazing and exclusion regimes in a montane grassland from Central Argentina. - Biodiversity Conservation 11: 407-420.

[11] Casasús, I., Bernués, A., Sanz, A., Villalba, D., Riedel, J.L., Revilla, R. (2007): Vegetation dynamics in Mediterranean forest pastures as affected by beef cattle grazing. - Agriculture, Ecosystems and Environment 121: 365-370.

[12] Flowerdew, J.R., Ellwood, S.A. (2001): Impacts of woodland deer on small mammal ecology. - Forestry 74: 277-287.

[13] Frank, D.A., Kuns, M.M., Guido, D.R. (2002): Consumer control of grassland plant production. - Ecology 83: 602-606.

[14] Frank, D.A., McNaughton, S.J., Tracy, B.F. (1998): The ecology of the Earth's grazing ecosystems. - Bioscience 48: 513-521.

[15] Gurnell, J., Flowerdew, J.R. (1994): Live trapping small mammals: a practical guide. 3rd Edition. - The Mammal Society, London.

[16] Hülber, K., Ertl, S., Gottfried, M., Reiter, K., Grabherr, G. (2005): Gourmets or gourmands? Diet selection by large ungulates in high-alpine plant communities and possible impacts on plant propagation. - Basic and Applied Ecology 6: 1-10.

[17] Hobbs, N.T. (1996): Modification of ecosystems by ungulates. - Journal of Wildlife Management 60: 695-713.

[18] Hołub-Pacewiczowa, Z. (1931): Osadnictwo pasterskie i wędrówki w Tatrach i na Podtatrzu. - Polska Akademia Umiejętności, Kraków. (In Polish)

[19] Hutterer, R. (1999): *Sorex minutus* Linnaeus, 1766. - In: Mitchell-Jones, A.J., Amori, G., Bogdanowicz, W., Kryštufek, B., Reijnders, P.J.H., Spitzenberger, F., Stubbe, M., Thissen, J.B.M., Vohralík, V., Zima, J. (eds.) The Atlas of European Mammals, Academic Press, London.

[20] Juchiewicz, M., Zemanek, M., Bieniek, M., Siuta, E. (1986): Small rodent communities in the Tatra Mountain forests. - Acta Theriologica 31: 433-447.

[21] Juškaitis, R. (2002): Spatial distribution of the yellow-necked mouse (*Apodemus flavicollis*) in large forest areas and its relation with seed crop of forest trees. - Mammalian Biology 67: 206-211.

[22] Kausrud, K., Mysterud, A., Rekdal, Y., Holand, Ø., Austrheim, G. (2006): Density-dependent foraging behaviour of sheep on alpine pastures: effects of scale. - Journal of Zoology 270: 63-71.

[23] Kaźmierczakowa, R. (1990): Impact of grazing on the biocenoses of forest glades in the Tatras (recapitulation). - Studia Naturae 34: 163-173. (In Polish)

[24] Keesing, F. (1998): Impacts of ungulates on the demography and diversity of small mammals in central Kenya. - Oecologia 116: 381-389.

[25] Kratochvíl, J., Pelikán, J. (1955): Poznámky o pronikàni hraboše polno do Tatranského národního parku. - Zoologicke a Entomologicke Listy 4: 303-312. (In Slovak)

[26] Kryštufek, B. (1999): *Microtus subterraneus* (de Sélys-Longchamps, 1836). - In: Mitchell-Jones, A.J., Amori, G., Bogdanowicz, W., Kryštufek, B., Reijnders, P.J.H., Spitzenberger, F., Stubbe, M., Thissen, J.B.M., Vohralík, V., Zima, J. (eds.) The Atlas of European Mammals, Academic Press, London.

[27] Lanta, V., Austrheim, G., Evju, M., Klimešová, J., Mysterud, A. (2014): Linking sheep density and grazing frequency to persistence of herb species in an alpine environment. - Ecological Research 29: 411-420.

[28] Loe, L. E., Mysterud, A., Stien, A., Steen, H., Evans, D.M., Austrheim, G. (2007): Positive short-term effects of sheep grazing on the alpine avifauna. - Biological Letters 3: 109-111.

[29] Mayer, A.C., Huovinen, Ch. (2007): Silvopastoralism in the Alp: Native plant species selection under different grazing pressure. - Ecological Engineering 29: 372-381.

[30] Mayer, R., Kaufmann, R., Vorhauser, K., Erschbamer, B. (2009): Effects of grazing exclusion on species composition in high-altitude grasslands of the Central Alps. - Basic and Applied Ecology 10: 447-455.

[31] Moore, E.K., Britton, A.J., Iason, G. Pemberton, J. Pakeman, R.J. (2015): Landscape-scale vegetation patterns influence small-scale grazing impacts. - Biological Conservation 192: 218-225.

[32] Moser, B.W., Witmer, G.W. (2000): The effects of elk and cattle foraging on the vegetation, birds, and small mammals of the Bridge Creek Wildlife Area, Oregon. - International Biodeterioration and Biodegradation 45: 151-157.

[33] Mysterud, A., Austrheim, G. (2008): The effect of domestic sheep on forage plants of wild reindeer; a landscape scale experiment. - European Journal of Wildlife Research 54: 461-468.

[34] Mysterud, A., Hansen, L.O., Peters, C., Austrheim, G. (2005): The short-term effect of sheep grazing on selected invertebrates (Diptera and Hemiptera) relative to other environmental factors in an alpine ecosystem. - Journal of Zoology, London 266: 411-418.

[35] Newton, A.C., Stewart, G.B., Myers, G., Diaz, A., Lake, S., Bullock, J.M., Pullin, A.S. (2009): Impact of grazing on lowland heathland in north-west Europe. - Biological Conservation 142: 935-947.

[36] Pedo, E., de Freitas T.R.O., Hartz S.M. (2010): The influence of fire and livestock grazing on the assemblage of non-flying small mammals in grassland-Araucaria Forest ecotones, southern Brazil. - Zoologia 27: 533-540.

[37] Piękoś-Mirkowa, H., Mirek, Z. (1996): Plant communities. - In: Mirek, Z. (ed.) Nature of the Tatra National Park. Tatry i Podtatrze, Vol. 3, The Tatra Mountains National Park, Kraków and Zakopane. (In Polish)

[38] Pucek, Z. (1999): *Sicista betulina* (Pallas, 1779). - In: Mitchell-Jones, A.J., Amori, G., Bogdanowicz, W., Kryštufek, B., Reijnders, P.J.H., Spitzenberger, F., Stubbe, M., Thissen, J.B.M., Vohralík, V., Zima, J. (eds.) The Atlas of European Mammals, Academic Press, London.

[39] Rolando, A., Dondero, F., Ciliento, E., Laiolo, P. (2006): Pastoral practices and bird communities in Gran Paradiso National Park: management implications in the Alp. - Journal of Mountain Ecology 8: 21-26.

[40] Rosický, B., Kratochvíl, J. (1955): Drobní savci Tatranského národního parku. - Ochrana Přírody 10: 34-47. (In Slovak)

[41] Saucy, F. (1999): *Arvicola terrestris* (Linnaeus, 1758). - In: Mitchell-Jones, A.J., Amori, G., Bogdanowicz, W., Kryštufek, B., Reijnders, P.J.H., Spitzenberger, F., Stubbe, M., Thissen, J.B.M., Vohralík, V., Zima, J. (eds.) The Atlas of European Mammals, Academic Press, London.

[42] Schmidt, N.M., Olsen, H., Bildsøe, M., Sluydts, V., Leirs, H. (2005): Effects of grazing intensity on small mammals population in wet meadows. - Basic and Applied Ecology 6: 57-66.

[43] Sebastiá, M.T., de Bello, F., Puig, L., Taull, M. (2008): Grazing as a factor structuring grasslands in the Pyrenees. - Applied Vegetation Science 11: 215-222.

[44] Skawiński, P. (1993): Kasprowy Wierch and Goryczkowa Valley: Human impact an nature in the Tatra Mountains. - In: Cichocki, W. (ed.) The endagered nature of the Polish Tatra Mountains, The Tatra Museum named after dr T. Chałubiński, The Tatra National Park, Zakopane. (In Polish)

[45] Spitzenberger, F. (1999): *Clethrionomys glareolus* (Schreber, 1780). - In: Mitchell-Jones, A.J., Amori, G., Bogdanowicz, W., Kryštufek, B., Reijnders, P.J.H., Spitzenberger, F., Stubbe, M., Thissen, J.B.M., Vohralík, V., Zima, J. (eds.) The Atlas of European Mammals, Academic Press, London.

[46] Spitzenberger, F. (1999): *Neomys fodiens* (Pennant, 1771). - In: Mitchell-Jones, A.J., Amori, G., Bogdanowicz, W., Kryštufek, B., Reijnders, P.J.H., Spitzenberger, F., Stubbe, M., Thissen, J.B.M., Vohralík, V., Zima, J. (eds.) The Atlas of European Mammals, Academic Press, London.

[47] Spitzenberger, F. (1999): *Sorex alpinus* Schinz, 1837. In: Mitchell-Jones, A.J., Amori, G., Bogdanowicz, W., Kryštufek, B., Reijnders, P.J.H., Spitzenberger, F., Stubbe, M., Thissen, J.B.M., Vohralík, V., Zima, J. (eds.) The Atlas of European Mammals, Academic Press, London.

[48] Steen, H., Mysterud, A., Austrheim, G. (2005): Sheep grazing and rodent populations: evidence of negative interactions from a landscape scale experiment. - Oecologia 143: 357-364.

[49] Štollmann, A., Dudich, A. (1985): Fauna of small ground mammals (Insectivora, Rodentia) of the Western Tatras. - Zbornik Prác o TANAP. 26: 161-172. (In Slovak)

[50] Torre, I, Díaz, M., Martínez-Padilla, J., Bonal, R., Viñuela, J., Fargallo, J.A. (2007): Cattle grazing, raptor abundance and small mammal communities in Mediterranean grasslands. - Basic and Applied Ecology 8: 565-575.

[51] Weigl, P.D., Knowles, T.W. (2014): Temperate mountain grasslands: a climate-herbivore hypothesis for origins and persistence. - Biological Reviews 89: 466-476.

[52] Wheeler, P. (2008): Effects of sheep grazing on abundance and predators of field vole (*Microtus agrestis*) in upland Britain. - Agriculture, Ecosystems and Environment 123: 49-55.

[53] Zima, J., Hrabě, V., Štěrba, O., Schlamp, M. (1984): Small mammals of the hollow of seven springs in the Belianske Tatras. - Zbornik Prác o TANAP. 25: 29-45. (In Slovak)

[54] Zima, J. (1999): *Microtus agrestis* (Linnaeus,1761). - In: Mitchell-Jones, A.J., Amori, G., Bogdanowicz, W., Kryštufek, B., Reijnders, P.J.H., Spitzenberger, F., Stubbe, M., Thissen, J.B.M., Vohralík, V., Zima, J. (eds.) The Atlas of European Mammals, Academic Press, London.

ANALYSING THE ATTRIBUTES OF ECOLOGICAL EVALUATION ON LOCAL AND REGIONAL LEVELS VIA WILLINGNESS TO PAY (WTP) – A HUNGARIAN CASE STUDY

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Abstract. The need for integrating natural resources into the development and decision-making processes of various sectors on a political level is becoming more and more apparent in the European Union. The number of ecological evaluation projects is on the rise, the areas of usage include anywhere from local, through national, to global levels. This study's results offer answers to: how local, and regional (national) Willingness to Pay (WTP for short), in other words, social interpretation of natural resources differ through Hungary; and how income, or personal connection becomes a defining factor for forming preferences regarding certain values. Local and regional WTP values were compared to the social-related costs of both a local, and a national ecological evaluation project. The analyses showed that different WTP evaluations yielded very different results, and that actual rehabilitation costs can be defined via different characteristics for various projects.

Keywords: *ecological evaluation, willingness to pay, ecosystem service, natural resources, water preservation*

Abbreviations:
CVM – Contingent Valuation Method
HCSO – Hungarian Central Statistical Office
WTP – Willingness to Pay

Introduction

Our research aims to economically evaluate a certain domestic sample area via methods applicable to converting natural resource capital to monetary values. The focus of our research is the revitalisation project of the Szabadság Island side branch of the Danube, in Mohács town. The goal of the research is to obtain information about the more widely interpreted results and social effects of the rehabilitation conducted mainly for environment protection reasons. The project from the very beginning was expected to have the surplus advantages of various social-economic benefits apart from the environmental perspective. This research aims to capture these benefits figures, two years after the project has concluded, by which the state of the environment becomes interpretable and analysable in economic terms. We also aim to examine if the locals' efforts to sustain the rehabilitated environment - without state intervention - may be successful in the next ten years.

The definition of *natural capital* is used differently by authors with varied approaches, yet often these are close to each other, and overlap. The source of the

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definition is said to be the usage of the basic term of economics, "capital", for nature (Boros, 2004; Brand, 2009; Teeb Foundations, 2010). Many argue that the definition was intended as a metaphor in the beginning, which could define the finiteness of physical and biological resources on Earth, also interpretable by economics (MA, 2005; Norgaard, 2010). However, the economics analogy holds true if we define natural capital as a stock which can create, and supply a constant flow of valuable products or services for society (Costanza, Daly, 1992; Bateman et al., 2006). A real economic valuation builds on the biophysical understanding and goals to measure people's preferences for the benefits from ecosystem processes. These advantages or benefits may accrue to different categories of population over different geographical and time scales (Pavan, 2008).

We may consider the evaluation a process of information compression, which can embrace the different attributes of any given object or definition into a collective, and by extension, comparative attribute, the *value* (Vatn and Bromley, 1994; Van Zanten et al., 2014). Economics usually handles this one-dimension value measurement indicator in the form of the price, defined by the market, and in money, which is determined during market transactions, along the preferences of market actors. Living nature, and its ecosystem services are usually public goods and quasi-public goods however, which don't have a market. This lack of markets - not including some well-defined capital elements – makes it difficult to define the natural capital's value (Turner et al., 1994; Koopman et al., 2015). The most significant challenge of today is to economically evaluate the ever-decreasing bio-diversity (Nijkamp et al., 2008). Evaluating the results of already realised projects - together with actual budget numbers - should be integrated into the protocol of WTP methodologies.

Materials and Methods

WTP analysis with contingent valuation

To translate into figures the effects changes in natural resources have on human welfare, the most used stated preference method is the Contingent Valuation Method (CVM for short). This method can translate both the usage-related, both the non-usage-related elements of total economic value to numbers (Pearce and Moran, 1994; Marjainé, 2005). An important benefit is that the possible errors and inconsistencies were unearthed during the three-decade development of the methodology, which can be accounted for, and corrected by implementing proper techniques. The NOAA Committee, organised in the USA evaluated the up-to-date CVM practice in 1993, and found the contingent valuation to be an applicable ecological evaluation method (Arrow et al., 1993). Their recommendations have since integrated into the methodology, to correct known hardships and setbacks it imposed.

Our survey was conducted on Mohács town sample and on a national one. So it required two different questionnaires on these levels. The opinions of Mohács town inhabitants were collected via face-to-face questionnaires in autumn 2014. The sampling was conducted on public areas of Mohács town (total population of the town was 19,000 heads in 2014), by asking easy to approach participants. The national questionnaire was strictly online. The national level questionnaire was filled out by 96 people in autumn 2014, everyone for their own will. Naturally, this sample number on its own isn't enough to draw general conclusions for the entire country's inhabitants, but this part of the research never intended to create a strictly representative sample.

We wanted to work with a sample which may help us in understanding the opinions of people living farther from the area in question. Cross-referencing our data with that of the Hungarian Central Statistical Office, we came to understand that the sample is representative for the entire population of Hungary in terms of gender ratio. As for the other indicators in the sample, the younger generation, those who underwent higher education, active workers, inhabitants of the capital, and those living in larger households are over-represented. Based on the HCSO data, the net average income per capita is about 1.5 times higher than the national average.

The local and national questionnaires were identical, apart from minimal changes needed due to different levels of knowledge on the area. A difference was that possible answers were pre-determined, instead of allowing free wording of their answers.

We were interested in the analysis of locals' and non-locals' willingness to pay basically because international case studies came up with significant differences between the two values before. This is natural, however, since locals consider environment protection projects more important, meaning they may possibly offer higher amounts for them, regardless of income (Boddington, 1993; Bateman et al., 2004). Meanwhile, the decision to offer money of those living farther from the location showed connection with the level of income.

Questionnaire

Questionnaires are useful in cases when we need data which can be statistically processed, and generalised. As for WTP analyses, this is a mandatory requirement, as we're not exclusively interested in indicators, which we can request from statistical databases (Koetse and Brouwer, 2015). The reason is that the basis of willingness to pay analyses is the personal questioning of people, during which we offer them an opportunity to estimate the value of nature for them in monetary terms. Therefore, even though WTP analyses are not really widespread in the scientific fields due to their inherent complications, they become the sole method of measuring the opinions of inhabitants in monetary units. It helps with decreasing the top-down nature of state interventions in long-term, and including society in decision-making gets a higher possibility (Van den Bergh and Botzen, 2015). Using questionnaires, we collected the opinions of the inhabitants of Mohács town in autumn 2014, as part of a contingent valuation analysis. Parallel to this, we conducted a national online questionnaire data collection, with the same content. Therefore, local and national WTP data became comparable, and possible to evaluate. In order to understand the economic value of WTP data as well, we conducted analyses on similar projects in different areas of the country as a reference check, and compared our calculated WTP value with the actual cost of the submitted rehabilitation project.

Results

Analysing the willingness to pay in Mohács town

When the local questionnaire was concluded, the sample consisted of 51 people. The primary results can be seen in *Table 1*. We can see that only about half, 26 people have a positive willingness to pay, who would offer 17,45 EUR on average for sustaining the local ecology.

***Table 1.** Non-cleaned results of Willingness to Pay in Mohács town (n=51)*

Indicator	**WTP**
Frequency, WTP>0	26 (51%)
Average WTP	17,45 EUR
Deviation	49,5 EUR
Median	3,3 EUR
Minimum (WTP>0)	3,3 EUR
Maximum	333,3 EUR
Modus (WTP>0)	6,6 EUR, 16,6 EUR

Naturally, we were also interested in what the negative and positive nature of willingness to pay are in connection with, therefore, we also asked our participants to state their reasons. 65% of those who were willing to contribute with money to the local environment protection goals chose to reason with 'conservation'. And in case of negative willingness to pay, the most frequent answer was the low income of the household (28%). Furthermore, 20% was a significant number, whereby people answered that public funds should cover such projects.

However, based on processed literature, we could see how primary results may contain contradictions and extreme values in many cases. The former would list cases such as someone begins the questionnaire by writing that they consider contributing to the area's nature conservation - meaning they consider it valuable - but by reaching monetary contribution, their answer given is 0. To reach valid values, we cleaned our sample of such cases, and also excluded the highest value, the 333,3 EUR contribution, after which we worked with a sample number of 44. *Table 2* shows the actual WTP value.

***Table 2.** Valid WTP values in Mohács town*

	Average WTP	**Median**	**Median; WTP for >0**
Total number of answers (n=51)	17,45 EUR	3,3 EUR	26 (51%)
Cleaned of extremities (n=44)	12,65 EUR	6,6 EUR	25 (57%)
Ratio of Total and Cleaned	72%	200%	112%

Another instance of literature highlights the phenomenon of the so-called "embedding effect". This means that though we analysed the opinions related to a given project (Szabadság Island), people are prone to generally interpret environment protection goals - or the given geographical area - thereby giving a different value in terms of their willingness to pay. This is why we need filter questions, by which we can refine the stated values by narrowing them down to the project and its geographical location. *Table 3* illustrates the valid WTP values, and their relation to the previously introduced base WTP values.

Table 3. *"Valid" WTP values cleaned of extremities, and the "embedding effect" in Mohács town (n=44)*

	Base WTP	**Valid WTP**	**Ratio of Valid and Base WTP values**
Frequency, WTP>0	25 (57%)	18 (41%)	72%
Avg. WTP	12,65 EUR	7,42 EUR	72%
Deviation	21,5 EUR	18,93 EUR	88%
Median	6,6 EUR	0 EUR	-
Minimum (WTP>0)	3,3 EUR	3,3 EUR	100%
Maximum	120 EUR	120 EUR	100%
Modus (WTP>0)	6,6 EUR, 16,6 EUR	16,6 EUR	-

The chart clearly shows that in the end, merely 41% of our valid sample number was willing to offer money to support the side branch we analysed, while the others would offer their contributions for more general environment protection goals, and other areas of the Danube. The next most important indicator - being the average amount of contributions - also changed, by 28%, which means a statistically significant value. This means that the primary data has to be cleaned, since the final results are quite different. Therefore, we also conducted the cleaning of data we gathered from the national level analyses.

Estimation of the national willingness to pay

The focus of the national level was also to economically evaluate the Szabadság Island project of Mohács town in order to determine how the local and global interpretation of an environment protection project differ from each other. Furthermore, the two samples' different values may be important for us, since we should select indicators which cause these differences. The results generated from out 96 sample number obviously can not be considered as a general conclusion for the entirety of Hungary, we were merely interested in the opinions of those living farther from the project area from an orientation perspective. Following the construction of the local evaluation, we began with absolute data seen in *Table 4*.

Table 4. *Non-cleaned results of Willingness to Pay in the national sample (n=96)*

Indicator	**WTP**
Frequency, WTP>0	35 (36%)
Avg. WTP	6,52 EUR
Deviation	14,5 EUR
Median	0 EUR
Minimum (WTP>0)	3,3 EUR
Maximum	100 EUR
Modus (WTP>0)	16,6 EUR

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The difference is quite significant, since of the non-resident we asked, only 36% have a positive willingness to pay, and the value of WTP is significantly lower as well. The reason behind the positive values results from a simple intention to conserve (74%), similarly to how it was in the local sample. However, 15% of the previous participants said that they are active users of the area, which obviously does not surface in this sample. Those who would not aid the cause of the side branch due to problematic income are again, similar in number (23%). However, those who said that these projects should be paid for by public funds were more in this sample (36%). This is another difference which may be related to geographical reasons, since in case of projects which they are not directly involved in, residents tend to reason that the state should have a bigger role.

We continued by filtering our sample with our previously used method for the valid WTP values (*Table 5*), during which the sample was reduced by 18. However, this time, the values did not really change that much.

Table 5. *Valid WTP values in the national sample*

	Avg. WTP	**Median**	**WTP for >0 frequency**
Total number of answers (n=96)	6,52 EUR	0 EUR	35 (36%)
Cleaned of extremities (n=77)	6,84 EUR	0 EUR	34 (44%)
Ratio of Total and Cleaned	105%	-	122%

However, filtering the embedding effect created a much higher turbulence in the results, since we can see that the valid WTP value became less than half of the original. Most of the participants who were willing to pay up to this point clearly stated that their contribution is meant strictly for general environment protection goals, and merely 20 people said that they would finance the Mohács town project explicitly (*Table 6*).

Table 6. *"Valid" WTP values cleaned of "first" values, and the "embedding effect" in the national sample (n=77)*

	Base WTP	**Valid WTP**	**Ratio of Valid and Base WTP values**
Frequency, WTP>0	34 (44%)	20 (26%)	59%
Avg. WTP	6,84 EUR	2,84 EUR	42%
Deviation	11,74 EUR	6,73 EUR	57%
Median	0 EUR	0 EUR	-
Minimum (WTP>0)	3,3 EUR	1,66 EUR	50%
Maximum	66,6 EUR	33,3 EUR	50%
Modus (WTP>0)	16,6 EUR	16,6 EUR	-

Therefore, the conclusion from the data collected during via the questionnaire clearly shows how people think about the same environment protection initiative locally and non-locally, after which we will look at the connections behind their answers. This is required because the value and geographical spread of the WTP value can not offer sufficient interpretative strength in and of itself, despite our ability to draw general conclusions. This is due to the possible importance of some qualitative indicators like knowledge of the area for the national sample, or the usage of the area for the local sample. Finally, one of the defining values of such an analysis - most notably in Central and Eastern Europe - is the state of income in general, and analysing contributions in relation to it. Therefore, we have to continue by finding the indicators, that proved to have a significant connection related to the contribution offers.

Connections between willingness to pay, and independent variables

During our analysis, we searched for connections between willingness to pay, and descriptive variables. We evaluated demographic, and cognitive- and attitude attributes, and cross-referenced them with the resulting WTP values. In the following part, we will introduce the matches where we found significant connections. Out of the independent variables, we used the answers to *sex, age, has children and people in the household* as they were in the questionnaire's answers. Other variables were derived from the answers we got for the questionnaire.

To understand connections between the frequency of positive willingness to pay, and nominal variables, we used a cross-tabulation, where the strength of the correlation was measured using Cramer's V. To evaluate individual correlations between nominal variables and the WTP sum, we used the Eta-statistics. The correlation between the interval scales and the WTP values, we used correlation calculations, and used linear regression for the income per capita in the end. *Tables 7* and *8* illustrate the results of the statistical analysis. These charts only hold the variables where at least weak correlation was found, and significant values were labelled with a *.

Table 7. *Correlation between Willingness to Pay, and relevant variables for the Mohács town sample*

Mohács town sample (n=44)		**Base WTP**		**Valid WTP**	
		Frequency (Cramer's V)	Sum (Eta / correlation coefficient)	Frequency (Cramer's V)	Sum (Eta / correlation coefficient)
Demographic variables	Age	-	-0,208	-	-0,130
	Sex (M=1, F=2)	-0,315**	-0,318*	-0,289*	-0,284
	Size of household	-	-0,216	-	-0,155
	Active earner in household	0,188	0,164	0,331**	0,158
Cognitive variables	Direct usage important	0,435*	0,188	0,221	0,234
	Had prior knowledge	0,229	0,175	0,370**	0,267*

** p < 0,05, and * p < 0,1

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Table 8. *Correlation between Willingness to Pay, and relevant variables for the national sample*

National sample (n=77)		**Base WTP**		**Valid WTP**	
		Frequency (Cramer's V)	Sum (Eta / correlation coefficient)	Frequency (Cramer's V)	Frequency (Cramer's V)
Demographic variables	Has higher education	0,107	0,153**	0,142	0,152**
	Size of household	-	-0,302**	-	-0,251**
	Has children	-0,273**	-0,245**	-0,304**	-0,268**
Cognitive variables	Positive attitude to environment	0,190*	0,200	0,019	0,062
	Visited Danube-Drava National Park	0,242**	0,283**	0,094	0,190*
	Had prior knowledge	0,246**	0,370	0,153	0,238
Income	Income of household by category[1]	0,262	0,262	0,227	0,123
	Income/capita[1], regression coefficient	-	0,012**	-	0,006*

** p<0,05 and * p<0,1

[1] Analysed with exclusion of Don't know / No data answers for income, n=66

We can see that the indicators which effect the results are different for the two cases. The demographic and cognitive variables are different due to the different geographical areas. Since the amount of usage may be important locally, meanwhile, even knowledge on the area may prove lacking nationally. Also, it is not surprising that the income indicators are only present in the national sample, since it is natural that while those living far away from the project will only offer contributions based on their income, locals might find it important enough to disregard that to certain levels. Since this indicator only proved important in case of the national analysis for determining the WTP value, it shall be the first one to be evaluated.

Income and willingness to pay

The question related to income had an 85% share for the national level online questionnaire. Both questionnaires' results showed that those who gave a positive offer, the average income of the household, and the average income per capita were higher, but only the national sample yielded a significant difference. In the case of average income per capita, the national questionnaire had a difference of 30%, both from the perspective of the basic, and the valid WTP.

In the selection by income categories, the frequency of positive offers for the national sample followed the increase in income (but not significantly). However, in Mohács town, income did not influence the frequency of the positive offers. The national sample showed a weak, but significant positive correlation between income per capita, and amount offered as contribution, both in the base WTP, and the valid WTP. The linear regression coefficient was 0,012 for the base WTP, while it was 0,006 for the valid WTP, meaning a 3,3 EUR increase in income yields a 0,02 EUR increase in contribution.

However, the descriptive strength of the indicator is low, as the values of the R^2 were 0,082 for the base WTP, and 0,051 for the valid WTP respectively. *Figure 1* shows the dot graph and the linear graph.

The lower than expected effect of the income may be explained by how people would spend only a small part of the household's total annual income for this purpose. The national sample's value was 0.02%, while the Mohács town sample's was 0.09.

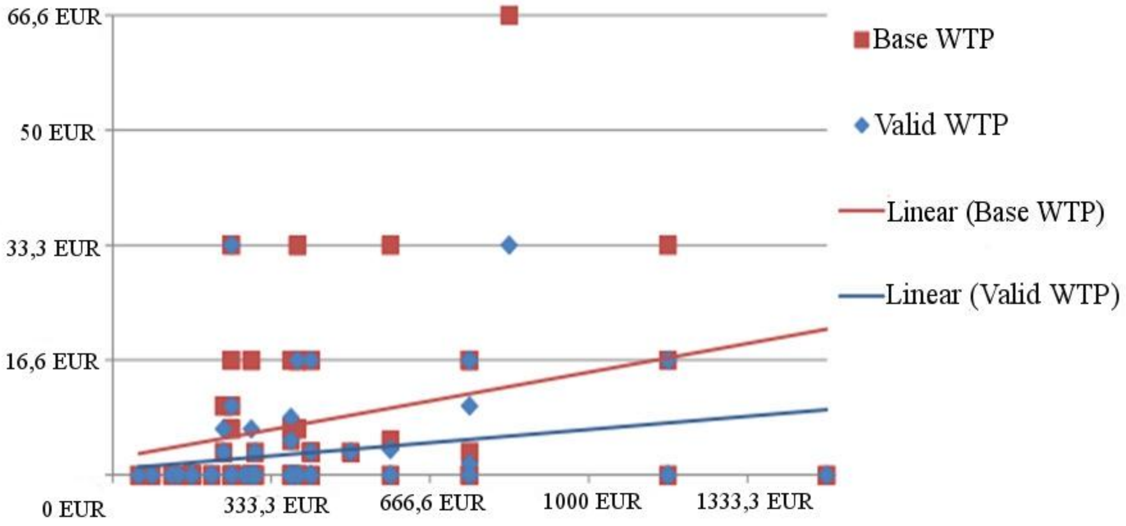

Figure 1. *Income per capita and WTP in the national sample (n=66)*

In Mohács town, neither the income per capita, nor the total income shows correlation to the WTP. However, out of all the indicators related to the monetary welfare of the household, the economic activity (active earner in household) had a significant correlation with the frequency of positive offers. Two-thirds of the participants living in an economically active household made positive offers, while the same could be said about 33% of the economically inactive households' members. The first WTP offer of active earners was more than 3,5 times more than that of households with no active earner (*Figure 2*). In the case of valid WTP, in households where no active earner lived, the contribution offers were always zero. The latter can also be said for the national sample.

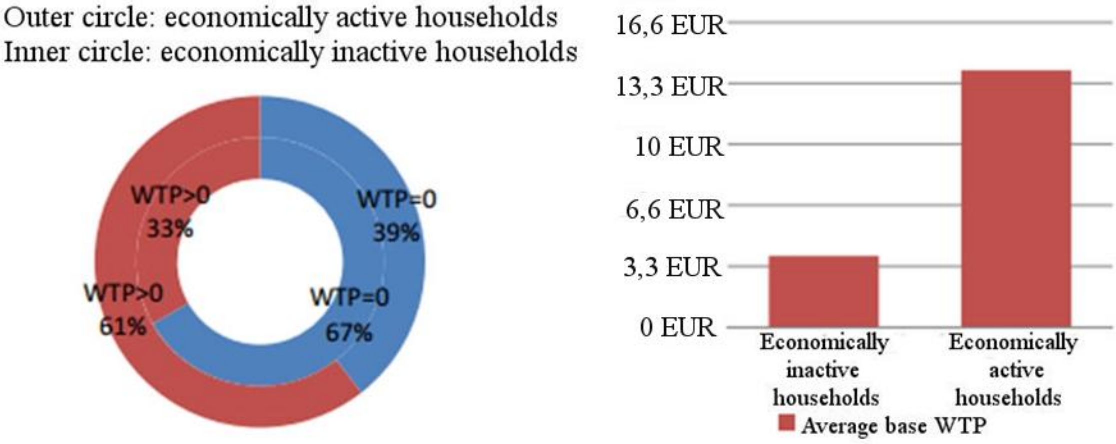

Figure 2. *Base WTP and economic activity in Mohács town (n=44)*

Correlation between demographic indicators and WTP

The Mohács town sample showed significant correlation between the WTP value, and the sex of the answering participant. Men had double the frequency of positive contribution offers compared to women, while the average of the WTP sum was four times as much. This may be explained by the significant difference in prior knowledge between sexes (71% of men, 37% of women). Therefore, the two sexes probably gave different offers due to different attitudes towards the area, stemming from different knowledge of said area.

The national sample showed significant difference between those with a higher education, and those without one. 29% of those with a diploma would offer contributions to protect the side branch, while only 13% of those who do not have a diploma. The ratio of the average WTP sum was four times as much. As we can observe in general trends, higher education and income also had a positive correlation within the sample. Without further analysis, we can not say if the higher WTP resulted from the denser knowledge and social responsibility coming with higher education, or if it is a result of a higher income. An interesting result is how the national sample yielded significant differences between participants with and without children. While nearly two-thirds of the former would pay for the side branch, only one out of eight of the parents said the same. Looking at the average WTP sum, the difference is four times, between households with and without a child. Contrary to this, the fact that a household has a child, or has none held no significance in the Mohács town sample.

Correlation between cognitive variables and the WTP

As expected, positive willingness to pay was in the strongest correlation with the prior knowledge about the area. In the Mohács town sample, both the basic WTP and the ones following the filter questions yielded significantly higher amounts of positive offers from those who knew the sample area, or visited the side branch before. The ratio with the average offer is twice for the basic WTP, and five times for the valid WTP. The national questionnaire also yielded similar results: those who heard of the case before were more willing to offer contributions, and higher amounts.

Yet another positive effect was if the participant visited the Danube-Drava National Park before, where the Szabadság Island can be found. This group gave an average 3,94

EUR / household / year in the national sample, while those who never visited the national park would only pay 1,37 EUR. Though the income of those who know the national park is higher in general, the difference is slim, which is why the higher WTP should be attributed to positive attitude, rather than differences in income.

Basically everyone in the Mohács town sample visited the protected Szabadság Island, barely a few kilometres from the city, which is why this variable is not relevant. We also found that the connection with environment protection was positive for both the Mohács town and the national samples, but the correlation was too weak, and non-significant between the offered contribution and the environmental attitude.

We evaluated the correlation between willingness to pay, and direct usage for the Mohács town sample as well. Out of the questions related to TEV (total economic value) elements' importance, only the recreational usage question's answers (on a 1-4 scale) were cross-tabulated with the WTP frequency, and the sum of contributions, which yielded a weak positive correlation in the end.

Comparison between results of the Mohács town and national samples

In accordance with prior expectations, the contingent valuation's results show that the Szabadság Island area had much more value for the inhabitants of Mohács town, than for those who live in other parts of Hungary. This became apparent in the frequency of positive offers, and the amount of contribution offered as well. The participants of the Mohács town sample gave an offer higher by an average of 85% for the first question than those living farther. After the filter questions, they were less frequent to change their offers, which means that their WTP value for the side branch was more than 2,5 times as much as the national value (*Figure 3*).

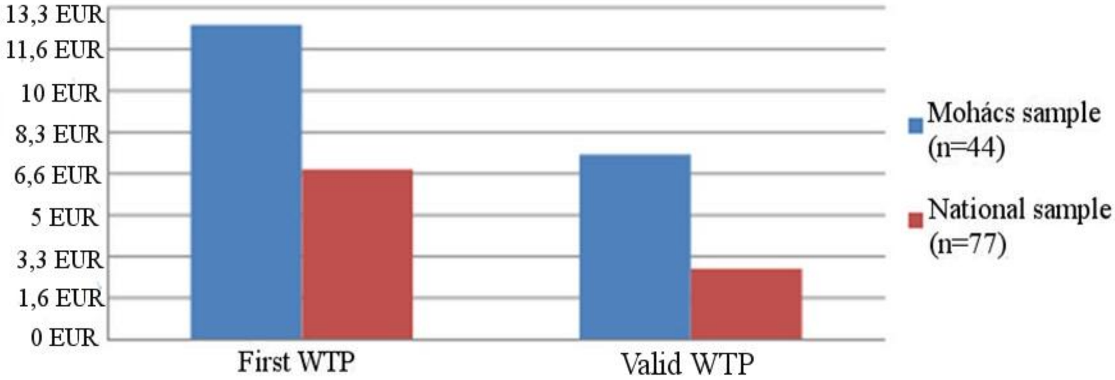

Figure 3. *Average willingness to pay for Mohács town and national samples*

The closer relationship of the locals to the area's natural values - which also appears in their willingness to pay - clearly aligns with the data from literature (Barton et al., 2011). However, the most important factors in support do not differ: both questionnaires yielded that participants selected the love for nature and the Danube, and conservation for future generations as the most notable factor. Therefore, the CVM method made it possible to evaluate at least partially the values unrelated to usage as well.

The third most notable reason in the national sample was to contribute to a good cause, while in the Mohács town sample, the opportunity for direct usage. Furthermore, every second participant of the Mohács town sample said that they had either partial, or complete prior knowledge of the Szabadság Island's history, while the ratio of this in the national sample was merely 12%. This was the factor with a significant effect on willingness to pay, apart from personal connection. The summarised values of the two questionnaires can be seen in *Table 9.*

Table 9. *Cross-referencing WTP and independent variables in the Mohács town and national samples*

	Mohács town sample (n=44)	**National sample (n=77)**	**National and Mohács town sample ratios**
Base WTP>0	57%	44%	77%
Valid WTP>0	41%	26%	63%
Avg. base WTP	12,65 EUR	6,84 EUR	54%
Avg. valid WTP	7,42 EUR	2,84 EUR	38%
Ratio of base and valid avg. WTP	59%	42%	71%
Minimum WTP	3,3 EUR	1,6 EUR	50%
Maximum WTP	120 EUR	33,3 EUR	28%
Avg. income of households	703,7 EUR	1047,62 EUR	149%
Avg. income per capita	270 EUR	416,5 EUR	154%
Prior knowledge	50%	12%	24%

Aggregating the willingness to pay

We aggregated the results of the local and national questionnaires, for both the inhabitants of Mohács town, and those of Hungary. Then, we summarised the two results. During the aggregation, we only included households with at least one earner, and calculated with the value corrected with this. Our resulting, one-year aggregated WTP was further re-calculated for a ten-year period, since the WTP question clearly asked for "contributions annually, for the next ten years". We used a 2% inflation and a 3% social discount value for the ten-year present value calculation. Our resulting values can be seen in *Table 10*, where we can clearly see that the average WTP value of the Mohács town sample is 2,6 times as much as the national value.

Table 10. *Aggregated values of the willingness to pay*

	Average valid WTP (EUR)	**Households with active earners (num)**	**Total WTP annually (EUR)**	**10-year sum on present value (EUR)**
Mohács town	7,42	4410	32.737	265.425
National (w/o Mohács town)	2,84	2.532.012	7.190.914	58.302.649
Sum	-	2.536.422	7.223.651	58.568.073

Marjainé and associates (2009) recommend three correctional points for the evaluation of rehabilitation of water bodies, with value transfer. These are the importance of the water body in question, the income of the inhabitants, and the direct usage. The income can be found in official databases, which means that it is useful not only to modify the results for value transfer with, but during primary analyses as well, as it can be used to rebalance non-representative sampling. If the participants of the sample have a much higher income than average, the average WTP can be corrected with the difference in income. The income and WTP were only in significant correlation in the national sample, therefore, the correction was only applied to the national value. The income per capita calculated from the HCSO database and the income per capita for the sample showed difference, hence the average WTP was modified with a regression coefficient. The modified aggregated results can be seen in *Table 11.*

Table 11. *Aggregated values of willingness to pay, modified by income*

	Average valid WTP (EUR) modified with income	**Households with active earners (num)**	**Total WTP annually (EUR)**	**10-year sum on present value (EUR)**
Mohács town	7,42	4410	32.737	265.425
National (w/o Mohács town)	1,99	2.532.012	5.038.704	40.852.912
Sum	-	2.536.422	5.071.441	41.118.337

If we look at willingness to pay as an interval, the value modified with income can be considered as the lower border value, and the original value as the upper border value. This means a 5.038.704 EUR – 5.071.441 EUR annual welfare increase for the entire Hungarian nation if the Szabadság Island side branch's rehabilitated state is sustained. The present value of this calculated for ten years is 40.852.912 – 41.118.337 EUR. Our contingent valuation's results suggest that the rehabilitated state of the side branch is worth at least this much to the entire society.

Cross-referencing local and national values with results of other research

We chose Marjainé's (2011) similar research as the reference to compare our local WTP values with, where she researched the willingness to pay of inhabitants in smaller areas with extreme water conditions related to local water management. *Figure 4* shows the cross-referencing of these three areas' average willingness to pay, and our own research results.

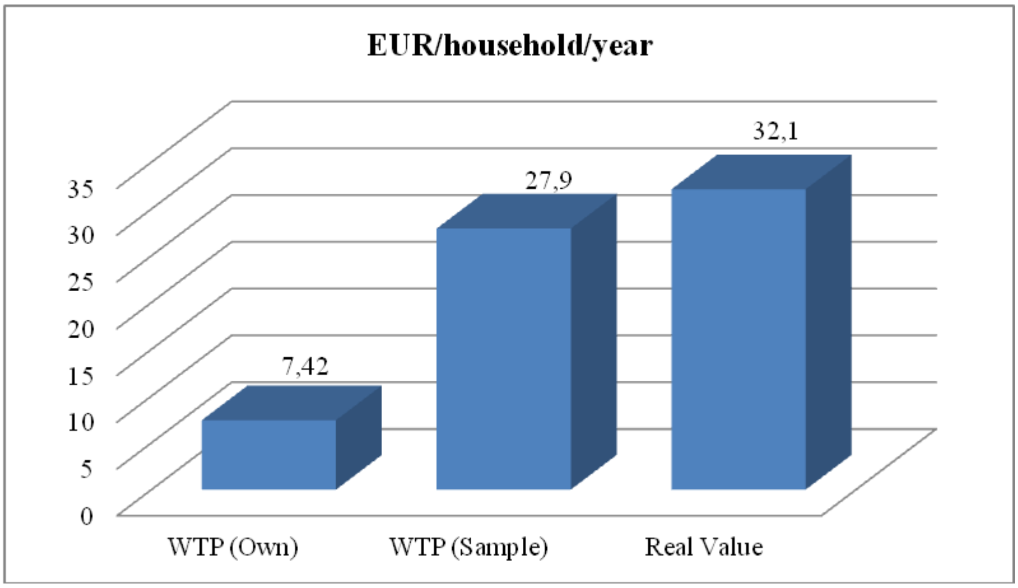

Figure 4. *Evaluation of the local results*
Source: Self-made, based on Marjainé (2011) and our own research

In the first column of the illustration [WTP (Own)], we can see that the inhabitants of Mohács town significantly undervalued the project compared to other local initiatives [WTP (sample)], where the locals would have paid higher contributions to make the initiative a success. The second column shows the willingness to pay of the three areas which were used as a reference basis. Finally, the third column shows our calculations of the actual costs of the side branch restoration project, which ran from 2009 to 2013, on future value. This shows what is the amount the households of Mohács town would've had to pay for 10 years, in order to cover all costs. If we also take this into consideration, we can say that the natural resources are quite underrated in our area of analysis compared to other Hungarian examples, and to the amounts spent.

To evaluate the results of the national questionnaire, we used the 2009 study "*Economic Valuation of Environmental and Resource Costs and Benefits in the Water Framework Directive: Technical Guidelines for Practitioners*" by Aquamoney, which measured the attitudes of the member states' inhabitants of the European nations towards water management and environment protection projects. The methodology of the research was based on the intent to unearth what amount of money the countries' inhabitants are willing to pay for the national water quality to be raised to 'ecologically good'. Their research resulted in that a Hungarian household's annual willingness to pay for this is 50 EUR each (Brouwer et al., 2009). If we include that Hungary's various streams are 52.335 km long in total, we can see that the Hungarian society is willing to pay 0,0009 EUR for the protection and conservation of a km of stream. During our analysis, we saw how the idea that locals value their own environment more than those living farther away holds true. However, in light of this data, we can see that the project realised in the Mohács town area (3,5 km long) was significantly overrated, since the average 1,99 EUR contribution offer of households amounts to 0,56 EUR for each km (*Figure 5*).

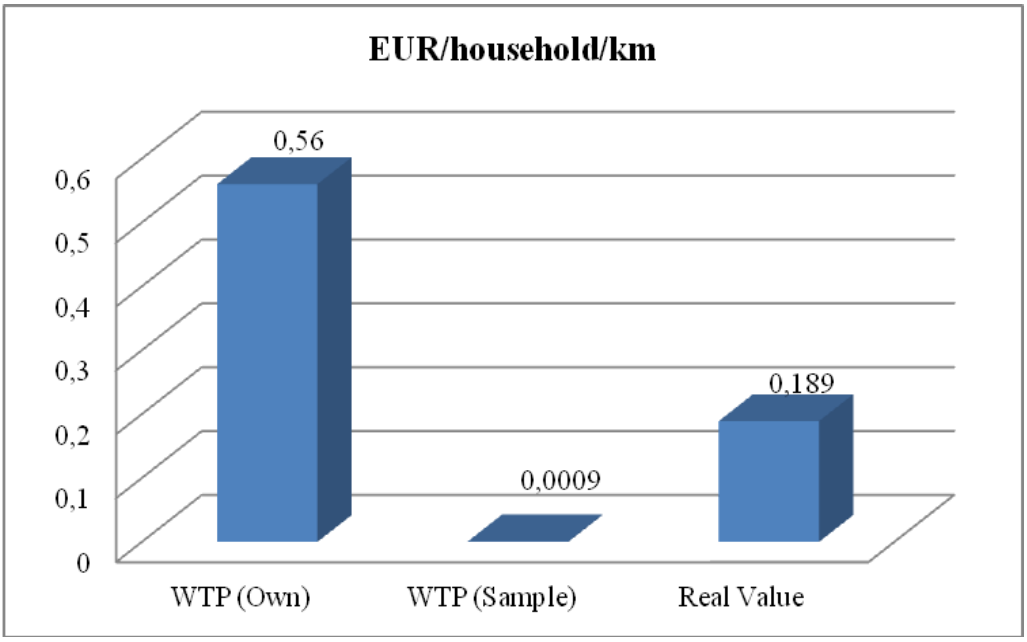

Figure 5. *Evaluation of the national results*
Source: Self-made, based on Brouwer et al. (2009) and our own research

The illustration shows out own national results with the WTP (Own) label, while the WTP (Sample) shows Aquamoney's project reference. Furthermore, based on the actual costs of the project, we once again looked at what Hungarian households would've had to be willing to pay to finance the initiative from their contributions. We have to state that in this case, we are not talking about a 10 year contribution process, but a single amount. And based on this, we can see that the national contributions observed during the course of our evaluation were enough to cover the project's actualisation multiple times.

Discussion

At this point, our research yielded an interesting result, since we were able to see our hypothesis hold true in the first round, meaning the locals were much more generous with the monetary evaluation of the rehabilitation, than those who lived farther away. The overview of literature, and the comparison with actual costs however showed that while the project proved underrated locally, the national willingness to pay would have generously financed the actualisation process with excess.

The research of the sample area proves that monetary environment evaluation methods are applicable to prove how valuable related people consider a given natural area. Based on the research, it became apparent that the locals may interpret their known natural resource up to twice and a half as valuable as any other unknown areas. We can assess a welfare increase for the entire society stemming from natural resource conservation or rehabilitation, which is in line with the value definition of the neoclassic economics. However, based on the literature, we should separately handle two of the many limits of the evaluation methods. One is that nature's value may only be expressed in monetary terms when in contact with humanity, as a human's perceived usefulness is something that can be translated to figures. Nature’s intrinsic values, unrelated to human society, its own value for itself stays unreachable within the boundaries of economics. The other is that values estimated through costs or hypothetic markets usually don't offer enough of a basis for correcting disadvantages of the lack of market, which is shown by different WTP values calculated for similar areas. Furthermore, to properly compensate for the ecosystem's services in a market framework and thereby rearranging negative development tendencies, we don't have well-working structures in the European policies for now.

However, based on our conducted analyses, we can say that:

- WTP methods and result elements should be corrected with details, which also represent actualised costs in similar projects,
- when talking about the results, we have to stress that the number of answers, in other words, the number of the sample is a significant factor of representativeness, but references to the driven process of the analysis, in other words, the actual, experience-based data also have to have proper weight during questioning,
- WTP answers also clearly show how actual costs and related social groups' environmental requirements may show great differences, which may question the validity of WTP.

According to the analyses, we can state that it is advised to add unit definitions to related costs during WTP questionnaires, because the results may contain significant errors otherwise, and therefore serve as a basis for wrong assumptions. In order to make adaptation possible, getting to know the mechanisms already working in market economies overseas based on paying for the ecosystem services is advised. The contingent valuation conducted for both the local and national sample clearly shows that people consider things more valuable if they know them. Therefore, one key to conserving natural capital is to develop knowledge related to it, and to spread knowledge to decision-makers and laymen about it. If decision-makers and citizens/consumers would know the mechanisms and resulting benefits of healthy natural systems, we'd need a shorter step to integrate this information into daily economic and political decisions' operating logic. This is why evaluating nature, and introducing its resources should get a bigger share of general coverage.

REFERENCES

[1] Arrow, K., Solow, R., Portney, P., Leamer, E., Radner, R., Schuman, H. (1993): Report of the NOAA Panel on Contingent Valuation. – US Federal Register 58 (10): 4602-4614.

[2] Barton, D. N., Lindhjen, H., NINA, Magnussen, K., Sweco, N., Holen, S., NIVA (2011): Valuation of Ecosystem Services from Nordic Watersheds. Nordic Council of Ministers, Copenhagen. doi: 10.6027/TN2012-506

[3] Bateman, I.J., Cole, M., Cooper, P., Georgiou, S., Hadley, D., Poe, G.L. (2004): On visible choice sets and scope sensitivity. – Journal of Environmental Economics and Management 47: 71–93. doi: 10.1016/S0095-0696(03)00057-3

[4] Bateman, I.J., Day, B.H., Georgiou, S. and Lake, I. (2006): The aggregation of environmental benefit values: Welfare measures, distance decay and total WTP. – Ecological Economics 60 (2): 450-460. doi: 10.1016/j.ecolecon.2006.04.003

[5] Boddington, M.A.B. (1993): Financial and Economic Measurement of Environmental Factors. – Journal of the Institute of Water and Environmental Management 7: 125-133.

[6] Brouwer, R., Barton, D., Bateman, I., Brander, L., Georgiou, S., Martín-Ortega, J., Navrud, S., Pulido-Velazquez, M., Schaafsma, M., Wagtendonk, A. (2009): Economic Valuation of Environmental and Resource Costs and Benefits in the Water Framework

Directive: Technical Guidelines for Practitioners. – Institute for Environmental Studies, VU University Amsterdam, the Netherlands.
[7] Boros, T. (2004): Két új ökológiai fogalom: a „természeti tőke" és a „környezeti fenntarthatóság" értelmezése és alkalmazása gyakorlati problémákra. – Műszaki információ. Környezetvédelem 9-10: 3-23.
[8] Brand, F.S. (2009): Critical natural capital revisited: ecological resilience and sustainable development. – Ecological Economics 68: 605-612. doi: doi:10.1016/j.ecolecon.2008.09.013
[9] Costanza, R., Daly, H.E. (1992): Natural capital and sustainable development. – Conservation Biology 6: 37–46.
[10] Koetse, M.J., Brouwer, R. (2015): Reference Dependence Effects on WTA and WTP Value Functions and Their Disparity. – Environmental and Resource Economics 61: 1-23. doi: 10.1007/s10640-015-9920-2.
[11] MA (2005): Millennium Ecosystem Assessment, 2005. Ecosystems and Human Well-being: Synthesis. – Island Press, Washington.
[12] Marjainé Sz.Zs. (2005): A természetvédelemben alkalmazható közgazdasági értékelési módszerek / Economic evaluation methods in the nature protection practice – Ministry of Environmental and Water Management, Special Edition, Budapest, 2005
[13] Marjainé Sz.Zs. (2011): Az ökoszisztéma-szolgáltatások közgazdaság-tudományi megközelítése / Economic aspects of ecosystem service and management. – Magyar Tudomány 172 (7): 788-795.
[14] Nijkamp P., Vindigni G., Nunes, P. A. L. D. (2008): Economic valuation of biodiversity: A comparative study. – Ecological Economics 67: 217-231. doi: 10.1016/j.ecolecon.2008.03.003
[15] Norgaard, R.B. (2010): Ökoszisztéma szolgáltatások – Hogyan vált egy szemléletes metafora a lényeg elhomályosítójává? – Kovász 15 (1-4): 61-92.
[16] Koopman, J.L., Kuik, O.J., Tol, R.S.J. Brouwer, R. (2015): Water scarcity from climate change and adaptation response in an international river basin context. – Climate Change Economics 6 (1): 1-22. doi: 10.1142/S2010007815500049
[17] Pavan, Sukhdev (2008) The economics of ecosystems and biodiversity. European Communities, Banson Production, Cambridge UK, 2008 p. 33 web: http://ec.europa.eu/environment/nature/biodiversity/economics/pdf/teeb_report.pdf
[18] Pearce, D., Moran, D. (1994): The Economic Value of Biodiversity. – IUCN, Earthscan Publications Ltd., London.
[19] TEEB Foundations (2010): The Economics of Ecosystems and Biodiversity Ecological and Economic Foundations. – Earthscan, London and Washington.
[20] Turner, R. K., Pearce, D., Bateman, I. (1994): Environmental Economics: An elementary introduction. – John Hopkins University Press, Baltimore.
[21] Van den Bergh, J.C.J.M., Botzen, W.J.W. (2015): Monetary valuation of the social cost of greenhouse gas emissions. – Ecological Economics 114: 33-46. doi: 10.1016/j.ecolecon.2015.03.015
[22] Van Zanten, B.T., Verburg, P.H., Koetse M.J., Van Beukering, P.J.H. (2014): Preferences for European Agrarian Landscapes: A Meta-Analysis of Case Studies. – Landscape and Urban Planning 132: 89–101. doi: 10.1016/j.landurbplan.2014.08.012
[23] Vatn, A., Bromley, D.V. (1994): Choices without Prices without Apologies. – Journal of Environmental Economics and Management 26 (2): 129–148. doi: 10.1006/jeem.1994.1008

WILLINGNESS TO PAY FOR SUSTAINABLE WATER USAGE IN HARRAN PLAIN-GAP REGION, TURKEY

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Abstract. The purpose of this study is to evaluate factors affecting farmers' willingness to pay (WTP) for sustainable water usage in terms of efficient irrigation training in the Harran Plain-Turkey and determine the coefficients, and affecting factors that contribute to it. The data used in this study come from a sample of 22,167 farmers in the Harran Plain, 675 of them were chosen via simple random sampling method and interviewed face to face by given questionnaires. Sampling was conducted in all water user associations' areas in 2014. The logistic regression and probit model were used for analysis. The results indicate that the average WTP was \$170.6 and yearly amount was \$3,781,690 for irrigation training in Harran plain. Farmers believe in the necessity of irrigation training for sustainable usage and accept for payment by 59% and WTP of the farmers' may further increase with good practices. Explanatory affecting factors, such as age, education level, land amount, ownership type, modern irrigation method and offered amount for training were significantly explained WTP and coefficients were determined. So, the probability of payment acceptance for irrigation training could be written for any farmers in Harran plain. This study is first of its type in GAP Region-Turkey.

Keywords: *sustainable water usage, irrigation training, willingness to pay, Harran Plain, Turkey*

Introduction

Water insufficiency is confronted with the problem in terms of quality and quantity based on many reasons such as mismanagement, unawareness, climate change and drought, growing population, urbanization, industrialization, agricultural irrigation and varying usage areas. The efforts for improving living standards of human beings are at the core of all problems related to ecology and environment. These actions also constitute the biggest threat of water resources. Water cannot be considered just as H_2O (Malt, 1994) and need more care, conservation and protection for today and future demands from all related parties in an integrated way. A reliable, adequate and sustainable water usage has become necessity for all nations at least for food safety. In this respect, water management has been one of the most important issues for decision makers to take into considerations for today and future needs.

Southeastern Anatolian Project (GAP, in its Turkish acronym) is a multi-sectorial regional sustainable development projects that is mainly based on soil and water resources. GAP is the largest investment for regional development in the history of Turkey and fourth largest irrigation project in the world (Miyata and Fujii, 2007; Yurekli, 2015). Sustainable development includes efficient multifunctional agriculture, using environment-friendly and saving technologies and attention to quality and a

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socially acceptable rural development, simultaneously and managed in harmony with the production and protection (Várallyay, 2010). The scope of the GAP is to eliminate regional disparities among the other regions and increase living standards of regions people. Within the GAP's scope, there are 22 dams, 19 hydroelectric power plants and irrigation of 1.822 million hectares (ha) of agricultural land (GAP, 2012). Harran Plain is the research field that has 150,000 ha of irrigation areas. Irrigation has begun first time in Harran plain within the scope of GAP at 1994. It is located at southeast part of Turkey at 375 meters of altitude that is the lowest altitude locations in the GAP. The average precipitation is between 300-365 mms and annual evaporation is 1,848 mms (State Meteorology Works, 2011).

The most water consumption in the world takes place in agricultural irrigation (Johansson et al., 2002) and consuming around 70% (Grafton and Hussey, 2011). The agricultural irrigation in Turkey is 72.27% among water usage by sectors (Aydogdu et al., 2015a). Water is the most important input in agriculture. Agricultural irrigations are important for sustainable farming and income for farmers who are aware that it is not possible to get a significant income from farming without irrigation. There is an increasing pressure on efficient use of water in irrigation. These pressures are based on the principles of efficiency and the use of modern saving techniques in irrigation. In this way, both water and land resources are taken into consideration for sustainability. Modern irrigation technology may cause increase in the production, while reducing the amount of water used. On the other hand the use of modern irrigation techniques are related to many factors such as age, education, number of households, experience, ability, knowledge, land size, income, and water fee. Modern irrigation has positive effects at reduction of payments to be made depending on the amount of used water and also to the problem of salinization which can occur in arid and semi-arid climate. There is a statistical significance between payment and used water amount to age, land amount, experience and education variables in Harran plain (Aydogdu et al., 2014a).

It is essential to use efficient irrigation technologies such as pressurized systems for water and soil savings. There are drainage problems due to improper irrigation methods that is mainly furrow irrigation in Harran plain (Yenigun and Aydogdu, 2010) due to excessive water usage. The Imambakir water user association (WUA) is located in the lowest altitude of the Harran plain where groundwater level is high, intensive salinity is observed around Bozyazi and Tuzluca areas due to excessive irrigation and results to significant yield losses (Aydogdu et al., 2014b) and salt effected area is around 10% in the plain (Cullu et al., 2002). Salinity is one of the major problem of the world that affects 20% of total irrigated areas (Ghassemi et al., 1995), 23% of total cultivated land has been degraded by salinity (Tanji, 2002) and affecting an area of 800 million ha that is equal to 6% of the total land area globally (FAO, 2008). Salinity affects plant growth and causing yield losses, and also adversely affects soil structure, fertility and soil life (Howari et al., 2002; Mohammed et al., 2008). Salinity decreases cotton yield 29.6% in Harran Plain (Cullu, 2003) and has led to a 1,840,625 kg cotton yield losses in a year, the resulting income loss was $935,711 in 2009 in the Akcakale, within GAP-Harran Plain (Aydogdu et al., 2014c). On the other hand, climate change and droughts have increased the importance of water and its usage by farmers. There are already water shortages in some parts of the plain mainly because of misusage and mismanagement. According to the results of the two studies, farmers have willing to pay 71.69% more than the existing price under certain conditions for irrigation water (Aydogdu, 2016) and 2.23 fold of current price in case of water shortages (Aydogdu et al., 2016) in Harran plain.

In order to save soil and water resources, training is necessary. If a suitable combination of crop pattern and irrigation methods can be supported through training programs for farmers, water efficiency, productivity and effectiveness can be increased significantly in the field (Aydogdu et al., 2015b) and results to sustainable natural resources. In this study, it is intended to measure probability of acceptance and the impact of the selected parameters on irrigation training both, in terms of the, willingness to accept and WTP of farmers for sustainable soil and water resources.

Materials and methods

The main material of this research comes from a sample of 675 farmers among 22,167 in the Harran Plain who were chosen via a simple random sampling method. There are 22 WUAs in the plain. Sampling was conducted in irrigation season of 2014 and interviewed face to face by given questionnaires. Within this scope, all the WAUs were visited in the Harran Plain. To maximize the reliability of the results, villages that represent the every WUA were purposefully selected, and local interviewers were used.

A particular theoretical market was formed based on question that was asked for acceptance of given payment amount on a yearly basis, regarding irrigation training either from public or private sector for safe and sustainable water usage to farmers. The payment amount is randomly selected for each questionnaire, starting with 100 Turkish Liras (TL, 1$=2.19 TL and 1€=2.91 TL at surveyed time, Anonymous, 2014), changing the rate of multiple of 50 TL, increasing to 1000 TL. The answer will be either yes or no to this question. Thus, acceptances of farmers and coefficients of effective factors by means of analysis are determined. Then, probability of payment acceptance could be written according to the obtained coefficients of variables for any farmers. Lastly, the regression analysis was applied to the scenarios in order to see the effects of improvements to be made on selected parameters in probit model.

SPSS and limited dependent variables (LIMDEP) package programs were used in analysis. Logistic regression, Odds, Omnibus, Cox and Snell R Square, Nagelkerke R Square, Wald test, Hosmer-Lemeshow fit tests, probit model and regression analysis were conducted. Logistic regression uses maximum likelihood (ML) estimation in multiple regressions. The general form of the distribution is assumed. Starting values of the estimated parameters are used and the likelihood that the sample came from a population with those parameters is computed. The values of the estimated parameters are adjusted iteratively until the ML value for the estimated parameters is obtained. That is, ML approaches try to find estimates of parameters that make the data actually most likely observed. Odds ratios in logistic regression can be interpreted as the effect of a one unit of change in the predicted odds ratio with the other variables in the model held constant. An important property of odds ratios is that they are constant. It does not matter what values the other independent variables take on (O’Halloran, 2015). It is defined as division of the probability of an event to the probability of not being event.

A Wald test can be used in a great variety of different models including models for dichotomous variables and models for continuous variables (Harrel, 2001). The test is used to determine whether a certain predictor variable is significant or not. Such as; constant term in the initial model, standard error for constant terms, degree of freedom, the level of significance and exponential logistic regression coefficient. The test consists of dividing the value of the coefficient by standard error. Omnibus test is implemented on an overall hypothesis that tends to find general significance between parameters' variance, while examining parameters of the same type. They test whether the explained variance in a set of data is significantly greater than the unexplained variance, overall.

There is not R-squared statistics in logistic regressions that is similar to the regression analysis. In SPSS, there are two modified R-squared values: one developed by Cox and Snell that never reaches 1 and the other developed by Nagelkerke. The correction increases the Cox and Snell version to make 1.0 a possible value for R-squared by modified it by Nagelkerke. These values indicate the amount of variance explained by the model. The Hosmer-Lemeshow fit test is designed to correct and use when there are discontinuous and continuous predictors, at the same time and not recommend the use of this test when sampling size less than 400 (Hosmer and Lemeshow, 2000). Sample size was 675 in this reserach. This test is performed by dividing the predicted probabilities into deciles and then computing a Pearson chi-square that compares the predicted to the observed frequencies. Lower values and insignificances indicate a good fit to the data and, therefore, good overall model fit.

These tests and analysis are explained more detailed at Maddala (1983), Takeshi (1985), Gujarati (1995; 2006), Hosmer-Lemeshow (2000), Harrell (2001), Thampapillai (2002), Greene (2003), Anonymous (2005), Stigler (2007), Kalaycı (2014), Anonymous (2015) and O'Halloran (2015).

Results and Discussion

Because of the region's patriarchal family structure, all survey respondents were male. The total amount of cultivated land, including second crop, was 10,294 ha in the surveyed area, and 52.1% of the farms are 10 ha or smaller. Of the producers, 64.7% farm only on their own land, and 35.3% own their farm land in addition to other rented lands and/or partnerships. 81.6% of lands are located in the gravity irrigation, and 18.4% are located in the pumping irrigation area. Cotton was the main crop by 51.9%, followed wheat by 30.4%, corn by 11.3% and other crops by 6.4%. The average income from agricultural activities was calculated as 32,925 TL/year and 2,159 TL/ha. The descriptive statistics of the model is given in *Table 1.*

Table 1. *Descriptive statistics of model*

Variables	Definition	Mean	Std. Deviation
AGE	Age of farmers (year)	46,13	10,635
EDUCTN	Education level: if literacy:1, primary school:2, secondary school:3, highschool:4, university graduated:4	2,66	1,134
EDYEAR	Years passed in education	6,84	3,916
MARITAL	Marital status: If married: 1, if not: 0	0,97	0,222
HOUSHLD	Number of households	7,15	3,838
AGWORK	Number of households working at agriculture	3,52	2,437
NONAGWRK	Number of households working at nonagricultural sector	0,46	0,848
EXPRNCE	Experienced years in farming	25,37	11,001

LAND	Land amount (hectares)	15,25	22,759
OWNRSHP	Ownership status, if privately owned:1, if share holder:2, if renter:3, if at least two of them:4	1,93	1,249
INCOME	Yearly income based on agriculture (TL)	32924,52	54021,324
MDRNIRR	If modern irrigation user: 1, if not: 0	0,19	0,390
WTP	Offered amount for training (TL)	368,17	179,165
WTPANS	If the farmer accepts to pay for training: 1, if not: 0	0,59	0,493

Of the farmers who accept to pay (coded as WTPANS) for irrigation training was 59%, means 398 and who don't accept to pay was 41%, means 277. The classification table based on step 0 is given in *Table 2.*

Table 2. *Classification table of step 0*

Observed			Predicted		
			WTPANS		
			No	Yes	Percentage Correct
Step 0	WTPANS	No	0	277	0,0
		Yes	0	398	100,0
	Overall Percentage				59,0

According to first classification table all the respondents were classified in yes and percentage of verification in this case was 59%. The statistics of the variables are located in the initial model, step 0, and is given in *Table 3.*

Table 3. *Coefficients of variables in the equation*

		B	S.E.(Standard Error)	Wald	df	Significance	Exp(B)
Step 0	Constant	0,321	0,093	11,841	1	0,001	1,379

According to significance level in table 3, p<%5, all of the independent variables are making meaningful contribution. The Omnibus tests of model coefficients are given *Table 4.*

Table 4. *Omnibus tests of model coefficients*

		Chi-square	df	Significance
Step 1	Step	203,079	13	0,000
	Block	203,079	13	0,000
	Model	203,079	13	0,000

Chi-square test showed that it's statistically significant, p<%5. This significance shows that existing of a relationship between dependent variable that is acceptance of payment for irrigation training, and combination of independent variables. The model summary is given in *Table 5.*

Table 5. *Model summary*

Step	-2 Log likelihood	Cox & Snell R Square	Nagelkerke R Square
1	437,872	0,350	0,471

Cox and Snell and Nagelkerke R square values indicate the amount of variance explained by the model. The variance of acceptance of payment for irrigation training was explained 35% by Cox & Snell and 47.1% by Nagelkerke R square. The test result of Hosmer and Lemeshow is located in *Table 6.*

Table 6. *Hosmer and Lemeshow test results*

Step	Chi-square	df	Significance
1	4,264	8	0,833

The test was assessed the compliance of the logistic regression model as a whole and result indicated that it was insignificant (p> 0.05) means that existing of an adequate level of model-data fit. The classification table that was obtained from logistic regression result is given in *Table 7.*

Table 7. *Classification table at step1*

Observed			Predicted		
			WTPANS		
			NO	YES	Percentage Correct
Step 1	WTPANS	NO	192	85	69,2
		YES	71	327	82,1
	Overall Percentage				76,6

The percentage of verification was increased from 58% to 76.6% that indicates that variables make meaningful contribution to the model. Initially more variables were used in model, given in descriptive statistics, and significant ones were selected in terms of contribution to the model after the first trial and run again. The most appropriate multivariable that are age, education, number of nonagricultural workmanship in farmers family, land, ownership, income, modern irrigation users and WTP were selected in logistic regression model as variables and second run result of model is given in *Table 8.*

One unit effects on numeric variables, such as age, land, nonagricultural workmanship, income and WTP were measured. Categorical, nominal, variables such as education levels, ownership and modern irrigation usage, were compared with the reference variable in order to observe the differences between them. In education level, university graduated selected as reference, education one indicates literacy, education two indicates primary school, education three indicates secondary school, education four indicates high school graduated farmers. In ownership level, few selected as reference means that farmer has either two of them, privately own land, share holder and renter in farming. The ownership one indicates privately own land, ownership two indicates share holders and ownership three indicates renter.

Table 8. *Logistic regression model's variables in the equation*

		B	S.E.	Wald	df	Sig.	Exp(B)	95% C.I.for Exp(B)	
								Lower	Upper
Step1	AGE	0,023	0,013	3,165	1	0,075	1,023	0,998	1,049
	EDUCTN			12,806	4	0,012			
	EDUCTN(1)	-1,920	0,665	8,338	1	0,004	0,147	0,040	0,540
	EDUCTN(2)	-1,516	0,569	7,092	1	0,008	0,220	0,072	0,670
	EDUCTN(3)	-0,726	0,613	1,401	1	0,237	0,484	0,145	1,610
	EDUCTN(4)	-1,403	0,601	5,445	1	0,020	0,246	0,076	0,799
	NONAGWRK	0,269	0,167	2,610	1	0,106	1,309	0,944	1,815
	LAND	0,005	0,002	7,319	1	0,007	1,005	1,001	1,008
	OWNRSHP			6,574	3	0,087			
	OWNRSHP(1)	0,058	0,355	0,026	1	0,871	1,059	0,528	2,124
	OWNRSHP(2)	-0,388	0,489	0,630	1	0,427	0,678	0,260	1,769
	OWNRSHP(3)	1,053	0,550	3,659	1	0,056	2,866	0,974	8,427
	INCOME	0,000	0,000	2,379	1	0,123	1,000	1,000	1,000
	MDRNIRR(1)	-0,711	0,324	4,827	1	0,028	0,491	0,260	0,926
	WTP	-0,007	0,001	92,916	1	0,000	0,993	0,992	0,995
	CONSTANT	2,559	0,849	9,091	1	0,003	12,927		

Significant variables in the model results will be interpreted. Age is an important factor on human behaviors and perceptions. Age increases payment acceptance of the farmers. A unit increases in age has an effect on acceptance of payment by 2.3% in a positive way. There is a relationship between attitudes to water management and age of farmers according to a survey conducted in Harran Plain (Aydogdu et al., 2015a). Education has an effect on payment acceptance (p<5%). Literate farmers have 85.3%, primary school graduated farmers have 78%, secondary school farmers have 51.6% and high school graduated farmers have 75.4% less acceptance as compared to university graduated farmers. The results are statistically significant and meaningful. When education level is increasing, concerns about sustainable water usage thus payment acceptance is increasing, too. The vice versa is true, too. According to the studies that were conducted in Harran plain are also showed that there is a significant relationship between education levels and WTP of the farmers and attitudes to water management (Aydogdu, 2016; Aydogdu et al., 2016; Aydogdu et al., 2015a). People who are educated, they are more prone to learning and innovation (Değirmenci et al., 2012) and more sensitive about natural resources. Land amount has positive effect on payment acceptance (p<5%). A unit increases in land amount increases the acceptance of payment by 1%. The ownership type has an effect on payment acceptance. Land amount and status of ownership have effect on attitudes of the farmers in Harran plain (Aydogdu et al., 2015a). Land owners have 5.9%, share holders have 2.87 fold more and renters have 32.2% less acceptance as compared to the farmers who have more than one type of ownership. Rent agreement is usually done on an annual basis. There is no guarantee for rental to next year. So, renters have less payment acceptance, this is an expected result. In this regard there is a correlation between rental period and acceptance. It was stated by farmers that in case of long-term rent agreements, acceptance for payment will increase.

Modern irrigation users have less payment amount as compared to furrow irrigation users by 49.1% and it is statistically significant ($p<5\%$). These farmers are already using water saving technologies and have some skills about it. The WTP has negative effect on payment acceptance ($p<5\%$), this is expected result too. Farmers are considered more payment as a welfare loss. A research conducted by Aydogdu (2016) is also proved that there was statistical significance between irrigation type and WTP in Harran plain. There was not significant relationship between the number of nonagricultural workmanship in farmers' family and income to payment acceptance, but there was proximity. The result of nonagricultural workmanship in farmers' family might be acceptable, because these people are already source of income for their family by working nonagricultural sectors. But the result based on income was unexpected. Income is the main factor of being ability to pay. The probability of payment acceptance for water saving irrigation training for sustainable farming can be written according to the coefficients of variables at table 8 for any farmers. For example: the payment acceptance probability of logistic regression equation of a high school graduated farmer who has privately own land and already using modern irrigation system can be written as follows:

Ln p/(1-p)=2.559+0.023(age)-1.403(education level)+0.005(land amount)+0.058 (ownership)-0.711 (modern irrigation)-0.007(offered WTP amount)

In order to see the effects on variables in case of positive improvements on some selected variables such as age, land and offered WTP amount; a scenario model is applied to probit by regression. Randomly five different proportions of 10%, 25%, 50%, 75% and 100% improvements were made and the effects on the acceptance for payment were observed. Age of farmers was increased in the first scenario and their impacts were observed in *Table 9*.

Table 9. *The change in the age scenario of farmers*

Age is increased by 10%			
Result	Main Case	In Scenario Case	Change
0	277 (41.04%)	268 (39.7%)	-9
1	398 (58.96%)	407 (60.3%)	9
Total	675 (100%)	675 (%100)	0
Age is increased by 25%			
Result	Main Case	In Scenario Case	Change
0	277 (41.04%)	261 (38.67%)	-16
1	398 (58.96%)	414 (61.33%)	16
Total	675 (100%)	675 (%100)	0
Age is increased by 50%			
Result	Main Case	In Scenario Case	Change
0	277 (41.04%)	207 (30.67%)	-70
1	398 (58.96%)	468 (69.33%)	70
Total	675 (100%)	675 (%100)	0
Age is increased by 75%			
Result	Main Case	In Scenario Case	Change
0	277 (41.04%)	203 (30.07%)	-74
1	398 (58.96%)	472 (69.93%)	74
Total	675 (100%)	675 (%100)	0

Age is increased by 100%			
Result	Main Case	In Scenario Case	Change
0	277 (41.04%)	195 (28.89%)	-82
1	398 (58.96%)	480 (71.11%)	82
Total	675 (100%)	675 (%100)	0

The positive correlation between age of farmers and acceptance was observed. The WTP increases with increasing age of farmers. The graph of the changes that occur with age is likely to take place in *Figure 1.*

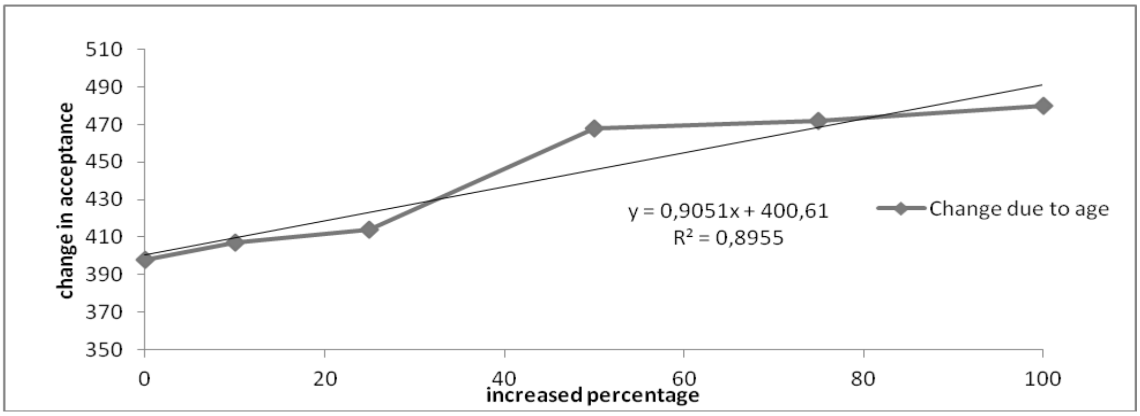

Figure 1. *The changes that occur due to age is likely to take place*

Responsibilities of older aged farmers are increasing. In order to fulfill the growing responsibilities it is needed more revenue. The main source of income for farmers is agriculture. Therefore, the farmer wants to get sustainable income from agriculture and look favorably at training to improve productivity of resources. Land of farmers was increased in the second scenario and their impacts were observed in *Table 10.*

Table 10. *The change in land scenario of farmers*

Land is increased by 10%			
Result	Main Case	In Scenario Case	Change
0	277 (41.04%)	272 (40.3%)	-5
1	398 (58.96%)	403 (59.7%)	5
Total	675 (100%)	675 (%100)	0
Land is increased by 25%			
Result	Main Case	In Scenario Case	Change
0	277 (41.04%)	261 (38.67%)	-16
1	398 (58.96%)	414 (61.33%)	16
Total	675 (100%)	675 (%100)	0
Land is increased by 50%			
Result	Main Case	In Scenario Case	Change
0	277 (41.04%)	197 (29.19%)	-80
1	398 (58.96%)	478 (70.81%)	80
Total	675 (100%)	675 (%100)	0
Land is increased by 75%			
Result	Main Case	In Scenario Case	Change
0	277 (41.04%)	185 (27.41%)	-92

1	398 (58.96%)	490 (72.59%)	92
Total	675 (100%)	675 (%100)	0
Land is increased by 100%			
Result	Main Case	In Scenario Case	Change
0	277 (41.04%)	178 (26.37%)	-99
1	398 (58.96%)	497 (73.63%)	99
Total	675 (100%)	675 (%100)	0

A positive correlation between land amount of farmers and acceptance was observed. WTP is increasing with increasing land amount of farmers. The graph of the changes that occur due to land amount increasing is likely to take place in *Figure 2*.

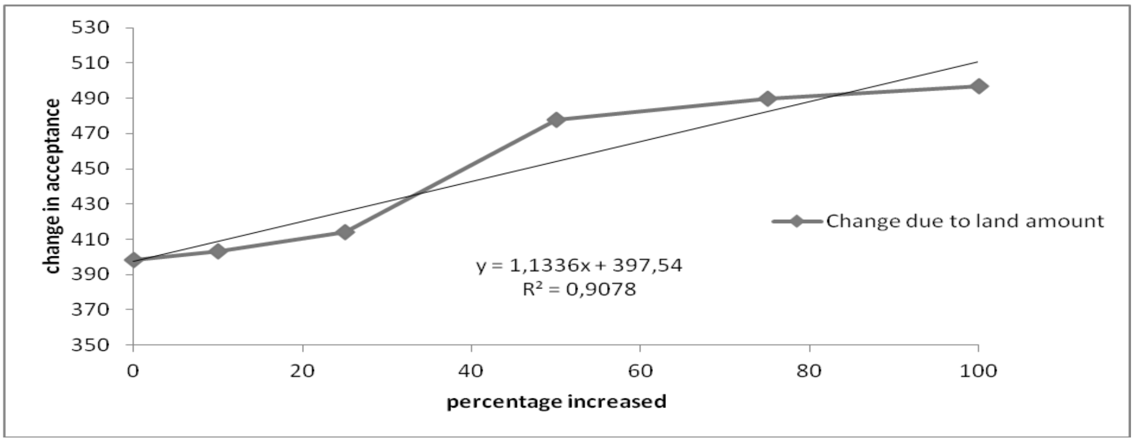

Figure 2. *The changes that occur due to land is likely to take place*

Depending on the increase in the amount of land, it will increase revenues to be derived from irrigated agriculture. When irrigation efficiency is achieved by training, both it will increase the revenues obtained from unit area and reduce the amount of water used. Both results will make positive contribution to farmers' welfare by means of additional income from land and reduce payment amount of water fee. The offered amount was increased in the third scenario and their impacts on WTP were observed in *Table 11.*

Table 11. *The change in WTP based on increased offered amount scenario of farmers*

Payment amount is increased by 10%			
Result	Main Case	In Scenario Case	Change
0	277 (41.04%)	285 (42.22%)	8
1	398 (58.96%)	390 (57.78%)	-8
Total	675 (100%)	675 (%100)	0
Payment amount is increased by 25%			
Result	Main Case	In Scenario Case	Change
0	277 (41.04%)	299 (44.3%)	22
1	398 (58.96%)	376 (55.7%)	-22
Total	675 (100%)	675 (%100)	0
Payment amount is increased by 50%			
Result	Main Case	In Scenario Case	Change
0	277 (41.04%)	374 (55.41%)	97
1	398 (58.96%)	302 (44.59%)	-97
Total	675 (100%)	675 (%100)	0

	Payment amount is increased by 75%		
Result	Main Case	In Scenario Case	Change
0	277 (41.04%)	464 (68.74%)	187
1	398 (58.96%)	211 (31.26%)	-187
Total	675 (100%)	675 (%100)	0
	Payment amount is increased by 100%		
Result	Main Case	In Scenario Case	Change
0	277 (41.04%)	476 (70.52%)	199
1	398 (58.96%)	199 (29.48%)	-199
Total	675 (100%)	675 (%100)	0

A negative correlation between increased offered amount and acceptance of the farmers were observed. The acceptance decreases with increasing offered amount for training. The graph of the changes that occur due to increased offered amount in WTP is likely to take place in *Figure 3*.

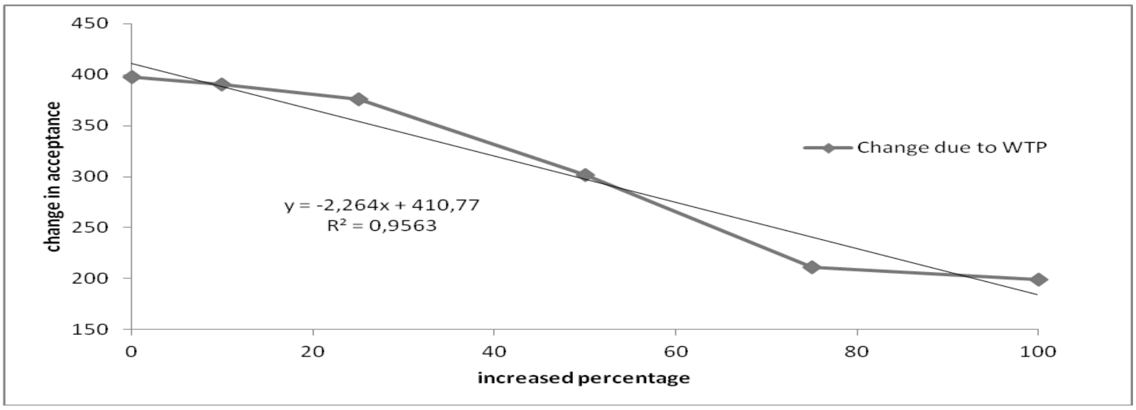

Figure 3. *The changes in WTP due to increased offered amount is likely to take place*

When the offered training fee is increasing, WTP is declining. Increased fee means to pay more for training, which will affect negatively the welfare of the farmers. The farmers do not want to pay more than their stated amount. More payment has negative effect on the farmers' budget. This is an expected result. Another research also concluded that increased price for payment, results to decrease in WTP of the farmers in Harran plain (Aydogdu, 2016).

Conclusions

The soil and water resources are not unlimited resources. The reduction and deterioration has begun in terms of the quality and quantity in natural resources. They need protection and conservation for sustainable environment. Sustainability of natural resources can be achieved with awareness, training and education. Agricultural extension and training activities have been implemented by the State in the form of public extensions in Turkey and still has major role in these services. Private sector has been involved to these services in recent times with a limited areas and staffs in Turkey. There is a trend to privatization of public services because of many reasons such as social, economic and political aspects; budget deficit, reducing public expenditures, inefficiency of public services and optimum use of resources in Turkey.

The average offered amount to pay was $240. The average accepted payment amount of farmers' is calculated as $170.6, which was 1.2% of their yearly income and means that farmers have ability to pay. The unaccepted average amount was calculated as $318. There were 22,167 farmers in Harran plain irrigation areas at surveyed time. Accepted payment amount of farmers' was calculated as $3,781,690 per year for irrigation training for efficient, effective and sustainable water usage for agriculture. According to the scenarios, age of the farmers has an effect by 12.15% and land amount by 14.67% on acceptance in a positive way. On the other hand, increased offered amount for training has an affect by 29.48% on acceptance in a negative way. There are already water shortages in some parts of the plain mainly because of drought, misusage and mismanagements. Water scarcity increases payment acceptance of the farmers for safe water. In this sense, locations of the farmers are important to receive safe water for sustainable usage and farming. If their fields located towards to donwstream of the plain, water constraints are likely to occur. So, such a training program will be accepted more in these areas. Aydogdu (2016) showed that location has an effect on WTP for the farmers in Harran plain. Training is necessary and should be given in the field by experienced and patient staffs before the irrigation season. It should not be expected from the farmers to understand everything which has been described and shown to them immediately. The education level of the farmers are not high in general and they use local languages, too. Therefore, the language, materials and contents of this training should be in a manner that would be acceptable, understandable and easily applicable by the farmers in the field. Farmers adopt innovations more quickly that coinciding with their experience. They prefer to see and try rather than listening. In this case, willingness to pay of the farmers' may further increase with good practices.

These results are meaningful and could be considered as guidelines for the public and private sectors' decision and policy makers for training of farmers about sustainable water usage. In this way, both natural resources will be protected as well as service provider could generate revenue that is simply win-win theory for all related parties. This study is the first of its type in GAP-Harran plain, Turkey.

REFERENCES

[1] Anonymous (2005): http://www.upa.pdx.edu/IOA/newsom/da2/ho_logistic3.doc Newsom, Data Analysis II, fall 2005. 12 Dec 2015
[2] Anonymous.(2014): web:http://www.isyatirim.com.tr/p_exchange_avarege.aspx, 31Dec 2014.
[3] Anonymous (2015): https://en.wikipedia.org/wiki/Omnibus_test/Wald_test. 12 Dec 2015
[4] Aydogdu, M.H. (2016): Evaluation of Willingness to Pay for Irrigation Water: Harran Plain Sampling in Gap Region-Turkey. - Applied Ecology and Environmental Research 14(1):349-365. Doi: http://dx.doi.org/10.15666/aeer/1401_349365
[5] Aydogdu, M. H., Karli, B., Aydogdu, M. (2015b): Evaluation of attitude of stakeholders for irrigation water management: A case study of Harran Plain, Turkey. - Journal of Environmental & Agricultural Sciences 4:42-47.
[6] Aydogdu, M. H., Karlı, B., Yenigün, K., Aydogdu, M. (2016): Evaluation of Farmers' Willingness to Pay for Water under Shortages: a case study of Harran Plain, Turkey. - Journal of Environmental & Agricultural Sciences 7:23-28.

[7] Aydogdu, M. H., Karlı, B., Yenigün, K., Manci, A. R., Aydogdu, M. (2014a): Pricing trends in agricultural irrigation; attitudes and perceptions of farmers to pricing; GAP-Harran Plain irrigations, Şanlıurfa. - The Journal of Academic Social Science Studies (29):165-188. http://dx.doi.org/10.9761/JASSS2529

[8] Aydogdu, M. H., Yenigun, K., Aydogdu, M. (2015a): Factors affecting farmers' satisfaction from water user associations in the Harran Plain-GAP Region, Turkey. - Journal of Agricultural Science and Techonology 17:1669-1684.

[9] Aydogdu, M., Aydogdu, M. H., Cullu, M. A. (2014b): The impact determination on cotton yields of soils which have different salt levels and its relation with satellite data (Şanlıurfa İmambakır Harran Plain Irrigation Association). - Turkish Studies 9(2):231-250. doi: 10.7827/TurkishStudies.6009.

[10] Aydogdu, M., Aydogdu, M. H., Cullu, M. A. (2014c): The determination of the cotton productivity and losses under the effect of salinity by using GIS and RS. (GAP Region, Akçakale Sampling) Turkey. - The Journal of Academic Social Science Studies 24:617-630. http://dx.doi.org/10.9761/JASSS2167.

[11] Cullu, M.A. (2003): Estimation of the effect of soil salinity on crop yield using remote sensing and geographic information systems. - Turkish Journal of Agriculture and Forestry 27:23-28.

[12] Cullu, M.A., Almaca, A., Sahin, Y. Aydemir, S. (2002): Application of GIS for monitoring soil salinization in the Harran Plain, Turkey. International meeting on sustainable land and management, Soil science society of Turkey. Canakkale.

[13] Değirmenci, Y., Manyaz, İ., Güzelaydin, İ., Erkuş, E., Koçak, F., Ari, B. (2012): Agricultural extension and consultancy. Gıda, Tarım ve Hayvancılık Bakanlığı, Eğitim, Yayım ve Yayınlar Dairesi Başkanlığı, Ankara. (In Turkish)

[14] FAO. (2008): FAO Land and Plant Nutrition Management Service. http://www.fao.org/ag/agll/spush (October 2008).

[15] GAP. (2012): GAP Administration, GAP'ta Son Durum, 2012, p.6-55 Sanliurfa, Turkey.

[16] Ghassemi, F., Jackeman, A. J., Nix, H.A. (1995): Salinization of land and water resources: Human causes, extent, management and case studies. - CAB International, Wallingford, Oxon, U.K.

[17] Grafton, Q. R., Hussey, K. (2011): Water Resources Planning and Management. - Cambridge University Press. New York.

[18] Greene, W. H. (2003): Econometric Analysis. - Prentice Hall, 1050p., New Jersey.

[19] Gujarati, D. G. (1995): Basic Econometrics. - Mc Graw-Hill Int Edit., 705p., NY, USA.

[20] Gujarati, D. G. (2006): Basic Econometrics. - Literatür Publication, Istanbul. (In Turkish)

[21] Harrell, F. E. Jr. (2001): "Sections 9.2, 10.5". Regression modeling strategies. - New York: Springer-Verlag .ISBN 0387952322

[22] Hosmer, D. W., Lemeshow, S. (2000): Applied logistic regression (2nd Edition). - New York: Wiley.

[23] Howari, F.M., Goodell, P.C., Miyamato, S. (2002): Spectral properties of salt crusts formed on saline soils. - Journal of Environmental Quality 31:1453-1461.

[24] Johansson, R. C., Tsur, Y., Roe, T. L., Doukkali, R., Dinar, A. (2002): Pricing irrigation water: a review of theory and practice. - Water Policy 4:173-199

[25] Kalaycı, Ş. (2014): SPSS applied multivariate statistical techniques. Asil Yayın Dağıtım. Ankara.

[26] Maddala, G. S. (1983): Limited Dependent and Qualitative Variables in Econometrics. Cambridge, 356 p., London.

[27] Malt, C. B. (1994): Water is not H_20. - Cognitive Psychology 27:41-70.

[28] Miyata, S., Fujii, T. (2007): Examining the socioeconomic impacts of irrigation in the Southeast Anatolia Region of Turkey. - Agricultural Water Management 8:247-252.

[29] Mohammed, S., Muller, T., Joergensen, R. G. (2008): Relationship between soil biological and other soil properties in saline and alkaline arable soils from Pakistani Punjab. - Journal of Arid Environments 72:448-457.

[30] O'Halloran, S. (2015): Sustainable Development U9611 Econometrics II. Lecture 10: Logistical Regression II— Multinomial Data p.5-8. 12 Dec 2015. http://www.columbia.edu/~so33/SusDev/Lecture_10.pdf

[31] State Meteorology Works. (2011): http://www.dmi.gov.tr/veridegerlendirme/il-veilceleristatistik.aspxm=Sanlıurfa Data evaluations 25 Sept 2015

[32] Stigler, S. M. (2007): The Epic Story of Maximum Likelihood. - Statistical Science 22(4):598-620, Doi:10.1214/07-STS249.

[33] Takeshi, A. (1985): Advanced Econometrics. Oxford: Basil Blackwell. ISBN 0-631-13345-3.

[34] Tanji, K. K. (2002): Salinity in the soil environment. pp.21-53, Kuwer Academic Publishers, Dordrechet, Boston, London.

[35] Thampapillai, D. J. (2002): Environmental Economics: Concepts, Methods, and Policies. - Oxford Univ. Press, pp. 73-91, Oxford.

[36] Várallyay, G. (2010): Role of Soil Multifunctionality in Sustainable Development. - Soil & Water Resources 3:102–107

[37] Yenigun, K., Aydogdu, M. H. (2010): Evaluation of Irrigation and Drainage Systems of GAP, the Turkey's Largest Integrated Water Resource Development Project. - Scientific Researches and Essays 5(21):3237-3253.

[38] Yurekli, K. (2015): Impact of climate variability on precipitation in the Upper Euphrates-Tigris Rivers Basins of Southeast Turkey. - Atmospheric Researches 154:25-38.

DOI: http://dx.doi.org/10.15666/aeer/1403_147160

THE COMPARISON OF THE POTENTIAL EFFECT OF CLIMATE CHANGE ON THE SEGMENT GROWTH OF *FRAXINUS ORNUS*, *PINUS NIGRA* AND *AILANTHUS ALTISSIMA* ON SHALLOW, CALCAREOUS SOILS

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Abstract. Growth patterns of different plant species are primarily determined by edaphic factors, climate conditions and their species-specific adaptation properties. Changing sub-regional aridity trends due to the projected climate change like soil erosion and the invasion of alien plant species threaten the ligneous vegetation of karst areas. We aimed to study and model the potential effect of aridity on the growth rate of young individuals of the native *Fraxinus ornus* and its two important competitors, the exotic *Pinus nigra* and the aggressive invader *Ailanthus altissima* in a karstic forest-steppe of the Veszprém plateau, Hungary on different soil depths. Mean soil depth and the Thornthwaite agrometeorological index were used as covariates. Climate data were gained from the E-OBS gridded dataset for the period of 1950 to 2013 and from the MPI Echam5 climate model for the period of 2081 to 2100. We found significant correlation between the soil depth values and the measured heights and the average of monthly Thornthwaite agrometeorological indices. In conclusion, aridity and soil-depth have significant, but different effect on the growth patterns of the studied species. While the annual growth season of *Pinus nigra* and *Fraxinus ornus* are determined by the aridity of the months of January to May, and from February to June, respectively, the growth of *Ailanthus altissima* is mainly determined by the period of March to August. The climate prediction-based growth model predicts the decline of the growth patterns of each species for the 2081-2100 period in Hungary due to climate change.

Keywords: *Fraxinus ornus, Pinus nigra, Ailanthus altissima, climate change, Thornthwaite agrometeorological index, aridity*

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Abbreviations: The used tree and stand abbreviations were based on the symbols of International Union of Forest Research Organizations (IUFRO) standardization (van Soest, 1965) except the values which were given in cm-^{2} based values adjusting to the young age of the stands and some new value was introduced: the standardized annual stem segment growth (hs), the the annual segment height (ha), the calculated total height of the same age trees (hc) and the measured total height of trees of similar age (hm). The following abbreviations were used in the text: ha: annual stem offshoots growth, hs: standardized annual stem segment growth, hc: the calculated total stem height, hm: the measured total stem height, t: age from individual [years], Sm: mean soil dept, P: monthly sum of precipitation [mm], T: monthly mean temperature [°C], r: the growth-soil depth coefficient, TAI: Thornthwaite agrometeorological index [mm °C^{-1}].

Introduction

The protection of the natural karst vegetation against the alien plant species is one of the most important elements of the conservation for preserving integrity of the soil cover on carbonate bedrocks and the karst aquifer systems. Recently, the native vegetation is threatened by invasive species like the tree of heaven, *Ailanthus altissima* (Mill.) Swingle (Simaroubaceae). Some methods have been developed to control of the invasive *A. altissima* recommending the use of pathogens as weevils, insects, fungi or webworms (Ding et al., 2006). The substitutability of the tree-of-heaven during the changing climate with other plant species as e.g. *Fraxinus ornus* L. (Oleaceae) has not been numerically analyzed. *F. ornus* is a characteristic, widespread native ash species of the natural vegetation on dolomite and limestone karst of the South-Central Europe and the Mediterranean (Kostova, 2001). Due to their significant drought and fire tolerance (Chiatante et al., 2006), *F. ornus* is an important species and codominate the low canopy with *Quercus pubescens* Willd. (Fagaceae) in the *Cotino-Quercetum pubescentis* association (Csontos et al., 2001) which is common in the studied karstland area of Veszprém. *Pinus nigra* Arnold (Pinaceae) is an exotic tree on the dolomite rocks of the Transdanubian hills and the closest natural occurrence is in the Vienna Basin, Austria, representing the northernmost occurrence of the species in Europe (Leal et al., 2008). The dolomite terrains of the Transdanubian Mountains were intensively afforested with *P. nigra* in the 20th century (Csontos et al., 1996). Occupying large areas on diverse dolomite rock grasslands, *P. nigra* plantations resulted in the local extinction of the original flora and fauna (Cseresznyés et al., 2006; Udvardy, 1998a) and is also known as a transformer species (Winkler and Tóth, 2012; Bódis, 1993). *Ailanthus altissima*, which is also a worldwide established transformer plant species (Udvardy, 1998b) is native to China, Taiwan and Northern Vietnam (Kowarik and Samuel, 2007; Ding et al., 2006). Although the members of the family of Simarubaceae are mostly tropical woody plants, *A. altissima* grows under several climatic conditions from humid or dry temperate to sub-tropical areas (Miller, 1990). As an invasive species, *A. altissima* occur in different habitats in the Mediterranean coniferous and broadleaved associations (Kowarik, 1983) and is a typical early-successional species in forest ecosystems (Robinson and Handel, 1993). Producing allopathic materials, *A. altissima* can suppress the growth of other angiosperms in their vicinity (Ding et al., 2006; Heisey, 1990). Although the fossil members of the *Ailanthus* genera are known from the Tertiary sediments of Hungary e.g. the *A. confucii* Unger form the Middle Miocene (Hably, 2001) and the *A. tardensis* Hably form Lower Oligocene (Corbett, 2004; Hably, 2001), the first specimens of *A. altissima* were planted in Europe only in the 1751 in France (Hu, 1979). *A. altissima* might have been introduced in Hungary in the late 18th or the early 19th century. According to Sárospataki (2014), the first individuals were planted in 1807 in Western Hungary, Keszthely then in 1814 in the archduke park of Sárvár. *A. altissima* has became the most important invasive species in the semi-natural forests, urban areas and dry deciduous woodlands in Hungary (Mihály and Botta-Dukát, 2004; Török et al., 2003; Udvardy, 1998b) as in many other areas of the world (Constán-Nava, 2010). *A. altissima* is a successful ligneous invader species on rock grasslands e.g. on the Szársomlyó Hill in the Duna-Dráva National Park in Southwestern Hungary (Erdős et al., 2005) and in the Aggtelek National Park in Northeastern Hungary (Váczi, 2001). It appears that *Pinus nigra* and *Ailanthus altissima* are the most important competitors of *Fraxinus ornus* in the natural rock grasslands and dry deciduous woodland habitats of Hungary. The rapid, athropogenic climate change has the potential to change the fitness of the native plant populations, and can trigger the area expansion of certain species (Huntley, 1991) dramatically altering their compositions (Jump and Peñuelas, 2005), and the degradation of the fragmented flora and fauna results in poor plant associations dominated by alien species (Gibbons et al., 2000). It is widely agreed that climate change will result expansion of many invasive species (Hellmann et al., 2008; Gritti et al., 2006; Beerling et al., 1995) as a consequence of elongation of the vegetation period, increasing winter temperatures and the increasing aridity. The process will restructure the natural vegetation with replacing natural elements by more warm-resistant, partly Mediterranean species.

Regional climate models predict an increasing aridity of the Carpathian Basin by the end of the 21st century particularly in the vegetation period (Pieczka et al., 2011; Bartholy and Gelybó, 2007; Bartholy et al., 2009). The studied Veszprém karstland has a continental climate with warm, dry summers and mild winters. The zonal vegetation is the transition of the forest and forest steppe zones. The average annual precipitation was 650 mm, the annual mean temperature was 9.4°C in the period of 1902-1950 (Ádám et al., 1987). During 1980-2009 the annual mean temperature increased by 1.3 to 1.4°C in the area. In 1960-2009 the annual precipitation decreased by -5- to -15 % (Bartholy et al., 2007). In the last three decades the number of days of mean temperature higher than 25°C increased by 6-8 days/year (Bartholy et al., 2007). Summer precipitation is very likely to decrease for the period of 2071-2100 by -25 to -30% according to IPCC SRES emission scenario A2 or -10 to -15% according to scenario B2 (Strenger et al., 2004). On the other hand, winter precipitation is likely to increase by 35% (A2) or 20% (B2) (Bartholy and Gelybó, 2007).

Model results showed that the rising decay frequency of *Pinus nigra* individuals in the last decades was a parallel change to the decreasing growth rate of pines due to the increasing aridity trend in the Veszprém karst plateau (Trájer et al. 2015). As drought inhibits growth (Chiatante *et al.,* 2006; Kramer and Turner 1980), in this paper, we investigate the effect of the sub-regional drought trends and the foreseen change of precipitation and temperature on the stem growth of young individuals of *Fraxinus ornus* and its two most important competitors: *Pinus nigra* and *Ailanthus altissima*. Our specific aims were: (1) to describe the temporal patterns of temperature and precipitation on the annual vertical growth of young individuals of the three species and (2) to model the past and predict their future vertical growth. We hypothesized that aridity in the growing period may have a great influence on the annual segment growth in the future. Since the more noticeable phase of the anthropogenic climate change started in the 1950's (King and Harangozó, 1998), we intended to model the whole period of the last six decades as well as the predicted future effect of climate change of the last two decades of the 21st century.

Materials and methods

Study site and and soil thickness measurements

The study was performed in the central part of the Transdanubian Highland in Hungary (*Fig.1A*). The bulk of the area built up mainly of Triassic carbonates which were formed on a tropical shallow carbonate platform (Balog *et al.,* 1997). The Triassic rocks of the Transdanubian Range belong to the Alpine Triassic type (Haas, 1992) which means that the analogies of the bedrock and the calcareous soils formed on the carbonates can be found in the entire Mediterranean Basin and the members of the Alpine orogeny as in the Alps (Goldhammer et al., 1990), in the Betic Cordillera in Spain (Martin et al., 1987). Genetically the karstic area of Veszprém belongs to the Transdanubian type which can be characterized by the significant affection of repeated tectonic movements and the faulting of the limestone and dolomite rocks into blocks (Bárány-Kevei, 2005). Due to the tectonic history, carbonate- and dolomite rubble-rich skeletal soils are characteristic to the area (Fodor *et al.,* 2005; Márton and Fodor, 2003). The bedrock of the shallow soils is hard, cemented dolomite and limestone breccias (*Fig.1B*). The thickness of the shallow, dolomite bedrock soil of the plant individuals were estimated by the averaging of four manual, perpendicular soil sounding measurements with an iron sounder from 30 cm of the basis of the stems in each case. The thickness of the soil was defined by the depths where the probe reached the firm bedrock carbonate rock. The soil thickness data were averaged in each case. Each of the studied tree individuals grows on well-lit, open area.

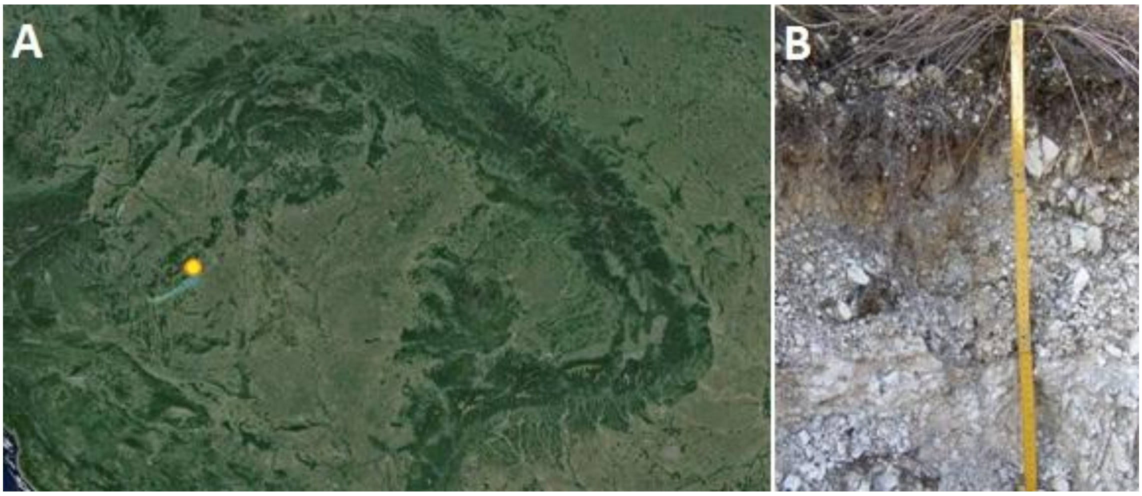

***Figure 1.** The studied karst region of the Carpathian Basin (yellow spot marks the place of Veszprém in the 'A' panel) and a typical section of a dolomite-based skeletal soil ('B' panel). The satellite picture was gained from the Google Earth^{TM} landscape visualization (Sheppard and Cizek, 2009).*

Measurement of the annual vertical growth of the species

The study was based on the measurement of 491 plant individuals. A total of 205 individuals of *Pinus nigra,* 140 of *Fraxinus ornus* and 146 of *Ailanthus altissima* were involved in the study. We measured the stem height and the corresponding soil thickness values of 81 individuals of *Pinus nigra,* the 40 of *Fraxinus ornus* and 63 of *Ailanthus altissima* and the annual stem segment heights of 124 individuals of *Pinus nigra,* 100 of *Fraxinus ornus* and 83 of *Ailanthus altissima*. The stem heights of the individuals were measured from the soil level to the top of the stem. The reliably measurable number of the years varies by species, according to our observations this number is more than 11 years in *Pinus nigra*, about 9 years for *Ailanthus altissima* and 7 for *Fraxinus ornus*. We determined the ‘segment’ as the annual vertical and radial internodes between the branching levels of two adjacent years in case of *Pinus nigra* and the vertical dimension of a segment was measured between the two adjacent levels of the last 11 years (2004-2013). In cases of *Fraxinus ornus* and *Ailanthus altissima* the conception of the annual segments were based on the annual internodes of the plants. Seven years (2007 to 2014) were determined in case of *Fraxinus ornus* and nine (2005 to 2013) in case of *Ailanthus altissima*. The measurements in case of *A. altissima* were partly based on root sprout clones. We measured the individuals on well-illuminated, open areas (*Fig.2A* and *2B*).

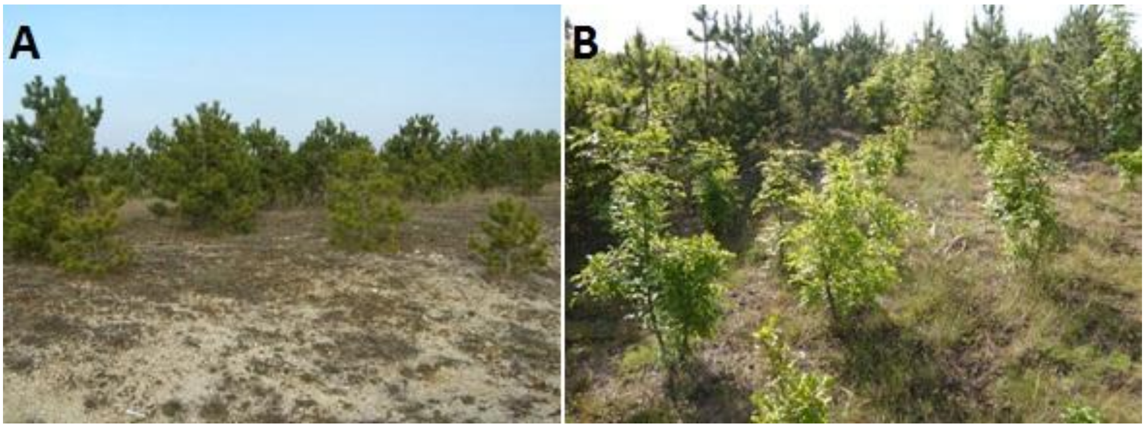

***Figure 2.** Characteristic landscape of the Pinus nigra (a) and Pinus nigra-Fraxinus ornus mixed plantations (b) in the studied sites.*

We standardized the annual segment growth values of the plants to eliminate the individual differences e.g. due to the different soil thickness, the age of the trees as the segment lengths were linearly regressed in case of each specimen and then the yearly residuals were averaged. The standardized annual stem segment growth (h_s) was defined as the difference from the growth trend of the annual stem segments of the specimens (*Fig.3A and 3B*).

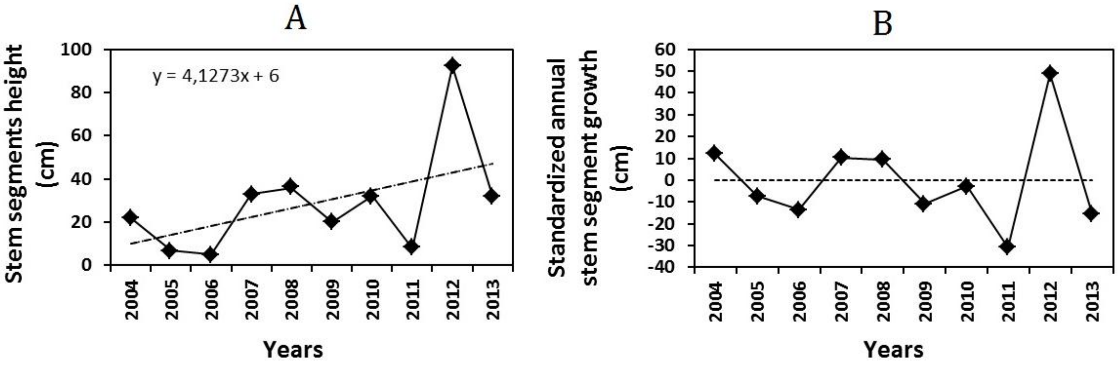

***Figure 3.** Example for the calculation of the used standardized annual stem segment growth (h_s): the annual stem segment height of a Pinus nigra individual with the equation of the growing trend (3A) and the differences from the trend (3B).*

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Climate data and data processing

The monthly mean daily surface air temperature and the monthly sum of daily precipitation data of the observed period were acquired from the E-OBS gridded dataset (source: E-OBS database 2014) for 0.25° grid resolution. The latitudinal expansion was 47.00°-47.50° N, and the longitudinal was 17.755°-18.25° E. The MPI Echam5 climate model was used (Jungclaus *et al.,* 2006) which was based on the SRES A1B scenario of the period January 2081 to December 2100 (Gaffin *et al.,* 2004). The latitudinal expansion was 46.632°-48.496° N, and the longitudinal was 17.813°-19.688° E. The slight difference of the grids was not arbitrary, originated from the differences between the databases of the observed and modelled climate. We aimed to find the most resemble grids which overlap the studied area. According to regional climate models the sum of the annual precipitation is not expected to change significantly for 2081-2100 in the region, but the modelled values of the seasonal precipitation sums show the significant redistribution of the annual precipitation patterns (*Fig.4A* and *4B*).

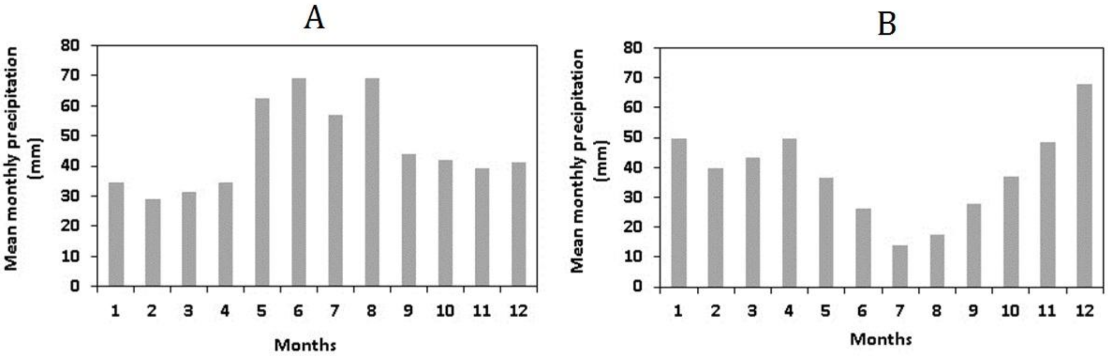

Figure 4. *The mean annual distribution of the precipitation for the period of 2004-2013 (A) and the projected mean annual distribution of the precipitation for the modelled period of 2081-2100 according to the used MPI Echam5 climate model (B).*

The Thornthwaite agrometeorological index (TAI) was employed as aridity index (Kemp, 1990). We averaged the monthly values during the correlations and the modeling steps.

$$TAI = 1.65 \bullet \left(\frac{P}{T + 12.2}\right)^{\frac{10}{9}} \quad \text{(Eq. 1)}$$

Correlation between the aridity index and standardized annual stem segment growth, modelling

We studied the association between of the mean of the TAI of the months for continuous periods within the vegetation season of the year and the standardized annual stem segment growth. Continuous periods of the aridity indices of 9th, 10th, 11th and 12th months of the previous year also were involved to the study. The correlation with the best fitted significance value and R^2 of the options was chosen. We used this simple method to avoid the collinearity due to the few numbers (7, 9 and 11) of the testable years and the relatively large number of the variables (12-14). Equation of the best fit linear regression correlation was used as the model of the aridity-based annual growth of the species. The years of 2004 to 2013 were used as the reference periods.

We used the associations between soil thickness and the total stem heights of the same year's old individuals (h) as the main factor which determines the absolute stem height. Due to the permanency of the soil thickness, in case of young individuals the soil thickness has no influence on the deviation of the individual trends. The estimation of the growth model provided a so-called "ideal" total vertical stem height according to the aridity values of the studied period starting from the height of 10 cm in case of *Fraxinus ornus* and *Pinus nigra*. Due to its clonal growth, in case of *Ailanthus altissima* we used the oldest 3 annual stem segment of the plants. Displaying the above described equations to the plantation of same aged individuals we calculated the correlation between the proportion of the measured (h_m) and the calculated (h_c) annual vertical growth values and the identical soil depth values.

$$r = \frac{h_m}{h_c} \qquad \text{(Eq. 2)}$$

The total annual vertical growth was calculated in different soil depths according to the Eq.3. Ten years of growth were modelled according to the projected monthly climate values for the soil depth of 5 to 20 cm.

$$h = r * h_c \qquad \text{(Eq. 3)}$$

Linear Correlation and Regression were performed by the online, free statistical program pack of VassarStats website (Lowry, 2012). The Fig.11 was prepared in ArcGIS 10.1 software (Longley *et al.,* 2001). For Fig.11-13, the different growth values of the years were sorted into attribute table. Values of the spatial data were interpolated by the IDW interpolation function of the Spatial Analyst Tool of ArcGIS.

Results

Young stems of the studied species showed rather different segment growths (*Fig.5*). The length of the annual stem offshoots of *Fraxinus ornus* showed a permanent growth (number of years=7, R^2=0.05, p= 0.625).

$$h_a=-0.0894 \cdot t+23.689 \qquad \text{(Eq. 4)}$$

In *Pinus nigra* a continuous growing trend was found (number of years=11, R^2=0.76, $p<0.001$).

$$h_a=1.925 \cdot t+12.567 \qquad \text{(Eq. 5)}$$

The length of the stem offshoots of *Ailanthus altissima* showed an exponentially decreasing trend (number of years=9, R^2=0.84, $p<0.001$).

$$h_a=1.925 \bullet t+12.567 \qquad \text{(Eq. 6)}$$

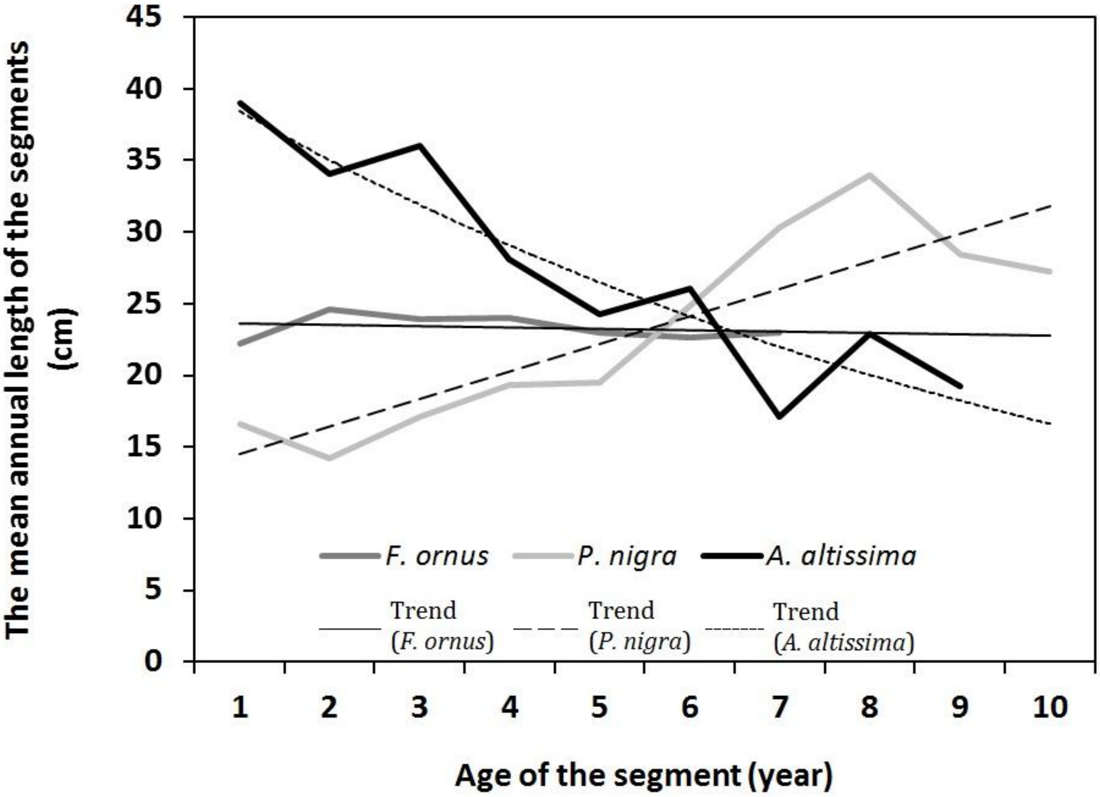

Figure 5. *The trend of the length of the same years old annual segments of Fraxinus ornus, Pinus nigra and Ailanthus altissima*

The averaged TAI of the following months were most significantly correlated to the standardized annual stem segment growth (h_s) in the period of 2007 to 2013: January to June (R^2=0.75, p=0.002), January to July (R^2=0.73, p=0.014) and February to July (R^2=0.70, p=0.018) in case of *Fraxinus ornus*; February to May (R^2=0.70, p=0.002, Fig.7), January to May (R^2=0.69, p=0.005) and March to May (R^2=0.62, p=0.011) in case of *Pinus nigra* and March to August (R^2=0.62, p=0.011), January to May (R^2=0.69, p=0.005) and March to May (R^2=0.62, p=0.011) in case of *Ailanthus altissima*. The following equations were used in the growth models: Eq.7 (*Fraxinus ornus*, *Fig.6A*), Eq.8 (*Pinus nigra*, *Fig.6B*) and Eq.9 (*Ailanthus altissima*, *Fig.6C*).

$$h_s=1.4451 \bullet \mathrm{TAI}_{\mathrm{avg}(1,2,3,4,5,6,7)}-5.7542 \qquad \text{(Eq. 7)}$$

$$h_s=1.0579 \bullet \mathrm{TAI}_{\mathrm{avg}(2,3,4,5)}-3.7276 \qquad \text{(Eq. 8)}$$

$$h_s=1.0579 \bullet \mathrm{TAI}_{\mathrm{avg}(3,4,5,6,7,8)}-3.7276 \qquad \text{(Eq. 9)}$$

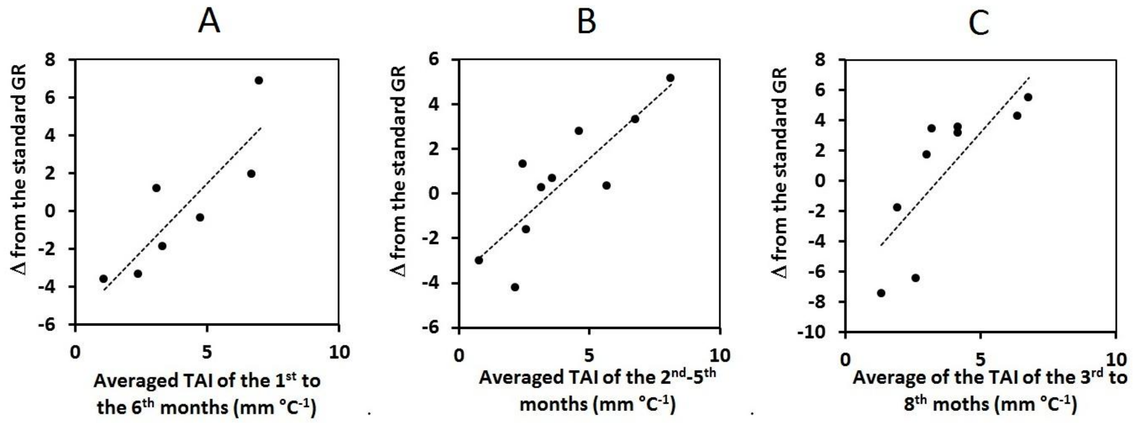

Figure 6. The association between the difference from the h_s and the averaged TAI in case of Fraxinus ornus (1st to the 6th months; 6A), Pinus nigra (2nd to the 5th; 6B), Ailanthus altissima (3rd to the 8th months; 6C).

Significant linear correlation was found between the mean soil depth and height of 7 years-old plantations of *Fraxinus ornus* (R^2=0.46, p<0.001, Eq. 10) and *Pinus nigra* (R^2=0.54, p<0.001, Eq.11). In case of *Ailanthus altissima* significant linear correlation was found between the mean soil depth and height of the oldest three annual segments (R^2=0.21, p=0.001, Eq.12; *Fig. 7*).

$$h_a=7.1568\bullet S_m-53.8385 \qquad \text{(Eq. 10)}$$

$$h=20.0345\bullet S_m-24.3157 \qquad \text{(Eq. 11)}$$

$$h=5.2715\bullet S_m-40.727 \qquad \text{(Eq. 12)}$$

We calculated the ratio between the measured and calculated height of individuals of each species of the same age (*hence: ratio*) according to the segment growth trend and the TAI-based growth model equations. The gained rations were regressed with the correspondent soil depth values. Significant correlation (R^2=0.54, p<0.001) were found between the ratios of 11 years old *Pinus nigra* individuals according to the Eq.4 and Eq.7 using the TAI values of 2004-2013 and soil depth values. We also found significant correlation (R^2=0.47, p<0.001) between the ratio of 7 years old *Fraxinus ornus* individuals according to the Eq.5 and Eq.8 using the TAI values of 2005-2013 and soil depth values. Lastly, significant correlation (R^2=0.21, p<0.001) was found between the ratio of the 3 years old *Ailanthus altissima* individuals according to the Eq.6 and Eq.9 using the TAI values of 2011-2013 and soil depth values (*Fig.8*).

Ailanthus altissima: $r=0.0719\bullet S_m+0.5552$ (Eq. 13)

Pinus nigra: $r=0.0746\bullet S_m-0.0905$ (Eq. 14)

$$\textit{Fraxinus ornus}: \mathrm{r}=0.0452\cdot S_m -0.3403 \qquad \text{(Eq. 15)}$$

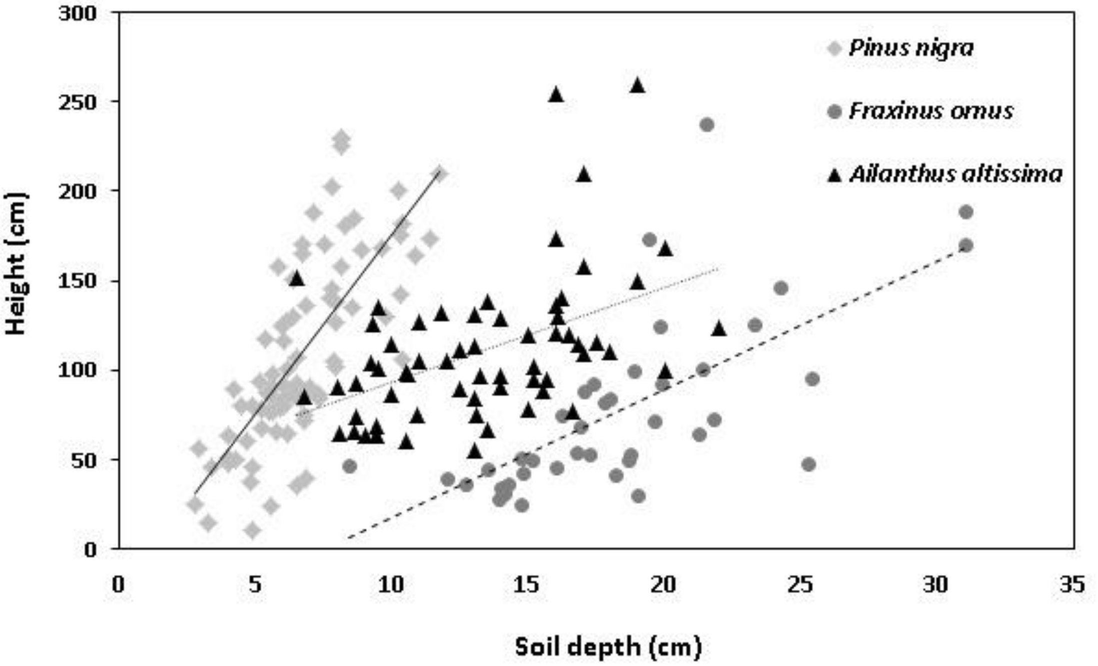

Figure 7. *The correlation between the mean soil depths and the heights of 9 years old Pinus nigra (light gray rhombus), Fraxinus ornus (dark gray circles) and the summarized length of the 3 years old Ailanthus altissima individuals (black triangles) to determine the growth-soil depth coefficient (r)*

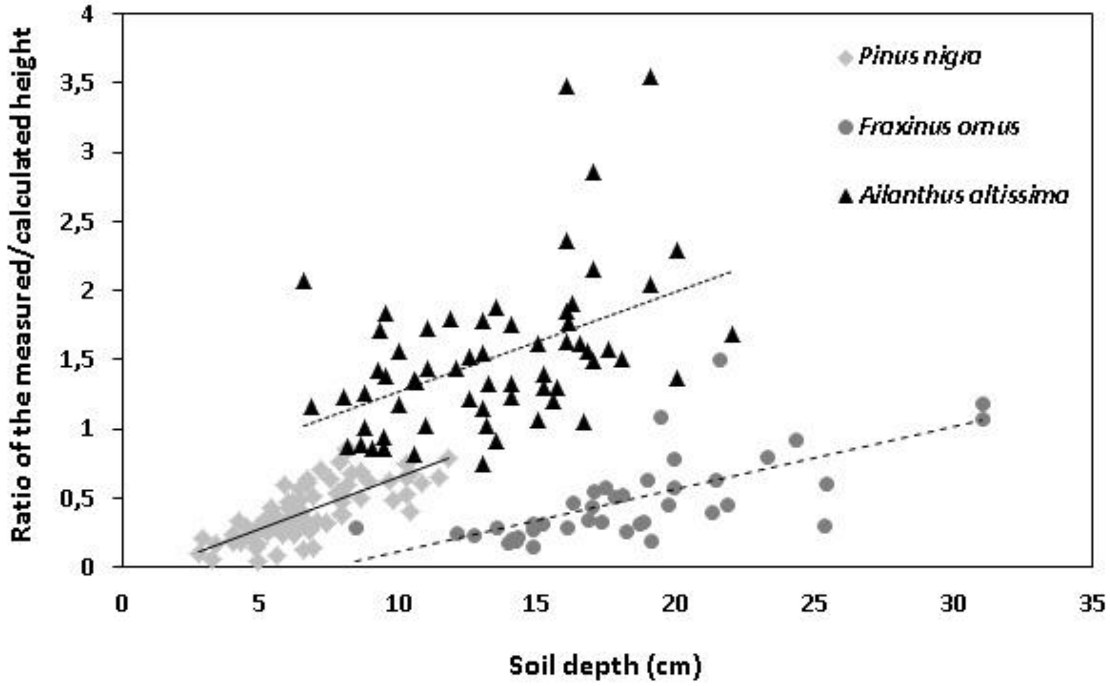

Figure 8. *The correlation between the mean soil depths and the calculated per measured ratio of Pinus nigra, Fraxinus ornus and Ailanthus altissima individuals*

The model predicts the significant decrease of standardized annual stem segment growth in case of *Fraxinus ornus* and *Ailanthus altissima* during the last five decades (1950-1999): [R^2=0.17, *p=0.001*; differences from the standardized stem segment growth: -0.99 (1950-1959), -1.27 (1960-1969), -1.76 (1970-1979), -1.93 (1980-1989), -2.89 (1990-1999)], [R^2=0.07, *p=0.045*; differences from the standardized stem segment growth: -2.09 (1950-1959), -2.05 (1960-1969), -2.33 (1970-1979), -2.56 (1980-1989), -2.97 (1990-1999)]. In case of *Pinus nigra* a barely significant decrease of the standardized annual stem segment growth was found during the last six decades (1950-2010): [R^2=0.07, *p=0.055*; differences from the standardized stem segment growth: -0.45 (1950-1959), -0.8 (1960-1969), -1.16 (1970-1979), -1.28 (1980-1989), -1.43 (1990-1999)]. *Fig.9* shows the modelled standardized annual stem segment growth of the species for the period of 1950 to 2003.

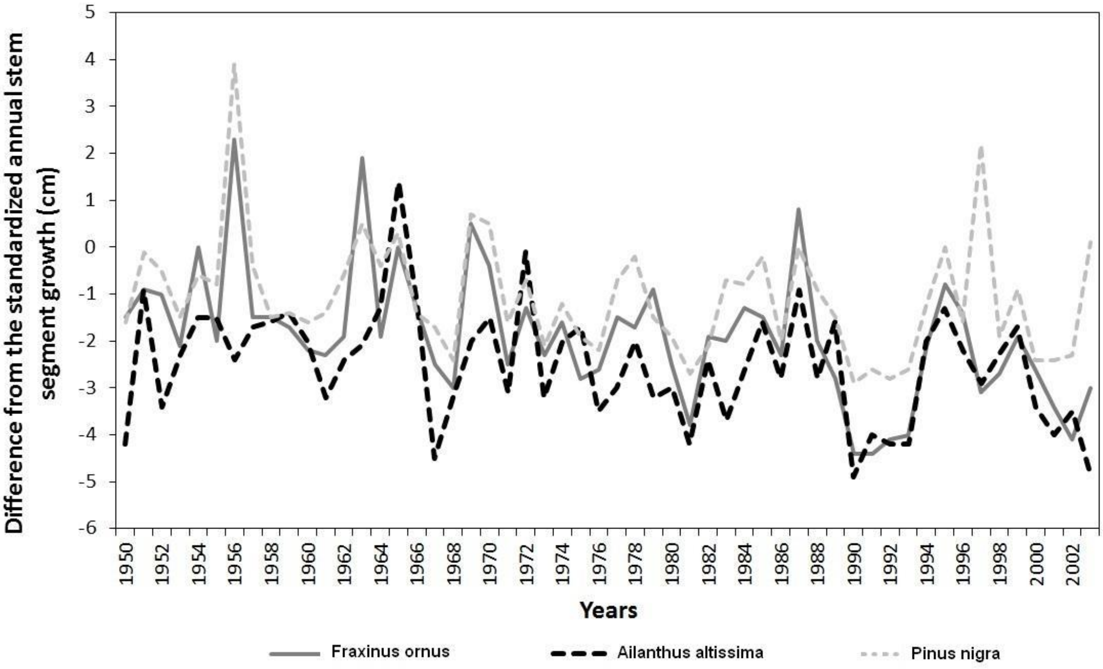

Figure 9. *The modelled difference values of the standardized annual stem segment growth of Fraxinus ornus, Pinus nigra and Ailanthus altissima in cm from 1950 to 2003*

The model predicts the decrease of standardized annual stem segment growth compared to the decades of the period for the future periods of 2081-2089 and 2090-2100: -2.54 and -2.54 (*Fraxinus ornus*), -4.29 and -4.64 (*Ailanthus altissima*), -1.55 and -1.50 (*Pinus nigra; Fig.10*).

The model predicts the highest decrease of the standardized annual stem segment growth in case of *Ailanthus altissima* in 1950-2010, including the future period of 2081-2100. In case of *Pinus nigra* the changing climate patterns will cause a relatively low decrease of the standardized annual stem segment growth, while the change in case of *Fraxinus ornus* can be moderate (*Fig.11)*.

DOI: http://dx.doi.org/10.15666/aeer/1403_161182

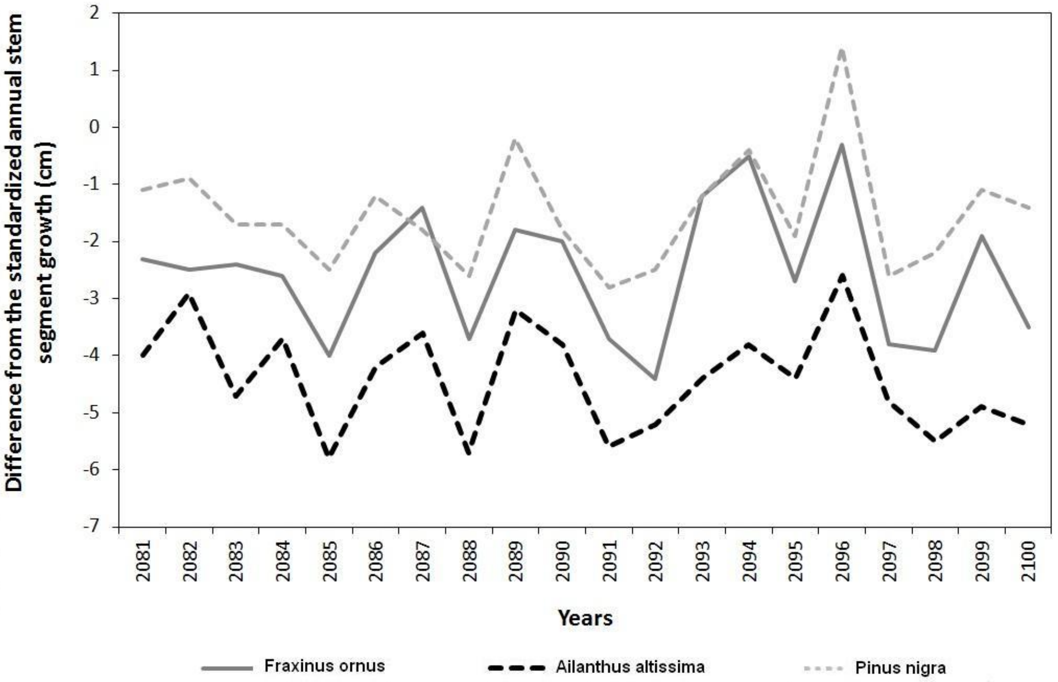

Figure 10. *The modelled difference values of the standardized annual stem segment growth of Fraxinus ornus, Pinus nigra and Ailanthus altissima in cm from 2081 to 2100*

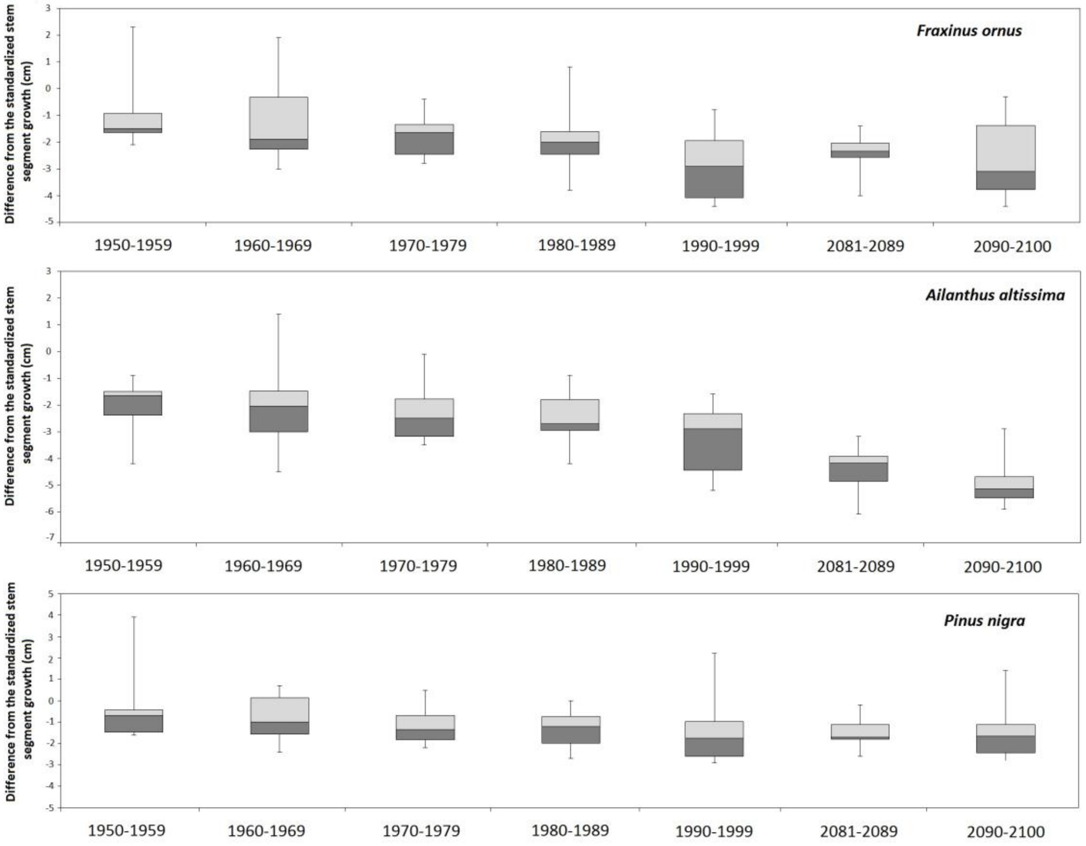

Figure 11. The box and whisker plots of the change of the modelled standardized annual stem segment growth.

DOI: http://dx.doi.org/10.15666/aeer/1403_161182

Fraxinus ornus shows the lowest absolute stem segment growth for each modelled soil depths in the period of 2091-2100 (*Fig.12*).

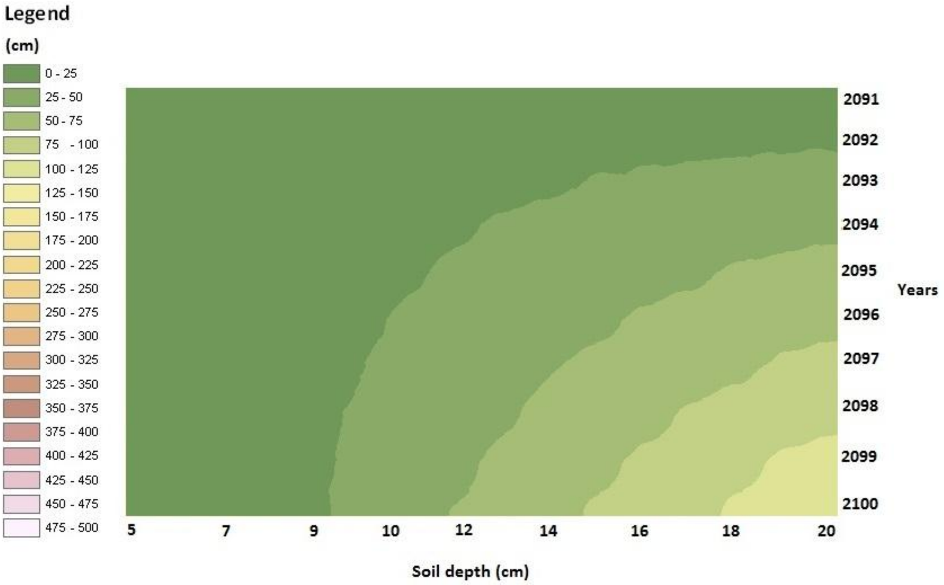

Figure 12. *The modeled future (2081-2100) growth of Fraxinus ornus on soils of different deepth*

The model predicts an intermediate segment growth of *Pinus nigra* (*Fig.13*).

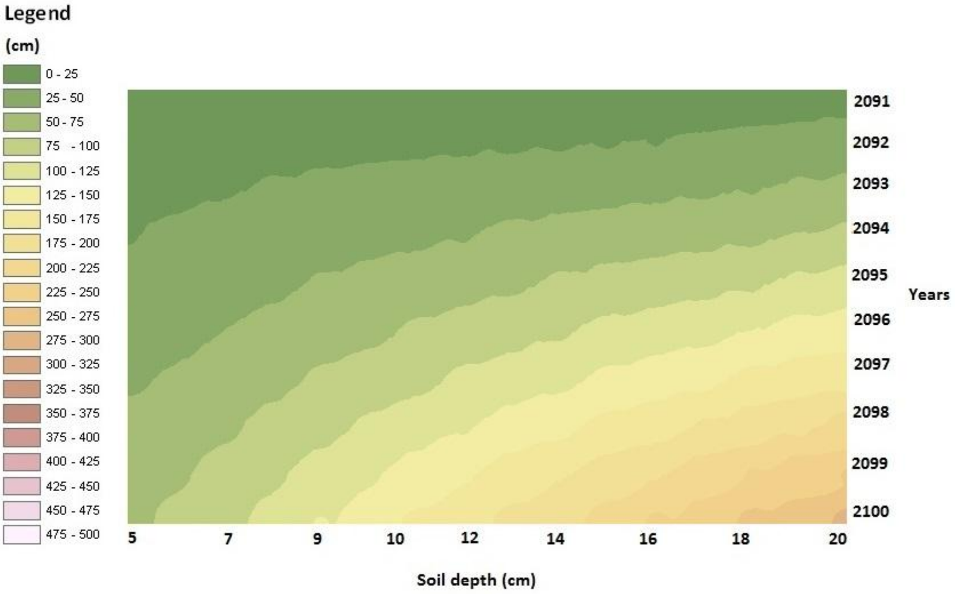

Figure 13. *The modeled future (2081-2100) growth of Pinus nigra on soils of different depth*

Ailanthus altissima seems to be the most rapidly growing tree species at same soil depths (*Fig.14*).

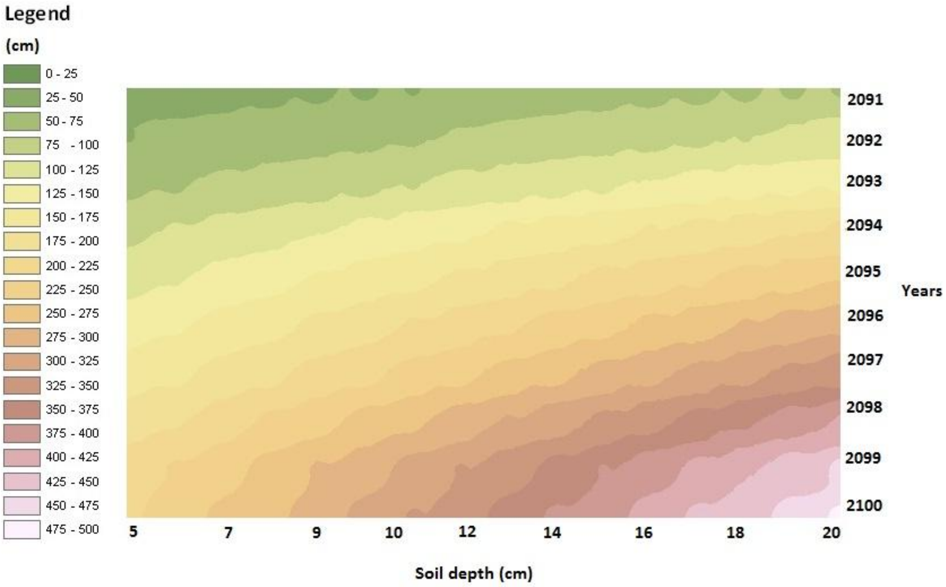

Figure 14. *The modeled future (2081-2100) growth of Ailanthus altissima on soils of different deepth*

Discussion

Significant differences were demonstrated between the aridity and soil-depth based growth patterns of the young specimens of *Fraxinus ornus*, *Pinus nigra* and *Ailanthus altissima* on the dolomite karst area of the Transdanubian Range, Hungary. While the annual growth season of the primarily South-Central European and Mediterranean *Pinus nigra* and *Fraxinus ornus* are determined by the aridity of the months of January to May or from February to June, the growth of *Ailanthus altissima* is mainly determined by the period of March to August. The obtained results showed similar seasonal association between the annual stem segment growth of *Pinus nigra* and the precipitation patterns to the ring-based study of Martín-Benito et al. (2008). Martín-Benito *et al.* (2000) described that the early offshoots of *Pinus nigra* is mostly influenced by climatic conditions prior to the growing season. Wimmer *et al.* (2000) found a very similar association between the false rings and the precipitation of May.

It is important to note, that our model is valid only for the 1 to 10 old plants since e.g. in case of *Ailanthus altissima* after 10–15 years the initially branching growth pattern turns into dichasial or monochasial (Kowarik and Säumel, 2007). The model results showed that the increasing summer-spring aridity will enhance more the difference between the growth capability of *Fraxinus ornus* and *Ailanthus altissima* than in case of *Fraxinus ornus* and *Pinus nigra* therefore; *Pinus nigra* may overgrow the individuals of *Fraxinus ornus*. Although we tried to study different, non-clonal specimens of *Ailanthus altissima* it is possible that this aim was not totally fulfilled and hence complicates modeling and the comparison since the root sprouting plays an important role in the establishment of *Ailanthus altissima* in new areas (Kowarik, 1995; Rabe, 1985). It is likely that the found initial rapid segment growth of the young *Ailanthus altissima* clones are in accordance with the specific canopy and light gap filling strategy of the species (Knapp and Canham, 2000; Espenschied-Reilly and Runkle, 2008; Gómez-Aparicio and Canham, 2008; Call and Nilsen, 2003;

Espenschied-Reilly and Runkle, 2008). Nevertheless, the present observations correspond to the fact that both root sprouts and seedlings can exceed a very rapid, even 1-2 m segment growth in their first year (Hunter, 1995; Miller, 1990).

The measured segment growth partly reflects the height of the species at their maturity: the average mature height of *Pinus nigra* ranges from 20 to 50 m in the natural occurrence of the species (Krugman and Jenkinson, 1974), *Ailanthus altissima* can reach the 18 to 21 m in height (Vines, 1960) and the maximum height of *Fraxinus ornus* is about 20 m on humid and rich soils (Oxford Herbaria, 2014), but it rarely exceeds 10-15 m (Kwantlen Plant Database, 2015). Although *Pinus nigra* reaches its mature size by approximately 80 years of age (Van Haverbeke and David, 1990), the life span of the *Ailanthus altissima* individuals ranges only from 30 to 70 years (Espenschied-Reilly and Runkle, 2008; Miller and James, 1990). The ramet bank of *A.s altissima* may explain the fast initial grow of the species in contrast to *Pinus nigra* and *Fraxinus ornus* (Kowarik, 1995). The allopathic compounds (Heisey, 1996; McFeeters, 1977) of the leaves and the barks might explain the successful colonization of the species during the decreased segment growth in later years. Kowarik (1983) described that while the distribution of *Ailanthus altissima* is mainly affected by site fertility in the Mediterranean, in Central Europe climate is the major factor that affects occurrence.

We found that aridity is a secondary factor of growing and soil depth is a much more important factor of absolute growth in case of all species. These results indicate that considering the climate/aridity requirements of the species, the climate of the studied area is closer to the Mediterranean climate than to the typical continental what corresponds to the sub-Mediterranean character of the Transdanubian Mountains (Németh, 2011; Bartha and Nagy, 2005). *A. altissima* is able to survive severe droughts on dry, rocky soils using an effective water-saving mechanism that includes decreasing the water loss by leaves and reducing the root hydraulic conductance (Trifilò *et al.,* 2004). Vertical growth of *A. altissima* is also sensitive to aridity, but *A. altissima* has advantage in the absolute annual growth against *Pinus nigra* and *Fraxinus ornus* due to its rapid growth in the first years. *A. altissima* showed the greatest growth on shallow soils which is in accordance with the observations that its seedlings often occupy the pavement cracks and other dry sites (Graves *et al.,* 1989), e.g. wall cracks and flat concrete roofs, moreover can tolerate compacted soils (Miller, 1990; Pan and Bassuk, 1985). *Pinus nigra* also showed great tolerance against the shallow, calcareous soil. Similar to *Ailanthus altissima*, *Pinus nigra* in their natural occurrence lives in various soil types and is able to live near the sea tolerating dry, shallow soils, sands, chalks, and limestone soils (Haverbeke, 2014). *P. nigra* is characterized by a relatively rapid, cca 15 to 34 cm annual growth which, moreover, showed an accelerating trend in the first 11 years. The maximum of the measured segment growth is very close to the growth of the *P. nigra* individuals of the Great Plain where approximately a 30 cm yearly segment growth was measured on "average sites" in the first 20 years after planting (Sander, 1963). The modeled 300 cm stem length of the 10-years old *P. nigra* individuals on 20 cm deep soils is comparable to the 5.3 m height of the same year's individuals which was measured in the Secrest Arboretum at Ohio State University on deep, rich soils (Aughanbaugh, 1958).

A. altissima is able to become a dominant species on disturbed calcareous vegetation (Patterson, 2008). Although *Fraxinus ornus* in Central and Eastern Europe also lives mainly on dry chalk and dolomite rocks soils (Oxford Herbaria, 2014) we found slightly slower segment growth on shallow soils than for *Pinus nigra* and *Ailanthus altissima* which can be a consequence the extensive root system of the *Fraxinus ornus* requiring deeper soils (Oxford Herbaria, 2014). Our findings confirm the importance of the effective treatment of re-naturalized areas at least in the first decades since the segment growth of the native *Fraxinus ornus* is significantly slower than that of the offshoots of *Ailanthus altissima*.

Emission models suggest the increase of the atmospheric carbon-dioxide concentration which may have beneficial effects on plant growth. For example, Kaushal et al. (1989) found that the height of the seedlings of *Pinus nigra* in their 2nd growth year was 10% more at 800 $\mu mol•mol^{-1}$ carbon-dioxide atmosphere than under the the 'normal' 350 $\mu mol•mol^{-1}$. Several further studies investigated the effects of elevated CO_2 level on tree growth (Kilpeläinen *et al.,* 2003; Broadmeadow *et al.,* 2000; Kaushal *et al.,* 1989), but these studies in general agreed that other factors (like low soil nutrient availability, decreasing precipitation, unbalanced water supply, increasing atmospheric ozone concentration (Broadmeadow *et al.,* 2000; Curtis, 1996; Lal, 2004; Nearing *et al.,* 2004), can reduce or reverse the proposed positive effect of the increasing CO_2 concentration on plant growth. This effect can be particularly relevant for dry deciduous woodland vegetation which *a priori* grows on shallow soils. Due to the inconsistent, complex influence and the lacking experimental data of the studied species, the effect of the elevating atmospheric carbon-dioxide concentration was omitted from the model presented in thes paper. Additionally, climate change may increase the risk of forest fires in the Mediterranean areas (Moriondo *et al.,* 2006) and the fitness of *Pinus nigra* individuals can be considerably influenced by fungal infections during severe droughts in Hungary (Koltay *et al.,* 2005).

Since climate models predict the Mediterranean-like rearrangement of the annual precipitation patterns in the Carpathian Basin (Bartholy *et al.,* 2009, 2007), the native *Fraxinus ornus* may have a slight long-term advantage in contrast to *Ailanthus altissima* since *Fraxinus ornus* also has the ability of rapid invasion (Thebaud and Debussche, 1991) what can be supported by the continuous growth strategy of the species. In long term, replacement of *Pinus nigra* by *Fraxinus ornus* may be successful. Although conifer*s* can successfully occupy canopy gaps from the seed bank (Daskalakou and Thanos, 1996), the seedling density strongly decreases with stand age (Augosto *et al.,* 2001) and some of the species of the original dolomite grassland and dry deciduous woodland vegetation have a long-term persistent seed bank under *Pinus nigra* plantations (Csontos *et al.,* 1996). The models predict that *F. ornus* will be a useful candidate of re-naturalizations if the necessary treatments will also be performed combined with the removal of *Pinus nigra* and *Ailanthus altissima.* Since *Fraxinus ornus* generally grows in mixed forests with oak and pine (Gratani and Foti, 1998) there is chance that the *F. ornus* will replace the *Pinus nigra* plantations because the invasion of *Ailanthus* is partly prohibited by the high light demand of the species in closed associations (Botta-Dukát, 2008; Kowarik and Säumel, 2007). It should be emphasized that we did not study the effect of the increasing shading on growth rates. The subsequent treatment is also important since *Ailanthus altissima* can survive in poorly lit environments while may be shrubby if suppressed beneath the canopy (Váczi, 2011). Our results showed that the reconstruction of the native dry deciduous woodland associations with *Fraxinus ornus* can be executed only with the continuous parallel removal of *Ailanthus altissima* due to the similar aridity tolerance and soil requirements of the species. The model predicts the similar affection of the annual growth of the species in the future due to the increasing aridity. Although *Pinus nigra* also threaten the re-naturalization of the dolomite grasslands, the removal of the pine species may be more easily executed than the eradication of *Ailanthus altissima*.

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REFERENCES

[1] Ádám, L., Marosi, S., Szilárd, J. (1987): A Dunántúli-középhegység. – Akadémiai kiadó, Budapest, p. 500. [in Hungarian]

[2] Aughanbaugh, J. E., Muckley H. R., Diller, O. D. (1958): Performance records of woody plants in the Secrest Arboretum. – Ohio Agricultural Experiment Station, Departmental Series 41. Columbus. 90 p.

[3] Augusto, L., Dupouey, J. L., Picard, J. F, Ranger, J. (2001): Potential contribution of the seed bank in coniferous plantations to the restoration of native deciduous forest vegetation. – Acta Oecologica 22: 87-98.

[4] Balog, A., Haas, J., Read, J. F., Coruh, C. (1997): Shallow marine record of orbitally forced cyclicity in a Late Triassic carbonate platform, Hungary. – Journal of Sedimentary Research 67: 661-675

[5] Bárány-Kevei, I. (2005): Genetic types, human impact and protection of Hungarian karsts. – Acta Climatol Chorol Univ Szegediensis 38-39: 17-23.

[6] Bartholy, J., Pongracz, R., Torma, C., Pieczka, I., Kardos, P., & Hunyady, A. (2009): Analysis of regional climate change modelling experiments for the Carpathian basin. – International Journal of Global Warming 1: 238-252.

[7] Bartha, D., Király, G., Vidéki, R., Nagy A., (2005): Occurrence of Rare Tree and Shrub Species in Hungary. – Acta Silvatica Lignaria Hungarica 1: 9-23.

[8] Bartholy, J., Pongrácz, R., Gelybó, Gy. (2007): Regional climate change expected in Hungary for 2071-2100. – Applied Ecology and Environmental Research 5: 1-17.

[9] Beerling, D. J., Huntley, B., Bailey, J. P. (1995): Climate and the distribution of Fallopia japonica: use of an introduced species to test the predictive capacity of response surfaces. – Journal of Vegetation Science 6: 269-282.

[10] Bódis, J. (1993): The effect of Pinus nigra on open dolomite grass-lands. I. Textural changes (A feketefenyő hatása nyílt dolomitsziklagyepre. I. Texturális változások.). – Botanikai Közlemények 80: 129–139.

[11] Botta-Dukát, Z. (2008): Invasion of alien species to Hungarian (semi-) natural habitats. – Acta Botanica Hungarica 5(Suppl): 219-227.

[12] Broadmeadow, M. S., Jackson, S. B. (2000): Growth responses of Quercus petraea, Fraxinus excelsior and Pinus sylvestris to elevated carbon dioxide, ozone and water supply. – New Phytologist 146: 437-451.

[13] Call, L. J., Nilsen, E. T. (2003): Analysis of spatial patterns and spatial association between the invasive tree-of-heaven (Ailanthus altissima) and the native black locust (Robinia pseudoacacia). – The American Midland Naturalist 150: 1-14.

[14] Corbett, S. L., Manchester, S. R. (2004): Phytogeography and fossil history of Ailanthus (Simaroubaceae). – International Journal of Plant Sciences 165: 671-690.

[15] Csontos, P., Tamás, J., Kalapos, T., (2001): Correlation between age basal diameter of Fraxinus ornus L. in three ecologically contrasting habitats. – Acta Botanica Hungarica 43: 127-136.

[16] Csontos, P., Horánszky, A., Kalapos, T., Lõkös, L. (1996): Seed bank of Pinus nigra plantations in dolomite rock grassland habitats, and its implications for restoring of the

grassland vegetation. – Hungarian Natural History Museum. In Annales Historico Naturales-Musei Nationalis Hungarici 88: 69-78.

[17] Cseresznyés, I., Csontos, P., Bózsing, E. (2006): Stand age influence on litter mass of Pinus nigra plantations on dolomite hills in Hungary. – Botany 84: 363-370.

[18] Chiatante, D., Iorio, A. D., Sciandra, S., Scippa, G. S., Mazzoleni, S. (2006): Effect of drought and fire on root development in Quercus pubescens Willd. and Fraxinus ornus L. seedlings. – Environmental And Experimental Botany 56: 190-197.

[19] Constán-Nava, S., Bonet, A., Pastor, E., Lledó, M. J. (2010): Long-term control of the invasive tree Ailanthus altissima: Insights from Mediterranean protected forests. – Forest Ecology and Management 260: 1058-1064.

[20] Curtis, P. S. (1996): A metaanalysis of leaf gas exchange and nitrogen in trees grown under elevated carbon dioxide. – Plant, Cell & Environment 19: 127-137.

[21] Daskalakou, E. N., Thanos, C. A. (1996): Aleppo pine (Pinus halepensis) postfire regeneration: the role of canopy and soil seed banks. – International Journal of Wildland Fire 6: 59-66.

[22] Ding, J., Wu, Y., Zheng, H., Fu, W., Reardon, R., Liu, M. (2006): Assessing potential biological control of the invasive plant, tree-of-heaven, Ailanthus altissima. – Biocontrol Science and Technology 16: 547-566.

[23] E-OBS database (2014): http://www.ecad.eu/download/ensembles/ensembles.php. Last accessed in 05 03 2015

[24] Espenschied-Reilly, A. L., Runkle, J. R. (2008): Distribution and Changes in Abundance of Ailanthus altissima (Miller) Swingle in a Southwest Ohio Woodlot. – Ohio Journal of Science 108: 16-22.

[25] Erdős, L., Márkus, A., Körmöczi, L. (2005): Consequences of an extirpation trial of Ailanthus altissima (Ailanthus altissima (Mill.) Swingle) on rock grasslands and slope steppes. – Tiscia (Szeged) 35: 3-7.

[26] Fodor, L., Csillag, G., Németh, K., Budai, T., Cserny,T., Martin, U., Dewey , J. (2005): Tectonic development, morphotectonics and volcanism of the Transdanubian Range: a field guide. – Occasional Papers of the Geological Institute of Hungary 204: 59-86.

[27] Gaffin, S. R., Rosenzweig, C., Xing, X., Yetman, G. (2004): Downscaling and geo-spatial gridding of socio-economic projections from the IPCC Special Report on Emissions Scenarios (SRES). – Global Environmental Change 14: 105-123.

[28] Gómez-Aparicio, L., Canham, C. D. (2008): Neighbourhood analyses of the allelopathic effects of the invasive tree Ailanthus altissima in temperate forests. – Journal of Ecology 96: 447-458.

[29] Goldhammer, R. K., Dunn, P. A., Hardie, L. A. (1990): Depositional cycles, composite sea-level changes, cycle stacking patterns, and the hierarchy of stratigraphic forcing: examples from Alpine Triassic platform carbonates. – Geological Society of America Bulletin 102: 535-562.

[30] Gratani, L., Foti, I. (1998): Estimating forest structure and shade tolerance of the species in a mixed deciduous broad-leaved forest in Abruzzo, Italy. – In Annales Botanici Fennici 35: 75-83

[31] Graves, W. R., Dana, M. N., Joly, R. J. (1989): Influence of Root-zone Temperature on Growth of Ailanthus altissima (Mill.) Swingle1. – Growth Regul 5: 163-167.

[32] Gritti, E. S., Smith, B., Sykes, M. T. (2006): Vulnerability of Mediterranean Basin ecosystems to climate change and invasion by exotic plant species. – Journal of Biogeography 33: 145-157.

[33] Gibbons, J. W., Scott, D. E., Ryan, T. J., Buhlmann, K. A., Tuberville, T. D., Metts, B. S., Winne, C. T. (2000): The Global Decline of Reptiles, Déjà Vu Amphibians Reptile species are declining on a global scale. Six significant threats to reptile populations are habitat loss and degradation, introduced invasive species, environmental pollution, disease, unsustainable use, and global climate change. – BioScience 50: 653-666.

[34] Haas, J. (1982): Facies analysis of the cyclic Dachstein Limestone Formation (Upper Triassic) in the Bakony Mountains, Hungary. – Facies 6: 75-83.

[35] Hellmann, J. J., Byers, J. E., Bierwagen, B. G., Dukes, J. S. (2008): Five potential consequences of climate change for invasive species. – Conservation Biology 22: 534-543.

[36] Hably, L. (2001): Fruits and leaves of Ailanthus Desf. from the Tertiary of Hungary. – Acta Palaeobotanica 41: 207-219.

[37] Heisey, R. M. (1996): Identification of an allelopathic compound from Ailanthus altissima (Simaroubaceae) and characterization of its herbicidal activity. – American Journal of Botany 83: 192-200.

[38] Heisey, R. M. (1990): Allelopathic and herbicidal effects of extracts from tree of heaven (Ailanthus altissima). – American Journal of Botany 77: 662-670.

[39] Haverbeke, D. F. V. (2014): European Pinus nigra.

[40] http://www.na.fs.fed.us/pubs/silvics_manual/Volume_1/pinus/nigra.htm. Last accessed in 23 05 2014

[41] Huntley, B. (1991): How plants respond to climate change: migration rates, individualism and the consequences for plant communities. – Annals of Botany 67(Suppl1): 15-22.

[42] Hunter, J. C. (1995): Ailanthus altissima (Miller) Swingle: its biology and recent history. – California Exotic Pest Plant Council News 3: 4-5.

[43] Jacob, D., Podzun, R. (1997): Sensitivity studies with the regional climate model REMO. – Meteorology and Atmospheric Physics 63: 119-129.

[44] Jump, A. S., Penuelas, J. (2005): Running to stand still: adaptation and the response of plants to rapid climate change. – Ecology Letters 8: 1010-1020.

[45] Jungclaus, J. H., Keenlyside, N., Botzet, M., Haak, H., Luo, J. J., Latif, M., Roeckner E. (2006): Ocean circulation and tropical variability in the coupled model ECHAM5/MPI-OM. – Journal of Climate 19: 3952-3972.

[46] Kaushal, P., Guehl, J. M., Aussenac, G. (1989): Differential growth response to atmospheric carbon dioxide enrichment in seedlings of Cedrus atlantica and Pinus nigra ssp. Laricio var. Corsicana. – Canadian Journal of Forest Research 19: 1351-1358.

[47] Kemp, D. (2002): Global environmental issues: a climatological approach. – Routledge, London, 217 p.

[48] Kilpeläinen, A., Peltola, H., Ryyppö, A., Sauvala, K., Laitinen, K., Kellomäki, S., (2003): Wood properties of Scots pines (Pinus sylvestris) grown at elevated temperature and carbon dioxide concentration. – Tree Physiology 23: 889-897.

[49] King, J. C., Harangozó, S. A. (1998): Climate change in the western Antarctic Peninsula since 1945: observations and possible causes. – Annals of Glaciology 27: 571-575.

[50] Koltay, A., Kövics, G. J., Dávid, I. (2005): Health condition of Hungarian forests-appearance of new disease. – In: 10. Tiszántúli Növényvédelmi Fórum, Debrecen, Hungary 130-140.

[51] Knapp, L. B., Canham, C. D. (2000): Invasion of an old-growth forest in New York by Ailanthus altissima: sapling growth and recruitment in canopy gaps. – Journal of the Torrey Botanical Society 127: 307-315.

[52] Kostova, I, (2001). Fraxinus ornus. L. Fitoterapia 72: 471-480.

[53] Kowarik, I., Säumel, I. (2007): Biological flora of Central Europe: Ailanthus altissima (Mill.) Swingle. – Perspectives in Plant Ecology, Evolution and Systematics 8: 207-237.

[54] Kowarik, I. (1995): Clonal growth in Ailanthus altissima on a natural site in West Virginia. – Journal of Vegetation Science 6: 853-856.

[55] Kowarik, I. (1983): Colonization by Ailanthus altissima (Ailanthus altissima) in the French mediterranean region (Bas-Languedoc), and its phytosociological characteristics. – Phytocoenologia 11: 389-405.

[56] Kramer, P. J., Turner, N. C. (1980): Drought, stress, and the origin of adaptations. − In: Turner N.C. Kramer P.J. (Eds.)- Adaptation of plants to water and high temperature stress, 7 Functional Plant Biology 13: 175-190.

[57] Krugman, S. L., Jenkinson, J. L. (1974): Pinaceae pine family. In: Schopmeyer, C.S., - Seeds of woody plants in the United States. − Agriculture Handbook, Washington, Department of Agriculture Forest Service 598-637.

[58] Kwantlen (Politechnic University) Plant Database. https://plantdatabase.kwantlen.ca/plant/plantDetail/925. Last accessed in 19 03 2015

[59] Lal, R. (2004): Soil carbon sequestration impacts on global climate change and food security. − Science 304: 1623-1627.

[60] Leal, S., Eamus, D., Grabner, M., Wimmer, R., Cherubini, P. (2008): Tree rings of Pinus nigra from the Vienna basin region (Austria) show evidence of change in climatic sensitivity in the late 20th century. − Canadian Journal of Forest Research 38: 744-759.

[61] Loáiciga, H. A., Maidment, D. R., Valdes, J. B. (2000): Climate-change impacts in a regional karst aquifer, Texas, USA. − Journal of Hydrology 227: 173-194.

[62] Longley, P. A., Goodchild, M. F., Maguire, D. J., Rhind, D. W. (2001). Geographic information system and Science, 2nd Edition. − England: John Wiley & Sons, Ltd,. Chichester 537 p.

[63] Lowry R., (2012): VassarStats: Website for statistical computation. Vassar College. http://vassarstats.net/. Last accessed in 19 03 2015

[64] Martin, J. M., Torres-Ruiz J., Fontboté, L. (1987): Facies control of strata-bound ore deposits in carbonate rocks: the F-(Pb-Zn) deposits in the Alpine Triassic of the Alpujárrides, southern Spain. − Mineralium Deposita 22: 216-226.

[65] Martín-Benito, D., Cherubini, P., del Río, M., Cañellas, I. (2008): Growth response to climate and drought in Pinus nigra Arn. trees of different crown classes. − Trees 22: 363-373.

[66] Márton, E., Fodor, L. (2003): Tertiary paleomagnetic results and structural analysis from the Transdanubian Range (Hungary): rotational disintegration of the Alcapa unit. − Tectonophysics 363: 201-224.

[67] Mc Feeters, R. F., Chichester, C. O., Whitaker, J. R. (1971): Purification and properties of chlorophyllase from Ailanthus altissima (Tree-of-Heaven). − Plant Physiology 47: 609-618.

[68] Mihály, B., Botta-Dukát, Z. (2004): Biológiai inváziók Magyarországon. Özönnövények. Biological invasions in Hungary. Invasive plants]. − Természetbúvár Alapítvány Kiadó, Budapest 407 p. [In Hungarian]

[69] Miller, J. H. (1990): Ailanthus altissima (Mill.) Swingle ailanthus. − Silvics of North America 2: 101-104.

[70] Moriondo, M., Good, P., Durao, R., Bindi, M., Giannakopoulos, C., Corte-Real, J. (2006): Potential impact of climate change on fire risk in the Mediterranean area. − Climate Research 31: 85-95.

[71] Nearing, M. A., Pruski, F. F., O'neal, M. R. (2004): Expected climate change impacts on soil erosion rates: a review. − Journal of Soil and Water Conservation 59: 43-50.

[72] Németh, C. (2011): Bryofloristical data from the Transdanubian Mountain ranges, Bakony and Vértes Mts (Hungary). − Studia Botanica Hungarica 42: 5-22.

[73] Oxford Herbaria (2014): Fraxinus ornus (Fraxinus ornus). Ch. 6th, 88-108.

[74] http://herbaria.plants.ox.ac.uk/fraxigen/pdfs_and_docs/book/fraxigen_c6.pdf. Last accessed in 23 05 2014

[75] Patterson, K. D. (2008): Vegetation classification and mapping at Petersburg National Battlefield, Virginia. − Technical Report NPS/NER/NRTR--2008/127. Philadelphia, PA: U.S. Department of the Interior, National Park Service, Northeast Region 235 p.

[76] Pan, E., Bassuk, N. (1985): Effects of soil type and compaction on the growth of Ailanthus altissima seedlings. − Journal of Environmental Horticulture 2: 13

[77] Pieczka, I., Pongrácz, R., Bartholy, J. (2011): Comparison of Simulated Trends of Regional Climate Change in the Carpathian Basin for the 21st Century Using Three Different Emission Scenarios. − Acta Silvatica et Lignaria Hungarica 7: 9-22.

[78] Rabe, E. P. (1985): Distribution and growth response of Ailanthus altissima in the urban environment. − Ithaca, NY: Cornell University 87 p. Thesis.

[79] Robinson, G. R., Handel, S. N. (1993): Forest restoration on a closed landfill: rapid addition of new species by bird dispersal. − Conservation Biology 7: 271-278.

[80] Sander, D. H. (1963): Height-age curves for Pinus nigra in windbreaks on loess soils of Nebraska. − USDA Forest Service, Research Note RM-13. Rocky Mountain Forest and Range Experiment Station, Fort Collins, CO. 2 p.

[81] Sárospataki, M. (2014): Dendrológiai kertek a 19. századi magyarországi kertépítészetben Dendrological gardens in 19th century garden architecture in Hungary (Doctoral dissertation, Budapesti Corvinus Egyetem). http://phd.lib.uni-corvinus.hu/753/1/Sarospataki_Mate.pdf. Last accessed in 23 05 2014.

[82] Sheppard, S. R., Cizek, P. (2009): The ethics of Google Earth: Crossing thresholds from spatial data to landscape visualisation. − Journal of Environmental Management 90: 2102-2117.

[83] Shiu-ying, H. (1979): „Ailanthus altissima”. Arnoldia 39: 29–50.

[84] Soest, J., Ayral, P., Schober, R., Hummel, F. C. (1965): The standardization of symbols in forest mensuration. − University of Maine Agricultural Experiment Station, Technical Bulletin, Orono 15p.

[85] Strengers, B., Leemans, R., Eickhout, B., de Vries, B., Bouwman, L. (2004): The land-use projections and resulting emissions in the IPCC SRES scenarios as simulated by the IMAGE 2.2 model. − GeoJournal 61: 381-393.

[86] Thebaud, C., Debussche, M. (1991): Rapid invasion of Fraxinus ornus L. along the Herault River system in southern France: the importance of seed dispersal by water. − Journal of Biogeography 18: 7-12.

[87] Török, K., Botta-Dukát, Z., Dancza, I., Németh, I., Kiss, J., Mihály, B., Magyar, D. (2003): Invasion gateways and corridors in the Carpathian Basin: biological invasions in Hungary. − Biological Invasions 5: 349-356.

[88] Trájer, A., Bede-Fazekas, Á., Hammer, T., Padisák, J. (2015): Modelling the growth of young individuals of *Pinus nigra* on thin carbonate soils under climate change in Hungary. − Acta Botanica Hungarica 57:419-442.

[89] Trifilò, P., Raimondo, F., Nardini, A., Gullo, M. L., Salle, S. (2004): Drought resistance of Ailanthus altissima: root hydraulics and water relations. − Tree Physiology 24: 107-114.

[90] Udvardy, L. (1998a): Classification of adventives dangerous to the Hungarian natural flora. − Acta Botanica Hungarica 41: 315–331

[91] Udvardy, L. (1998b): Spreading and coenological circumstances of Ailanthus altissima (Ailanthus altissima) in Hungary. − Acta Botanica Hungarica 41: 299–314

[92] Váczi, B. (2011): A bálványfa (Ailanthus altissima) hatása az Aggteleki Nemzeti Park érintett társulásaira: http://dea.unideb.hu/dea/handle/2437/115179. Last accessed in 23 05 2014 [In Hungarian]

[93] Van Haverbeke, D. F. (1990): Pinus nigra Arnold European Pinus nigra. In: Burns, Russell M.; & Honkala, Barbara H., technical coordinators. − Silvics of North America. Volume 1. Conifers. Agric. Handb. 654.Washington, DC: U.S. Department of Agriculture, Forest Service 395-404.

[94] VassarStat: http://vassarstats.net/corr_big.html. Last accessed in 23 05 2014

[95] Wang, J., Meng, J. J., Cai Y. L. (2008): Assessing vegetation dynamics impacted by climate change in the southwestern karst region of China with AVHRR NDVI and AVHRR NPP time-series. − Environmental Geology 54: 1185-1195.

[96] Wimmer, R., Strumia, G., Holawe, F. (2000): Use of false rings in Pinus nigra to reconstruct early growing season precipitation. – Canadian Journal of Forest Research 30: 1691-1697.
[97] Winkler, D., Tóth, V. (2012): Effects of Afforestation with Pines on Collembola Diversity in the Limestone hills of Szárhalom (West Hungary). – Acta Silvatica et Lignaria Hungarica 8: 9-20.
[98] Zunya, W., Yihui, D., Jinhai, H., Jun ,Y. (2004): An updating analysis of the climate change in China in recent 50 years. – Acta Meteorologica Sinica 62: 228-236.

A CASE STUDY OF THE BEHAVIOR OF AEROSOL OPTICAL PROPERTIES UNDER THE INCIDENCE OF A SAHARAN DUST INTRUSION EVENT

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Abstract. In this paper a local-scale study of Saharan dust loading over Iasi city, Romania, during July 2012 is reported. The intrusion of Saharan dust above the monitoring site [Iasi_LOASL, Romania (47.19N, 27.55E)] and the variations of ozone and NO_2 concentrations are estimated by using advanced remote sensing techniques (i.e. Aerosol Robotic NETwork – AERONET, Cloud-Aerosol Lidar and Infrared Pathfinder Satellite Observations – CALIPSO) and forecast models (i.e. DREAM and MAP 3D). The time interval was appropriately selected, to remove the influence of precipitation and clouds on photochemical processes. During intrusion events, the Saharan dust represents the main source for changes in optical and chemical parameters of the atmosphere.

Keywords: *remote sensing, AERONET, Saharan dust, aerosols, ozone*

Introduction

Taking into account the major role of aerosols in absorption and scattering of solar radiation, which alters the optical properties of Earth's atmosphere, remote sensing techniques and modeling approach have been recently developed, providing complementary information about temporal and spatial variation of aerosol optical parameters (e.g. Single Scattering Albedo, Ångstrom exponent, Lidar ratio etc.). Recent researches confirmed that in Romania, especially in urban areas, the main sources of air pollution amend the complex mechanisms of photochemical oxidants; they produce an adverse effect on the optical properties of Earth's atmosphere and global climate change (Cazacu et al., 2015, 2011; Papayannis et al., 2014). Of all the classes of photochemical oxidants in the atmosphere, ozone and nitrogen dioxide are among the most extensively studied; recent studies focused on the possible influence of Saharan dust on them (Ohde and Siegel, 2012).

Actual monitoring and modeling techniques for the Earth's atmosphere (Light Detection And Ranging – LIDAR, sun photometer, UV cameras, MAP3D etc.) provide useful information on changes in the weather conditions and the climate (Belegante et al., 2011; Cazacu et al., 2011; Nicolae et al., 2010; Timofte et al., 2011).

The Saharan dust significantly influences the radiative transfer through absorption, scattering and reflection phenomena, strongly affecting the energy fluxes and spectral distribution of solar radiation (Earth's radiation budget), as well as photosynthesis processes (Haywood et al., 2008; Papayannis et al., 2014). In addition to the influence of Saharan dust, radiative transfer is also affected by anthropogenic sources of nitrogen oxides (NO and NO_2) and volatile organic compounds (VOCs) under the influence of solar ultraviolet (UV) radiation, favouring photochemical reactions (Ohde and Siegel, 2012; Shon et al., 2008).

This paper presents a study of Saharan dust loading over long distances, as well as monitoring of possible influences on the atmosphere over Romania. The analysis of experimental data and theoretical models specific to Romania have shown fairly low concentrations of Saharan dust, but large enough to influence the optical parameters, so that the transfer of solar radiation is affected even during sunny days.

Some research and development projects, such as ROmanian LIdar NETwork (ROLINET) and Romanian Atmospheric research 3D Observatory (RADO) investigate the air quality in the North-East region of Romania, in the proximity of the Iasi city; several research papers reported on this topic. Starting from the end of spring to early fall, over Romania, multiple Saharan dust events occur (Cazacu et al., 2015, 2012, 2011; Gothard et al., 2014; Labzovskii et al., 2014; Papayannis et al., 2014; Unga et al., 2013). In this paper, we report the influence of Saharan dust during July 2012 on the local air quality. Besides, we compare the air quality forecast given by MAP3D with the experimental data acquired through remote sensing techniques available on the site, the Atmospheric Research 3D Observatory RADO Station of the Alexandru Ioan Cuza University of Iasi (sun photometry). Other experimental and theoretical approaches in the quantification of air quality were also used, i.e. Cloud-Aerosol Lidar and Infrared Pathfinder Satellite Observations (CALIPSO), Dust REgional Atmospheric Model (DREAM) and Hybrid Single Particle Lagrangian Integrated Trajectory Model (HYSPLIT). Evidence of Saharan dust intrusion was provided by sun photometry and by calculus methods of aerosols dispersion (based on databases of various meteorological survey stations).

Methodology

Mesoscale Air Pollution 3D Modeling (MAP3D) developed at the Laboratory of Environmental Fluid Mechanics and Hydrology of École Polytechnique Fédérale de Lausanne, Switzerland, is capable of providing daily forecasts for air pollutants (O_3, NO, NO_2, PM_{10} particles) (Couach et al., 2004). The MAP3D is based on the chemical model MM5/CHIMERE, which has been successfully applied for the entire Romanian territory [with a resolution of 15x15 km^2 (Cazacu et al., 2011)].

Complementary researches were based on the following techniques:

- Aerosol Robotic NETwork (AERONET), a NASA network for monitoring and characterizing atmospheric aerosols using a Cimel Automatic Sun Tracking Photometer

CE 318 ground-based, solar- powered, weather-hardy, robotically-pointed sun and sky spectral photometer (Holben et al., 1998). Starting from 7 May 2012, the monitoring station Iasi_LOASL located in Iasi, Romania (47.19N, 27.55E) became active in this network, giving researchers immediate access to the quantitative values for various types of aerosols (Unga et al., 2013). In order to provide optical aerosol properties the Ångstrom exponent, Single Scattering Albedo (*SSA*), Lidar report calculated by AERONET ($S_{AERONET}$) and size distribution were considered from level 1.5 with zenith angle larger than 50°, retrieval error less than 5% and AOT (aerosol optical thickness) at 440 nm greater than 0.2. The Lidar ratio is defined as ratio between extinction coefficient and backscatter coefficient and can be calculated using the SSA parameter (at 532 nm) and phase function at 180 degree (Cattrall et al., 2005).

- Dust REgional Atmospheric Model (DREAM), an operational website developed by Earth Sciences Division of the Barcelona Supercomputing Center, used to confirm the presence of Saharan dust over Romania (Basart et al., 2012; Nickovic et al., 2001; Pérez et al., 2006).
- Hybrid Single-Particle Lagrangian Integrated Trajectory (HYSPLIT) model, a complete system for computing both simple air parcel trajectories and complex dispersion, also comprising capabilities for deposition simulations. The model uses the existing meteorological forecast fields from regional or global models to compute advection, stability and subsequent dispersion (Draxler and Rolph, 2015);
- The Cloud Aerosol Lidar with orthogonal Polarization (CALIOP) system, on board of the Cloud-Aerosol Lidar and Infrared Pathfinder Satellite Observations (CALIPSO) platform, is based on the elastic-backscatter Lidar used for aerosols and cloud investigation. The CALIOP is providing level 1 products, high-resolution profiles of the attenuated backscattered radiation by aerosols and clouds at visible (532 nm) and near-infrared wavelengths (1064 nm) and depolarized backscattered radiation using the visible channel (at 532 nm). With previously developed algorithms (Winker et al., 2009), level 2 products [classification of different features by layer type, e. g. clouds, aerosols type like Saharan or mineral dust, biomass burning, urban/industrial, the extinction coefficient profile and total column aerosol optical depth (AOD) for a defined Lidar ratio for each detected aerosol layer] are estimated. Mamouri et al (Mamouri et al., 2009) and Pappalardo et al (Pappalardo et al., 2010) have shown that level 1 attenuated backscatter profiles are in reasonable agreement with ground-based Lidar measurements.

Results and discussions

The data from July 2012, considered as a hot summer month, were acquired since the aerosols optical properties vary from season to season, as shown by Mihai and Stefan (Mihai and Stefan, 2011). Using data from AERONET and analysing the Ångstrom and SSA parameters, we selected the possible Saharan dust intrusion events. A decrease in the Ångstrom parameter (specially for values lower than 1.5 at 440–870 nm) indicates an increase of number of particles with a diameter greater than 1 μm (coarse mode) from the volumetric distributions retrieval while an increase of SSA with increasing wavelength (from 440 nm to 1020 nm, with SSA values between 0.91 and 0.99) indicates a dust influence (Dubovik et al., 2002; Giles et al., 2012). Thus, an analysis of selected days (the 3rd, 4th, 15th and 25th of July 2012) presenting this trend was made, and we validated the presence of Saharan dust with data from the HYSPLIT and BSC

DREAM models, as well as data acquired through the AERONET, as will be seen below.

As previously reported by Unga et al. (2013), July 2012 studies on the tropospheric aerosols above Iasi revealed major influence of urban and industrial aerosols on the air quality in this region. Afterwards, Cazacu et al. have proven that the Iasi_LOASL monitoring site is specific for urban/industrial aerosols type with influences of biomass burning and mineral dust especially when Saharan dust events occur (Cazacu et al., 2015). Thus, in all AERONET data the presence of urban/industrial aerosols was always registered over the studied site.

Concerning the present study, by applying the HYSPLIT model for the days of 3rd, 4th, 15th and 25th of July 2012 in which the Ångstrom coefficient had a decreasing behavior below 1.4 (the highlighted dates in *Fig. 1*) and the SSA parameter varied from 0.91 (at 440 nm) up to 0.99 (at 1020 nm) with a very low spherical particle fraction (0.2÷6.6), results indicating the presence of Saharan dust have been obtained (*Fig. 2*).

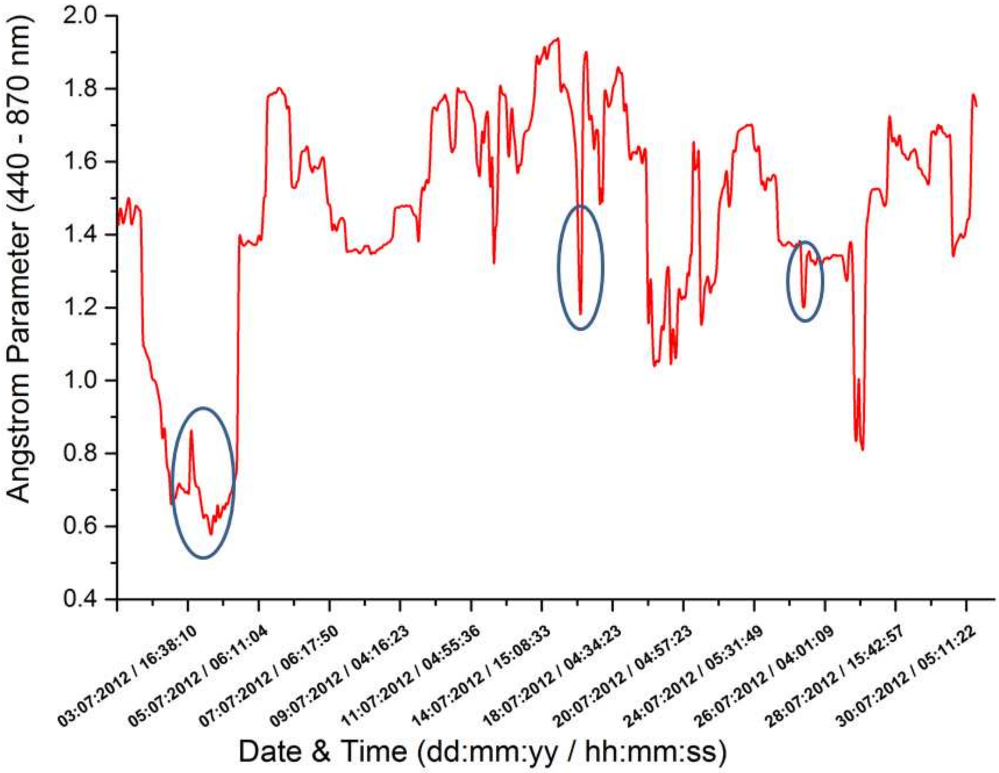
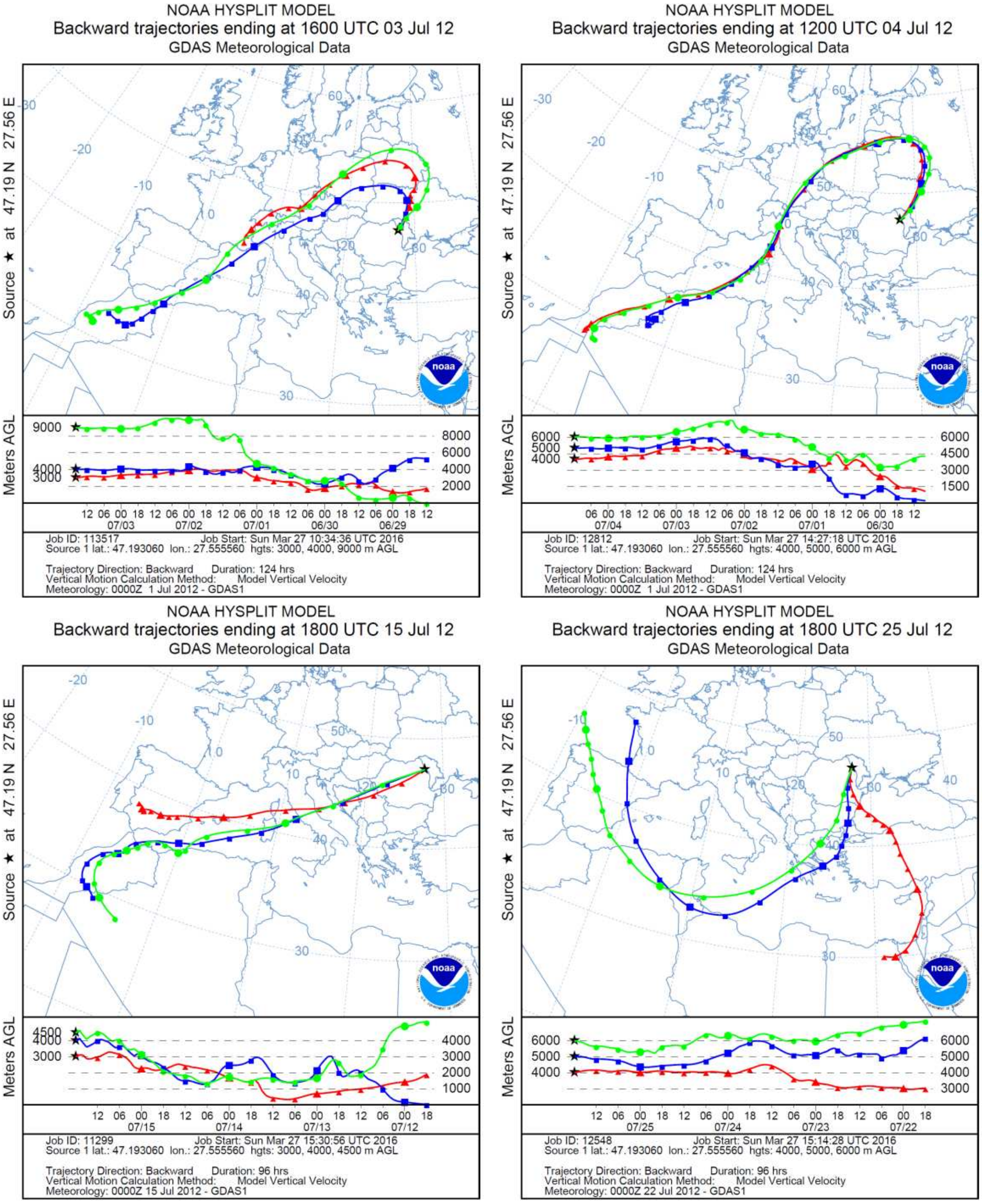


Figure 1. *Variation of Ångstrom parameter during July 2012.*

It can be observed that these intrusion events are originating from Sahara desert. These results are in agreement with the BSC DREAM model, confirming the presence of Saharan dust for 15th and 25th of July 2012 (*Fig. 3*). Even though for the days of 3rd and 4th of July 2012 the BSC DREAM model does not agree with the presence of Saharan dust above the observation site, particle trajectories indicated by HYSPLIT suggest the Sahara desert as possible origin of measured. The presence of Saharan dust for these days was previously shown by Unga et al (2013) taking into account the Lidar ratio from AERONET data. The given uncertainties are ruled out by comparison with previous observation quantities such as: the Ångstrom coefficient, the Single Scattering Albedo (*SSA*), and the LIDAR report calculated by AERONET.


NOAA HYSPLIT MODEL
Backward trajectories ending at 1600 UTC 03 Jul 12
GDAS Meteorological Data

NOAA HYSPLIT MODEL
Backward trajectories ending at 1200 UTC 04 Jul 12
GDAS Meteorological Data

NOAA HYSPLIT MODEL
Backward trajectories ending at 1800 UTC 15 Jul 12
GDAS Meteorological Data

NOAA HYSPLIT MODEL
Backward trajectories ending at 1800 UTC 25 Jul 12
GDAS Meteorological Data

Figure 2. *Air mass trajectories from HYSPLIT model.*

All the above-mentioned parameters indicate that in fact we are dealing with Saharan dust, since significant drops of the Ångstrom coefficient from 1.5 to 0.5 and $S_{AERONET}$ from 50 sr to 30 sr are recorded. Although the concentration is rather small, the optical quantities measured by the solar photometer correspond to those reported for Saharan dust class-type dust (Ansmann et al., 2011, 2009; Muller et al., 2010, 2009; Unga et al., 2013).

By analyzing data gathered for high altitudes, the local influence can be disregarded, thus obtaining additional information on the influence of Saharan dust over the local air quality. The concentration is rather different and quantitative analysis provided by the BSC DREAM indicates variations of the surface density from 0.05 to 0.50 g/m^2 over the observation site.

As can be seen from *Figure 3*, 15 and 25 July 2012 as clear days and Saharan dust influence days are confirmed by AERONET (*Fig. 1*) and HYSPLIT (*Fig. 2*). The absence of cloudiness is confirmed by both the BSC DREAM model and the meteorological data using the EUMETSAT satellite database - European Organization for the Exploitation of Meteorological Satellites (EUMETSAT, 2012). The above-mentioned models and experimental data are required to use the MAP3D chemical model.

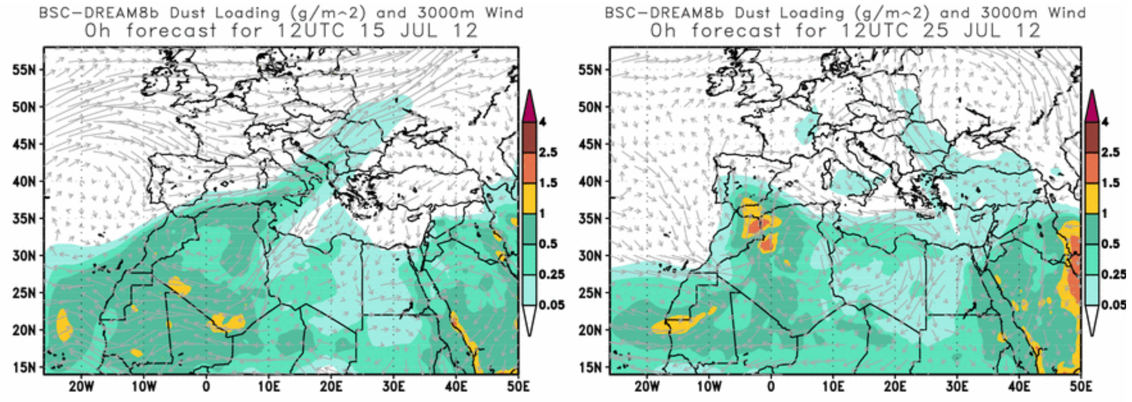


Figure 3. *Saharan dust loading by DREAM forecast for July 15th and July 25th, 2012.*

As it can be shown for the 3rd, 4th, 15th and 25th July 2012, the MAP3D model forecasts an increase of the PM$_{10}$ concentration (*Fig. 4*). These results are calculated for troposphere, indicating the daily maximum values for altitudes up to 8 km. The complete series from July 2012, indicates that PM$_{10}$ concentration increased over the entire tropospheric column (up to 8 km).

Besides, as it can be easily observed, a new concentration increase is forecasted starting with 19 July. By appealing to HYSPLIT and BSC DREAM models, we cannot confirm any additional intrusion of Saharan dust. The data acquired on 19 July indicate the strong necessity of gathering data from as many sources as possible.

Since the MAP3D model indicated an increase in PM10 concentration for 24 to 25 July and the DREAM model forecasted a powder density similar to that recorded on the 15 July (*Fig. 3*), these data were completed with products derived from the CALIPSO Lidar measurements. The total backscattering coefficient recorded by CALIPSO for the 00:35 to 00:48 interval (UTC) is given (*Fig. 5 - up*). The marked area represents the Lidar signal from atmospheric constituents. Appealing to inversion methods for calculation of optical parameters using the Lidar signal, a classification of the aerosols is given (*Fig. 5 - down*). From the data gathered by CALIPSO, a dispersion of the Saharan dust up to 5 km altitude can be emphasized. One can state that acquired experimental data match the forecast data.

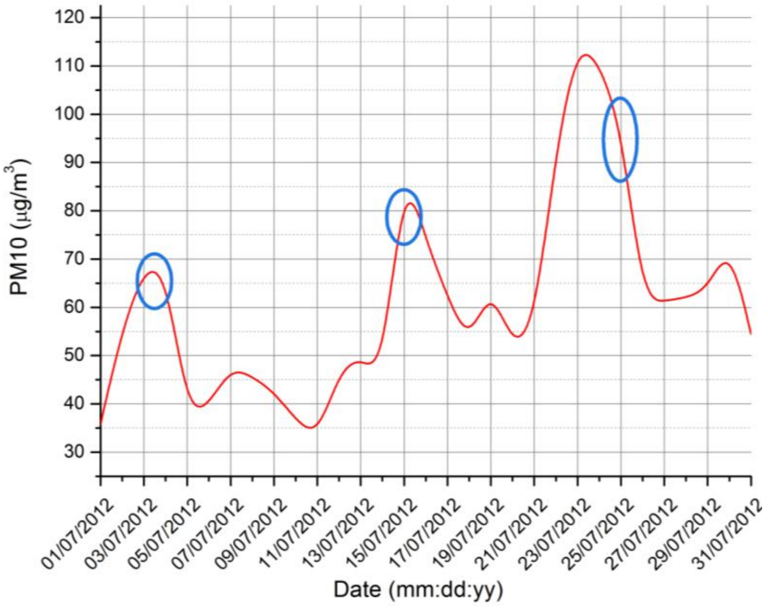


Figure 4. *Variation of daily maximum concentration of* PM_{10} *for July 2012 from MAP3D model.*

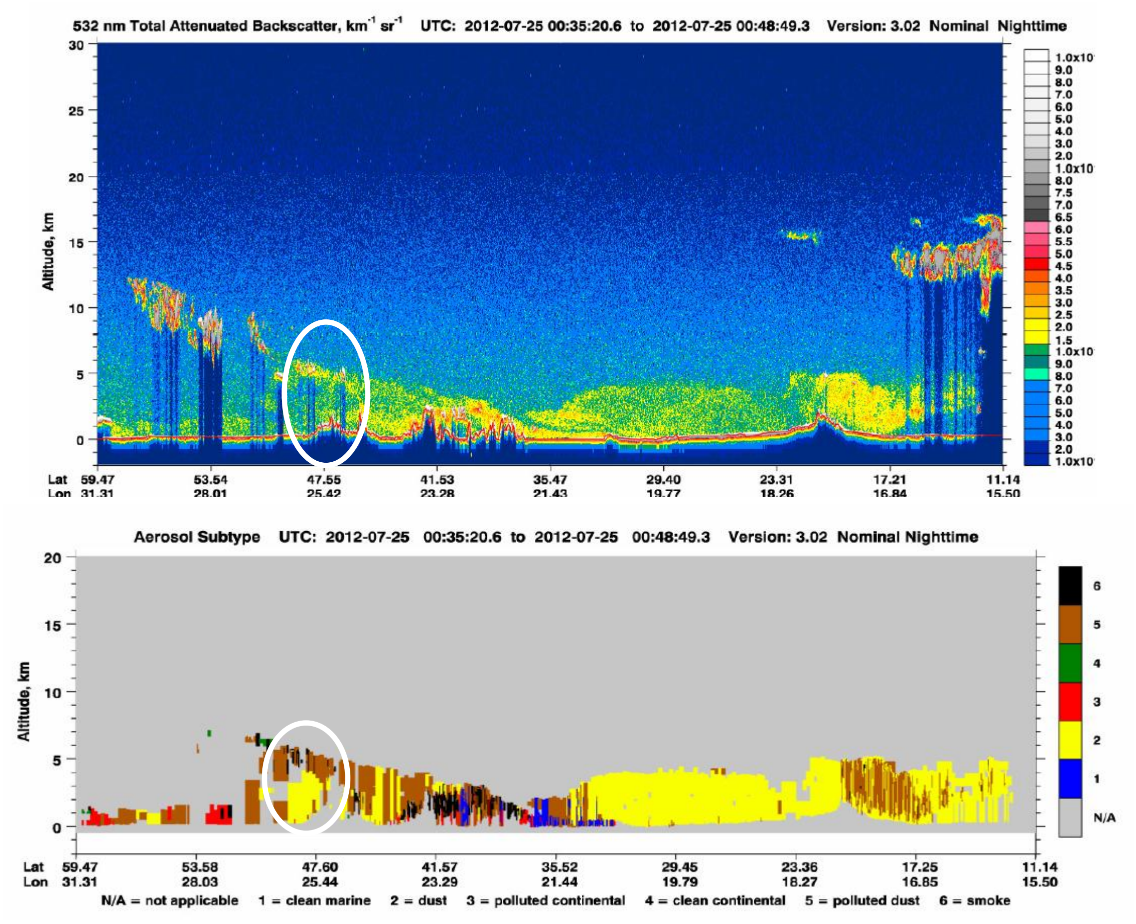


Figure 5. *(up): 532 nm backscatter Lidar profile from CALIPSO and (down): aerosol type classification.*

The influence of the Saharan dust storms over the local climate can be quantified at last. Recent studies indicate variations of ozone concentrations during the Saharan dust events (Frouin and McPherson, 2012; Ohde and Siegel, 2012). A decrease in the ozone concentration was explained by the fact that Saharan dust absorbs in the wavelength range below 440 nm. Another observation indicates a decrease in UV solar radiation intensity reaching the ground level, while the infrared spectrum is not affected. Based on these observations, a parameter closely monitored was the tropospheric ozone concentration.

In our case, the influence of Saharan dust over the tropospheric ozone production was not observed. Given that the data were gathered during clear days, the influence of atmospheric clouds can be excluded. As can be inferred from *Figure 6*, the variation of the tropospheric ozone concentration may be explained by the following equations (Cazacu et al., 2011; Seinfeld and Pandis, 2006):

$$
\begin{aligned}
&[NO_3 + h\nu(\lambda < 700nm)] \rightarrow NO + O_2 \\
&[NO_3 + h\nu(\lambda < 7580nm)] \rightarrow NO_2 + O \\
&[NO_2 + h\nu(\lambda < 420nm)] + O_2 \rightarrow NO + O_3
\end{aligned}
$$

As instance, the 3D distribution of the ozone given by the MAP3D model is not uniform up to altitudes as high as 8 km, as a consequence of the typical chemical reaction of ozone in the atmosphere (*Fig. 7*). Furthermore, no observable influence of the Saharan dust was noticed over our ground observatory, due to its small concentration.

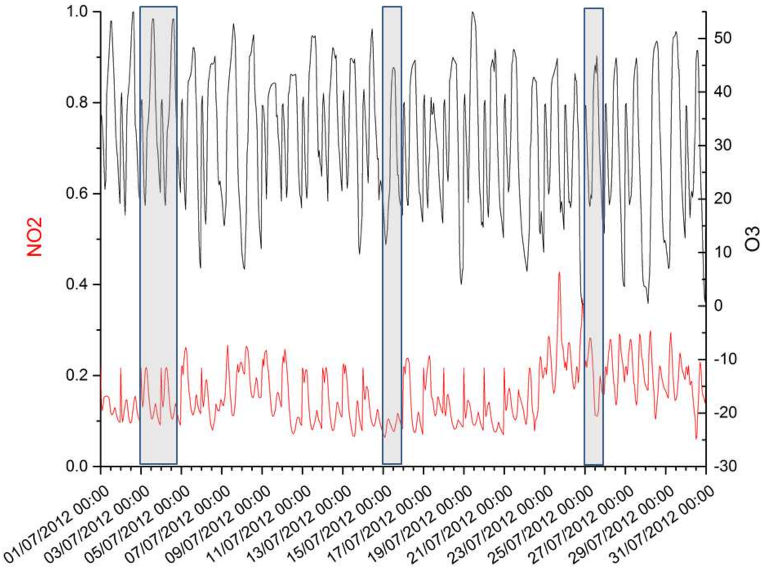


Figure 6. *Variation of NO_2 and O_3 concentrations during July 2012 forecasted by MAP3D model (ground level).*

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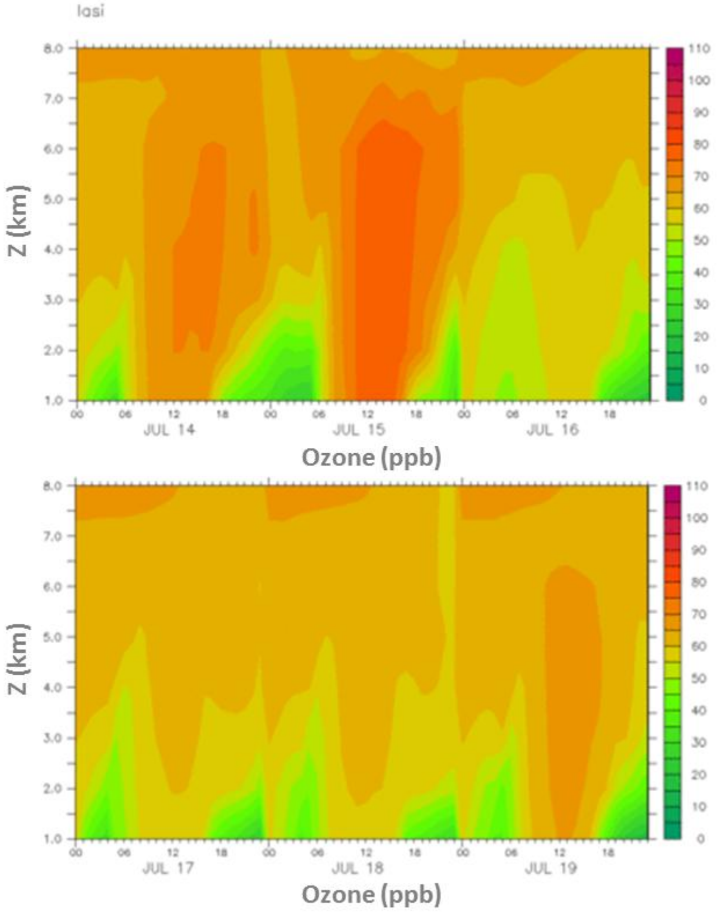

Figure 7. *Three-dimensional distribution of O_3 computed by MAP3D model (up to 8 km).*

Conclusions

The impact of Saharan dust in low and middle troposphere on the behavior of aerosol optical properties was emphasized using modern remote sensing techniques (sun photometer, CALIPSO) and modelling approaches (MAP3D, HYSPLIT, DREAM models).

On the 3rd, 4th, 15th and 25th of July 2012, AERONET data show values between 0.6 – 1.5 of Ångstrom parameter and values between 0.91 ÷ 0.99 of SSA at an increase of wavelengths from 440 nm to 1020 nm. Although these values indicate the presence of mineral dust, the AERONET Lidar ratio (e.g. 30 sr), HYSPLIT data, DREAM model and CALIPSO satellite data confirmed also the influence of Saharan dust load over Iasi_LOASL monitoring site.

The influence of Saharan dust on the tropospheric ozone production has not been clearly observed, probably because of strong absorption of UV-vis radiation ($\lambda<420$ nm) by dust particles. The variations of ozone and nitrogen dioxide concentrations are not directly related to Saharan dust loadings and only some seasonal variations are emphasized.

Further complementary measurements are required to fully understand the impact of Saharan dust on the ozone molecules at different altitudes in the troposphere using UV-molecular emission spectroscopy.

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REFERENCES

[1] Ansmann, A., Petzold, A., Kandler, K., Tegen, I., Wendisch, M., Muller, D., Weinzierl, B., Muller, T., Heintzenberg, J. (2011): Saharan Mineral Dust Experiments SAMUM-1 and SAMUM-2: What have we learned?. – Tellus, Series B: Chemical and Physical Meteorology 63(4): 403–429.

[2] Ansmann, A., Tesche, M., Knippertz, P., Bierwirth, E., Althausen, D., Müller, D., Schulz, O. (2009): Vertical profiling of convective dust plumes in southern Morocco during SAMUM. – Tellus, Series B: Chemical and Physical Meteorology 61(1): 340–353.

[3] Basart, S., Pérez, C., Nickovic, S., Cuevas, E., Baldasano, J.M. (2012): Development and evaluation of the BSC-DREAM8b dust regional model over Northern Africa, the Mediterranean and the Middle East. – Tellus B 64: 18539.

[4] Belegante, L., Talianu, C., Nemuc, A., Nicolae, D.N. (2011): Detection of local weather events from multiwavelength lidar measurements during the EARLI09 campaign. – Romanian Journal of Physics 56(3-4): 484–494.

[5] Cattrall, C., Reagan, J., Thome, K., Dubovik, O. (2005): Variability of aerosol and spectral lidar and backscatter and extinction ratios of key aerosol types derived from selected Aerosol Robotic Network locations. – Journal of Geophysical Research D: Atmospheres 110(10): 1–13.

[6] Cazacu, M.M., Timofte, A., Balin, I., Dimitriu, D.G., Gurlui, S. (2011): Complementary atmospheric urban pollution studies in the North-East region of Romania, Iasi county. – Environmental Engineering and Management Journal 10 (1): 139–145.

[7] Cazacu, M.M., Timofte, A., Talianu, C., Nicolae, D., Danila, M.N., Unga, F., Dimitriu, D.G., Gurlui, S. (2012): Grimsvotn Volcano: atmospheric volcanic ash cloud investigations, modelling-forecast and experimental environmental approach upon the Romanian area. – Journal of Optoelectronics and Advanced Materials 14(5-6): 517–522.

[8] Cazacu, M.M., Timofte, A., Unga, F., Albina, B., Gurlui, S. (2015): AERONET data investigation of the aerosol mixtures over Iasi area, One-year time scale overview. – Journal of Quantitative Spectroscopy and Radiative Transfer 153: 57–64.

[9] Couach, O., Kirchner, F., Jimenez, R., Balin, I., Perego, S., Van Den Bergh, H. (2004): A development of ozone abatement strategies for the Grenoble area using modeling and indicators. – Atmospheric Environment 38(10): 1425–1436.

[10] Draxler, R.R., Rolph, G.D. (2015): HYSPLIT (HYbrid Single-Particle Lagrangian Integrated Trajectory) Model access via NOAA ARL READY Website. [Online]. Available from: http://ready.arl.noaa.gov/HYSPLIT.php.

[11] Dubovik, O., Holben, B., Eck, T.F., Smirnov, A., Kaufman, Y.J., King, M.D., Tanré, D. Slutsker, I. (2002): Variability of Absorption and Optical Properties of Key Aerosol Types Observed in Worldwide Locations. – Journal of the Atmospheric Sciences 59(3): 590–608.

[12] EUMETSAT (2012): Exploitation of Meteorological Satellites, Satellite images database from July of 2012. [Online]. Available from: http://www.eumetsat.int.

[13] Giles, D.M., Holben, B.N., Eck, T.F., Sinyuk, A., Smirnov, A., Slutsker, I., Dickerson, R.R., Thompson, A.M., Schafer, J.S. (2012): An analysis of AERONET aerosol absorption properties and classifications representative of aerosol source regions. – Journal of Geophysical Research: Atmospheres 117: D1720.

[14] Gothard, M., Nemuc, A., Radu, C., Dascalu, S. (2014): An intensive case of saharan dust intrusion over south east Romania. – Romanian Reports in Physics 66(2): 509–519.

[15] Haywood, J.M., Pelon, J., Formenti, P., Bharmal, N., Brooks, M., Capes, G., Chazette, P., Chou, C., Christopher, S., Coe, H., Cuesta, J., Derimian, Y., Desboeufs, K., Greed, G., Harrison, M., Heese, B., Highwood, E.J., Johnson, B., Mallet, M., et al. (2008): Overview of the Dust and Biomass-burning Experiment and African Monsoon Multidisciplinary Analysis Special Observing Period. – Journal of Geophysical Research 113: D00C17.

[16] Holben, B.N., Eck, T.F., Slutsker, I., Tanré, D., Buis, J.P., Setzer, A., Vermote, E., Reagan, J.A., Kaufman, Y.J., Nakajima, T., Lavenu, F., Jankowiak, I., Smirnov, A. (1998): AERONET—A Federated Instrument Network and Data Archive for Aerosol Characterization. – Remote Sensing of Environment 66(1): 1–16.

[17] Labzovskii, L., Toanca, F., Stefan, S. (2014): Determination of saharan dust properties over bucharest, Romania part 1: Procedures and algorithms. – Romanian Journal of Physics 59(9-10): 1084–1096.

[18] Mamouri, R.E., Amiridis, V., Papayannis, A., Giannakaki, E., Tsaknakis, G. and Balis, D.S. (2009): Validation of CALIPSO space-borne-derived attenuated backscatter coefficient profiles using a ground-based lidar in Athens, Greece. – Atmospheric Measurement Techniques 2(2): 513–522.

[19] Mihai, L., Stefan, S. (2011): Temporal Variation of Aerosol Optical Properties at Măgurele, Romania. – Journal of Atmospheric and Oceanic Technology 28: 1307-1316.

[20] Muller, D., Ansmann, A., Freudenthaler, V., Kandler, K., Toledano, C., Hiebsch, A., Gasteiger, J., Esselborn, M., Tesche, M., Heese, B., Althausen, D., Weinzierl, B., Petzold, A. and Von Hoyningen-Huene, W. (2010): Mineral dust observed with AERONET Sun photometer, Raman lidar, and in situ instruments during SAMUM 2006: Shape-dependent particle properties. – Journal of Geophysical Research Atmospheres 115(11): 1–18.

[21] Muller, D., Heinold, B., Tesche, M., Tegen, I., Althausen, D., Arboledas, L.A., Amiridis, V., Amodeo, A., Ansmann, A., Balis, D., Comeron, A., D'amico, G., Gerasopoulos, E., Guerrero-rascado, J.L., Freudenthaler, V., Giannakaki, E., Heese, B., Iarlori, M., Knippertz, P., et al. (2009): EARLINET observations of the 14-22-May long-range dust transport event during SAMUM 2006: Validation of results from dust transport modelling. – Tellus, Series B: Chemical and Physical Meteorology 61(1): 325–339.

[22] Nickovic, S., Kallos, G., Papadopoulos, A., Kakaliagou, O. (2001): A model for prediction of desrt dust cycle in the atmosphere. – Journal of Geophysical Research 106(D16): 18113.

[23] Nicolae, D., Belegante, L., Nemuc, A. (2010): Laser remote sensing in atmosphere investigation. – Optoelectronics and Advanced Materials – Rapid Communications 4(12): 1946–1951.

[24] Ohde, T., Siegel, H. (2012): Impacts of Saharan dust and clouds on photosynthetically available radiation in the area off Northwest Africa. – Tellus B 64: 17160.

[25] Papayannis, A., Nicolae, D., Kokkalis, P., Binietoglou, I., Talianu, C., Belegante, L., Tsaknakis, G., Cazacu, M.M., Vetres, I. and Ilic, L. (2014): Optical, size and mass properties of mixed type aerosols in Greece and Romania as observed by synergy of lidar and sunphotometers in combination with model simulations: A case study. – Science of The Total Environment 500-501:277–294.

[26] Pappalardo, G., Wandinger, U., Mona, L., Hiebsch, A., Mattis, I., Amodeo, A., Ansmann, A., Seifert, P., Linné, H., Apituley, A., Alados Arboledas, L., Balis, D., Chaikovsky, A., D'Amico, G., De Tomasi, F., Freudenthaler, V., Giannakaki, E., Giunta, A., Grigorov, I., et al. (2010): EARLINET correlative measurements for CALIPSO: First intercomparison results. – Journal of Geophysical Research 115: D00H19.

[27] Pérez, C., Nickovic, S., Pejanovic, G., Baldasano, J.M. and Özsoy, E. (2006): Interactive dust-radiation modeling: A step to improve weather forecasts. – Journal of Geophysical Research 111: D16206.

[28] Seinfeld, J.H., Pandis, S.N. (2006): Atmospheric Chemistry and Physics: From Air Pollution to Climate Change, 2nd Edition. – In: John Wiley and Sons Inc., New Jersey.

[29] Shon, Z.-H., Madronich, S., Song, S.-K., Flocke, F.M., Knapp, D.J., Anderson, R.S., Shetter, R.E., Cantrell, C.A., Hall, S.R., Tie, X. (2008): Characteristics of the NO-NO_2-O_3 system in different chemical regimes during the MIRAGE-Mex field campaign. – Atmospheric Chemistry and Physics 8(23): 7153–7164.

[30] Timofte, A., Cazacu, M.M., Radulescu, R., Belegante, L., Dimitriu, D.G., Gurlui, S. (2011): Romanian lidar investigation of the Eyjafjallajokull volcanic ash. – Environmental Engineering and Management Journal 10(1): 91–97.

[31] Unga, F., Cazacu, M.M., Timofte, A., Bostan, D., Mortier, A., Dimitriu, D.G., Gurlui, S., Goloub, P. (2013): Study of tropospheric aerosol types over Iasi , Romania , during summer of 2012. – Environmental Engineering and Management Journal 12(2):297–303.

[32] Winker, D.M., Vaughan, M.A., Omar, A., Hu, Y., Powell, K.A., Liu, Z., Hunt, W.H., Young, S.A. (2009): Overview of the CALIPSO Mission and CALIOP Data Processing Algorithms. – Journal of Atmospheric and Oceanic Technology 26(11): 2310–2323.

SYNERGY OF SECONDARY COMPOUNDS IN THE ARTIFICIAL FOODS OF THE LAST INSTAR OF *HYPHANTRIA CUNEA* (DRURY) (LEPIDOPTERA: ARCTIIDAE)

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Abstract. This study aims at putting forward the synergy of secondary compounds on total food consumption, the amount of pupal lipid and protein, weight of pupae, and development time in the last instar of *Hyphantria cunea*. A non-choice feeding experiment was applied with a total of 14 foods; one of them was the control food, 9 of them were prepared by adding different concentrations of tannic acid, gallic acid and *p*-coumaric acid (1, 3, 5% of dry weight) to the control food, 3 foods with 2 combinations prepared by adding 3% concentration tannic acid, gallic acid and *p*-coumaric acid, and 1 food with three combinations.The total food consumption of the foods with double combinations was different when compared to control food. The pupal weights of the larvae which were fed with food containing two combinations (tannic acid+ *p*-coumaric acid) and the ones fed with food containing three combinations (tannic acid + gallic acid + *p*-coumaric acid) were different from the ones in the control group. The pupal protein content in food containing triple combinations was lower than the control food. The pupal lipid content in food containing triple combinations was more than the control food. It was found that the development time increased as the tannic acid concentration of the food increased; on the contrary, the development time decreased as the gallic acid concentration of the food containing gallic acid increased.

Keywords: *Hyphantria cunea, secondary compounds, tannic acid, gallic acid, p-coumaric acid*

Introduction

Secondary plant compounds serve different ecological functions such as allelopathy, herbivore deterrence, attraction of pollinators, predation of organisms on herbivores, and antifungal effect (Chou and Kuo, 1986; Harborne, 1977; Baas, 1989; Dicke and Sabelis, 1989). Through the observations deduced from the feeding behaviours of herbivore insects, it is known that these compounds perform the function of being deterrent to the herbivores or being toxic against them (Fraenkel, 1959; Ehrlich and Raven, 1964).

Phenolic compounds, which are a group of secondary compounds, are aromatic compounds containing one or more hydroxyl groups. There are more than 8000 known phenolic compounds in nature and 14 groups are categorized depending on the arrangements of these compounds; a small group of these compounds functions in plant-herbivore interactions (Strack, 1997). The physiological roles of phenolic compounds vary depending on their chemical structure and physicochemical environment (such as PH, redox potential, oxidase concentration, oxidants and antioxidants) (Larson, 1995; Metadiewa et al., 1999; Sugihara et al., 1999; Galati et al., 2002; Sakihama et al., 2002; Hagerman et al., 2003).

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Tannins which are accumulated against herbivorous animals in many plant species and especially in trees (Bernays et al., 1989; Peters and Constabel, 2002) show their biological effects through protein binding. It is shown that they have affected a great number of herbivores ranging from insects to mammals (Bernays et al., 1989). Gallic acid is a phenolic whose molecular weight is small. Gallic acid is derived from chrosmic acid by means of shikimic acid. It is known that gallic acid has antioxidant, antibacterial, anti-inflammatory and chemical protective properties (Kang et al., 2008; Kim et al., 2006; Giftson et al., 2009). *p*-Coumaric acid is an intermediate in biosynthesis of lignin and has a broad distribution in angiosperm plants (Berenbaum, 1982). *p*-Coumaric acid is of great importance because of its protective chemical and antioxidant properties (Torres and Rosazza, 2001).

This research aims at studying the synergistic effect of secondary compounds on *H. cunea*. For this purpose, 3 phenolic compounds (tannic acid, gallic acid and *p*-coumaric acid) with different molecular weights have been used and the synergistic effect of these phenolics on the nutrition and growth of the last instar has been studied.

Materials and methods

Collection of the larvae

H. cunea larvae were collected during the field research from hazelnut gardens on the coast within the Çarşamba border in Samsun in July, 2011.

Feeding experiment

10 different data were collected from the last instar larvae with an aim to be associated with a number of earlier publications in literature (Telang et al., 2001; Lee et al., 2002; Henriksson et al., 2003; Lee et al., 2004). For each food group, 10 larvae were put in plastic cases (sized 5cmx10cmx2cm) one by one. The larvae in the cases were put in an incubator adjusted to 27 ^{0}C heat and 70% humidity within a period of 16 h light - 8 h dark. Each molting was noted and the data obtained from the last instar to pupae were evaluated. In feeding experiments, each day after weighed in 0,001 gr sensitive scale a new food, it was given and after the remaining food was dried in the incubator, and their dry weight was calculated. Moreover, each day the changes in the weight and their feces were noted and these measurements were followed until the larvae became pupae.

Secondary compounds

The first factor in choosing the secondary compounds is whether these compounds react with each other when they are included within food in double and triple combinations. As three of the secondary compounds have an acidic property- though the reaction was a weak possibility- tannic acid, gallic acid and *p*-coumaric acid were mixed in double and triple combinations in aqueous media at room temperature with a proportion of 1:1. However, no chemical reaction or change was observed. The other reason for choosing these 3 secondary compounds (tannic acid ($C_{76}H_{52}O_{46}$), gallic acid ($C_7H_6O_5$), *p*-coumaric acid ($C_9H_8O_3$)) is that they have different molecular weights.

Content of artificial foods

In this research, artificial foods with different contents were prepared with the aim of determining the effects of different secondary compounds and their different combinations on the nutrition and the growth of *H. cunea*. The artificial food which was developed by Yamamoto (1969) to feed the larvae was modified (only the protein and carbohydrate amounts were changed) and used as the control food and foods were prepared by using the secondary compounds such as tannic acid (TA) (Sigma, 16201), gallic acid (GA) (Sigma, G 7384) and *p*-coumaric acid (PCA) (Sigma, C 9008) suitable for the purpose of this research. By adding an amount of 1, 3 and 5% of tannic acid, gallic acid and *p*-coumaric acid of total dry weight to artificial food, 9 foods were prepared and by adding an amount of 3% of tannic acid, gallic acid and *p*-coumaric acid of dry weight to artificial food, 3 foods with double combination, a food with triple combination (TA+GA+PCA) and a control food were prepared. Therefore, 14 foods were prepared. These foods are shown in *Table 1.*

Table 1. *Food types and contents*

Food types	Food contents
A	Control Food (CF)
B	CF + %1 T.A.
C	CF + %3 T.A.
D	CF + %5 T.A.
E	CF + %1 G.A.
F	CF + %3 G.A.
G	CF + %5 G.A.
H	CF + %1 P.C.A.
K	CF + %3 P.C.A.
L	CF + %5 P.C.A.
M	CF + %3 T.A. + %3 G.A.
N	CF + % 3 T.A. + % 3 P.C.A.
P	CF + %3 G.A. + %3 P.C.A.
R	CF + % 3 T.A. + %3 G.A. + %3 P.C.A.

The content of Yamamoto's artificial food is wheat germ (Sigma, W-0125), casein as the protein (Sigma, C 6554), (30g/kg (modified amount)); saccharose as the carbohydrate (Sigma, S 1888), (30g/kg (modified amount)), torula yeast (Sigma, Y 4625), vitamin mixture (Sigma, V-1007), salt mixture (Sigma, W 1374), cholesterol (Sigma, C 2044), sorbic acid (Sigma, S 1626), methyl paraben (Sigma, H 3647), linseed oil (Sigma, L 3026), agar and water.

Statistical analyses

In this research, whether there was a difference between the data of total food consumption, pupal weight, the amount of pupal lipid and protein, and the development time was determined by ANOVA (Dunnet test). For these tests, SPSS 17 version from a minicomputer was used.

Results

In accordance with the feeding experiment carried out on *H. cunea* larvae and with 14 artificial foods, 13 of these were prepared by adding secondary compounds at different rates and 1 as control group, the consumption amount of larvae, protein and lipid amounts of pupae, pupal weight and development time were explained under each heading. The data obtained from the Dunnet test is shown in *Table 2*.

Total food consumption

When compared to control food, total food consumption was statistically different in larvae that were fed in the other food groups. Compared with the control group, when 3 different concentrations of tannic acid, gallic acid and *p*-coumaric acid were considered, it is certain that there was a difference in the effect of 3 secondary compounds on the total food consumption. It was found that in comparison to the control group, as the secondary compound concentration increased in the food containing both tannic and *p*-coumaric acid (except for H food), there was a decrease in the total food consumption. On the contrary, as the secondary compound concentrations increased in food containing gallic acid, the total food consumption also increased.

The lowest total food consumption was found in the larvae fed by food containing 5% of tannic acid. When compared to the control group, the groups that had the maximum total food consumption contained food that had 1, 3 and 5 % gallic acid. The total food consumptions of the larvae fed on double (M, N, P foods) and triple (R food) combinations were different when compared to control food (A). The total food consumption in M food was much more than the control food. However, the total food consumption in N food was less than that of the control group. The total food consumption in the larvae that were fed with P food increased when compared to control group. Moreover, it should be pointed out that there was no statistically, significant difference between the larvae feeding on R food and the control food.

Pupal weight

The pupal weight of the larvae feeding on the food containing 1, 3 and 5% concentration of TA and GA and on the food containing 5% of PCA were statistically different from the control group. In comparison to the control food, when tannic acid (except for 3% of TA) and *p*-coumaric acid concentrations in foods increased, the pupal weight decreased. On the contrary, when the gallic acid concentrations increased, the pupal weight also increased.

The lowest pupal weight was found in the larvae feeding on N food. The highest pupal weight was obtained from the larvae feeding on G food. N food among the food containing double combinations and the R food containing triple combinations were statistically different from those in the control food. The pupal weight of the larvae feeding on N food among the double combinations was lower than larvae feeding on control food. Similarly, the pupal weight of the larvae feeding on R food was lower than those in the control food.

Pupal protein content

There was no statistical difference between the control food and the pupal protein content of the larvae feeding on H and P foods. When the secondary compounds which were added to the foods containing both tannic and *p*-coumaric acid increased, the pupal weight decreased. On the contrary, when the gallic acid concentration increased, the pupal weight also increased.

Table 2. *The development time, total food consumption, pupal weight, the amount of pupal protein and lipid of H.cunea in the no-choice feeding experiment.*

	Food types	Total food consumption (mg)	Pupal weight (mg)	Amount of pupal protein (mg)	Amount of pupal lipid (mg)	Development time (day)
	A	223,8±2,4	25,1±1,0	7,3±0,2	8,9±0,3	3,2±0,1
AVARAGE±STANDARD ERROR	B	210,1±1,4	20,2±0,6	6,0±0,2	7,7±0,3	3,3±0,2
	C	200,6±1,8	20,3±0,7	5,7±0,1	6,2±0,1	4,2±0,1
	D	182,0±1,7	16,4±0,5	5,3±0,1	4,7±0,2	4,8±0,3
	E	294,4±1,9	33,8±0,7	8,2±0,2	9,4±0,3	3,3±0,2
	F	306,5±1,8	35,0±0,5	11,0±0,1	12,9±0,3	3,2±0,1
	G	321,5±1,8	36,1±0,6	11,6±0,1	12,1±0,3	3,1±0,1
	H	243,0±3,1	25,5±0,6	7,5±0,2	6,8±0,2	3,2±0,1
	K	213,0±3,1	23,4±0,4	6,4±0,2	6,0±0,2	3,1±0,1
	L	197,0±1,7	20,2±0,3	5,7±0,1	5,4±0,1	3,1±0,1
	M	236,0±6,2	25,9±0,7	8,3±0,1	9,5±0,2	4,1±0,2
	N	206,6±1,8	15,4±0,6	5,9±0,1	5,3±0,2	3,6±0,2
	P	274,7±1,3	26,2±0,3	7,7±0,1	7,4±0,2	3,3±0,2
	R	220,0±2,2	20,6±0,8	6,1±0,6	6,2±0,1	4,1±0,2
ANOVA	df	139	139	139	139	139
	F	278.5	114.2	204.9	105.3	11.0
	P	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	Dunnet test	B < 0,05 C < 0.001 D < 0.001 E < 0.001 F < 0.001 G < 0.001 H < 0.001 K < 0,05 L < 0.001 M < 0,05 N < 0.001 P < 0.001	B <0,001 C < 0.001 D < 0.001 E < 0.001 F < 0.001 G < 0.001 L < 0.001 N < 0.001 R < 0.001	A < 0,05 B < 0.001 C < 0.001 D < 0.001 E < 0.001 F < 0.001 K < 0,05 L < 0.001 M < 0,05 N < 0.001 R < 0.001	B < 0,05 C < 0.001 D < 0.001 F < 0.001 G < 0.001 K < 0,001 L < 0.001 N < 0.001 P < 0.001 R < 0.001	C < 0.001 D < 0.001 M < 0,05 R < 0.05

Statistically significant means according to Dunnet's Multiple Range Test (p<0.05)

When compared to control food, the minimum pupal protein content was obtained from the larvae feeding on the food (D) containing 5% of TA. The highest pupal protein content was obtained from the larvae feeding on the food (G) containing 5% of GA. Control food was lower than the foods (E, F and G) containing 1, 3 and 5% of GA, but higher than the foods (B, C and D) containing 1, 3 and 5% of TA. The pupal protein content of the larvae feeding on control food (except for H food) was more than the larvae feeding on K and L foods. It was found that of all the pupal protein content obtained from the larvae feeding on the foods containing double combinations, only the pupal protein content of the larvae feeding on N food was lower than the control food. The pupal protein content in R food was lower than the control food. However, it is significant that there was no statistical difference in the larvae feeding on H and P foods when compared to the control group.

Pupal lipid content

The pupal lipid content of the larvae feeding on the control food was statistically different from other groups. There was no statistical difference in the pupal lipid content of the larvae feeding on the food (M) containing TA+GA and the food (E) containing 1% of GA when compared to the control food. Compared to control food, as the secondary compounds which were added to the foods containing both tannic and *p*-coumaric acid increased, the pupal lipid content decreased. On the contrary, when the gallic acid concentration in foods containing gallic acid (except for the food containing 5% of GA) increased, the pupal lipid content also increased.

The minimum pupal lipid content was found in the larvae feeding on the food containing 5% of TA. When compared to the control group, the group that had the maximum pupal and lipid content was obtained from the larvae feeding on the food (F) containing 3% of GA. It was found that lipid contents obtained from the pupae which fed on the foods containing 1, 3 and 5% of GA (except for M food) were more than the other groups. The pupal lipid content in R food was more than the control food. When compared to the control food, there was not any statistical difference in the larvae feeding on E and M foods.

Development time

It was found that the development time of the larvae feeding on the control food was different from those feeding on C, D, M and R foods, but there was no statistical difference from the larvae feeding on the others foods. When tannic acid concentration increased, the development time was prolonged. On the contrary, when the gallic acid concentration in food containing gallic acid increased, the development time shortened. The shortest development time was observed in the larvae feeding on K, L and G foods. The longest development time was observed in the larvae feeding on D food.

Discussion

When three different concentrations of tannic acid, gallic acid and *p*-coumaric acid (1, 3 and 5%) are taken into consideration, it can be seen that these three secondary compounds had different effects on the total food consumption of *H. cunea* larvae. When the secondary compounds in the foods (except for H food) containing both tannic and *p*-coumaric acid increased, the total food consumption of the larvae decreased. On the contrary, when the gallic acid concentration increased, the total food consumption of the larvae also increased.

Even though tannic acid, gallic acid and *p*-coumaric acid are three phenolic compounds, each of them had a different effect on *H. cunea*. Therefore, the result supports the statement in literature, which is, the chemical structure of phenolic compounds and the physiological roles vary with the phenolic in relation to the physicochemical environment (for example, pH, redox potential, oxidase concentrations, oxidants and antioxidants) (Larson, 1995; Metadiewa et al., 1999; Sugihara et al., 1999; Galati et al., 2002; Sakihama et al., 2002; Hagerman et al., 2003). In this research, it was found that as the tannic acid concentration in the food increased, the total food consumption decreased. This result is opposite to the fact that the effects of the low levels of tannin on choosing foods and consumption amounts are hyperbole (Klocke and Chan, 1982).

Obtaining the minimum total food consumption from the larvae feeding on the food (D) containing 5% of tannic acid resemble the results of the previous research in literature (Simpson and Raubenheimer, 2001; Hemming and Lindroth, 1995; Hemming and Lindroth, 2000). The reason for the decrease in total food consumption can result from the deterrent property of tannic acid on feeding.

The highest total food consumption (1, 3 and 5%) were the artificial foods (E, F and G foods) containing gallic acid. It is pointed out that free amino acids and trehalose concentration of basic blood glucose of the insects have an importance in feedback mechanism (Simpson and Raubenheimer, 1993; Simpson and Simpson, 1992; Friedman et al., 1991; Thompson and Redak, 2000). Therefore, the results may show that the gallic acid affected these two mechanisms.

The total food consumption of the larvae feeding on M food (CF+ 3% of TA+ 3% of GA) was much more than the control group. While the total food consumption obtained from the larvae feeding on C food was less than the control group, it is significant that two secondary compounds (tannic acid and gallic acid) in M food with synergistic effect were much more than the control group. Similarly, while the total food consumption of the larvae feeding on P food (CF+ 3% of GA+ 3% of PCA) increased, it is significant that the total amount decreased in the larvae feeding on K food (CF+ 3% of PCA) in comparison to the control group. The total food consumption in N food (CF+ 3% of TA+ 3% of PCA) decreased when compared to the control group. The total food consumption in the larvae feeding on C and K food was less than the control group. It is an important result that there was no difference between the larvae feeding on R food and the control group. The results that were found in the larvae feeding on M, P and R foods in particular may mean that the synergistic effect is different.

The pupal lipid contents in B, C and D foods decreased when compared to the control group. These foods all contained tannic acid (1, 3 and 5% in order). Simpson and Raubenheimer (2001) have found in their study with *Locusta migratoria* that tannic acid has not prevented the carbohydrates in the foods from turning into fats in the body.

It is significant that the pupal lipid content of the larvae fed on M food was not different from the control group. However, while the pupal lipid content of the larvae feeding on C food decreased, the pupal lipid content of those feeding on F food increased. The similarity of the larvae feeding on M food that contains two secondary compounds shows the difference of the synergistic effects. On the other hand, the decrease in the pupal lipid content in the larvae feeding on R food that contains three secondary compounds and the difference from the results obtained from M food can show how triple synergistic effect can change. The foods containing the highest amount of pupal lipid content when compared to the control group were F (CF+ 3% of GA) and G (CF+ 5% of GA) foods. There was no difference between the larvae feeding on E food (CF + 1% of GA) and the control group. This result may mean that gallic acid can show its effects in higher concentrations.

The lipids used during the adult period were derived from the lipids stored during the pre-adult phases (Giron and Casas, 2003). When the concentration increased in the larvae feeding on the foods that contained only tannic acid (1, 3 and 5%) and *p*-coumaric acid (1, 3 and 5%) concentration, the decrease in the lipid content could be a disadvantage for *H. cunea* and the highest lipid content of the larvae feeding on the foods with gallic acid could be an advantage.

The two highest amounts of pupal protein content (F and G foods) when compared to the control group were found in the larvae feeding on the foods that contained gallic acid. While there was no difference between the control group and the larvae feeding on P food (synergy), it is significant that the lipid content in the larvae feeding on F food was high. The low amount of pupal protein content in triple combinations (R food) when compared to the control group may mean that the negative effect of tannic acid and *p*-coumaric acid in the food transcended the positive effect of gallic acid. The stored proteins carried from the larval period to the adult period can play an important role especially due to the limited nitrogen consumption of adult herbivore insects (Hahn, 2005). Therefore, the gallic acid in the foods can be an advantage in stored protein. In this study, when tannic acid and *p*-coumaric acid concentrations in the foods (except for H food) increased, pupal protein content decreased. However, it was pointed out that the protein content of *L. migratoria* was higher than those feeding on foods without tannic acid (Simpson and Raubenheimer, 2001). In the single, double and triple combinations of tannic acid (except for TA+GA combination), all the groups have a low amount of pupal protein content. In this study, the reason for the pupal protein content to be low in the food groups containing tannic acid when compared to the other secondary compounds can result from the tannic acid having a more negative effect**.**

Simpson and Raubenheimer (2001) have pointed out when the amount of tannic acid increases in the grasshoppers feeding on foods with tannic acid, the weight decreases in *L. migratoria*. Similarly in this study, when the tannic acid concentration increased (except for C food), the pupal weight decreased. This result is similar to the statement that both in insects and vertebrates, the negative effects of tannins can be related to the high tannin concentration (Aerts et al., 1999). The lowest pupal weight was found in the larvae feeding on N (CF +3 % of TA + 3% of PCA) food. Even though there was no difference between the larvae feeding on K food (CF+ 3% of PCA) when compared to the control group, the lowest amount in the larvae feeding on N food can show the synergistic effect.

The previous research pointed out that the differences in the secondary compounds content change the food choice and performance of *Malacosoma disstria* larvae in a definite way (Hemming and Lindroth, 1995; Hemming and Lindroth, 2000). The highest amounts of pupal weight were found in the larvae feeding on E, F and G foods. The low weight of the pupae can affect the fecundity of the adult females (Honek, 1993). The higher amount of weight of the larvae feeding on foods containing gallic acid (E, F and G foods) can affect fecundity positively.

The longest development time was observed in the larvae feeding on C and D foods. Simpson and Raubenheimer (2001) have found that the larval periods extend when tannic acid is added to the food in *L. migratoria*. The extension of development time, the chance of facing the natural enemies during feeding or searching for food (Bernays, 1997) or the increase of predator/parasitism risk with a longer development and feeding time (Moran and Hamilton, 1980; Loader and Damman, 1991; Benrey and Denno, 1997) are also available for this species. One of the significant results of this study is that the development time of the larvae fed on M and R foods also lengthened out. It can be said that tannic acid in both foods had a stronger effect on the synergistic effect than gallic acid and *p*-coumaric acid. Ehrlich and Raven (1964) expressed that during the co-evolution process, following the spread and speciation of insects, new defense substances emerge on the parts of plants and then defenses against insects evolve. Even though some of the herbivore insects are adapted to newly produced defensive compounds sooner or later (Bernays, 1998); in this study the negative effect of tannic acid on the development time of *H. cunea* larvae is obvious.

Conclusions

It can be said that *H. cunea* larvae, a polyphagous insect, have a great ecological tolerance to feeding and secondary compounds. Having a great ecological tolerance to secondary compounds may mean that it is hard to struggle with *H. cunea* during population explosion.

In this research, the reaction of *H. cunea* to different secondary compounds was determined and it was observed that the larvae were resistant to concentrations of the secondary compounds and could reach the pupal period. Even though it is believed that plant phenolics play an important role in chemical defense against herbivores, some specific physiological effects of them can be changed on insects (Harborne, 1991; Appel, 1993). It can be said that *H. cunea* larvae are physiologically resistant to secondary compounds. Moreover, the results obtained from the larvae fed on double and triple combinations of secondary compounds used in this research show that the synergistic effect is different. Further research with other species will make the significance of the synergistic effect become clearer.

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REFERENCES

[1] Aerts, R.J., Barry, T.N., McNabb, W.C. (1999): Polyphenols and agriculture: beneficial effects of proanthocyanidins in forages. - Agriculture Ecosystem and Environment 75: 1-12.

[2] Appel, H. (1993): Phenolics in ecological interactions: The importance of oxidation. - Journal of Chemical Ecology 19: 1521-1552.

[3] Baas, W.J. (1989): Secondary plant compounds, their ecological significance and consequences for the carbon budget. - In: Lambers, H., Cambridge, M.L., Konings, H., Pons, T.L. (ed.) Causes and consequences of variation in growth rate and productivity of higher plants, Introduction of the carbon/nutrient cycle theory, Netherlands, 313-340.

[4] Benrey, B., Denno, R.F. (1997): The slow-growth-high-mortality hypothesis: a test using the cabbage butterfly. - Ecology 78: 987-999.

[5] Berenbaum, M. (1982): Coumarins and Caterpillars: A Case for Coevolution. - Evolution 37: 163-179.

DOI: http://dx.doi.org/10.15666/aeer/1403_195205

[6] Bernays, E.A., Driver, G.C., Bilgener, M. (1989): Herbivores and plant tannins. - Advances in Ecological Research 19: 263-302.

[7] Bernays, E.A. (1997): Feeding by lepidopteran larvae is dangerous. - Ecological Entomology 22: 121-123.

[8] Bernays, E.A. (1998): Evolution of feeding behaviour in insect herbivores. - Bioscience 48: 35-45.

[9] Chou, C.H., Kuo, Y.L. (1986): Allelopathic research of subtropical vegetation in Taiwan. Allelopathic exclusion of understorey by *Leucaena leucophylla* (Lam) de Wit. - Journal of Chemical Ecology 12: 1431-1448.

[10] Dicke, M., Sabelis, M.W. (1989): Does it pay plants to advertise for bodyguards? - In: Lambers, H., Konings, H., Cambridge, M.L., Pons, Th.L. (ed.) Consequences of Variation in Growth Rate and Productivity of Higher Plants, Towards a cost-benefit analysis of induced synomone production, Netherlands, 341-358.

[11] Ehrlich, P.R., Raven, P.H. (1964): Butterflies and plants: a study in co-evolution. - Evolution 18: 586-608.

[12] Fraenkel, G.S. (1959): The raison d'être of secondary plant substances. The odd chemicals arose as a means of protecting plants from insects and now guide insects to food. - Science 129: 1466-1470.

[13] Friedman, S., Waldbauer, G.P., Eertmoed, J.E., Naeem, M., Ghent, A.W. (1991): Blood trehalose levels have a role in the control of dietary self-selection by *Heliothis zea* larvae. - Insect Physiology 37: 919-928.

[14] Galati, G., Sabzevari, O., Wilson, J.X., O'brien, P.J. (2002): Prooxidant activity and cellular effects of the phenoxyl radicals of foodary flavonoids and other polyphenolics. - Toxicology 177: 91-104.

[15] Giftson, J.S., Jayanthi, S., Nalini, N. (2009): Chemopreventive efficacy of gallic acid, an antioxidant and anticarcinogenic polyphenol, against 1, 2-dimethyl hydrazine induced rat colon carcinogenesis. - Investigation New Drugs 28: 251-259.

[16] Giron, D., Casas, J. (2003): Lipogenesis in adult Parasitic Wasp. - Journal of Insect Physiology 49: 141-147.

[17] Hagerman, A.E., Dean, R.T., Davies, M.J. (2003): Radical chemistry of epigallocatechin gallate and its relevance to protein damage. - Archives of Biochemistry and Biophysics 414: 115-120.

[18] Hahn, D.A. (2005): Larval nutrition affects lipid storage and growth, but not protein or carbohydrate storage in newly eclosed adults of the grasshopper *Schistocerca americana*. - Journal of Insect Physiology 51: 1210-1219.

[19] Harborne, J.B. (1977): Introduction to Ecological Biochemistry. - Academic Press, London, 70-95.

[20] Harborne, J.B. (1991): Flavonoid Pigments. – In: Rosenthal G.A., Berenbaum M.B. (ed.) Herbivores: Their Interactions with Secondary Plant Metabolites, Vol I., Academic Press, New York, 389-429.

[21] Hemming, J.D.C., Lindroth, R.L. (1995): Intraspecific variation in aspen phytochemistry-effects on performance of gypsy moths and forest tent caterpillars. - Oecologia 103: 79-88.

[22] Hemming, J.D.C., Lindroth, R.L. (2000): Effects of phenolic glycosides and protein on gypsy moth (Lepidoptera: Lymantriidae) and forest tent caterpillar (Lepidoptera: Lasiocampidae) performance and detoxication activities. - Environmental Entomology 29: 1108-1115.

[23] Henriksson, J., Haukioja, E., Ossipov, V., Ossipova, S., Sillanpa, A.S., Kapari, L., Pihlaja, K. (2003): Effects of host shading on consumption and growth of the geometrid *Epirrita autumnata*: interactive roles of water, primary and secondary compounds. - Oikos 103: 3-16.

[24] Honek, A. (1993): Intraspecific variation in body size and fecundity in insects-a general relationship. - Oikos 66: 483-492.

[25] Kang, M.S., Oh, J.S., Kang, I.C., Hong, S.J., Choi, C.H. (2008): Inhibitory effect of methyl gallate and gallic acid on oral bacteria. - Journal of Microbiology 46: 744-750.

[26] Kim, S.H., Jun, C.D., Suk, K., Choi, B.J., Lim, H., Park, S., Lee, S.H., Shin, H.Y., Kim, D.K., Shin, T.Y. (2006): Gallic acid inhibits histamine release and pro-inflammatory cytokine production in mast cells. - Toxicological Sciences 91: 123-131.

[27] Klocke, J.A., Chan, B.G. (1982): Effects of cotton condensed tannin on feeding and digestion in the cotton pest, *Heliothis zea*. - Journal of Insect Physiology 28: 911-916.

[28] Larson, R.A. (1995): Antioxidant mechanisms of secondary natural products. - In: Ahmad, S. (ed.) Oxidant-induced Stress and Antioxidant Defenses in Biology, Chapman and Hall, New York, 210-237.

[29] Lee, K.P., Behmer, S.T., Simpson, S.J., Raubenheimer, D. (2002): A geometric analysis of nutrient regulation in the generalist caterpillar *Spodoptera littoralis* (Boisduval). - Journal of Insect Physiology 48: 655-665.

[30] Lee, K.P., Raubenheimer, D., Simpson, S.J. (2004): The effects of nutritional imbalance on compensatory feeding for cellulose-mediated foodary dilution in a generalist caterpillar. - Physiological Entomology 29: 108-117.

[31] Loader, C., Damman, H. (1991): Nitrogen content of food plants and vulnerability of *Pieris rapae* to natural enemies. - Ecology 72: 1586-1590.

[32] Metadiewa, D., Jaiswal, A.K., Cenas, N., Dickancaite, E., Segura-Auilar, J. (1999): Quercetin may act as a cytotoxic prooxidant after its metabolic activation to semiquinone and quinoidal product. - Free Radical Biology and Medicine 26: 107-116.

[33] Moran, N., Hamilton, W.D. (1980): Low nutritive quality as defense against herbivores. - Journal of Theoretical Biology 86: 247-254.

[34] Peters, D.J., Constabel, C.P. (2002): Molecular analysis of herbivore-induced condensed tannin synthesis: cloning and expression of dihydroflavonol reductase from trembling aspen (*Populus tremuloides*). - The Plant Journal 32: 701-712.

[35] Sakihama, Y., Cohen, M.F., Grace, S.C., Yamasaki, H. (2002): Plant phenolic antioxidant and prooxidant activities: phenolics-induced oxidative damage mediated by metals in plants. - Toxicology 177: 67-80.

[36] Simpson, S.J., Raubenheimer, D. (1993): The central role of the haemolymph in the regulation of nutrient intake in insects. - Physiological Entomology 18: 395-403.

[37] Simpson, S.J., Raubenheimer, D. (2001): The geometric analysis of nutrient-allelochemical interactions: a case study using locusts. - Ecology 82: 422-439.

[38] Simpson, S.J., Simpson, C.L. (1992): Mechanism controlling modulation by amino acids of gustatory responsiveness in the locust. - Journal of Experimental Biology 168: 269-287.

[39] Strack, D. (1997): Phenolic metabolism. - In: Dey, P.M., Harborne, J.B. (ed.) Plant biochemistry, Academic Press, New York, 387-416.

[40] Sugihara, N., Arakawa, T., Ohnishi, M., Furuno, K. (1999): Anti- and pro-oxidative effects of flavonoids on metal-induced lipid hydroperoxide-dependent lipid peroxidation in cultured hepatocytes loaded with a-linolenic acid. - Free Radical Biology and Medicine 27: 1313-1323.

[41] Telang, A., Booton, V., Chapman, R.F., Wheeler, D.E. (2001): How female caterpillars accumulate their nutrient reserves. - Journal of Insect Physiology 47: 1055-1064.

[42] Thompson, S.N., Redak, R.A. (2000): Interactions of dietary protein and carbohydrate determine blood sugar level and regulate nutrient selection in the insect *Manduca sexta* L. - Biochimica et Biophysica Acta-General Subjects 1523: 91-102.

[43] Torres, J., Rosazza, J.P.N. (2001): Microbial transformations of *p*-coumaric acid by *Bacillus megaterium* and *Curvularia lunata*. - Journal of Natural Products 64(11): 1408-1414.

[44] Yamamoto, R.T. (1969): Mass rearing of tobacco hornworm. II. Larval rearing and pupation. - Journal of Economic Entomology 62: 1427-1431.

CUTTING MANAGEMENT VERSUS BIOMASS AND CARBON STOCK OF OAK UNDER HIGH DENSITY PLANTATION IN CENTRAL HIMALAYA, INDIA

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Abstract. Tree growth and productivity are influenced by silvicultural strategies such as tree density and cutting management, which determine the potential of tree species to store carbon in the vegetation. A field experiment was conducted at Vivekananda Institute of Hill Agriculture, Almora, India in a 30-year-old *Quercus leucotrichophora* to evaluate the changes in vegetation carbon pools as a function of four cutting management (1m, 2m, local practice, and above 1/3rd part undisturbed), under high density plantation (1.0m × 1.0m). Tree cutting management significantly (<0.01) influenced the carbon stocks of *Q. leucotrichophora* trees. Total vegetation (aboveground + below ground) carbon stock was highest for the above 1/3rd part undisturbed (356.9 Mg ha^{-1}) and lowest in the cutting at 1m (86.7 Mg ha^{-1}). Overall, less disturbance to stands promote carbon storage, but intense cutting management (e.g., cutting at 1m) may adversely affect tree growth and productivity, reducing vegetation carbon pools. Cutting management effects are very much visible on carbon stock of *Q. leucotrichophora* under high density plantation. By extension, stand cutting exerts negative or positive feedbacks on biomass accretion, which may also influence the amount of carbon sequestered by the trees.

Keywords: *biomass, carbon stock, cutting management, high density plantation, Himalaya, oak plantation*

Introduction

The United Nations Framework Convention on Climate Change (UNFCCC) is one of the instruments of the global efforts to stabilize the greenhouse gases (GHGs) in the atmosphere (UNFCCC, 1993). Among the climate change lessening strategies trees are the economical means to capture and store atmospheric carbon dioxide (CO_2) in vegetation (Malhi et al., 2008) which has encouraged screening of trees with high carbon (C) storage potential (Delaney et al., 1997; Paquette et al., 2009). Multipurpose tree species such as *Quercus leucotrichophora* A. Camus have an imperative position for C storage potential (Singh et al., 2014). Tree management practices and site characteristics (Nair et al., 2010) in addition to the inherent production potential of the species affect C sequestration (removal of CO_2 from the atmosphere). In a given species, according to edapho-climatic conditions various silvicultural practices are adopted to improve tree productivity, which also alters C sequestration potential (Dixon, 1997; Jiménez et al., 2011; Coletta et al., 2016).

Oaks (*Quercus* spp.) are the dominant, climax tree species of the moist temperate forests of the Indian Himalayan region (Troup, 1921), where about 35 species of *Quercus* are extensively distributed between 1000-3500 m elevations. *Q. leucotrichophora* is an evergreen tree, locally known as “Banj oak” and it is a valuable

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keystone species with great societal relevance (Ramakrishnan, 2001). Banj is among the main forest-forming species in the densely populated mid-altitudinal zones of the Central Himalaya and provides a variety of ecosystem services (Zobel and Singh, 1997; Sharma et al., 2009). The oaks, particularly *Q. leucotrichophora* is intricately associated not only with agro-ecosystems but also with the life support systems of the inhabitants of the hills in the Himalaya (Makino, 2011). High calorific value of banj wood makes it an excellent fuel, its leaves are palatable which makes them major source of cattle fodder in the winter and dry seasons (Negi et al., 1997; Yadav and Bisht, 2013) and can be correlated with natural springs and wildlife (Singh, 1981).

Information on the effects of planting density and thinning on C sequestration by multipurpose tree species is scarce (Jandl et al., 2007) but on conifers particularly in Europe are available (Marziliano et al., 2015; Coletta et al., 2016); similarly, thinning and pruning interaction may influence growth and development of tree by affecting resources availability (Beadle et al., 2007). However, information on the impact of various cutting management levels in combination with high density plantation (HDP) on the biomass and carbon stock of woody perennial based land use systems is still unavailable. Despite the widespread use of *Q. leucotrichophora* in the Himalayan region (Yadav and Bisht, 2013) species-specific information on HDP and cutting management regimes and its impact on C sequestration are deficient. The *Q. leucotrichophora* plantation, like farm plantations of other species in Himalayan region, is multipurpose. Besides timber products and diversification of farm income the objective is to deliver a range of environmental benefits.

The focus of this article is cutting management influence in *Quercus leucotrichophora* on biomass and carbon stock under HDP of the Central Himalaya. A field investigation was carried out to evaluate the aboveground and belowground (root) C stocks of this species under HDP with tree cutting management practices (i.e. fodder harvesting techniques) in the Central Himalayan region of India. In this paper, we report growth, biomass C stocks and C sequestration rate of HDP of *Q. leucotrichophora* under different cutting management practices.

Materials and Methods

Study area

The study was conducted at experimental farm Hawalbagh (29° 36' N and 79° 40' E, 1250 m amsl) of Vivekananda Parvatiya Krishi Anusandhan Sansthan, Almora, India. The site experiences a sub-temperate climate and annual rainfall of 1100 mm with 96 + rainy days most of which is received during the monsoon season (June–September) and the remaining from October to May. Temperature ranges between 32 °C during summer and the minimum temperature from below freezing during winter. The experimental site was neutral in pH (6.6) and had 0.85% organic C (0–15 cm). The pH of each soil was determined using a digital pH meter and Walkey's and Blacks titration method (Jackson, 1967) was used to measure soil organic carbon.

Plantation

Q. leucotrichophora was planted at this site in 1983 with high density at a spacing of 1 × 1 m and managed properly for many years. Seedling survival was excellent and mortality, if any, was replaced immediately. In the year of 1997 four cutting management, i.e. coppicing at 1 m height, pollarding at 2 m height, local practice, and keeping above 1/3rd part undisturbed practices introduced in the plantation for fodder harvesting. So, in this article cutting management practices means fodder harvesting techniques which are being used in this trial. Fodder harvested during lean period, i.e. winter season due to drying of grasses and rainfed farming no source of fodder than trees in mid hills of Himalaya. Here in this article we have used some terms such as ‘least disturbed tree’ synonymous with ‘less intense cutting’ means minimum biomass removed from the trees such as local practice and keeping above 1/3rd part undisturbed of the trees and ‘increased disturbance’ synonymous with ‘high intense cutting’ means maximum biomass of the trees removed such as coppicing at 1 m height and pollarding at 2 m height of the trees. The trial was laid out in a randomized block design with replicates block wise.

Estimation of carbon stocks

In the plantation a total of 240 trees, comprising 60 trees from each plot of cutting management practices were measured for girth at breast height (gbh) except for basal area in 1 m height cutting management plots and height with the help of measuring tape and Ravi multimeter, respectively. The gbh and basal area is than converted to diameter at breast height (dbh) and basal diameter. Equation, $\text{Volume}/d^2=5009470+0.00563/d^2$ (FSI, 2009) was used to calculate volume and biomass measured by multiplying volume with tree density. Below ground biomass of trees was calculated by Cairns (1997) and as per the IPCC guidelines. Carbon stock was estimated by considering carbon content 0.45 (Magnussen and Reed, 2004) and biomass carbon dioxide by multiplying with a factor 3.67. Standing stock of biomass and C was converted on area basis (per ha) by multiplying the mean tree values with the corresponding density of trees.

Statistical analysis

Data from the completely randomized block design experiment were analyzed using one way analysis of variance (AGRES ANOVA).

Results and Discussion

Growth

The mean height and diameter per tree among different cutting management practices was significantly highest (10.14 m and 12.21 cm) for the trees which were kept 1/3rd top portion undisturbed followed by others and lowest (2.02 m and 5.35 cm) for trees coppiced at 1 m height, respectively (*Table 1*). Though, the height of trees coppiced at 1 m was on par with trees pollarded at 2 m height. Similar results were also obtained for mean volume per tree significantly highest (0.0833 m^3) for the trees kept 1/3rd top portion undisturbed followed by other practices and lowest (0.0202 m^3) for trees which were coppiced at 1 m height which was on par with the pollarding at 2 m height among different cutting management practices.

Table 1. *Effect of cutting management on the growth of oak in Central Himalaya*

Treatment	**Height (m)**	**Diameter (cm)**	**Mean volume (m^3)**
Coppicing at 1m	2.02	5.35	0.0202
Local	6.76	8.11	0.0396
Pollarding at 2m	2.93	6.70	0.0288
1/3rd top portion undisturbed	10.14	12.21	0.0833
LSD (.01)	1.32	1.77	0.0212

The mean height, diameter and volume decreased with increasing cutting intensity, meaning growth parameters were less in more intense cutting management practices, i.e. cutting at 1 m and 2 m height as compared to for the trees which were managed with less intense cutting, i.e. 1/3rd top portion undisturbed and local practice. In general, removal of higher proportion of leaves during pruning operations may adversely affect photosynthetic rates and would depress tree growth including decrease in annual diameter growth (Gyenge et al., 2010). The volume on area basis showed the same trend as the mean height and diameter and ranged from 202 to 833 m^3 ha^{-1} for different cutting management practices (*Figure 1*).

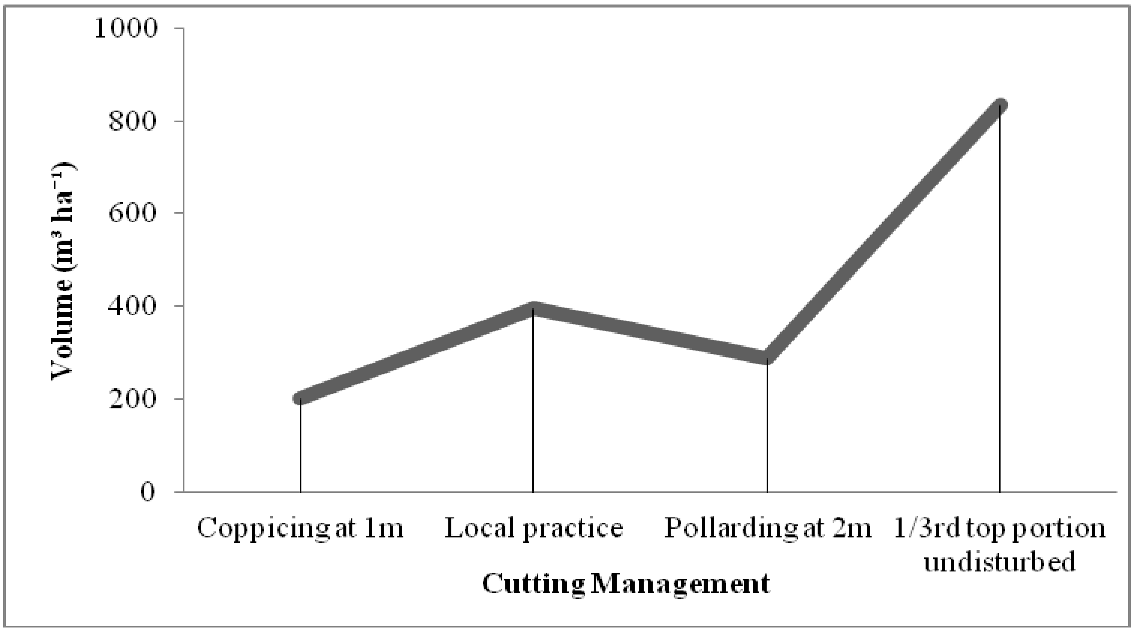

Figure 1. *Total volume of oak influenced by cutting management in Central Himalaya*

Biomass accumulation

Highly significant ($p < .01$) variation in total biomass was observed among different cutting management practices. Total tree biomass including aboveground and belowground biomass, ranged from 192.6 to 793.2 Mg ha^{-1} (*Table 2*). The Least disturbed trees, i.e. 1/3rd top portion undisturbed practice recorded the significantly highest (793.2 Mg ha^{-1}) total tree biomass accumulation and it declined with increasing tree disturbance, i.e. coppicing at 1 m recorded significantly lowest (192.6 Mg ha^{-1}) in the form of cutting management practices. The results are in conformity with the findings of Singh et al. (2014); Yadav and Bisht (2014). It is well known that limitations imposed by cutting management of trees on the lateral expansion and roots may restrain resource acquisition potential of trees leading to reductions in their biomass production. Similarly in a five year old plantation of Shisham with 625 trees ha^{-1} the total vegetation biomass was 58.7 Mg ha^{-1} and annual biomass accumulation 12.6 Mg ha^{-1} yr^{-1} (Lodhiyal et al., 2002), which is quite low than this study.

Table 2. *Cutting management influence on biomass of oak in Central Himalaya*

Treatment	**Biomass (Mg ha^{-1})**		
	Aboveground	**Belowground**	**Total**
Coppicing at 1m	151.6	40.9	192.6
Local	296.7	80.1	376.8
Pollarding at 2m	215.8	58.3	274.1
1/3rd top portion undisturbed	624.6	168.6	793.2
LSD (.01)	158.8	42.8	201.7

Carbon stock

Total plantation C stocks (aboveground + belowground) followed a reverse trend and it decreased with increased disturbance in the form of cutting management practices (*Table 3*). Almost one and a half-fold increase in C stocks have been observed in the pollarding at 2 m (123.4 Mg ha^{-1}) compared to that of the cutting at 1m (86.7 Mg ha^{-1}) and two fold in the 1/3rd top portion undisturbed (356.9 Mg ha^{-1}) as compared to local practice (169.6 Mg ha^{-1}).

Table 3. *Carbon stocks of oak as influenced by cutting management in Central Himalaya*

Treatment	**Carbon stock (Mg ha^{-1})**			**C sequestration rate (Mg ha^{-1} yr^{-1})**
	Aboveground	**Belowground**	**Total**	
Coppicing at 1m	68.1	18.4	86.7	2.9
Local	133.4	36.1	169.6	5.7
Pollarding at 2m	97.2	26.2	123.4	4.1
1/3rd top portion undisturbed	281.2	75.9	356.9	11.9
LSD (.01)	71.4	19.3	90.7	3.4

Cutting at 1 m height depressed two fold and four fold C stock and pollarding at 2 m height of the tree reduced half fold and almost three fold C stock in comparison to local and 1/3rd top portion undisturbed practices, respectively. Carbon stocks being a function of the overall tree growth (*Table 1*), this is not surprising. Singh et al. (2014) reported the impact of human-induced small-scale disturbances (lopping of branches and leaf removal) adversely impacts the functioning of banj oak (*Quercus leucotrichophora* A. Camus) forests of Central Himalaya. Majid and Paudyal (1992) noted reductions in tree growth when crown length removal from below exceeded 40% in the *A. mangium* plantation of peninsular Malaysia. The biomass carbon Co_2 mitigation by the Oak high density plantation followed the trend of biomass and carbon stock and varied between 317-1306 Mg ha^{-1} among different cutting management practices (*Figure 2*).

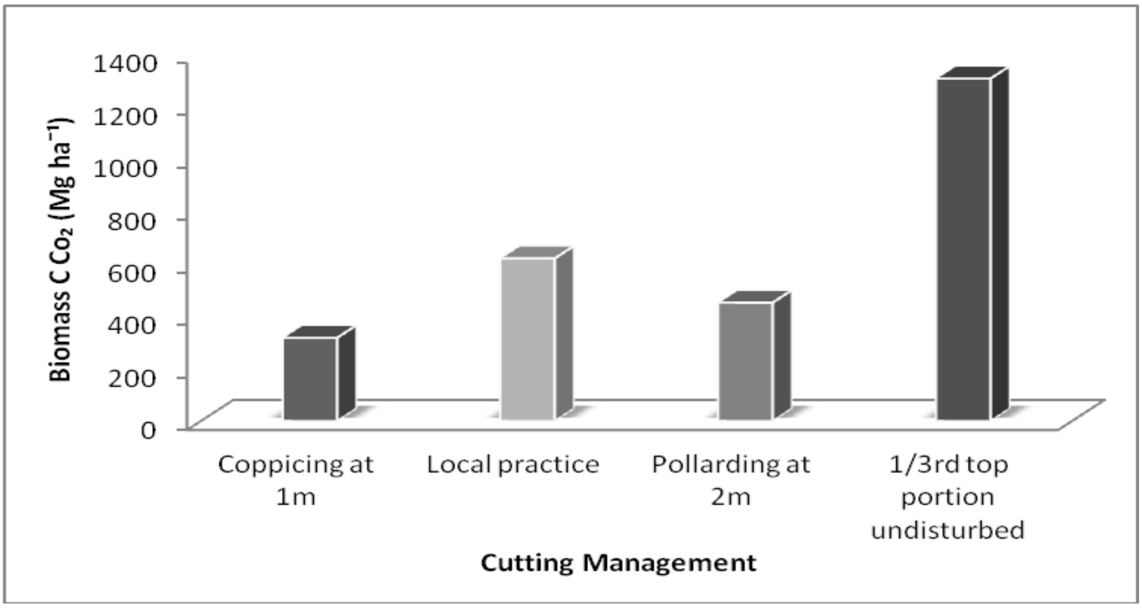

Figure 2. *Cutting management influence on total biomass CO_2 in Central Himalaya*

Biomass and carbon stock estimates for 30 year old *Q. leucotrichophora* in the present study reveal its potential as a promising tree species for C sequestration. Higher rate of total C sequestration was with the minimal disturbance cutting management practice, i.e. in $1/3^{rd}$ top portion undisturbed (11.9 Mg ha^{-1} yr^{-1}) followed by other practices and least in cutting at 1 m height (2.9 Mg ha^{-1} yr^{-1}) which was the intense cutting management practice (*Table 3*). The varying rate of C sequestration of plantations can be attributed to their differential cutting management practices. Results of the C sequestration rates were almost within range to those obtained in earlier studies (KTGAL, 2004; Yadav and Bisht, 2014; Yadav et al., 2015). According to *Table 4* pearson correlation shows that diameter is highly correlated to volume, total biomass and carbon sequestration rate of oak under high density plantation.

Table 4. *Pearson correlations among different parameters of oak under high density plantation*

Parameters	Height	Diameter	Volume	Total biomass	C sequestration rate
Height	1	.970*	.951*	.951*	.951*
Diameter	.970*	1	.995**	.995**	.995**
Volume	.951*	.995**	1	1.000**	1.000**
Total biomass	.951*	.995**	1.000**	1	1.000**
C sequestration rate	.951*	.995**	1.000**	1.000**	1

Conclusion

This paper shows the potential of *Q. leucotrichophora*, a Himalayan woody species, to sequester atmospheric CO_2 and influence of cutting management practices on high density plantation C pools. Intense cutting management practices, i.e. cutting at 1 m and 2 m height depressed overall tree growth, biomass and C stocks of this species. On the other hand the less disturbed, i.e. 1/3rd top portion undisturbed and local practices which stored good amount of C in plantation. Local inhabitants of Himalayan region can harvest fodder from this species while still allowing the storage of carbon by planting and choosing appropriate cutting management practices in agroforest for sustainable development. Overall, species, site, age and good silvicultural practices may favor tree growth and productivity and promote plantation CO_2 sequestration and C conservation.

REFERENCES

[1] Beadle, C., Barry, K., Hardiyanto, E., Irianto, R., Junarto Mohammed, C., Rimbawanto, A. (2007): Effect of pruning *Acacia mangium* on growth, form and heart rot. -For. Ecol. Manage. 238:261–267.
[2] Cairns, M.A., Brown, S., Helmer, E.H., Baumgardner, G.A. (1997): Root biomass allocation in the world's upland forests. -Oecologia 111:1–11.
[3] Coletta, V., Menguzzato, G., Pellicone, G., Veltri, A. Marziliano, P.A. (2016): Effect of thinning on above-ground biomass accumulation in a Douglas-fir plantation in southern Italy. -Journal of Forestry Research. In press
[4] Delaney, M., Brown, S., Lugo, A.E., Torres-Lezama, A., Bello-Quintero, N. (1997): The distribution of organic carbon in major components of forests located in five life zones of Venezuela. -Journal of Tropical Ecology 13: 697-708.
[5] Dixon, R.K. (1997): Silvicultural options to conserve and sequester carbon in forest systems: Preliminary economic assessment. -Crit. Rev. Environ. Sci. Technol. 27:139–149.
[6] FSI [Forest Survey of India]. (2009): Forest Survey of India-Annual Report, Ministry of Environment and Forest, Dehradun.
[7] Gyenge, J.E., Fernańdez, M.E., Schlichter, T.M. (2010): Effect of stand density and pruning on growth of ponderosa pines in NW Patagonia, Argentina. -Agroforest. Syst. 78:233–241.
[8] Jackson, M.L. (1958): Soil chemical analysis. -Asia Publishing House, New Delhi, p 498.
[9] Jandl, R., Lindner, M., Vesterdal, L., Bauwens, B., Baritze, R., Hagedorn, F., Johnsong, D.W., Minkkinen, K., Byrne, K.A. (2007): How strongly can forest management influence soil carbon sequestration? -Geoderma 137(3–4):253–268.
[10] Jiménez, E., Vega, J.A., Fernández, C., Fonturbel, T. (2011): Is pre-commercial thinning compatible with carbon sequestration? A case study in a maritime pine stand in northwestern Spain. -Forestry 84:149–157.
[11] KTGAL [Kyoto Think Global Act Local]. (2004): Project Report: 2003–2004 [submitted to International Centre for Integrated Mountain Development, Kathmandu, Nepal]. Nainital, India: Central Himalayan Environment Association.
[12] Lodhiyal, N., Lodhiyal, L.S., Pangtey, Y.P.S. (2002): Structure and function of Shisham forests in Central Himalaya, India: dry matter dynamics. -Ann. Bot. 89 (41): 54.
[13] Magnussen, S., Reed, D. (2004): Modelling for estimation and monitoring (FAO-IUFRO, 2004).
[14] Majid, N.M., Paudyal, B.K. (1992): Pruning trial for *Acacia mangium* Wild. plantation in Peninsular Malaysia. -For. Ecol. Manage. 47:285–293.
[15] Makino, Y. (2011): Lopping of Oaks in Central Himalaya, India. -Mountain Research and Development 31(1):35-44.
[16] Malhi, Y., Roberts, J.T., Betts, R.A., Killeen, T.J., Li, W., Nobre, C.A. (2008): Climate change, deforestation, and the fate of the Amazon. -Science 319:169–172.

[17] Marziliano, P.A., Coletta, V., Menguzzato, G., Nicolaci, A., Pellicone, G., Veltri, A. (2015): Effects of planting density on the distribution of biomass in a douglas-fir plantation in southern Italy. -iForest 8:368-376.

[18] Nair, P.K.R., Nair, V.D., Kumar, B.M., Showalter, J.M. (2010): Carbon sequestration in agroforestry systems. -Adv. Agron. 108:237–307.

[19] Negi, A.K., Bhatt, B.P,, Todaria, N.P., Saklani, A. (1997): The effects of colonialism on forests and the local people in the Garhwal Himalaya, India. -Mountain Research and Development 17:159–168.

[20] Paquette, A.J., Hawryshyn, A., Senikas, V., Potvin, C. (2009): Enrichment planting in secondary forests: a promising clean development mechanism to increase terrestrial carbon sinks. -Ecol. Soc. 14(1):31.

[21] Ramakrishnan, P.S. (2001): Ecology and sustainable development. New Delhi, India: National Book Trust of India.

[22] Sharma, C.M., Gairola, S., Ghildiyal, S.K., Suyal, S. (2009): Forest resource use patterns in relation to socioeconomic status. -Mountain Research and Development 29:308–319.

[23] Singh, J.S. (1981): Rural ecosystems and development in the Himalaya. - In: Singh, J.S., Singh, S.P., Shastri, C. (Eds.) Science and Rural Development in Mountains, Gyanodaya Prakashan, Nainital, India, pp 74-87.

[24] Singh, V., Thadani, R., Tewari, A., Ram, J. (2014): Human Influence on Banj Oak (*Quercus leucotrichophora* A. Camus) Forests of Central Himalaya. -Journal of Sustainable Forestry 33:373–386.

[25] Troup, R.S. (1921): Silviculture of Indian Trees. Vol. I-III. -Clarendon Press, Oxford

[26] UNFCCC [United Nations Framework Convention on Climate Change]. (1993): The United Nations Framework Convention on Climate Change, UN

[27] Yadav, R.P., Bisht, J.K., Pandey, B.M. (2015): Above ground biomass and carbon stock of fruit tree based land use systems in Indian Himalaya. -The Ecoscan 9(3&4): 779-783.

[28] Yadav, R.P., Bisht, J.K. (2013): Agroforestry: A way to conserve MPTs in North Western Himalaya. -Res. J. Agriculture and Forestry Sci. 1(9): 1-7.

[29] Yadav, R.P., Bisht, J.K. (2014): Agroforestry to combat climate change. -Kheti 66: 11-14.

[30] Zobel, B.D., Singh, S.P. (1997): Himalayan Forests and Ecological Generalizations. -Bio Science 47(11):735-745.

POPULATION VARIABILITY IN *QUERCUS BRANTII* LINDL. BASED ON THE ACORN MORPHOMETRY AND CHOMPOSITION OF PHENOLIC COMPOUNDS

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Abstract. Persian Oak (*Quercus brantii* Lindl.) is one of the most important woody species of Zagros forests in western and southwestern Iran. In order to determine the variation of morphometry and total phenolic, flavonoid and tannin contents, ripen acorns from 27 populations of *Q. brantii* growing wild in nine regions of western and southwestern Iran were collected and examined. The amounts of total phenolic/tannin and flavonoid contents were determined by “Folin-Ciocalteu” and “Aluminum Chloride Colorimetric” methods, respectively. Significant differences in acorn morphometry were observed between the populations and regions, and the higher values of acorn morphometry belonged to four regions of Khuzestan province. Similarly, there also were significant differences in amounts of total phenolic, flavonoid and tannin contents between different populations and regions, and the higher amount of total phenolic, flavonoid and tannin contents were observed in populations from Khuzestan province. Different degrees of correlations were observed between the morphometery, total phenolics, flavonoids and tannins, and geographical and climatical factors. Cluster analysis and principle component analysis (PCA) based on the morphometric and phytochemical data resulted in detecting two major groups including: 1) populations from Khuzestan province, and 2) populations from adjacent provinces. Results of present study showed that morphology and amounts of total phenolic, flavonoid and tannin contents of *Q. brantii* acorns are significantly influenced by environmental factors of their original provenances.
Keywords: *Persian oak, phenolic compounds, environmental factors*

Introduction

The genus *Quercus* L. (English: “Oak”, Persian: “Baloot”) from the family Fagaceae has ca. 500 species of trees and shrubs in the Northern Hemisphere (except for the Arctic). Taxonomy of the genus has been controversial due to the widespread inter-species hybridization and introgression (Bajalan, 2013). The Zagros Mountain range, which is extended northwest-southeast from the border areas of eastern Turkey and northern Iraq to the southern Iran is a typical example of the continental variation of the Mediterranean climate pattern, with a snowy, cold winter and mild rainy spring followed by a dry summer and autumn (Frey and Probst, 1986). *Quercus brantii* Lindl. (Persian oak) is the most important tree species of the Zagros Mountains in Iran and covers more than 50% of the western forests of the country (Heydari et al., 2013). With average length of 1300 km and average width of 200 km, Zagros forests are mainly

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consisted of Persian oak as dominant species mixed with other deciduous broad-leaved trees (Mehrnia et al., 2013).

The Oak forests of western Iran are also very important to the local communities from different aspects including soil and water conservation, byproducts and environmental values (Taleshi and Babarabi, 2013). Reports from the past decades show that ripen acorns of *Q. brantii* used to be the most important gathered food for the local communities of Zagros Mountains (Shiran et al., 2011). Chemical constitutions of acorns of different *Quercus* species have disinfectant, antioxidant and antimicrobial properties and the decoctions of acorn peels have been used to heal burns and cuts. (Şöhretoğlu et al., 2014; Borges et al., 2013; Salminen et al., 2004).

The most important chemical components of Oak acorns are related to the various classes such as saponins and phenolic compounds (including flavonoids and tannins) (Şöhretoğlu et al., 2014). It is shown that environmental factors such as altitude, latitude, precipitation, temperature, soil properties, and aspect would affect the synthesis of secondary metabolites. Amount of phenolic compounds can be influenced by developmental stage of the plant and different environmental factors such as soil composition, temperature, rainfall and ultraviolet radiation incidence, so that phenolic compounds and the other secondary metabolites show a chemical interface between plants and environment (Borges et al., 2013). Phytochemical compounds such as cuticular waxes, antifreeze polyols, and aromatics lead to acclimation to abiotic burdens, and some secondary metabolites such as aromatic alkaloids, furanocoumarins, flavonoids, phenylpropenoids and tannins may result in protection against both biotic and climatic effects (Alonso-Amelot et al., 2004).

Review of literature

Anatomical, morphological and chemical markers, enzyme activity indicators and DNA marker techniques are widely used to demonstrate the high variability and polymorphism in different *Quercus* species (Galván et al., 2012). Reports on the variability of chemical composition of oak acorns at the population-level are few (Galván et al., 2012; Brookes and Wigston, 1979; Shahian-Jahromi, 1999). Brookes and Wigston (1979) found that acorn shape and size and amounts of different element (N, P, K, Ca, Mg and Na) contents are unreliable discriminants between *Q. petraea* (Matt.) Liebl., *Q. robur* L. and their hybrids. Galván et al. (2012) found negative correlations between morphometric characteristics of the acorn with the altitude in different populations of *Q. ilex* L. Despite the fact that chemical composition of different oak organs could be affected by environmental factors, variation of phenolic compounds might be useful in taxonomy of different *Quercus* species (Shahian-Jahromi, 1999). Singh and Todaria (2012) showed that nutrient composition of leaves in *Q. semecarpifolia* Sm. is correlated to seasonal changes and also altitude. Salminen et al. (2004) demonstrated a seasonal variation in the phenolic contents of *Q. robur*'s leaves. In *Q. variabilis* Blume seedlings, variation of nutrients and carbohydrates are related to environmental factors (Lei et al., 2013). Martin-StPaul et al. (2012) observed different degrees of photosynthetic sensitivity to drought among the populations of *Q. ilex* during a rainfall gradient. Morphology and chemical contents of leaves taken from different species of oak might be affected by a rainfall gradient. It is suggested that each species has a different mechanism to cope with water shortage, which is related to its structure and habit (Castro-Díez et al., 1997). Quantity of the secondary metabolites changes under drought stress in seedlings of *Q. brantii*, *Q. infectoria* Oliv. and *Q. libani* Oliv. (Nazari et al., 2013). Significant differences were also found in quality and quantity of protein profile of the acorns in different populations of *Q. ilex* (Galván et al., 2011).

In this work we first aim to evaluate the amounts of total phenolic, flavonoid and tannin contents of acorns collected from different populations of *Q. brantii* growing wild in SW Iran. Beside the chemical evaluations, some morphologic characters will also be measured. Finally, we aim to find the possible correlations between chemical contents of acorns and morphometric data, and also different ecological factors.

Material and methods

Plant materials

This study was performed on the acorns collected from 27 populations of the Persian oak (*Q. brantii*) growing wild in nine different regions of SW Iran. These materials were from provinces Lorestan (region KHA), Khuzestan (regions ALG, GOL, MOG, BAB, and EMA), Chaharmahal and Bakhtiari (region MON), Kohgiluyeh and Boyer-Ahmad (region DIS) and Fars (region BAA) (*Fig.1*). Geographical coordinates, altitude, aspect, average annual precipitation and average annual temperature corresponding to each region are presented in *Table 1.* Collection of acorns was carried out in three altitudes (low, middle and high) in each site during the autumn 2014. Each population was represented by at least 5 trees (individuals) with at least 50 m distance from each other.

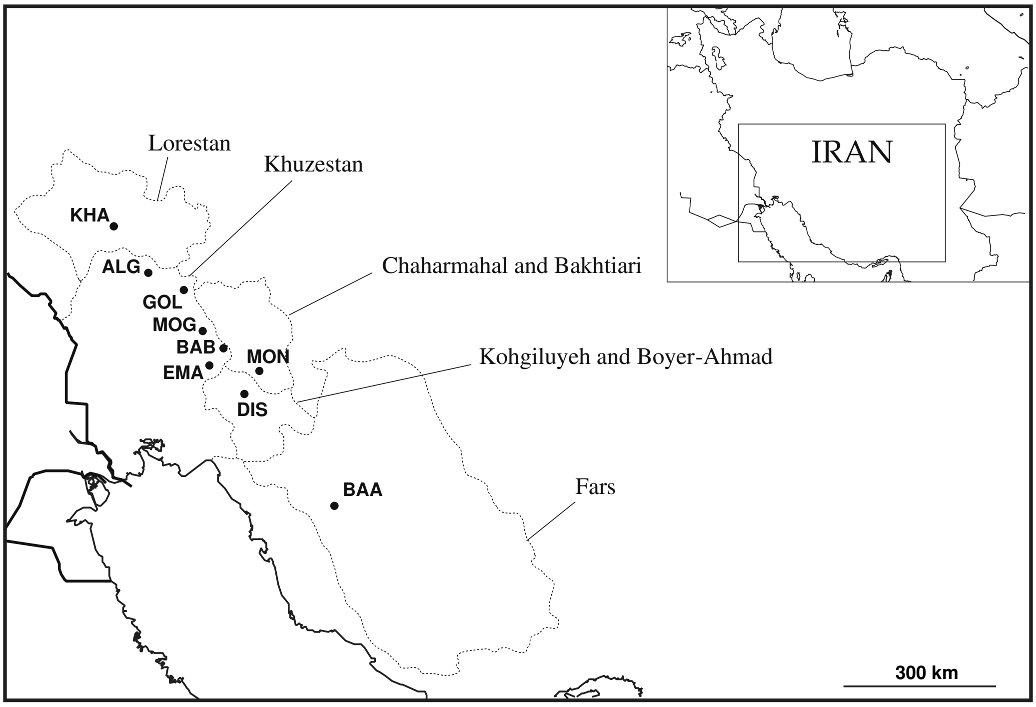

***Figure 1**. Map of Iran indicating localities of collected plant material. Each point represents the region in which three populations are collected (further details in Table 1). Abbreviations: GOL, Golzar; EMA, Emamzadeh-Abdollah; BAB, Baloot-Boland; MOG, Mongasht; ALG, Alvar-Garmsiri; DIS, Dishmook; MON, Monj; KHA, Khorram-Abad; BAA, Barm-Arjan.*

***Table 1.** Geographical and climatic details of 27 populations of Quercus brantii collected for this study.*

Pop.	*Reg.*	*Prov.*	*Abr.*	*Alt.*	*Lat.*	*Long.*	*Asp.*	*AAP*	*AAT*
1	Golzar	Khuzestan	GOL	850	32°12′54.3″	49°43′40.1″	South	35.27	21.4
2				1350	32°11′9.3″	49°42′46.2″	West		
3				1600	32°9′16.4″	49°43′36.5″	East		
4	Emamzadeh - Abdollah	Khuzestan	EMA	1630	31°24′46.1″	50°9′54.1″	East	132.6	27.5
5				1801	31°23′38.6″	50°9′46.6″	West		
6				2086	31°23′2.1″	50°9′21.9″	West		
7	Baloot-Boland	Khuzestan	BAB	910	31°45′26.4″	50°8′47.1″	South	34.27	19.02
8				1540	31°41′51.1″	50°18′25.7″	South		
9				2030	31°39′43.5″	50°23′26.6″	North		
10	Mongasht	Khuzestan	MOG	950	31°47′54.9″	49°59′9.3″	East	35.27	21.4
11				1285	31°46′30.1″	49°59′37.0″	West		
12				1621	31°46′15.7″	50°0′23.7″	West		
13	Alvar-Garmsiri	Khuzestan	ALG	650	32°50′24.7″	48°22′34.5″	East	20.26	26.55
14				800	32°49′45.4″	48°16′2.8″	West		
15				1000	32°53′8.2″	48°15′53.0″	East		
16	Dishmook	Kohgiluyeh and Boyer-Ahmad	DIS	700	31°14′29.1″	50°18′0.2″	East	372.3	25.0
17				1435	31°17′8.5″	50°16′51.8″	West		
18				2000	31°16′20.8″	50°22′12.1″	East		
19	Monj	Chaharmahal and Bakhtiari	MON	1550	31°30′27.1″	50°38′55.5″	South	35.7	16.21
20				1830	31°29′45.9″	50°365′3.8″	West		
21				2245	31°30′10.3″	50°35′29.0″	West		
22	Khorram-Abad	Lorestan	KHA	1162	33°26′18.1″	48°12′5.3″	South	30.57	17.28
23				1420	33°20.5′2″	48°25.18′9″	East		
24				1874	33°17.29′4″	48°26.15′1″	East		
25	Barm-Arjan	Fars	BAA	1249	29°33′46.6″	51°51′19.5″	North	13.9	22.58
26				1655	29°29′28.7″	51°58′22.4″	North		
27				2101	29°32′19.3″	51°57′31.8″	West		

Abbreviations: Pop., population; Reg., Region; Prov., Province; Abr., Abbreviation; Alt., Altitude; Lat., Latitude; Long., Longitude; Asp., Aspect; AAP, Average Annual Precipitation (mm), AAT, Average Annual Temperature (°C).

Climatic Data were obtained from the Iran Agency of Meteorology (IRIMO) (http://www. irimo.ir).

Acorn morphometry

In order to measure size and weight, at least 10 healthy and fully-ripen acorns from each tree were selected and examined. Length (cupule excluded) and diameter at the widest part of acorn were measured by using a digital caliper (Mitutolyo). Fresh weight of acorns was measured on a digital precision balance (JL603-C, Mettler TOLEDO).

Preparation of acorn flour

In order to remove the acorn's pericarp, longitudinal cuts were made using a sharp knife. This was repeated on 20-30 acorns collected from each tree. Cotyledons were converted to powder using a blade mill. The obtained powders were dried in a forced-air drier at 45°C for 48 hours, and then homogenized in a Waring Blender and sieved on a 1 mm mesh to get a fine homogeneous flour. Samples were kept in a refrigerator (4°C) until chemical analyses.

Preparation of the extracts

Maceration method was used for extraction (Khoddami et al., 2013). 500 ml hidroalcoholic (MeOH) solvent was added to 100 g of each sample (acorn powder). Samples were kept at room temperature and away from sunlight for 72 hours. They were shaken and mixed several times and then were filtered. A rotary set was applied in order to thicker the extract. In order to remove the solvents, extracts were kept in freeze-drier for 48 hours. Finally, the extracts were tightly covered and were kept in the refrigerator until analysis.

Determination of total contents of phenolic compounds

"Folin-Ciocalteau" method (Makkar et al., 1993) with slight modifications was used for determination of total contents of phenolic compounds. Suitable aliquots of the tannin-containing extract (0.1, 0.5 and 1 ml), 2.5 ml of the diluted Folin-Ciocalteu reagent (10:1 ratio) and then 2 ml of the 7.5% Sodium carbonate solution were added in tubes. After shaking the tubes on vortex and incubating for 2 hours, absorbance of their contents were recorded at 765 nm on a Spekol spectrophotometer (Analytik, Jena). The amount of total phenolics was calculated as tannic acid equivalent from the calibration curve (mg tannic acid equivalent/g dry extract). In order to prepare the calibration curve, concentrations of 0.02, 0.04, 0.06, 0.08 and 0.1 mg/ml of tannic acid were used. Experiments repeated three times for each sample.

Determination of total tannin contents

1 ml distilled water plus 1 ml of the tannin-containing extract was added to 100 mg Polyvinylpolypyrrolidone (PVPP) in a 100 × 12 mm test tube. Then the tube was shaken, roomed for 15 min at 4°C, was shaken again, and then centrifuged at 3000 g for 10 min. The supernatant containing simple phenolic compounds was then collected and its content was measured spectrophotometry (Makkar et al., 1995). The difference between primary and secondary total phenolic contents (or non-tannin phenols) of each extract showed the content of tannins that express as tannic acid equivalent/g dry extract. Experiments repeated three times for each sample.

Determination of total flavonoid contents

"Aluminum Chloride Colorimetric" method as described by Chang et al. (Chang et al., 2002) with slight modifications was used to determine the total flavonoid contents. 2 ml methanolic extract was mixed with 2 ml $AlCl_3.6H_2O$ 2% and was incubated at room temperature for 15 min. The absorbance of this mixture was measured at 430 nm. Finally, the amount of total flavonoid as routine equivalent was calculated from the calibration curve (mg routine equivalent/g dry extract). The calibration curve was prepared by using 0.0025, 0.005, 0.01, 0.02, 0.1 and 0.5 mg/ml dilutions. Experiments repeated three times for each sample.

Statistical analysis

Kolmogorov–Smirnov test was used to determine the normality of frequency distributions. One-way ANOVA test were used to compare means of normal distributions (Galván et al., 2012). Duncan's test was used to analyses the differences in acorn morphometry and chemical data (*Table 2*). Pearson's correlation was carried out to test for correlation between acorn size, weight, chemical composition, climate and geographical data. Ward's cluster method using squared Euclidian distances was used for ascertainment of distances based on acorn morphometry and chemical data. Principal component analysis (PCA) was used for determine the most important factors of acorn for preparing of graph and compare with cluster analysis. Statistical analysis was carried out using SPSS v. 22 (IBM Inc, Chicago, IL).

Table 2. *Studied characteristics of Quercus brantii acorn*

Studied characters	**Abbreviation letters**
Total phenolic content	TPC
Total flavonoid content	TFC
Total tannin content	TTC
Acorn weight	AW
Acorn length	AL
Acorn diameter	AD

Results

Morphometry of acorn

The results of morphometrical measurements of different oak populations are shown in *Table 3* and *Table 4*. Acorn length ranged from 3.41 ± 0.045 cm (reg. BAB, pop. 9, high altitude) to 6.38 ± 0.032 cm (reg. BAB, pop. 8, middle altitude) in populations. The mean of acorn length also ranged from 4.82 ± 0.262 cm (reg. BAA) to 5.87 ± 0.519 cm (reg. ALG) in regions. Acorn diameter ranged from 1.60 ± 0.025 cm (reg. BAB, pop. 9, high altitude) to 2.74 ± 0.025 cm (reg. EMA, pop. 5, middle altitude) in populations, and from 1.81 ± 0.144 cm (reg. MON) to 2.23 ± 0.381 cm (reg. EMA) in regions. Acorn weight ranged from 3.63 ± 0.025 g (reg. BAB, pop. 9, high altitude) to 11.53 ± 0.015 g (reg. GOL, pop. 2, middle altitude) in populations and from 5.49 ± 0.492 g (reg. BAA) to 8.49 ± 2.37 g (reg. GOL) in regions. Statistically significant differences ($P < 0.05$) were observed in acorn length, maximum diameter and weight between different populations.

Table 3. *Acorn dimensions and weight of the 27 different populations of Quercus brantii collected from 9 regions in SW Iran. Minimum and maximum measurements are written in* ***Bold-italic*** *and* **Bold**, *respectively.*

Reg.	***Pop.***	***AL (cm)***	***AD (cm)***	***AW (g)***
GOL	1	5.60 ± 0.040 n	1.93 ± 0.020 fgh	6.22 ± 0.025 h
	2	5.46 ± 0.066 kl	2.58 ± 0.030 o	**11.53 ± 0.015** y
	3	4.75 ± 0.055 d	2.04 ± 0.035 kl	7.72 ± 0.021 o

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EMA	4	5.25 ± 0.045 hi	1.98 ± 0.010 ij	7.33 ± 0.030 m
	5	4.94 ± 0.040 e	**2.74 ± 0.025** p	9.73 ± 0.025 w
	6	4.86 ± 0.066 e	1.97 ± 0.020 hij	7.82 ± 0.020 p
BAB	7	5.52 ± 0.040 klmn	2.10 ± 0.015 m	8.13 ± 0.025 r
	8	**6.38 ± 0.032** q	2.07 ± 0.020 lm	8.43 ± 0.026 t
	9	***3.41 ± 0.045*** a	***1.60 ± 0.025*** a	***3.63 ± 0.025*** a
MOG	10	5.44 ± 0.060 k	1.74 ± 0.015 d	7.83 ± 0.031 p
	11	5.33 ± 0.055 j	2.02 ± 0.020 k	9.43 ± 0.025 v
	12	5.05 ± 0.055 f	1.70 ± 0.055 b	7.53 ± 0.025 n
ALG	13	6.06 ± 0.060 p	1.83 ± 0.030 e	6.04 ± 0.040 f
	14	6.36 ± 0.045 q	2.04 ± 0.035 kl	7.04 ± 0.025 l
	15	5.21 ± 0.060 ghi	1.85 ± 0.020 e	6.18 ± 0.031 g
DIS	16	5.50 ± 0.055 klm	1.95 ± 0.015 ghi	6.90 ± 0.031 k
	17	5.86 ± 0.040 o	2.04± 0.036 kl	8.39 ± 0.031 s
	18	4.53 ± 0.060 b	1.72 ± 0.020 cd	4.29 ± 0.031 b
MON	19	5.29 ± 0.045 ij	1.74 ± 0.010 d	6.61 ± 0.015 j
	20	5.58 ± 0.034 mn	2.00 ± 0.010 jk	10.70 ± 0.015 x
	21	5.35 ± 0.050 j	1.69 ± 0.010 c	6.63 ± 0.015 j
KHA	22	5.15 ± 0.035 g	2.33 ± 0.025 n	9.30 ± 0.020 u
	23	5.55 ± 0.032 lmn	1.91 ± 0.020 fg	7.89 ± 0.010 q
	24	4.89 ± 0.023 e	1.82 ± 0.030 e	6.41 ± 0.020 i
BAA	25	4.65 ± 0.065 c	1.89 ± 0.021 f	5.88 ± 0.018 e
	26	5.16 ± 0.040 gh	1.83 ± 0.020 e	4.84 ± 0.015 c
	27	4.64 ± 0.050 c	1.81 ± 0.030 e	5.75 ± 0.017 d

Abbreviations: Reg., Region; Pop., Population; AL, Acorn length; AD, Acorn diameter; AW, Acorn weight; GOL, Golzar; EMA, Emamzadeh-Abdollah; BAB, Baloot-Boland; MOG, Mongasht; ALG, Alvar-Garmsir; DIS, Dishmook; MON, Monj; KHA, Khorram-Abad; BAA, Barm-Arjan.

The descriptive statistics are presented in terms of the Mean ± SD (Standard Deviation). Mean values with the same letters indicate homogeneous subsets for P ≤ 0.05 according to Duncan test.

Table 4. *Acorn dimensions and weight of Quercus brantii collected from 9 regions in SW Iran. Minimum and maximum measurements are written in* ***Bold-italic*** *and* **Bold,** *respectively.*

Reg.	*AL (cm)*	*AD (cm)*	*AW (g)*
GOL	5.27 ± 0.397 a	2.18 ± 0.302 c	**8.49 ± 2.37** d
EMA	5.02 ± 0.182 a	**2.23 ± 0.381** c	8.29 ± 1.10 cd
BAB	5.10 ± 1.32 a	1.93 ± 0.243 ab	6.73 ± 2.33 abc
MOG	5.27 ± 0.179 a	1.82 ± 0.207 ab	8.26 ± 0.884 cd
ALG	**5.87 ± 0.519** b	1.91 ± 0.102 ab	6.42 ± 0.470 ab
DIS	5.30 ± 0.598 a	1.90 ± 0.144 ab	6.53 ± 1.79 ab
MON	5.41 ± 0.138 ab	***1.81 ± 0.144*** ab	7.98 ± 2.04 bcd
KHA	5.19 ± 0.289 a	2.02 ± 0.235 bc	7.87 ± 1.25 bcd
BAA	***4.82 ± 0.262*** a	1.84 ± 0.043 ab	***5.49 ± 0.492*** a

Abbreviations: Reg., Region; AL, Acorn length; AD, Acorn diameter; AW, Acorn weight; GOL, Golzar; EMA, Emamzadeh-Abdollah; BAB, Baloot-Boland; MOG, Mongasht; ALG, Alvar-Garmsir; DIS, Dishmook; MON, Monj; KHA, Khorram-Abad; BAA, Barm-Arjan.

The descriptive statistics are presented in terms of the Mean ± SD (Standard Deviation). Mean values with the same letters indicate homogeneous subsets for P ≤ 0.05 according to Duncan test.

Phytochemical contents of acorn

The results showed that amounts of total phenolic, flavonoid and tannin contents varied among populations and regions (*Table 5 and Table 6*). Amounts of total phenolic contents ranged from 210.18 ± 8.24 (reg. MON, pop. 21, high altitude) to 791.70 ± 3.49 mg tannic acid equivalent/g dry extract (reg. GOL, pop. 1, low altitude) in populations and from 324.17 ± 95.16 (reg. MON) to 727.93 ± 78.99 mg tannic acid equivalent/g dry extract (reg. GOL) in regions. Amounts of flavonoid contents ranged from 15.96 ± 0.027 (reg. BAA, pop. 27, high altitude) to 21.70 ± 0.126 mg routine equivalent/g dry extract (reg. MOG, pop. 11, medium altitude) in populations and from 16.60 ± 0.517 (reg. BAA) to 21.56 ± 1.03 mg routine equivalent/g dry extract (reg. MOG) in regions. Total tannin contents ranged from 161.95 ± 6.03 (reg. MON, pop. 21, high altitude) to 648.78 ± 3.09 mg tannic acid equivalent/g dry extract (reg. GOL, pop. 1, low altitude) in populations and from 277.46 ± 93.05 (reg. MON) to 581.55 ± 67.38 mg tannic acid equivalent/g dry extract (reg. GOL) in regions. Statistically significant differences ($P < 0.05$) were observed in phytochemical contents between 27 populations (*Table 5*) and 9 regions (*Table 6*).

Table 5. *Amounts of total phenolic, flavonoid and tannin contents in 27 population of Quercus brantii collected from 9 regions in SW Iran. Minimum and maximum measurements are written in **Bold-italic** and* **Bold,** *respectively.*

Reg.	***Pop.***	***TPC***[a]	***TFC***[b]	***TTC***[c]
GOL	1	**791.70 ± 3.49** m	21.42 ± 0.252 kl	**648.78 ± 3.09** p
	2	755.56 ± 70.35 m	18.43 ± 0.270 de	578.33 ± 71.61 o
	3	636.53 ± 14.88 k	18.53 ± 0.225 de	517.55 ± 9.16 lm
EMA	4	555.70 ± 2.26 hi	18.10 ± 0.055 cd	439.81 ± 4.36 h
	5	686.46 ± 5.97 l	21.36 ± 0.600 kl	558.91 ± 3.13 no
	6	569.11 ± 48.01 hij	18.45 ± 0.360 de	462.79 ± 33.57 hijk
BAB	7	596.86 ± 22.24 ijk	19.44 ± 0.120 hi	486.67 ± 13.60 jkl
	8	642.31 ± 30.18 kl	19.75 ± 0.312 i	531.31 ± 23.66 mn
	9	636.91 ± 2.01 k	21.04 ± 0.126 k	532.83 ± 2.61 mn
MOG	10	617.17 ± 8.03 jk	20.33 ± 0.381 j	531.24 ± 7.47 mn
	11	605.80 ± 5.73 jk	**21.70 ± 0.126** l	498.95 ± 3.41 klm
	12	617.17 ± 7.5 jk	20.20 ± 0.265j	484.53 ± 5.27 ijkl
ALG	13	547.88 ± 1.16 h	18.80 ± 0.190 efg	454.62 ± 3.71 hij
	14	558.50 ± 0.645 hi	19.42 ± 0.126 hi	469.56 ± 0.805 hijk
	15	645.85 ± 30.47 kl	20.28 ± 0.171 j	487.86 ± 22.48 jkl
DIS	16	474.67 ± 27.61 g	21.63 ± 0.345 l	402.98 ± 21.38 g
	17	391.04 ± 34.23 cde	18.64 ± 0.198 ef	286.34 ± 19.21 bc
	18	367.01 ± 3.91 bc	19.07 ± 0.427 fgh	279.05 ± 2.07 b
MON	19	426.24 ± 28.62 def	18.10 ± 0.027 cd	371.81 ± 23.53 fg
	20	336.09 ± 0.853 b	19.09±0.126 fgh	298.61 ± 2.53 bcd
	21	***210.18 ± 8.24*** a	19.15 ± 0.180 gh	***161.95 ± 6.03*** a
KHA	22	432.02 ± 0.645 efg	17.74 ± 0.427 c	375.76 ± 2.46 fg
	23	375.95 ± 48.93 bc	16.80 ± 0.372 b	321.67 ± 41.09 cde
	24	440.77 ± 15.12 fg	18.10 ± 0.344 cd	380.74 ± 12.72 fg

BAA	25	536.89 ± 42.31 h	16.74 ± 0.180 b	444.57 ± 40.04 hi
	26	434.25 ± 26.31 efg	17.10 ± 0.120 b	355.82 ± 18.62 ef
	27	382.29 ± 31.34 bcd	***15.96 ± 0.027*** a	326.81 ± 23.26 de

Abbreviations: Reg., Region; Pop., Population; TPC, Total phenolic content; TFC, Total flavonoid content; TTC, Total tannin content; GOL, Golzar; EMA, Emamzadeh-Abdollah; BAB, Baloot-Boland; MOG, Mongasht; ALG, Alvar-Garmsir; DIS, Dishmook; MON, Monj; KHA, Khorram-Abad; BAA, Barm-Arjan.

[a] *mg tannic acid equivalent/g dry extract.*

[b] *mg routine equivalent/g dry extract.*

[c] *mg tannic acid equivalent/g dry extract.*

The descriptive statistics are presented in terms of the Mean ± SD (Standard Deviation). Mean values with the same letters indicate homogeneous subsets for P ≤ 0.05 according to Duncan test.

Table 6. *Amounts of total phenolic, flavonoid and tannin contents of Quercus brantii collected from 9 regions in SW Iran. Minimum and maximum measurements are written in* ***Bold-italic*** *and* **Bold,** *respectively.*

Reg.	***TPC*** [a]	***TFC*** [b]	***TTC*** [c]
GOL	**727.93 ± 78.99** d	19.46 ± 1.48 bc	**581.55 ± 67.38** d
EMA	603.76 ± 66.84 c	19.30 ± 1.59 bc	487.17 ± 57.30 c
BAB	625.36 ± 28.54 c	20.08 ± 0.757 cd	516.94 ± 26.52 c
MOG	613.38 ± 8.41 c	**21.56 ± 1.03** d	504.91 ± 21.28 c
ALG	584.07 ± 48.99 c	19.5 ± 0.658 bc	470.68 ± 18.38 c
DIS	410.91 ± 53.69 b	19.78 ± 1.43 bcd	322.79 ± 61.93 ab
MON	***324.17 ± 95.16*** a	18.78 ± 0.520 b	***277.46 ± 93.05*** a
KHA	416.25 ± 39.79 b	17.55 ± 0.670 a	359.39 ± 35.62 b
BAA	451.14 ± 74.21 b	***16.60 ± 0.517*** a	375.73 ± 58.70 b

Abbreviations: Reg., Region; TPC, Total phenolic content; TFC, Total flavonoid content; TTC, Total tannin content; GOL, Golzar; EMA, Emamzadeh-Abdollah; BAB, Baloot-Boland; MOG, Mongasht; ALG, Alvar-Garmsiri; DIS, Dishmook; MON, Monj; KHA, Khorram-Abad; BAA, Barm-Arjan.

[a] *mg tannic acid equivalent/g dry extract.*

[b] *mg routine equivalent/g dry extract.*

[c] *mg tannic acid equivalent/g dry extract.*

The descriptive statistics are presented in terms of the Mean ± SD (Standard Deviation). Mean values with the same letters indicate homogeneous subsets for P ≤ 0.05 according to Duncan test.

Correlation between the characters and geographical/climatical data

The correlation between the morphometric/phytochemical measurements and geographical/climatical data are shown in *Table 7*. Acorn length, diameter and weight showed statistically significant negative correlation with longitude ($P \leq 0.01$ or $P \leq 0.05$), and positive correlation with latitude range of seed collection ($P \leq 0.01$ or $P \leq 0.05$). Also, acorn length and diameter showed statistically significant negative correlation with altitude ($P \leq 0.01$ or $P \leq 0.05$). Acorn weight and diameter had a positive correlation ($P \leq 0.01$ or $P \leq 0.05$) with Average Annual Precipitation (AAP). Except for the acorn weight ($P \leq 0.01$), the aspect showed no correlation with other factors (*Table 7; Fig.2*).

***Table 7.** Correlation between the morphometric and phytochemical measurements and geographical/climatical data.*

	Morphometric measurements			**Geographical data**				**Climate data**	
	AW	**AL**	**AD**	**Alt.**	**Lat.**	**Long.**	**Asp.**	**AAP**	**AAT**
AW	1.00	0.442^{**}	0.727^{**}	-0.078	0.257^{*}	-0.257^{*}	0.506^{**}	0.336^{**}	-0.046
AL	0.442^{**}	1.00	0.350^{**}	-0.520^{**}	0.321^{**}	-0.321^{**}	0.125	-0.018	-0.047
AD	0.727^{**}	0.350^{**}	1.00	-0.243^{*}	0.262^{*}	-0.262^{*}	0.140	0.224^{*}	0.126
TPC	0.167	-0.004	0.304^{**}	-0.439^{**}	0.284^{*}	-0.284^{*}	-0.126	0.053	0.386^{*}
TFC	0.158	0.216	-0.003	-0.252^{*}	0.164	-0.164	0.173	0.422^{**}	0.142
TTC	0.210	0.037	0.307^{**}	-0.448^{**}	0.275^{*}	-0.275^{*}	-0.177	0.0240	0.327^{**}

Abbreviations: AW, Acorn Weight; AL, Acorn Length; AD, Acorn Diameter; TPC, Total Phenolic Content; TFC, Total Flavonoid Content; TTC, Total Tannin Content; Alt., Altitude; Lat., Latitude; Long., Longitude; Asp., Aspect; AAP, Average Annual Precipitation (mm); AAT, Average Annual Temperature (°C).

Pearsons's correlation coefficient is indicated with level of significance ($P \leq 0.05$ and $P \leq 0.01$). Minus sign (-) shows the negative and plus sign (+) shows the positive correlation between factors.

**Correlation is significant at the 0.05 level (2-tailed).*

***Correlation is significant at the 0.01 level (2-tailed).*

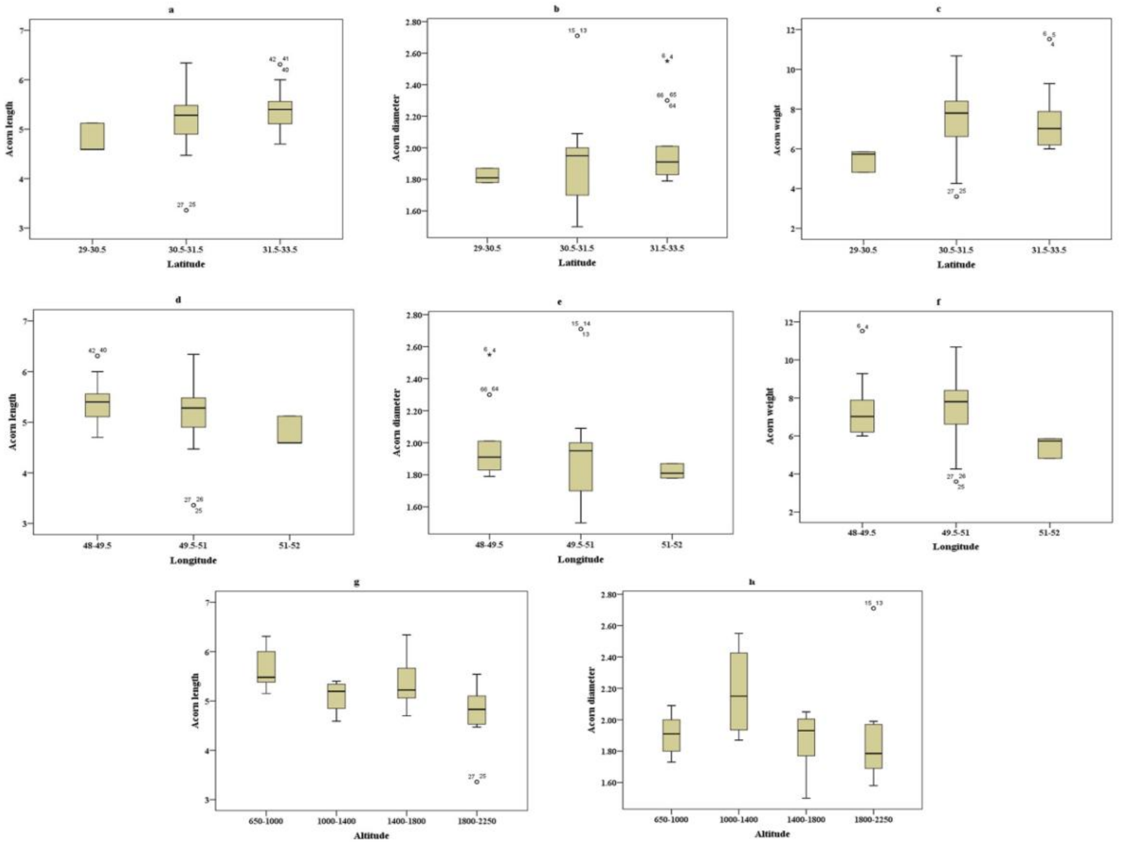

***Figure 2.** Correlation between acorn morphometry and geographical data. **a,** Acorn length and latitude; **b,** Acorn diameter and latitude; **c,** Acorn weight and latitude; **d,** Acorn length and longitude; **e,** Acorn diameter and longitude; **f,** Acorn weight and longitude; **g,** Acorn length and altitude; **h,** Acorn diameter and altitude.*

Total phenolic and tannin contents were positively correlated with latitude (P ≤ 0.05), acorn diameter (P ≤ 0.01), and Average Annual Temperature (P ≤ 0.05), and negatively correlated with altitude (P ≤ 0.01) and longitude (P ≤ 0.05). Total flavonoid content was positively correlated with Average Annual Precipitation (P ≤ 0.01) and negatively correlated with altitude (P ≤ 0.05). It is showed that aspect did not affect the chemical contents (*Table 7; Fig.3).*

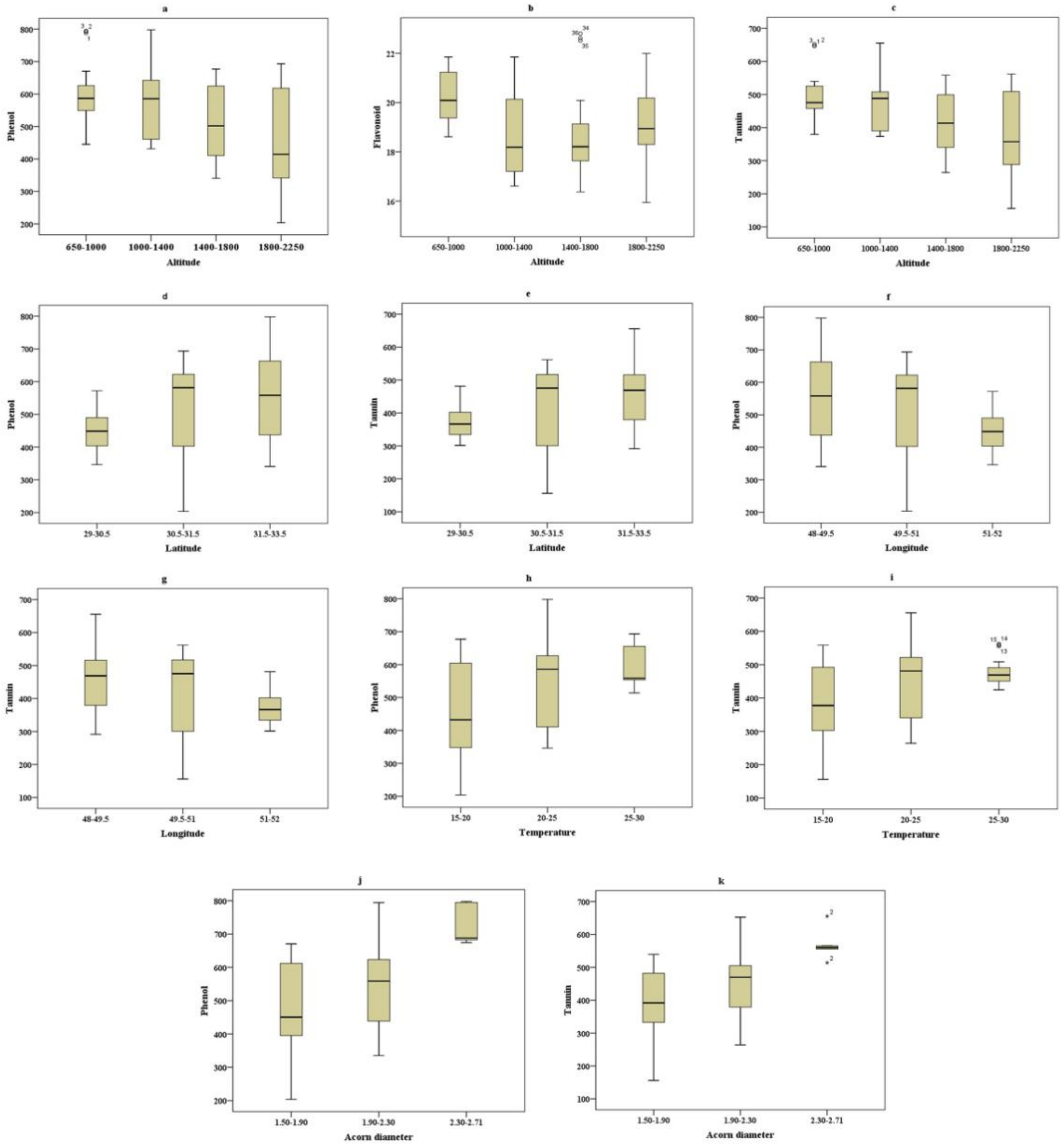

Figure 3. *The correlation between the phytochemical and morphometric data and geographical/climatical data.* ***a,*** *Phenolic content and altitude;* ***b,*** *Flavonoid content and altitude;* ***c,*** *Tannin content and altitude;* ***d,*** *Phenolic content and latitude;* ***e,*** *Tannin and latitude;* ***f,*** *Phenol and longitude;* ***g,*** *Tannin content and longitude;* ***h,*** *Phenolic content and temperature;* ***i,*** *Tannin content and temperature;* ***j,*** *Phenolic content and acorn diameter;* ***k,*** *Tannin content and acorn diameter.*

Cluster analysis

A hierarchical dendrogram obtained from the cluster analysis of morphometric and phytochemical data of 27 populations using Ward method is shown in *Fig. 4*. Populations grouped into two main clusters A and B at an average distance value (ADV) of 18. Cluster A is divided into two smaller sub-clusters A1 (consisted by populations of 3, 4, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15) and A2 (consisted by populations of 1, 2, 5) at ADV of 3. Except for the population 25, all other populations belong to regions GOL, EMA, BAB, MOG and ALG (all from Khuzestan province). This cluster of populations showed a tendency to present higher values in acorn length, acorn diameter, acorn weight, and amounts of total phenolic, flavonoid and tannin contents. These populations grow in regions with an annual temperature ranging between 19.02 and 27.5°C and an altitude ranging between 650 and 2086 m. The cluster B includes population 21 plus a nested sub-clade including populations 16, 17, 18, 19, 20, 23, 24, 26 and 27, all from regions DIS, MON, KHA, and BAA outside the provinces Khuzestan. They showed to have lower amount of phenolic compounds and similar values in total phenolic, flavonoid and tannin contents. These populations grow in regions with an annual temperature ranging between 16.21 and 25.0°C and an altitude ranging between 700 and 2245 m.

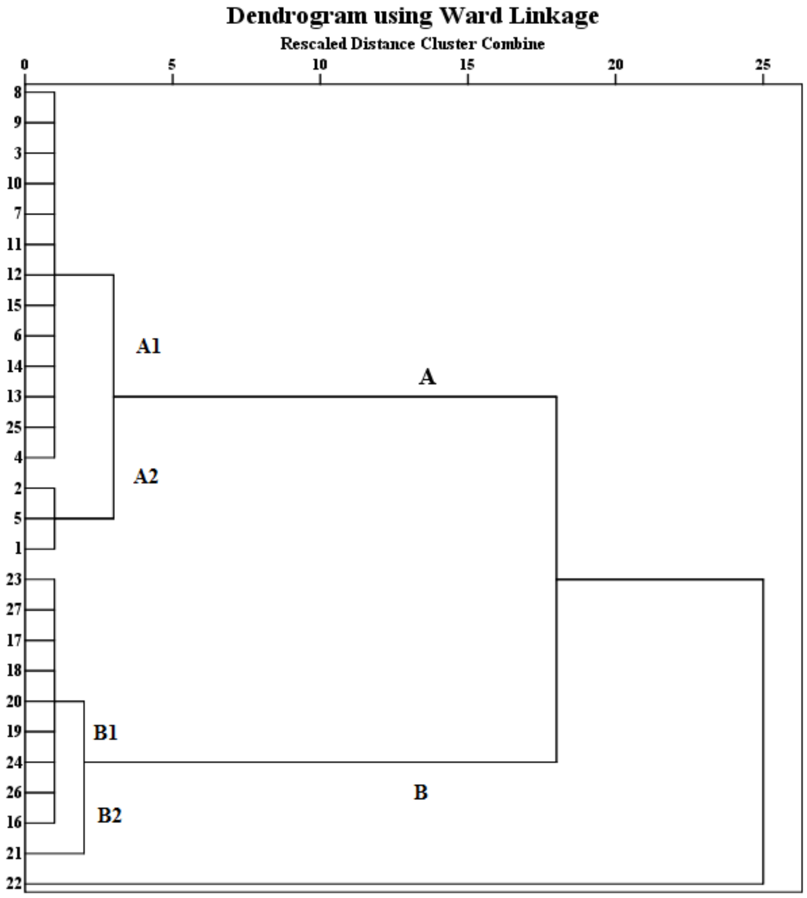

Figure 4. *A hierarchical dendrogram obtained from the cluster analysis of morphometric and phytochemical data of 27 populations of Quercus brantii using Ward method with squared Euclidian distance. 1-3: GOL, Golzar; 4-6: EMA, Emamzadeh-Abdollah; 7-9: BAB, Baloot-Boland; 10-12: MOG, Mongasht; 13-15: ALG, Alvar-Garmsir; 16-18: DIS, Dishmook; 19-21: MON, Monj; 22-24: KHA, Khorram-Abad; 25-27: BAA, Barm-Arjan.*

Principal component analysis (PCA)

Principal component analysis (PCA) showed that the variation explained by the first component was mostly accounted for by variables related to the TPC (Total Phenolic Content) and TTC (Total Tannin Content) with 42% of total variation. On the other hand, these traits had the greatest impact in ordination. The second component explained 23% of the total variation, which was mostly due to the variation in AW (Acorn Weight) and AD (Acorn Diameter). Thus, these four traits had the greatest impact in the ordination. The PCA ordination based on first two components derived from principal components analysis could separate the populations on the base of acorn characteristics (*Fig. 5*). The symbols shown in the upper half of the graph illustrate the populations mainly growing in Khuzestan province and the signs in the lower half of the graph illustrate the populations mainly growing in other four provinces. As illustrated in *Fig. 5*, PCA results conform to those of cluster analysis.

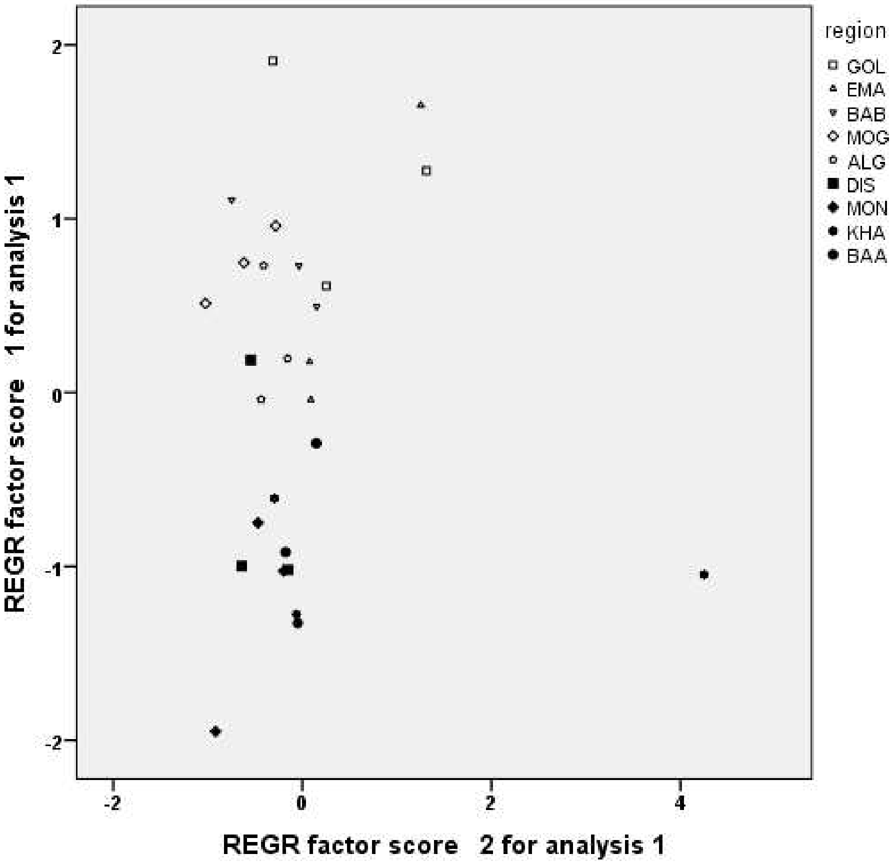

Figure 5. *Ordination of 27 populations on the basis of first two component of PCA. Abbreviations: GOL, Golzar; EMA, Emamzadeh-Abdollah; BAB, Baloot-Boland; MOG, Mongasht; ALG, Alvar-Garmsiri; DIS, Dishmook; MON, Monj; KHA, Khorram-Abad; BAA, Barm-Arjan.*

Discussion

We have observed variability in acorn morphometry and phytochemical contents of 27 populations of Persian oak (*Q. brantii*) in southwestern Iran. The correlations between these variables and some geographical/climatical factors were also evaluated.

Morphometrical characteristics of Persian oak acorn exhibited variation at the population's level (*Table 3 and Table 4*). These variations in acorn size were influenced by environmental factors *(Table 7; Fig.2)*. Populations from the DIS, BAA, KHA, and MON had the smallest average acorn size, while Populations from the GOL, EMA, BAB, MOG, and ALG had the longest and broadest acorns. Populations growing in lowest altitudes showed the longest and broadest acorns; on the contrary, populations that grow in highest altitudes showed the smallest and thinnest acorns. Also, our study showed the positive correlation between acorn dimentions and latitude range of acorn collection. Variation in acorn morphometry at population's level had earlier been reported for some *Quercus* spp. In case of *Q. ilex,* acorn weight, length and diameter exhibited significant inverse correlation with altitude and significant positive correlation with latitude of acorn origin (Galván et al., 2012). Reports showed that acorn weight and length have significant inverse correlation with altitude of acorn origin of *Q. glauca* (Singh and Todaria, 2012). Furthermore, acorn morphometry of *Q. suber* also has showed a positive correlation with high latitudes of acorn origin of this species (Ramírez-Valiente et al., 2009). There is also a positive relationship between the acorn size of different American oaks and latitude (Aizen and Woodcock, 1992). Significant differences between the populations of *Q. ilex* based on their phytochemical compositions are shown in different studies (Galván et al., 2012; Galván et al., 2011). Phenolic compounds could be regarded as important markers in taxonomy of oaks (Shahian-Jahromi, 1999).

Based on our results, amounts of phenolic content of Persian oak exhibited variation and statistically significant differences at the population's level (*Table 5*). This is the first report of relationship between geographical/climatical data and acorn chemical composition of *Q. brantii* in Iran. It is shown that phenolic compounds of acorns in Persian oak populations are influenced by altitudinal, latitudinal, longitudinal, Average Annual Precipitation and Average Annual Temperature variation (*Table 7; Fig.3*). However, in this study, four populations from Khuzestan province (GOL, EMA, BAB, MOG, and ALG) showed a tendency toward presenting higher acorn size and phenolic compounds. The effect of some geographical/environmental factors on acorn chemical composition of *Q. ilex* (Galván et al., 2012) and *Q. semecarpifolia* (Singh and Todaria, 2012) is proved. Latitude variations play an important role in physiological performance of *Q. variabilis* plants (Lei et al., 2013). Tannins can help *Q. brantii* to resist under drought stress (Nazari et al., 2013). Different environmental conditions affect the phytochemical composition of different plant species (Borges et al., 2013; Lantemona et al., 2013; Bezerra et al., 2013; Mpofu et al., 2006; Ghafour et al., 2010; Alonso-Amelot et al., 2007; Hemati et al., 2012). Dissimilar to our results, a positive correlation exists between the tannins content of *Glycyrrhiza glabra* L. and mean annual precipitation, and a negative correlation exists between its tannins content and latitude (Oloumi and Hassibi, 2011). Similarly, mixed results were observed in *Juglans regia* L. (Ghasemi et al., 2011), *Arnica montana* (Spitaler et al., 2008) and *Nepeta nuda* (Kofidis and Bosabalidis, 2008). It seems that different species respons differently to the geographical and climatical factors. Furthermore, it is indicated that environmental stresses play important roles in genes expression, product and activity of enzymes involved in plant metabolism (Lantemona et al., 2013). The production of secondary metabolites in plants can be assumed as the result of plant development including metabolism changes, morphogenesis and differentiation. On the other hand, synthesis of secondary metabolites is the expression of a plant genome under developmental control. The relation of climatic factors with secondary metabolite production is necessary for understanding the biology of secondary metabolites in plants with protective role in relation to abiotic stresses such as temperature, water status, UV exposure, etc. (Oloumi and Hassibi, 2011). Differences in the phytochemical contents of the acorns could be related to some factors such as (1) weather conditions (raining and temperature), (2) the acorns physiological stages (ripening or germination) and (3) sanitary conditions (dryness, dampening, decaying, and attacks from pests) (Galván et al., 2012). The chemical structure of phenolic compounds provides the plant with a strong filtration power against the destructive ultra-violet (UV) light. So, the main importance of phenolic compounds may be related to the plants exposing to the excess solar high frequency radiation. Synthesis of phenolic and tannin compounds in plants can be affected by some factors such as the influence of environmental stress, disturbance, drought, and electromagnetic radiation, beside the main affecting factors like genetic determinants, physiological demands and evolution-controlled needs (Alonso-Amelot et al., 2007).

Conclusion

In conclusion, studied SW Iranian populations of *Q. brantii*, differ from each other in their acorn morphometry and the amounts of phytochemical contents. Acorn dimensions and weight and phenolic compounds showed to be significantly different between populations. Based on the results of this study, acorn size and weight and phenolic compounds are affected by biotic and abiotic factors including genetic structure of plants, plant growth and physiological conditions, geographical and climate conditions, and attacks from pests. *Quercus brantii* is a dominant species of oak in Zagros forests and further studies would help to provide efficient and useful conservation strategies for this species.

REFERENCES

[1] Aizen, M.A., Woodcock, H. (1992): Latitudinal trends in acorn size in eastern North American species of *Quercus*. - Canadian Journal of Botany 70: 1218-1222.

[2] Alonso-Amelot, M.E., Oliveros-Bastidas, A., Calcagno-Pisarelli, M.P. (2007): Phenolics and condensed tannins of high altitude *Pteridium arachnoideum* in relation to sunlight exposure, elevation, and rain regime. - Biochemical Systematics and Ecology 35 (1): 1-10.

[3] Alonso-Amelot, M.E., Oliveros, A., Calcagno-Pisarelli, M.P. (2004): Phenolics and condensed tannins in relation to altitude in neotropical *Pteridium* spp. A field study in the Venezuelan Andes. - Biochemical Systematics and Ecology 32 (11): 969-981.

[4] Bajalan, I. (2013): Leaves of Oak (*Quercus brantii* var. *persica*) as a rich source of phenolic compounds. - International Journal of Farming and Allied Sciences 2 (19): 734-736.

[5] Bezerra, A.S., Nörnberg, J.L., Lima, F.O., Rosa, M.B., Carvalho, L.M. (2013): Climatic parameters and variation of phenolic compounds in barley. - Ciência Rural 43 (9): 1546-1552.

[6] Borges, L.L., Alves, S.F., Sampaio, B.L., Conceição, E.C., Bara, M.T.F., Paula, J.R. (2013): Environmental factors affecting the concentration of phenolic compounds in *Myrcia tomentosa* leaves. - Brazilian Journal of Pharmacognosy 23 (2): 230-238.

[7] Brookes, P.C., Wigston, D.L. (1979): Variation of morphological and chemical characteristics of acorns from populations of *Quercus petraea* (Matt.) Liebl., *Q. robur* L.and their hybrids. - Watsonia 12: 315-324.

[8] Castro-Díez, P., Villar-Salvador, P., Pérez-Rontomé, C., Maestro-Martínez, M., Montserrat-Martí, G. (1997): Leaf morphology and leaf chemical composition in three *Quercus* (*Fagaceae*) species along a rainfall gradient in NE Spain. - Trees 11 (3): 127-134.

[9] Chang, C.C., Yang, M.H., Wen, H.M., Chern, J.C. (2002): Estimation of total flavonoid content in propolis by two complementary colorimetric methods. - Journal of Food and Drug Analysis 10 (3):178-182.

[10] Frey, W., Probst, W. (1986): A synopsis of the vegetation in Iran. - In: Kurschner, H. (ed.), Wiesbaden, Germany.

[11] Galván, J.V., Novo, J.J.J., Cabreta, A.G., Ariza, D., García-Olmo, J., Navarro-Cerillo, R.M.N. (2012): Population variability based on the morphometry and chemical composition of the acorn in Holm oak (*Quercus ilex* subsp. *ballota* [Desf.] Samp.). - European Journal of Forest Research 131 (4): 893–904.

[12] Galván, J.V., Valledor, L., Cerrillo, R.M.N., Pelegrín, E.G., Jorrín-Novo, J.V. (2011): Studies of variability in Holm oak (*Quercus ilex* subsp. *ballota* [Desf.] Samp.) through acorn protein profile analysis. - Journal of Proteomics 74 (8): 1244-1255.

[13] Ghafour, N., Hoshyar, A., Raad, M. (2010): Determination of some chemical constitutes of oak plants *(Quercus spp.)* in the mountain oak forest of Sulaimani governorate. - Journal of Zankoy Sulaimani 13 (1): 129-142.

[14] Ghasemi, K., Ghasemi, Y., Ehteshamnia, A., Nabavi, S.M., Nabavi, S.F., Ebrahimzadeh, M.A., Pourmorad, F. (2011): Influence of environmental factors on antioxidant activity, phenol and flavonoids contents of walnut (*Juglans regia* L.) green husks. - Journal of Medicinal Plants Research 5 (7): 1128-1133.

[15] Hemati, Kh., Ghasemnezhad, A., Mashayekhi, K., Bashiri-Sadr, Z. (2012): Site effect on some important flavonoid compounds of Linden tree (*Tilia platifolia* L.). - Journal of Plant Production 19 (2): 141-148 [Farsi with English abstract].

[16] Heydari, M., Poorbabaei, H., Rostami, T., Begim-Faghir, M., Salehi, A., Ostad-Hashmei, R. (2013): Plant species in oak (*Quercus brantii* Lindl.) understory and their relationship with physical and chemical properties of soil in different altitude classes in the Arghavan valley protected area, Iran. - Caspian Journal of Environmental Sciences 11 (1): 97-110.

[17] Khoddami, A., Wilkes, M.A., Roberts, T.H. (2013): Techniques for analysis of plant phenolic compounds. - Molecules 18 (2): 2328-2375.

[18] Kofidis, G., Bosabalidis, A.M. (2008): Effects of altitude and season on glandular hairs and leaf structural traits of *Nepeta nuda* L. - Botanical Studies 49: 363-372.

[19] Lantemona, H., Abadi, A.L., Rachmansyah, A., Pontoh, J. (2013): Impact of altitude and seasons to volume, brix content, and chemical composition of Aren Sap in North Sulawesi. - Journal of Environmental Science, Toxicology and Food Technology 4 (2): 42-48.

[20] Lei, J.P., Xiao, W., Liu, J.F., Xiong, D., Wang, P., Pan, L., Jiang,Y., Li, M.H. (2013): Responses of nutrients and mobile carbohydrates in *Quercus variabilis* seedlings to environmental variations using in situ and ex situ experiments. - Plos one 8 (4) e61192: 1-9.

[21] Makkar, H.P.S., Blümmel, M., Becker, K. (1995): Formation of complexes between polyvinyl pyrrolidones and polethylene glycols with tannins and their implications in gas production and true digestibility in *in vitro* techniques. - British Journal of Nutrition 73 (06): 897–913.

[22] Makkar, H.P.S., Blümmel, M., Borowy, N.K., Becker, K. (1993): Gravimetric determination of tannins and their correlations with chemical and protein precipitation methods. - Journal of the Science of Food and Agriculture 61 (2):161–165.

[23] Martin-StPaul, N.K., Limousin, J.M., Rodríguez-Calcerrada, J., Ruffault, J., Rambal, S., Letts, M.G., Misson, L. (2012): Photosynthetic sensitivity to drought varies among populations of *Quercus ilex* along a rainfall gradient. - Functional Plant Biology 39 (1): 25–37.

[24] Mehrnia, M., Nejadsattari, T., Assadi, M., Mehregan, I. (2013): Taxonomic study of the genus *Quercus* L. Sect. *Quercus* in the Zagros forests of Iran. - Iranian Journal of Botany 19 (1): 62-74.

[25] Mpofu, A., Sapirstein, H.D., Beta, T. (2006): Genotype and environmental variation in phenolic content, phenolic acid composition, and antioxidant activity of hard spring wheat. - Journal of Agricultural and Food Chemistry 54 (4): 1265-1270.

[26] Nazari, M., Zolphaghari, R., Fayyaz, P. (2013): Secondary compositions variations under drough stress in *Q.brantii*, *Q.infectoria* and *Q.libani* seedling. - Journal of Forests and Wood Productes 66 (1): 1-14. [Farsi with English abstract].

[27] Oloumi, H., Hassibi, N. (2011): Study the correlation between some climate parameters and the content of phenolic compounds in roots of *Glycyrrhiza glabra.* - Journal of Medicinal Plants Research 5 (25): 6011-6016.

[28] Salminen, J.P., Roslin,T., Karonen, M., Sinkkonen, J., Pihlaja, K., Pulkkinen, P. (2004): Seasonal variation in the content of Hydrolyzable tannins, flavonoid glycosides, and proanthocyanidins in oak leaves. - Journal of Chemical Ecology 30 (9): 1693-1711.

[29] Shahian-Jahromi, M., Siami, A., Heydari, R. (1999): Morphological and chemotaxonomical evaluation of some *Quercus* species of Western Azerbaijan. M.Sc thesis, Department of Biology, University of Urmia, Iran [Farsi with English abstract].

[30] Shiran, B., Mashayekhi, S., Jahanbazi, H., Soltani, A., Bruschi, P. (2011): Morphological and molecular diversity among populations of *Quercus brantii* Lindl. in western forest of Iran. - Plant Biosystems 145 (2): 452-460.

[31] Singh, B., Todaria, N.P. (2012): Nutrients composition changes in leaves of *Quercus semecarpifolia* at different seasons and altitudes. - Annals of Forest Research 55 (2): 189-196.

[32] Şöhretoğlu, D., Kuruüzüm-Uz, A., Simon, A., Patócs, T., Dékány, M. (2014): New secondary metabolites from *Quercus coccifera* L. - Records of Natural Products 8 (4): 323-329.

[33] Spitaler, R., Winkler, A., Lins, I., Yanar, S., Stuppner, H., Zidorn, C. (2008): Altitudinal variation of phenolic contents in flowering heads of *Arnica montana* cv. ARBO: a 3-year comparison. - Journal of Chemical Ecology 34 (3): 369-375.

[34] Taleshi, H., Maasoumi-Babarabi, M. (2013): Leaf morphological variation of *Quercus brantii* Lindl. along an altitudinal gradient in Zagros forests of Fars Province, Iran. - European Journal of Experimental Biology 3 (5): 463-468.

EFFECT OF CONSERVATION MANAGEMENT PRACTICES ON SAND GRASSLAND VEGETATION IN BUDAPEST, HUNGARY

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Abstract. Despite the large-scale human presence in cities, valuable natural habitat fragments survived, displaying outstanding biodiversity. In and around Budapest (Hungary), species-rich sandy grassland vegetation has shrunk drastically and has remained only in small patches and fragments. The presence of invasive plants is a serious problem in all protected sites of Budapest because they endanger the flora and fauna of the isolated habitats. At the Újpest Homoktövis Nature Conservation Area, conservation interventions have been taking place since 2006 to preserve the grassland fragments in the long term. We followed the effects of this management by surveying coenological relevés in ten permanent quadrats of 1 × 1 m each in seven sample sites in 2012 and 2013. We evaluated the coenological records according to the relative ecological indicators (water and nitrogen) of the species. Detrended correspondence analysis (DCA) was also used for data analysis. Changes of the vegetation could be clearly observed during a 7-year-long period, because the species of the sandy grassland have become dominant and this was favourable regarding the aims of the nature conservation. In the stands where management had been carried out for 7 years, a natural or nearly natural vegetation developed, and invasive species and weeds disappeared.

Keywords: *habitat reconstruction, grassland restoration, nature conservation management, sandy grasslands, invasive plant species, habitat in urban environment, Újpest Homoktövis Természetvédelmi Terület*

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Introduction

Geographically, the area of Budapest lies in a varied area, where different types of habitat can be found within relatively small distances (Pécsi, 1958, 1959). Due to its diversity, the area of the city has been populated for a long time, however, from administrative aspects, Budapest – as the result of the unification of three individual towns - can look back only to 140 years of history. From the XVII. century a growing number of studies appeared that aimed to discover wildlife (Bajor, 2009), and reached the same conclusion: thanks to its geographical features, the original wildlife of the present capital is unique and rich in species. In order to implement adequate nature conservation management today, it is essential to know the structure and changes of the original flora and fauna, based on former surveys.

In the first half of the XVIII. century only the hydrography of the Danube was studied (Marsigli, 1726). Afterwards, a prosperity of floristic studies could be observed; its first pioneers were the employees of the Botanical Gardens (Winterl, 1788). Apart from the present area of Budapest, the role of the surrounding areas became more remarkable and a plant identification handbook (Sadler, 1818, 1826, 1840) was prepared based on the studies in the area. Professional publications originally written in Latin were also published or updated in Hungarian (Gönczy, 1864). Parallel to the increasing dominance of Linnaeus' approach, the collective examination of species and their environment started to separate in surveys in Budapest (Bajor, 2011). Later a publication emphasized the unique character of the flora of the landscape (Borbás, 1872). However, an increasing number of scientists have drawn attention to the contraction and degradation of the natural wildlife in the area of Budapest since the early 1990s (Pénzes, 1942; Pénzes and Csízy, 1956; Pécsi, 1958, 1959). By the end of the 20th century, studies of the remaining local fragments has become characteristic because large, coherent areas practically had been abolished by then (Rakonczay, 1992; Simon, 1994; Tardy, 1996; Seregélyes et al., 1996-1997; Pintér 2008).

Research on invasive plants also started in the beginning of 2000s. The presence of invasive plants is a serious problem in all protected sites of Budapest because they endanger the flora and fauna of the isolated habitats (Bajor, 2011). The most dangerous of these species are *Robinia pseudoacacia, Ailantus altissima, Acer negundo*, *Eleagnus angustifolia*, *Solidago canadensis* and *Asclepias syriaca,* and their spread is becoming more intense not only in Budapest but also nationwide. Based on surveys, the extent of the area infested by invasive plants exceeds 1 million hectares in Hungary. Due to their uncontrolled diffusion, the costs of their suppression are increasing from year to year (Mihály and Botta-Dukát, 2004; Udvardy, 1998, 2008a, 2008b). Therefore, the importance of the habitat reconstruction activities is increasing, which usually aim at preserving the isolated but still remaining habitat fragments and, also at reducing the isolation and increasing the extent of grassland patches, if possible (Critchley et al., 2003). These aims can often be reached by habitat restoration, the main objective of which is to restore the original, initial state of the habitat, regarding the present possibilities (Clewell, 2000).

The Újpest Homoktövis Nature Conservation Area ("Természetvédelmi Terület", hereafter Újpest Homoktövis TT) represents one of the last fragments of the former rich sandy grassland vegetation that has shrunk drastically in the last centuries and was thus selected as our study site. Although Raymund Rapaics carried out the first coenological relevés in the area, the actual surveys became regular only after the first half of the 1970s, as the result of the activity of Tibor Simon and his colleagues (Seregélyes et al., 1996-1997). They recognized the outstanding importance of this area, which resulted in the area being declared a nature conservation area in 1974. However, the first studies revealed that the most valuable parts were left out from the reserve and the protection was extended in 1999. Although this area was a closed military area, the degradation and fragmentation of the sandy grassland vegetation had already become significant and was further enhanced by the construction of the Budapest Waterworks by 1999 (Seregélyes et al., 1996-1997). Since then the advance of non-native, mainly woody, species has been observed. These detrimental processes warranted conservation actions, which targeted the restoration of the site. The general aim of this study was to follow the effects of habitat restoration. More specifically, we asked the following questions:

1. How do the restoration activities affect the species number, diversity and species composition of the vegetation?
2. How did the amount of dominant species change during the examined interval (7 years)?
3. How did the vegetation change from 2012 to 2013 in the same quadrats?

Material and methods

Study site

The study area lies on the Pest plain near the left bank of the Danube (Dövényi, 2010). The study area is found within the boundaries of Budapest in the northern part of the Újpest district, and represents a unique natural value of the capital (Verseczki et al., 2007).

The bedrock is decisively river sand brought by the Danube, and also contains calcium, magnesium and bicarbonate. The grain size of the sand, which played an important role in the emergence of the area, varies between 0.02 and 2 mm (Stefanovits et al., 1999; Poller, 2012). A small amount (<1%) of organic material is also present in the sandy habitats, however, its volume increased strongly in the edge zone of the sandy grass remains due to the invasive woody plants.

The Újpest Homoktövis TT was first proposed for protection in the 1950s, but was finally designated in 1974 (Pécsi, 1959; Seregélyes et al., 1996-1997). The protection originally covered 5.7 ha, and it was extended to 24 ha in 1999 and to 40 ha in 2013. Currently the occurrence of 22 protected and specially protected plants is known here. The area was named after the sea buckthorn *(Hippophae rhamnoides)*, because it is the only registered wild occurrence of this species in Hungary, although some researchers debate this (Papp, 1982; Babulka and Turcsányi, 1987; Farkas, 1998; Láng, 2002; Szalay, 2010). From invasive species, *Robinia pseudoacacia, Pinus nigra*, *Eleagnus angustifolia* and *Acer negundo* cover the largest area in the nature reserve (Seregélyes et al., 1996-1997).

Conservation management

In order to preserve the grassland fragments in the long term, conservation interventions have been taking place in the habitat of *Hippophae rhamnoides* since 2006. We have performed this activity with official permission and with the help of volunteers from the Budapest Group of the Hungarian Ornithological and Nature Conservation Society and students from several educational institutions (1200 volunteers so far).

We carried out habitat management strictly after the growing season on 28 occasions between 2006 and 2013.

Management included the mechanical beating down of sprouts of invasive species, and chemical parching of their stems. Management actions were planned systematically, and we managed to clear invasive plants in an area of c. 5 ha in the last seven years. As a result, c. 40% of the treated area regenerated and became sand grassland again. In the first period this value was only about half of the current rate, i.e. only 20% of the habitat was free of woody invasive plants.

Data collection

Reflecting the sites of the yearly treatments between 2006 and 2013, we divided the area into seven sample areas. After the first treatment, maintenance activities took place in every sample area for the following four years, and in addition, eradication and mowing was performed, if necessary.

Accordingly, we studied different phases of sandy grasslands in the following seven sample areas: I: no treatment, the area has been a natural grassland for more than 20 years, II: treated (invasive woody species cleared) in 2006, III: treated in 2007, IV: treated in 2008, V: treated in 2009, VI: treated in 2010 (it emerged after the shrub eradication, that this area was filled with debris and on this surface shrubs and trees began to grow), VII: treated in 2011.

We designated coenological relevés in ten permanent quadrats of 1 x 1 m in each sample area, and recorded cover values of all vascular plant species in percentages. We defined the bottom left corners of the quadrats by GPS coordinates. Sampling was carried out in 2012 and 2013. For plant species nomenclature, we used Király (2009), and for plant communities, we followed Borhidi et al. (2012).

Data analysis

We evaluated the coenological records according to the relative ecological indicators (here we used water and nitrogen demand) of the vascular plant species (Borhidi, 1995) based on the database „Flóra" (Horváth et al., 1995). We calculated the Shannon diversity index for all 70 quadrats, covering both sampling dates. We calculated with the species whose estimated cover exceeded 8% as per the averaged quadrats as dominant.

In order to present the similarities and differences between the areas, we used a cluster analysis based on the Bray-Curtis matrix of dissimilarity, and an ordination (Pavlů et al., 2011). Hierarchical clustering with complete linkage methods were computed for dendrograms in which each element classify of its own. Each clusters combined sequentially into larger clusters until these elements are in the same clusters. Always the shortest distances are combined. Detrended correspondence analysis (DCA) was used for data analysis.

We combined the data of each species recorded in the quadrats, and if one species was found simultaneously in several various areas during one year, we considered the highest cover value for DCA analysis. We used this method of simplification instead of the averaging method, because using the averaging method, the determinant dissimilarities and the higher average cover values of the portions of areas might have disappeared. Further, this method was the best one to conform with our sampling method. After elimination of outliers two main axes represented with this multivariate technique: observed species and divergent areas taken into investigation. DCA is superior in nonmetric multidimensional scaling hence its an improved reciprocal averaging technique. DCA limitations are related with extreme outliers so the best to remove them before analysis.

We conducted all analyses using the program R 3.02. (R Development Core Team 2015).

Results

Vegetation of the areas

The number of species decreased significantly from 2012 to 2013 in areas 1-5 (Mann-Whitney test, $p<0.05$). The smallest decrease was in area I. There was no clear tendency in species numbers among areas when years were considered separately (*Table 1*).

Table 1. *Average species number (± SD) in the study areas*

	I	II	III	IV	V	VI	VII
2012	9±3	15±2	11±2	13±3	16±3	13±4	12±3
2013	6±2	8±2	8±2	9±3	10±3	12±4	14±4

However, species compositions changed in various directions between years and among areas. In areas I and II, species composition was nearly identical in every relevé, and the common dominant species were *Festuca vaginata, Stipa borysthenica* and *Peucedanum arenarium.* While *Fumana procumbens* was dominating in site I, its cover values decreased in site II. In site II, cover values of *Festuca pseudovaginata* increased similarly to *Festuca vaginata,* which is a characteristic and dominant species for sandy grasslands.

The dominance relationships of the two *Festuca* species changed among areas because *Festuca pseudovaginata* had relatively high cover values in site II and V, while *Festuca vaginata* was dominant in site I and III. *Stipa borysthenica* occurred in sites I-V and VII and missing in site VI. *Stipa capillata* was present in sites I-IV with relatively low cover values and it did not occur in sites V-VII (*Table 2.*).

Table 2. *Mean cover values of the dominant species in the study areas (FES_PSE: Festuca pseudovina, FES_VAG: Festuca vaginata, STI_BOR: Stipa borysthenica, STI_CAP: Stipa capillata)*

	FES_PSE	FES_VAG	STI_BOR	STI_CAP
I/2012	0,00	12,10	3,00	0,00
I/2013	0,00	8,70	4,70	0,40
II/2012	4,20	5,10	7,80	0,50
II/2013	5,00	0,80	10,90	0,00
III/2012	1,00	11,50	2,50	0,40
III/2013	0,44	16,11	2,22	3,33
IV/2012	0,00	3,90	1,80	1,30
IV/2013	0,00	1,00	2,00	0,30
V/2012	4,10	0,00	0,60	0,00
V/2013	8,10	2,50	2,60	0,00
VI/2012	0,00	0,00	0,00	0,00
VI/2013	0,00	0,00	0,00	0,00
VII/2012	0,20	0,00	0,00	0,00
VII/2013	2,48	0,50	0,64	0,00

The distribution of the woody species is also important since it indicates the success of the conservation treatment. Six invasive woody species occurred mostly in sites V - VII: *Ailanthus altissima, Acer negundo, Populus canadensis, Robinia pseudoacacia, Fraxinus pennsylvanica* and *Celtis occidentalis* (*Fig. 1.*). The native shrubs and trees were almost missing from areas I-IV, whereas their amount was higher in sites V-VII.

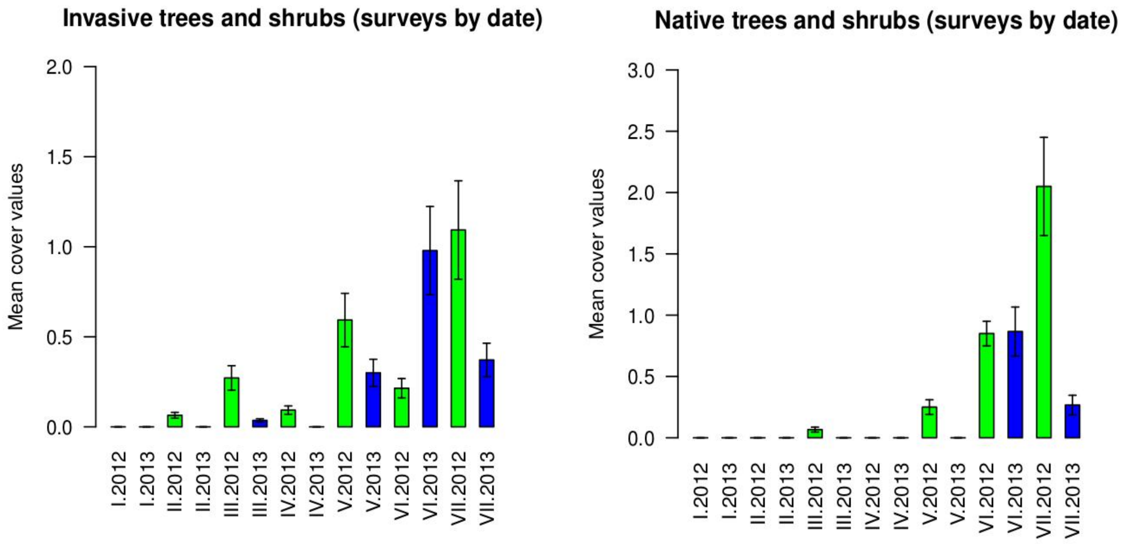

Figure 1. *Mean cover of invasive and native woody species in the study areas*

The cover of weed species was high in areas VI and VII, and in areas which had been treated for a longer period of time, the proportion of weeds was reduced (*Fig. 2*). Significant difference were determined among 14 distinct examined groups with Kruskall-Wallis test (chi-squared = 102.7425, df = 13, p-value = 4.871e-16). Coverage of weeds significantly decreased in area VI (p=0.0480) and VII. (p=0.0177) between years 2012 and 2013 (*Fig. 2.*).

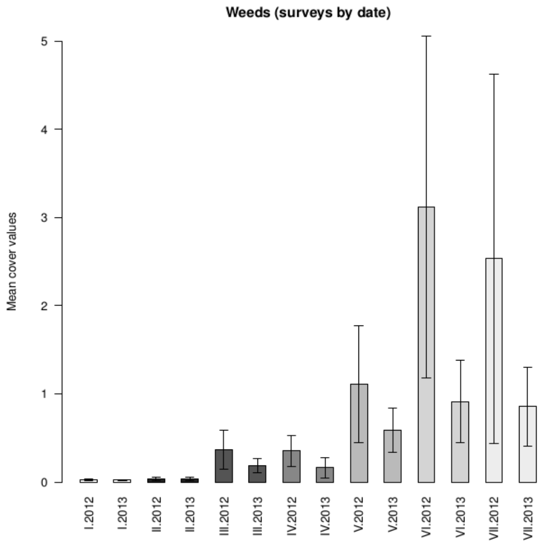

Figure 2. *Occurrence of weed species in the study areas*

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The relevés of 2013 showed a less diverse state, except two areas (I and VII). In areas II-V, nearly the same diversity values were observed in 2012. In these areas, diversity was higher than in site I (natural grassland) because the presence of weed species caused an increase in diversity. The lowest diversity was found in area I in 2012 and in area VI in 2013. In area I, diversity was relatively low in both years and showed no change, in contrast to the other, treated areas (*Fig. 3.*). Kruskall-Wallis test showed significant difference among distinct examined groups (chi-squared = 48.1906, df = 13, p-value = 6.073e-06). Shannon diversity values showed significant ($p<0.05$) differences among VI (2013) and II (2012 and 2013), VII (2013). There was no significant differences among groups in every other comparisons.

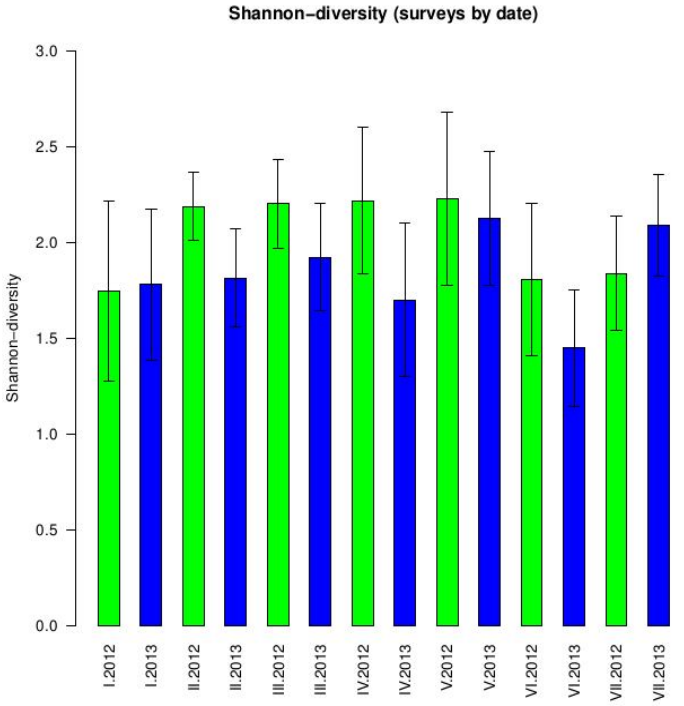


Figure 3. *Diversity values in the seven areas in 2012 and 2013*

Based on the classification of the coenological relevés (*Fig. 4.*), species of areas VI and VII showed the highest deviation from those of the other areas because of the higher amount of weed species. The quadrats of area VI formed a relatively distinct group. Records from area I and II were also separated from the other groups (these are the relevés from the natural grassland and the area treated for the longest time). The fragmentation is based on the amount of the dominant species (*Festuca vaginata, F. pseudovaginata, Poa bulbosa, Carex liparicarpos, Fumana procumbens).*

The DCA analyses showed that *Clematis vitalba*, *Melandrium album* and *Oenothera biennis* occuring in areas VI and VII were clearly separated from the rest of the species. Characteristic sandy grassland species (*Festuca pseudovaginata, Festuca vaginata, Potentilla arenaria, Peucedanum arenarium and Fumana procumbens*) formed another group. These species occurred both in the natural grasslands or in previously treated areas (areas I-V.). The invasive species (*Robinia pseudoacacia, Ballota nigra,* *Ambrosia artemisifolia, Conyza canadensis*) also formed a sharply separated group. These species were also found in the recently treated areas VI and VII. Area VII differed greatly between the two years, in 2012 it was very different from the other relevés, whereas in 2013 it was very similar to them. The reason can be that after shrub eradication the species composition of weeds changed rapidly, weed species occurred in the first year were disappeared one year later.

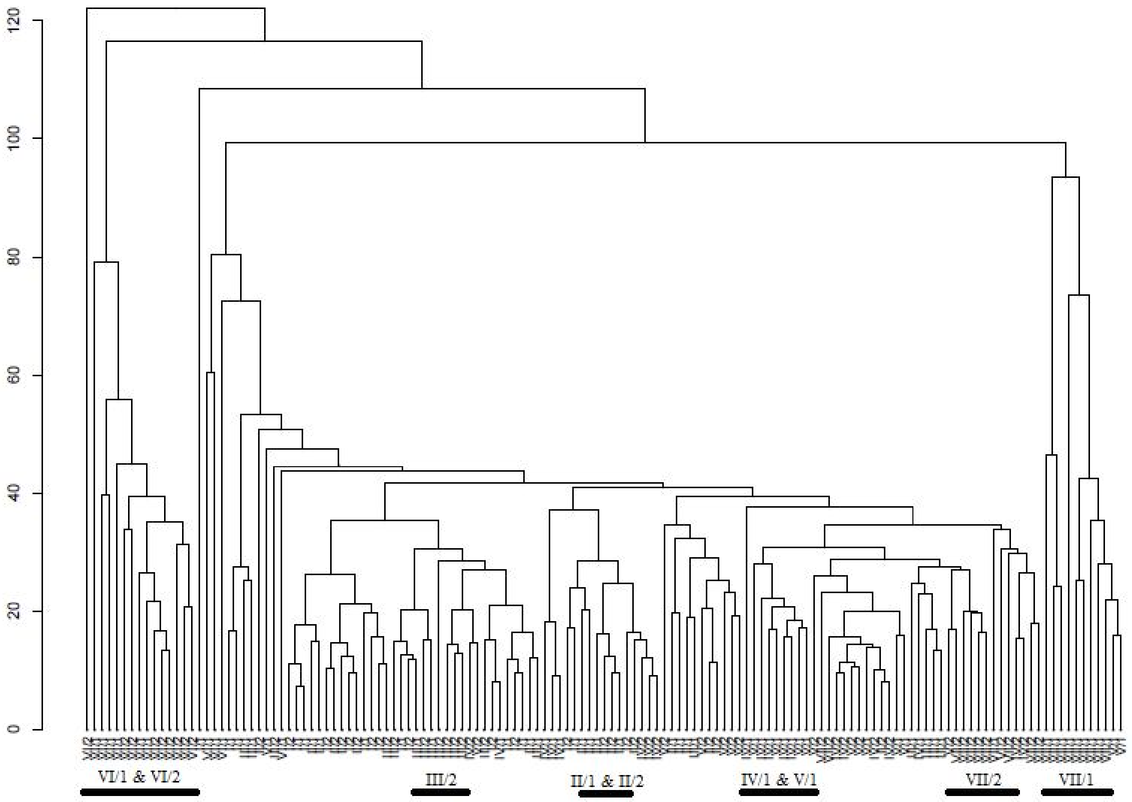
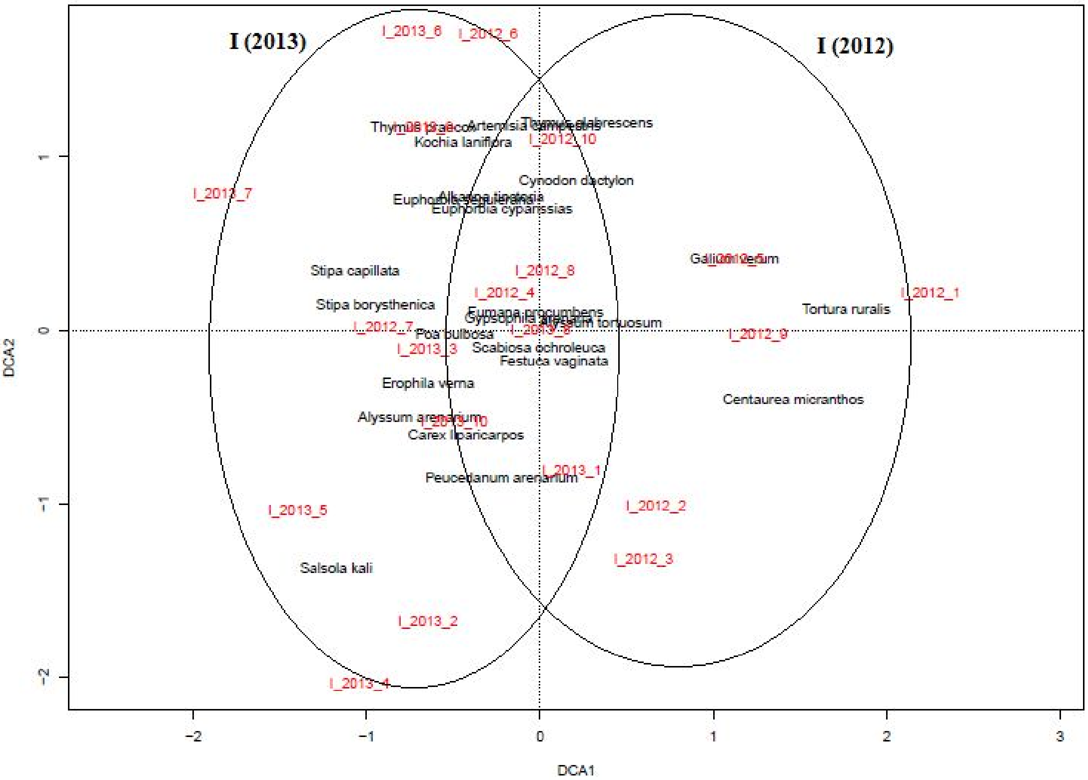
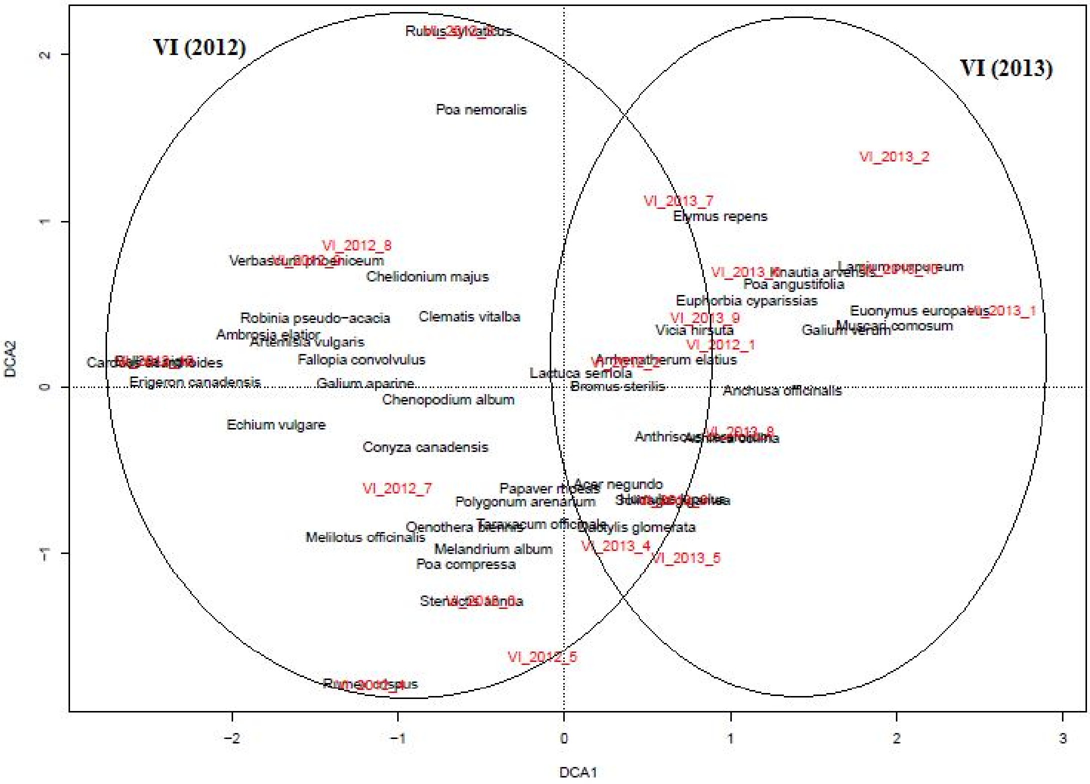


Figure 4. *Dendrogram of the coenological relevés of the study sites (1: 2013, 2: 2012)*

Mainly areas I and VII were separated. Invasive species were also found in areas V, VI and VII; however the analysis showed that they disappeared in areas I-IV. The aggregation of areas I, II, III and IV demonstrates the adequate environmental conditions of the sand species. Areas VI and VII were mostly covered by weeds and invasive species, because regeneration of the sandy grassland had started there only a short time ago.

Fig. 5. shows the shift in the vegetation. In area I, the records of 2012 and 2013 overlapped substantially. In contrast, records from the two years separated greatly from one another in area VI and particularly in area VII, demonstrating that the vegetation was going through a transformation in these recently managed areas. Species composition of area I can be regarded stable from a coenological point of view. In contrast, area VII is in the beginning of the regeneration process and in course of this, rapid changes in the weed species composition can cause coenological variation between years.


I (2013)
I (2012)
I_2013_6
I_2012_6
Thymus praecox
Artemisia campestris
Thymus glabrescens
Kochia laniflora
I_2012_10
I_2013_7
Cynodon dactylon
Euphorbia seguieriana
Alkanna tinctoria
Euphorbia cyparissias
Stipa capillata
I_2012_8
Galium verum
I_2012_5
I_2012_4
Stipa borysthenica
Fumana procumbens
Gypsophila arenaria
Alyssum tortuosum
I_2012_1
Tortura ruralis
I_2012_7
Poa bulbosa
I_2012_9
I_2013_3
Scabiosa ochroleuca
Festuca vaginata
Erophila verna
Centaurea micranthos
Alyssum arenarium
I_2013_10
Carex liparicarpos
I_2013_1
Peucedanum arenarium
I_2012_2
I_2013_5
I_2012_3
Salsola kali
I_2013_2
I_2013_4
DCA2
DCA1
−2
−1
0
1
2
3


VI (2012)
VI (2013)
Rubus caesius
Poa nemoralis
VI_2013_2
VI_2013_7
Elymus repens
VI_2012_8
Verbascum phoeniceum
Chelidonium majus
VI_2013_6
Knautia arvensis
Lamium purpureum
Poa angustifolia
Euphorbia cyparissias
Euonymus europaeus
VI_2013_1
Robinia pseudo−acacia
Clematis vitalba
VI_2013_9
Vicia hirsuta
Galium verum
Muscari comosum
Ambrosia elatior
Artemisia vulgaris
VI_2012_1
Carduus acanthoides
Fallopia convolvulus
Arrhenatherum elatius
VI_2012_2
Lactuca serriola
Erigeron canadensis
Galium aparine
Bromus sterilis
Anchusa officinalis
Chenopodium album
Echium vulgare
Anthriscus cerefolium
VI_2013_8
Conyza canadensis
VI_2012_7
Acer negundo
Papaver rhoeas
Polygonum arenarium
Oenothera biennis
Taraxacum officinale
Dactylis glomerata
Melilotus officinalis
Melandrium album
VI_2013_4
VI_2013_5
Poa compressa
VI_2012_5
DCA2
DCA1
−2
−1
0
1
2
3

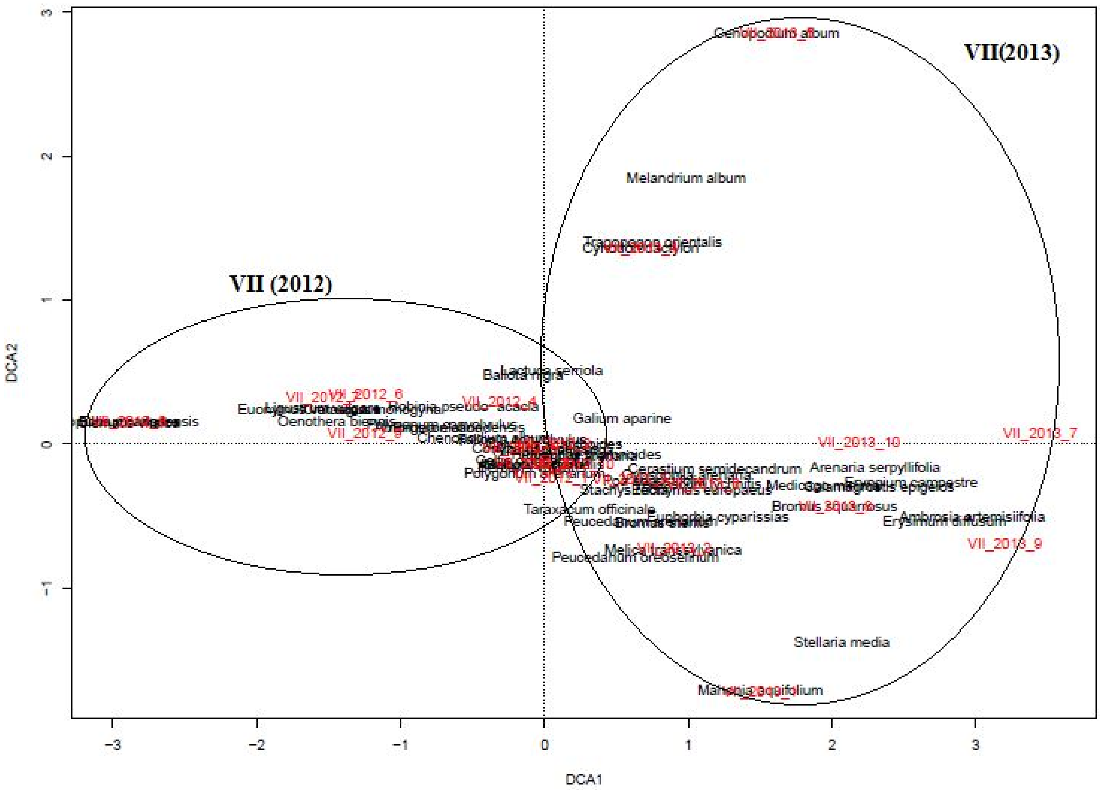


Figure 5. DCA (Detrended Correspondence Analysis) representation of the frequent plant species on the relevés of 2012 and 2013 in sites I, VI and VII

Analysis of the vegetation of sites based on relative ecological indicator values

Areas I and II had the highest cover of species requiring nutrient-poor soils (categories 1 and 2) on the basis of their relative nitrogen demand, followed by areas III and IV (*Fig. 6*). Area V was a submesotrophic and slightly nutrient-rich habitat (categories 3 and 4). In areas VI and VII, the cover of plants indicating medium (category 5) and high nitrogen volume (categories 7 and 8) was also high. From 2012 to 2013, the proportion of plants indicating different levels of nitrogen volume shifted. Although the cover of plants in categories 1 and 2 was similarly high in 2012 and 2013 in area I, it increased from 2012 to 2013 in areas II to V, and the difference was larger in more recently managed areas (*Fig. 6*). In area IV, a spectacular shift appeared toward species demanding less nitrogen; the cover of species in categories 1 and 2 increased, while that of species in category 3 decreased. In area V, this process just started (*Fig. 6*).

On the basis of the relative water demand of the species, drought indicator plants (categories 1 and 2) had the highest proportion in area I in 2012 (*Fig. 7.*). In area II, species typical in habitats of long drought periods (category 2) increased considerably from 2012 to 2013, resulting in a similar cover of category 1 and 2 plants in areas I and II in 2013. In site I, the cover of rocky, semi-desert species was highest in 2013. The proportion of drought-tolerant species decreased in areas with more recent management and the proportion of species of more humid areas, including forest species, increased as advancing from area III through VII. The proportion of species with different levels of relative water demand also shifted from 2012 to 2013. Again, the increase in the proportion of drought indicator species (categories 1 and 2) was greater in areas with more recent management (*Fig. 7*), i.e. the earlier the shrub eradication, the higher the proportion of drought indicator plants. On the basis of these data, area VI represents an outstanding peak, similarly to the case with relative nitrogen demand.

Figure 6. *Average cover values of species according to their relative nitrogen demand.*

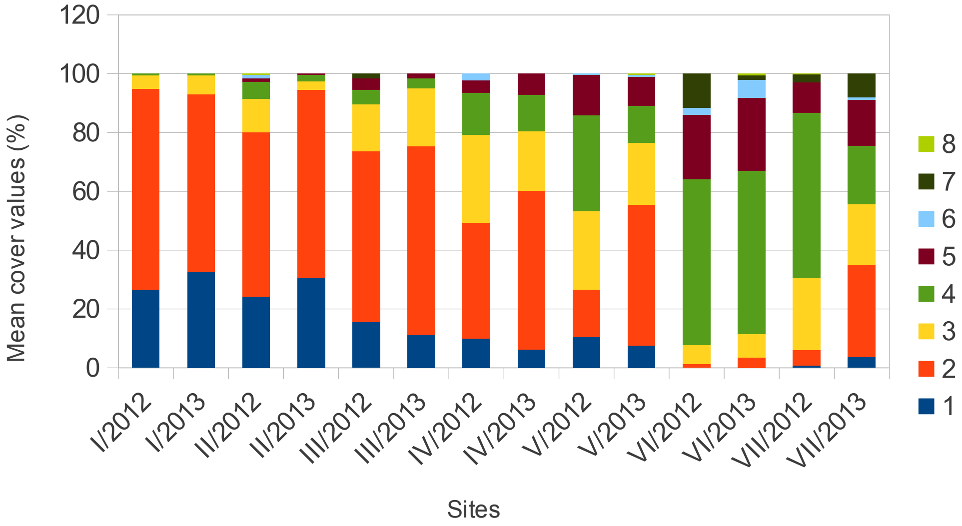


Figure 7. *Average cover values of species according to the relative water demand*

Discussion

Nowadays the issue of habitat reconstruction is becoming more significant, and an increasing number of studies deal with the active restoration of valuable habitats (Török et al. 2009; Vida et al., 2008; Penksza et al., 2011). However, the restoration of natural and semi-natural habitats in urban environments is almost unknown in Hungary (Kézdy and Tóth, 2013). In order to accelerate the restoration process, over-sowing

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with seed-mixture is used in many cases (Bakker, 1989; Jongepierová et al., 2007; Török et al., 2009). In this study, overseeding did not seem appropriate because fragments of the original plant communities existed as a propagulum source (Pintér, 2008).

Changes in the vegetation could be clearly observed during a 7-year-long period. The most important change was that species of sandy grasslands have become dominant, which can generally be regarded as positive for nature conservation (Borhidi et al., 2012). Regarding our first question, our study also shows that as a result of the gradual removal of invasive woody plants, favourable conditions can be established for species of sandy grasslands in the long term. Based also on the findings of other studies, the short-term post-treatment (mowing) caused significant changes and a further increase in species richness can be expected (Ilmarinen and Mikola, 2009; Török et al., 2007; Vida et al., 2008).

Our results showed that continuous sandy grassland regenerated in study areas II and III, where conservation management has taken place for 7 years. Our coenological data showed that weeds and invasive species have gradually disappeared from the natural reserve area, and that species composition has also become more similar to the sandy vegetation. In contrast to other data indicating that the spontaneous restoration of grasslands can be very slow (Török et al., 2008), the process is taking place at a rapid pace in the study area.

Regarding our second question, we found that in stands where conservation management had been carried out for seven years, a natural or nearly natural vegetation developed. In these quadrats, invasive species and weeds have practically disappeared. Our investigations revealed that as a consequence of the treatments the stands of *Festuca vaginata* and *Festuca pseudovaginata* have been continuously increasing. Instead of *Festuca vaginata, Festuca pseudovaginata* is becoming dominant in disturbed areas (Penksza, 2003), which has been confirmed by our results. On the basis of the ecological indicators, area VI (where management had been carried out earlier) is displaying a more unfavourable view than site VII. The most important change was detected in case of indicator values of nitrogen demand. After shrub eradication topsoil contains more humus and nutrients yet which originated from the leaves of the former woody vegetation. However, in course of leaching of soil amount of nutrients probably decreasing rapidly year by year, which can cause that less and less species with high nitrogen demand occur.

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REFERENCES

[1] Babulka, P., Turcsányi, G. (1987): Természetvédelmi terület vagy szemétlerakóhely? Veszélyben az újpesti homoktövis [Nature conservation area or dump? Sea buckthorn in danger in Újpest] - Búvár 42(3): 38-39 (in Hungarian)

[2] Bajor, Z. (2009): Budapest természeti kalauza [Nature guide of Budapest] - Kossuth Kiadó, Budapest (in Hungarian)

[3] Bajor, Z. (2011): Özönnövények és egyéb tájidegen növényfajok előfordulása Budapest helyi védettségű természeti értékein [Occurence of invasive plant species in the local nature conservation areas in Budapest] - Fővárosi Önkormányzatnak készített tanulmánykötet, Budapest (in Hungarian)

[4] Bakker, J. P. (1989): Nature management by grazing and cutting. On the ecological significance of grazing and cutting regimes applied to restore former species rich grassland communities in the Netherlands - Kluwer Academic Publishers, Dordrecht

[5] Borbás, V. (1872): Pest-megye flórája Sadler óta és újabb adatok [New data to the flora of Pest county] - Math. Term. Közlem. 9:15-54. (in Hungarian)

[6] Borhidi, A. (1995): Social behaviour types, the naturalness and relative ecological indicator values of the higher plants in the Hungarian flora. - Acta Botanica Hungarica 39(1-2): 97-181.

[7] Borhidi, A., Kevey, B., Lendvai, G. (2012): Plant communities of Hungary - Akadémiai Kiadó, Budapest

[8] Clevell, A. F. (2000): Restoring for natural authenticity - Ecological Restoration 18: 216-217.

[9] Critchley, C. N. R., Burke, M. J. W., Stevens, D. P. (2003): Conservation of lowland semi- natural grasslands in the UK: a review of botanical monitoring results from agri-environment schemes - Biological Conservation 115: 263-278.

[10] Dövényi, Z. [ed.] (2010): Magyarország kistájainak katasztere [Inventory of microregions of Hungary] - Magyar Tudományos Akadémia, Földrajztudományi Kutatóintézet, Budapest (in Hungarian)

[11] Farkas, S. (1998): Magyarország védett növényei [Protected plants of Hungary] - Mezőgazda kiadó, Budapest (in Hungarian)

[12] Gönczy, P. (1879): Pest megye és tájéka viránya [Flora of Pest county and surroundings] - Magyar Királyi Egyetemi Ny., Budapest (in Hungarian)

[13] Horváth, F., Dobolyi, Z. K., Morschhauser, T., Lőkös, L., Karas, L., Szerdahelyi, T. (1995): Flóra adatbázis 1.2. Taxonlista és attribútum állomány. [Data base of flora 1.2. Taxonomy list and attributes.] - MTA ÖBKI, Vácrátót (in Hungarian)

[14] Ilmarinen, K., Mikola, J. (2009): Soil feedback does not explain mowing effects on vegetation structure in a semi-natural grassland - Acta Oecologica 35(6): 838-848.

[15] Jongepierova, I., Mitchley, J., Tzanopoulos, J. (2007): A field experiment to recreate species rich hay meadows using regional seed mixtures - Biological Conservation 3: 297-305.

[16] Kézdy, P., Tóth, Z. [eds.] (2013): Természetvédelem és kutatás a budai Sas-hegyen. Tanulmánygyűjtemény [Nature conservation and research in Sas-hegy. Case studies] - Duna-Ipoly Nemzeti Park Igazgatóság, Budapest (in Hungarian)

[17] Király, G. [ed.] (2009): Új magyar füvészkönyv. Magyarország hajtásos növényei. Határozókulcsok [New Hungarian herbal. The vascular plants of Hungary. Identification key] - ANP Igazgatóság, Jósvafő (in Hungarian)

[18] Láng, I. [EIC] (2002): Környezet- és Természetvédelmi Lexikon I. [Encyclopedia of nature conservation and environment protection] - Akadémia Kiadó, Budapest (in Hungarian)

[19] Marsigli, L.F. (1726): Danubius pannonico-mysicus. Reprint by Andras, D.A. 2004. - Vízügyi Múzeum, Budapest

[20] Mihály, B., Botta-Dukát, Z. [eds] (2004): Biológiai inváziók Magyarországon – Özönnövények [Biological invasions in Hungary – Invasive plants] - Természetbúvár Alapítvány Kiadó, Budapest (in Hungarian)

[21] Papp, L. (1982): A homoktövis jelentősége és szaporítása [Importance and propagation of *Hippophae rhamnoides*] - Az erdő, 7: 309-312. (in Hungarian)

[22] Pavlů, L., Pavlů, V., Gaislera, J., Hejcman, M., Mikulka, J. (2011): Effect of long-term cutting versus abandonment on the vegetation of a mountain hay meadow (*Polygono-Trisetion*) in Central Europe - Flora 206: 1020-1029.

[23] Penksza, K. (2003): Festuca pseudovaginata, a new species from sandy areas of the Carpathian Basin. – Acta Bot. Hung. 45: 356-372.

[24] Penksza, K., Szentes, Sz., Loksa, G., Dannhauser, C., Házi, J. (2011): A legeltetés hatása a

gyepekre és természetvédelmi vonatkozásai a Tapolcai- és a Káli-medencében [Nature conservation aspects of grazing in the Tapolca and Káli Basins] - Természetvédelmi Közlemények 16: 33-57 (in Hungarian)

[25] Péchy, M. [ed.] (1958): Budapest természeti képe [Environmental characteristics of Budapest] - Akadémiai Kiadó, Budapest (in Hungarian)

[26] Péchy, M. [ed.] (1959): Budapest természetföldrajza [Geography of Budapest] - Akadémiai Kiadó, Budapest (in Hungarian)

[27] Pénzes, A. (1942): Budapest élővilága [Wildlife of Budapest] - Királyi Magyar Természettudományi Társulat, Budapest (in Hungarian)

[28] Pénzes, A., Csizy, F. (1956): Budapest élővilága [Wildlife of Budapest] - Társadalom- és Természettudományi Ismeretterjesztő Társulat, Budapest (in Hungarian)

[29] Pintér, B. (2008): Budapest védelemre javasolt területei [Proposed protected areas in Budapest] - Tölgy Természetvédelmi Egyesület tanulmánykötete, Gödöllő (in Hungarian)

[30] Poller, E. F. (2012): Feldarabolódó homokpusztagyepek Dunakeszin és környékén [Fragmented sandy grasslands in Dunakeszi and sorroundings] - BSc. szakdolgozat, SZIE MKK-TTT, Gödöllő (in Hungarian)

[31] Rakonczay, Z. (1992): Sas-hegytől a Kálvária-dombig; Az Észak-Dunántúl természeti értékei [From Sas-hegy to Kálvária-domb; Natural values of the northern part of Dunántúl] - Mezőgazda Kiadó, Budapest (in Hungarian)

[32] R Development Core Team (2015). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.r-project.org/

[33] Sadler, J. (1818): Verzeichniss der um Pesth und Ofen wildwachsenden phanerogamischen Gewächse mit Angabe ihrer Standorte und Blüthezeit - Pesth

[34] Sadler, J. (1826): Flora comitatus pestiensis - Pestini

[35] Sadler, J. (1840): Flora comitatus Pesthinensis, Ed. 2. – Pesthini

[36] Seregélyes, T., S. Csomós, Á., Szél, Gy., Szollát, Gy. (1996-97): Budapest Főváros természetvédelmi területeinek élővilága 10-7; Az Újpesti Homoktövis Élőhelye [Flora and fauna of the nature conservation areas of Budapest; Habitat of *Hippophae rhamnoides* in Újpest] - Botanikus Bt., Budapest, 38 p. (in Hungarian)

[37] Simon, T. [ed.] (1994): Természeti kincsek Dél-Budán; A Tétényi-fennsík és a Háros-sziget növény- és állatvilága, természetvédelme [Natural values in Dél-Buda; Flora, fauna and nature conservation of the Tétényi-fennsík and Háros-sziget] - Cserépfalvi Kiadó - Zöld Jövő, Budapest (in Hungarian)

[38] Stefanovits, P., Filep Gy, Fileky Gy. (1999): Talajtan [Pedology] - Mezőgazdasagi Kiadó, Budapest (in Hungarian)

[39] Szalay, L. (2010): Páratlan gyógyító hatás: A homoktövis [Unique healing effect: The sea buckthorn] - Biokultúra: A Magyar Biokultúra Szövetség szakfolyóirata, 3: 21. (in Hungarian)

[40] Tardy, J. [ed.] (1996): Magyarországi települések védett természeti értékei [Protected natural values of Hungarian settlements] - Mezőgazda Kiadó, Budapest (in Hungarian)

[41] Török, P., Arany, I., Prommer, M., Valkó, O., Balogh, A., Vida, E., Tóthmérész, B., Matus, G. (2007): Újrakezdett kezelés hatása fokozottan védett kékperjés láprét fitomasszájára, faj- és virággazdagságára [Above ground vegetation and phytomass of strictly protected abandoned hay-making Molinion meadows in Zemplén Mountains (Hungary) after restored management] - Természetvédelmi Közlemények 13: 187-197 (in Hungarian)

[42] Török, P., Matus, G., Papp, M., Tóthmérész, B. (2008): Secondary succession in overgrazed Pannonian sandy grasslands - Preslia 80: 73-85

[43] Török, P., Arany, I., Prommer, M., Valkó, O., Balogh, A., Vida, E., Tóthmérész, B., Matus, G. (2009): Vegetation, phytomass and seed bank of strictly protected hay-making Molinion meadows in Zemplén Mountains (Hungary) after restored management - Thaiszia - J. Bot., Košice, 19, Suppl. 1: 67-78.

[44] Udvardy, L. (1998): Classification of adventives dangerous to the Hungarian natural flora. Acta Botanica Hungarica 40: 315-331.
[45] Udvardy, L. (2008a): Boxelder (Acer negundo L.). In: Botta-Dukát Z., Balogh L. (szerk): The most important invasive plants in Hungary. Hungarian Academy of Sciences, Institute of Ecology and Botany, Vácrátót. pp. 121-127.
[46] Udvardy, L. (2008b): Tree of Heaven (Ailanthus altissima (Mill.) Swingle). In: Botta-Dukát Z., Balogh, L.(szerk): The most important invasive plants in Hungary. Hungarian Academy of Sciences, Institute of Ecology and Botany, Vácrátót. pp. 121-127.
[47] Verseczki, N., Lendvai, Cs., Pintér, B. (2007): Az Újpesti homoktövis és élőhelyének védelme [Habitat and protection of *Hippophae rhamnoides* in Újpest] - Madártávlat, 1: 18- 20. (in Hungarian)
[48] Vida, E., Török, P., Deák, B., Tóthmérész, B. (2008): Gyepek létesítése mezőgazdasági művelés alól kivont területeken: a gyepesítés módszereinek áttekintése [A review and assessment of grassland restoration techniques in arable lands] - Botanikai Közlemények 95 (1-2): 115-125. (in Hungarian)
[49] Winterl, J. J. (1972): Index Horti Botanici Universitatis Hungaricae, quae Pestini est. 1788. - Facsimile edition, Akadémiai Kiadó, Budapest

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THE EFFECT OF RIPARIAN FOREST SHADE ON THE STRUCTURAL CHARACTERISTICS OF MACROPHYTES IN A MID-FOREST LAKE

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Abstract. The aim of the study was to evaluate whether riparian trees limit growth of aquatic plants in lakes. In the studied lake, sections of the littoral zone were shaded to different degrees. The maximum extent of shading in the studied lake did not exceed 15 m. Regardless of the amount of light reaching the lake three groups of macrophytes developed. The data suggest that shading by riparian vegetation was not limiting to growth of all aquatic plants in the studied lake but did significantly limit development of macrophyte communities. The extent of shading was correlated with both the structure of macrophyte communities in addition to their range. In shady areas density, colonization depth, biomass and diversity index of emergent macrophytes were significantly lower. Submerged macrophytes in the lake were generally outside the scope of shadow impact. Only macrophytes occurring between 0.5 and 1 m depth were influenced by shade. Generally, submerged macrophyes were outside of the influence of shade. However, along the northern shore, lush growth of adjacent rushes may have effected submerged macrophytes and in these areas their measured values (biomass, density, abundance) were lower. In the southern part of the lake, rushes achieve lower density and extent of occurrence, but submerged macrophytes grew better, and both biomass and species richness were higher than along northern shores.

Keywords: *macrophytes, mid - forest lake, riparian vegetation, biomass, shading*

Introduction

Light is a key driving factor of life in aquatic ecosystems. Light penetration influences many processes regulated by “e.g. water density and viscosity, the solubility of solids and gases and the rate of metabolic processes in plants (Chełmicki, 2001; Squires et al., 2002; Sevindik and Celik, 2014). The temperature tolerance range of individual plant species is genetically fixed and most freshwater macrophytes appear to be eurythermic (Madsen and Brix, 1997; Feldmann, 2012).

Light availability is a critical environmental factor determining the growth of macrophyte communities and their species richness (Lacoul and Freedman, 2006). Light can also influence community composition, as well as zonation within a water body (Wersal and Madsen, 2013). In some cases, shading leads to the elimination of both emergent and submerged macrophytes (Bernatowicz, 1966). Submerged aquatic plants have morphological adaptations to colonizing and establishing populations in low light environments (Barko et al., 1986). The quantity of light reaching the water is especially important for these species, because in aquatic habitats light rays reaching plants is significantly reduced (Chambers and Kalff, 1985; Lehmann and Lachavanne, 1999; Sender, 2012). The anatomical and morphological differences of submersed and emergent macrophytes may result in specific adaptations to environments conditions.

Differences created by littoral geomorphology, water level fluctuation, variable light, and anthropogenic influences create a wide range of habitats for emergent and submerged water plants, as well as for floating-leaved plants (Feldmann and Nöges, 2007; Sender, 2010). Research suggests that greatly limits primary production in lakes thus it is speculated that managing for light could aid in the technical fight against overgrowing (Dawson, 1981; Spence, 1981; Ali et al., 2011).

The aim of the study was to investigate whether decreases in light from riparian trees shading limits growth of aquatic plants in lakes. In addition, data was collected to assess the effects of duration of sun exposure on species composition, zonation and biomass of different groups of macrophytes in the studied lake. These data will aid in understanding shifts in the structure and composition not only of submerged macrophytes plant communities that are often described in the literature (Lu et al., 2013; Squires et al., 2002; Wersal and Madsen, 2013), but also communities of emergent macrophytes which are much less studied. Determining correlations between trends light conditions and colonization patters would inform management and restoration of eutrophic lakes being overgrown by aquatic vegetation.

Materials and Methods

Lake Czarne Sosnowickie (51 ° 31' N, 23° 02' E) is located in the north-western part of Sosnowica commune, in the foothills of the western slope of the Garb Włodawski in north eastern Poland. It is located in the basin of rivers North - Piwonia – Tyśmienica - Wieprz. The lake’s catchment area is formed in the Quaternary formations, set on the Calcareous, reaching 23 m below water surface. Shallow banks are covered with a thin layer of sand (Harasimiuk et al., 1998). This lake is one of the seven deepest lakes in the area of the Łęczna-Włodawa Lakeland (eastern Poland). The surface area of the lake is 37,6 ha and the maximum depth is 15,6 m.

The north and east shores of the lake are surrounded by pine forests mixed with birch and the south and southwest there is a swampy forest. The lake includes two small drainage ditches, which are the main cause of anthropogenic pressure on the lake. The reservoir is used for storage of water used by the fish farm in Sosnowica. Mid-forest location, paludification of the south shore and the difficult availability of the banks, makes it slightly seasonally used for tourist purposes (*Fig. 1*).

Because the lake was located in the midst of a forest, determination of research positions with various degrees of shading of the littoral zone was possible. The study was performed during the summer (in June and July) of 2013. The study was conducted in six transects I-III in the northern part (I - north - west, II - north, III - north - east) and IV-VI in the southern part of the lake (IV - south east, V - south, VI - south - west) (*Fig. 1*). The number and location of transects was associated with the greatest likeness of morphometric conditions in north and south parts of lake, as well as diversification of insolation conditions. Each of the transects started from the shoreline, and the distance into the lakes center depended on the range of macrophytes occurrence. In addition, in order to determine the total area occupied by macrophytes in the analyzed lake, another four transects on the eastern and western shores of the lake were established. GPS and sonar Elit no. 5 was used to determine the extent of macrophytes occurrence. Total phytolittoral cover was calculated on the basis of macrophyte analysis in horizontal transects, extending from the shoreline to the maximum colonization depth. The distribution of transects estimated by application of the formula MLT – minimal required number of transects (Jensén, 1977).

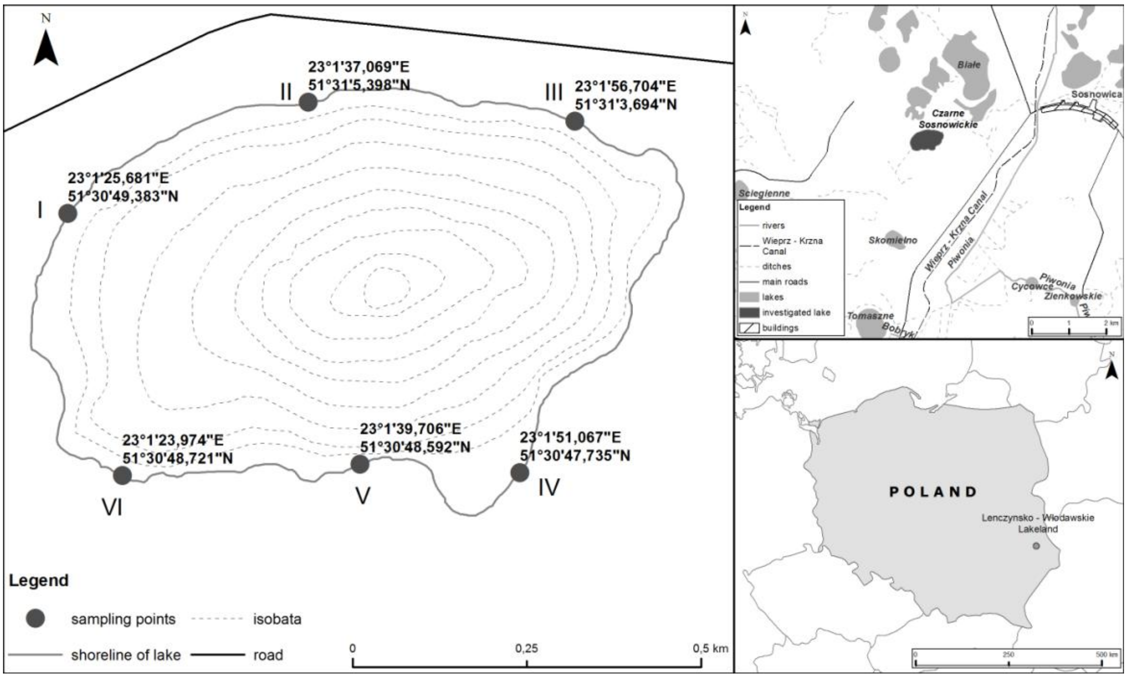

Figure 1. *Localization of investigated Lake Czarne Sosnowickie and sampling points*

Data from topographic maps at a scale of 1:10 000 and orthophotomaps, as well as field data were collected and processed using the software ArcGIS for Desktop 10.1.

The length of shadow, for every single sampling point, five times during the day (at 7am, 9am, 12am, 2pm and 4pm) was also calculated. The shadow model was made using GIS techniques (the Solar analysis of ArcGis desktop software). Moreover, the degree of shading (S_n – shading rate) of the horizontal transects was determined at 12 am, 15th of each month on the basis of the formula (adopted from Mander, 1995):

$$S_n = \frac{sr}{B}\left[\left(Hd\ ctg\alpha / sin\varphi /\right) - \left(Ld - \frac{sk}{2}\right)\right] \qquad \text{(Eq. 1)}$$

where:
sr – degree of belt plant compactness (%)
B – width of the littoral zone (m)
Hd – height of trees (m)
α – angle of sunlight (^{o})
φ – the angle between the north-south direction, and the direction of the line the banks (^{o})
Ld – distance of trees belt from water surface (m)
sk – factor depending on the shape and compactness the tree canopy (-)

At the same time in each transect qualitative and quantitative structure of emergent and submerged macrophytes (cover, abundance, biomass, number of species) and basic physical and chemical parameters of water (temperature, pH, electrolytic conductivity, nitrate, phosphate, oxygen, visibility) were determined. The following instruments were used for determination of physical and chemical properties of water: OXI 330-oxymeter made by WTW (oxygen content, temperature), electronic conductivity meter made by Hanna (electrolytic conductivity), microchip Slandi SP300 pH-meter (water reaction). The content of biogenic nitrogen and phosphorus compounds was determined using the microchip Slandi photometer LF 205.

Analysis of emergent macrophytes included: species composition, density (ind. per m^2), mean length and biomass of the species occurring in all research sites *Phragmites australis (Cav.) Trin. ex Steud.*, as well as, total biomass (g_{dw} per m^2), width and extent of rushes (m). Measurements of submerged macrophytes were conducted at 0.5 m intervals from the shoreline to the maximum depth of their occurrence. Monitoring included analysis of species, total biomass (g_{dw} per m^2) and share of each species in biomass, as well as the extent of occurrence. Each measurement was repeated 3 times.

Biodiversity of each group of macrophytes was supported by the index of Shannon-Weaver (Hs) (Hennink and Zeven, 1991):

$$H_s = -\Sigma(N_i \times \ln N_i) \quad \text{(Eq. 2)}$$

where N_i is the relative abundance of species i.

Floristic studies were carried out using the Bernatowicz floral rake, the floristic anchor and the sonar Elite no. 5 of LOWRENCE (Sender, 2012b).

The collected data set was analyzed statistically to determine arithmetic mean and standard deviation (SD). The parameters were compared with the use of t-test. To determine the strength of the relationship among factors Pearson's correlation coefficient was used. For the statistical analysis Statistica 5.1 StstSoft software was used.

Results

The analyzed positions with two distinguished parts of the lake were characterized by a small diversity of physico-chemical parameters. Higher values of the Secchi disk visibility and water temperature were found on the north side of the Lake, which was more exposed to the sun. The pH of the water was clearly alkaline. The rest of the analyzed physical and chemical parameters including: conductivity, hardness, PO_4, NH_4 and oxygen content achieved slightly higher values in the southern shore of the lake (*Table 1*). Differences between distinguished transects and physical and chemical parameters were not statistically significant ($T=0.02<T\alpha=2.14$, $p<0.05$).

Table 1. *Mean values of selected physical and chemical parameters in analyzed transects of Lake Czarne Sosnowickie*

Part of the lake	**North shore**		**South shore**	
Factor	**mean**	±	**mean**	±
Visibility (m)	0.86	0.04	0.705	0.055
Temperature (°C)	26.55	1.25	25.05	0.05
Water reaction (pH)	7.675	0.505	8.455	0.005
Conductivity ($\mu S \cdot dm\text{-}^3$)	297.9	9	312	0.3
O_2 ($mg \cdot dm^{-3}$)	6.29	3.56	12.6	0.4
Hardness	10.905	0.725	9.495	0.045
PO_4 ($mg \cdot dm^{-3}$)	0.02	0.012	0.048	0.005
NH_4 ($mg \cdot dm^{-3}$)	0.457	0.033	0.7895	0.0725

In the investigated lake, macrophytes occupied 11 ha, which accounted for 29.9% of surface area of lake. The main component of phytolittoral was emergent macrophytes consisted almost 70% of total cover of macrophytes (*Table 2*).

Table 2. *Characteristic of phytolittoral in Lake Czarne Sosnowickie*

Factor	Value	
	ha	**%**
Total phytolittoral surface	11	29.2
Share of emergent macrophytes in phytolittoral	7.51	68.3
Share of submerged macrophytes in phytolittoral	3.52	32.09
Colonization depth of macrophyte occurence (m)	2.5	
Maximum depth of emergent macrophyte occurence (m)	1.3	
Mean width of rushes (m)	19	

Shading of littoral zone of the lake Czarne Sosnowickie was clearly differentiated. The area covered by shadows was highly dependent on the height of riparian trees and time of day. In the north, the trees reached a maximum height of 26 m, and in the south 30 m. The length of the shadow in the northern shore of the lake was low and ranged from 0.9 m to 1.77 m. However, in the southern shore the shadow of the trees reached up to 15 m (*Table 3*).

Table 3. *Length of the shadow (standard deviation, SD) in the following hours (15 days of the month 2013)*

	JUNE					**JULY**				
	7am	**9am**	**12am**	**2pm**	**4pm**	**7am**	**9am**	**12am**	**2pm**	**4pm**
I	1.7 ±0.9	1.5 ±0.3	1.0 ± 0.7	1.25 ±0.02	1.66 ±0.4	1.9 ±0.6	1.4 ±0.03	0.9 ±1.01	1.1 ±0.7	1.7 ±1.01
II	1.6 ±0.1	1.56 ±0.02	1.4 ±0.5	1.6 ±0.3	1.63 ±0.8	1.6 ±0.5	1.5 ±1.1	1.3 ±0.4	1.45 ±1.0	1.77 ±1.3
III	1.7 ±0.2	1.4 ±0.2	1.2 ±0.09	1.22 ±0.1	1.31 ±0.2	1.4 ±1.1	1.2 ±0.7	1.0 ±0.2	1.1 ±0.5	1.6 ±1.1
IV	7.9 ±2.1	7.8 ±1.7	7.2 ±0.9	7.9 ±1.4	8.9 ±1.3	7.5 ±1.1	7.2 ±1.0	7 ±0.5	7.3 ±1.4	7.9 ±2.3
V	7.7 ±1.1	7.2 ±0.1	6.8 ±1.1	7.3 ±2.9	7.6 ±1.0	7 ±1.8	6.2 ±1.6	6 ±0.8	6.5 ±1.1	8.1 ±0.7
VI	12.9 ±3.2	11 ±2.5	10.1 ±3.5	10.5 ±4.3	12.9 ±1.2	14 ±2.6	11.6 ±5.8	10 ±3.8	11 ±0.6	15.9 ±3.5

The degree of shading of the zone covered by macrophytes was varied in the different transects of the lake. In the northern shore of the lake shading rate was about 3%, while in the southern approximately 30%. Along the northern shore, on average only 6% of rushes were covered by tree shade, in contrast, on the southern shore shade covered up to 89% of rushes.

In all transects, shade completely covered the zone occupied by all groups of macrophytes. Only at one investigated site was the range of the rushes smaller than the area of shading. The length of transects, covering with colonization depth of macrophytes, was much greater than the shade of nearby riparian trees and ranged from 20 m in the southern shore to 65 m in the northern shore (*Fig. 2*). There was strong correlation between the length of the littoral zone shading, and the colonization of emergent macrophytes ($R^2 = -0.84$; $p<0.05$).

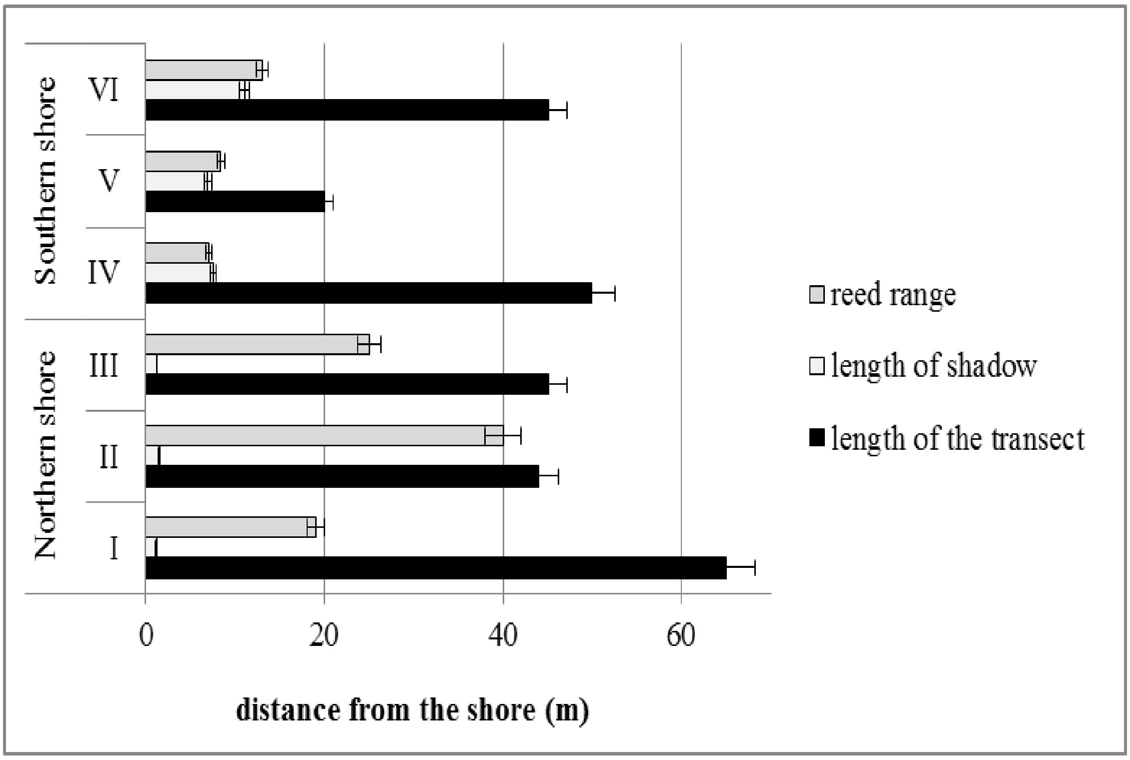

Figure 2. *The length of shadow, rushes width and length of the analyzed transects (mean values) in Lake Czarne Sosnowickie*

The entire shallow littoral zone was inhabited by rushes. Emergent macrophytes developed from the shoreline, until up to 1.3 m depth in the northern shore (*Fig. 3*). The width of belt rushes was clearly differentiated in the northern shore of reaching an average width of 28 m (± 8.8), while in the southern shore 9 m (± 2.6) and these differences were statistically significant ($T=2.85>T\alpha=2.77$, $p<0.05$).

As colonization depth increased so did diversity of species and total community biomass of macrophyte in the analyzed parts of the lake Czarne Sosnowickie. Emergent and floating plants achieved significantly higher values in the northern shore of the lake. Only submerged macrophytes, despite a small range of distribution, in the southern shore achieved higher values of biomass. Differences in their biomass were not statistically significant ($T=1.35<T\alpha=2.57$, $p<0.05$) (*Fig. 4*).

Plants with floating leaves grew equally well in both of the analyzed sections of the lake. In the northern part of the lake (7 species), there was a slightly greater numbers of free floating and floating-leaved plants and biomass of emergent macrophytes ranged from 21 $g_{dw}\cdot m^{-2}$ (III transect) to 34 $g_{dw}\cdot m^{-2}$ (II transect). In contrast, on the southern shore there were only 4 species and their biomass ranged from 10 $g_{dw}\cdot m^{-2}$ (VI transect) to 19 $g_{dw}\cdot m^{-2}$ (V transect) (*Fig. 4*). Differences in biomass and the colonization depth of these groups of macrophytes at different positions were not statistically significant ($T=1.75<T\alpha=2.57$, $p<0.05$).

Both the northern and southern shore of the lake was under the influence of riparian trees shade. The amount of light had a significant impact on the structure of emergent macrophytes. It turned out that in the more shady places (southern shore) helophytes density and biomass was lower. But in these transects shoots of emergent macrophytes were longer (Pearson 0.92, the correlation very large) suggesting high competition for light.

In the northern shore of the lake rushes species diversity, biomass and colonization depth were generally higher than along the southern shore (*Table 4*).

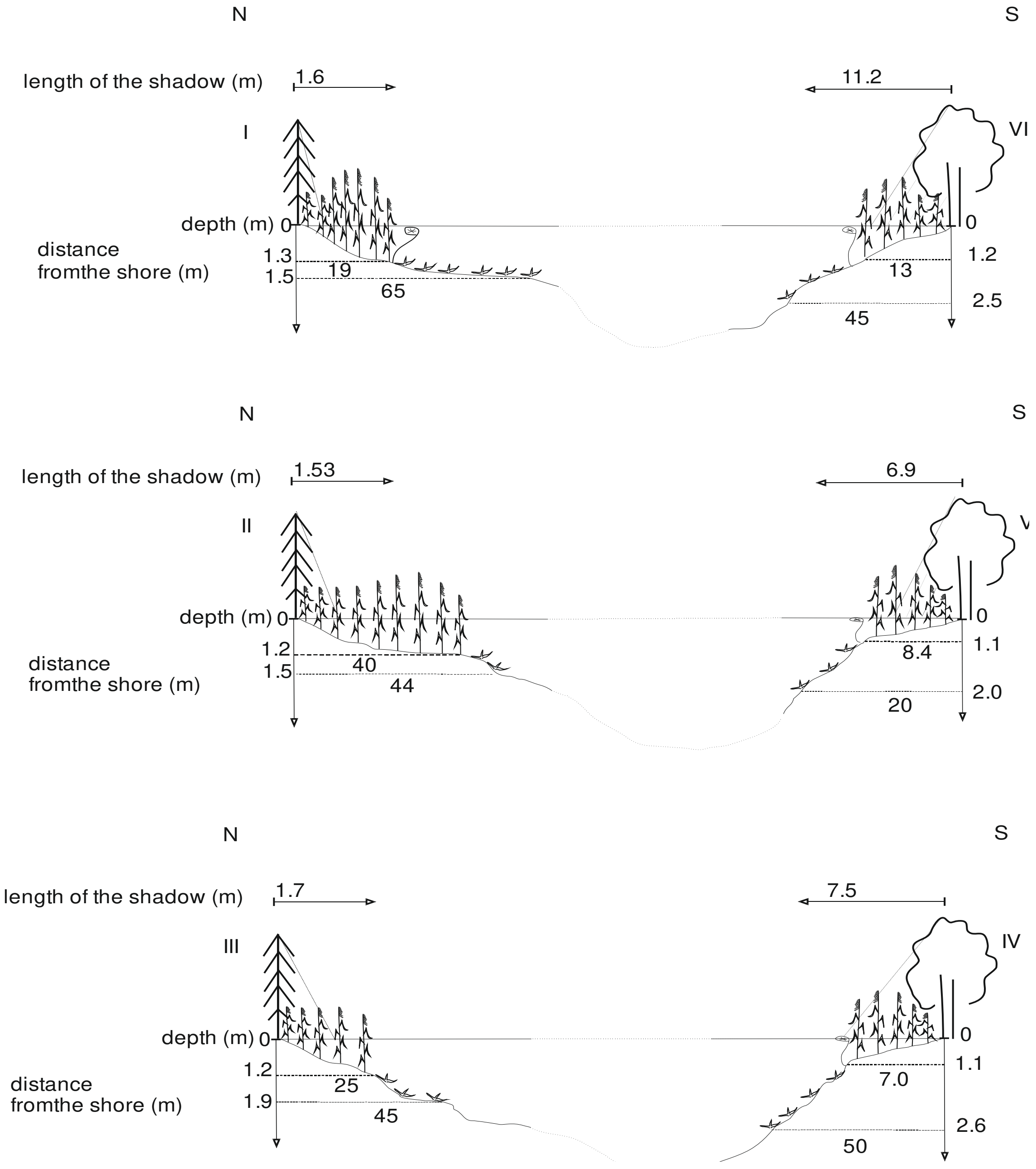

Figure 3. *Distribution of macrophytes in the northern and southern shore of the Lake Czarne Sosnowickie*

Differences of some morphometrical features of *Phragmites australis (Cav.) Trin. ex Steud.*, and the fact that the species occurs in all positions of study were clear and statically significant ($T = 10.3 > T\alpha = 4.3$, $p < 0.05$) (*Table 4*).

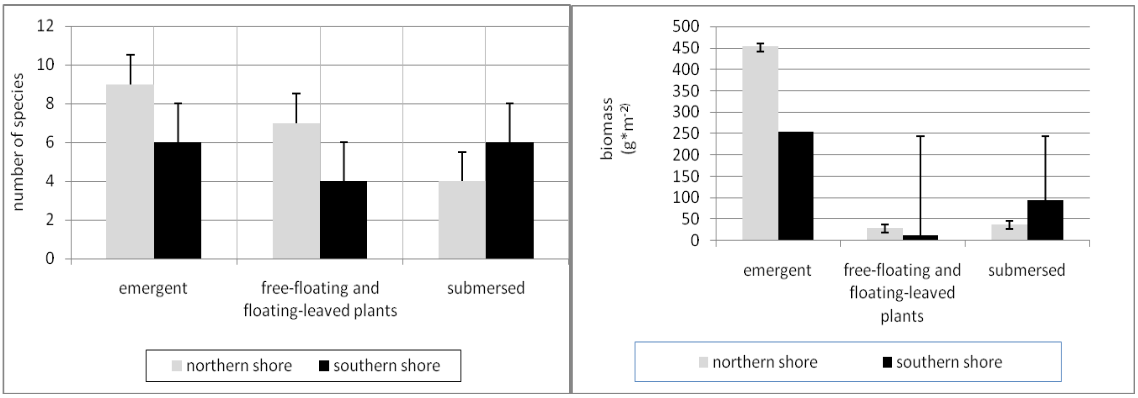

Figure 4. *Mean number of macrophyte species and their biomass in distinguish parts of shore*

Table 4. *The structure of emergent macrophytes in studied transects of Lake Czarne Sosnowickie*

Transects	**Northern shore**			**Southern shore**		
Feature	**North-west (I)**	**North (II)**	**North-east (III)**	**South-west (VI)**	**South (V)**	**South-east (IV)**
Dominant species in biomass	*Typha angustifolia L.*			*Phragmites australis (Cav.) Trin. ex Steud.*		
Width of belt rushes (m)	19 ±2.8	40 ±5.1	25 ± 2.2	13 ±1.9	9,4 ± 0.9	7 ±1.1
Max depth of rushes occurrence (m)	1.3	1.2	1.2	1.2	1.1	1.1
Density of helophytes (ind. per m^2)	76	64	60	56	38	48
Number of species	9	7	5	4	3	2
Mean length of *Phragmites australis* (m)	1.8 ±0.2	1.8 ±0.5	1.9 ±1.2	2.1 ±0.7	2 ±0.1	2.3 ±0.4
Biomass of single shoot of *Phragmites australis* (g_{dm})	15.2 ±2.4	13.9 ±1.3	14.2 ±1.7	17.4 ±3.4	18.2 ±2.2	18.8 ±4.1
Total biomass of rushes ($g_{sm}\cdot m^{-2}$)	516.8 ± 22	435 ±19.8	408 ±25.6	302.4 ±17.7	199.2 ±11.5	259 ±10.4
Index of Shannon-Weaver	1.5	1.4	1.4	1.1	0.9	0.6

The low density of emergent macrophytes caused that biomass of shoots was higher. Dependency statistically significant ($T = 5.2 > T\alpha = 3.3$, $p < 0.05$).

There was a strong correlation between length of shadow and biomass of emergent macrophytes (Pearson 0.99). The smaller shadow caused the higher total biomass of rushes. Higher correlation was also noted between the width of helophytes and the length of shadow. If the range of helophytes decrease, shadow will increase (Pearson 0.76).

Shannon-Weaver index confirmed significant variation within this group of macrophytes (*Table 4*).

Higher colonization depth of submerged macrophytes was in transects on the south side of lake (average 2.5 m). On the northern shore the maximum depth of macrophytes occurrence was only 1.5 m (*Fig. 3*). The highest diversity of species (7) and biomass (178.8 $g_{dw}\cdot m^{-2}$) occurred in the southern shore in a depth ranging from 1.5 to 2.0 m. In the northern shore the highest number of submerged species (5) was observed in a depth ranging from 1 to 1.5 m and their biomass was 157.3 $g_{dw}\cdot m^{-2}$ (*Fig. 5*).

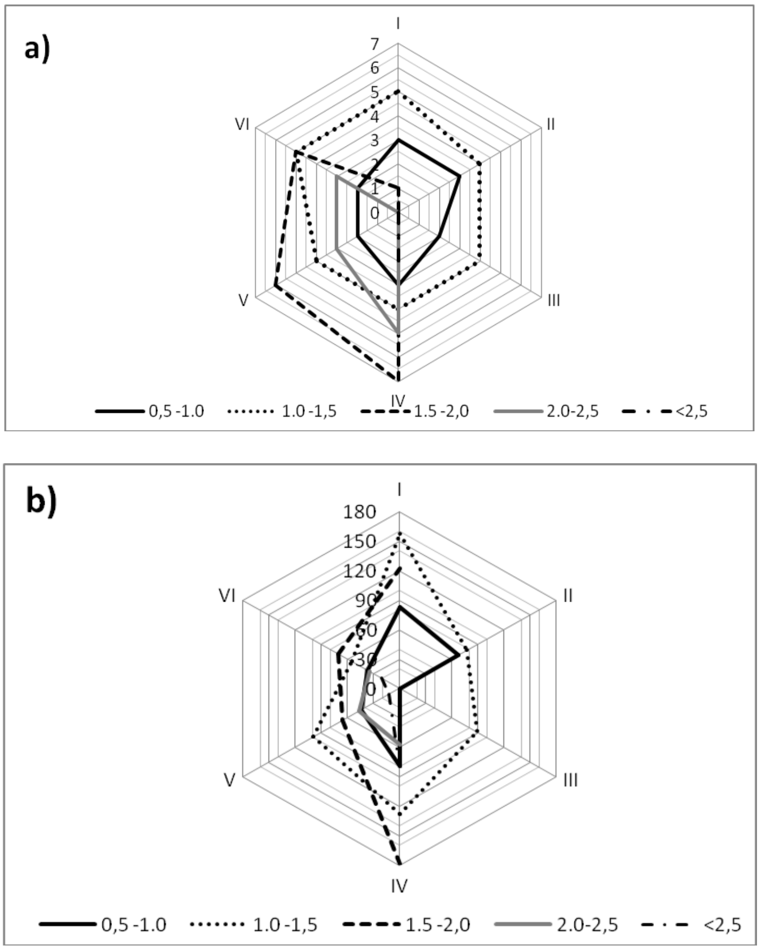

Figure 5. ***a)*** *number of plant species and* ***b)*** *biomass of submerged macrophytes in particular depths of Lake Czarne Sosnowickie*

The same submerged plant species occurred in each investigated part of the lake. Only their share in the biomass on particular depths and transects was different (*Fig. 5, Table 5*).

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Table 5. Share of submerged macrophyte species in biomass and Shannon-Weawer index for individual depths

Feature	**Stands**	**Transect**	**Depth (m)**				
			0.5 -1.0	**1.0 -1.5**	**1.5 -2.0**	**2.0-2.5**	**<2.5**
Shannon-Weaver index	Northern shore	I	1.6	1.1	-	-	-
		II	1.3	0.7	-	-	-
		III	1.6	0.5	0.9	-	-
	Souther shore	IV	1	0.9	1.6	1.1	0.6
		V	0.7	1	1.3	1.2	-
		VI	0.6	1.3	1.1	1.3	-
Share in biomass	Northern shore	I	*Myriophyllum spicatum L.*	*Myriophyllum spicatum L.*			
		II	*Hydrocharis morsus-ranae L.*	*Ceratophyllum demersum L.*			
		III	*Nuphar lutea (L.) Sibth.&Sm.*	*Chara fragilis Desv.*	*Chara fragilis Desv.*		
	Souther shore	IV	*Lemna minor L.*	*Chara fragilis Desv.*	*Nuphar lutea (L.) Sibth.&Sm.*	*Nuphar lutea (L.) Sibth.&Sm.*	*Nuphar lutea (L.) Sibth.&Sm.*
		V	*Ceratophyllum demersum L.*	*Myriophyllum spicatum L.*	*Ceratophyllum demersum L.*	*Nuphar lutea (L.) Sibth.&Sm.*	
		VI	*Ceratophyllum demersum L.*	*Nuphar lutea (L.) Sibth.&Sm.*	*Nuphar lutea (L.) Sibth.&Sm.*	*Nuphar lutea (L.) Sibth.&Sm.*	

Discussion

The main question of this study was to determine whether riparian trees limit growth of aquatic plants in lakes. Data showed that it depends on the group of macrophytes and range of shadow impact. The whole lake was surrounded by forest and similar water conditions on all shores were confirmed by chemical analysis. Slightly higher values of the analyzed physical and chemical parameters were in the southern shore of the lake. The probable cause of this phenomenon was an extinct peat bog, transformed by drainage (Szymczyk and Szyperek, 2005).

An unambiguous determination of the impact of shading on the growth of macrophytes is very difficult. Natural conditions are variable and usually applied to a wide variety of factors. Riparian vegetation limits access of light to the lake only in the shallow part of the littoral zone. Studies have shown that a large amount of penetrating sunlight positively affects emergent aquatic vegetation. First of all, less shading is conducive to the development of emergent and free-floating plants. In these low-light stands there was a higher species diversity, colonization depth and biomass. They were much higher than recorded for rivers in south Estonia with similar shade conditions (Mander et al., 1995). Despite the lower visibilities, higher species diversity and biomass, the submerged macrophytes occurred the southern shore of the investigated lake. Experimental studies on the effects of shading carried out on two species of submerged macrophytes confirmed their disappearance in complete shade, as well as relatively good growth under moderate shading (Lu et al., 2013). Generally, in the studied lake~~,~~ submerged macrophytes disappeared in the area of floating leaves' plants. The most deeply penetrated macrophyte species tolerant of low light conditions were *Myriophyllum spicatum L.* and *Ceratophyllum demersum L.* (Lougheed et al., 2001). Submerged macrophytes in investigated lake with an area approximately 4 ha were not dependent on the shading caused by the surrounding forest. Their structure and development are related to the conditions in the lake. As a rule, submerged macrophytes grow to a depth of two to three times the Secchi disc depth (Chambers and Kalff, 1985). In the studied lake almost all zones of submerged macrophyte occurrence were not dependent on shading from shoreline vegetation. Only macrophytes occurring in the area of 0.5-1 m depth were influenced by shading. In the northern shore, in spite of better conditions of sunlight, due to the lush growth of rushes, submerged macrophytes were losing the competition. In the southern shore where the rush vegetation achieve lower density and extent of occurrence, submerged macrophytes developed better. In these areas, there was a greater variety of species and biomass. Apart from the impact of the shadow zone, along the northern shore of the lake the number of species decreased, while in rose along the southern shore, just like biomass. Free-floating plants may grow well in low transparency of water (Feldmann and Nöges, 2007). In the investigated lake, this group of plants developed better (higher species richness and higher biomass) along the northern shore, with better light condition. This group of macrophytes with dense reed stands can modify habitat conditions for other species in a very strong way and even eliminate them (Feldmann and Nöges, 2007). Emergent macrophytes are a group of aquatic plants less dependent on the light conditions occurring in water (Lacoul and Freedman, 2006). The amount of light reaching them depends on the environment of the lake.

Usually, higher rushes species diversity, biomass and extent of occurrence were reached in the northern shore of the lake. This result suggests more favourable conditions for this group of plants developing in shadier areas. *Typha angustifolia L.* was a dominant species in the northern shore. This species is usually considered an indicator of mineral substrate in meso- to eutrophic water bodies (Zarzycki, 1984). In the southern shore of the lake, the organic substrate was dominated by *Phragmites australis* (Cav.) Trin. Ex Steud. In general, the best habitat conditions for the development of this group of macrophytes were in the northwestern and southwestern part of the lake. It was supported by the configuration of lake basin, creating favourable conditions for their spread (Gasith and Hoyer, 1998).

Thus, the possibility of using coastal vegetation as a tool for dealing with lake overgrowth may not be reasonable as data from this study suggests that shading does seem not to play a significant role in development of macrophyte communities. In bigger lakes interactions are local, whereas in rivers the management of tree vegetation might control their overgrowing (Mander et al., 2005).

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REFERENCES

[1] Ali, M. M., Hassa, S. A., Shaheen, A. M. (2011): Impact of riparian trees shade on aquatic plant abundance in conservation islands. - Acta Botanica Croatica 70 (2): 245-258.

[2] Barko, J. W., Adams, M. S., Clesceri, N. L. (1986): Environmental factors and their consideration in the management of submersed aquatic vegetation: review. - Journal of Aquatic Plant Management 24: 1-10.

[3] Bernatowicz, S. (1966): The effect of shading on the growth of macrophytes in lakes. - Polish Journal of Ecology (former Ekol. Pol.) 2431, 14, 32: 605-616.

[4] Chambers, P. A., Kalff, J. (1985): Depth distribution and biomass of submerged aquatic macrophytecommunities in relation to Secchi depth. - Canadian Journal of Fisheries and Aquatic Sciences 42: 701-709.

[5] Chełmicki, W. (2001): Wather: resurs, degradation, protection (Woda: zasoby, degradacja, ochrona). -Scientific Publisging PWN Warszawa, 305.

[6] Dawson, F. H. (1981): The reduction of light as a technique for the control of aquatic plants an assessment. - Proceedings of the Association of Applied Biologists Symposium Aquatic Weeds and their Control, Oxford, 157-164.

[7] Feldmann, T. (2012): The structuring role of lake conditions for aquatic macrophytes. A thesis for applying for the degree of doctor of Philosophy in Hydrobiology. Estonian University of Life Sciences, Tartu.

[8] Feldmann, T., Nöges, P. (2007): Factors controlling macrophyte distribution in large shallow Lake Vortsjarv. - Aquatic Botany 87: 15-21.

[9] Gasith, A., Hoyer, M. V. (1998): Structuring role of macrophytes in lakes: changing influence along lake size and depth gradients. In: Jeppesen, E., Sondergaard, M., Sondergaard, M., Christoffersen, K., (eds.) The Structuring Role of Submerged Macrophytes in Lakes. - Springer, New York, 381–392.

[10] Harasimiuk, M., Michalczyk, Z., Turczyński, M. (1998): Łęczna-Włodawa Lakes. Natural monography (Jeziora łęczyńsko-włodawskie. Monografia przyrodnicza). - Publishing. Biblioteka Monitoringu Środowiska Lublin.

[11] Hennink, S., Zeven, A. C. (1991): The interpretation of Nei and Shannon-Weaver within the population variation indices. Euphitica 51: 235-240.

[12] Jensén, S. (1977): An objective method for sampling the macrophyte vegetation in lakes. - Vegetatio 33:107-118

[13] Lacoul, P., Freedman, B. (2006): Environmental influences on aquatic plants in freshwater ecosystems. - Environmental Reviews 14: 89-136.

[14] Lehmann, A., Lachavanne, J. (1999): Changes in the water quality of Lake Geneva indicated by submerged macrophytes. Freswater Biology 42: 457-466.

[15] Lougheed, V. L., Crosbie, B., Chow-Fraser, P. (2001): Primary determinants of macrophyte community structure in 62 marsh across the Great Lakes basin: latitude, land use and water quality effects. - Canadian Journal of Fisheries and Aquatic Sciences 58: 1603-1612.

[16] Lu, J., Wang, Z., Xing, W., Lui, G. (2013): Effect of substrate and shading on growth of two submerged macrophytes. - Hydrobiologia 700: 157-167.

[17] Madsen, T. V., Brix, H. (1997): Growth, photosynthesis and acclimation by two submerged macrophytes in relation to temperature. - Oecologia 110: 320-327.

[18] Mander, Ü. (1995): Riparian buffer zones and buffer strips on stream banks: dimensioning and efficiency assessment from catchments in Estonia. In: Eiseltová, M., Biggs, J., (Eds.), Restoration of Stream Ecosystems.- IWRB Publication Slimbridge, Gloucester, UK, 37: 45–64.

[19] Mander, Ü., Hayakawa, Y., Kuusemets, V. (2005): Riparian buffer zone in agricultural watersheds. Purification processes, ecological functions, planning and design of riparian buffer zones in agricultural watersheds.- Ecological Engineering 24(5):421-432.

[20] Mander, Ü., Kuusemets, V., Ivask, M. (1995): Nutrient dynamics of riparian ecotones: a case study from the Porijõgi River catchment, Estonia. - Landscape and Urban Planning 31 (1-3): 333-348.

[21] Sedvik, T. O., Celik, K. (2014): The effects of certain physical and chemical variables on the succession of the phytoplankton in the shallow Cagis pond (Balıkesir, Turkey). - Ekoloji 23(93): 27-35

[22] Sender, J. (2010): Long - term changes of plant water vegetation in the macrophyte tape lakes on Łęczna-Włodawa Lakeland (Długoterminowe zmiany struktury roślinności wodnej w makrofitowych jeziorach na Pojezierzu Łęczyńsko-Włodawskim) - Annales UMCS, Agricultura 65(2):58-67.

[23] Sender, J. (2012): Quantitative investigations of vascular flora in deep and shallow eutrophic lake. - TEKA Commission of Protection and Formation of Natural Environment – OL PAN, 9: 206-214.

[24] Sender, J.(2012b): The dynamics of macrophytes in a lake in an agricultural landscape.- Limnological Review DOI 10.2478/v10194-011-0049-6; 2: 93-100.

[25] Spence, D. H. N. (1981): Light quality and plant response under water. In: Smith H. (ed.), Plants and the day light spectrum. - Academic Press, New York, 245-275.

[26] Squires, M. M., Lesack, L., Huebert, D. (2002): The influence of water transparence on the distribution and abundance of macrophytes among lakes of Mackenzie Delta, Western Canadian Arctic.- Freshwater Biology 47: 2123-2135.

[27] Szymczyk, S., Szyperek, U. (2005): The role of peatlands in reducing nutrient runoff from agricultural catchment, (Rola torfowiska w ograniczaniu odpływu składników biogennych ze zlewni rolniczej).- Zeszyty Problemowe Nauk. Rolniczych 506.

[28] Wersal, R. M., Madsen, J. D. (2013): Influence of light intensity variations on growth characteristics of *Myriophyllum aquaticum*. - Journal of Freshwater Ecology 28(2): 147-164.

[29] Zarzycki, K. (1984): Ecological indicator numbers of vascular plants in Poland, (Ekologiczne liczby wskaźnikowe roślin naczyniowych Polski). - Publising PAN. Instytut Botaniki Kraków.

USING LONG-TERM FIELD EXPERIMENT DATA TO PREPARE A CROP SIMULATION MODEL FOR CLIMATE IMPACT STUDIES

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Abstract. This study presents the performance of the 4M crop model when simulating the effects of different fertilizer levels. Calculated biomass formation data were compared to observed data collected in a long-term field experiment launched in 1958 at Martonvásár, Hungary. After calibration, the model adequately simulated the differences between the effects of the different fertilization levels, as well as the long-term trends and averages of biomass productions. On the other hand, the model underestimated the inter-annual variability of the yield revealing a fundamental problem of crop modeling. A new formula was proposed to enable the model to simulate the increased leaf decay rate induced by heat and water stresses. The calibrated model was used to estimate the yields of 294 representatively selected Hungarian farms. After validation, the model was used for projecting the effects of fertilization in a future climate scenario. 4M predicted a considerable decrease of maize yields as well as yield safety for the future. The comparison of the observed and simulated data suggested two ways of model improvements which facilitate a more accurate projection of the impact of environmental stress factors.

Keywords: *long-term experiment, maize, fertilization, crop modeling, climate change*

Introduction

The Carpathian basin is an important area of crop production in Europe. Annually, around ten million tons of yields of different crops are produced here for 8 countries of the basin. The majority of the agricultural land in the basin (5.7 million hectares) is located in Hungary which ranks among the best in the world concerning the average yields of her main crops (maize, winter wheat and sunflower).

Since the 1960s in many countries in the world, including Hungary, agricultural production has gone through tremendous improvement. In Hungary the average yields of maize and winter wheat, were tripled in three decades compared to those of the 1950s. Together with introducing new, intensive cultivars and hybrids, one of the main factors behind this development was the enormous increase in fertilizer use. Compared to 1960, by 1980 Hungarian arable land was given 10 times as much fertilizer. The same trend could be observed worldwide.

In the meantime, field experiments were launched to investigate the effect of different fertilization practices on yield characteristics (quantity, stability, etc.). In the early 1960s, a network of experimental stations (OMTK) was set up in Hungary to explore the potential (benefits and drawbacks) of fertilization in agricultural production (Kismányoky and Jolánkai, 2009). Locations were selected in a way to represent all major soil types and climatic zones of the country. Including the stations of OMTK, there are ongoing long-term experiments at more than 20 locations in Hungary with the oldest one launched in 1929.

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Although many times the initiators of the long-term experiments had only a quite limited idea what the collected data could be used for, during the past decades, many important applications have emerged from or been supported by these initiatives, such as fertilizer advisory systems (Fodor at al., 2011), soil carbon and nitrogen turnover models (Jenkinson, 1990), decision support tools (Gijsman et al., 2002), early warning systems of soil nutrient depletion (Korsaeth, 2012) and even new statistical methods (Fisher, 1921). Long-term experimentation is particularly important in understanding the changes that are occurring in the environment. Using the knowledge acquired in these living laboratories the effects of a certain manipulation may be separated from other variables, making it possible to manipulate almost any component and to be confident that one is able to predict the possible impacts (Southwood, 1994).

Process-based crop models are widely used in decision support systems or to assess impacts of climate change on agriculture at different spatial scales (Rosenzweig and Parry, 1994; Olesen and Bindi, 2002; Supit et al., 2010; Klein et al., 2012; Fodor et al., 2014). Since the projected changes of climate in the 21st century show geographical patterns (IPCC, 2007) the impact studies provided region specific results (Semenov and Shewry, 2011; Ray et al., 2013; Ramirez-Cabral et al., 2016). For Europe, the case studies show an obscure picture for the future (Audsley et al., 2006; Supit et al., 2010). Despite the uncertainties climate change will most likely favour the winter crops and grass lands but introduces yield losses for spring crops in Southern and Central Europe.

According to Knox et al. (2012) and Fezzi et al. (2014) the potential major threats for agriculture include: 1) increased biotic stresses: pest, disease and weed problems; 2) increased abiotic stresses: heat, drought, water logging; 3) increased soil and wind erosion as well as nutrient loss (runoff and leaching); 4) higher GHG emission rates due to enhanced soil biological activity; 5) decreased water availability and quality. In addition to these, farmers will need to adapt to climate change mitigation policies, likely to involve increased costs of carbon emissions reflected in both their inputs (e.g. fertilisers) and outputs (e.g. higher transport costs). Alongside the threats, it seems that climate change will generate opportunities, as well (e.g. Peltonen-Sainio et al., 2009), including: 1) accelerated and more water-use-efficient crop growth; 2) lengthened growing and grazing season; 3) increased carbon sequestration; 4) expanded climatic suitability for agriculture; 5) introduction of new crops; 6) changes in crop rotation.

Long-term experiments provide excellent sources of data for developing, calibrating and validating crop simulation models (Jamieson et al., 1998; Eitzinger et al., 2004). Deterministic cropping system models could be successfully used in Education (e.g. with the aid of computers, virtual plant production competition could be organized among the students), in Research (e.g. the effects of yield-determining, -limiting and -reducing factors could be disentangled and the results of observations could be extrapolated in time and space), in Practice (e.g. model results could be used in decision support systems as well as for supporting policy making). These impact models are the only tools (after careful calibration) for predicting the possible, agriculture-related effects of climate change. A comprehensive review of the usefulness of long-term experiments can be found in Science (Rasmussen et al., 1998).

In this study the 4M crop simulation model is presented and tested against measured data. Observed and simulated biomass formation data (LAI, yield, etc.) measured in a long-term fertilization experiment are compared. After calibration and validation the model is used for projecting the effects of fertilization in a future climate scenario.

Materials and Methods

Crop model

The 4M crop simulation model (Fodor et al., 2002; Fodor and Pásztor, 2010) was used in the study. 4M is a daily-step, deterministic model. Its computations are determined by the numerical characteristics (defined by input parameters) of the atmosphere–soil–plant system. Besides the data that describe the physical, chemical and biological profile of the system, it is also necessary to set its initial, boundary and constraint conditions in the input file of the model. The parameters regulate the functions and equations of the model: the development and growth of plants and the heat, water and nutrient balance of the soil. The initial conditions are the measured system variables at the beginning of the simulation run such as the water and nutrient content of the soil. The boundary conditions are primarily the daily meteorological data such as global radiation, temperature and precipitation. The constraint conditions cover the numerical expressions of human activities such as data about planting, harvest, fertilization or irrigation. Besides plant development and growth, the model calculates the water, heat and nitrogen flow as well as the nitrogen transformation processes of the soil: e.g. the amount of nitrate that percolates down under the root zone and the amount of the NO_x gases released from the soil due to denitrification (*Fig 1*). More detailed description of the model can be found in Fodor et al. (2012) and Sándor and Fodor (2012).

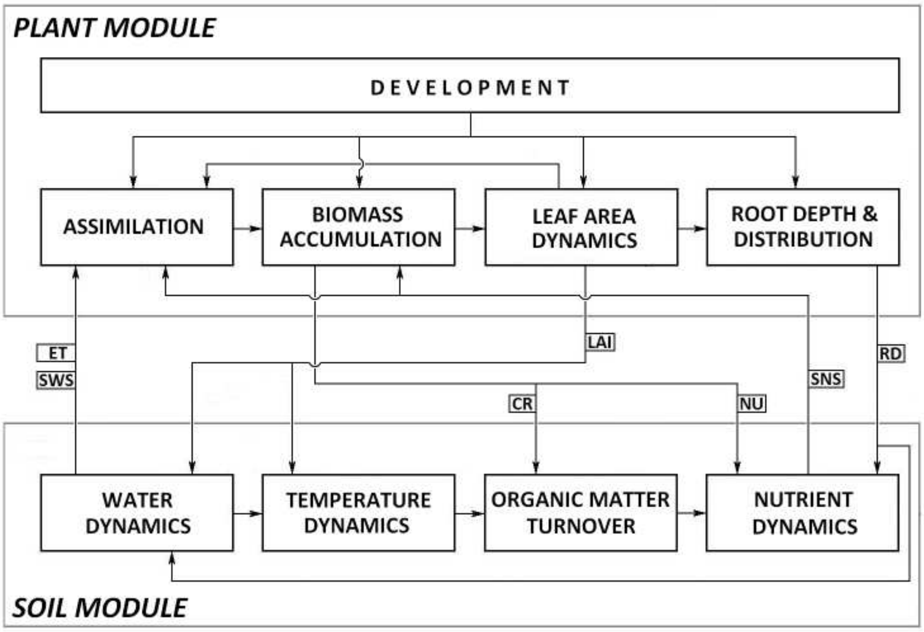

Figure 1. *Flow chart of the 4M crop simulation model. Indicated system variables: ET – evapotranspiration, SWS – soil water status, LAI – leaf area index, CR – crop residue, NU – nutrient uptake, SNS – soil nutrient status, RD – rooting depth.*

4M is basically a CERES [12] clone, rewritten in JAVA. Since its first release in 2002, three major developments have been made to the original version: 1) a new module was added to calculate the growth and the maintenance respiration as well as the CO_2 emission of plants (Fodor et al., 2014), 2) a phosphorus module was added to calculate soil P dynamics and plant P uptake (Máthé-Gáspár and Fodor, 2014), 3) a new function was added to account for the heat and water stress induced canopy decay. In addition to these developments, in 4M, practically every model parameter is accessible by the user through a graphical user interface.

Model improvement

Owing to the continuous development efforts in the past decades crop models are adequately able to predict the long-term trends and averages of biomass production. Despite this fact, at their present stage, crop models are unable to handle the effects of environmental stress factors (heat waves, droughts, etc.) adequately. The year 2007 provided an excellent possibility to improve the leaf senescence module of the model. The CERES model, and consequently the 4M model, does not take the effect of extreme air temperature and water shortage into account when calculating the daily rate of leaf senescence. Due to this fact, these models tend to overestimate the maize leaf area in the post-flowering period. 4M calculates the age of the leaf in thermal time measured in degree days (°Cd). When the leaf age exceeds the lifespan of the leaf (input parameter) the leaf dies. Based on observations a simple equation is proposed to enable the model to simulate the increased leaf decay rate (LDR) induced by heat (H_{str}) and water (W_{str}) stresses (Eq. 1). These two factors are simply added to the original formula.

$$LDR = DTT \cdot (1 + a \cdot H_{str} + b \cdot W_{str}) \qquad \text{(Eq. 1)}$$

Where DTT denotes the daily thermal time (°Cd) which is the difference between the daily average temperature and the base temperature with the condition of $DTT \geq 0$. The value of the a and b parameters is to be determined during the model calibration. Both stress factors range between zero and one. H_{str} is zero if the daily maximum temperature (T_{max}) is below 35 °C and equals one if T_{max} reaches or exceeds 40 °C. Between the two limits a linear increase is postulated. W_{str} is zero if the soil is wet enough to enable the plants to take up sufficient amount of water for the daily transpiration. Supposing a linear trend, W_{str} increases to one as the soil water content drops to the wilting point in the root zone.

Long-term experiment

The maize (*Zea mays*) monoculture experiment was launched in 1958 with seven fertilizer treatments. In this study, only the treatments with inorganic fertilizers have been investigated: control, $N_1P_1K_1$, $N_2P_2K_2$. Each treatment was represented in seven replicates using 8×10 m = 80 m^2 size parcels in a Latin square arrangement. The P (superphosphate, $Ca(H_2PO_4)_2$) and K (potassium chloride, KCl) fertilizers were applied in every fourth year (started in 1958), before the autumn tillage. N fertilizer (ammonium nitrate, NH_4NO_3) was applied every spring before making the seedbed. Autumn soil disinfections and pre-flowering pesticide spraying were applied against pests (e.g. maize bug (*Diabrotica virgifera virgifera*)) to minimize their damage. Pre- and post-emergent herbicides were applied annually to keep the parcels clean from weeds.

Meteorological data

A database of the Hungarian Meteorological Service for the 1961-2010 period was used in the study: including daily global radiation, maximum and minimum temperature and precipitation, covering the area of Hungary with an 10×10 km resolution grid. The MISH interpolation technique (Szentimrey et al. 2011) was used for producing the grid of meteorological data from the local observations. The country is covered by 466 rectangles considered meteorologically homogenous. 50-year long dataseries of the grid cell in which the long-term experiment is located (Martonvásár, long: 49°19'28", lat: 18°47'26") were used as model inputs (*Fig. 2*).

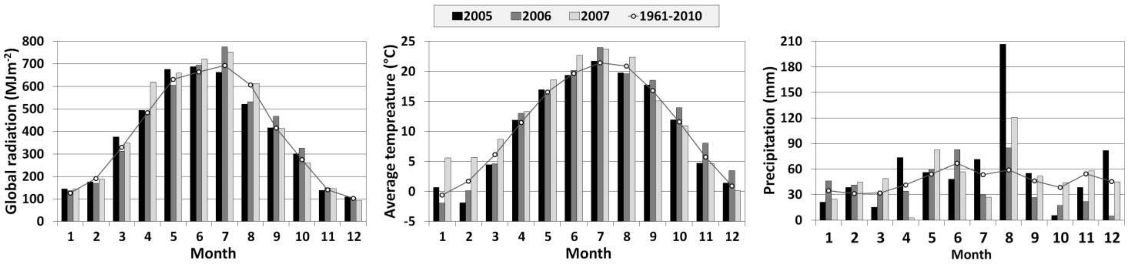

Figure 2. *Characteristics of the meteorological data series used in the study.*

Soil data

The soil of the experiment belongs to the chernozem FAO soil unit as well as to the medium textural class (sand fraction: 51.4%, silt fraction: 34.0%, clay fraction: 14.6%) with a deep (>40 cm) A horizon. Characteristic values of the soil water retention curve were determined with an Eijkelkamp sand/kaolin box as well as with a pressure membrane apparatus. For this measurement, five 100 cm^3 undisturbed core samples were collected from the topsoil (0-20 cm depth). According to the measurements the saturated water content, the field capacity and the wilting point water content of the soil is 0.49, 0.31 and 0.09 cm^3cm^{-3}, respectively. Bulk density was determined using the same core samples resulting in a 1.47 gcm^3 average value. Saturated hydraulic conductivity of the soil was measured in situ with a Decagon mini disc infiltrometer. The measurements were carried out in five replicates providing a 9 cmd^{-1} average value. Other important soil characteristics are summarized in *Table 1*. Soil humus as well as ammonium lactate (AL) soluble P and K contents were determined according to the method of Tyurin (1937) and Egner et al. (1960), respectively. The AL method is a quite widespread soil analysis method used in more than 10 countries in Europe including the Netherlands, Portugal, and Sweden. At the beginning of the long-term experiment the average soil humus, AL-P_2O_5 and AL-K_2O content was 2.9 %, 45 $mgkg^{-1}$ and 240 $mgkg^{-1}$, respectively.

Table 1. *Soil chemical characteristics of the field experiment, 30 years after the start, in 1990.*

Treatments	Humus content (%)	pH_{KCl}	AL-P_2O_5 ($mgkg^{-1}$)	AL-K_2O ($mgkg^{-1}$)
Control	2.82	6.08	32.4	218
$N_1P_1K_1$	2.91	5.85	69.0	251
$N_2P_2K_2$	2.92	5.42	125.3	380

Agromanagement data

Soil cultivation, planting, fertilization, spraying and harvest dates, as well as the amount of the applied materials (seeds, fertilizers, etc.) have been carefully recorded since the beginning of the long-term experiment. The control, the $N_1P_1K_1$ and the $N_2P_2K_2$ treatments received zero, 66/38/75 and 132/76/150 $N/P_2O_5/K_2O$ $kgha^{-1}y^{-1}$ active ingredients, respectively. In the first period (1959-1974) of the experiment late varieties (FAO 500-600) were sown, while in the second period (1975-1990) early hybrids (FAO 300-400) were preferred. It has to be emphasized, that since 1991, the NORMA hybrid (FAO 380) has been used. Sowing was scheduled to the second half of April (depending on the weather and soil conditions) every year with 7 plants per m^2 density and 70 cm row distance. After harvest, the crop residue was always incorporated into the soil with the autumn tillage. The changes of cultivars during the experiment were taken into account in the simulations in a simple way. For the cultivars of the late FAO group longer phenological stages were set in the input files. Since the newer cultivars do not have a significantly higher radiation use efficiency (RUE), thus a single, fixed RUE value was used throughout the 50 year-long simulated period.

Data used for model calibration (2005-2007)

The observed meteorological, soil and agromanagement data were used as model inputs. The approximate values of the plant specific parameters (phenological characteristics, maximum root depth, light use efficiency, specific leaf area, specific N content, etc.) were determined based on the relevant scientific literature (Hodges, 1990), (Stockle and Nelson, 1996). Then, the parameters were fine-tuned using an inverse modeling technique (Soetaert and Petzoldt, 2010) so that the observed and simulated biomass formation dynamics would be as similar as possible. For this comparison, three plants were cut out from the replicates of all treatments (control, $N_1P_1K_1$ and $N_2P_2K_2$) every two weeks starting at the 4-leaf stage and finishing at physiological maturity. The parcels were sampled ten times during each vegetation period. The sample plants were cut into pieces and the parts were separated into ‘stem’, ‘leaf’ and ‘yield’ groups. The area of the leaves was measured with a LI-3100A leaf area meter. Stem, leaf and yield dry weights were measured after 48 hours of drying at 105 °C temperature. In addition to these, the occurrence dates of the main phenological stages were recorded. Specific leaf area (SLA) was determined by using the observed leaf area and the corresponding leaf dry weight data. The calculated SLA value (15.4 m^2kg^{-1}) was provided for the model as an input. Base temperature and the maximum root depth were set to 8 °C and 1.7 m, respectively. The following parameters were fine-tuned (determined with inverse modeling) by minimizing the absolute error between the observed and the simulated flowering and maturity dates as well as the LAI, biomass and yield data for the year 2005, 2006 and 2007:

- Cumulative thermal time from emergence to flowering (°Cd)
- Cumulative thermal time from flowering to physiological maturity (°Cd)
- Radiation use efficiency ($kgMJ^{-1}$)
- Mass partition among root/stem/leaf in the early growth stage (%)
- Mass partition among root/stem/leaf after the early growth stage till flowering (%)
- Lifespan of leaves (°Cd)
- Parameters (a and b) of the leaf senescence equation (Eq. 1)

The first two parameters govern the phenological development of the simulated plants, while the rest of the parameters determine the plant growth: biomass formation, allocation and senescence. Within the long-term experiment dataset we had the details on plant growth only for these three years (2005-2007). Minolta SPAD 502 portable chlorophyll-meter was also used to determine leaf chlorophyll content in the grain filling period according to Schepers et al. (1992). This data was not used for calibrating the model, just for interpreting the calibration results. The model performance was evaluated by using the mean relative error and the Nash–Sutcliffe model efficiency coefficient, ENS (Nash and Sutcliffe, 1970).

$$ENS = 1 - \frac{\sum_{n=1}^{N}(V_n^o - V_n^c)^2}{\sum_{n=1}^{N}(V_n^o - M^o)^2} \qquad \text{(Eq. 2)}$$

where M^o is the mean of observed values, V^o and V^c are the observed and calculated values, respectively, and N is the number of observations.

ENS can range from $-\infty$ to 1. ENS = 1 corresponds to a perfect model performance. An efficiency less than zero ($ENS < 0$) occurs when the observed mean is a better predictor than the model meaning that the model is practically useless. The closer the efficiency coefficient is to 1, the more accurate the model is.

Data used for model validation (1961-2010)

After calibrating the model, it was validated in a two-way process. First, the model performance was assessed with the help of the 50-year long data series of the long-term experiment. Though the calibration and validation datasets were not entirely independent, for the sake of continuity, data from the 2005-2007 period was included in the investigated 50-year long period. Since this 3-year long period gives only 6% of the 1961-2010 period, it was assumed that this 'malpractice' would not distort the validation results. The observed and simulated cumulated yields were compared for the control, the $N_1P_1K_1$ and the $N_2P_2K_2$ treatments using simple graphs, while for the annual yields simple statistical indicators (e.g. RMSE and t-value) were used. Paired t-tests were carried out with $\alpha = 0.05$ confidence level. Second, the model performance was evaluated using real farm data. Data of 294 representatively selected Hungarian agricultural enterprises (regarding their spatial distribution within the country) were used from the period of 2001-2010. The available data included the geographical location of the parcels with maize, time of the agro-technical operations, amount of the applied fertilizers and final yields. The required soil input parameters were retrieved from the 0.1×0.1 km resolution soil database of Hungary (Pásztor et al., 2014). Daily meteorological inputs were retrieved from 10×10 km resolution CarpatClim database (Spinoni et al., 2014). The observed and simulated yields were compared using graphs and indicators similar to those of the first validation step.

Impact projection of fertilizer practices using a future climate scenario

A climate scenario was constructed based on the outputs of the ARPEGE-CLIMATE global circulation model (Déqué et al., 1998), which was then dynamically downscaled for Hungary with the ALADIN-Climate V4.5 regional climate model (RCM) (Wang et al., 2011). The IPCC SRES A1B scenario (Nakicenovic and Swart, 2000) was used in the ALADIN simulations. RCMs, similarly to models used in numerical weather prediction, create realistic daily meteorological data taking into account the future changes (defined by the IPCC SRES A1B scenario in our case) in the chemical composition of the atmosphere and the consequent alteration of the radiation balance of the atmosphere. Thus, no additional statistical/synthetic method (e.g. weather generator) is required for producing daily time-step meteorological data. The 10 km horizontal resolution outputs of the RCM (daily maximum/minimum temperature and precipitation data for the 1951-2110 period) were validated using the interpolated gridded dataset of observed data over Hungary for the 1961-1990 reference period (CECILIA 2013). The average prospective changes of the monthly climatic variables were estimated by comparing the data of the 1961-1990 (measured) and the 2061-2090 (climate model output) periods (*Fig 3*).

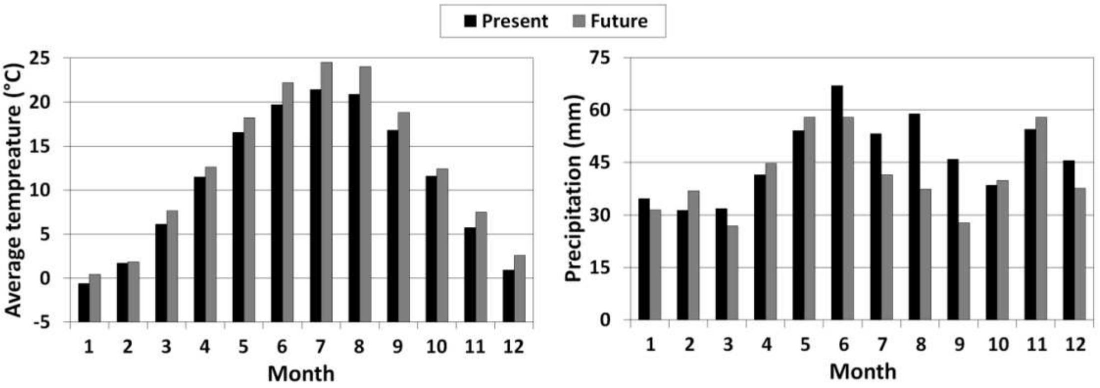

Figure 3. *Climatic characteristics of weather data used in the study. Present: 1961-1990, Future: 2061-2090.*

Model simulations were carried out for the present (1961-2010) and for the future (2061-2110) using measured and projected meteorological data, respectively. It was postulated that the atmospheric CO_2 concentration was 350 and 700 ppm in the present and future period, respectively (IPCC 2014).

Results and Discussion

Observed data clearly show that small leaves (shooting out in the early generative stage) have considerably larger specific leaf area than that of the large leaves (*Fig. 4.*). This characteristic difference was taken into account during the simulations by setting different SLA values in the model for the early and the late growth stages.

Model calibration

Plant parameter values obtained during the model calibration are summarized in *Table 2*. For the sake of clarity only the control and the $N_2P_2K_2$ treatments are presented in the graphs that summarize the observed and simulated LAI, biomass and yield data for the calibration period (2005-2007).

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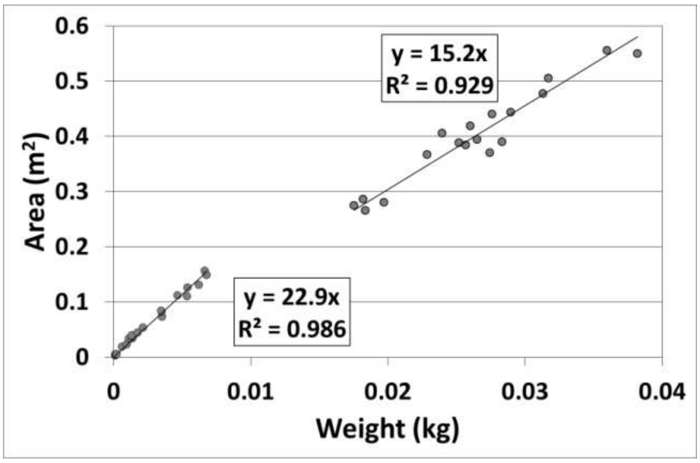

Figure 4. *Leaf area vs. leaf weight in the year 2005, 2006 and 2007. The slope of the fitted line provides the specific leaf area parameter for the crop model.*

Table 2. *Plant parameters of the early hybrids (FAO 300-400) obtained during the calibration of the model.*

Parameter	Value
Cumulative thermal time from emergence to flowering (°Cd)	800
Cumulative thermal time from flowering to physiological maturity (°Cd)	1000
Radiation use efficiency ($kgMJ^{-1}$)	0.003
Mass partition among root/stem/leaf in the early generative stage (%)	30 / 5 / 65
Mass partition among root/stem/leaf in the late generative stage (%)	25 / 50 / 25
Lifespan of leaves (°Cd)	1100
Leaf senescence parameters (Eq. 1.))	1.0 / 0.6

The model tends to underestimate the leaf area, especially for the control treatment where the plants were growing in a nitrogen limited environment as this treatment received zero N fertilizer in the past 50 years. Despite of this, the model gave fairly good estimations for the biomass and the yield even for 2007, when the model considerably underestimated the LAI for all of the treatments (*Fig. 5.*). This apparent contradiction could be resolved with the following argumentation. In nitrogen limited environments maize plants tend to allocate proportionally more mass to the leaves than in an environment with optimal nutrient supply. According to the observations, in the period of the most intensive growth period (50-70 days after sowing), the leaf growth rate accounted for 43.4 and 34.8 % of the biomass growth rate for the control and the $N_2P_2K_2$ treatments, respectively. On the other hand, leaves of the plants in the control treatment had significantly lower chlorophyll content (smaller corresponding radiation use efficiency) according to the observations (*Fig. 6.*). Since these two reversely acting phenomenon are not taken into account in the model, the simulated plants with smaller leaf area, but with higher radiation use efficiency might be able to produce (virtually) similar amounts of biomass to that of the real ones. That is to say that, though the model considerably underestimated the leaf area expansion of the plants in the control treatment, the calculated biomass production was similar to that of the real one since the photosynthetic activity in the model does not decrease with the lower nitrogen availability, unlike in real plants.

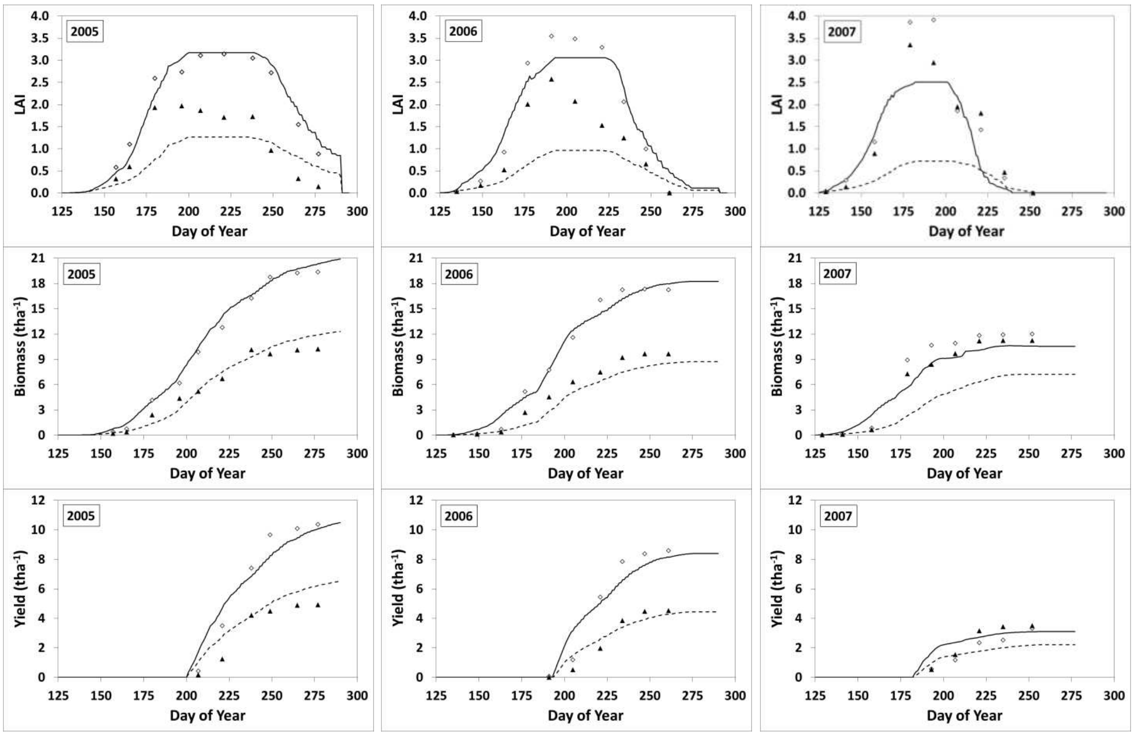

Figure 5. *Observed (dots) and simulated (lines) data for the 2005-2007 period. Diamonds and continuous lines denote the N2P2K2 treatment while triangles and dashed lines represent the control treatment. Plants were sampled 9-10 times in each vegetation period and dots present the average of the measured values for each sampling dates.*

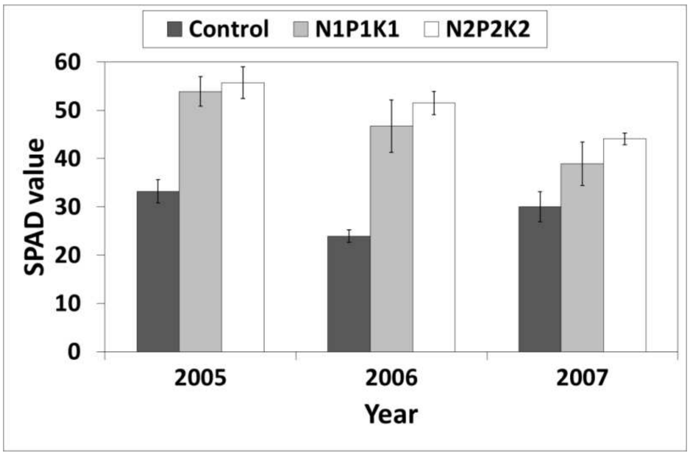

Figure 6. *Effect of fertilization level on the leaf chlorophyll content. SPAD denotes the relative chlorophyll content index. Bars denote the standard deviation of the measurement replicates. The annual averages were significantly different according to the t-tests (α = 0.05) for all treatments and for all pair of years. The only exception was the 2005-2007 comparison for the control treatment.*

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The model was able to simulate the differences between the years, as well as the effects of the different fertilization levels. 4M gave better biomass and yield predictions for the higher N-level treatments: The mean relative errors of the biomass (yield) estimations were 22 (22), 8 (9) and 6 (4) % for the control, $N_1P_1K_1$ and $N_2P_2K_2$ treatments, respectively. The Nash–Sutcliffe model efficiency coefficients were summarized in *Table 3*. Except for the leaf area index in 2007 the NSE values were positive. Additionally, NSE values were close to 1 for biomass and yield in most of the cases meaning that the model is able to provide reliable estimates of these parameters. In addition, the model provided better estimations for years with more or with more evenly distributed precipitation (2006 vs 2007 in *Fig. 2.*). The mean relative errors of the biomass (yield) estimations were 9 (11), 7 (5) and 20 (23) % for the year 2005, 2006 and 2007, respectively.

In 2007 there was practically zero precipitation in April and only 35 mm of rain (mainly from sporadic light showers, >5 mmd^{-1} rain was recorded only on two days) was registered between the 10th of June and the 10th of August. Despite of this the highest LAI was recorded in this year. Leaf area climaxed in the first decade of July that was followed by a week-long heat wave with maximum temperatures above 35 °C. The extreme temperature and the water deficit stress caused rapid leaf senescence when the leaf area fell by one-half within two weeks. The model, with the enhanced leaf senescence method, simulated fairly well the declining section of the leaf area curves (*Fig. 5.*) even for the extreme weather conditions in 2007. Since the model failed to adequately calculate the leaf area maximum for this year, the LAI values were seriously underestimated for the rest of the vegetation period, even though the tangents of the observed and simulated leaf area declination were similar (*Fig. 5.*). Obviously, the proposed leaf senescence method is unable to simulate the rate of the canopy decay for the control treatment where most likely the nitrogen deficit stress increased the senescence rate as the observed data suggest (*Fig. 5.*).

Table 3. *Nash–Sutcliffe model efficiency coefficients (NSE) obtained during the model calibration.*

Parameter	Year	NSE (control / $N_2P_2K_2$ treatment)
LAI	2005	0.651 / 0.978
Biomass		0.939 / 0.990
Yield		0.911 / 0.983
LAI	2006	0.190 / 0.932
Biomass		0.904 / 0.983
Yield		0.924 / 0.979
LAI	2007	-0.201 / 0.733
Biomass		0.415 / 0.885
Yield		0.909 / 0.967

Model validation

The root mean squared error (RMSE), the mean absolute error (MAE) and the mean relative error (MRE) of the yield simulations were the following for the three investigated treatments for the 1961-2010 period. Control: RMSE = 1.19 tha^{-1}, MAE = 0.94 tha^{-1}, MRE = 29%; $N_1P_1K_1$: RMSE = 1.35 tha^{-1}, MAE = 1.13 tha^{-1}, MRE = 23%; $N_2P_2K_2$: RMSE = 1.43 tha^{-1}, MAE = 1.23 tha^{-1}, MRE = 20%. The expected value of the observed and the *simulated* yields were 3.6, 5.8, 6.3 tha^{-1} and *3.3, 5.6, 6.0 tha^{-1}* for the control, $N_1P_1K_1$ and $N_2P_2K_2$ treatments, respectively. According to the paired t-tests, no significant differences were found between the observed and simulated yields. The annual yields of the control and $N_2P_2K_2$ treatments for the 1961-2010 period are presented in *Fig 7.*, while the cumulative yields are presented in *Fig. 8*. The model underestimated the observed yields especially for the high-yield years. In the low-yield years this tendency was reversed especially for the fertilized treatments.

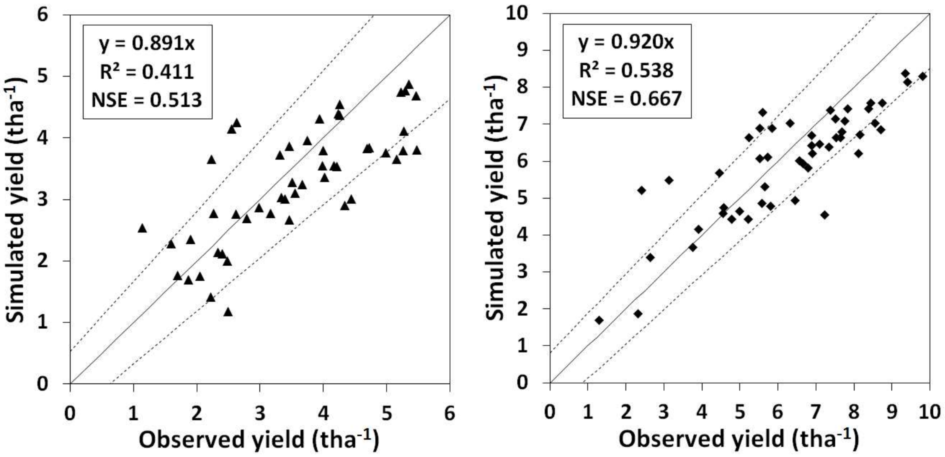

Figure 7. *Simulated vs observed yields for the control (left) and the $N_2P_2K_2$ treatments (right) for the 1961-2010 period. Dotted lines indicate the 20% relative error threshold.*

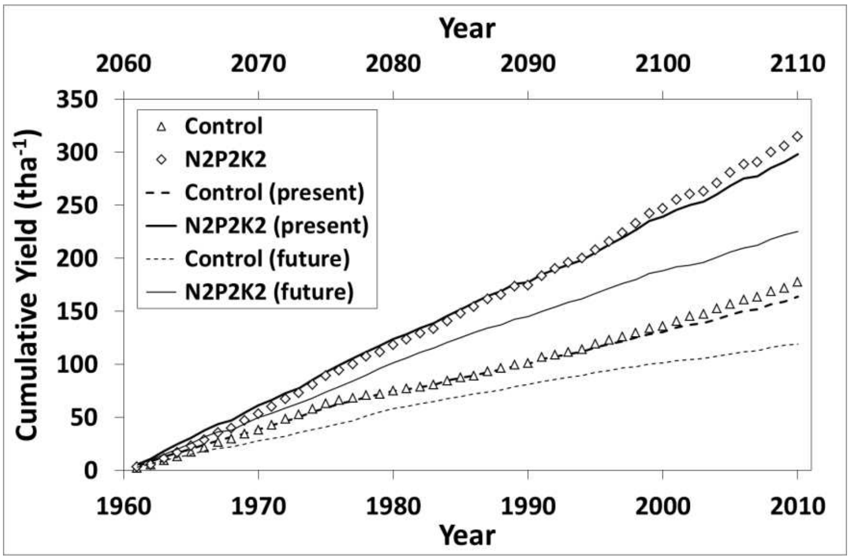

Figure 8. *Observed (dots) and simulated (lines) cumulative yields for the control and the $N_2P_2K_2$ treatments, for the 1961-2010 (heavy lines) and the 2061-2110 (thin lines) periods.*

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The model underestimated the inter-annual variability of the yield for all of the treatments. The standard deviation of the observed and the *simulated* yields was 1.19 and *0.62*, 1.84 and *0.97*, 1.98 and *1.08* tha^{-1} for the control, $N_1P_1K_1$ and $N_2P_2K_2$ treatments, respectively. This result emphasizes the fact that the model is still not capable of estimating the effects of yield-limiting and yield-reducing factors adequately. Most likely, the parameters used to simulate heat and water stress induced senescence (Eq. 1.) do not completely explain variations of leaf decay rate. 4M is not the only model with this type of weakness as recent studies have revealed that the response of plants to a combination of two different abiotic stresses is unique and cannot be directly extrapolated from the response of plants to each of the different stresses applied individually (Mittler, 2006). 4M was able to predict the long-term trends and averages of biomass productions adequately (*Fig. 8.*) for the investigated treatments. However, there were two periods when the model considerably and systematically over- (1961-1963) or underestimated (1995-2010) the observed yields. The first deficiency could be explained by the fact that these were the 3rd to 5th years of the long-term experiment when the effects of the treatments had not been consolidated yet and the spatial variability of the soil might have significantly distorted the observed data. This result supports the practice that data of the first 4-6 years of long-term experiments are usually not used when observations are evaluated. The systematic underestimation of the model for the last decade of the 1961-2010 period could be an indirect evidence for climate change or (at least) for the effect of the elevated air CO_2 concentration. The postulated 350 ppm atmospheric CO_2 concentration was most likely not valid for the end of the investigated period (CO2now, 2015). The capability of long-term experiments to scientifically demonstrate that climate change is actually happening has already been presented (Henkin et al., 2010). Another possible origin of the underestimation is the increased efficiency of the modern cultivars. Though their specific radiation use efficiency is not higher but their architecture (leaf angle) and stress tolerance is clearly better than those of the older hybrids. The Norma hybrid that had been bred in the Agricultural Institute, has been sown every year in the experiment since 1991. This variety has specifically high average harvest index and drought tolerance, neither of which has been simulated sufficiently by the model.

The root mean squared error (RMSE), the mean absolute error (MAE) the mean relative error (MRE) and the Nash–Sutcliffe model efficiency coefficient (NSE) of the yield simulations for the parcels of the 294 farms in the 2001-2010 period were the following: RMSE = 1.28 tha^{-1}; MAE = 1.03 tha^{-1}; MRE = 15.7 %; NSE = 0.641. These error indicators represent a quite good model performance. The observed vs. simulated yield data points are scattered symmetrically along the 1:1 lines on *Fig. 9.* and *Fig. 10.* except for sandy soils and low yielding years, as it can be especially seen on *Fig. 10*, in 2007 which was an extremely droughty year.

The results of the model validation confirm the fact that the 4M model still has the weakness of simulating the yield formation in extreme environmental conditions: e.g. droughts and/or soils with poor water holding capacity. On the other hand, in average, the model is capable to simulate the biomass formation with acceptable accuracy.

According to the simulation projections prospective yields will considerably decrease due to climate change (*Fig. 8.*). One hundred years from now average yields will be some 25% lower than the present yields. Even though higher CO_2 concentration in the atmosphere results in an increased plant stomatal resistance and in a better water use efficiency (Anda and Kocsis, 2008), this effect cannot counterbalance the summer water shortage. The coefficient of variation (cv) of the simulated yields was considerably higher for the future climate scenario (control: 32%, $N_2P_2K_2$: 25%) than for the present (control: 19%, $N_2P_2K_2$: 18%). These figures predict a considerable decrease of yield safety. These findings are in line with the results of earlier studies that also predict the decrease of yields (Bacsi et al., 1991; Harnos et al., 2002) owing to the increase of water limitation in crop production. According to Olesen et al. (2011) the increased risk of hail, pest and weed damages will further decrease the agricultural productivity in the Carpathian basin.

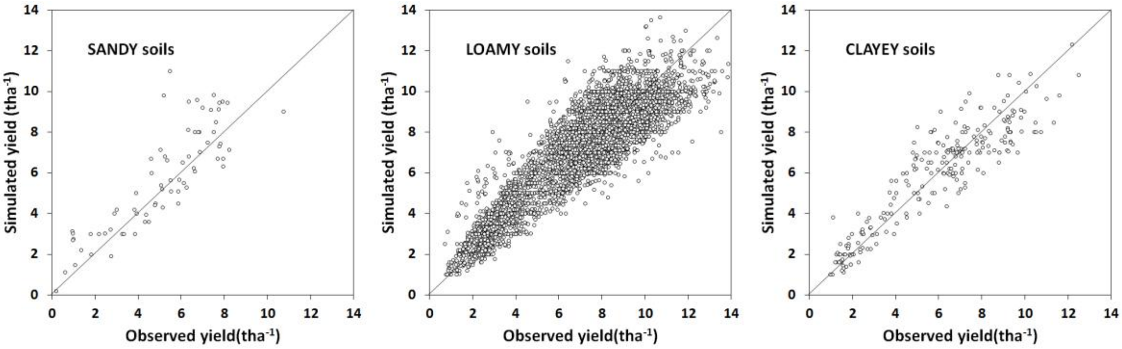

***Figure 9.** Observed and simulated yields of the representatively selected 294 Hungarian farms between 2001 and 2010 as a function of the soil type of the parcels.*

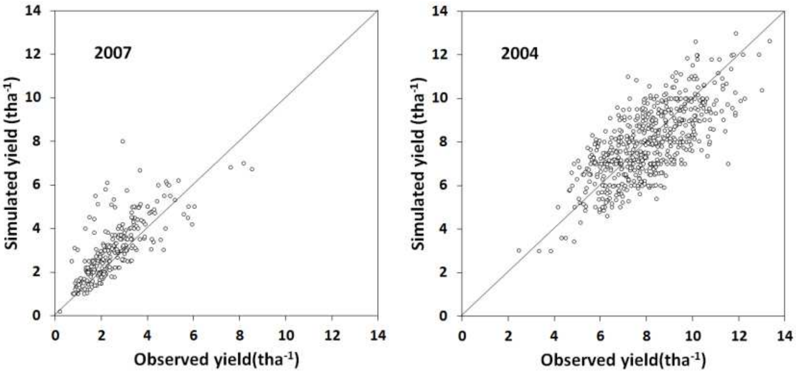

***Figure 10.** Observed and simulated yields of the representatively selected 294 Hungarian farms in 2007 (very droughty year) and 2004 (above average year).*

According to the observations, the 50-year average yield of the $N_2P_2K_2$ treatment is only 0.5 tha^{-1} higher than that of the $N_1P_1K_1$ treatment. To achieve this yield surplus, 66/38/75 $N/P_2O_5/K_2O$ $kgha^{-1}$ more active ingredient mineral fertilizer was applied to the $N_2P_2K_2$ treatment. Based on the average fertilizer and crop prices for the 1961-2010 period, the average income for the less intensively fertilized treatment would have been 1300 $€h^{-1}y^{-1}$ more than that of the more intensively fertilized treatment. For the climatic zone and for the soil type of the long-term experiment, which gives a considerable portion of the arable land in Hungary, it is not profitable to intensively fertilize the maize crop. This piece of information serves as an excellent example of how long-term experiments may support decision makers and even policy makers.

Conclusions

The efficiency of the 4M crop model in simulating the effects of different fertilizer levels was presented in this study. Calculated biomass formation data were compared to observed data which were collected in a long-term field experiment. The model successfully simulated the differences between the years, as well as the effects of the different fertilization levels. According to the results, using the more detailed observation data of three years, the model could be successfully calibrated to predict the long-term trends and averages of biomass productions adequately. On the other hand, the model underestimated the inter-annual variability of the yield which is an indicator of a more fundamental problem of crop modeling. At their present stage, crop models are unable to handle the effects of environmental (biotic and abiotic) stress factors adequately. Given that the current projections predict the increase of frequency of some extreme weather phenomena under climate change, this may lead to a severe underestimation of yield losses and yield variability when crop models are combined with climate change scenarios. Consequently, even though the 4M projection predicts considerable decrease of maize yields, as well as yield safety, the future reality could be considerably worse. The observations suggest two possible ways of model improvement. The model should have the ability 1) to change the assimilate partition and 2) to change the light use efficiency in nitrogen limited situations. More generally, model developers should aim at improving the capacity of crop models to project the impact of environmental (biotic and abiotic) stress factors especially focusing on their interdependent actions.

Concerning the projected maize yield losses, which may exceed 1,000,000 ton per year for the Carpathian basin according to the predictions, Hungarian farmers and agricultural enterprises should consider investing in irrigation to mitigate the harmful effects of severe summer hot and dry periods. The projected increase in food demand, as well as the need for increasing yield safety definitely substantiate investments in irrigation in Hungary similarly to the Mediterranean countries whose present climate is similar to the future climate of the Carpathian basin (Horváth, 2008). One possible way of accommodating the climatic changes is to increase the ratio of crops sown in the autumn in the crop rotation since these crops are less affected by the summer droughts. Another alternative for Hungarian agriculture is to start experimenting with alternative crops, such as energy crops (robinia (*Robinia pseudoacacia*), poplar (*Populus*), etc.) or crops native to or successfully produced in Mediterranean areas (fenugreek (*Trigonella foenum-graecum* L.), lady's thistle (*Silybum marianum* (L.) Gaernt.) or cotton (*Gossypium*), etc.). Well calibrated crop simulation models could support these testing efforts by carrying out virtual experiments.

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REFERENCES

[1] Anda, A., Kocsis, T. (2008): Impact of atmospheric CO_2 enrichment on some elements of microclimate and physiology of locally grown maize. – Applied Ecology and Environmental Research 6: 85–94.

[2] Audsley, E., Pearn, K. R., Simota, C., Cojocaru, G., Koutsidou, E., Rounsevell, M. D. A., Alexandrov, V. (2006): What can scenario modelling tell us about future European scale agricultural land use, and what not? – Environmental Science & Policy 9(2): 148–162.

[3] Bacsi, Zs., Thornton, P.K., Dent, J.B. (1991): Impacts of future climate change on Hungarian crop production: An application of crop growth simulation models. – Agricultural Systems 37: 435–450.

[4] CECILIA (2009): Deliverable 3.3: Assessment of the applicability of RCM and SDS models in the impact target areas by their validation according to relevant criteria; ranking of the models if possible pp. 34-40. http://www.cecilia-eu.org/restricted/deliverables.php. (Accessed: 08 Oct 2013)

[5] CO2now (2013): http://co2now.org/ (Accessed: 07 May 2015)

[6] Déqué, M., Marquet, P., Jones, R.G. (1998): Simulation of climate change over Europe using a global variable resolution general circulation model. – Climate Dynamics 14: 173–189.

[7] Egner, H., Riehm, H., Domingo, W. (1960): Unterschuchungenüber die chemische Bodenanalyseals Grundlage für die Beurteilung des Nährstoffzustandes der Böden II. Chemische Extraktions methoden zur Phosphor- und Kaliumbestimmung. – Kungliga Lantbrukshögskolans Annale 26: 199–215. (In German)

[8] Eitzinger, J., Trnka, M., Hösch, J., Žalud, Z., Dubrovský, M. (2004): Comparison of CERES, WOFOST and SWAP models in simulating soil water content during growing season under different soil conditions. – Ecological Modelling 171(3): 223–246.

[9] Fezzi, C., Bateman, I., Askew, T., Munday, P., Pascual, U., Sen, A., Harwood, A. (2014): Valuing provisioning ecosystem services in agriculture: The impact of climate change on food production in the United Kingdom. – Environmental and Resource Economics 57(2): 197–214.

[10] Fisher, R.A. (1921): Studies in crop variation. I. An examination of the yield of dressed grain from Broadbalk. – Journal of Agricultural Science 11: 107-135.

[11] Fodor, N., Csathó, P., Árendás, T., Németh, T. (2011): New environment-friendly and cost-saving fertiliser recommendation system for supporting sustainable agriculture in Hungary and beyond. – Journal of Central European Agriculture 12(1): 53–69.

[12] Fodor, N., Máthéné-G.G., Pokovai, K., Kovács, G.J. (2002): 4M - software package for modelling cropping systems. – European Journal of Agronomy 18: 389–393.

[13] Fodor, N., Pásztor, L. (2010): The agro-ecological potential of Hungary and its prospective development due to climate change. – Applied Ecology and Environmental Research 8: 177–190.

[14] Fodor, N, Pásztor, L, Németh, T. (2014): Coupling the 4M cropmodel with national geo-databases for assessing the effects of climate change on agro-ecological characteristics of Hungary. – International Journal of Digital Earth 7(5): 391–410.

[15] Gijsman, A.J., Hoogenboom, G., Parton, W.J., Kerridge, P.C. (2002): Modifying DSSAT for low-input agricultural systems, using a soil organic matter residue module from CENTURY. – Agronomy Journal 94(3): 462–474.

[16] Harnos, N., Bencze, S., Janda, T., Juhasz, A., Veisz, O. (2002): Interactions between elevated CO_2 and water stress in two winter wheat cultivars differing in drought resistance. – Cereal Research Communications 30: 359–366.

[17] Henkin, Z., Perevolotsky, A., Sternberg, M. (2010): Vulnerability of Mediterranean grasslands to climate change: What can we learn from a long-term experiment? – In: Porqueddu, C., Ríos, S. (eds) The Contributions Of Grasslands To The Conservation Of

Mediterranean Biodiversity. Zaragoza: CIHEAM/CIBIO/ FAO/SEEP, 2010. p. 167–174 (Options Méditerranéennes: Série A. Séminaires Méditerranéens; n. 92).

[18] Hodges, T. (1990): Predicting Crop Phenology. – CRC Press, Boca Raton, London, New York.

[19] Horváth, L. (2008): Use of spatial analogy method to analyze to possible land use change in Hungary. – CLIMA-21 Brochures 55: 23–27.

[20] IPCC (2007): Intergovernmental Panel on Climate Change (IPCC) WG1 Fourth Assessment Report, Climate Change: The Physical Science Basis, Summary For Policymakers.

[21] IPCC (2014): http://www.ipcc-data.org/observ/ddc_co2.html. (Accessed: 20 Feb 2014)

[22] Jamieson, P. D., Porter, J. R., Goudriaan, J., Ritchie, J. T., van Keulen, H., Stol, W. (1998): A comparison of the models AFRCWHEAT2, CERES-Wheat, Sirius, SUCROS2 and SWHEAT with measurements from wheat grown under drought. – Field Crops Research 55(1-2): 23–44.

[23] Jenkinson, D.S. (1990): The turnover of organic carbon and nitrogen in soil. – Philosophical Transactions of the Royal Society B 329, 361–368.

[24] Kismányoky, T., Jolánkai, M. (2009): Long-term fertilization experiments in Hungary. In Debreczeni, Bné., Németh, T. (eds). Scientific Results Of The Hungarian Long-term Fertilization Experiments (1967-2001). – Akadémiai Publisher, Budapest, 25–33.

[25] Klein, T., Calanca, P., Holzkämper, A., Lehmann, N., Roesch, A., Fuhrer, J. (2012): Using farm accountancy data to calibrate a crop model for climate impact studies. – Agricultural Systems 111: 23–33.

[26] Knox, J.W., Hurford, A., Hargreaves, L., Wall, E. (2012): Climate Change Risk Assessment for the Agriculture Sector. DEFRA report.

[27] Korsaeth, A. (2012): N, P, and K budgets and changes in selected topsoil nutrients over 10 years in a Long-Term Experiment with conventional and organic crop rotations. – Applied and Environmental Soil Science, Article ID 539582, 17 p, 2012. doi:10.1155/2012/539582.

[28] Máthé-Gáspár, G., Fodor, N. (2012): Modeling the phosphorus balance of different soils using the 4M crop model. – Plant, Soil and Environment 58(9): 391–398.

[29] Mittler, R. (2006): Abiotic stress, the field environment and stress combination. – Trends Plant Science 11(1): 15–19.

[30] Nakicenovic, N., Swart, R. (eds) (2000): Special report on emission scenarios. – Cambridge University Press, Cambridge.

[31] Nash, J.E., Sutcliffe, J.V. (1970): River flow forecasting through conceptual models part I – A discussion of principles. – Journal of Hydrology 10(3): 282–290.

[32] Olesen, J.E., Bindi, M. (2002). Consequences of climate change for European agricultural productivity, land use and policy. – European Journal of Agronomy 16(4): 239–262.

[33] Olesen, J.E., Trnka, M., Kersebaum, K.C., Skjelvag, A.O., Seguin, B., Peltonen-Sainio, P.,Rossi, F., Kozyra, J., Micale, F. (2011): Impacts and adaptation of European crop production systems to climate change. – European Journal of Agronomy 34, 96–112.

[34] Pásztor, L., Szabó, J., Bakacsi, Z., Laborczi, A., Dobos, E., Illés, G., Szatmári, G. (2014): Elaboration of novel, countrywide maps for the statisfaction of recent demands on spatial, soil related information in Hungary. – In: Arrouays, D., McKenzie, N. et al. (eds.) Global Soil Map: Basis Of The Global Spatial Soil Information System. Taylor & Francis Group, London, 207–212.

[35] Peltonen-Sainio, P., Jauhiainen, L., Hakala, K., Ojanen, H. (2009): Climate change and prolongation of growing season: Changes in regional potential for field crop production in Finland. – Agricultural and Food Science 18: 171–190.

[36] Ramirez-Cabral, N.Y.Z., Kumar, L., Taylor, S. (2016): Crop niche modeling projects major shifts in common bean growing areas. – Agricultural and Forest Meteorology 218-219: 102–113.

[37] Rasmussen, P.E., Goulding, K.W.T., Brown, J.R., Grace, P.R., Janzen, H.H., Körschens, M. (1998): Long-Term Agroecosystem Experiments: Assessing Agricultural Sustainability and Global Change. – Science 282: 893–896.

[38] Ray, D.K., Mueller, N.D., West, P.C., Foley, J.A. (2013): Yield trends are in sufficient to double global crop production by 2050. – PLoS ONE 8: e66428. doi:10.1371/journal.pone.0066428.

[39] Ritchie, J.T., Singh, U., Godwin, D.C., Bowen, W.T. (1998): Cereal growth, development and yield. – In: Tsuji, G.Y., Hoogenboom, G., Thornton, P.K. (eds): Understanding Options For Agricultural Production. Kluwer Academic Publishers, Dordrecht. 79-98.

[40] Rosenzweig, C. Parry, M.L. (1994): Potential impacts of climate change on world food supply. – Nature 367: 133–138.

[41] Sándor, R., Fodor, N. (2012): Simulation of soil temperature dynamics with models using different concepts. – The Scientific World Journal. Article ID 590287, 8 pages. DOI:10.1100/2012/590287.

[42] Schepers, J.S., Francis, D.D., Virgil, M., Below, R.E. (1992): Comparison of corn leaf nitrogen concentration and chlorophyll meter readings. – Communication in Soil Science and Plant Analysis 23: 2173–2187.

[43] Semenov, M.A., Shewry, P.R., (2011): Modelling predictsthat heat stress, not drought, will increase vulnerability of wheat in Europe. – Natural Sciences Rep. 1. http://dx.doi.org/10., 1038/srep00066.

[44] Soetaert, K., Petzoldt, T. (2010): Inverse modelling, sensitivity and Monte Carlo analysis in R Using Package FME. – Journal of Statistical Software 33(3): 1–28.

[45] Southwood, T.R.E. (1994): The importance of long-term experimentation. – In: Leigh, R.A., Johnston, A.E. (eds) Long-Term Experiments In Agricultural And Ecological Sciences. CAB International, Wallingford, 3–8.

[46] Spinoni, J. and the CARPATCLIM project team (39 authors) (2014): Climate of the Carpathian Region in 1961-2010: Climatologists and Trends of Ten Variables. – International Journal of Climatology. Article first published online: 12 June 2014. 2014. DOI: 10.1002/joc.4059.

[47] Stockle, C.O., Nelson, R.L. (1996): Cropsyst User's manual (Version 2.0). Biological Systems Engineering Dept., Washington State University, Pullman, Washington.

[48] Supit, I., van Diepen, C.A., de Wit, A.J.W., Kabat, P., Baruth, B., Ludwig, F., (2010): Recent changes in the climatic yield potential of various crops in Europe. – Agricultural Systems 103: 683–694.

[49] Szentimrey, T., Bihari, Z., Lakatos, M., Szalai S. (2011): Mathematical, methodological questions concerning the spatial interpolation of climate elements. – Időjárás 115, 1–11.

[50] Tyurin, I.V. (1937): Organic matter of soils. – Szelhozgiz. Moscow (in Russian).

[51] Wang, Y., Bellus, M., Wittmann, C., Steinheimer, M., Weidle, F., Kann, A., Ivatek-Sahdan, S., Tian, W., Ma, X., Bazile, E. (2011): The Central European limited area ensemble forecasting system: ALADIN-LAEF. – Quarterly Journal of the Royal Meteorological Society 137: 483–502.

ELECTRONIC APPENDIX

Appendix 1. Long-term Field Experiment, Martonvásár, Hungary
Appendix 2. N1P1K1 treatment, 22. 06. 2006
Appendix 3. N2P2K2 treatment, 22. 06. 2006
Appendix 4. Control and N2P2K2, 25. 06. 2006
Appendix 5. N2P2K2 stages, 2006
Appendix 6. Control treatment, 2006

HOW INITIAL COMPOSITION AFFECTS THE LATER DEVELOPMENT? - A SECONDARY SUCCESSIONAL STUDY IN DIFFERENTLY MANAGED AGRICULTURAL SITES

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Abstract. Plant succession is a well-studied phenomenon in plant ecology, yet the effects of disturbance and its influence on initial plant composition received little attention in this process. From many of the potential reasons we investigated the effect of soil fungi on early succession. At each site a total of 60 permanent plots were established and fungicide treatment was used on half of the plots, the other half served as control. We found that management by influencing initial plant communities, determine the way how forb and grass dominancy changes in time. In addition, our findings support that the applied fungicide treatment influence successional development of plant communities. Specifically, the elimination of fungi from an annual-dominated (highly disturbed) site led to stronger grass dominance, while decreased the presence of forbs. Nevertheless in the perennial-dominated (less disturbed) site fungicide treatments favored to forbs, but this effect was not significant. Our results suggest that previously used management regimes strongly affect the later secondary successional events that can also be strongly influenced by the soil fungal community.

Keywords: *fungicide, grassland, secondary succession, disturbance, soil, annual, perennial ratio*

Introduction

Succession is a well-studied process in ecology (Van der Putten et al., 2000). The term refers to the development of species composition in a community, which is associated with a sequence of changes in its structural and functional properties (Drury and Nisbet, 1973). Following abandonment, there is a large number of arable fields in Europe, where developmental processes appear, thus it is unequivocal that several studies have focus on secondary succession in these habitats (Collins, 2001; Rehounková, 2010). In these studies, successional processes tended to return plant communities to the state they were in prior to disturbance (Horn, 1974). In the last decades, the effect of disturbance has been a major topic in succession studies (Perry and Millington, 2008; Prach et al., 2014), suggesting that disturbance is a main driving factor in vegetation changes. Nevertheless, disturbance-induced changes in the

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biophysical environment are related to disturbance size (Dölle and Schmidt, 2009, Burt and Clary, 2015). Different disturbance history creates different circumstances, which alter the way of plant communities' development. From this aspect the initial status of communities can play a major role in succession processes.

Soil biota is another important factor influencing the successional development of plant assemblages. Soil mutualists and pathogenic microorganisms are crucial in early stages of both primary and secondary successions by influencing many important ecosystem processes. Including direct effects like supporting or impairing plants (Rillig and Mummey, 2006; van der Heijden et al., 2008); and indirect effects, like the altering of nutrient cycles (nitrogen, carbon) and soil formation.

One of the most important positive relationship between plant species and soil fungi, is the mycorrhizal connection. A large number of papers have dealt with the effects of mycorrhizal fungi on individual plant species (Emery and Rudgers, 2012; Rydlová et al., 2015), but also there is a growing number of studies that reveal their role in shaping natural plant communities (van der Heijden et al., 1998). A diverse soil community can increase species richness and productivity of the vegetation (Schnitzer et al., 2010), moreover soil fungi not only affects the diversity of biosystems, but also controls succession. They are very important determinants on the earlier successional phase by stabilizing perennial forb seedlings (Gange et al., 1993). It triggers this effect by reducing competition among species that facilitate coexistence in the critical phase of community development (Mariotte et al., 2013). Furthermore there are evidences showing that, not only the vegetation can change during succession, but simultaneously soil fungal community, suggesting a sensitive feed-back between plants and soil fungi (Sikes et al., 2012, Cutler et al., 2014).

In contrast, negative influences of soil fungi on plants control the later diversification of developing plant communities by species replacements (Reynolds et al., 2003). With negative feedback soil fungi can maintain plant species diversity within a community, by reducing the dominance of certain species (Mills and Bever, 1998). The presence or absence of certain soil biota can also explain why some exotic plant species are able to occur at higher abundance in the non-native versus native range. Maron et al., (2014) found that soils from the native range of some invasive plant species had a strong suppressive potential on these plants, whereas this is not the case in soils in the non-native range. This suggests that different composition of soil community can strongly alter the structure of vegetation.

Besides the previously mentioned direct effects, indirect effects of soil fungi could also be crucial in shaping community composition. Higher microbial biomass may negatively influence plant communities in competition for nutrients (Dunn et al., 2006). Another way for fungi to reduce available soil nutrients is by transforming them. Certain denitrifying fungi can transform nitrate into N gases causing high loss of soil nitrogen content (Waring et al., 2013).

During our research we tested the indirect effects of combined fungicide treatment on plant community composition of two differently managed sites, which were dominated by annual forbs or perennial forbs, and asked the following questions: a.) how does successional development differ in plant communities with different initial disturbance history; b.) what is the effect of the elimination of soil fungi on the species composition in different successional stages?

Materials and methods

Field experiment

We performed our field experiments from 2008 to 2011, at two differently managed sites in South Western Hungary. One of our sites (near the town of Siklós; coordinates: 45.881500, 18.254238) was a recently (2-3 years) abandoned vineyard (area 7.000 m^2) in which seasonal management still occurred (hoeing, mowing). We called this site annual-dominated, because, the site was dominated mainly by annuals (*Stellaria media*, *Lamium purpureum*, *Bromus sterilis*, *Arenaria serpyllifolia, Vicia angustifolia*) and some perennials (*Elymus repens*, *Poa pratensis; Appendix Table 1*). The second site (near the town of Máriakéménd; coordinates: 46.026577, 18.470698) was a diverse meadow steppe (area 16.000 m^2), that was also actively managed as an orchard 30 years ago and is now mown once a year. The site is called perennial-dominated, because the site was dominated mainly by perennials (*Achillea collina, Agrimonia eupatoria, Calamagrostis epigeios, Centaurea stoebe, Coronilla varia, Dactylis glomerata, Euphorbia cyparissias, Galium mollugo, Knautia arvensis, Poa angustifolia, Scabiosa ochroleuca, Appendix Table 2*). Both sites had similar geographic and climatic conditions. The region is hilly (120-180 m) with an average elevation of 150 m. The mean annual precipitation is 650-700 mm and the mean annual temperature is 10-11 ºC (averages between 1971-2000) (Bihari et al., 2009).

In both sites, we selected 60 (50×50 cm) fixed position quadrates for four years. Position of the quadrates was designated semi-systematically; both sites were divided into 60 equal sized blocks grid (85 m^2 in the annual-dominated and 270 m^2 in the perennial-dominated sites), and in each blocks one quadrate were posited randomly. In the first year we surveyed the baseline conditions of the quadrates (created a species list, estimated the cover of forbs and grasses, along with the estimation of bare ground and leaf litter). Scientific names of plant species follow Király (2009). From the beginning of the experiment we excluded every kind of management in the fields, for being able to observe successional differences. To detect the effects of soil fungi on the development of plant communities we applied a Mefenoxam (56,304 mg/l) and Thiophanate-methyl (1250 mg/l) fungicide mixture on half of the plots (30). The other half of the plots served as control. The fungicide treatment was applied twice a year in spring and in summer. Each treated plot received 250 ml of the fungicide mixture while control plots received the same amount of water.

We collected data from the plots once a year, in late spring (around May). In each plot, the number of all vascular plant species was recorded and their coverage (%) estimated. We also estimated litter coverage and bare ground surface. We distinguished two groups of vascular plants, (grasses and forbs) for the statistical analysis.

Data analysis

For the statistical analysis we carried out built linear model for each measured parameters, using function lm (based on Chambers, 1992) in R environment (R version 2.15.1; R Development Core Team, 2011), function anova were used to visualize ANOVA table. The models also included two and three-way interactions. Our dependent variables were the measured attributes (coverage of grasses, forbs, leaf litter and bare ground), while the independent variables were the fungicide treatment, disturbance, and year. All independent variables were treated as fixed factors and were log transformed. Transformation was based on graphical evaluation according to

Crawley (2014). For pair-wise comparisons, Tukey post-hoc tests were conducted in both cases with multcomp-package (Hothorn et al., 2008).

Results

We detected significant differences between the field sites with different initial plant dominancy (*Appendix Table 1* and *2*). The results of the ANOVA are shown in *Table 1.*

The coverage of forb species differed highly at the initial stage, depending on the management regime of the plant community (*Figure 1*). Significantly higher (16%) dominance of forbs were detected at the annual-dominated site compared to the perennial-dominated one ($t = -4.163$; $P < 0.01$). In the following two years a different trend was detected at both places: at the annual-dominated site we registered decreasing dominance of forbs, while at the perennial-dominated site their dominance increased. During this period the differences between the two sites disappeared. In the fourth year the tendencies strengthened and showed stronger significant differences between the sites (35%) ($t = 9.40800$; $P < 0.001$). In the perennial-dominated site, the coverage of forbs became higher than in the annual-dominated site by 40%. The fungicide treatment did not cause differences in the first three years, but at the fourth year the elimination of soil fungi affected forbs positively the annual-dominated site (14%) ($t = -3.708$; $P = 0.0204$). In the non-fungicided quadrates the number of forbs significantly decreased by 30% during the three years of the experiment, at the same time the fungicide treatment moderated the loss of forb coverage.

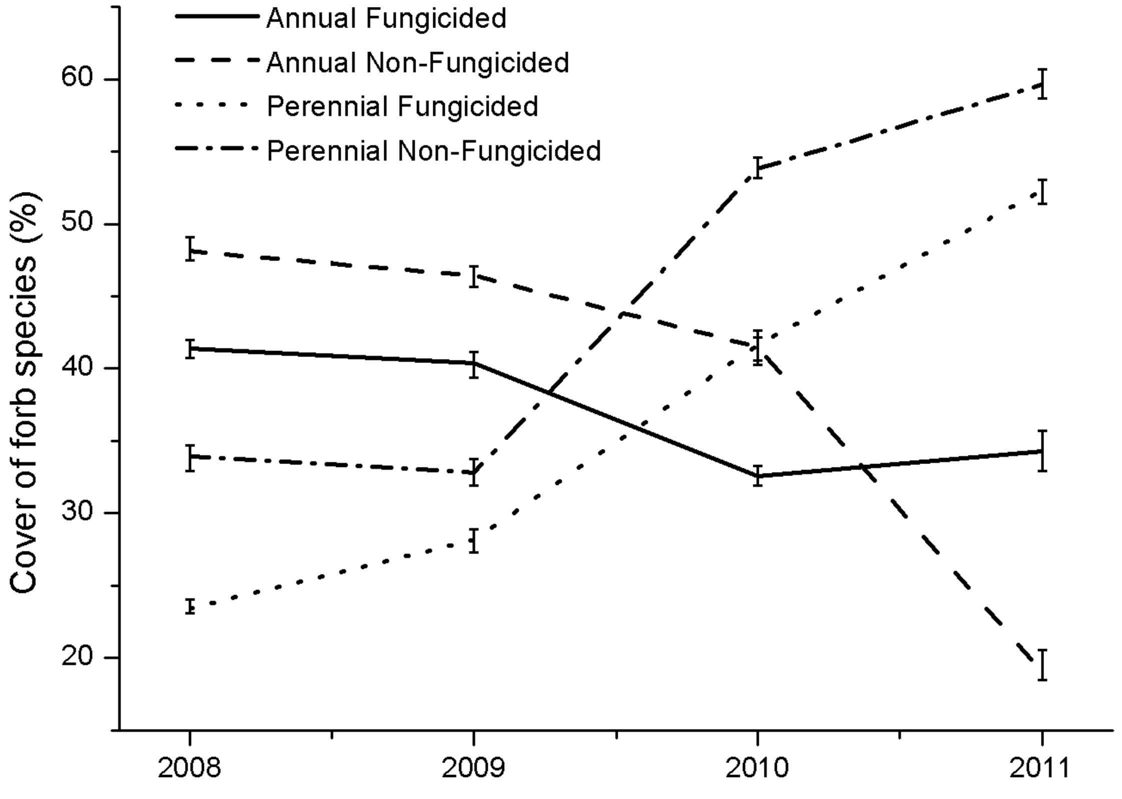

Figure 1. Changes in the coverage of forb species from 2008-2011. Error bars represent S.E.M.

Table 1. *ANOVA results of the applied linear models of the effects of different sites (annual-dominated and perennial-dominated), fungicide treatment and time on the forb cover, grass cover, bare ground cover, and leaf litter cover.*

	Forb cover (%)			Grass cover (%)			Bareground (%)			Leaf litter (%)		
	df	F-value	P	df	F-value	P	df	F-value	P	df	F-value	P
Site	1	5.894	0.0155 *	1	49.425	6.68e-12 ***	1	3.5749	0.028719 *	1	598.018	< 2,2e-16 ***
Fungicide treatment	2	13.198	2.58e-06 ***	2	21.602	9.91e-10 ***	2	1.568	0.211080	2	6.667	0.00139 **
Year	3	10.597	9.07e-07 ***	3	3.978	0.00807 **	3	13.380	2.04e-08 ***	3	16.388	3.52e-10 ***
Site:Year	3	52,7877	< 2,2e-16 ***	3	73.510	< 2,2e-16 ***	3	52.048	< 2,2e-16 ***	3	370.456	< 2,2e-16 ***
Treatment:Year	6	2.420	0.0257 *	6	2.601	0.01716 *	6	3.921	0.000767 ***	6	0.999	0.42517
Site:Treatment:Year	15	13,069	< 2,2e-16 ***	15	21,451	< 2,2e-16 ***	15	23,211	< 2,2e-16 ***	15	46,772	< 2,2e-16 ***

Significance level codes: 0 ‘***’ 0,001 ‘**’ 0,01 ‘*’ 0,05 ‘!’ >0,1 ‘ ’

The grass coverage showed contrary results to the forbs (*Figure 2*). In the annual-dominated site the dominance of grasses were low, while at the perennial-dominated site they were significantly (11%) higher (t = 3.606; P = 0.0292). In the second year of the experiment these differences further increased (20%) (t = 4.591; P < 0.001). Namely at the perennial-dominated site the coverage of grasses increased while at the more disturbed site it decreased. In the third year we did not experience changes at the perennial-dominated site, but detected strong increase in grass coverage at the annual-dominated site. In the fourth year of the experiment the dominance of grasses at the highly disturbed annual-dominated site increased further, while at the perennial-dominated site it strongly decreased, causing significant differences between the two sites (34%) (t = -6.128; P <0.001). The fungicide treatment did not cause differences during the first three years, but in the fourth year a significant effect was detected at the annual-dominated site (t = 4.195; P <0.01). The elimination of soil fungi caused lower increase (15%) of grass dominance than in the control plots.

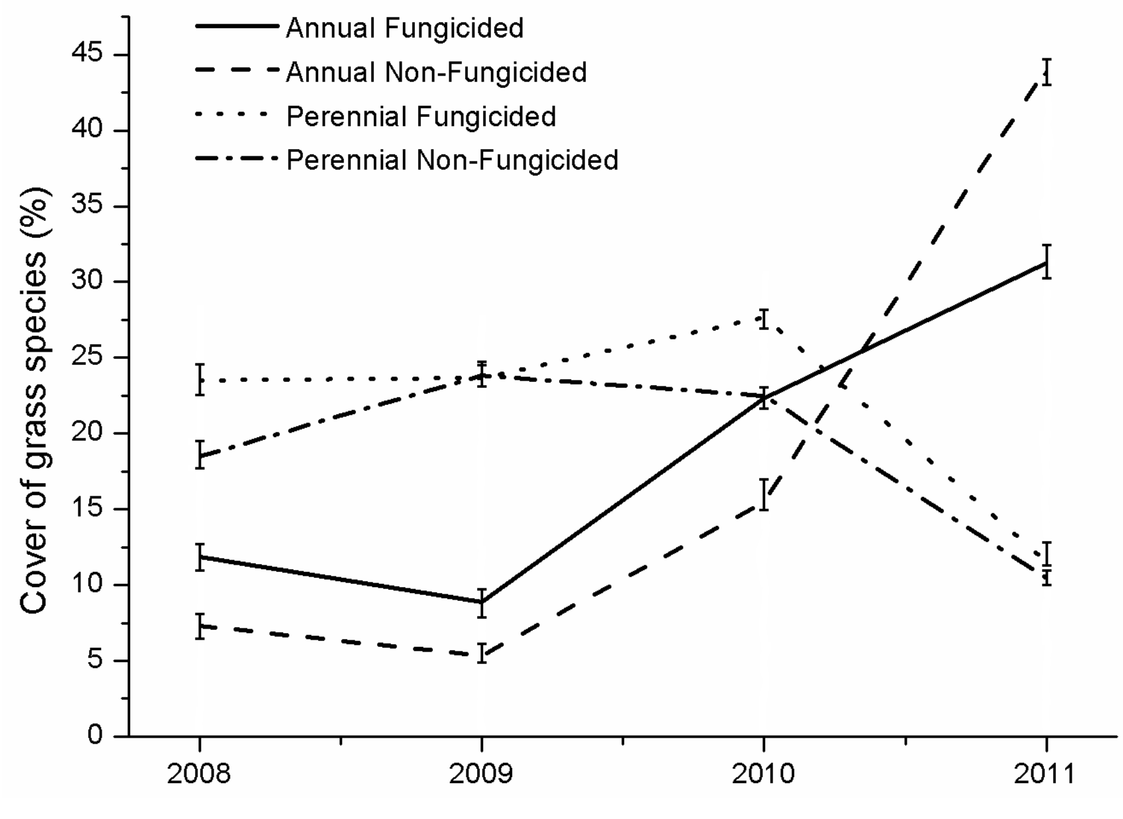

Figure 2. *Changes in the coverage of grass species from 2008-2011. Error bars represent S.E.M.*

The number of species changed at both sites significantly. In the second year the number of species on perennial-dominated site did not change, but in the third year it increased significantly by three new colonizer (*Crepis biennis, Rosa canina, Scabiosa ochroleuca*) (t = -5.5284; P < 0.001). In the last year no differences were detected. At the annual-dominated site the number of species increased significantly in the first two year, while stagnated at the last two years. Fungicide treatment did not affect this attribute at any of the experimental sites.

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Changes in bare ground surface were also observed (*Figure 3*). The two sites significantly (27%) differed in their initial stage (t = -8.109; P <0.01). At the perennial-dominated field the coverage of bare ground was low at the first year, while it was high at the annual-dominated site due to the frequent management. In the second year of the experiment we recorded changes at both sites. At the annual-dominated site bare ground decreased by 7%, but at the perennial-dominated site it increased by 17%. The development of the two experimental sites was different. The bare ground at the annual-dominated site reduced from year to year down to 0%. At the perennial-dominated site the coverage decreased at the third year, and at the fourth year it increased again. We did not detect differences between the two sites at the second and third year, but there was a significant (12%) difference (t = 3.573; P = 0.0331) in the fourth year of the experiment. The fungicide treatment caused no differences in this attribute at any of the sites.

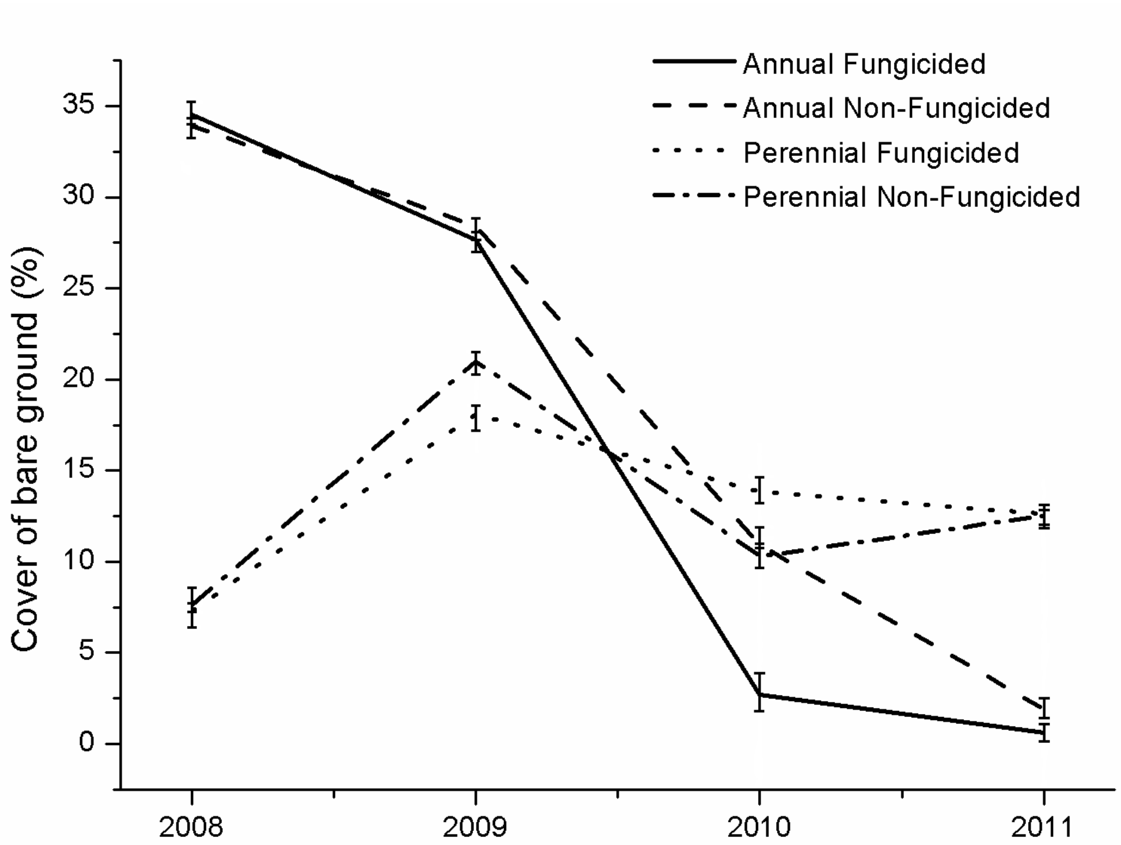

Figure 3. *Changes in the coverage of bare ground from 2008-2011. Error bars represent S.E.M.*

The quantity of litter also showed differences between the two experimental sites (*Figure 4*). At the initial stage we detected strong deviations (t = 17.466; P <0.001). At the annual-dominated site we observed 0% coverage of leaf litter, while at the perennial-dominated site the quantity was high (37%). After the management had been stopped for the experiment, the litter content changed significantly at both sites. The coverage on the perennial-dominated site decreased in the second year, while the coverage of annual-dominated site increased. The coverage of leaf litter did not change in the last two years. This value varied around 20%. Fungicide treatment did not affect this attribute at any of the experimental sites.

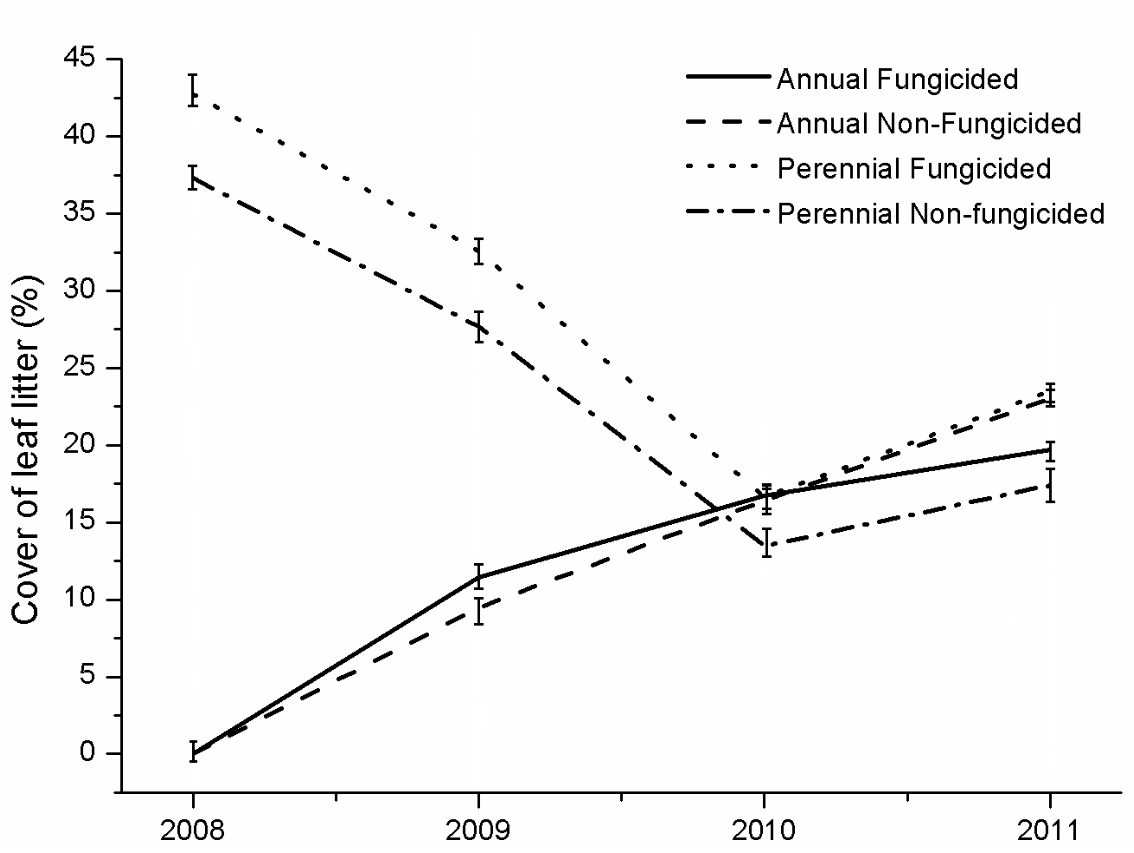

Figure 4. Changes in the coverage of leaf litter from 2008-2011. Error bars represent S.E.M.

Discussion

Our results show that the prevailing disturbance types of an area can significantly determine the later process of succession. Two sites were selected with different management history to conduct our experiment. These sites differed in their composition already in the initial phase. In the more heavily disturbed, annual-dominated site, the dominance of annuals was stronger, while in the less disturbed, perennial-dominated site, the grass species were dominant in the first year. This could be explained by the stronger disturbance tolerance of annual forb species, which usually dominate pioneer sites, while perennial grasses are more sensitive to intensive disturbance (Wilcox, 1998; Vaszari and Kukorelli, 2010). Our results support the findings of Porcova and Wikler (2014), who explained the dominance of annuals over perennials with the extensive management of vineyards. In contrast, the annual mowing and grazing in the perennial-dominated site strengthened the perennial grass dominance and stabilized the community, by supporting their reproduction (Meckenzie et al., 2006; Gao et al., 2014).

After abandonment of sites, secondary succession started to change the structure of the communities differently. During the experimental time, on the annual-dominated site, the dominance of forbs decreased, while perennial grasses took over their place. It is essential, because grass cover has an important role in succession by stabilizing the community's structure in the early phase (Prach and Pysek, 2001). In contrast, at the perennial-dominated site, grass dominancy decreased, proportion of perennial forbs increased significantly and at the same time shrub species started to appear, showing a further step of its succession (Kahmen and Poschold, 2004).

Changing percentage of bare ground is also an important factor in the process of succession. Increasing and decreasing of gaps can open or even close the way to new occupants, helps the development of communities and the maintenance of inner dynamics (Zhang et al., 2012). In the perennial-dominated site, bare ground cover increased in the first year, by the alteration of structure which causes changes in dominance. During this period the coverage of forbs and grasses suppressed both promoting the later forb dominancy. To the third and fourth year the bare ground did not change further. In the annual-dominated site the reduction of bare ground is the consequence of the termination of intensive hoeing. The release from disturbance resulted the reduction of gaps, and nearly a 100% plant coverage developed; (Fahey and Puettmann, 2007).

Change in the amount of leaf litter was similar, but had a different direction in the two sites. The lack of management caused leaf litter reduction in the second and third year in the perennial-dominated meadow, which is the result of the cessation of mowing. In contrast in the annual-dominated vineyard, the lack of soil surface disturbance resulted natural litter formation. The two sites with different history reached similar coverage, which suggests us a balanced quantity of litter. Composition of litter plays an important role in the development of communities by direct allelopathic effects of dead plant materials, which can alter species composition and dominancy of plant assemblages (Saito and Tsuyuzaki, 2012). Moreover it can influence the success of new occupants by altering the light and microclimatic conditions (Bosy and Reader, 1995), and also has strong impact on chemical and microbial composition of soils (Hobbie, 2015; Rinkes et al., 2014)

In our research we investigated the effect of fungicide treatment on the development of plant communities in the early phase of succession. We found a strong effect only in the case of the highly disturbed, annual-dominated community. As Jasper et al., (1991) showed, soil disturbance can have a significant impact on fungal communities, but this effect is strongly linked with the vegetation type. In disturbance-associated communities the fungal species richness can be largely explained by richness of plant species (König et al., 2010) and even by the quantity and quality of litter input into soil (Frossard et al., 2013). The fungicide treatment in our experiment had positive effects on forbs and negative effects on grass species. Here the soil fungi might have helped the expansion of grasses against forbs, which was also confirmed by McCain et al. (2011). Although these results contradict the findings of Gange et al., (1993), that clarifies the role of soil biota in the stabilization of forb species in the early succession. Dostálek et al., (2013) found that grass species are more independent from fungal diversity, while they assist perennial forb species in non-disturbed vegetation types. Similarly to his findings, in the less disturbed annual-dominated site we found higher forb density in non-fungicided plots, but the difference was not significant. Therefore our results and the scientific literature can highlight the soil fungal community can have different effects in highly and slightly disturbed communities, depending on its species composition. Along with our findings, it has to be mentioned that, we only found differences among the differently treated sites in the fourth year of the experiment. This finding suggests, that natural development of fungal community in soils, feed-back among vegetation and soil community jointly interacted with the applied treatment, and required a longer time to express in the vegetation composition (Wang et al., 2011; Cutler et al., 2014).

Our study revealed that different disturbance levels strongly influence early succession processes by altering initial composition. We detected significant changes in forb-grass ratio and also in other measured variables (bare ground and leaf litter coverage) during the experimental time. On the annual-dominated site the development resulted an increase in grass cover and a decrease in bare ground. In contrast the perennial-dominated site stepped into a new successional phase, which in the first period opened up, providing space for upcoming changes, including a decrease in grass dominancy, and an increase in forb dominancy, and an intensive shrub growth. Soil fungi appear to have a big impact on highly disturbed, annual-dominated communities, where they can decrease the dominance of annual forbs, resulting a more grass dominated assemblages. While in the less frequently disturbed site, perennial-dominated communities, and the soil community facilitates the dominance of forbs species.

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REFERENCES

[1] Bihari, Z., Szalai, S., Bozó, L. (2009): Natural Environment – Climate. In: Kocsis, K., Schweitzer, F., editors. Hungary in Maps. - HAS Geographical Research Institute, Budapest, Hungary 45–50.
[2] Bosy, J.L., Reader, R.J. (1995): Mechanisms Underlying the Suppression of Forb Seedling Emergence by Grass (*Poa pratensis*) Litter. - Functional Ecology 9: 635.
[3] Burt, J. W., Clary, J. J. (2014): Initial disturbance intensity affects recovery rates and successional divergence on abandoned ski slopes. - Journal of Applied Ecology 53: 607-615.
[4] Chambers, J. M. (1992): Linear models. Chapter 4 of Statistical Models in S eds J. M. Chambers and T. J. Hastie, Wadsworth & Brooks/Cole.
[5] Collins, B., Wein, G., Philippi T. (2001): Effects of disturbance intensity and frequency on early old-field succession. - Journal of Vegetation Science 12: 721-728.
[6] Crawley, M.J. (2014): Statistics: An Introduction Using R, 2nd Edition. - John Wiley and Sons, Chichester.
[7] Cutler, N. A., Chaput, D. L., van der Gast, C. J. (2014): Long-term changes in soil microbial communities during primary succession. - Soil Biology and Biochemistry 69: 359–370.
[8] Dostálek, T., Pánková, H., Münzbergová, Z., Rydlovál, J. (2013): The Effect of AMF Suppression on Plant Species Composition in a Nutrient-Poor Dry Grassland. - PLoS One 8: e80535.
[9] Dölle, M., Schmidt, W. (2009): The relationship between soil seed bank, above-ground vegetation and disturbance intensity on old-field successional permanent plots. - Applied Vegetation Science 12: 415–428.
[10] Drury, W.H., Nisbet, I.C.T. (1973): Succession. - Journal of the Arnold Arboretum 54: 331-368.
[11] Dunn, R.M., Mikola, J., Bol, R., Bardgett, R.D. (2006): Influence of microbial activity on plant–microbial competition for organic and inorganic nitrogen. - Plant and Soil 289: 321–334.

[12] Emery, S. M., Rudgers, J. A. (2012): Impact of competition and mycorrhizal fungi on growth of *Centaurea stoebe*, an Invasive Plant of Sand Dunes. - The American Midland Naturalist 167: 213-222.

[13] Fahey, R. T., Puettmann, K. J. (2007): Ground-layer disturbance and initial conditions influence gap partitioning of understorey vegetation. - Journal of Ecology 95: 1098–1109.

[14] Frossard, A., Gerull, L., Mutz, M., Gessner, M. (2013): Shifts in microbial community structure and function in stream sediments during experimentally simulated riparian succession. - FEMS Microbiology Ecology 84: 398-410.

[15] Gange, A.C., Brown, V.K., Sinclair, G.S. (1993): Vesicular-Arbuscular Mycorrhizal Fungi: A Determinant of Plant Community Structure in Early Succession. - Functional Ecology 7: 616–622.

[16] Gao, H., Gao, Y. B , He, X. D. (2014): Impacts of grazing and mowing on reproductive behaviors of *Stipa grandis* and *Stipa krylovii* in a semi-arid area. - Journal of Arid Land 6: 97-104.

[17] Goldberg, D.E., Werner, P.A. (1983): The Effects of Size of Opening in Vegetation and Litter Cover on Seedling Establishment of Goldenrods (*Solidago* spp.). - Oecologia 60: 149–155.

[18] Hobbie, S. E. (2015): Plant species effects on nutrient cycling: revisiting litter feedbacks. - Trends in Ecology and Evolution 30: 357–363.

[19] Hothorn, T., Bretz, F. & Westfall, P. (2008): Simultaneous Inference in General Parametric Models. – Biometrical Journal 50: 346–363.

[20] Jasper, D.A., Abbott, L.K., Robson, A.D. (1991): The effect of soil disturbance on vesicular-arbuscular mycorrbizal fungi in soils from different vegetation types. - New Phytologist 118: 471-476.

[21] Kahmen, S., Poschold, P. (2004): Plant functional trait responses to grassland succession over 25 year. - Journal of Vegetation Science 15: 21-32.

[22] Király, G (ed) (2009): Új magyar füvészkönyv. Magyarország hajtásos növényei. Határozókulcsok. (New Hungarian herbal. The vascular plants of Hungary. Identification key). - Aggteleki Nemzeti Park Igazgatóság. Jósvafő.

[23] König, S., Wubet, T., Dormann, C.F., Hempel, S., Renker, C., Buscot, F. (2010): TaqMan real-timePCR assays to assess arbuscular mycorrhizal responses to field manipulation ofgrassland biodiversity: effects of soil characteristics, plant species richness, andfunctional traits. - Applied and Environmental Microbiology 76: 3765–75.

[24] McCain, K. N. S., Wilson, G. W. T., Blair, J. M. (2011): Mycorrhizal suppression alters plant productivity and forb establishment in a grass-dominated prairie restoration. - Plant Ecology 212: 1675-1685.

[25] McKenzie, F. R., Jacobs, J. L., Kearney, G. (2006): Effects of spring grazing on dryland perennial ryegrass/white clover dairy pastures. 2. Botanical composition, tiller, and plant densities. Australian Journal of Agricultural Research 57: 555–563.

[26] Mariotte, P., Meugnier, C., Johnson, D., Thébault, A., Spiegelberger, T., Buttler, A. (2013): Arbuscular mycorrhizal fungi reduce the differences in competitiveness between dominant and subordinate plant species. - Mycorrhiza 23: 267–277.

[27] Maron, J.L., Klironomos, J., Waller, L. Callaway, R.M. (2014): Invasive plants escape from suppressive soil biota at regional scales. - Journal of Ecology 102: 19-27.

[28] Mills, K.E., Bever, J.D. (1998): Maintenance of Diversity within Plant Communities: Soil Pathogens as Agents of Negative Feedback. - Ecology 79: 1595–1601.

[29] Muller, C.H. (1966): The Role of Chemical Inhibition (Allelopathy) in Vegetational Composition. - Bulletin of the Torrey Botanical Club 93: 332-351.

[30] Perry, G.L.W., Millington, J.D.A. (2008): Spatial modelling of succession-disturbance dynamics in forest ecosystems: Concepts and examples. - Perspectives in Plant Ecology, Evolution and Systematics 9: 191–210.

[31] Porcova, L., Wikler J. (2014): he effect of the different vineyard management to composition of weed species. Conference MendelNet, 19-20th November 2014, Brno, Czech Republic. Book of articles p.: 90-94.

[32] Prach, K., Pyšek, P. (2001): Using spontaneous succession for restoration of human-disturbed habitats: Experience from Central Europe. - Ecological Engineering 17: 55–62.

[33] Prach, K., Řehounková, K., Lencová, K., Jírová, A., Konvalinková, P., Mudrák, O., Študent, V., Vaněček, Z., Tichý, L., Petřík, P., Šmilauer, P., Pyšek, P. (2014): Vegetation succession in restoration of disturbed sites in Central Europe: the direction of succession and species richness across 19 seres. - Applied Vegetation Science 17: 193–200.

[34] R DEVELOPMENT CORE TEAM. (2011): R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna.

[35] Řehounková, K., Pracha, K. (2001): Life-history traits and habitat preferences of colonizing plant species in long-term spontaneous succession in abandoned gravel–sand pits. - Basic and Applied Ecology 11: 45–53.

[36] Reynolds, H.L., Packer, A., Bever, J.D., Clay, K. (2003): Grassroots ecology: plant–microbe–soil interactions as drivers of plant community structure and dynamics. - Ecology 84: 2281–2291.

[37] Rillig, M.C., Mummey, D.L. (2006): Mycorrhizas and soil structure. - New Phytologist 171: 41–53.

[38] Rinkes, Z. L., DeForest, J. L., Grandy, A. S,, Moorhead, D. L., Weintraub, M. N. (2014): Interactions between leaf litter quality, particle size, and microbial community during the earliest stage of decay. - Biogeochemistry 117: 153-168.

[39] Rydlová, J., Sýkorová, Z., Slavíková, R., Turis, P. (2015): The importance of arbuscular mycorrhiza for Cyclamen purpurascens subsp. immaculatum endemic in Slovakia. _ Mycorrhiza 25: 599-609.

[40] Saito, T. I., Tsuyuzaki, S. (2012): Response of riparian vegetation to the removal of the invasive forb, Solidago gigantea, and its litter layer. - Weed Biology and Management 12: 63–70.

[41] Schnitzer, S.A., Klironomos, J.N., Hille Ris Lambers, J., Kinkel, L.L., Reich, P.B., Xiao, K., Rillig, M.C., Sikes, B.A., Callaway, R.M., Mangan, S.A., van Nes, E.H., Scheffer, M. (2010): Soil microbes drive the classic plant diversity–productivity pattern. - Ecology 92: 296–303.

[42] Sikes, B.A., Maherali, H., Klironomos, J.N. (2012): Arbuscular mycorrhizal fungal communities change among three stages of primary sand dune succession but do not alter plant growth. - Oikos 121: 1791–1800.

[43] Van der Heijden, M.G.A., Boller, T., Wiemken, A., Sanders, I.R. (1998): Different Arbuscular Mycorrhizal Fungal Species Are Potential Determinants of Plant Community Structure. - Ecology 79: 2082–2091.

[44] Van der Heijden, M.G.A., Bardgett, R.D., van Straalen, N.M. (2008): The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. - Ecology Letters 11: 296–310.

[45] Van der Heijden, M.G.A., Klironomos, J.N., Ursic, M., Moutoglis, P., Streitwolf-Engel, R., Boller, T., Wiemken, A., Sanders, I.R. (1998): Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. - Nature 396: 69–72.

[46] Van der Putten, W.H, Mortimer, S., Hedlund, K., Van Dijk, C., Brown, V.K., Lepš, J., Rodriguez-Barrueco, C., Roy, J., Diaz Len, T.A., Gormsen, D., Korthals, G.W., Lavorel, S., Santa Regina, I., Smilauer, P. (2000): Plant species diversity as a driver of early succession in abandoned fields: a multi-site approach. - Oecologia 124:91-99.

[47] Vaszari, Sz., Kukorelli, G. (2010): Gyomcönológiai vizsgálatok a Pannonhalmi borvidéken kettő-, három- és négyéves szőlőültetvényekben. Magyar Gyomkutatás és Technológia 11:3-14.

[48] Wang B., Liu G. B., Xue S., Zhu B. (2011): Changes in soil physico-chemical and microbiological properties during natural succession on abandoned farmland in the Loess Plateau. - Environmental Earth Sciences 62: 915-925.

[49] Waring, B. G., Averill, C., Hawkes, C. V., (2013): Differences in fungal and bacterial physiology alter soil carbon and nitrogen cycling: insights from meta-analysis and theoretical models. – Ecology Letters 16: 887–894.

[50] Zhang, M. Y., Huang, D., Wang, K., Zhang, Y. J., Wang, C. J., Wang, R. C., You, Y. L. (2012): Grassland gap effects on *Elymus dahuricus* Turcz. seedling emergence, survival and growth. - Journal of Arid Environments 77: 11–16.

APPENDIX

Appendix Table 1. *Species list and location data of the annual-dominated site. Dominancy of each species in the initial year (2008) is marked (+, 1, 2)*

Species name	**Location**	**GPS (°N,°E)**
	Annual-dominated; Máriagyűd	45.881500, 18.254238
Forb species		
Arenaria serpyllifolia	2	
Capsella bursa-pastoris	1	
Erigeron annuus	2	
Fumaria officinalis	+	
Galium aparine	1	
Geranium pusillum	1	
Holosteum umbellatum	+	
Lactuca serriola	1	
Lamium purpureum	2	
Myosotis arvensis	+	
Stellaria media	2	
Taraxacum officinale	1	
Veronica arvensis	1	
Veronica hederifolia	1	
Veronica polita	1	
Vicia angustifolia	1	
Vicia grandiflora	1	
Grass species		
Bromus sterilis	2	
Calamagrostis epigeios	1	
Cynodon dactylon	1	
Elymus repens	1	
Poa annua	1	
Poa pratensis	1	
Setaria viridis	1	

+	sporadic
1	frequent
2	dominant

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Appendix Table 2. *Species list and location data of the perennial-dominated site. Dominancy of each species is in the initial year (2008) marked (+, 1, 2)*

Species name	Location	GPS (°N,°E)
	Perennial-dominated; Máriakéménd	46.026577, 18.470698
Forb species		
Achillea collina	1	
Acinos arvensis	+	
Agrimonia eupatoria	+	
Ambrosia artemisiifolia	+	
Arrhenatherum elatius	+	
Artemisia vulgaris	+	
Astragalus glycyphyllos	+	
Carduus acanthoides	+	
Carlina vulgaris	+	
Centaurea banatica	+	
Centaurea micranthos	1	
Centaurea scabiosa agg.	1	
Cerastium fontanum	+	
Cerastium semidecandrum	+	
Chondrilla juncea	+	
Chrysanthemum leucanthemum	+	
Cichorium intybus	+	
Cirsium vulgare	+	
Coronilla varia	1	
Crataegus monogyna	1	
Daucus carota	1	
Dipsacus laciniatus	+	
Erigeron annuus	1	
Eryngium campestre	+	
Euphorbia cyparissias	+	
Euphorbia virgata	1	
Galium mollugo	2	
Galium verum	1	
Glechoma hederacea	+	
Hieracium pilosella agg.	+	
Hypericum perforatum	+	
Knautia arvensis	1	
Lathyrus tuberosus	+	
Linum austriacum	+	
Medicago lupulina	+	
Melilotus officinalis	+	
Mentha arvensis	+	
Mentha longifolia	+	
Nonea pulla	+	
Ononis spinosa	+	
Picris hieracioides	1	
Plantago lanceolata	+	
Ranunculus acris	+	
Ranunculus bulbosus	+	
Rubus caesius	+	
Salvia pratensis	+	

+	sporadic
1	frequent
2	dominant

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Taraxacum officinale	+
Thlaspi perfoliatum	+
Thymus glabrescens	+
Torilis arvensis	+
Tragopogon orientalis	+
Trifolium pratense	+
Valerianella locusta	+
Veronica chamaedrys ssp. vindobonensis	+
Vicia angustifolia	1
Grass species	
Alopecurus pratensis	+
Bromus hordaceus ssp. hordaceus	+
Calamagrostis epigeios	2
Cynodon dactylon	+
Dactylis glomerata s.str.	1
Elymus repens	+
Koeleria cristata s.str.	+
Poa angustifolia	2
Poa annua	+

PREDATION IMPACT OF CATTLE EGRET (*BUBULCUS IBIS*) ON MIGRATORY LOCUST (*LOCUSTA MIGRATORIA CAPITO*) AND RED LOCUST (*NOMADACRIS SEPTEMFASCIATA*) IN SOUTH AND SOUTHWEST REGIONS OF MADAGASCAR

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Abstract. Locust outbreaks in Madagascar cause economic damage, and biological control is an environment friendly option. This paper determined the predation values of *Bubulcus ibis* bird species as a measure that could contribute to an effective locust outbreak preventive control strategy. The study was conducted in the south and southwest regions of Madagascar that are heavily infested by red locust (*Nomadacris septemfasciata*) and the Migratory Locust (*Locusta migratoria capito*). Three types of experiments were done that included observed predation, potential predation and pecking frequency. The observed predation was 19 locusts per day in the outbreak period higher than recession period with a value of 3 locusts per day. The experimental predation was 54 locusts per day, and was higher than the observed predation in the outbreak period. The highest pecking frequencies were observed in Isoanala (mean = 20.34) followed by Bekily (mean = 8.34) and Tulear (mean = 5.75). The observations from Bekily and Tulear were significantly different (t (56) = 2.49, p = 0.000), because of vegetation type. In Tulear the vegetation was bushy and thick, which provided canopy for the locust unlike the savannah environment in Bekily. The use of birds in prevention of locust outbreak during recession was found to be interfered by other insects that were fed on by birds. Thus locusts can best be controlled by birds when their population increases in an outbreak. However, there is a need to assess the population of birds to determine if they were enough to predate on a locust outbreak.

Keywords: *pest management; gregarious and solitarious phase; pecking frequency; tropic agriculture; biological control*

Introduction

Locusts are a different kind of grasshopper that is specifically differentiated from other grasshoppers by their phenotypic plasticity, which causes the locusts to develop various phenotypic phases (Simpson and Sword, 2008). The various phases of locusts are responsible for determining the common population densities. There are two main locust densities that are characterized by low population known as the ‘solitarious’ phase and the swarm population called the ‘gregarious’ phase (Sánchez-Zapata et al., 2007). The solitarious form of locusts is found in isolation of one another, which is unlike the gregarious form that is attracted to each other to form swarms moving together. The gregarious locusts migrate in their large populations in search for food and the swarms contain immature and mature locusts. When locusts travel in such large numbers, they are known to cause damage to agriculture crop fields; making locusts the most destructive pest in tropical regions (Scanlan et al., 2001).

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There are locust species known to have a restricted geographical outbreak pattern, which has led to continuous locust outbreaks in specific areas (Magor et al., 2008). In Madagascar, the south and southwest regions have for decades had locust outbreaks that have devastated crop fields (Lecoq et al., 2011). The locust species dominant in this area are the red locust (*Nomadacris septemfasciata*) and the Migratory Locust (*Locusta migratoria capito*), both of which have geographically restricted outbreaks (Magor et al., 2008). Control of locust outbreaks in Madagascar has proven to be very difficult to control. Control methods of locust outbreaks in Madagascar have leaned towards use of insecticides, and use of organochlorine insecticide was preferred by farmers before it was banned. This kind of locust outbreak control is both expensive and leads to environmental pollution (Holt and Cooper, 2006; Gill and Garg, 2014).

The locust behavior in south and southwest regions of Madagascar follows the observed trends of locust plagues that breed successfully from solitarious to gregarious phase. To control locust plagues that have a geographical restriction, like in Madagascar, preventive control against pre-plague swarms is recommended (Steedman, 1999). There are different types of preventive control methods that have been used in different areas (Lecoq, 2001). Biological control of locust is deemed as a cheap and environmental friendly method, because it uses the pest's natural enemies as a population control mechanism. Many bird species feed on locusts, but their ability to keep the locust population in check so as to avoid economic damage is not clearly known (Kirk et al., 1996). In this study, we considered the *Bubulcus ibis* (Cattle Egret) bird species in the area as a natural enemy for locusts. This was because the *Bubulcus ibis*, which is dominant in south and southwest of Madagascar, feed on locusts; hence act as a biological preventive control method. However, questions existed on the locust feeding capability of the *Bubulcus ibis* to be considered as an effective biological preventive option. This paper aimed at determining the predation values from the *Bubulcus ibis* as a contribution towards effective locust outbreak prevention. Furthermore, the study determined the predation values during both solitarious and gregarious phases.

Materials and methods

Study area

South and southwest regions of Madagascar are constituted by mosaic vegetation units that include Savannas, steppes, forest galleries, wastelands, and bushy areas. This makes these regions of Madagascar to be the hub for locust outbreaks. These regions, which are adjacent to each other, are bordered by the Onilahy River to the north, Mandrare River to the south, and Horombe plateau in the east. Based on the annual rainfall amounts, the area is divided into three ecological units that support growth and development of locust swarms. These three ecological units are initial multiplication area, multiplication transitional area, and final multiplication area. The initial multiplication area has sub-humid to semi-arid climate, and over 6 million hectares of herbaceous plant cover that receives annual rainfall of 750-800 mm. The multiplication transitional area has a semi-arid climate and over 2 million hectares of diverse graminaceous spectrum that is favorable for breeding of locusts; the area receives an annual rainfall amount of 400-500 mm. The final multiplication area in the southern coastal area has an arid climate, and covers an area of two million hectares of grass vegetation, which has an annual rainfall amount of 350-400 mm.

Three sampling stations were selected on the three ecological units associated with locust swarm development. The three stations were Bekily,Tulear and Isoanala. Isoanala station located 23°50' south and 45°43' east, occupies an area of 3.75 km^2. The Isoanala station was an open environment that consisted of anthropic mosaic polyculture fields of various plant species such as *Acacia sp., Indigofera sp., Heteropogon contortus* (Poaceae), *Cynodon dactylon* (Poaceae) *Hyhaena Shatan* (Arecaceae) and *Eucalyptus sp*. This station had several water stations and was favorable area for locust to propagate.

The other stations of Tulear and Bekily are found between coordinates 23°21' to 24°05'14' south and 44°8'59' and 44°12'05' east, and covered a combined total of 1000 ha. These stations had locust exploration stations that have fertile soils that favor pasture growth and transhumance for farmers in surrounding areas. From the vegetation point of view, Tulear was green, however, the Bekily had a dry bush land dominated by *Heteropogon contortus* grass species. The Tulear station was classified as the multiplication area and Bekily was the gregarizing area.

Bird sampling

There are over 40 bird species in the study area of which 11 are known locust predators. Of these bird species, the *Bubulcus ibis* was selected because it was abundant in the study area and is a known biological pest control agent (Patankar et al., 2007). The *Bubulcus ibis* is resident to the study area and its population is spread over the entire area; this species is protected in Madagascar and has a rapid growth. The *Bubulcus ibis* is known to be a major predator of nymph and winged locusts.

Data collection and calculation

Studies of bird predation on locust were conducted from October 2012 to May 2013. Three types of experiments were done that included (i) experimental predation to estimate potential predation rate of birds on locusts; (ii) examination of captured bird stomach content; and (iii) determine the frequency of bird pecking per time unit. The results from these experiments were statistically measured for their significance by using t-test analysis.

Experimental predation (EP)

This experiment was performed at Antsakoaky station and involved capturing breeds that were placed in semi-natural cages. The cages were made of wire mesh installed directly on the ground, and were 2m x 1m x 1.5 in size. In each cage, individual birds of the two species were fed locusts of different developmental stages (nymph and winged); the locusts were fed in three different combinations of only nymph, only adult locusts, and a combination of hoppers and adults. The locusts fed to the bird were counted before being placed in the cages and the remainders were also counted to know the number of locusts not fed on. The birds in the cages were fed every morning and the remainders were removed in the evening of each day. This captive-feeding method was used to determine predation of an individual bird species on locusts of different development stages; calculated by using Equation 1.

$$\mathrm{EP} = \frac{\sum \text{Amount of locusts consumed per day}}{\text{Total days of experimentation}} \qquad \text{(Eq.1)}$$

Observed predation (OP)

Observed predation was done by examining stomach contents of hunted wild birds. It was based on determining the number of locust fed but also the development stage of the locust (whether nymph or adult). The stomach contents of birds were examined at different times of the day, and from different locations within the study area either in solitarious or gregarious periods. During stomach content examination, the presence of non-digestible components such as head, abdomen, femur, and wings were used to identify the captured locusts. This method also provided information on the period of day when the bird's predation of locust was highest. Equation (2) was used to calculate observed predation for individual bird per day.

$$\mathrm{OP} = \frac{\sum \text{Total amount of locusts in the digestive tracts}}{\text{Total amount of birds captured}} \qquad \text{(Eq.2)}$$

Pecking frequency

To determine the frequency of bird pecking on locust per time unit, observations were made with the aid of Canon binoculars (model: 10x42 L-IS-WP). The birds were observed in the hunting grounds to record individual bird attempts, but also record locust capture attempts for birds while in flight. This observation was used to check consistency between number of pecks and observed stomach contents of the studied bird species. In good viewing conditions, this method represented the real predation of *Bubulcus ibis*. Each bird was observed for a time unit of five (5) minutes and the observations were done during different times of the day. Equation (3) was used to calculate the total number of bird pecks per day.

$$\frac{\text{Number of Pecks in 5 min x Average High feeding Period in a Day}}{\text{5 min (period of counting number of perks)}} \qquad \text{(Eq.3)}$$

Results

Observed predation

Observed predation (OP) of *Bubulcus ibis* was done by examining the bird's stomach contents to determine the number of locusts that were preferred and eaten. The OP was performed on the birds during two different periods of outbreak and recession of the two studied locust populations. In this paper, locusts refers to red locust (*Nomadacris septemfasciata*) and the Migratory Locust (*Locusta migratoria capito*); grasshoppers refers to locusts of other types and other forms of grasshoppers, while other insects refers to non-grasshopper/locusts. In each period, data were collected in the morning and afternoon in order to compare the time of day when the birds preferred to feed the most. In the locust outbreak period, the results showed that the 17 birds studied ate a total of 512 insects; 64 percent were the two studied locusts, 27 percent were other grasshopper species and 8 percent were other types of insects (*Table 1*). These results showed that during the locust outbreak period the birds ate a high proportion of these locusts than any other insect, because they were most in number and so easy to catch.

The calculated OP for the eaten insects in total was 30 insects per bird in a day, while in the morning and afternoon the OP's were 31 and 29 respectively (*Table 1*). The t-test statistic showed that there was no significant difference (t (10) = 0.483, p = 0.639) in the total number of insects eaten in the morning and afternoon, which meant that the birds had a constant eating pattern. However, the OP's for the birds on locusts was found to be significantly different (t (14) = 2.71, p = 0.017) between morning (OP = 23) and afternoon (OP = 14) feeding times (*Table 1*). This observation meant that the birds fed on the locusts mostly in the morning than they did in the afternoon. This locust eating pattern was because on the day of bird hunting there was rainfall in the afternoon, which prevented the bird's flight ability hence low OP. The OP's for grasshoppers was higher in the afternoon (12) than in the morning (6), although there was no significant difference. This was a response to the lower OP for locusts in the afternoon, which led to the birds to increase their feeding on other grasshoppers.

Table 1. *Observed predation of Bubulcus ibis during outbreak period*

Hunting time	**Bubulcus killed**	**Insects in stomach**				**Percentage (%)of stomach content**		
		Total insects	*Locusts*	*Grasshoppers*	*Others*	*Locusts*	*Grasshoppers*	*Others*
MORNING	OB1	31	29	2	0	93.98	6.45	0
	OB2	36	22	14	0	61.12	38.88	0
	OB3	24	23	0	1	95.83	0	4.17
	OB4	43	38	3	2	88.38	6.97	4.46
	OB5	35	33	0	2	94.28	0	5.72
	OB6	33	24	8	1	72.73	24.24	3.03
	OB7	31	28	3	0	90.33	9.67	0
	OB8	29	8	14	7	27.59	48.27	24.14
	OB9	18	10	3	5	55.55	16.67	27.78
	OB10	30	18	9	3	60	30	10
AFTERNOON	OB11	41	8	33	0	19.51	80.49	0
	OB12	13	6	3	4	46.16	23.07	30.77
	OB13	35	17	13	5	48.58	37.14	14.28
	OB14	37	19	16	2	51.36	43.24	5.4
	OB15	33	18	6	9	54.55	18.18	27.27

	OB16	23	13	8	2	56.53	34.78	8.69
	OB17	20	15	5	0	75	15	0
Total		512	329	140	43	64	27	8
Calculation	**Parameter**		**Insects**		**Locusts**		**Grasshoppers**	
Observed Predation (OP)	Morning		31		23		6	
	Afternoon		29		14		12	
	Day		30		19		8	
t-test (*morning vs afternoon*)	t-stat		0.483		2.71		-1.51	
	Degrees of freedom		10		14		8	
	P values (two-tail)		0.639		0.017		0.171	

In the locust recession period, the results showed that the 19 birds captured ate a total of 619 insects; 86 percent of the bird's stomach contents were grasshoppers, 9 percent were the studied locusts and 5 percent were other types of insects (*Table 2*). These results showed that during the recession period the birds ate a high proportion of other grasshopper species than the *Nomadacris septemfasciata* and *Locusta migratoria capito*. Similar to the outbreak period, the birds also did not eat significantly different (t (11) = 1.55, p = 0.148) between the morning and afternoon parts of the day (*Table 2*). A striking difference however existed between the OP's for the studied locusts and other grasshopper species, because there was a reduced population of the studied locusts it gave rise to other insects to dominate and increase their populations. This would be linked to the reduced competition that these other grasshopper species enjoyed in the absence of the studied locusts, which was not the case during the outbreak period. A comparison between OP's of the total insects between outbreak and recession periods showed no significant difference (t (23) = -0.425, p = 0.675) existed, supporting the observation that the birds fed the same amount in both periods.

Table 2. *Observed predation of Bubulcus ibis during recession period*

Hunting time	**Bird ID**	**Insects in Stomach**				**Percentage (%) of stomach content**		
		Total Insects	*Locusts*	*Grasshoppers*	*Others*	*Locusts*	*Grasshoppers*	*Others*
MORNING	RB1	58	11	47	0	18.97	81.03	0
	RB2	73	0	70	3	0	95.89	4.11
	RB3	36	7	29	0	19.45	80.55	0
	RB4	27	0	27	0	0	100	0
	RB5	73	0	71	2	0	97.26	2.74
	RB6	27	0	27	0	0	100	0
	RB7	11	0	9	2	3.7	85.18	11.12
AFTERNOON	RB8	14	3	11	0	21.43	78.57	0
	RB9	3	3	0	0	100	0	0
	RB10	4	3	1	0	75	25	0
	RB11	12	1	11	0	8.34	91.66	0

	RB12	4	1	3	0	25	75	0
	RB13	36	7	18	11	19.43	50	30.5
	RB14	55	3	45	4	5.45	82.27	7.27
	RB15	8	8	0	0	100	0	0
	RB16	28	3	25	0	10.72	89.28	0
	RB17	49	2	46	1	4.8	93.88	2.04
	RB18	64	0	57	7	0	89.19	10.81
	RB19	37	1	36	0	1.56	98.44	0
		619	**53**	**534**	**30**	**9**	**86**	**5**

Calculation	**Parameter**	**Insects**	**Locusts**	**Grasshoppers**
Observed Predation (OP)	Morning	44	3	40
	Afternoon	26	3	21
	Day	33	3	28
t-test (*morning vs afternoon*)	t-stat	1.55	-0.15	1.77
	Degrees of freedom	11	8	11
	P values (two-tail)	0.148	0.883	0.104

Experimental predation

The experimental predation (EP) was done to determine the feeding potential of the *Bubulcus ibis* on the locusts. This experiment was important as it took out any natural limiting factors that existed in the natural environment, in order to estimate how much the bird could predate on the locusts. During the experiment duration of 36 days, a total of 1282 larval and 1025 fledglings were fed to the caged birds. The results showed that 82 percent of the larval and 85 percent of the fledglings were fed on during this duration (*Table 3*). The EP for the nymph was calculated to be 39, while for the fledglings it was 38. The total EP was calculated to be even higher at 54 locusts, and most importantly was higher than the OP calculations. This was not surprising because the birds in the cages had no limiting environmental factors in the cage that could limit the bird's ability to hunt locusts. The environmental factors may include the bushy terrain, weather conditions such as precipitation limiting flight and competition just to mention a few. The EP results also showed that there was no preference for nymph or fledglings in the cage and that the bird's fed on the different combinations equally (*Table 3*).

Pecking frequency

The pecking frequency (PF) involved observing the birds in their natural environments, with the aid of binoculars, to determine the number of times a bird pecked on the studied locusts in a five minute interval. This observation was used to check consistency between number of pecks and observed stomach contents. Additionally, the observations were made in three different locations of Tulear, Isoanala and Berkily, which had different vegetation types (*Table 4*). The importance of observing birds at different locations was to assess the influence of vegetation type on locust predation by the *Bubulcus ibis*. The observations were also made in the outbreak and recession periods to further have quality comparisons. The highest PF were observed in Isoanala (mean = 20.34) followed by Bekily (mean = 8.34) and Tulear (mean = 5.75) in that order (*Figure 1; Table 4*). Isoanala observations were made during the fledgling's outbreak periods of *Locusta* and *Nomadacris* in the savannah fields of Madagascar. Apart from the increased population of the locusts that would make it easy for the *Bubulcus ibis* to feed, the savannah vegetation also plays an important role. The savannah vegetation is mostly grass with sparse tree canopies that do not provide an efficient ground cover to protect the locust or limit the birds hunting ability.

Table 3. *Potential predation of Bubulcus ibis*

Day	**Amount of locusts caged**		**Amount of locusts eaten**		**Percent eaten**	
	Larval	*Fledglings*	*Larval*	*Fledglings*	*Larval*	*Fledglings*
1	40		18		45	
2	40		23		58	
3	40		19		48	
4	40		26		65	
5	50		35		70	
6	50		38		76	
7	40	10	30	6	75	60
8	40	20	28	17	70	85
9	30	30	30	24	100	80
10		40		40		100
11		45		42		93
12	30	40	30	36	100	90
13	60		29		48	
14	50	10	30	8	60	80
15	50	20	50	10	100	50
16	50		38		76	
17	40		6		15	
18	48		46		96	
19	20	30	30	20	150	67
20	20	40	20	40	100	100
21	46	50	46	50	100	100
22		74		74		100
23		20		20		100
24		28		28		100
25	70	100	70	94	100	94
26	67	114	67	104	100	91
27		56		46		82
28		32				
29		51		51		100
30	60		60		100	
31	59		59		100	
32	30	36	18	36	60	100
33	12	49	11	49	92	100

34	100	4	100	4	100	100
35	100	30	100	10	100	33
36		96		60		63
Total	**1282**	**1025**	**1057**	**869**	**82**	**85**
Expected predation	Larval		39			
	Fledglings		38			
	Total		54			
t-test (Larval vs Fledglings)	t-stat		0.189			
	Degrees of freedom		45			
	P values (two-tail)		0.851			

The observations from Bekily and Tulear were significantly different (t (56) = 2.49, p = 0.000), which meant the birds were feeding differently in the two locations. In Tulear the vegetation was bushy and thick, which provided canopy for the locust and made it difficult for the birds to effectively hunt. Contrary to the bushy environment in Tulear, the savannah environment in Bekily could not provide this vegetative cover and the bird's predation on locusts was higher even though it was during recession period. From these results, it can be concluded that using birds to predate in environments that provide good vegetative cover for locusts would not be effective to reduce the locust populations.

Table 4. *Summary of pecking frequency calculations based on sampling location*

Location	**Average pecking frequency per 5 min**	**Average pecking frequency per day**	**Type of locust population**	**Vegetation Type**
Tulear	5.75	249.55	Outbreak nymph of Locusta with fledglings	Bushy wasteland
Isoanala	20.34	882.47	Fledglings outbreak of Locusta and Nomadacris	Savannah with field of polyculture
Bekily	8.34	357.33	Locusta and Nomadacris in recession period and outbreak of secondary species	Tree savannah with grass meadow
Calculation	**Parameter**		**Values**	
t-test (Tulear vs Isoanala)	t-stat		-11.9	
	Degrees of freedom		34	
	P values (two-tail)		0.000	
t-test (Tulear vs Bekily)	t-stat		4.29	
	Degrees of freedom		56	
	P values (two-tail)		0.000	
t-test (Isoanala vs Bekily)	t-stat		9.67	
	Degrees of freedom		37	
	P values (two-tail)		0.000	

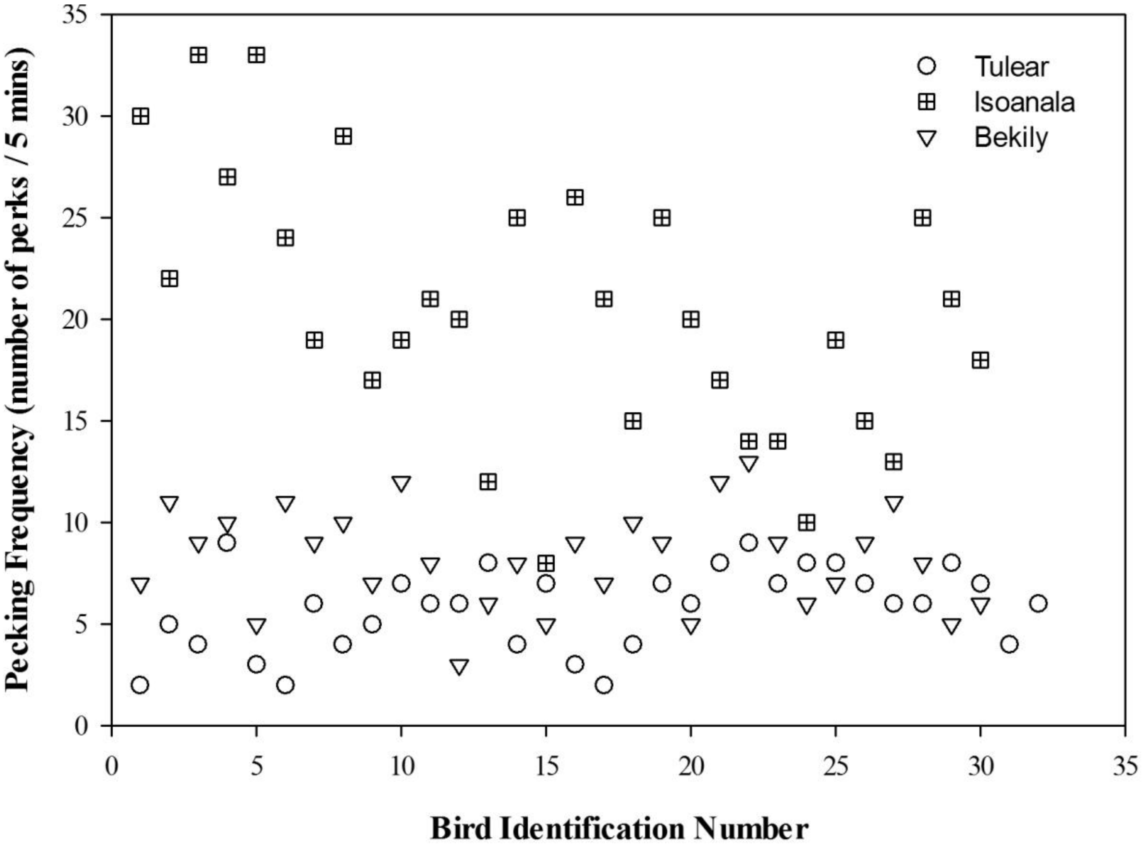

Figure 1. *Bubulcus ibis pecking frequency values in three locations of Tulear, Isoanala and Bekily.*

Discussion

Locust outbreaks in Madagascar, just like in many parts of the world need to be reduced to prevent the economic damage they cause through crop destruction. However, control of locust population in a cheap and environmental friendly manner is a challenge for agriculture pest management. Integrated pest management (IPM) is a known environmentally friendly method for pest management, which centers on the pests life cycle and their interaction with both living and non-living organisms in an ecosystem (Ahuja et al., 2015). The IPM approach is not a single method for control of pests, but rather a combination of environmentally friendly methods that together can be used effectively as pest management strategy. For instance studies on the use of entomopathogenic fungus known as *Metarhizium acridum* as a biological control has found it as environmentally good as it is highly selective with no side effects; however it was found to be slow acting and unreliable in its use to control locust outbreaks (Lomer and Langewald, 2001; Jin et al., 2008; Fang et al., 2014). In this study, the use of birds to predate on locusts has been evaluated as a locust mechanical control method that could be supplemented to already tried and effective strategies like the use of *M. acridum*. Three indicators (OP, EP and PF) were observed to determine the effectiveness of the *Bubulcus ibis* bird species in prevention of locust outbreaks. The results have shown that the birds do not feed on the locusts to their maximum ability in the natural conditions, because of limiting factors. In this study, rainfall and vegetative cover were the limiting factors that can also be expected in other similar locations. For birds to feed on the locusts, they target the insects that can easily be caught without spending much energy. However, in the presence of cover for the locusts the birds prefer to capture other types of insects. The type of vegetation plays a role in attracting the birds to feed on insects that feed on it (Jones and Sieving, 2006). Jones et al., (2005) in their study conducted on north-central Florida farmlands insectivorous bird activity was higher in polyculture crops than in monocultures. Thus it was normal to observe in our study that birds caught in savannah and partly dry forest had a high number of locusts in their stomachs. In some cases, such as where rainfall is a limiting factor, birds do not fly at all hence reducing their ability to predate on the locusts. The use of birds in prevention of locusts was found to be interfered by other insects available in the area. The presence of other insects meant that birds would not reduce a locust outbreak, because the birds predate on insects that can be easily caught. During the solitarious' phase the locusts population is lower than other insects and the numbers cannot be compared to an outbreak period. Most avian species have opportunistic traits in their feeding habits, switching food types to take advantage of readily available food sources. For instance, during an insect outbreak in a Polish forest, predation of passerine nests declined, as predators switched to feeding on caterpillars (Tomialojc and Wesolowski, 1990). Thus locust populations can be reduced by birds when their population increases in an outbreak or if there are no other insects for birds to feed on. Based on the perking frequency results, a large bird population would be needed to predate on millions of locusts that make a swarm. This study has shown that birds can effectively reduce locust population in an outbreak when they are easy to predate on. However, using the IPM concept of combining several methods, the use of environmentally friendly chemicals like *Metarhizium acridum* can be used because it makes hoppers more easily available to birds (Lomer and Langewald, 2001). Despite the discovery of this study, there is a need to assess the population of birds in locust infested areas to determine if the bird population is enough to predate on a locust outbreak and make an effective contribution to an integrated locust control approach.

REFERENCES

[1] Ahuja, D. B., Ahuja, U. R., Singh, S. K., Singh, N. (2015): Comparison of Integrated Pest Management approaches and conventional (non-IPM) practices in late-winter-season cauliflower in Northern India. - Crop Protection 78: 232-238.

[2] Fang, W., Lu, H., King, G.F., St. Leger, R.J. (2014): Construction of a Hypervirulent and Specific Mycoinsecticide for Locust Control. - Scientific Reports 4, article number: 7345.

[3] Gill, H. K., Garg, H. (2014): Pesticide: Environmental Impacts and Management Strategies, pp. 187-230. - In: Solenski, S., Larramenday, M. L. (eds.) Pesticides - Toxic Effects. Intech. Rijeka, Croatia. (http://www.intechopen.com/books/pesticides-toxic-aspects/pesticides-environmental-impacts-and-management-strategies).

[4] Holt, J., Cooper, J.F. (2006): A model to compare the suitability of locust hopper targets for control by insecticide barriers. - Ecological Modelling 195: 273–280.

[5] Jin, S.F., Feng, M.G., Chen, M.Q. (2008): Selection of global Metarhizium isolates for the control of the rice pest Nilaparvata lugens (Homoptera: Delphacidae). - Pest management science 64(10): 1008-14.

[6] Jones, G.A., Sieving, K.E. (2006): Intercropping sunflower in organic vegetables to augment bird predators of arthropods. - Agriculture, Ecosystems and Environment 117:171–177.

[7] Jones, G. A., Sieving, K. E., Jacobson, S. K. (2005): Avian Diversity and Functional Insectivory on North-Central Florida Farmlands. - Conservation biology 19(4): 1234-1245.

[8] Kirk, D.A., Evenden, M.D., Mineau, P. (1996): Past and Current Attempts to Evaluate the Role of Birds as Predators of Insect Pests in Temperate Agriculture. - Current Ornithology 13: 175-269.

[9] Lecoq, M. (2001): Recent progress in Desert and Migratory Locust management in Africa. Are preventative actions possible? - Journal of Orthoptera Research 10(2): 277-291.

[10] Lecoq, M., Andriamaroahina, T. R., Solofonaina, H., Gay, P. E. (2011): Ecology and population dynamics of solitary Red Locusts in southern Madagascar. - Journal of Orthoptera Research 20(2): 141-158.

[11] Lomer, C., Langewald, J. (2001): What is the place of biological control in acridid integrated pest management? - Journal of Orthoptera Research 10(2): 335-341.

[12] Magor, J.I., Lecoq, M., Hunter, D.M. (2008): Preventive control and Desert Locust plagues. - Crop Protection 27: 1527–1533.

[13] Sánchez-Zapata, J.A., Donázar, J. A., Delgado, A., Forero, M.G., Ceballos, O., Hiraldo, F. (2007): Desert locust outbreaks in the Sahel: resource competition, predation and ecological effects of pest control. - Journal of Applied Ecology 44: 323–329.

[14] Scanlan, J. C., Grant, W. E., Hunter, D. M., Milner, R. J. (2001): Habitat and environmental factors influencing the control of migratory locusts (*Locusta migratoria*) with an entomopathogenic fungus (*Metarhizium anisopliae*). - Ecological Modelling 136: 223–236.

[15] Simpson, S. J., Sword, G. A. (2008): Locusts. - Current Biology 18(9): R364-R366.

[16] Steedman, A. (1999): The Locust Handbook (third ed.) - Natural Resources Institute, Chatham, UK.

[17] Tomialojc, L., Wesolowski, T. (1990): Bird Communities of the Primaeval Temperate Forest of Bialowieza, Poland – In: Keast, A. (Eds.) Biogeography and Ecology of Forest Bird Communities. The Hague, Netherlands:SPB Academic Publishing.

[18] Patankar, P., Desai, I., Shinde, K., Suresh, B. (2007): Ecology and breeding biology of the Cattle Egret *Bubulcus ibis* in an industrial area at Vadodra, Gujarat. - Zoos' Print Journal 22(11): 2885-2888.

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EFFECT OF DIFFERENT N NUTRIENT CONTENTS ON BIOMASS OF GREEN MANURE AS SECOND CROP, UNDER UNFAVORABLE CLIMATE CONDITIONS IN HUNGARY

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Abstract. The growth and nutrient composition of three plant species produced as second crop for green manure purposes (phacelia – *Phacelia tanacetifolia* Benth., mustard – *Sinapis alba* L. and oilseed radish – *Raphanus sativus* L. *var. oleiformis* Pers.) were studied between 2007 and 2011 at the Crop Production and Biomass Utilisation Demonstration Centre (Növénytermesztési és Biomassza-hasznosítási Bemutató Központ) of Szent István University under unfavourable site conditions on Luvic Kastanozems soil (WRB 2014), subject to two different levels of fertiliser supply (0 kg/ha N; 50 kg/ha N).

The application of 50 kg/ha nitrogen active ingredient multiplied the biomass of each of the three plant species – as an average of the five years of the study to 2.86, 3.07 times and 2.51 times the amount produced without treatment, in the case of phacelia, mustard and oilseed radish, respectively. The rates of the increase of the dry matter content were somewhat lower than those of the green mass increase because the nitrogen improved supply also resulted in an increase in the water content of the green manure plants. One kilogram per hectare added nitrogen increased the green mass and the dry matter content by 412.5 kg and by 37.9 kg as an average of the five years of the study in the case of phacelia, while in the case of mustard the green mass and the dry matter content increased by 431.4 kg and 59.1 kg, respectively. In the case of oilseed radish the corresponding figures were 386.7 kg and 34.1 kg, respectively. Without added nitrogen however, the generally acceptable 10 t/ha biomass could not always be harvested from the given site of relatively unfavourable conditions.

The amount of NPK absorbed per hectare also increased in response to the added nitrogen – to 2.60/2.36/2.13 times the in the case of phacelia over 2007-2011, 2.81/2.29/2.29 times in the case of mustard and 2.72/2.09/1.95 times in the case of oilseed radish. Each kilogram of added nitrogen enabled the uptake of an added amount of 1.6 kg, 2.3 kg and 1.8 kg of additional nitrogen in the case of phacelia, mustard and oilseed radish, respectively, as an average of the period between 2007 and 2011. The nitrogen applied made a significant contribution to the uptake of phosphorous and potassium as well. Each kilogram of added nitrogen increased the uptake of P_2O_5 by 0.5 kg. 0.5 kg and 0.6 kg in the case of phacelia, mustard and oilseed radish, respectively and it boosted the uptake of K_2O by 1.8 kg, 2.1 kg and 1.4 kg in the case of phacelia, mustard and oilseed radish, respectively.

It may be concluded from the above results that when a second crop is grown for use as green manure at the given cropping site the application of nitrogen is always recommended, to the extent possible, but if the straw is left on the soil surface after cereals, it is definitely strongly recommended.

Keywords: *application of green manure, green manure crops, phacelia, mustard, oilseed radish, N nutrient content*

Introduction

Green manure plants are fresh green, non-woody plants, rich in water, sugar, starches, protein and nitrogen, worked into the soil (Kahnt, 1981). In analysing the effects of green manure plants on the next crop Kahnt (1981) identified a total of 14 factors of relevance. Owing to the large number of factors the effectiveness of the use of

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green manure can only be assessed on the basis of precise information of the given site's parameters.

Mustard (*Sinapis alba* L.) and oilseed radish (*Raphanus sativus* L. *var. oleiformis* Pers.) (both belonging to the *Brassicaceae* family) are particularly suitable for these purposes. They play a major role in soil protection (Brown and Morra, 1995; Grossman, 1993; Boydston and Hang, 1995) and in controlling soil-borne pathogens (Papavizas, 1966; Papavizas and Lewis, 1971; Ramirez and Villapudua Munnecke, 1988; Muelchen et al., 1990; Mayton et al., 1996; Croft et al., 1993; Deng et al., 1993), including nematodes (Mojtahedi et al., 1991, 1993), and soil-borne insects that are harmful to crops (Brown et al., 1991).

The soil disinfecting effects are caused by root excretions and, after their incorporation in the soil, the compounds released during their decomposition, even in small concentrations (Bialy et al., 1990; Lazzeri et al., 1993; Williams et al., 1993). Consequently, they inhibit the germination of weed seeds, along with the initial growth of the seedlings (Gardner et al., 1990; Bradow, 1991; Vaughn and Boydston, 1997).

Such positive effects have been observed in relation to phacelia (*Phacelia tanacetifolia* Benth.) as well (Dhima et al., 2009).

Phacelia and mustard are even suitable for the remediation of soils contaminated with heavy metals (Kim et al., 2010; Foucault et al., 2013).

Green manure plants absorb nitrogen from the soil, preventing its leaching out of the soil (Martinez and Guiraud, 1990; Jackson et al., 1993). The C:N ratio in the soil after the incorporation of the green manure plant depends on when it is worked into the soil, the type, moisture content and temperature of the soil (Cadisch and Giller, 1997). These are some of the parameters on which it depends whether the impact on the next crop is positive (Derpsch et al., 1986; Catt et al., 1992; Thorup-Kristensen, 1993; Kara and Penezoglu, 2000; Zhang and Fang, 2007; Collins, 2007), neutral (Allison et al., 1998a, 1998b; Richards et al., 1996), or negative (Allison and Armstrong, 1992; Clark et al., 1997a, 1997b; Vaughan and Evanylo, 1998).

The most important characteristics of a good green manure plant include rapid growth, a large amount of biomass and inexpensive sowing seeds. The aim of our research was to examine the performance of three green manure plants possessing each of the above characteristics, under the given unfavourable cropping site conditions. We sought for answers also to whether successful application of green manure is possible even without added nitrogen, in view of the expected pentosan effect or whether the application of nitrogen is an indispensable prerequisite. The amount by which a unit of added nitrogen increased the amount of the green mass and the dry matter was also examined, along with the increase in the per-hectare NPK uptake.

Material and methods

The trials were set up in the town of Gödöllő, in the Crop Production and Biomass Utilisation Demonstration Centre of Szent István University (47° 34' 43'' N; 19° 22' 39'' E; 229 m above sea level). The experiment site is in a low hilly area, on a gentle north-western slope. The area is rather heterogeneous, comprising spots of varying degrees of erosion and sedimentation.

The annual mean temperature is 9.4 °C, the annual average amount of precipitation is 590 mm. The average rainfall during the months of August to October that is the most important period for the second crop in a given year is 150 mm.

Crop-years 2007 and 2008 were of average pattern from the aspect of the second crop in those years, with 169.4 mm and 130.8 mm rainfall between August and October, respectively. 2009 and 2011 however, were dry years, with merely 32.0 mm and 30.0 mm rainfall during the growing season of the green manure plants grown as second crop. 2010 on the other hand, was a rather wet year with 198.6 mm rain during the months of August to October.

The site is located in the micro-region called Gödöllő Hilly Region. The hilly region is characterised by eroded carbonate soils that are typical of hilly regions, elevated to varying extents and broken up in a checker pattern. In the area around the town of Gödöllő upper Pannonian freshwater limestone and chalky clay (marl) are to be found on the surface at various spots among the loess, sand and slope clay settled on the upper Pannonian sandy clay and river sediment. The area elevated during the Pleistocene and a heavily fragmented hilly region with rather steep slopes developed where soil erosion and deflation have resulted in the redeposition of large amounts of materials. The soil in the experiment site is a rust-brown forest soil developed, for the most part, on sand (luvic calcic phaeozem) according to the Hungarian genetic soil classification system. The rust-brown forest soil subtype that has evolved on Tertiary sand and marl belongs to the Ramann's brown forest soil type. As a result of the degradation processes a slightly humic variant of a fertile layer of a medium depth has developed (Stefanovits, 1999a, 1999b; Füleky, 1999; Máté, 2005). The area is exposed to erosion and the soil is sensitive to compaction.

The key soil parameters of the experimental plot are set out in *Tables 1-2*. According to the standards laid down in the MÉM-NAK (the Crop Protection and Agricultural Chemistry Centre of the Ministry of Agriculture and Food) (MÉM, 1979) the soil contains a low level of N, a very high level of P_2O_5 and a good supply of K_2O.

Table 1. *The soil profile of the experimental site*

Ap layer (0-25 cm)	Brown (10YR 3/3), fresh, loose, slightly structured soil comprising predominantly small crumbs, densely interwoven with root residues, with a high proportion of sand but no chalk. Rich in earthworm channels. The transition into the layer below is sharp, a straight line in the soil profile.
A_2 layer (25-40 cm)	Brown (10YR 3/3), humid, slightly compacted, moderately structured, comprising small crumbs, interwoven with root residues, with some earthworm channels, containing no chalk. The transition into the accumulation (subsoil) layer is gradual, showing a wavy line.
B layer (40-60 cm)	Red-brown (2.5YR 3/6), humid, compacted, of a granular structure, less densely interwoven with roots, loam. The transition into the layer below is gradual, showing a wavy line.
BC layer (60-70 cm)	Mixed colour (10YR 3/3 and 10YR 7/4), fresh, slightly compacted, a clay of no particular structure. The transition into the layer below is gradual, showing a baggy pattern.
C layer (70-100 cm)	Light yellowish brown (10YR 7/4), dry, tightly packed, with no structure, a silted clay.

Table 2. *Important pedological data of experiment*

Genetic soil level	pH (H_2O)	Arany-type plasticity index	humus (%)	$CaCO_3$ (%)	total salt (%)	total N mg kg^{-1}	AL-P_2O_5 mg kg^{-1}	AL-K_2O mg kg^{-1}
A (0-40 cm)	6.76	30	1.32	0.00	0.044	16.8	371.1	184.0
B (40-60 cm)	7.08	40	1.04	0.00	0.052	11.9	33.0	112.0
BC (60-70 cm)	7.66	61	0.88	0.00	0.060	2.0	123.0	127.1
C (70-100 cm)	8.10	60	0.54	5.57	0.075	16.8	107.5	110.8

The green manure experiments with the second crops were conducted in 2007-2011. The seeds of the plants grown as second crops in the experiments were sown after harvesting the previously grown winter wheat, where harvest was immediately followed by stubble stripping. In each year the second crop was sown right after stubble treatment, on 15 August split-plot experimental design. One plot size was 10 x 100 meters. Three plant species (phacelia – *Phacelia tanacetifolia* Benth., mustard – *Sinapis alba* L, oilseed radish – *Raphanus sativus* L. *var. oleiformis* Pers.) were grown and two different doses of nutrients (0 kg/ha N; 50 kg/ha N) were applied in three iterations. Ammonium-nitrate was used as fertiliser, incorporated in the soil as part of stubble treatment. The seeds of the green manure plants were drilled in accordance with the sowing seed norms found in technical literature (Antal, 2000) (*Table 3*).

Table 3. *The seeds requirement of green manure plants*

plants	germinal number ha^{-1}	seeds requirement (kg ha^{-1})
phacelia	5.000.000	10
mustard	2.000.000	15
oilseed radish	2.500.000	25

The biomass so produced was measured and samples for the analysis of chemical composition of the biomass were taken in early November before freezing of the vegetation.

The NPK content was established from 1 g finely ground absolute dry sample with sulphuric acid digestion and 30 % hydrogen-peroxide thermal decomposition. After decomposition the N, P and K content was established from samples diluted up to 100 cm^3. The nitrogen content was measured using a Parnass-Wagner water steam distiller apparatus.

The phosphorous content was measured using the vanadate-molybdate technique. The extinction of the yellow-colour solution was measured with a spectrophotometer (Spekol 221).

The potassium content was measured in the solutions used for the establishment of the phosphorous content as well, with a series of dilutions with a flame photometer (Jenway PFP 7).

The statistical evaluation was based on ANOVA test, one- and a two-factor variance analysis and post hoc tests.

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Results and discussion

The biomass of the green manure plants was heavily affected by the amount of precipitation (*Table 4*). The crop-year effect was particularly pronounced in the case of the plots where no fertiliser had been applied. Phacelia without fertiliser produced only 4.8 t/ha green mass in 2009, in contrast to the 20.3 t/ha produced in 2011. Phacelia did not produce the minimum 10 t/ha green mass (Késmárki and Petróczki, 2003) without added nitrogen in 2008 and 2009. Nitrogen top dressing increased the green mass to 1.91-3.95 times – 2.85 times, as an average of five years – the amount produced without added nitrogen. Phacelia was hard hit by the drought of 2009, yet the application of nitrogen improved the plants' stress tolerance substantially, along with the efficiency of its utilisation of what little water there was, resulting ultimately in a 16.7 t/h green mass. The shortage of air in the soil, caused by the heavy rains in 2010 had a negative impact on the growth of the plants as well. This is considered to be the reason for the larger green mass produced in 2011. Similar results were also observed by Blazewick-Wozniak and Wach (2012).

In the case of mustard, the green mass produced without added nitrogen was below 10 t/ha only in 2008 and 2010 (7.3 t/ha and 7.7 t/ha, respectively). The application of 50 kg/ha nitrogen active ingredient boosted the amount of biomass produced to 3.07 times the amount produced without added nitrogen (that is, to 33.1 t/ha), as an average of the 5 years of the experiment. The poor yield recorded in 2008 was caused by the drought, while in 2010 it was a result of the pentosan effect caused by the excessive rains.

Oilseed radish produced 9.8-22.5 t/ha biomass without and 27.6-43.4 t/ha with added fertiliser. Added nitrogen boosted the green mass produce to 1.93-3.33 times the amount produced without the application of fertiliser.

As an average of the five years of the experiment each of the three green manure plants produced similar amounts of biomass. Studies conducted by Stivers-Young (1998) however, showed that mustard and oilseed radish are capable of producing larger amounts of biomass than phacelia.

In terms of dry mass produced per hectare of land similar trends were observed to those found in regard to green mass, however, the increase in dry mass after the application of nitrogen fell short of the increase in green mass by 75.6 % in the case of phacelia, 52.2 % in the case of mustard and 54.3 % in the case of oilseed radish, as an average of five years (*Table 5*). The reason for this was that the application of nitrogen resulted in an increase in the plants' water content as well. Phacelia, though the plants were not as tall as the mustard plants, produced as much or even more dry mass than the latter. Similar results were observed by Asagi and Ueno (2009) as well.

The nitrogen content per hectare increased after the application of nitrogen fertiliser to 2.6 times the amount found without added nitrogen in phacelia as an average of five years, from 57.3 kg/ha to 136/1 kg/ha. The 50 kg/ha nitrogen fertiliser enabled the uptake of an additional 28.8 kg/ha of nitrogen (*Table 6*). Although Asagi and Ueno (2009) found that phacelia was capable of fixing as much nitrogen as mustard, our results showed that this is significantly affected by the so-called crop-year effect. Each of phacelia, mustard and oilseed radish was, however, capable of fixing N even at low temperatures (Stivers-Young, 1998).

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Table 4. *The green mass of green manure plants (t ha^{-1}) (Gödöllő, 2007-2011)*

treatments		2007	2008	2009	2010	2011	mean	$\mathrm{LSD}_{5\%}$
phacelia	no fertilisation	18.4±2.9Ac	9,7±1.5Ab	4,8±0.7Aa	12,8±3.4Abc	20,3±1.3Ac	13,2	4.0
	with 50 kg N ha^{-1} fertiliser	35.2±2.3Bb	38,5±4.8Bbc	16,7±2.5Ba	37,3±2.6Bbc	41,5±1.4Bc	33,8	5.3
	change in biomass (%)	191%	395%	345%	292 %	204%	286%	
	$\mathrm{LSD}_{5\%}$	5.9	8.0	4.1	6.8	3.1		
mustard	no fertilisation	12.9±2.8Ab	7,3±2.5Aa	16,6±3.8Ab	7,7±2.4Aa	13,3±1.1Ab	11,6	4.8
	with 50 kg N ha^{-1} fertiliser	26.3±1.0Ba	31,7±3.8Ba	48,2±5.7Bb	29,4±2.7Ba	30,0±0.9Ba	33,1	6.1
	change in biomass (%)	204%	433%	291%	383%	225%	307%	
	$\mathrm{LSD}_{5\%}$	4.7	7.3	11.0	5.7	2.3		
oilseed radish	no fertilisation	13.9±1.0Ab	9,8±0.5Aa	10,3±2.4Aa	13,6±1.6Ab	22,5±1.2Ac	14,0	2.7
	with 50 kg N ha^{-1} fertiliser	30.4±0.2Bab	27,6±2.5Ba	34,4±7.5Bb	31,0±1.3Bab	43,4±0.9Bc	33,3	6.5
	change in biomass (%)	219%	282%	333%	228%	193%	251%	
	$\mathrm{LSD}_{5\%}$	1.6	4.1	12.6	3.2	2.4		

***Table 5.** The dry mass of green manure plants (t ha^{-1}) (Gödöllő, 2007-2011)*

treatments		2007	2008	2009	2010	2011	mean	LSD$_{5\%}$
phacelia	no fertilisation	3.2±0.6Ac	1.3±0.1Aab	0.9±0.2Aa	2.9±0.8Ac	1.8±0.1Ab	2.0	0.8
	with 50 kg N ha^{-1} fertiliser	4.5±0.3Bd	3.2±0.3Bb	2.3±0.1Ba	5.8±0.4Be	3.8±0.1Bc	3.9	0.5
	change in biomass (%)	141%	242%	267%	200%	205%	211%	
	LSD$_{5\%}$	1.0	0.5	0.3	1.4	0.3		
mustard	no fertilisation	3.0±0.7Ac	1.1±0.3Aa	2.1±0.4Ab	2.3±0.7Ab	1.8±0.05Aab	2.1	0.9
	with 50 kg N ha^{-1} fertiliser	4.9±0.2Bb	3.6±0.5Ba	6.7±1.0Bc	6.0±0.5Bc	3.9±0.1Ba	5.0	1.0
	change in biomass (%)	165%	310%	323%	266%	202%	255%	
	LSD$_{5\%}$	1.1	0.9	1.8	1.4	0.2		
oilseed radish	no fertilisation	2.8±0.2Ab	1.3±0.1Aa	1.6±0.2Aa	3.1±0.4Ab	1.6±0.1Aa	2.1	0.4
	with 50 kg N ha^{-1} fertiliser	4.0±0.03Bb	2.8±0.3Ba	4.8±1.0Bc	4.3±0.2Bbc	3.1±0.1Ba	3.8	0.8
	change in biomass (%)	141%	212%	293%	140%	193%	196%	
	LSD$_{5\%}$	0.4	0.6	1.6	0.6	0.2		

Table 6. *Effect of different nutrient levels on the uptake of N amount of green manure plants (kg ha^{-1}) (Gödöllő, 2007-2011)*

treatments		2007	2008	2009	2010	2011	mean	LsD$_{5\%}$
phacelia	no fertilisation	73.7±13.8Ac*	30.1±4.7Aa	31.7±5.4Aa	103.4±21.7Ad	47.8±3.1Ab	57.3	21.9
	with 50 kg N ha^{-1} fertiliser	164.4±11.4Bc	118.2±12.0Bb	86.1±10.7Ba	214.1±29.7Bd	97.9±3.8Bab	136.1	29.2
	change in nutrient content (%)	223%	393%	271%	207%	205%	260%	
	LSD$_{5\%}$	28.7	20.7	19.2	59.0	7.9		
mustard	no fertilisation	88.1±17.5Ab	33.6±8.7Aa	85.2±18.9Ab	96.6±22.4Ab	47.7±2.7Aa	70.2	28.7
	with 50 kg N ha^{-1} fertiliser	188.0±6.7Bb	138.8±21.0Ba	283.2±42.4Bc	220.3±9.8Bb	103.0±3.1Ba	186.7	39.8
	change in nutrient content (%)	213%	413%	333%	228%	216%	281%	
	LSD$_{5\%}$	30.0	36.5	74.4	39.2	6.6		
oilseed radish	no fertilisation	64.1±12.6Ab	31.1±6.8Aa	57.4±9.4Ab	103.3±6.5Ac	51.5±3.9Ab	61.5	15.3
	with 50 kg N ha^{-1} fertiliser	174.9±9.0Bb	127.0±13.7Ba	172.0±33.1Bb	180.7±5.8Bb	102.7±2.2Ba	151.5	30.5
	change in nutrient content (%)	273%	409%	299%	175%	199%	271%	
	LSD$_{5\%}$	24.9	24.4	55.2	13.9	7.2		

*Capital letter: post hoc test between treatments
Small letter: post hoc test between years

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As an average of the five years of the experiment the amount of absorbed nitrogen per hectare increased to 2.81 % the amount fixed without added nitrogen. The application of 50 kg/ha nitrogen enabled the utilisation of an additional 66.5 kg nitrogen active ingredient. Of the plants used in the experiment mustard contained the largest amount of nitrogen. Its efficiency in fixing nitrogen had been observed by Baggs et al. (2000) as well. The growth of mustard sown among short-day plants remained vegetative, producing a substantial mass of foliage, and it is the very leaves of the plant that can store the largest quantities of nitrogen (Chaves et al., 2004).

In the case of oilseed radish the active ingredient uptake increased by 2.7 times as an average over five years, i.e. the delivery of 50 kg/ha nitrogen enabled the uptake of an additional 40.0 kg/ha. Like mustard, oilseed radish also produced a large mass of foliage, and of all plant organs leaves contain the largest quantities of nitrogen (Rogasik et al., 1992; Muller et al., 1988).

The per-hectare P_2O_5 contents are illustrated by the figures presented in *Table 7*. The P_2O_5 content of phacelia increased to 2.36, that of mustard grew to 2.29 times and that of oilseed radish increased to 2.09 times the amounts measured on plots without fertiliser, as an average of the five years of the experiment. The application of nitrogen fertiliser enabled, as an average of five years, the uptake of an additional 26.6 kg of P_2O_5 in the case of phacelia, while the increase in the case of mustard and oilseed radish amounted to 27.0 kg and 30.4 kg, respectively. The increase in the per-hectare amount of P_2O_5 absorbed by plants is explained by Liebig's minimum law.

The per-hectare K_2O contents are illustrated by the figures presented in *Table 8*. The K_2O content of phacelia increased to 2.13, that of mustard grew to 2.29 times and that of oilseed radish increased to 1.95 times the amounts measured on plots without fertiliser, as an average of the five years of the experiment. The application of nitrogen fertiliser enabled, as an average of five years, the uptake of an additional 88.1 kg of P_2O_5 in the case of phacelia, while the increase in the case of mustard and oilseed radish amounted to 102.9 kg and 69.2 kg, respectively.

Significant differences between the biomass increase caused by the application of one unit (1 kg) of nitrogen active ingredient and the crop-year effect could be detected in all cases (*Table 9*). The adding of one unit of nitrogen resulted in a significant increase in the biomass produced. One kg of nitrogen active ingredient increased the green mass by 412.5 kg and the dry mass by 37.9 kg as an average of the five years of the experiment. In the case of mustard the green mass grew by 431.4 kg, the dry mass increased by 59.1 kg, while in the case of oilseed radish the corresponding figures were 386.7 kg and 34.1 kg.

The crop-year effect did not always have a statistically proven impact on the per-hectare NPK uptake increase caused by the application of a unit of fertiliser active ingredient (*Table 10*). The quantity of nutrients absorbed increased regardless of the amount and distribution of precipitation. One kilogram of nitrogen active ingredient enabled, the uptake of an additional 1.6 kg of nitrogen in the case of phacelia, 2.3 kg in the case of mustard and 1.8 kg in the case of oilseed radish, as an average of the period between 2007 and 2011. This is of particular importance in view of the fact that the soil of the experiment site has a low nitrogen content. The added nitrogen increased the availability of P_2O_5 as well, as each kg of added nitrogen resulted in a 0.5 kg. 0.5 kg and 0.6 kg of additional P_2O_5 uptake in phacelia, mustard and oilseed radish, respectively. Each kg of added active ingredient resulted in an 1.8 kg, 2.1 kg and 1.4 kg additional K_2O uptake in phacelia, mustard and oilseed radish, respectively.

Table 7. *Effect of different nutrient levels on the uptake of P_2O_5 amount of green manure plants (kg ha^{-1}) (Gödöllő, 2007-2011)*

treatments		2007	2008	2009	2010	2011	mean	$LSD_{5\%}$
phacelia	no fertilisation	39.2±16.3Abc*	13.9±2.9Aab	8.7±2.4Aa	26.0±6.2Ab	29.0±1.8Abc	23.4	14.6
	with 50 kg N ha^{-1} fertiliser	61.0±4.1Bc	43.5±3.9Bb	23.2±3.7Ba	64.8±7.7Bc	57.4±2.2Bc	50.0	8.5
	change in nutrient content (%)	156%	313%	265%	198%	198%	236%	
	$LSD_{5\%}$	26.9	7.8	7.2	15.8	4.6		
mustard	no fertilisation	27.3±4.7Abc	12.7±2.8Aa	22.7±7.1Ab	30.9±3.6Ac	25.5±3.0Abc	23.4	8.2
	with 50 kg N ha^{-1} fertiliser	46.2±1.5Bb	32.3±4.3Ba	61.8±9.3Bc	62.7±9.4Bc	49.0±1.5Bb	50.4	11.4
	change in nutrient content (%)	169%	254%	272%	203%	192%	229%	
	$LSD_{5\%}$	7.9	8.2	18.7	16.1	5.4		
oilseed radish	no fertilisation	38.8±2.8Ab	18.3±1.8Aa	17.3±0.5Aa	35.1±3.3Ab	50.5±9.3Ac	32.0	8.5
	with 50 kg N ha^{-1} fertiliser	51.9±4.7Bb	34.1±5.5Ba	58.4±12.1Bb	65.1±9.1Bb	102.7±2.2Bc	62.4	13.8
	change in nutrient content (%)	134%	186%	338%	185%	203%	209%	
	$LSD_{5\%}$	8.8	9.3	19.4	15.6	15.3		

* Capital letter: post hoc test between treatments
Small letter: post hoc test between years

Table 8. *Effect of different nutrient levels on the uptake of K_2O amount of green manure plants (kg ha^{-1}) (Gödöllő, 2007-2011)*

treatments		2007	2008	2009	2010	2011	mean	$LSD_{5\%}$
phacelia	no fertilisation	190.8±9.9Ad*	75.2±4.9Ab	34.0±3.7Aa	111.3±17.0Ac	92.3±10.5Abc	100.7	18.8
	with 50 kg N ha^{-1} fertiliser	251.7±15.6Bcd	179.5±17.6Bb	97.8±23.6Ba	219.4±24.1Bc	195.8±7.7Bbc	188.8	34.1
	change in nutrient content (%)	132%	239%	287%	197%	212%	213%	
	$LSD_{5\%}$	29.7	29.3	38.3	47.3	20.8		
mustard	no fertilisation	156.4±24.7Ac	52.3±18.6Aa	91.6±23.4Ab	98.7±19.2Ab	73.1±6.6Aab	94.4	35.6
	with 50 kg N ha^{-1} fertiliser	253.8±23.4Bcd	183.2±33.2Bab	218.4±27.0Bb	169.3±16.0Ba	161.7±4.8Ba	197.3	42.0
	change in nutrient content (%)	162%	350%	238%	172%	221%	229%	
	$LSD_{5\%}$	54.6	61.0	57.3	40.0	13.2		
oilseed radish	no fertilisation	121.8±6.6Ad	58.0±2.7Aa	62.1±6.4Aa	111.1±3.4Ac	81.0±4.4Ab	86.8	9.1
	with 50 kg N ha^{-1} fertiliser	178.9±13.8Bb	126.8±27.2Ba	175.6±42.1Bab	132.4±17.9Bab	166.4±19.7Bab	156.0	47.5
	change in nutrient content (%)	147%	219%	283%	119%	205%	195%	
	$LSD_{5\%}$	24.5	43.9	68.2	29.4	32.4		

*Capital letter: post hoc test between treatments
Small letter: post hoc test between years

Table 9. *Specific biomass increasing effect of 1 kg additional N (kg ha^{-1})*

plants	biomass	2007	2008	2009	2010	2011	mean	LSD$_{5\%}$
phacelia	green mass	336.1±69.0ab	575.8±114.0d	237.5±57.3a	490.3±90.4c	423.0±37.2bc	412.5	111.9
	dry mass	26.2±12.2a	37.4±4.4a	29.1±4.8a	58.2±18.2b	38.5±3.8a	37.9	14.8
mustard	green mass	268.5±36.8a	487.3±56.9d	633.3±38.8e	434.2±12.6c	333.6±25.1b	431.4	53.1
	dry mass	38.9±9.4a	48.1±7.6a	93.0±13.1c	74.9±4.2b	40.7±2.9a	59.1	11.9
oilseed radish	green mass	329.8±20.9a	356.1±60.2ab	480.8±102.6c	349.0±6.8ab	417.7±7.9b	386.7	77.5
	dry mass	23.1±4.2a	30.2±9.6a	63.0±14.6b	24.8±3.7a	29.5±0.5a	34.1	11.7

***Table 10.** Specific NPK content increasing effect of 1 kg additional N (kg ha^{-1})*

plants	NPK	2007	2008	2009	2010	2011	mean	LSD$_{5\%}$
phacelia	N	1.8±0.3	1.8±0.3	1.1±0.3	2.2±1.0	1.0±0.1	1.6	ns
	P_2O_5	0.4±0.2a	0.6±0.1a	0.3±0.1a	0.8±0.2a	0.6±0.03a	0.5	0.8
	K_2O	1.2±0.4a	2.1±0.4b	1.3±0.5a	2.2±0.8b	2.1±0.2b	1.8	0.5
mustard	N	2.0±0.3b	2.1±0.4b	4.0±1.2d	2.5±0.4c	1.1±0.1a	2.3	0.2
	P_2O_5	0.4±0.1	0.4±0.1	0.8±0.3	0.6±0.2	0.5±0.1	0.5	ns
	K_2O	1.9±0.8b	2.6±0.5c	2.5±0.9c	1.4±0.1a	1.8±0.1b	2.1	0.2
oilseed radish	N	2.2±0.2	1.9±04	2.3±0.6	1.5±0.2	1.0±0.1	1.8	ns
	P_2O_5	0.3±0.1	0.3±01	0.8±0.2	0.6±0.1	1.0±0.1	0.6	ns
	K_2O	1.1±0.4ab	1.4±0.6b	2.3±1.0c	0.4±0.4a	1.7±0.3bc	1.4	0.8

Conclusions

Each of the three plant species involved in the experiment was suitable for use as green manure under the given unfavourable site conditions, fulfilling their roles in terms of soil protection and organic matter conserving. In regard to the per-hectare biomass and chemical composition, particularly in view of the uptake of nitrogen, the cruciferous mustard and oilseed radish were found to perform better than phacelia.

The 50 kg/ha nitrogen active ingredient made a significant contribution to the increase in the total amount of biomass produced and improvement in its chemical composition, while without added nitrogen the crop was badly affected by what is known as pentosan effect. The application of nitrogen fertiliser multiplied the per-hectare nitrogen content in each of the three plant species. The added nitrogen also greatly facilitated the uptake of phosphorous and potassium.

After the delivery of 50 kg/ha nitrogen each of the three plant species produced a stable green mass with substantial amounts NPK absorbed, but without added nitrogen the poorly endowed site could not always produce the amount that would have been considered as adequate. It is concluded from the results of the experiment that when growing second crops for green manure at the given site nitrogen should always be applied but when the straw is left on the field to be incorporated into the soil after the harvest of cereal crops, the application of nitrogen is indispensable.

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REFERENCES

[1] Allison, M. F., Armstrong, M. J., Jaggard, K. W., Todd, A. D. (1998a.): Integration of nitrate cover crops into sugarbeet (Beta vulgaris) rotations. I. Management and effectiveness of nitrate cover crops. - Journal of Agricultural Science 130: 53-60.

[2] Allison, M. F., Armstrong M. J., Jaggard, K. W., Todd, A. D. (1998b): Integration of nitrate cover crops into sugarbeet (Beta vulgaris) rotations. II. Effect of cover crops on growth, yield and N requirement of sugarbeet. - Journal of Agricultural Science 130: 61-67.

[3] Allison, M. F., Armstrong, M. J. (1992): The integration of cover crops into sugar beet (Beta vulgaris) rotations. - Aspects of Applied Biology 30: 301–308.

[4] Antal, J. (2000): Növénytermesztők zsebkönyve (*Crop producers' pocket book*). - Mezőgazda Kiadó. Budapest.

[5] Asagi, N., Ueno, H. (2009): Nitrogen dynamics in paddy soil applied with various ^{15}N-labelled green manures. - Plant Soil 322: 251-262.

[6] Baggs, E. M., Watson, C. A., Rees, R. M. (2000): The fate of nitrogen from incorporated cover crop and green manure residues. - Nutrient Cycling in Agroecosystems 56: 153-163.

[7] Bialy, Z., Oleszek, W., Lewis, J., Fenwick, G. R. (1990): Allelopathic potential of glucosinolates (mustard oil glycosides) and their degradation products against wheat. - Plant Soil 129: 277-281.

[8] Blazewick-Wozniak, M., Wach, D. (2012): The fertiliser value of summer catch crops preceding vegetables and its variation in the changing weather conditions. - Acta Scientinarium Polonorum Hortorum Cultus 11(3): 101-116.

[9] Boydston, R. A., Hang, A. (1995): Rapeseed (Brassica napus) green manure crop suppresses weeds in potato (Solanum tuberosum). - Weed Technology 9: 669-675.

[10] Bradow, J. M. (1991): Relationships between chemical structure and inhibitory activity of C_6 through C_9 volatiles emitted by plant residues. - Journal of Chemistry Ecology 17: 2193-2212.

[11] Brown, P. D., Mora M. J. (1995): Gluosinolate-containing plant tissues as bioherbicides. - Journal of Agricultural and Food Chemistry 43: 3070-3074.

[12] Brown, P. D., Morra M. J., Mccaffrey, J. P., Auld, D. L., Williams, L., I. (1991): Allelochemicals produced during glucosinolate degradation in soil. - Journal of Chemistry Ecology 17: 2021-2034.

[13] Cadisch, G., Giller, K. E. (1997): Driven by Nature: Plant Litter Quality and Decomposition. - Wallingford: CAB International. 409.

[14] Catt, J.A., Christian, D.G., Goss, M.J., Harris, G.C., Howse, K.R. (1992): Strategies to reduce nitrate leaching by crop rotation, minimal cultivation and straw incorporation in the Brimstone Farm Experiment, Oxfordshire. - Aspects of Applied Biology 30: 255–262.

[15] Chaves, B., Neve, S., Hofman, G., Boeckx, P., Cleemput, O. (2004): Nitrogen mineralization of vegetable root residues and green manures as related to their (bio)chemical composition. - European Journal of Agronomy 21(2): 161-170.

[16] Clark, A. J., Decker, A. M., Meisinger, J. J., McIntosh, M. S. (1997a): Kill date of vetch, rye, and a vetch-rye mixture: I. Cover crop and corn nitrogen. - Agronomy Journal 89: 427-434.

[17] Clark, A. J., Decker, A. M., Meisinger, J. J., McIntosh, M. S. (1997b): Kill date of vetch, rye, and a vetch-rye mixture II. Soil moisture and corn yield. - Agronomy Journal 89: 434-441.

[18] Collins, H. P., Delgado, J. A., Alva, A. K., Follett, R. F. (2007): Use of nitrogen-15 isotopic techniques to estimate nitrogen cycling from a mustard cover crop to potatos. - Agronomy Journal 99: 27-35.

[19] Croft, K. P. C., Juttner, F., Slusarenko, A. J. (1993): Volatile products of the lipoxygenase pathway evolved from Phaseolus vulgaris (L.) leaves inoculated with Pseudomonas syringae pv phaseolicola. - Plant Physiology 101:13-24.

[20] Deng, W., Hamilton-Kemp, T. R., Nielsen, M. T., Andersen, R. A., Collins, G. B., Hildebrand, D. F. (1993): Effects of six-carbon aldehydes and alcohols on bacterial proliferation. - Journal of Agricultural and Food Chemistry 41: 506-510.

[21] Derpsch, R., Sidiras, N., Roth, C. H. (1986): Results of studies made from 1977 to 1984 to control erosion by cover crops and no-tillage techniques in Paraná, Brazil. - Soil and Tillage Research 8: 253-263.

[22] Dhima, K. V., Vasilakoglou, I. B., Gatsis, T. D., Panou-Philotheou, E., Eleftherohorinos, I. G. (2009): Effects of aromatic plants incorporated as green manure on weed and maize development. - Field Crops Research 110: 235-241.

[23] Foucault, Y., Lévéque, T., Xiong, T., Schreck, E., Austruy, A., Shahid, M., Dumat, C. (2013): reen manure plants for remediation of soils polluted by metals and metalloids (Ecotoxicity and human bioavailability assessment. - Chemosphere. 93. 7. 1430-1435.

[24] Füleky, Gy. (1999): Tápanyag-gazdálkodás (*Nutrient management*). - Mezőgazda Kiadó. Budapest.

[25] Gardner, H. W., Dornbos, D. L., Jr., Desjardins, A. E. (1990): Hexanal, trans 2-hexenal, and trans-2-noncnal inhibit soybean, Glycine max, seed germination. - Journal of Agricultural and Food Chemistry 38: 1316-1320.

[26] Grossman, J. (1993): Brassica alternatives to herbicides and soil fumigants. Integrated Pest Management Practicum. 15.1- 10.

[27] Jackson, L. E., Wyland, L. J., Stivers, L. J. (1993): Winter cover crops to minimise nitrate losses in intensive lettuce production. - Journal of Agricultural Science 121: 55–62.

[28] Kahnt, G. (1981): Gründüngung. - DLG-Verlag. Frankfurt.

[29] Kara, E., Penezoglu, M. (2000): The effect of green manuring on soil organic content and soil biological avctivity. Anadolu. 10. 1. 73-86.

[30] Késmárki, I., Petróczki, F. (2003): Komposztálás-zöldtrágyázás (*Composting, green manuring*). - Agro Napló 7(7): 11-13.

[31] Kim, K. R., Owens, G., Kwon, S. I. (2010): Influence of Indian mustard (Brassica juncea) on rhizosphere soil solution chemistry in long-term contaminated soils: a rhizobox study. - Journal of Environmental Science 22: 98-105.

[32] Lazzeri, L., Tacconi,R., Palmieri, S. (1993): In vitro activity of some glucosinolates and their reaction products toward a population of the nematode Heterodera schachtii. - Journal of Agricultural and Food Chemistry 41: 825-829.

[33] Martinez, J., Guiraud, G. (1990): A lysimeter study of the effects of a ryegrass catch crop during a winter wheat/maize rotation, on nitrate leaching and on the following crop. - Journal of Soil Science 41: 5-16.

[34] Máté, A. (2005): Növénytermesztési Tanüzem (*Cropping study farm*). Tanulmány. Gödöllő.

[35] Mayton, H. S., Oliver, C., Vaughn, S. F., Loria, R. (1996): Correlation of fungicidal activity of Brassica species with allyl isothiocyanate production in macerated leaf tissue. - Phytopathology 86: 267-271.

[36] MÉM-NAK (1979): A műtrágyázás üzemi számítási módszere. MÉM, Budapest

[37] Mojtahedi, H., Santo, G. S., Hang, A., Wilson, J. H. (1991): Suppression of root-knot nematode populations with selected rapeseed cultivars as green manure. - Journal of Nematology 23: 170-174.

[38] Mojtahedi, H., Santo, G. S., Wilson, J. H., Hang, A. (1993): Managing Melodogyne chitwoodi on potato with rapeseed as green manure. - Plant Disease 77: 42-46.

[39] Muelichen, A. M., Rand, R. E., Parke, J. L. (1990): Evaluation of crucifer green manuresfor controlling Aphanomyces root rot of peas. - Plant Disease 74: 651-654.

[40] Muller, J. C., Denys, D., Morlet, G., Mariotta, A. (1988): Influence of catch crops on mineral nitrogen leaching and its subsequent plant use, pp. 8598. - In: Jenkinson, D.S., Smith, K.A. (eds.) Nitrogen efficiency in agricultural soils, vol. 2. Elsevier Applied Science, New York.

[41] Papavizas, G. C., Lewis, J. A. (1971): Effect of amendments and fungicides on Aphanomycesroot rot of peas. - Phytopathology 61: 215-220.

[42] Papavizas, G. C. (1966): Suppression of Aphanomyces root rot of peas by cruciferous soil amendments. - Phytopathology 56: 1071-1075.

[43] Ramirez-Villapudua, J., Munecke., D. E. (1988): Effect of solar heating and soil amendments of cruciferous residues on Fusarium oxysporum f. sp. conglutinans and other organisms. - Phytopathology 78: 289-295.

[44] Rogasik, J., Smukalski, M., Obenauf, S. (1992): Cover crops on sandland in Germany: Husbandry and fate of nitrogen. - Aspects of Applied Biology 30: 309-316.

[45] Richards, I. R., Wallace, P.A., Turner, I. D. S. (1996): A comparsion of six cover crop types in terms of nitrogen uptake and effect on response to nitrogen by a subsequent spring barley crop. - Journal of Agricultural Science 127: 441–449.

[46] Stefanovits, P. (1999a): Főtipusok, típusok és altípusok (*Main types, types and subtypes*). - In: Stefanovits, P., Filep, Gy., Füleky, Gy.: Talajtan. Mezőgazda Kiadó. Budapest.

[47] Stefanovits, P. (1999b): A tájak talajviszonyai (*Soil conditions in Hungary's regions*). - In: Stefanovits, P., Filep, Gy., Füleky, Gy.: Talajtan. Mezőgazda Kiadó. Budapest.

[48] Stivers-Young, L. (1998): Growth, Nitrogen Accumulation, and Weed Suppression by Fall Cover Crops Following Early Harvest of Vegetables. - Hortscience 33(1): 60-63.

[49] Thorup-Kristensen, K. (1993): The effect of nitrogen catch crops on the nitrogen nutrition of a succeeding crop 1. Effects through mineralisation and pre-emptive competition. - Acta Agriculturae Scandinavica Section B, Soil and Plant Science 43:74-81.

[50] Vaughan, J. D., Evanylo, G. K. (1998): Corn response to cover crop species, spring dessication time, and residue management. - Agronomy Journal 90:536-544.

[51] Vaughn, S. F., Boydston, R. A. (1997): Volatile allelochemicals released by Crucifer green manures. - Journal of Chemical Ecology 23(9): 2107-2116.

[52] Williams, L., Morra, M. J., Brown, P. D., McCaffrey, J. P. (1993): Toxicity of allyl.isothiocyanate-amended soil to Limonius californicus (Mann.) (Coleoptera: Elateridae) wireworms. - Journal of Chemical Ecology 19:1033-1046.

[53] Zhang, M. K., Fang, L. P. (2007): Effect of tillage, fertiliser and green manure cropping on soil quality at an abandoned brick making site. - Soil and Tillage Research 93:87-93.

ELECTRONIC APPENDIX

This article has an electronic appendix with basic data.

CARBON STOCKS IN A 20-YEAR-OLD CONIFEROUS PLANTATION – A CASE STUDY IN FANDOGHLOO REGION, NORTHWESTERN IRAN

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Abstract. In Fandoghloo region, northwestern Iran, tree plantations were established on a degraded rangeland, which was previously mainly used for grazing. We assessed the above- and belowground ecosystem biomass and carbon (C) pools of pure and mixed *Pinus nigra*, *Cedeus libani* and *Picea abies* plantation stands (20 years-old) in Ardabil, Iran. Biomass and C stocks of above- and belowground tree biomass and litter were determined from plot-level inventories. Mineral soil C stocks were estimated from soil cores at three depths 0-15, 15-30 and 30-50 cm. Our results showed an increase in the total ecosystem carbon stock after the afforestation. The highest total carbon stock was 55.57 Mg ha^{-1} in the mixed *P. nigra - C. libani* and it was 25.006 Mg ha^{-1} in the degraded rangeland. The carbon stock in the aboveground biomass ranged from 2.4 Mg ha^{-1} in the degraded rangeland to 19.28, 15.1, 6.17, 4.55, and 3.87 Mg ha^{-1} in the *P.nigra - C. libani*, *P.nigra*, *P. abies- P.nigra*, *C. libani* and *P. abies* stands, respectively. The amount of litter carbon stock ranged from 0.53 Mg ha^{-1} in the degraded rangeland to 3.51, 3.06, 2.03, 1.55, 1.41 Mg ha^{-1} in the *P. abies- P.nigra*, *P.nigra-C. libani*, *P.nigra*, *C. libani* and *P. abies* stands, respectively. The soil carbon stock increased from 21.41 to 30.11, 28.58, 28.41, 27.45 and 25.43 Mg ha^{-1} in the *C. libani*, *P.nigra*, *P.nigra - C. libani*, *P. abies - P.nigra* and *P. abies* stands, respectively. Significant interactions were observed between stand and soil depth on carbon stock after rangeland afforestation with coniferous species. Aboveground tree biomass became the major ecosystem C pool. The difference in total ecosystem C between the degraded rangeland and mixed *P.nigra - C. libani* stand was 30.56 Mg ha^{-1}. The highest SOC accumulation was observed in the surface layer of the *C. libani* (34.95 Mg ha^{-1}), but the accumulation rate is species dependent. Our results highlight the importance of coniferous plantations on degraded rangeland that will most probably increase the rate of C sequestration and thereby mitigate the negative effects of increasing atmospheric CO_2 concentrations. As a matter of fact, the selection of appropriate species and plantation will be considered in the next afforestation projects.

Keywords: *carbon sequestration, pure and mixed plantations, Pinus nigra, Cedeus libani, Picea abies*

Introduction

Nowadays humanity faces important global challenges in the form of climate change, which is a result of increasing greenhouse gases (GHGs), mainly carbon dioxide (CO_2) (Nakakaawa et al., 2010). Biomass and soil are considered to have a large potential for temporary and long-term carbon (C) storage (Gower, 2003; Houghton, 2005). Enhancing C sequestration by increasing forested land area (e.g. afforestation) has been suggested as an effective factor to mitigate elevated atmospheric carbon dioxide (CO_2) concentrations and hence contribute towards the prevention of global warming (Watson,

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2000; IPCC, 2001). The United Nations Framework Convention on Climate Change (UNFCCC) also promotes afforestation programs as strategies to reduce net CO_2 emissions (UNFCCC, 1997). The study of the effects of afforestation on the ecosystem carbon stock has been widely reported in the recent years (Zinn et al., 2002; Lemma et al., 2006; Nosetto et al., 2006; Yüksek and Yüksek, 2011; Hansson et al., 2013; Zhag et al., 2013; Varamesh et al., 2014), although the contribution of afforestation was known in biomass carbon sequestration, the effects of tree plantation on soil carbon stock were less certain because of effective factors such as rainfall, soil texture, stand age (Guo and Gifford, 2002; Paul et al., 2002). However, reports from various studies on the effects of afforestation on net SOC accretion are inconsistent. Due to these inconsistencies, more studies seem needed to evaluate the potential of SOC accumulation under productive plantation forests on degraded rangelands (Varamesh et al., 2014). The afforestation could be a very useful method for restoring degraded soils and ecosystems (IPCC, 2000) and in the global scale, this method is considered as soil conservation, desertification, and an increase of carbon sequestration (Grunzweig et al., 2003; Kumar et al., 2001; Maestre and Cortina, 2004; Nosetto et al., 2006). Giuffre et al. (2003) obtained significant differences by evaluating of soil organic carbon between the afforested regions compared with the rangeland in the Patagonia, Argentina. Nosetto et al. (2006) found that the afforestation with *Pinus ponderosa* in grassland of Patagonia increases above- and belowground carbon stocks, and Mireia et al. (2010) noted a significant increase in soil organic carbon in the plantation of *Pinus halepensis*. Fonseca et al. (2012) found that carbon in the biomass was over 78 Mg/ha but the soil represents the main carbon sink at an ecosystem level with more than 85 Mg/ha. Mean annual increments for carbon in the biomass was above 5.3 Mg/ha and over 1.3 Mg/ha in the soil. Heras et al. (2013) claimed that in the short-term, the carbon stored in the live biomass at afforested stand level was reduced, but the positive effect on productivity permits to restore and exceed the initial amount of carbon in the medium-term.

Among the different ecosystems, coniferous forests were considered as great resources of carbon stocks (Gucinski et al., 1995). Several researchers (Akala and Lal, 2001; Xiao-Wen et al., 2009) have focused on the importance of afforstration and suitable species selection to enhance the carbon sequestration. Thus attention to forest ecosystem compartments contribution in carbon sequestration is important, because it is essential to understand the potential of carbon sequestration of forest ecosystem to consider complex of trees, understory, soil and litter (Uri et al., 2012).

The *Pinus nigra*, *Cedrus libani*, and *Picea abies* are known for afforstation in Iran. However, there are few studies about the effects of afforestation on the total carbon stock of ecosystem with these species in the degraded rangelands of Iran. As a result, specific objectives were to investigate the distribution of carbon stuck in the compartments (soil, above-, belowground biomass and litters) of coniferous planted stands and degraded rangelands, assess the depth distribution of SOC concentration up to 50 cm depth, determine the trends and variations in C pool at the scale of the plantation and establish the association of SOC with selected physical and chemical soil properties. The basic assumption of this study was relatively homogeneous biophysical conditions of the stands.

Materials and Methods

Study area

The study area is located in the northwest of Iran (northeast of Ardabil), between latitudes 38° 22' and 38° 24' N and longitudes 48° 31' and 48° 34' E (*Fig. 1*). It covers an area of about 50 ha. According to Namin Meteorological Station, the mean annual precipitation and temperature were 379 mm and 8.8°C, respectively. The elevation of the forested area ranges from 1350 to 1500 m above sea level. The study area includes pure and mixed *Pinus nigra*, *Cedeus libani* and *Picea abies* plantation stands. In the past, afforested areas were barren lands which were previously mainly used for grazing and were planted with the mentioned species almost 20 years ago. The soil texture of the area is loam and clay loam.

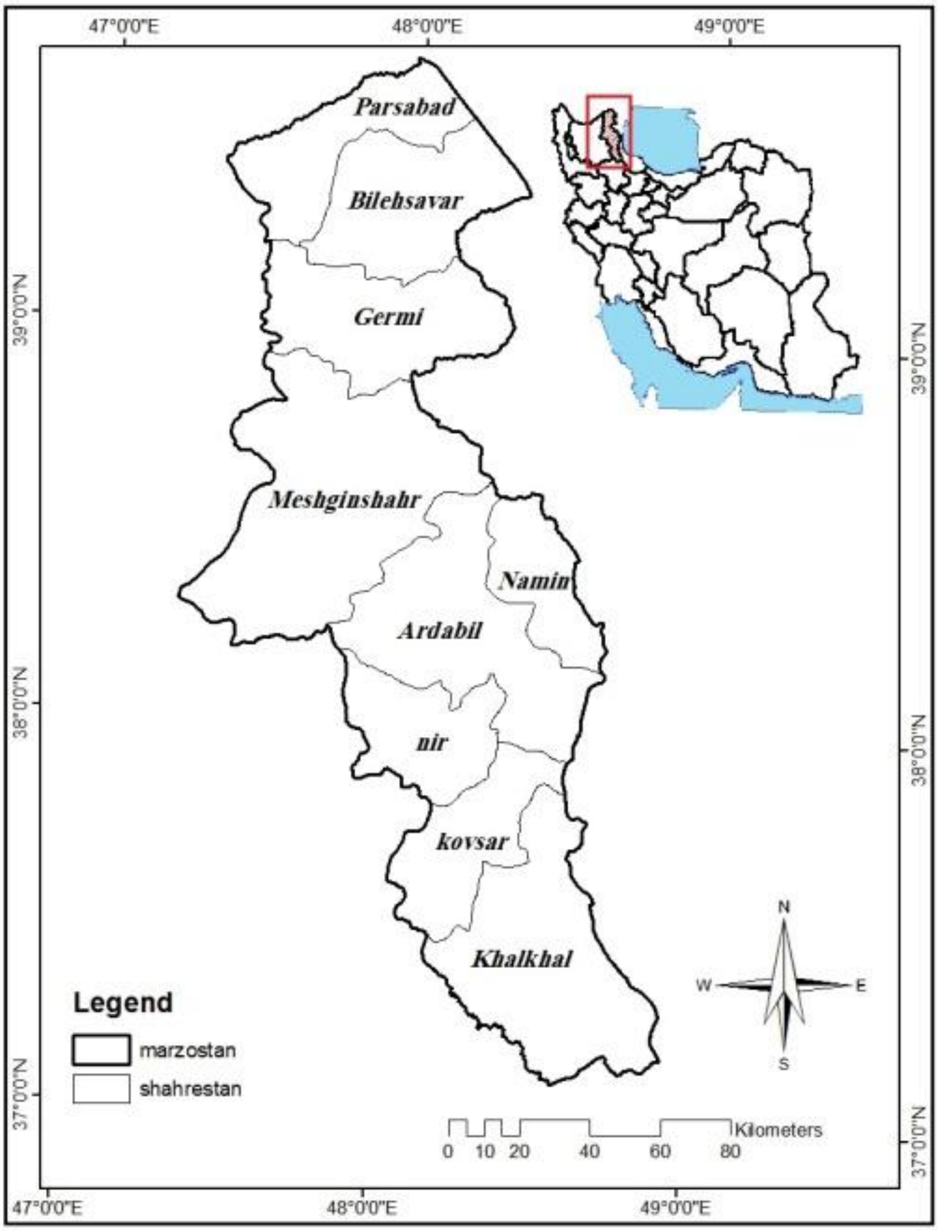

Figure 1. *Study area*

Sampling and inventory method of soil litter and trees

10 hectare areas were selected for each stand and in order to decrease the bordering effects, surrounding rows of stands were not considered during sampling (Varamesh et al., 2014). In order to characterize carbon storage and distribution, we sampled soil and litter pools where organic carbon accumulates. To evaluate carbon stocked in tree compartments we used tree allometric equations. Sampling and inventory were carried out during October (2013) using a randomly systematic method from six 400 m^2 squares (20 x 20 m) in each type of plantation system, i.e. *Picea abies*, *Cedrus libani* and *Pinus nigra*.

Calculation of above- and belowground biomass

In 20 × 20 m plots, quantitative properties of all trees within the plot were measured including diameter at breast height (DBH), total height (H) and trunk height (HC), and two perpendicular diameters of the canopy (W and L). Other reservoirs, like fine roots, fruits, coarse woody debris or understorey species, were considered negligible and therefore, not measured.

To calculate tree biomass and to compute trunk, canopy and root volume, the following steps were done according to prescription (Hernandez et al., 2004):

First, the basal area of tree was computed using Equation 1, and then the tree volume was gained using Equation 2. Finally, the biomass of trunk (kilogram) was computed according to the Equation 3.

$$Ab = \pi x r^2 \qquad \text{(Eq. 1)}$$

Where π= 3.1415927 and *r* is the radius of the tree at breast height (0.5 DBH).

$$V = Ab \times H \times Kc \qquad \text{(Eq. 2)}$$

Where, Ab is the basal area; H is the height, and Kc is a site-dependent constant in standard cubing practice used in forest inventory (0.5463).

$$Biomass = V \times WD \times 1000 \qquad \text{(Eq. 3)}$$

V= volume of the trunk. WD= wood density.

Since the full sampling of tree roots is time demanding and expensive and also in order to avoid destructive sampling methods, the root biomass was calculated using Equation number 4 (Hernandez et al., 2004):

$$BGB = VolumeAGB \times 0.2 \qquad \text{(Eq. 4)}$$

BGB = Belowground biomass. AGB = Aboveground biomass.

The canopy volume of trees was computed by the Equation 5.

$$V\left(m^3\right) = \frac{\Pi \times Db^2}{12} \qquad \text{(Eq. 5)}$$

Note: $\pi = 3.141592$; Db = diameter of the crown (to calculate Db, the average of the field measurements L and W is taken and used as the diameter of the crown: $Db = (L + W)/2$); Hc = height from the ground to the base of the crown.

The carbon Stock of biomass was calculated based on Equation 6. below:

$$C = 0.55 \text{ x Biomass}_{(\text{total})} \qquad \text{(Eq. 6)}$$

Soil and litter sampling

Four soil profiles were dug in the four corners of the 400 m^2 plot, then soil samples were collected at 0-15, 15-30, and 30-50 cm depths using a core (Ø 35 mm) sampler, thus resulting in 72 soil samples for each stand at three different depths. For calculation of the soil carbon, nitrogen and phosphorus storage, bulk density of soil was determined. From the soil pit, bulk density samples were taken from different soil layers (0–50 cm) with a stainless steel cylinder (d = 40 mm and volume = 50 cm^3) avoiding compression of the soil and preserving soil structure (Uri et al., 2012). Litter was removed from each profile, as well as, large plant material (e.g. root and shoots) occurring in each soil sample. Then soil samples were air-dried and 2 mm sieved (Lemma, et al., 2006). Litter, as well as grass, shrubs and forbs biomass was collected in 0.25 m^2 plots (0.5 x 0.5 m^2). Sub samples of each compartment were oven dried and the dry matter coefficients were used to transform fresh weight into dry matter.
The organic carbon content of litter was calculated by the following Equation (7):

$$OC = \tfrac{1}{2} \text{ x } OM \qquad \text{(Eq. 7)}$$

Laboratory analysis

Dry bulk density was calculated by dividing the oven dry mass at (105 °C) of the <2 mm fraction by the volume of the core. The soil texture was determined by the Bouyoucos hydrometer method (Bouyoucos, 1962). Soil pH was determined potentiometrically in a 1:2.5 (v/v) soil:water suspension. Electrical conductivity (EC) was characterized with (soil:water ratio, 1:2). For testing of total N (Kjeldahl) in the soil samples, Tecator ASN 3,313 was employed. Available phosphorus (ammonium lactate extractable) in the soil was determined by flow injection analysis using Tecator ASTN 9/84 and total organic C determined by the Walkey and Black method.

The total SOC (Mg ha^{-1}) stock within a certain soil layer was calculated according to the following equation (Lemma et al., 2006):

$$SOC\ (Mg\ ha^{-1}) = SOC\ (g\ kg^{-1})\ z\ þ_b\ 10 \qquad \text{(Eq. 8)}$$

Where, z is soil layer thickness (m), and $þ_b$ is dry bulk density (Mg m^{-3}).

Statistical analysis

All of data were analyzed using the SPSS 19.0. The variable normality test was checked by the Kolmogorov-Smirnov test, meanwhile, Levene's test was used to examine the equality of the variances. Differences in soil characteristics among afforested stands and depths were tested with two-way analysis (ANOVA) using the General Linear Model (GLM) procedure, with different stands and depths (0–15, 15–

30, and 30–50 cm) as independent factors. Interactions between independent factors were also tested. Duncan's test was used to separate the averages of the dependent variables which were significantly affected by treatment. Significant differences among treatment averages for different parameters were tested at $p \leq 0.05$.

Some selected physico-chemical properties of the soil are presented in *Table 1.* Soil pH (H_2O, 1:1) ranges from 5.43 to 5.85 and EC ranges from 0.1 to 0.0063 dS m^{-1}. Soil texture was loam in *P. nigra* stand and clay-loam in the other stands. The increase in bulk density with increasing depth was gained in more stands.

Table 1. *Some soil properties in stands and Rangeland*

Soil properties	Depth (cm)	Stand					
		Pinus	*Cedrus*	*Picea abies*	*Pinus-Cedrus*	*Picea- Pinus*	*Rangeland*
PH_{H2O}	0-15	5.63±0.03	5.85±0.13	5.49±0.058	5.81±0.05	5.51±0.05	5.53±0.07
	15-30	5.65±0.03	5.40±0.026	5.54±0.086	5.83±0.12	5.45±0.01	5.72±0.1
	30-50	5.50±0.03	5.43±0.0066	5.59±0.057	5.62±0.07	5.61±0.0057	5.71±0.06
Bulk density (g/cm^{-3})	0-15	1.44±0.003	1.51±0.0033	1.49±0.030	1.54±0.078	1.41±0.003	1.37±0.041
	15-30	1.51±0.003	1.50±0.012	1.55±0.041	1.62±0.05	1.31±0.006	1.50±0.044
	30-50	1.61±0.009	1.53±0.0003	1.59±0.036	1.46±0.015	1.53±0.003	1.57±0.050
EC (dS m^{-1})	0-15	0.023±0.0007	0.0063±0.0012	0.02±0.0023	0.022±0.0006	0.02±0.0007	0.02±0.003
	15-30	0.031±0.0006	0.0073±0.0003	0.02±0.0026	0.021±0.0037	0.02±0.0003	0.03±0.337
	30-50	0.018±0.0003	0.011±0.0033	0.02±0.0023	0.021±0.0012	0.01±0.0003	0.03±0.003
Sand (%)	0-15	25.67±0.3	28.33±0.88	33.42±1.82	25.33±2.33	26.33±0.33	40.33±4.26
	15-30	29.67±0.3	26.00±0.21	33.75±2.25	35.00±0.31	29.00±0.58	44.33±7.17
	30-50	29.67±2.18	31.00±0.57	32.92±2.43	29.00±1	29.33±0.33	38±4.51
Clay (%)	0-15	32.00±0.6	31.00±0.57	28.17±1.13	32.33±1.20	31.00±0.58	23.67±0.33
	15-30	28.67±0.33	30.67±0.66	28.67±1.16	28.33±0.33	31.67±0.33	21 ±1.0
	30-50	31.67±0.33	27.33±0.33	30.33±1.01	32.67±1.20	34.00±0.25	22.33±2.6
Silt (%)	0-15	42.33±0.7	40.67±0.33	38.42±1.81	42.33±1.20	42.67±0.33	36±4.58
	15-30	41.67±0.33	43.33±0.66	37.58±1.42	36.67±0.33	39.33±0.67	34.67±7.7
	30-50	38.67±1.85	41.67±0.33	36.75±2.14	38.33±0.33	36.67±0.33	39.67±6.7

Values are means(±SE) of triplicate soil analysis.

Results

Some of the quantitative parameters of the stands are given in *Table 2*. Pure stand of *P. nigra* in most parameters had higher values compared to other stands. The highest amount of trunk height (m) was in the *C. libani* and greatest canopy volume was observed in the *P. nigra - C. libani.*

Table 2. *Stand characteristics of the Pinus nigra, Cedrus, Picea abies, Pinus-Cedrus and Picea- Pinus stands*

Stand parameter	Stands				
	Pinus nigra	*Cedrus*	*Picea abies*	*Pinus-Cedrus*	*Picea- Pinus*
DBH (cm)	17.27±0.305a	12.47±0.018d	13.14±0.706c	15.75±0.027b	15.67±0.088b

Tree height (m)	7.61± 0.315a	6.25±0.123d	6.96±0.070bc	6.73±0.093cd	7.28±0.041ab
Trunk Height(m)	3.17±0.018d	3.68±0.043a	3.48±0.038b	3.28±0.006c	3.28±0.015c
Canopy volume (m^3/ha)	9.72±0.069b	3.58±0.006d	6.54±1.094c	12.49±0.012a	5.73±0.033c
basal area (m^2/ha)	0.024±0.000a	0.01±0.000d	0.013±0.001d	0.02±0.001b	0.018±0.000c
Stand volume (m^3 /ha)	0.13±0.015a	0.04±0.003d	0.051±0.006cd	0.09±0.001b	0.072±0.003bc

Values followed by the same letter within a row are not statistically different (Duncan, $P < 0.05$).

Total carbon stock in the ecosystem

The total carbon stock of ecosystem has changed from 25.006 Mg ha^{-1} in degraded rangeland to 55.57 Mg ha^{-1} in the mixed *P.nigra - C.libani*, 49.01 Mg ha^{-1} in the *P.nigra*, 38.73 Mg ha^{-1} in the mixed *P.abies - P.nigra*, 37.84 Mg ha^{-1} in the pure *C.libani* and 31.68 Mg ha^{-1} in the pure *P.abies* stands (*Fig. 2*).

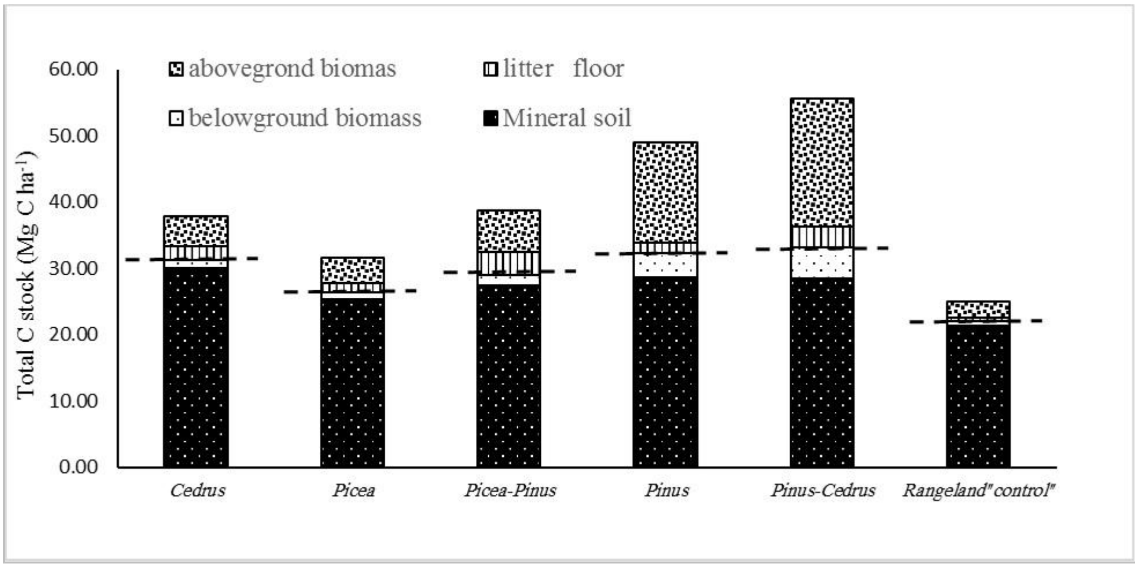

Figure 2. *C content of total above- and belowground tree biomass, floor litter, and mineral soil in the stands (dashed line separates above- from belowground C stock)*

The carbon stock in the aboveground biomass showed significant increase after the afforestartion in the degraded rangeland ($p < 0.001$), so that it has increased from 2.4 Mg ha^{-1} in the rangeland to 3.87, 4.55, 6.17, 15.1 19.28 Mg ha^{-1} in the *P.nigra - C.libani*, *P.nigra*, *P.abies - P.nigra*, *C.libani* and *P.abies* stands, respectively (*Fig. 2*). The carbon stock in the belowground biomass showed significantly higher values after degraded rangeland afforestration in all stands ($p < 0.001$). So, it increased from 0.67 in the degraded rangeland to 1.15, 1.60, 3.79, 4.82, and 10.97 Mg ha^{-1} in the *P.nigra - C.libani*, *P.nigra*, *P.abies - P.nigra*, *C.libani* and *P.abies* stands, respectively (*Fig. 2*). The carbon stock in the ecosystem litter had the same trend mentioned above. Therefore, a significant difference ($p < 0.001$) was observed in the carbon stock, the value of which ranged from 0.53 in the rangeland to 1.41, 1.55, 2.027, 3.07, 3.51 Mg ha^{-1} in the *P.abies - P.nigra*, *P.nigra - C.libani*, *P.nigra*, *C.libani* and *P.abies* stands, respectively (*Fig. 2*). The amount of carbon stock in the soils showed significant differences in the range of 21.41 in the rangeland and 25.43, 27.45, 28.41, 28.58, 30.11

Mg ha^{-1} in the *C.libani*, *P.nigra*, *P.nigra - C.libani*, *P.abies - P.nigra* and *P.abies* stands, respectively (*Fig. 2*). In general, the impacts of the degraded rangeland afforestation on the carbon stock in the ecosystem compartments showed significant difference increase.

The carbon stock in the soil

After rangeland afforestation with coniferous species, significant ($p < 0.05$) interactions were observed between stand and soil depth carbon stock. In general, the soil carbon stocks increased in all layers, namely upper (0-15 cm), middle (15-30 cm) and lower (30-50 cm) layers (*Fig. 3*). The highest carbon stock in the mineral soil was observed in the surface layer of the *C. libani* (34.95 Mg ha^{-1}), and the middle and lower layer of *P. nigra* stands (28.98 and 33.23 Mg ha^{-1}, respectively) (*Fig. 3*). The carbon stock in the upper, middle and lower layer of each stand showed statistically significant differences among the *P. nigra, P. abies - P. nigra*, and *C. libani* stands (*Fig. 3*). The rangeland, *P. nigra – C. libani* and *P. abies* stands showed no significant differences in the depths considered. The rangeland afforestation effects on the carbon stock presented differences with the depth changes.

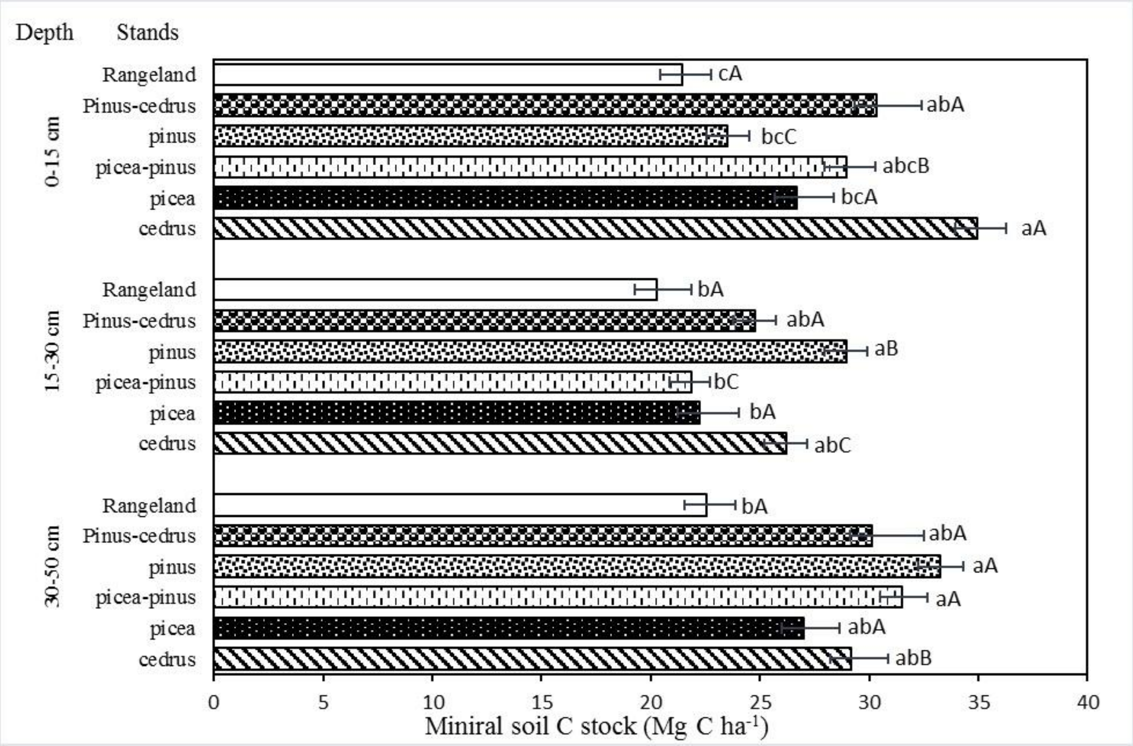

Figure 3. *Distribution of C stocks in mineral soil of stands (Mean±SE). Different lowercase letters within each soil depth indicate the significant difference of mean values ($p<0.05$) among the stands. Different capital letters within same stand indicate the significant difference of mean values ($p<0.05$) among the depths.*

Discussion

Establishment of coniferous species on the degraded rangeland caused an increase in the carbon stock of ecosystem. Considering 20 years of establishment of coniferous

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species in the degraded rangeland, the amount of annual sequestration in the mixed *P. nigra* - *C. libani*, *P. nigra*, mixed *P. abies* - *P. nigra*, pure *C. libani*, and *P. abies* stands were 1.60, 1.26, 0.72, 0.67, 0.35 Mg C ha^{-1} y^1, respectively.

One of the controversial issues was the proportion of the carbon stock in the soil and biomass (Uri et al., 2012). In this study, the contribution of soil in the carbon stock was higher than that of the other sections (85.60, 80.28, 79.58, 70.85, 58.30 and 51.11 in the *P.abies*, *C.libani*, *P.abies* - *P.nigra*, *P.nigra*, and *P.nigra* – *C.libani* stands, respectively). These results indicated the integral importance of soil in the carbon sequestration of ecosystem. Peltoniemi et al. (2004) expressed that a more significant amount of carbon was stored in the forest soils than in the forest biomass. Similar results were described by Garten and Charles (2002).

According to the EC / UN-ECE (2003), the carbon stock of forest soils in Europe was 1/5 times more than that of the biomass. In contrast, other studies indicated the high proportion of biomass in the total ecosystem carbon stock (Uri et al., 2012; De Wit et al., 2006; Peichl and Arain, 2006). Conflicting results on the soil carbon stock could be due to the influence of various factors on this matter. It was apparent that the type of tree species affected the carbon stock by the amount and quality of organic matter input by litter, root activity and microclimate change (Jandl et al., 2007; Lugo and Brown, 1993).

Also, Osher et al. (2003) expressed that the change of soil carbon stock associated with land use changes is in relation to soil mineralogy. In our study, it was found that the type of tree species had a direct impact on the carbon stock in the mineral sector of soil. Furthermore, a significant correlation was found among the clay, clay- silt, and soil carbon stock (*Fig. 4a, c*), while a significant negative correlation was found between the percentages of sand and soil carbon stock (*Fig. 4b*).

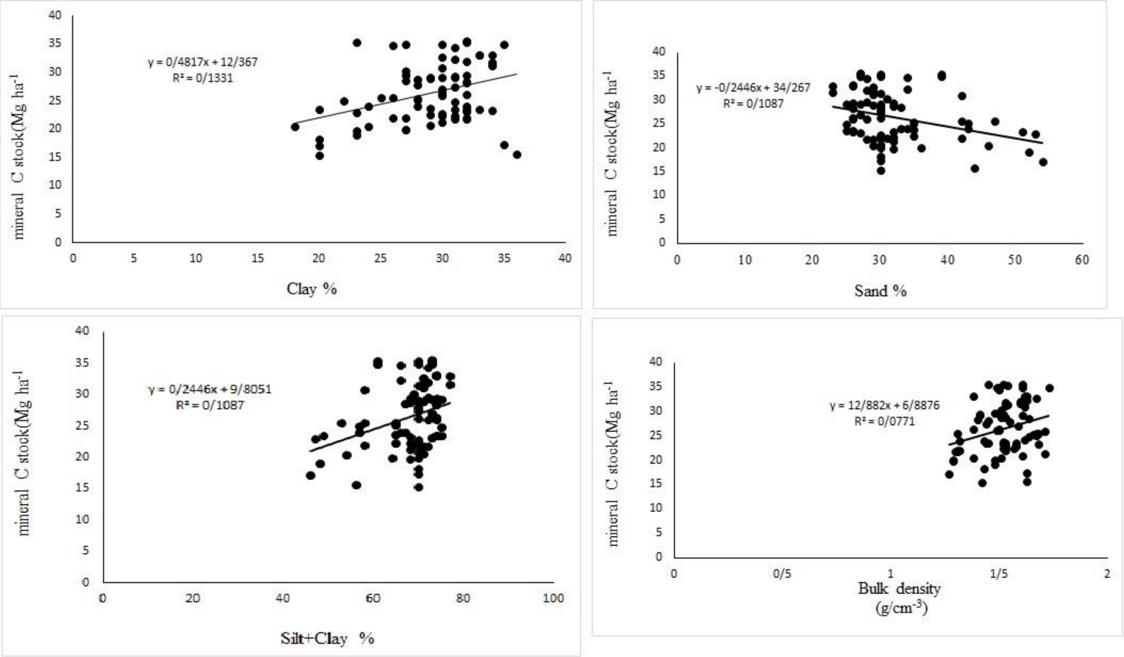

Figure 4. *Correlation between C content (Mg ha^{-1}) and soil textural (Clay, Clay+silt and Sand) and bulk density, all are significant in 0.001 level except bulk density in 0.05 level.*

Bauer et al. (1987) believed that the soil organic carbon was related to the clay of soil. Garten (2002), Powers (2002) and Matus et al. (2008) confirm our findings. They found that carbon sequestration correlated with the silt and clay of soil. A high percentage of sand in the spruce stand (*Table 1*) could be a more significant factor in the low carbon stock than the other stands. Rapid carbon aggregation and turnover in the coarse-grained soils (Richter et al., 1999) could be effective to decrease the soil carbon stock in the pure spruce stand (*Fig. 2*).

The clay and organic carbon were often formed as aggregate, and therefore they had much more affinity to bind organic matter compared to the sand (El Tahira et al., 2009). In this regard, Borchers and Perry (1992) observed that the concentration of organic matter was less in the sandy soils than in the clay and silt soils.

In this study, different distributions of the soil organic carbon were determined in the soil layers of considered stands (*Fig. 2*). The increasing trend of carbon stock was observable in the pure pine (*Fig. 2*). Such a trend was seen to augment the bulk density with the increase of depth in this stand (*Table 1*). In general, a positive correlation between bulk density and the soil carbon stock was found in this study (*Fig. 3d*).

However, the differences in the amount of sand, clay and silt in the different depths could be the reason of different carbon distribution in the soil layers. The differences among tree species in terms of root activity region had remarkable impacts on these differences. The root decompositions (especially thin roots) were important processes affecting the density and carbon stock (Steele et al., 1997).

pH was one of the main soil properties that affected the availability of the soil nutrients (Beery and Wilding, 1971), thus higher soil pH in the surface soil layer of *C. libani* stand than other stands could cause higher mineral carbon stock of soil.

Because there was a more appropriate condition for degradation in the surface layer of *C. libani* stand than the other stands which in turn increased the carbon stock in the higher levels of soil.

Thuille and Schulze (2006) noted the decrease of fauna activity of acidic soil which could also result in high carbon stock in the surface layer of pine - cedar stand.

The litter production and its degradation had a significant effect on the soil fertility (Pragasan and Parthasaratly, 2005) and it was considered as a notable factor by which tree species could affect soil organic matter (Finzi et al., 1998). The high carbon stock in the biomass and litter of mixed *P. nigra* - *C. libani* (*Fig. 2*) probably was due to the high canopy density of this stand compared to the other stands. (*Table 1*).

The litter resulted from trees were the main source of carbon in the forest ecosystems (De Marco, 2013) and the high density of canopy could be an integral source of litter and aboveground biomass in *P. nigra* - *C. libani* stand. In total, 40.21 percent of the total carbon stock was in the aboveground *P. nigra* - *C. libani* stand. Accordingly, development of root would increase by the increase of canopy. The highest belowground carbon stock included roots in *P. nigra* - *C. libani* stand had significant difference than the other stands and it possessed 8.67 percent contribution to total carbon stocks of ecosystem.

In this study, the properties of species in terms of growth of stem, branch, and leaf had significant impact on the rate of carbon accumulation in the above- and belowground biomass. In total, the amount of carbon accumulation in the coniferous ecosystems had significant increase in the degraded rangeland.

Conclusions

Our study shows the potential of fast growing coniferous plantations in degraded rangelands to mitigate the effect of high CO_2 concentrations over a short time span. Further knowledge was found during the calculation process and future research areas were suggested.

Coniferous plantations on degraded rangeland will most probably increase the rate of C sequestration and thereby mitigate the negative effects of increasing atmospheric CO_2 concentrations. The sequestration rates will be affected by tree species, former land use, soil mineralogy, climate and management of the plantations. Those effects on C sequestration rates need further investigations and more long-term studies are desirable. Moreover we can say that the maintenance or increase in carbon stock of forests has been linked to economic and external benefits, such as climate change mitigation, biodiversity improvement, water storage and regulation of local climate at landscape level.

REFERENCES

[1] Akala, V.A., Lal, R. (2001) : Soil organic carbon pools and sequestration rates in reclaimed minesoils in Ohio. – Journal of Environmental Quality 30: 2098–2104.

[2] Bauer, A., Cole, C.V., Black, A.L. (1987): Soil property comparisons in virgin grasslands between grazed and nongrazed management systems. – Soil Science Society of America Journal 51: 176–182.

[3] Beery, M., Wilding, L.P. (1971): The Relationship between Soil pH and Base-Saturation Percentage for Surface and Subsoil Horizons of Selected Mollisols, Alfisols, and Ultisols in Ohio. – Ohio J. Sci. 71:43-55

[4] Borchers, J.G., Perry, D.A. (1992): The influence of soil texture and aggregation on carbon and nitrogen dynamics in southwest Oregon forests and clear cuts. – Can. J. Forest Res. J. Can. Rech.Forest 22: 298–305.

[5] Bouyoucos GJ. (1962): Hydrometer method improved for making particle size analysis of soils. – Argon J 56: 464–465.

[6] De Marco, A., Esposito, F., Berg, B., Giordano, M., Virzo De Santo, A. (2013): Soil C and N sequestration in organic and mineral layers of two coeval forest stands implanted on pyroclastic material (Mount Vesuvius, South Italy). – Geoderma 209–210: 128–135.

[7] De Wit, H.A., Palosuo, T., Hylen, G., Liski, J. (2006): A carbon budget of forest biomass and soils in southeast Norway calculated using a widely applicable method. – Forest Ecology and Management 225: 15–26.

[8] EC/UN-ECE, de Vries, W., Reinds, G.J., Posch, M., Sanz, M.J., Krause, G.H.M., Calatayud, V., Renaud, J.P., Dupouey, J.L., Sterba, H., Vel, H.M., Dobbertin, M., Gundersen, P., Voogd, J.C.H. (2003): Intensive monitoring of forest ecosystems in Europe, 2003. Technical report. EC/UN-ECE 2003, Brussels, Geneva (p. 163).

[9] El Tahira, B.A., Ahmedb, D.M., Ardo, J., Gaafard, A.M., Salih, A.A. (2009): Changes in soil properties following conversion of Acacia senegal plantation to other land management systems in North Kordofan State, Sudan. – Journal of Arid Environments 73 : 499–505.

[10] Fataei, E., Varamesh, S., Behtari, B., Seyyed Safavian, S.T. (2013): Soil Carbon and Nitrogen Stocks under *Pinus nigra* and *Cedrus libani* afforestation in the Northwestern Highlands of Iran. – Advances in Environmental Biology 7(13): 4316-4325.

[11] Finzi, A.C; Canham, C.D; van Breemen, N. (1998): canopy tree soil interactions within temperature forests: species effects on pH and cations. – Ecological Applied 8: 447-454.

[12] Garten Jr., C. T. (2002): Soil carbon storage beneath recently established tree plantations in Tennessee and South Carolina, USA. – Biomass and Bioenergy 23 (2): 93–190.

[13] Giuffre L., Heredia O., Pascale C., Cosentino D., Conti M., Schnug E. (2003): Land use and carbon sequestration in arid soils of northern Patagonia (Argentina). – Landbauforschung Volkenrode 53(1): 13-18.

[14] Gower, S.T. (2003): Patterns and mechanisms of the forest carbon cycle. – Ann. Rev. Environ. Resour. 28: 169–204.

[15] Grunzweig, J.M., Lin, T., Rotenberg, E., Schwartz, A., Yakir, D. (2003): Carbon sequestration in arid-land forest. – Global Change Biology 9: 791–799.

[16] Gucinski, H., Vance, E., Reiners, W.A. (1995): Potential effects of global climate change. – In: Smith, W.K., Hinckley, T.M. (Eds.) Ecophysiology of Coniferous Forests. Academic Press, New York, Chapter 10, pp. 309–331.

[17] Guo, L.B., Gifford, R.M. (2002): Soil carbon stocks and land use change: a meta analysis. – Global Change Biology 8, 345-360.

[18] Hansson, K., Fröberg, M., Helmisaari, H.S., Kleja, D.B., Olsson, B.A., Olsson, M., Tryggve, P. (2013): Carbon and nitrogen pools and fluxes above and below ground in spruce, pine and birch stands in southern Sweden. – Forest Ecology and Management . (in press). http://dx.doi.org/10.1016/j.foreco.2013.05.029.

[19] Hernandez, R., Koohafkan, P., Antoine, J. (2004): Assessing Carbon Stocks and modeling win-win Scenarios of carbon sequestration through land-use changes. – Food and Agriculure Organization, p.166.

[20] Houghton, R.A. (2005): Aboveground forest biomass and the global carbon balance. – Global Change Biol. 11: 945–958.

[21] IPPC, 2000. Land use, Land-Use Change And Forestry. – Cambridge University Press, Cambridge, 375 pp.

[22] IPCC, 2001. Climate change Mitigation. http://www.grida.no/climate/ipcc_tar/wg3/pdf/TAR-total.pdf.

[23] Jandl, R., Lindner, M., Vesterdal, L., Bauwens, B., Baritz, R., Hagedorn, F., Johnson, D.W., Minkkinen, K., Byrne, K.A. (2007): How strongly can forest management influence soil carbon sequestration? – Geoderma 137: 253-268.

[24] Kumar, S., Datta, R., Sinha, S., Kojima, T., Katoh, S., Mohan, M. (2001): Carbon stock, afforestation and acidic deposition: an analysis of inter-relation with reference to arid areas. – Water, Air, and Soil Pollution 130: 1127–1132.

[25] Lemma, B., Berggren, D., Nilssonc, I., Olsson, M. (2006): Soil carbon sequestration under different exotic tree species in the southwestern highlands of Ethiopia. – Geoderma 136:886 – 898.

[26] Lugo, A.E., Brown, S. (1993): Management of tropical soils as sinks or sources of atmospheric carbon. – Plant Soil 149: 27–41.

[27] Maestre, F.T., Cortina, J. (2004) : Are Pinus halepensis plantations useful as a restoration tool in semiarid Mediterranean areas? – Forest Ecology and Management 198: 303–317.

[28] Matus, F. J., Lusk, C. H., Maire, C. R. (2008): Effects of soil texture, carbon input rates, and litter quality on free organic matter and nitrogen mineralization in Chilean rain forest and agricultural soils. – Communications in Soil Science and Plant Analysis 39: 187–201.

[29] McDicken, K. G. (1997): A Guide to Monitoring Carbon Storage in Forestry and Agro forestry Projects. – Winrock International Institute for Agricultural Development, Forest Carbon Monitoring Program. pp: 91.

[30] Mireia, L., Bruno, G.M., Belén, T. (2010): Storage of organic carbon and black carbon in density fractions of calcareous soils under different land uses. – Geoderma 159: 31-38.

[31] Nosetto, M.D., Jobbágy, E.G., Paruelo, J.M. (2006): Carbon sequestration in semi-arid rangelands: Comparison of Pinus ponderosa plantations and grazing exclusion in NW Patagonia. – J. Arid Environ. 67: 142–156.

[32] Osher, L.J., Matson, P.A., Amundson, R. (2003): Effect of land-use change on soil carbon in Hawaii. Biogeochemistry 65:213–232.

[33] Paul, K. I., Polglase, P. J., Nyakuengama, J. G., Khanna, P. K. (2002): Change in soil carbon following afforestation. – Forest Ecology and Management 168: 241-257.

[34] Peichl, M., Arain, M. A. (2006): Above- and belowground ecosystem biomass and carbon pools in an age-sequence of temperate pine plantation forests. – Agricultural and Forest Meteorology 140: 51–63.

[35] Peltoniemi, M., Makipaa, R., Liski, J., Tamminen, P. (2004): Changes in soil carbon with stand age–an evaluation of a modelling method with empirical data. – Global Change Biol. 10:2078–2091.

[36] Powers, J.S., Schlesinger, W.H. (2002): Relationships among soil carbon distributions and biophysical factors at nested spatial scales in rainforests of northeastern Costa Rica. – Geoderma 109(3-4):165-190.

[37] Pragasan, L.A; Parthasarathy, N. (2005): Litter production in tropical dry evergreen forests of south India in relation to season, plant life forms and physiognomic groups. CURRENT SCIENCE, VOL. 88, NO. 8: 1255-1263

[38] Richter, D.D., Markewitz, D., Trumbore, S.E., Wells, C.G. (1999): Rapid accumulation and turnover of soil carbon in a re-establishing forest. – Nature 400: 56–58.

[39] Steele, S.J., Gower, S.T., Vogel, J.G., Norman, J.M. (1997): Root mass, Root mass, net primary production and turnover in aspen, jack pine and black spruce forests in Saskatchewan and Manitoba, Canada. – Tree Physiol. 17:577-587.

[40] Thuille, A., Schulze, E.D. (2006): Carbon dynamics in successional and afforested spruce stands in Thuringia and the Alps. – Global Change Biology 12: 325–342.

[41] Uri, V., Varik, M., Aosaar, J., Kanal, A., Kukumägi, M., Lõhmus, K. (2012):Biomass production and carbon sequestration in a fertile silver birch (*Betula pendula* Roth) forest chronosequence. – Forest Ecology and Management 267: 117–126.

[42] United Nations Framework Convention on Climate change (UNFCCC). 1997. Kyoto protocol to the UnitedNations framework convention on climate change. Available on the Internet: http://unfccc.int/resource/ docs/convkp/kpeng.htlm.

[43] Varamesh, S., Hosseini, S.M, Abdi, N., Akbarinia, M. (2010): Effects of afforestation on Soil Carbon Sequestration in an Urban Forest of arid zone in Chitgar forest park of Tehran. – Forest Science 3: 75-90.

[44] Varamesh, S., Hosseini, S.M., Abdi, N. (2011): Estimating Potential of Urban Forests for Atmospheric Carbon Sequestration. – Journal of Environmental Studies 37 (57): 113-120.

[45] Varamesh, S., Hosseini, S.M., Keivan Behjou, F., Fataei, E. (2014): The impact of land afforestation on carbon stocks surrounding Tehran,Iran. – Journal of Forestry Research 25(1): 135−141.

[46] Watson, R.T. (2000): Land Use, Land-Use Change, and Forestry: A Special Report of the IPCC. – Cambridge University Press, Cambridge, p. 377.

[47] Xiao-Wen, D., Shi-Jie, H., Yang-Ling, H., Yu-Mei, Z. (2009): Carbon and nitrogen transformations in surface soils under Ermans Birch and Dark coniferous forests. – Pedosphere 19(2): 230-237.

[48] Yüksek, T., Yüksek, F. (2011): The effects of restoration on soil properties in degraded land in the semi-arid region of Turkey. – Catena 84:47–53.

[49] Zhang, C., Liu, G., Xue, S., Sun, C. (2013): Soil organic carbon and total nitrogen storage as affected by land use in a small watershed of the Loess Plateau, China. – European Journal of Soil Biology 54: 16-24.

[50] Zinn, Y.L., Resck, D.V.S., Silva, J.E. (2002): Soil organic carbon as affected by afforestation with Eucalyptus and Pinus in the Cerrado region of Brazil. – Forest Ecology and Management 166: 285–294.

ADVANCES IN CROP RESPONSES TO ENHANCED UV-B RADIATION

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Abstract The increased ultraviolet-B (UV-B) radiation (280–320 nm) on the Earth's surface is one of the most important concerns of global change. This concern is primarily because increased UV-B radiation has been unambiguously shown to be responsible for the majority of harmful effects on aquatic as well as terrestrial organisms, and thus influence ecological interactions. For the past 4 plus decades, many studies have been conducted on the damaging effects of elevated UV-B radiation on plants. These studies have shown a diverse range of responses to UV-B radiation, and might be in general arbitrarily divided into two classes, photomorphogenic and stress responses at the morphological, physiological, biochemical and molecular levels. Crop plants evolved different adaptive or defensive mechanisms to UV-B radiation, including accumulation of UV-absorbing sunscreens, production of enzymatic and non-enzymatic antioxidants, changes of phytohormones or activation of DNA-repairing enzymes. A diagram illustrating the general responses of UV-B radiation and complexity of the interactions among factors was developed. Three urgent specific researches are proposed, which might provide opportunities for genetic engineering and possibility of breeding to deal with potential crop yield reductions due to elevated UV-B in agricultural systems, and thus will play a major role in determining the crops future.

Keywords: *global change, UV-absorbing compounds, antioxidants, phytohormones, DNA-repai*r

Introduction

There has been significant interest in documenting the potential impacts of long-term increases in ultraviolet-B (UV-B, 280–320 nm) radiation on crop plants over the last several decades (Germ et al., 2006; Caldwell et al., 2007; Ballaré et al., 2011; Häder et al., 2011; Liu et al., 2013). Great intraspecific variation in the responses of plants to UV-B radiation has been observed in main crops, e.g., wheat (Li et al., 2010; Zu et al., 2010; Singh et al., 2012; Schreiner et al., 2012), maize (Biggs and Kossuth, 1978; Correia et al., 1999; Cartwright et al., 2001), soybean (Sullivan and Teramura, 1990; D'surney et al., 1993; Caldwell et al.,1994; Feng et al., 2001; Li et al., 2002; Zu et al., 2003; Baroniya et al., 2013), rice (Barnes et al., 1990; Dai et al., 1994; Kumagai et al., 2001; Hidema and Kumagai, 2006) and sorghum (Ambasht and Agrawal, 1998; Kataria and Guruprasad, 2012a). There has been significant interest in documenting the potential impacts of long-term increases in ultraviolet-B (UV-B, 280–320 nm) radiation on crop plants over

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the last several decades (Germ et al., 2006; Caldwell et al., 2007; Ballaré et al., 2011; Häder et al., 2011; Liu et al., 2013).

Direct effects of natural or enhanced levels of UV-B radiation on plant yield have been detected under field conditions. However, in quantitative terms, these effects tend to be modest, with growth reductions rarely exceeding 20% (Searles et al., 2001; Newsham and Robinson, 2009; Wargent et al., 2009; Ballaré et al., 2011). These differences in UV-B sensitivity among crops and cultivars can be due to different adaptive or defensive mechanisms to UV-B radiation, which provides opportunities for genetic engineering and possibility of breeding to deal with potential crop yield reductions due to elevated UV-B in agricultural systems, and thus will play a major role in determining the crops future.

Relative to the 1979–1992 conditions, for the 2010–2020 time period, the GISS model results indicate a springtime enhancement of erythemal UV doses of up to 14% in the Northern hemisphere and 40% in the Southern hemisphere (Taalas et al., 2000). Therefore, the discovery of how crop plants interact with UV-B radiation, and what kind of protective strategies or mechanisms they possess in order to cope with the harmful UVB radiation, is essential to a better understanding of the balance between damage and protection.

Earlier review on field crop responses to UV-B reported the effects of UV-B radiation on visual symptoms, cell and its components, leaf ultrastructure and anatomy, photosynthesis, growth and development, transpiration and stomatal conductance, production and yield, as well as interactions of UV-B with abiotic and biotic factors of crop plants such as low temperature and drought (Kakani et al., 2003; Breznik et al., 2009). This article summarized much-needed and useful information to researchers regarding the general consequences of ultraviolet-B radiation on crop plants from the morphological, physiological, especially biochemical, cellular and molecular levels, and intended to raise genetic modification questions for molecular biologists and geneticists to address, which will aid future climate negotiations and support growers to maintain high productivity.

Leaf morphology, anatomy and UV-absorbing compounds

Photomorphogenic responses result in altered architecture or chemical composition and may be thought to be adaptive responses of plants to the incident radiation micro-climate, which may ultimately modify the penetration of UV radiation into plants (Beggs and Wellman, 1994; Ballaré et al., 1992, 2011). UV-induced morphological changes include thicker leaves, shorter petioles, shorter stems, increased axillary branching and altered root: shoot ratios (Robson et al., 2015). The epidermal attenuation appears to be the dominant UV-B screening mechanism in the majority of plants. In order for UV radiation to be effective in plants, it must effectively penetrate into the tissues and be absorbed. Ultraviolet penetration varies with plant species. Penetration of UV-B was found to be the greatest in herbaceous dicotyledons (broad-leaf plants) and was progressively less in woody dicotyledons, grasses and conifers (Day et al., 1992, 1993).

Enhanced UV-B reduced leaf area and leaf thickness (indicated by specific leaf weight) has been reported in maize, *Amaranthus tricolor* and sorghum varieties (Correia et al., 1999; Kataria and Guruprasad, 2012), while specific leaf area and length of internodes and petiole in Indian cress (*Tropaeolum majus*) were unaffected by enhanced UV-B radiation (Germ et al., 2015). The decrease in leaf thickness may have increased the UV-B penetration within leaves and decreased photosynthetic rates and dry weight accumulation. Increased epidermal cell wall thickness was also found in loblolly pine (*Pinus taeda*) and Scots pine (Laakso et al., 2000). Qi et al. (2003) found that there was a good correlation between total leaf thickness and total concentration of leaf UV-B absorbing compounds in southern broadleaf tree species in USA, and a strong presence of UV-absorbing compounds in the upper and lower epidermis, the vascular bundles and the leaf hairs, if present. However, the main site of UV-B attenuation took place within the upper leaf epidermis (Qi et al., 2003).

The accumulation of flavonoids in the epidermis has been shown to reduce epidermal transmittance of UV-B radiation (Tevini et al., 1991), and those exhibited high epidermal transmittance may be less UV-B tolerant. Feng et al. (2003) found that the greater tolerance of soybean cultivar ‘Jindou’ to elevated UV-B exposures was attributed partly to its higher foliar flavonoid content, smaller leaf size, thicker leaf cuticle and scabrous (hairy) lamina. Differences in UV-absorption characteristics between a barley (*Hordeum vulgare*) mutant and the mother line indicated that the flavonoid mutant exhibits increased sensitivity to UV-B radiation, though the content of flavonoid in the mutant was only 7% compared to the mother variety in the primary leaf (Reuber et al., 1996). In addition, cuticular waxes and lignins may also serve protective roles by absorbing UV radiation (Caldwell et al., 1983). Wax content increased in tolerant genotypes while it decreased in the susceptible genotypes, because wax layer is an important surface character that responds to UV-B radiation (Kakani et al., 2004).

UV-B radiation is an important environmental factor for many plants with remarkable influence on defence-related secondary metabolite biosynthesis (Germ et al., 2015). Many studies suggest that the accumulation of UV-absorbing phenylpropanoid compounds, mainly flavonoids, anthocyanins and related phenolics in cell vacuoles and/or cell walls of the leaf epidermis is a protective measure against UV-B effects on mesophyll tissue of a leaf (Robberecht and Caldwell, 1978; Schmelzer et al., 1988; Day, 1993; Li et al., 1993; Beggs and Wellman, 1994; Rozema et al., 1997; Hutzler et al., 1998; Bornman, 1999; Mazza et al., 2000; Bieza and Lois, 2001; Flint et al., 2004; Sullivan et al., 2007; Izaguirre et al., 2007). In fact, in a meta-analysis, Searles et al. (2001) found that an increase in UV-absorbing compounds in response to supplemental UV-B was the most consistently reported response to UV-B radiation. UV-B absorbing compounds are present throughout the leaf, but accumulate significantly in leaf trichomes and epidermal cells (Zancan et al., 2008). They have effective radical scavenging capabilities, and can contribute directly to enhance photoprotection against UV-B radiation (Smith and Markham, 1998; Karioti et al., 2008; Li et al., 2012).

Arabidopsis flavonoid mutants are hypersensitive to UV-B radiation, thus confirming the role of flavonoids and other phenolic compounds in the UV-B protection of plants (Li

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et al., 1993). In addition, another *Arabidopsis* mutant reported to be tolerant to lethal UV-B levels showed constitutively elevated accumulation of flavonoids and other phenolic compounds (Bieza and Lois, 2001). Flavonoids not only served as UV-B filters, but also were hypothesized to act as antioxidants, by absorbing UV-B radiation in the upper tissue layer and thus preventing damage to sensitive targets, their absence could lead to greater oxidative stress (Peng et al., 2003). Changes in flavonoid contents in wheat leaves have been observed under field conditions (Li et al., 2000). In soybean, increases in total leaf phenolics exposed to ambient UV radiation were demonstrated by Mazza et al. (2000).

Levizou and Manetas (2002) showed significant correlations between total phenolic levels and UV-B absorbing capacity (simple methanolic absorbance at 300 nm). Enhanced UV-B radiation induced increased synthesis of total phenolic compounds in leaves, but not in flowers of Indian cress (*Tropaeolum majus*) (Germ et al., 2015). Koti et al. (2007) observed genotypic variation in the production of these compounds at high UV-B levels. However, opposite results were reported by different authors. Kreft et al. (2002) showed that exposure of buckwheat plants (*Fagopyrum esculentum* Moench) to elevated UV-B radiation reduced the accumulation of rutin, a flavonoid with antioxidant properties. Yao et al. (2006) found that effect of UV-B radiation on the concentration of the flavonoid rutin in buckwheat leaves depended on leaf position and the level of UV-B radiation. Rutin concentration was higher in top leaves than in lower ones regardless of the UV-B level, top leaves typically receive more radiation than lower leaves. Sullivan et al. (2007) did not find that even though UV-B absorbing compounds accumulated with an imposed stress, but these compounds were not directly related to sensitivity/tolerance of soybean genotypes.

Intraspecific differences in the composition and concentration of flavonoids have been found among five cultivars of Cucumis sativus (Murali and Teramura, 1986) and in two cultivars of soybean (Murali et al., 1988), 20 cultivars of wheat (Li et al., 2000), 10 soybean cultivars (Feng et al., 2001), and 20 soybean cultivars (Li et al., 2002). Screening of the total flavonoid contents in 20 Chinese soybean cultivars in a field study using UV-B lamps revealed that seven cultivars had increased total flavonoid levels while five showed decreased levels, and no changes were observed in eight cultivars (Zu et al., 2003). Since alterations in the levels of individual flavonoids were not taken into account, UV-B could have had an impact on certain compounds without increasing the total level. Warren et al. (2003) found that certain flavonoids were selectively produced after UV-B exposure.

A series of experiments provided convincing evidence that plants subjected to UV-B radiation responded to changes in the content and ratios of different flavonoid in leaf epidermal cells, wax, and hairs (Harborne and Williams, 2000). Some flavonoid increased much more than others, especially the flavonoids with ortho-hydroxy structures in B-ring such as quercetin and quercetin glycoside in Trifolium repens (Hofmann et al., 2000), luteolin in Marchantia polymorpha (Markham et al., 1998), chlorogenic acid in Cucumis sativus (Kondo and Kawashima, 2000), isoorientin acylated glucosides in Oryza sativa (Markham et al., 1998).

Winter and Rostas (2008) confirmed that three of the analyzed flavonoids showed a significant increase in plants receiving full ambient radiation. Of these, two compounds were quercetin based flavonols, resulting in a shift in the relative flavonol content in favor of the quercetin glycosides and at the expense of kaempferol glycosides. As quercetin flavonols are known to have an improved ability as free radical scavengers due to the additional ortho-dihydroxyl group in the B-ring (Harborne and Williams, 2000) compared to kaempferol flavonols, it might be of advantage for the plants to invest more in quercetin flavonols under UV stress. Due to our lack in understanding of flavonoid function in plants, further studies would be worthwhile. Laboratory studies have demonstrated that the regulation of flavonoid biosynthesis may involve multiple photoreceptors, including the phytochromes, blue-absorbing photoreceptors, and one or more UV photoreceptors (Beggs and Wellmann, 1994).

Genetic blocks in the synthesis of phenolic sunscreens in phenylpropanoid mutants are known to result in increased susceptibility to UV (e.g. Li et al., 1993; Lois and Buchanan, 1994; Stapleton and Walbot, 1994; Landry et al., 1995; Reuber et al., 1996), however, it is not yet clear whether the slight variations in levels of UV-absorbing compounds that are commonly detected among varieties of the same species or between plants subjected to different UV regimes are physiologically significant under field conditions.

The accumulation of anthocyanins in the vacuoles of epidermal cells where they attenuate the UV component of sunlight with minimal absorption of photosynthetically active radiation has also been suggested (Stapleton and Walbot, 1994; Landry et al., 1995). Gould et al. (2002) reported that purified anthocyanin extracts showed strong antioxidant properties in vitro, and they can also scavenge reactive oxygen in living cells. By real-time imaging of H2O2 in cells after mechanical injury, they found that anthocyanins, among various flavonoids, were the only molecules suitably located to account for the enhanced rates of H2O2 scavenging, suggesting that anthocyanins have elevated antioxidant capabilities in vivo (Gould et al., 2002). Therefore, the mechanism by which anthocyanins confer UV protection may involve UV absorption or scavenging of reactive oxygen species (ROS), or both. In Arabidopsis thaliana, sinapate esters also provide UV-B attenuation, but this biosynthetic pathway is not present in corn (*Zea mays*) (Sheahan, 1996).

Proline is regarded as an osmoprotectant, however, several authors implicated a role for proline in the detoxification of ROS (Saradhi et al., 1995; Matysik et al., 2002), and an enhanced accumulation of proline in soybean leaves could be linked with detoxification against Ni and UV-B induced oxidative stress (Prasad et al., 2005).

A similar manner as ascorbate or glutathione and function as an electron donor for the peroxidase reaction was assumed (Takahama and Oniki, 2000). A key function of ascorbic acid in the apoplast is redox buffering, which protects the plasmalemma from oxidative damage. It has been demonstrated that the symplastic ascorbate redox state is relatively constant throughout the life of a cell, despite large changes in apoplastic ascorbate. Moreover, the ascorbate redox state in the apoplast is largely independent of that in the symplasm and the ascorbate pool in the apoplast is flexible. This flexibility allows the cell to sense the environment and contribute to trigger molecular responses (Pignocchi and Foyer, 2003).

Due to our lack in understanding of functional significance of natural variations in phenylpropanoid levels, there is a knowledge gap regarding the photocontrol of phenylpropanoid accumulation under field conditions, and the dynamics of specific compound accumulation, localization patterns and constitutive or background levels of UV-screening compounds warrant further studies.

Besides the compounds mentioned above, recently, Smrkolj et al. (2006) proposed that selenium (Se) could protect plants from the harmful effects of UV-B radiation. They observed that enhanced UV-B radiation leads to higher selenium accumulation in flowers compared to ambient UV-B radiation conditions in buckwheat. Germ et al. (2009) found that for St. John's wort (*Hypericum perforatum* L.) herb, the highest concentration of Se was found in plants exposed to reduced UV-B radiation, which might be a self-denfence mechanism involved in this plant for antioxidative effects. Therefore, Selenium can increase the tolerance of plants to UV-induced oxidative stress, and there could be a similar connection between radiation and selenium as that known for flavonoids and radiation.

The antioxidant defense system

To keep UV-B damage to a minimum, plants possess enzymatic and non-enzymatic antioxidative defense systems in cellular compartments (Bowler et al., 1992). UV-B exposure is known to lead to the generation of active oxygen species (AOS) and eventually results in oxidative stress in plants (Arnott and Murphy, 1991; Dai et al., 1997; Hideg et al., 2003; Kalbina and Strid, 2006). AOS not only function as destructive radicals, but also as signaling molecules during UV-B responses (Green and Fluhr, 1995; Mackerness et al., 1997, 2001; Mackerness and Jordan, 1999).

The inhibition effect on plant growth and development was mainly due to enhanced oxidative stress caused by UV radiation (Jansen et al., 1998). It has also been reported that UV-B can promote the formation of lipid oxidation products, destroy the natural lipid soluble antioxidants (Salmon et al., 1990), and induce the expression of the genes which encode for antioxidants (Strid et al., 1994).

It has already been demonstrated that plant cells and tissues protect themselves against oxidative insults through the up-regulation of a wide variety of antioxidants enzymes to UV-B exposure (Davies, 1986; Beligni and Lamattina, 1999; Chen et al., 2003). The main enzymatic antioxidant defense system includes enzymes such as superoxide dismutase (SOD; EC 1.15.1.1), catalase (CAT; EC 1.11.1.6), guaiacol peroxidase (POD; EC 1.11.1.7), ascorbate peroxidase (APX; EC1.11.1.11), glutathione reductase (GR; EC1.6.4.2), and dehydroascorbate reductase (DHAR; EC1.8.5.1) (Bowler et al., 1994; Kondo and Kawashima, 2000).

SOD rapidly converts O_2 to H_2O_2 which can then be converted to water and oxygen by CAT (Noctor and Foyer, 1998). Contrasting responses of SOD to UV-B exposure have been reported revealing no uniform responses. For example, SOD activity was increased

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by UV-B radiation in pea and wheat (Alexieva et al., 2001), *Arabidopsis* (Rao and Ormord 1995), *Lemna gibba* (Babu et al., 2003) and rice (Dai et al., 1997), but was not affected in barley (Mazza et al., 1999) and soybean (Malanga et al., 1999), and was decreased in sunflower cotyledon (Costa et al., 2002).

Also, SOD expression was not affected by UV-B radiation in *Nicotiana plumbaginifolia* L. (Willekens et al., 1994), but was decreased in *Pisum sativum* (Strid et al., 1994). In a field study, supplemental UV-B increased SOD activity in wheat and mung bean (Agrawal and Rathore, 2007).

CAT is a constitutive component of peroxisomes and has a low substrate affinity (Corpas et al., 1999). An alternative mode of H_2O_2 destruction is via APX which is found throughout the cell (Jimenez et al., 1997). APX is a specific peroxidase that catalyzes the breakdown of H_2O_2 at the expense of oxidizing ascorbate to monodehydroascorbate. APX isozymes are distributed in at least four cells compartments: the stroma, the thylakoid membrane, the microbody, and the cytosol (Asada, 1992). The removal of H_2O_2 through series of reactions is known as the ascorbate–glutathione cycle (Noctor and Foyer, 1998).

Synthesis of antioxidant enzymes like POD, APX and SOD have been observed in UV-B treated *Arabidopsis thalian*a seedlings (Rao et al., 1996). Liu and McClure (1995) revealed that POD enzyme activities were increased under UV-B irradiation to adapt to the oxidative stress, and the SOD activities were changed differently according to the UV-B irradiation intensities (Tekchandani and Guruprasad, 1998).

Although it is not known how plants irradiated with UV-B generate AOS, it is thought that NADPH oxidase may be involved in the generation of AOS (Rao et al., 1996). Direct evidence of induction of NADP-malic enzyme by UV-B radiation was observed in leaves, stems and roots of three bean cultivars (Pinto et al., 2002). These results suggest that NADP-malic enzymes play an active role in plant defense responses against UV-B, possibly by providing NADPH for lignin and flavonoid biosynthesis. It is also possible that measures of only total activities of enzymes may not adequately reflect UV-induced compartment-specific changes or enzyme alterations that do not change total activity. For example, UV-B could differently regulate enzyme isoforms as reported for POD (Murali et al., 1988), CAT (Willekens et al., 1994), SOD (Babu et al., 2003; Rao et al., 1996) and APX (Yannarelli et al., 2006a) in previous studies. More studies are needed to resolve these issues.

Logemann et al. (1995) found UV-induction of enzymes can provide carbon substrates for the shikimate pathway, while Casati and Walbot (2003) proposed that induction of enzymes that can also provide energy in the form of ATP for the synthesis of these and other molecules necessary for cell functions under UV-B stress. Shweta and Agrawal (2006) have shown that simultaneous exposure of UV-B+Cd and UV-B+Ni caused increased accumulation of malondiadehyde (MDA) content in spinach. Increased MDA content caused by UV-B indicated a loss of membrane function and induction of oxidative damage (Li et al., 2012). Elevated MDA content is regarded as a sensitive indicator of oxidative stress in plants exposed to different stresses including Cd and UV-B (Wang et al., 2008).

A hierarchical cluster analysis by Zu et al. (2003) indicated that the contribution of each physiological indicator (% change) to the overall sensitivity of soybean cultivars to enhanced UV-B radiation had the following sequence: SOD activity, membrane permeability, flavonoid contents, malonaldehyde (MDA) contents, chlorophyll a contents, chlorophyll b contents. Zu et al. (2010) further demonstrated that UV-B induced oxidative stress via indirect mechanisms such as inhibition of antioxidative defense systems, or via the activation of ROS-producing enzymes such as NADPH oxidases.

Studies on the effects of UV-B on the enzymatic antioxidants at both the activity level (Agrawal and Rathore, 2007; Yannarelli et al., 2006b) and the mRNA level (Willekens et al., 1994) have yielded inconsistent results so it is not clear how uniform this response is among plant species and how this may be modified by concurrent environmental conditions. Yannarelli et al. (2006b) demonstrated that increased HO^- activity was associated with augmented protein expression and transcript levels.

The non-enzymatic defense system consists of low molecular weight antioxidants such as proline, ascorbate, glutathione, α-tocopherol, and carotenoids (Larson, 1988; Rao et al., 1996; Arora et al., 2002; Matysik et al., 2002; Giordano et al., 2004; Jain et al., 2004; Shiu and Lee, 2005). Ascorbic acid (AsA) is a major primary antioxidant reacting directly with hydroxyl radicals, superoxide and singlet oxygen, and also a powerful secondary antioxidant reducing the oxidized form of α-tocopherol. Increases in the AsA pool in response to UV-B exposure have been observed in several species (Galatro et al., 2001; Dai et al., 1997; Takeuchi et al., 1996; Rao and Ormord, 1995). However, in maize seedling, UV-B exposure had no effect on the AsA content (Carletti et al., 2003). Glutathione is the major low molecular weight thiol compound in most plants (Foyer et al., 1994). Overall, glutathione (or homoglutathione) appears to play a role in protection against oxidative damage arising from a number of stresses such as irradiation, heat, and exposure to heavy metals (Grill et al., 1985). Moreover, ascorbic acid and glutathione may be involved in several types of protective mechanisms (Wefers and Sies, 1988). The reduced and oxidized forms of ascorbate and glutathione are transported across the chloroplast envelope (Anderson et al., 1983; Beck et al., 1983) by transporters whose activity may be changed in response to stress.

Ultraviolet radiation has been shown to be very effective in inducing lipid oxidation of biological membranes (Kochevar, 1990; Foyer et al., 1994), polyunsaturated fatty acids (Yamashoji et al., 1979) and phospholipid liposomes (Pelle et al., 1990). There is a considerable amount of data that demonstrates ways in which UV radiation alters membrane structure or function: changes in membrane permeability, inhibited K-ATPase and peroxidized lipids in wheat (*Triticum aestivum*) (Li et al., 2000; Wright et al., 1981) and decreased membrane resistance in *Chara coralline* (Doughty and Hope, 1973). The damage to nonphotosynthetic membranes that are detected by electron microscopy generally requires high fluence or occurs only after a long lag time following irradiation. In the latter case, the effect of UV can be regarded as an acceleration of normal senescence processes (Skokut et al., 1977). The physiological effects of UV stimulated membrane changes are uncertain. There is little evidence that the UV damage to membranes is responsible for cell death. UV stimulated membrane changes may play a role in the UV-induced synthesis of anthocyanins (Murphy, 1983).

Hydrogen peroxide is known to diffuse across biological membranes and causes cellular damage. An increase in lipid peroxidation and H_2O_2 was demonstrated following UV-B treatments (Mishra et al., 2011). Understanding the mechanisms for removal of AOS is important for UV studies because increasing evidence suggests that AOS are involved in the damage caused by UV-B radiation. For example, UV-B radiation has been shown to increase AOS levels (Kalbina and Strid 2006; Hideg et al., 2003) and lipid peroxidation (Yannarelli et al., 2006b; Yang et al., 2005) in plants.

Therefore, adaptation or acclimation to photooxidative stress is multifactorial and many factors are involved in the overall defense strategy of the plant. A more indepth understanding of the generation and scavenging of AOS is needed before this relationship can be fully understood. However, very few studies have been conducted on the impacts of solar UV-B radiation on enzymes and antioxidants under natural and UV-B exclusion conditions (Mazza et al., 1999; Agrawal and Rathore 2007; Xu et al., 2008a).

Phytohormones responses to UV-B

It is well known that phytohormones play a vital role in the regulation of the growth and development of higher plants, as they are involved in controlling the ongoing process in the cell division, elongation and development, morphogenesis, and biological production (Beffa et al., 1990; Liu et al., 2010). The importance of the five “classical” classes of phytohormones in higher plants is well established. More recently, several other molecules have also been recognized as phytohormones. These include jasmonic acid (JA), salicylic acid (SA), brasinosteroids (BR) and polyamines (PA) (Saruhan et al., 2012).

The synthesis and action of phytohormones are modulated by environmental factors (Lachno and Baker, 1986). Plant hormones are the initiation factor of adversity-responsive gene expression (Zaffari et al. 1998). Studies indicated that a relatively small increase in UV-B can have dramatic effects on synthesis, transport, and allocation of plant endogenous hormones such as indole-3-acetic acid (IAA), cytokinin (CTK), and abscisic acid (ABA) etc., which resulted in inhibition of cell elongation, stomatal closure, and decreasing photosynthetic rate (Keiller and Holmes, 2001).

Photooxidation free-radical damage caused by strengthening UV-B radiation decreased IAA and gibberellins (GA) content, but increased indole acetic acid oxidase (IAAO) activity, which reinforced the harm from free radical induced by UV-B stress (Huang et al., 1997; Wang and Li, 2000; Huang et al., 2005).

Reduction in plant height has often been used as an index to assess the degree of UV-B radiation sensitivity (Biggs and Kossuth, 1978). UV-B radiation significantly dwarfed soybean, primarily due to shorter internodes rather than smaller node number (Teramura, 1980). This could be due to photo-oxidative destruction of the phytohormone IAA followed by reduced cell wall extensibility as demonstrated in sunflower seedlings (Ros and Tevini, 1995). The levels of ethylene, which promote radial growth and reduce elongation, are increased after irradiation with UV-B (Caldwell et al., 1995). However, the mechanism for UV-B radiation to increase plant height is still not clear. UV-B radiation may directly affect cell division and some intrinsic growth characteristics (Beggs et al., 1985).

It is believed that GA signaling is essential for internode elongation, cambial activity, and fiber differentiation, which has been documented in tobacco stems (Dayan et al., 2012). Phytochromes regulate GA synthesis during germination and seedling establishment. However, in the UV region of the spectrum, the absorption spectra for Pr and Pfr exhibit little discrimination (Lagarias et al., 1987; Chen et al., 2004) so this family of photoreceptors may not be involved in controlling soybean internode elongation.

Luo et al. (2006) and Zhu et al. (2006) demonstrated that several GA hormones were present in high concentrations in the upper-most internode of a mutant rice plant and were involved in the elongation of this internode. Also Sharma and Guruprasad (2009) demonstrated similarities in response of young *Amaranthus caudatus* plants to exclusion of UV-B and exclusion of both UV-A and UV-B with responses to external application of GA3, including increased hypocotyl lengths.

Changes in plant height caused by increases in the internode lengths due to UV-B radiation were likely mediated by a change in the presence of phytohormones or plant growth regulators, but the genetic mechanisms and biochemical syntheses that cause the changes are not known. Several experiments suggest the causal phytohormone is likely to be a GA, judged from the effect of exogenous applied GA upon soybean. Mislevy et al. (1988) applied GA3 to soybean at seedling emergence and found hypocotyl elongation and elongation of the 1st and 2nd internodes

Peng and Zhou (2009) using hydroponics culture investigated the effects of La III on the contents of endogenous hormone in soybean seedlings under elevated ultraviolet-B radiation (280–320 nm). They showed that the content of IAA in soybean seedlings decreased initially and then increased when the seedlings underwent UV-B treatment during the stress and convalescent period, while indole acetic acid oxidase (IAAO) activity increased at first and then decreased. A similar change of ABA content and IAAO content in soybean seedlings occurred; GA content decreased during the experiment compared with control. They also found that the content of IAA and GA in soybean seedlings with La III + UV-B treatment was higher than those of UV-B treatment; IAAO activity and GA content in soybean seedlings with La III + UV-B treatment were lower than those of UV-B treatment.

One function of ABA is to regulate activity of the stomatal guard cells. In bad conditions, accumulation of ABA in plant tissue can reduce stomatal conductance, caused stomatal closure, and inhibit photosynthesis. Studies have showed that UV-B leads to stomatal closure or incremented stomatal resistance (Tevini and Teramura, 1989; Bjorn, 1996), which resulted from leakage of K from the guard cell or changes of the stomatal regulated hormone ABA content (Yang et al., 2000).

The process of ABA induced stomatal closure required H_2O_2, and NO to attend, and the accumulation of ABA content by UV-B radiation originated from increased chloroplast membrane permeability, turgor loss, and disengaged inhibitory action of an

ABA synthetic (Burnett et al., 2000; Wang et al., 2001). Alonso et al. (2015) found that the triterpene squalene and the diterpene phytol were significantly higher in the treatment with combinations of water deficit, solar UV-B and ABA applications, and two application of ABA on leaves and berries, at veraison and 15 days after, were enough to activate compounds with antioxidant and antifungal properties, and thus proposed it as a possible acclimation mechanism that modifies membrane fluidity under environmental signals both biotic and abiotic.

Salicylic acid (SA) is considered to be an important signalling molecule, which plays an important role in regulating a number of physiological processes and plant resistances to stresses (Saruhan et al., 2012). Studies have demonstrated that SA can ameliorate the injurious effects of abiotic stresses on crops (Nazar et al., 2011; Bandurska and Cieślak 2013). Belkhadi et al. (2010) found that SA pre-soaking counteracted Chl destruction, and the foliar application of SA proved to be equally fruitful in increasing the pigment content (Hayat et al., 2005).

Plants accumulate large amounts of SA when exposed to UV radiation and SA is thought to be directly involved in signalling various antioxidant responses (Larkindale and Knight 2002; Bandurska and Cieślak 2013). Several reports show that SA can induce antioxidant activity under multiple stresses (Mutlu et al., 2009; Saruhan et al., 2012). A decline in activities of CAT, POD, and SOD was observed in plants treated with SA (Choudhury and Panda, 2004).

Li et al. (2014) showed that SA alleviated the adverse effects of Cd and/or UV-B on growth, and pigment content, but did not mitigate the inhibitory effect of Cd on chlorophyll fluorescence parameters in soybean seedlings. Cd and/or UV-B induced oxidative stress and increased lipid peroxidation that was significantly decreased by SA pre-treatment. They also showed that the Cd and/or UV-B increased SOD activity, decreased POD activity, and CAT activity was mostly unaltered. They thus proposed that SA might act as one of the potential antioxidants as well as a stabilizer of membrane integrity to improve plant resistance to the Cd and/or UV-B stress. Ervin et al. (2004) found that exogenous SA application alleviated the damaging effects induced by UV-B radiation in Kentucky bluegrass. SA stimulates photosynthetic machinery by increasing the content of chlorophyll in UV-stressed plants (Mahdavian et al., 2008). Stratmann (2003) reported that UV radiation may influence JA levels and lead to an overlap in gene expression caused by UV-B and herbivory.

However, the mechanism of plant hormones including SA- and JA-induced resistance to UV-B radiation is still unclear.

DNA damage and genetic consequences

Because of its absorption spectrum, DNA is a major target of UV-B damage; even low doses of radiation can kill mutants lacking specific DNA repair pathways (Britt et al., 1993; Britt, 1996; Landry et al., 1997). UV-B radiation is reported to cause cellular damage by generating photoproducts in DNA and direct damage to proteins (Gerhardt et al., 1999; McNamara and Hill, 2000; Bray and West, 2005).

A variety of DNA damage caused by UV radiation is due to direct absorption of UV-B radiation by the native DNA molecule or indirectly by oxidative stress via free radicals and reactive oxygen species (ROS) (Latifi et al., 2009). Hargreaves et al. (2007) proposed that UV-A radiation that is not directly absorbed by DNA, can still induce DNA damage either by producing a secondary photoreaction of existing DNA photoproducts or via indirect photosensitizing reactions. The measurements of DNA damage by Mazza et al. (2000) showed that the UV-B component of sunlight induced greater perturbations in the cells of those leaves that scored as more UV transparent in the fluorescence determinations. They also determined that, under field conditions, most of the sunscreen response induced by solar UV in soybean can be attributed to the UV-B component.

Repair mechanism of plants includes repair of DNA damages by excision repair or by repair of pyrimidine dimers as photolyase, activated by UV-A and photosynthetically active radiation (Britt, 1996; Taylor et al., 1997). Absorption of UV-B radiation by DNA causes phototransformations resulting in the production of cyclobutane pyrimidine dimers (CPDs) and pyrimidine (6–4) pyrimidona dimers (6–4 PPs). Because DNA and RNA polymerases are not able to read through these photoproducts, their elimination by CPD photolyases is essential for DNA replication and transcription (Britt and May, 2003).

It is well-documented that elevated UV-B radiation causes an up-regulation of genes and enzymes involved in the phenylpropanoid pathway (Chappell and Hahlbrock, 1984; Rozema et al., 1997; Ryan et al., 2002). Chalcone synthase (CHS; EC 2.3.1.74) catalyzes the first step reaction of the flavonoid biosynthesis, it may be possible to increase the production of UV-B-protective flavonoids by genetically improving the expression of CHS. Soybean CHS is encoded by a multigene family (GmCHS) of at least eight members (GmCHS1-GmCHS8) (Akada and Dube, 1995). Shimizu et al., (2000) reported that the expression of every member of the family was induced by exposure to white light and was enhanced further by supplemental UV-B radiation, except for that of GmCHS2. It has been shown that under realistic UV-B conditions, reduction in Rubisco levels is the primary cause for the decline in photosynthetic rate (Allen et al., 1997; Baker et al., 1997). Similarly, reduction in the expression and synthesis of Lhcb, encoding the harvesting complex proteins, and psbA, encoding the D1 polypeptide of PSII, could have potential impacts on the efficiency of the photosynthetic system (Jordan et al., 1998; Mackerness et al., 1997). Photosynthetic genes may be down-regulated (Surplus et al., 1998; Mackerness et al., 2001; Jordan, 2002).

Casati and Walbot (2003) examined the response of gene expression in maize to solar UV-B under field conditions, and found several photosynthesis-associated genes were decreased and antioxidant-associated genes were increased. Also, the genes involved in fatty acid metabolism and oxylipin biosynthesis were increased by solar UV-B (Izaguirre et al., 2003). Using microarray analysis, Casati and Walbot (2004) and Ulm et al. (2004) identified more than 100 UV-B responsive genes in maize and *Arabidopsis*, respectively. Yannarelli et al. (2006b) indicated that the up-regulation of HO-1 mRNA occurs in a manner similar to that found in other genes implicated in the

UV-B response. These results are the most comprehensive data currently available on the effects of solar UV-B on plant gene expression, and the actual signal transduction pathways activated by UV-B radiation are not yet well defined (Stratmann et al., 2000; Miles et al., 2002).

Earlier research indicated that plant MYB transcription factors regulate plant anthocyanin and phlobaphene biosynthesis (Dooner et al., 1991), trichome differentiation (Oppenheimer et al., 1991), epidermal cell shape determination (Noda et al., 1994), and gibberellin-regulated gene activation (Gubler et al., 1995). Shimizu et al. (2000) isolated and characterized a subfamily of GmMYB29 genes whose expression was found to be significantly upregulated upon UV-B irradiation. GmMZB29 consists of at least four closely related genes, which were classified into two groups based on their sequence similarity; groups A, and B. Expression of the group A members of the GmMYB29 subfamily was found to reach its peak within two hours after the onset of UV-B irradiation when the accumulation of GmCUS mRNA was still increasing. Similar time lag in the induction of an activator and its target genes has been reported in some other cases. For example, the expression of *Arabidopsis thatiana* Lhcb3 (Light-harvesting chlorophyll a/b binding protein) is induced by light irradiation for 1 hour and its mRNA accumulation increased even under continuous light irradiation up to 12 hours, whereas in CC41, which encodes a putative transcription factor of Lhcb3, the peak of mRNA accumulation was reached after irradiation for 1 hour (Wang et al., 1997).

Spraying plants with antioxidants prior to UV-B treatment can block the increase in pathogen-related transcripts and the decrease in photosynthetic transcripts (Surplus et al., 1998; Mackerness et al., 1999). This was an indication that ROS were involved in the pathway leading to changes in the level of these transcripts in response to UV-B radiation. To assess the role of ROS in the induction of HO-1 transcript levels, Yannarelli et al. (2006a) evaluated the action of AsA pre-treatment on the effects of UV-B. Consistent with the involvement of ROS in the regulation of HO-1 gene expression in response to UV-B, the increase in transcripts was blocked by pre-treatment with AsA.

Measuring DNA damage in higher plants is important in assessing the impacts of increased UV, and in testing the relationship of productivity to DNA damage and repair (Bennett et al., 2001). We still do not have a complete understanding of the molecular bases of these responses, but they generally are the result of signal perception by receptor molecules and transduction of a response signal to the cellular machinery, a part of which may regulate gene expression. However, Xu et al. (2008b) did not detect protein effects involved in the signal transduction, because many of the proteins involved in the signal transduction occurred in too low abundance in crude extracts and membrane proteins were usually under-represented on 2-D PAGE gels.

Also research at the mRNA level may not necessarily translate into the quantity and quality of the final gene products, i.e. the proteins. There is a loose correlation between mRNA and protein levels, especially for chloroplast genes, which are usually controlled at the post-transcriptional level (Mackerness et al., 1997). Moreover, many proteins undergo post-translational modifications (PTM) such as removal of signal peptides, phosphorylation and glycosylation, which are extremely important for protein activities and subcellular localizations. Therefore, changes at the mRNA level alone may not adequately assess the response to UV-B, and it is necessary to study the effects of UV-B at the protein level. There has been only limited research on the effects of UV-B on proteins, and most of this research focused on a single protein, such as PR-1 (Green and Fluhr, 1995), glutathione reductase, ascorbate peroxidase, superoxide dismutase (Rao et al., 1996) or nitrite reductase (Migge et al., 1998), and heme oxygenase (Yannarelli et al., 2006b).

Suchar and Robberecht (2014) developed a process-based model integrating the effects of UV-B radiation through epidermis, cellular DNA, and its consequences to the leaf expansion. They found that enhanced UV-B radiation induced DNA damage significantly delayed cell division, resulting in significant reductions in leaf growth and development. Leaf expansion was highly dependent on the number of cyclobutane pyrimidine dimers (CPD) present in the DNA, as a result of UV-B radiation dose, quantitative and qualitative absorptive properties of epidermal pigments, and repair mechanisms. Therefore, a thorough understanding the molecular basis of the UV-B response needs in depth research on proteome.

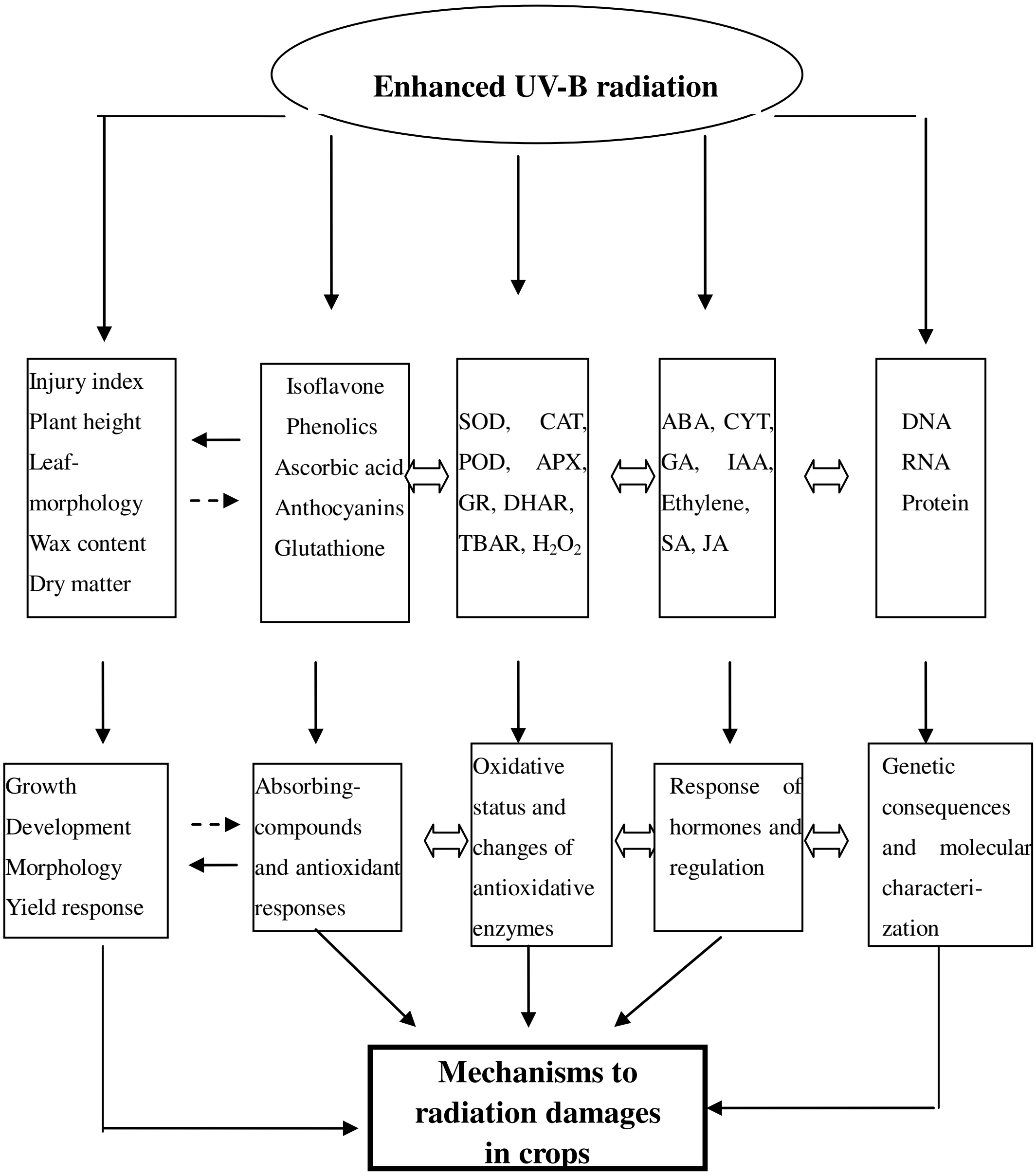
Summary

Based on available information in regard to the effects of the enhanced UV-B radiation on crops, the general responses of UV-B radiation could be proposed as in *Figure 1* to illustrate the complexity of the interactions among factors. The adaptation or acclimation to photooxidative stress is multifactorial and many factors are involved in the overall defense strategy of the plant. A more indepth understanding of the generation and scavenging of AOS is needed before this relationship can be fully understood.

Notwithstanding substantial new knowledge of molecular, cellular and organismal UV-B responses, there remains a clear gap in our understanding of the interactions between these organizational levels, and how they control plant architecture. It remains unproven whether UV-induced morphological changes have a protective function involving shading and decreased leaf penetration of UV-B, counterbalancing trade-offs such as decreased photosynthetic light capture and plant-competitive abilities. Future research will need to disentangle seemingly contradictory interactions occurring at the threshold UV dose where regulation and stress-induced morphogenesis overlap.

Due to our lack in understanding of functional significance of natural variations in phenylpropanoid levels, there is a knowledge gap regarding the photocontrol of phenylpropanoid accumulation under field conditions, and the dynamics of specific compound accumulation, localization patterns and constitutive or background levels of UV-screening compounds warrant further studies. The mechanism of plant hormones including SA- and JA-induced resistance to UV-B radiation is still unclear. A thorough understanding the molecular basis of the UV-B response needs in depth research on proteome.

Figure 1. *Interactions among factors and mechanism involved in preventing UV-B radiation damages*



Three specific researches are urgently needed, they are: (1) to differentiate the main UV-absorbing compounds and non-enzymatic antioxidants in contributing to defense system, (2) to investigate the specific role of phytohormones in response to UV-B radiation, and (3) to identify genetic consequences caused by full-season UV-B radiation and fill in the knowledge gap regarding the photocontrol mechanisms of UV-B to crops under field conditions.

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REFERENCES

[1] Agrawal, S.B., Rathore, D. (2007): Changes in oxidative stress defense system in wheat (*Triticum aestivum* L.) and mung bean (*Vigna radiata* L.) cultivars grown with and without mineral nutrients and irradiated by supplemental ultraviolet-B. -Environmental and Experimental Botany 59: 21-33.

[2] Akada, S., Dube, S.K. (1995): Organization of soybean chalcone synthase gene clusters and characterization of a new member of the family. -Plant Molecular Biology 29: 189-199.

[3] Alexieva, V., Sergiev, I., Mapelli, S., Karanov, E. (2001): The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat. -Plant Cell and Environment 24: 1337-1344.

[4] Allen, D.J., McKee, I.F., Farage, P.K., Baker, N.R. (1997): Analysis of limitations to CO_2 assimilation on exposure of leaves of two *Brassica napus* cultivars to UV-B. -Plant Cell and Environment 20: 633-640.

[5] Alonso, R., Berli, F.J., Bottini, R., Piccoli, P. (2015): Acclimation mechanisms elicited by sprayed abscisic acid, solar UV-B and water deficit in leaf tissues of field-grown grapevines. -Plant Physiology and Biochemistry 91: 56-60.

[6] Ambasht, N.K., Agrawal, M. (1998): Physiological and biochemical responses of *Sorghum vulgare* plants to supplemental ultraviolet-B radiation. -Canadian Journal of Botany-Revue Canadienne De Botanique 76: 1290-1294.

[7] Anderson, J.W., Foyer, C.H., Walker, D.A. (1983): Light-dependent reduction of dehydroascorbate and uptake of exogenous ascorbate by spinach-chloroplasts. -Planta 158: 442-450.

[8] Arnott, T., Murphy, T.M. (1991): A comparison of the effects of a fungal elicitor and ultraviolet-radiation on ion-transport and hydrogen-peroxide synthesis by rose cells. -Environmental and Experimental Botany 31: 209-216.

[9] Arora, A., Sairam, R.K., Srivastava, G.C. (2002): Oxidative stress and antioxidative system in plants. -Current Science 82: 1227-1238.

[10] Asada, K. (1992): Ascorbate peroxidase - a hydrogen peroxide-scavenging enzyme in plants. -Physiologia Plantarum 85: 235-241.

[11] Babu, T.S., Akhtar, T.A., Lampi, M.A., Tripuranthakam, S., Dixon, D.G., Greenberg, B.M. (2003): Similar stress responses are elicited by copper and ultraviolet radiation in the aquatic plant Lemna gibba: Implication of reactive oxygen species as common signals. -Plant and Cell Physiology 44: 1320-1329.

[12] Baker, N.R., Nogues, S., Allen, D.J. (1997): Photosynthesis and Photo Inhibition. -In: Lumsden, P.J. (ed.) Plants and UV-B: Responses to Environmental Change, Cambridge University Press, Cambridge

[13] Ballare, C.L., Caldwell, M.M., Flint, S.D., Robinson, A., Bornman, J.F. (2011): Effects of solar ultraviolet radiation on terrestrial ecosystems. Patterns, mechanisms, and interactions with climate change. -Photochemical & Photobiological Sciences 10: 226-241.

[14] Ballare, C.L., Scopel, A.L., Sanchez, R.A., Radosevich, S.R. (1992): Photomorphogenic processes in the agricultural environment. -Photochemistry and Photobiology 56: 777-788.

[15] Bandurska, H., Cieslak, M. (2013): The interactive effect of water deficit and UV-B radiation on salicylic acid accumulation in barley roots and leaves. -Environmental and Experimental Botany 94: 9-18.

[16] Barnes, P.W., Flint, S.D., Caldwell, M.M. (1990): Morphological responses of crop and weed species of different growth forms to ultraviolet-B radiation. -American Journal of Botany 77: 1354-1360.

[17] Baroniya, S.S., Kataria, S., Pandey, G.P., Guruprasad, K.N. (2013): Intraspecific variations in antioxidant defense responses and sensitivity of soybean varieties to ambient UV radiation. -Acta Physiologiae Plantarum 35: 1521-1530.

[18] Beck, E., Burkert, A., Hofmann, M. (1983): Uptake of ascorbate by intact spinach-chloroplasts. -Plant Physiology 73: 41-45.

[19] Beffa, R., Martin, H.V., Pilet, P.E. (1990): *In vitro* oxidation of indoleacetic-acid by soluble auxin-oxidases and peroxidases from maize roots. -Plant Physiology 94: 485-491.

[20] Beggs, C.J., Schneider-Zeibert, R., Wellman, E. (1985): UV-B Radiation and Sdaptive Mechanisms in Plants. -In: Worrest, R.C. (ed.) Stratospheric Ozone Reduction, Solar Ultraviolet Radiation and Plant Life, Springer Verlag, Berlin

[21] Beggs, C.J., Wellman, E. (1994): Photocontrol of flavonoid synthesis. -In: Kendrick, R.E., Kronenberg, G.H.M. (ed.) Photomorphogenesis in Plants, Kluwer Academic Publishers, Boston

[22] Beligni, M.V., Lamattina, L. (1999): Nitric oxide protects against cellular damage produced by methylviologen herbicides in potato plants. -Nitric Oxide-Biology and Chemistry 3: 199-208.

[23] Belkhadi, A., Hediji, H., Abbes, Z., Nouairi, I., Barhoumi, Z., Zarrouk, M., Chaibi, W., Djebali, W. (2010): Effects of exogenous salicylic acid pre-treatment on cadmium toxicity and leaf lipid content in *Linum usitatissimum* L. -Ecotoxicology and Environmental Safety 73: 1004-1011.

[24] Bennett, P.V., Hada, M., Hidema, J., Lepre, A.M., Pope, L.C., Quaite, F.E., Sullivan, J.H., Takayanagi, S., Sutherland, J.C., Sutherland, B.M. (2001): Isolation of high molecular length DNA: Alfalfa, pea, rice, sorghum, soybean, and spinach. -Crop Science 41: 167-172.

[25] Breznik, B., Grem, M., Kreft, I., Gaberscik, A. Crop responses to enhanced UV-B radiation. In: Singh, S. N. (ed.). Climate change and crops (Environmental science and engineering). Berlin: Springer, 2009, 269-281.

[26] Bieza, K., Lois, R. (2001): An Arabidopsis mutant tolerant to lethal ultraviolet-B levels shows constitutively elevated accumulation of flavonoids and other phenolics. -Plant Physiology 126: 1105-1115.

[27] Biggs, R.H., Kossuth, S.V. (1978): Effect of ultraviolet-B radiation enhancement under field conditions on potatoes, tomatoes, corn, rice, southern peas, peanut, squash, mustard and radish. UVB Biological and Climatic Effects Research, Final Report, EPA, Washington, D.C.

[28] Bjorn, L.O. (1996) Effects of ozone depletion and increased UVB on terrestrial ecosystems. -International Journal of Environmental Studies 51: 217-243.

[29] Bornman, J.F. (1999): Localisation and functional significance of flavonoids and related compounds. -Stratospheric Ozone Depletion: The Effects of Enhanced UV-B Radiation on Terrestrial Ecosystems: 59-69.

[30] Bowler, C., Vancamp, W., Vanmontagu, M., Inze, D. (1992): Superoxide dismutase and stress tolerance. Annual Review of Plant Physiology and Plant Molecular Biology 43; 83-116.

[31] Bowler, C., Vancamp, W., Vanmontagu, M., Inze, D. (1994): Superoxide-dismutase in plants. -Critical Reviews in Plant Sciences 13: 199-218.

[32] Bray, C.M., West, C.E. (2005): DNA repair mechanisms in plants: crucial sensors and effectors for the maintenance of genome integrity. -New Phytologist 168: 511-528.

[33] Britt, A.B. (1996): DNA damage and repair in plants. -Annual Review of Plant Physiology and Plant Molecular Biology 47: 75-100.

[34] Britt, A.B., Chen, J.J., Wykoff, D., Mitchell, D. (1993): A UV-sensitive mutant of arabidopsis defective in the repair of pyrimidine-pyrimidinone(6-4) dimers. -Science 261: 1571-1574.

[35] Britt, A.B., May, G.D. (2003): Re-engineering plant gene targeting. -Trends in Plant Science 8: 90-95.

[36] Burnett, E.C., Desikan, R., Moser, R.C., Neill, S.J. (2000): ABA activation of an MBP kinase in Pisum sativum epidermal peels correlates with stomatal responses to ABA. -Journal of Experimental Botany 51: 197-205.

[37] Caldwell, M., Teramura, A.H., Tevini, M., Bornman, J.F., Bjorn, L.O., Kulandaivelu, G. (1995): Effects of increased solar ultraviolet-radiation on terrestrial plants. -Ambio 24: 166-173.

[38] Caldwell, M.M., Ballare, C.L., Bornman, J.F., Flint, S.D., Bjorn, L.O., Teramura, A.H., Kulandaivelu, G., Tevini, M. (2003): Terrestrial ecosystems increased solar ultraviolet radiation and interactions with other climatic change factors. -Photochemical & Photobiological Sciences 2: 29-38.

[39] Caldwell, M.M., Flint, S.D., Searles, P.S. (1994): Spectral balance and UV-B sensitivity of soybean - a field experiment. -Plant Cell and Environment 17: 267-276.

[40] Caldwell, M.M., Robberecht, R., Flint, S.D. (1983): Internal filters-prospects for UV-acclimation in higher-plants. -Physiologia Plantarum 58: 445-450.

[41] Carletti, P., Masi, A., Wonisch, A., Grill, D., Tausz, M., Ferretti, M. (2003): Changes in antioxidant and pigment pool dimensions in UV-B irradiated maize seedlings. -Environmental and Experimental Botany 50: 149-157.

[42] Cartwright, H.N., Baucom, C., Singh, P., Smith, K.L., Stapleton, A.E. (2001): Intraspecific comparisons reveal differences in the pattern of ultraviolet radiation responses in four maize (*Zea mays* L.) varieties. -Journal of Photochemistry and Photobiology B-Biology 62: 88-96.

[43] Casati, P., Walbot, V. (2003): Gene expression profiling in response to ultraviolet radiation in maize genotypes with varying flavonoid content. -Plant Physiology 132: 1739-1754.

[44] Casati, P., Walbot, V. (2004): Rapid transcriptome responses of maize (*Zea mays*) to UV-B in irradiated and shielded tissues. -Genome Biology 5.

[45] Chappell, J., Hahlbrock, K. (1984): Transcription of plant defense genes in response to UV-light or fungal elicitor. -Nature 311: 76-78.

[46] Chen, K., Feng, H., Zhang, M., Wang, X. (2003): Nitric oxide alleviates oxidative damage in the green alga Chlorella pyrenoidosa caused by UV-B radiation. -Folia Microbiologica 48: 389-393.

[47] Chen, M., Chory, J., Fankhauser, C. (2004): Light signal transduction in higher plants. -Annual Review of Genetics 38: 87-117.

[48] Choudhury, S., Panda, S.K. (2004) Role of salicylic acid in regulating cadmium induced oxidative stress in *Oryza sativa* L. roots. -Bulgarian Journal of Plant Physiology 30: 95-110.

[49] Corpas, F.J., Palma, J.M., Sandalio, L.M., Lopez-Huertas, E., Romero-Puertas, M.C., Barroso, J.B., Del Rio, L.A. (1999): Purification of catalase from pea leaf peroxisomes:

Identification of five different isoforms. -Free Radical Research 31: S235-S241.

[50] Correia, C.M., Areal, E.L.V., Torres-Pereira, M.S., Torres-Pereira, J.M.G. (1999): Intraspecific variation in sensitivity to ultraviolet-B radiation in maize grown under field conditions - II. Physiological and biochemical aspects. -Field Crops Research 62: 97-105.

[51] Costa, H., Gallego, S.M., Tomaro, M.L. (2002): Effect of UV-B radiation on antioxidant defense system in sunflower cotyledons. -Plant Science 162: 939-945.

[52] Dai, Q.J., Peng, S.B., Chavez, A.Q., Vergara, B.S. (1994): Intraspecific responses of 188 rice cultivars to enhanced UVB radiation. -Environmental and Experimental Botany 34: 433-442.

[53] Dai, Q.J., Yan, B., Huang, S.B., Liu, X.Z., Peng, S.B., Miranda, M.L.L., Chavez, A.Q., Vergara, B.S., Olszyk, D.M. (1997): Response of oxidative stress defense systems in rice (*Oryza sativa*) leaves with supplemental UV-B radiation. -Physiologia Plantarum 101: 301-308.

[54] Davies, K.J. (1986): Intracellular proteolytic systems may function as secondary antioxidant defenses: an hypothesis. -Journal of Free Radicals in Biology & Medicine 2: 155-173.

[55] Day, T.A. (1993): Relating UV-B radiation screening effectiveness of foliage to absorbing-compound concentration and anatomical characteristics in a diverse group of plants. -Oecologia 95: 542-550.

[56] Day, T.A., Vogelmann, T.C., Delucia, E.H. (1992): Are some plant life forms more effective than others in screening out ultraviolet-B radiation. -Oecologia 92: 513-519.

[57] Dayan, J., Voronin, N., Gong, F., Sun, T.-p., Hedden, P., Fromm, H., Aloni, A. (2012): Leaf-Induced Gibberellin Signaling Is Essential for Internode Elongation, Cambial Activity, and Fiber Differentiation in Tobacco Stems. -Plant Cell 24: 66-79.

[58] Dooner, H.K., Robbins, T.P., Jorgensen, R.A. (1991): Genetic and developmental control of anthocyanin biosynthesis. -Annual Review of Genetics 25: 173-199.

[59] Doughty, C.J., Hope, A.B. (1973): Effects of ultraviolet radiation on the membranes of *Chara corallina*. -The Journal of Membrane Biology 13**:** 185-198.

[60] Dsurney, S.J., Tschaplinski, T.J., Edwards, N.T., Shugart, L.R. (1993): Biological responses of 2 soybean cultivars exposed to enhanced UVB radiation. -Environmental and Experimental Botany 33: 347-356.

[61] Ervin, E.H., Zhang, X.Z., Fike, J.H. (2004): Ultraviolet-B radiation damage on Kentucky Bluegrass II: Hormone supplement effects. -Hortscience 39: 1471-1474.

[62] Feng, H.Y., An, L.Z., Chen, T., Qiang, W.Y., Xu, S.J., Zhang, M.X., Wang, X.L., Cheng, G.D. (2003): The effect of enhanced ultraviolet-B radiation on growth, photosynthesis and stable carbon isotope composition (δC-13) of two soybean cultivars (*Glycine max*) under field conditions. -Environmental and Experimental Botany 49: 1-8.

[63] Feng, H.Y., Chen, T., Xu, S.J., An, L.Z., Qiang, W.Y., Zhang, M.X., Wang, X.L. (2001): Effect of enhanced UV-B radiation on growth, yield and stable carbon isotope composition in *Glycine max* cultivars. -Acta Botanica Sinica 43: 709-713.

[64] Flint, S.D., Searles, P.S., Caldwell, M.M. (2004): Field testing of biological spectral weighting functions for induction of UV-absorbing compounds in higher plants. -Photochemistry and Photobiology 79: 399-403.

[65] Foyer, C.H., Lelandais, M., Kunert, K.J. (1994): Photooxidative stress in plants. -Physiologia Plantarum 92: 696-717.

[66] Galatro, A., Simontacchi, M., Puntarulo, S. (2001): Free radical generation and antioxidant content in chloroplasts from soybean leaves exposed to ultraviolet-B.

-Physiologia Plantarum 113: 564-570.

[67] Gerhardt, K.E., Wilson, M.I., Greenberg, B.M. (1999): Tryptophan photolysis leads to a UVB-induced 66 kDa photoproduct of ribulose-1, 5-bisphosphate carboxylase/oxygenase (Rubisco) in vitro and in vivo. -Photochemistry and Photobiology 70: 49-56.

[68] Germ, M., Mazej, Z., Gaberšcik, A., Trošt Sedej, T. (2006): The response of *Ceratophyllum demersum* L. and *Myriophyllum spicatum* L. to reduced, ambient, and enhanced ultraviolet-B radiation. -Hydrobiologia (Den Haag) 570: 47–51.

[69] Germ, M., Stibilj, V., Kreft, S., Gaberšcik, A., Pajk, F., Kreft, I. (2009): Selenium concentration in St. John's wort (*Hypericum perforatum* L.) herb after foliar spraying of young plants under different UV-B radiation levels. -Food Chemistry 117: 204-206.

[70] Germ, M., Spahic, I., Gaberšcik, A. (2015): Morphological, biochemical and physiological responses of Indian cress (*Tropaeolum majus*) to elevated UV-B radiation. -Periodicum Biologorum 117 (3): 357-364.

[71] Giordano, C.V., Galatro, A., Puntarulo, S., Ballare, C.L. (2004): The inhibitory effects of UV-B radiation (280-315 nm) on Gunnera magellanica growth correlate with increased DNA damage but not with oxidative damage to lipids. -Plant Cell and Environment 27: 1415-1423.

[72] Gould, K.S., McKelvie, J., Markham, K.R. (2002): Do anthocyanins function as antioxidants in leaves? Imaging of H_2O_2 in red and green leaves after mechanical injury. -Plant Cell and Environment 25: 1261-1269.

[73] Green, R., Fluhr, R. (1995): UV-B-induced PR-1 accumulation is mediated by active oxygen species. -Plant Cell 7: 203-212.

[74] Grill, E., Winnacker, E.L., Zenk, M.H. (1985): Phytochelatins - the principal heavy-metal complexing peptides of higher-plants. -Science 230: 674-676.

[75] Gubler, F., Kalla, R., Roberts, J.K., Jacobsen, J.V. (1995): Gibberellin-regulated expression of a myb gene in barley aleurone cells - evidence for myb transactivation of a high-pl alpha-amylase gene promoter. -Plant Cell 7: 1879-1891.

[76] Häder, D.P., Helbling, E.W., Williamson, C.E., Worrest, R.C. (2011): Effects of UV radiation on aquatic ecosystems and interactions with climate change. -Photochemical & Photobiological Sciences 10: 242-260.

[77] Harborne, J.B., Williams, C.A. (2000): Advances in flavonoid research since 1992. -Phytochemistry 55: 481-504.

[78] Hargreaves, A., Taiwo, F.A., Duggan, O., Kirk, S.H., Ahmad, S.I. (2007): Near-ultraviolet photolysis of beta-phenylpyruvic acid generates free radicals and results in DNA damage. -Journal of Photochemistry and Photobiology B-Biology 89: 110-116.

[79] Hayat, S., Maheshwari, P., Wani, A.S., Irfan, M., Alyemeni, M.N., Ahmad, A. (2012): Comparative effect of 28 homobrassinolide and salicylic of NaCl stress in Brassica juncea L. -Plant Physiology and Biochemistry 53: 61-68.

[80] Hideg, E., Nagy, T., Oberschall, A., Dudits, D., Vass, I. (2003): Detoxification function of aldose/aldehyde reductase during drought and ultraviolet-B (280-320 nm) stresses. -Plant Cell and Environment 26: 513-522.

[81] Hidema, J., Kumagai, T. (2006): Sensitivity of rice to ultraviolet-B radiation. -Annals of Botany 97: 933-942.

[82] Hofmann, R.W., Swinny, E.E., Bloor, S.J., Markham, K.R., Ryan, K.G., Campbell, B.D., Jordan, B.R., Fountain, D.W. (2000): Responses of nine Trifolium repens L. populations to ultraviolet-B radiation: Differential flavonol glycoside accumulation and biomass

production. -Annals of Botany 86: 527-537.

[83] Huang, S.B., Dai, Q.J., Peng, S.B., Chavez, A.Q., Miranda, L.L., Visperas, R.M., Vergara, B.S. (1997): Influence of supplemental ultraviolet-B on indoleacetic acid and calmodulin in the leaves of rice (*Oryza sativa* L). -Plant Growth Regulation 21: 59-64.

[84] Huang, X.H., Zhou, Q., Zhang, G.S. (2005): Advances on rare earth application in pollution ecology. -Journal of Rare Earths 23: 5-11.

[85] Hutzler, P., Fischbach, R., Heller, W., Jungblut, T.P., Reuber, S., Schmitz, R., Veit, M., Weissenbock, G., Schnitzler, J.P. (1998): Tissue localization of phenolic compounds in plants by confocal laser scanning microscopy. -Journal of Experimental Botany 49: 953-965.

[86] Izaguirre, M.M., Mazza, C.A., Svatos, A., Baldwin, I.T., Ballare, C.L. (2007): Solar ultraviolet-B radiation and insect herbivory trigger partially overlapping phenolic responses in Nicotiana attenuata and Nicotiana longiflora. -Annals of Botany 99: 103-109.

[87] Izaguirre, M.M., Scopel, A.L., Baldwin, I.T., Ballare, C.L. (2003): Convergent responses to stress. Solar ultraviolet-B radiation and Manduca sexta herbivory elicit overlapping transcriptional responses in field-grown plants of Nicotiana longiflora. -Plant Physiology 132: 1755-1767.

[88] Jain, K., Kataria, S., Guruprasad, K.N. (2004): Effect of UV-B radiation on antioxidant enzymes and its modulation by benzoquinone and alpha-tocopherol in cucumber cotyledons. -Current Science 87: 87-90.

[89] Jansen, M.A.K., Gaba, V., Greenberg, B.M. (1998): Higher plants and UV-B radiation: Balancing damage, repair and acclimation. -Trends in Plant Science 3: 131-135.

[90] Jimenez, A., Hernandez, J.A., delRio, L.A., Sevilla, F. (1997): Evidence for the presence of the ascorbate-glutathione cycle in mitochondria and peroxisomes of pea leaves. -Plant Physiology 114: 275-284.

[91] Jordan, B.R. (2002): Molecular response of plant cells to UV-B stress. -Functional Plant Biology 29: 909-916.

[92] Jordan, B.R., James, P.E., Mackerness, S.A.H. (1998): Factors affecting UV-B-induced changes in *Arabidopsis thaliana* L. gene expression: the role of development, protective pigments and the chloroplast signal. -Plant and Cell Physiology 39: 769-778.

[93] Kakani, V.G., Reddy, K.R., Zhao, D., Gao, W. (2004): Senescence and hyperspectral reflectance of cotton leaves exposed to ultraviolet-B radiation and carbon dioxide. -Physiologia Plantarum 121: 250-257.

[94] Kalbina, I., Strid, A. (2006): Supplementary ultraviolet-B irradiation reveals differences in stress responses between *Arabidopsis thaliana* ecotypes. -Plant Cell and Environment 29: 754-763.

[95] Karioti, A., Kitsaki, C.K., Zygouraki, S., Ziobora, M., Djeddi, S., Skaltsa, H., Liakopoulos, G. (2008): Occurrence of flavonoids in Ophrys (*Orchidaceae*) flower parts. -Flora 203: 602-609.

[96] Kakani, V.G., Reddy, K.R., Zhao, D., Sailaja, K. (2003): Field crop responses to ultraviolet-B radiation: a review. Agricultural and Forest Meteorology 120: 191-218.

[97] Kataria, S., Guruprasad, K.N. (2012a): Intraspecific variations in growth, yield and photosynthesis of sorghum varieties to ambient UV (280-400 nm) radiation. -Plant Science 196: 85-92.

[98] Kataria, S., Guruprasad, K.N. (2012b): Solar UV-B and UV-A/B exclusion effects on intraspecific variations in crop growth and yield of wheat varieties. -Field Crops Research

125: 8-13.

[99] Keiller, D.R., Holmes, M.G. (2001): Effects of long-term exposure to elevated UV-B radiation on the photosynthetic performance of five broad-leaved tree species. -Photosynthesis Research 67: 229-240.

[100] Kochevar, I.E. (1990): UV-induced protein alterations and lipid oxidation in erythrocyte-membranes. -Photochemistry and Photobiology 52: 795-800.

[101] Kondo, N., Kawashima, M. (2000): Enhancement of the tolerance to oxidative stress in cucumber (*Cucumis sativus* L.) seedlings by UV-B irradiation: Possible involvement of phenolic compounds and antioxidative enzymes. -Journal of Plant Research 113: 311-317.

[102] Koti, S., Reddy, K.R., Kakani, V.G., Zhao, D., Gao, W. (2007): Effects of carbon dioxide, temperature and ultraviolet-B radiation and their interactions on soybean (*Glycine max* L.) growth and development. -Environmental and Experimental Botany 60: 1-10.

[103] Kreft, S., Strukelj, B., Gaberscik, A., Kreft, I. (2002): Rutin in buckwheat herbs grown at different UV-B radiation levels: comparison of two UV spectrophotometric and an HPLC method. -Journal of Experimental Botany 53: 1801-1804.

[104] Kumagai, T., Hidema, J., Kang, H.S., Sato, T. (2001): Effects of supplemental UV-B radiation on the growth and yield of two cultivars of Japanese lowland rice (*Oryza saliva* L.) under the field in a cool rice-growing region of Japan. -Agriculture Ecosystems & Environment 83: 201-208.

[105] Laakso, K., Sullivan, J.H., Huttunen, S. (2000): The effects of UV-B radiation on epidermal anatomy in loblolly pine (*Pinus taeda* L.) and Scots pine (*Pinus sylvestris* L.). -Plant Cell and Environment 23: 461-472.

[106] Lachno, D.R., Baker, D.A. (1986): Stress induction of abscisic-acid in maize roots. -Physiologia Plantarum 68: 215-221.

[107] Lagarias, J.C., Kelly, J.M., Cyr, K.L., Smith, W.O. (1987): Comparative photochemical analysis of highly purified 124 kilodalton oat and rye phytochromes in *vivo. Photochemistry and Photobiology* 46: 5-13.

[108] Landry, L.G., Chapple, C.C.S., Last, R.L. (1995): *Arabidopsis* mutants lacking phenolic sunscreens exhibit enhanced ultraviolet-b injury and oxidative damage. -Plant Physiology 109: 1159-1166.

[109] Landry, L.G., Stapleton, A.E., Lim, J., Hoffman, P., Hays, J.B., Walbot, V., Last, R.L. (1997): An *Arabidopsis* photolyase mutant is hypersensitive to ultraviolet-B radiation. -Proceedings of the National Academy of Sciences of the United States of America 94: 328-332.

[110] Larkindale, J., Knight, M.R. (2002): Protection against heat stress-induced oxidative damage in arabidopsis involves calcium, abscisic acid, ethylene, and salicylic acid. -Plant Physiology 128: 682-695.

[111] Larson, R.A. (1988): The antioxidants of higher-plants. -Phytochemistry 27: 969-978.

[112] Latifi, A., Ruiz, M., Zhang, C.-C. (2009): Oxidative stress in cyanobacteria. -FEMS Microbiology Reviews 33: 258-278.

[113] Levizou, E., Manetas, Y. (2002): Spectrophotometric assessment of leaf UV-B absorbing compounds and chemically determined total phenolic levels are strongly correlated. -Canadian Journal of Botany-Revue Canadienne De Botanique 80: 690-694.

[114] Li, J.Y., Oulee, T.M., Raba, R., Amundson, R.G., Last, R.L. (1993): *Arabidopsis* flavonoid mutants are hypersensitive to UV-B irradiation. -Plant Cell 5: 171-179.

[115] Li, X.M., Ma, L.J., Bu, N., Li, Y.Y., Zhang, L.H. (2014): Effects of salicylic acid

pre-treatment on cadmium and/or UV-B stress in soybean seedlings. -Biologia Plantarum 58: 195-199.

[116] Li, X.M., Zhang, L.H., Li, Y.Y., Ma, L.J., Bu, N., Ma, C.Y. (2012): Changes in photosynthesis, antioxidant enzymes and lipid peroxidation in soybean seedlings exposed to UV-B radiation and/or Cd. -Plant and Soil 352: 377-387.

[117] Li, Y., He, L.L., Zu, Y.Q. (2010): Intraspecific variation in sensitivity to ultraviolet-B radiation in endogenous hormones and photosynthetic characteristics of 10 wheat cultivars grown under field conditions. -South African Journal of Botany 76: 493-498.

[118] Li, Y., Zu, Y.Q., Chen, J.J., Chen, H.Y. (2002): Intraspecific responses in crop growth and yield of 20 soybean cultivars to enhanced ultraviolet-B radiation under field conditions. -Field Crops Research 78: 1-8.

[119] Li, Y., Zu, Y.Q., Chen, J.J., Chen, H.Y., Yang, J.L., Hu, Z.D. (2000): Intraspecific differences in physiological response of 20 wheat cultivars to enhanced ultraviolet-B radiation under field conditions. Environmental and Experimental Botany 44: 95-103.

[120] Liu, B., Liu, X.B., Li, Y.S., Herbert, S.J. (2013): Effects of enhanced UV-B radiation on seed growth characteristics and yield components in soybean. -Field Crops Research 154: 158-163.

[121] Liu, B., Liu, X.B., Wang, C., Jin, J., Herbert, S.J. (2010): Endogenous hormones in seed, leaf, and pod wall and their relationship to seed filling in soybeans. -Crop & Pasture Science 61: 103-110.

[122] Liu, L., McClure, J.W. (1995): Effects of UV-B on activities of enzymes of secondary phenolic metabolism in barley primary leaves. -Physiologia Plantarum 93: 734-739.

[123] Logemann, E., Wu, S.C., Schroder, J., Schmelzer, E., Somssich, I.E., Hahlbrock, K. (1995): Gene activation by UV light, fungal elicitor or fungal infection in Petroselinum crispum is correlated with repression of cell cycle-related genes. -Plant Journal 8: 865-876.

[124] Lois, R., Buchanan, B.B. (1994): Severe sensitivity to ultraviolet-radiation in an arabidopsis mutant deficient in flavonoid accumulation .2. mechanisms of uv-resistance in *Arabidopsis*. -Planta 194: 504-509.

[125] Luo, A.D., Qian, Q., Yin, H.F., Liu, X.Q., Yin, C.X., Lan, Y., Tang, J.Y., Tang, Z.S., Cao, S.Y., Wang, X.J., Xia, K., Fu, X.D., Luo, D., Chu, C.C. (2006): EUI1, encoding a putative cytochrome P450 monooxygenase, regulates internode elongation by modulating gibberellin responses in rice. -Plant and Cell Physiology 47: 181-191.

[126] Mackerness, S.A.H., John, C.F., Jordan, B., Thomas, B. (2001): Early signaling components in ultraviolet-B responses: distinct roles for different reactive oxygen species and nitric oxide. -FEBS Letters 489: 237-242.

[127] Mackerness, S.A.H., Surplus, S.L., Blake, P., John, C.F., Buchanan-Wollaston, V., Jordan, B.R., Thomas, B. (1999): Ultraviolet-B-induced stress and changes in gene expression in *Arabidopsis thaliana*: role of signalling pathways controlled by jasmonic acid, ethylene and reactive oxygen species. -Plant Cell and Environment 22: 1413-1423.

[128] Mackerness, S.A.H., Thomas, B., Jordan, B.R. (1997): The effect of supplementary ultraviolet-B radiation on mRNA transcripts, translation and stability of chloroplast proteins and pigment formation in Pisum sativum L. -Journal of Experimental Botany 48: 729-738.

[129] Mahdavian, K., Kalantari, K.M., Ghorbanli, M., Torkzade, M. (2008): The effects of salicylic acid on pigment contents in ultraviolet radiation stressed pepper plants. -Biologia Plantarum 52: 170-172.

[130] Malanga, G., Kozak, R.G., Puntarulo, S. (1999): N-acetylcysteine-dependent protection against UV-B damage in two photosynthetic organisms. -Plant Science 141: 129-137.

[131] Markham, K.R., Ryan, K.G., Bloor, S.J., Mitchell, K.A. (1998): An increase in the luteolin : apigenin ratio in Marchantia polymorpha on UV-B enhancement. -Phytochemistry 48: 791-794.

[132] Matysik, J., Alia, Bhalu, B., Mohanty, P. (2002): Molecular mechanisms of quenching of reactive oxygen species by proline under stress in plants. -Current Science 82: 525-532.

[133] Mazza, C.A., Battista, D., Zima, A.M., Szwarcberg-Bracchitta, M., Giordano, C.V., Acevedo, A., Scopel, A.L., Ballare, C.L. (1999): The effects of solar ultraviolet-B radiation on the growth and yield of barley are accompanied by increased DNA damage and antioxidant responses. -Plant Cell and Environment 22: 61-70.

[134] Mazza, C.A., Boccalandro, H.E., Giordano, C.V., Battista, D., Scopel, A.L., Ballare, C.L. (2000): Functional significance and induction by solar radiation of ultraviolet-absorbing sunscreens in field-grown soybean crops. -Plant Physiology 122: 117-125.

[135] McNamara, A.E., Hill, W.R. (2000): UV-B irradiance gradient affects photosynthesis and pigments but not food quality of periphyton. -Freshwater Biology 43: 649-662.

[136] Miles, G.P., Samuel, M.A., Ellis, B.E. (2002): Suramin inhibits oxidant signalling in tobacco suspension-cultured cells. -Plant Cell and Environment 25: 521-527.

[137] Mishra, V., Mishra, P., Srivastava, G., Prasad, S.M. (2011): Effect of dimethoate and UV-B irradiation on the response of antioxidant defense systems in cowpea (*Vigna unguiculata* L.) seedlings. -Pesticide Biochemistry and Physiology 100: 118-123.

[138] Mislevy, P., Boote, K.J., Martin, F.G. (1988): Soybean response to gibberellic-acid .1. time of application relative to emergence. -Field Crops Research 19: 113-121.

[139] Murali, N.S., Teramura, A.H. (1986): Effects of supplemental ultraviolet-B radiation on the growth and physiology of field-grown soybean. -Environmental and Experimental Botany 26: 233-242.

[140] Murali, N.S., Teramura, A.H., Randall, S.K. (1988): Response differences between 2 soybean cultivars with contrasting UV-B radiation sensitivities. -Photochemistry and Photobiology 48: 653-657.

[141] Murphy, T.M. (1983): Membranes as targets of ultraviolet-radiation. -Physiologia Plantarum 58: 381-388.

[142] Mutlu, S., Atici, O., Nalbantoglu, B. (2009): Effects of salicylic acid and salinity on apoplastic antioxidant enzymes in two wheat cultivars differing in salt tolerance. -Biologia Plantarum 53: 334-338.

[143] Nazar, R., Iqbal, N., Syeed, S., Khan, N.A. (2011): Salicylic acid alleviates decreases in photosynthesis under salt stress by enhancing nitrogen and sulfur assimilation and antioxidant metabolism differentially in two mungbean cultivars. -Journal of Plant Physiology 168: 807-815.

[144] Newsham, K.K., Robinson, S.A. (2009): Responses of plants in polar regions to UVB exposure: a meta-analysis. -Global Change Biology 15: 2574-2589.

[145] Noctor, G., Foyer, C.H. (1998): Ascorbate and glutathione: Keeping active oxygen under control. -Annual Review of Plant Physiology and Plant Molecular Biology 49: 249-279.

[146] Noda, K., Glover, B.J., Linstead, P., Martin, C. (1994): Flower color intensity depends on specialized cell-shape controlled by a MYB-related transcription factor. -Nature 369: 661-664.

[147] Oppenheimer, D.G., Herman, P.L., Sivakumaran, S., Esch, J., Marks, M.D. (1991): A MYB gene required for leaf trichome differentiation in arabidopsis is expressed in

stipules. -Cell 67: 483-493.

[148] Pelle, E., Maes, D., Padulo, G.A., Kim, E.K., Smith, W.P. (1990): An *invitro* model to test relative antioxidant potential - ultraviolet-induced lipid-peroxidation in liposomes. -Archives of Biochemistry and Biophysics 283: 234-240.

[149] Peng, Q., Zhou, Q. (2009): The endogenous hormones in soybean seedlings under the joint actions of rare earth element La(III) and ultraviolet-B stress. -Biological Trace Element Research 132: 270-277.

[150] Peng, Z.F., Strack, D., Baumert, A., Subramaniam, R., Goh, N.K., Chia, T.F., Tan, S.N., Chia, L.S. (2003): Antioxidant flavonoids from leaves of *Polygonum hydropiper* L. -Phytochemistry 62: 219-228.

[151] Pignocchi, C., Foyer, C.H. (2003): Apoplastic ascorbate metabolism and its role in the regulation of cell signalling. -Current Opinion in Plant Biology 6: 379-389.

[152] Pinto, M.E., Edwards, G.E., Riquelme, A.A., Ku, M.S.B. (2002): Enhancement of nodulation in bean (*Phaseolus vulgaris*) by UV-B irradiation. -Functional Plant Biology 29: 1189-1196.

[153] Prasad, S.M., Dwivedi, R., Zeeshan, M. (2005): Growth, photosynthetic electron transport, and antioxidant responses of young soybean seedlings to simultaneous exposure of nickel and UV-B stress. -Photosynthetica 43: 177-185.

[154] Qi, Y.D., Bai, S.J., Heisler, G.M. (2003): Changes in ultraviolet-B and visible optical properties and absorbing pigment concentrations in pecan leaves during a growing season. -Agricultural and Forest Meteorology 120: 229-240.

[155] Rao, M.V., Ormrod, D.P. (1995): Impact of uvb and O_3 on the oxygen-free radical scavenging system in *Arabidopsis-thaliana* genotypes differing in flavonoid biosynthesis. -Photochemistry and Photobiology 62: 719-726.

[156] Rao, M.V., Paliyath, C., Ormrod, D.P. (1996): Ultraviolet-B- and ozone-induced biochemical changes in antioxidant enzymes of *Arabidopsis thaliana*. -Plant Physiology 110: 125-136.

[157] Reuber, S., Bornman, J.F., Weissenbock, G. (1996): A flavonoid mutant of barley (*Hordeum vulgare* L.) exhibits increased sensitivity to UV-B radiation in the primary leaf. -Plant Cell and Environment 19: 593-601.

[158] Robberecht, R., Caldwell, M.M. (1978): Leaf epidermal transmittance of ultraviolet-radiation and its implications for plant sensitivity to ultraviolet-radiation induced injury. -Oecologia 32: 277-287.

[159] Robson, T.M., Klem, K., Urban, O., Jansen, M.A.K. (2015): Re-interpreting plant morphological responses to UV-B radiation. -Plant Cell and Environment 38: 856-866.

[160] Ros, J., Tevini, M. (1995): Interaction of UV-radiation and IAA during growth of seedlings and hypocotyl segments of sunflower. -Journal of Plant Physiology 146: 295-302.

[161] Rozema, J., vandeStaaij, J., Bjorn, L.O., Caldwell, M. (1997): UV-B as an environmental factor in plant life: Stress and regulation. -Trends in Ecology & Evolution 12: 22-28.

[162] Ryan, K.G., Swinny, E.E., Markham, K.R., Winefield, C. (2002): Flavonoid gene expression and UV photoprotection in transgenic and mutant *Petunia* leaves. -Phytochemistry 59: 23-32.

[163] Salmon, S., Maziere, J.C., Santus, R., Morliere, P., Bouchemal, N. (1990): UVB-induced photoperoxidation of lipids of human low and high-density lipoproteins - a possible role of tryptophan residues. -Photochemistry and Photobiology 52: 541-545.

[164] Saradhi, P.P., Alia, Arora, S., Prasad, K. (1995): Proline accumulates in plants exposed to

UV-radiation and protects them against UV induced peroxidation. -Biochemical and Biophysical Research Communications 209: 1-5.

[165] Saruhan, N., Saglam, A., Kadioglu, A. (2012): Salicylic acid pretreatment induces drought tolerance and delays leaf rolling by inducing antioxidant systems in maize genotypes. -Acta Physiologiae Plantarum 34: 97-106.

[166] Schmelzer, E., Jahnen, W., Hahlbrock, K. (1988): Insitu localization of light-induced chalcone synthase messenger-RNA, chalcone synthase, and flavonoid end products in epidermal-cells of parsley leaves. -Proceedings of the National Academy of Sciences of the United States of America 85: 2989-2993.

[167] Searles, P.S., Flint, S.D., Caldwell, M.M. (2001): A meta analysis of plant field studies simulating stratospheric ozone depletion. -Oecologia 127: 1-10.

[168] Sharma, A., Guruprasad, K.N. (2009): Similarities in the biochemical changes between solar UV exclusion and GA application in *Amaranthus caudatus*. -Physiology and Molecular Biology of Plants 15: 367-370.

[169] Sheahan, J.J. (1996): Sinapate esters provide greater UV-B attenuation than flavonoids in *Arabidopsis thaliana* (Brassicaceae). -American Journal of Botany 83: 679-686.

[170] Shimizu, T., Fujibe, R., Senda, M., Ishikawa, R., Harada, T., Niizeki, M., Akada, S. (2000): Molecular cloning and characterization of a subfamily of UV-B responsive MYB genes from soybean. -Breeding Science 50: 81-90.

[171] Shiu, C.T., Lee, T.M. (2005): Ultraviolet-B-induced oxidative stress and responses of the ascorbate-glutathione cycle in a marine macroalga *Ulva fasciata*. -Journal of Experimental Botany 56: 2851-2865.

[172] Shweta, M., Agrawal, S.B. (2006): Interactive effects between supplemental ultraviolet-B radiation and heavy metals on the growth and biochemical characteristics of *Spinacia oleracea* L. -Brazilian Journal of Plant Physiology 18: 307-314.

[173] Singh, M., Singh, S., Agrawal, S.B. (2012): Intraspecific responses of six cultivars of wheat (*Triticum aestivum* L.) to supplemental ultraviolet-B radiation under field conditions. -Acta Physiologiae Plantarum 34: 65-74.

[174] Skokut, T.A., Wu, J.H., Daniel, R.S. (1977): Retardation of ultraviolet-light accelerated chlorosis by visible-light or by benzyladenine in *Nicotiana glutinosa* leaves - changes in amino-acid content and chloroplast ultrastructure. -Photochemistry and Photobiology 25: 109-118.

[175] Smith, G.J., Markham, K.R. (1998): Tautomerism of flavonol glucosides: relevance to plant UV protection and flower colour. -Journal of Photochemistry and Photobiology a-Chemistry 118: 99-105.

[176] Smrkolj, P., Stibilj, V., Kreft, I., Germ, M. (2006): Selenium species in buckwheat cultivated with foliar addition of Se(VI) and various levels of UV-B radiation, Food Chemistry 96: 675-681.

[177] Stapleton, A.E., Walbot, V. (1994): Flavonoids can protect maize DNA from the induction of ultraviolet-radiation damage. -Plant Physiology 105: 881-889.

[178] Stratmann, J. (2003): Ultraviolet-B radiation co-opts defense signaling pathways. -Trends in Plant Science 8: 526-533.

[179] Strid, A., Chow, W.S., Anderson, J.M. (1994): UV-B damage and protection at the molecular-level in plants. -Photosynthesis Research 39: 475-489.

[180] Suchar, V.A., Robberecht, R. (2015): Integration and scaling of UV-B radiation effects on plants: from DNA to leaf. -Ecology and Evolution 5: 2544-2555.

[181] Sullivan, J.H., Gitz, D.C., III, Liu-Gitz, L., Xu, C., Gao, W., Slusser, J. (2007): Coupling

short-term changes in ambient UV-B levels with induction of UV-screening compounds. -Photochemistry and Photobiology 83: 863-870.

[182] Sullivan, J.H., Teramura, A.H. (1990): Field-study of the interaction between solar ultraviolet-b radiation and drought on photosynthesis and growth in soybean. -Plant Physiology 92: 141-146.

[183] Surplus, S.L., Jordan, B.R., Murphy, A.M., Carr, J.P., Thomas, B., Mackerness, S.A.H. (1998): Ultraviolet-B-induced responses in *Arabidopsis thaliana*: role of salicylic acid and reactive oxygen species in the regulation of transcripts encoding photosynthetic and acidic pathogenesis-related proteins. -Plant Cell and Environment 21: 685-694.

[184] Taalas, P., Kaurola, J., Kylling, A., Shindell, D., Sausen, R., Dameris, M., Grewe, V., Herman, J. (2000): The impact of greenhouse gases and halogenated species on future solar UV radiation doses. -Geophysical Research Letters 27: 1127-1130.

[185] Takahama, U., Oniki, T. (2000): Flavonoids and some other phenolics as substrates of peroxidase: Physiological significance of the redox reactions. -Journal of Plant Research 113: 301-309.

[186] Takeuchi, Y., Kubo, H., Kasahara, H., Sakaki, T. (1996): Adaptive alterations in the activities of scavengers of active oxygen in cucumber cotyledons irradiated with UV-B. -Journal of Plant Physiology 147: 589-592.

[187] Talor, R.M., Tobin, A.K., Bray, C.M. (1997): DNA Damage and Repair in Plants. -In: Lumsden, P.J. (ed.) Plants and UV-B Responses to Environmental Change, Cambridge University Press, Cambridge

[188] Tekchandani, S., Guruprasad, K.N. (1998): Modulation of a guaiacol peroxidase inhibitor by UV-B in cucumber cotyledons. -Plant Science 136: 131-137.

[189] Teramura, A.H. (1980): Effects of ultraviolet-B irradiances on soybean .1. importance of photosynthetically active radiation in evaluating ultraviolet-b irradiance effects on soybean and wheat growth. -Physiologia Plantarum 48: 333-339.

[190] Tevini, M., Braun, J., Fieser, G. (1991): The protective function of the epidermal layer of rye seedlings against ultraviolet-b radiation. -Photochemistry and Photobiology 53: 329-333.

[191] Tevini, M., Teramura, A.H. (1989): UV-B effects on terrestrial plants. -Photochemistry and Photobiology 50: 479-487.

[192] Ulm, R., Baumann, A., Oravecz, A., Mate, Z., Adam, E., Oakeley, E.J., Schafer, E., Nagy, F. (2004): Genome-wide analysis of gene expression reveals function of the bZIP transcription factor HY5 in the UV-B response of *Arabidopsis*. -Proceedings of the National Academy of Sciences of the United States of America 101: 1397-1402.

[193] Wang, C.X., Liu Y.L., Li, F.M., Wang, Z.J., Liu, X.G., Peng, A. (2000): Inhibition effect of rare earth nitrates on superoxide anion radical by pulse radiolysis. -Journal of the Chinese Rare Earth Society 18: 286-290.

[194] Wang, G.H., Chen, K., Chen, L.Z., Hu, C.X., Zhang, D.L., Liu, Y.D. (2008): The involvement of the antioxidant system in protection of desert cyanobacterium *Nostoc* sp against UV-B radiation and the effects of exogenous antioxidants. -Ecotoxicology and Environmental Safety 69: 150-157.

[195] Wang, X.Q., Ullah, H., Jones, A.M., Assmann, S.M. (2001): G protein regulation of ion channels and abscisic acid signaling in *Arabidopsis* guard cells. -Science 292: 2070-2072.

[196] Wang, Z.Y., Kenigsbuch, D., Sun, L., Harel, E., Ong, M.S., Tobin, E.M. (1997): A Myb-related transcription factor is involved in the phytochrome regulation of an *Arabidopsis* Lhcb gene. -Plant Cell 9: 491-507.

[197] Wargent, J.J., Moore, J.P., Roland Ennos, A., Paul, N.D. (2009): Ultraviolet Radiation as a Limiting Factor in Leaf Expansion and Development. -Photochemistry and Photobiology 85: 279-286.

[198] Warren, J.M., Bassman, J.H., Fellman, J.K., Mattinson, D.S., Eigenbrode, S. (2003): Ultraviolet-B radiation alters phenolic salicylate and flavonoid composition of *Populus trichocarpa* leaves. -Tree Physiology 23: 527-535.

[199] Wefers, H., Sies, H. (1988): The protection by ascorbate and glutathione against microsomal lipid-peroxidation is dependent on vitamin E. -European Journal of Biochemistry 174: 353-357.

[200] Willekens, H., Vancamp, W., Vanmontagu, M., Inze, D., Langebartels, C., Sandermann, H. (1994): Ozone, sulfur-dioxide, and ultraviolet-B have similar effects on messenger-RNA accumulation of antioxidant genes in *Nicotiana plumbaginifolia* L. -Plant Physiology 106: 1007-1014.

[201] Winter, T.R., Rostas, M. (2008): Ambient ultraviolet radiation induces protective responses in soybean but does not attenuate indirect defense. -Environmental Pollution 155: 290-297.

[202] Wright, L.A., Murphy, T.M., Travis, R.L. (1981): The effect of ultraviolet-radiation on wheat root vesicles enriched in plasma membrane. -Photochemistry and Photobiology 33: 343-348.

[203] Xu, C.P., Natarajan, S., Sullivan, J.H. (2008a): Impact of solar ultraviolet-B radiation on the antioxidant defense system in soybean lines differing in flavonoid contents. -Environmental and Experimental Botany 63: 39-48.

[204] Xu, C.P., Sullivan, J.H., Garrett, W.M., Caperna, T.J., Natarajan, S. (2008b): Impact of solar Ultraviolet-B on the proteome in soybean lines differing in flavonoid contents. -Phytochemistry 69: 38-48.

[205] Yamashoji, S., Yoshida, H., Kajimoto, G. (1979): Photooxidation of linoleic acid by ultraviolet light and effect of superoxide anion quencher. -Food and Nutrition 43: 1249–1254.

[206] Yang, J.H., Chen, T., Wang, X. (2000): Effect of enhanced UVB radiation on endogenous ABA and free proline in wheat (*Triticum asetivum* L.). -Acta Ecologica Sinica 20: 39-42.

[207] Yang, Y.Q., Yao, Y., Xu, G., Li, C.Y. (2005): Growth and physiological responses to drought and elevated ultraviolet-B in two contrasting populations of *Hippophae rhamnoides*. -Physiologia Plantarum 124: 431-440.

[208] Yannarelli, G.G., Gallego, S.M., Tomaro, M.L. (2006a): Effect of UV-B radiation on the activity and isoforms of enzymes with peroxidase activity in sunflower cotyledons. -Environmental and Experimental Botany 56: 174-181.

[209] Yannarelli, G.G., Noriega, G.O., Batlle, A., Tomaro, M.L. (2006b): Heme oxygenase up-regulation in ultraviolet-B irradiated soybean plants involves reactive oxygen species. -Planta 224: 1154-1162.

[210] Yao, Y., Xuan, Z., Li, Y., He, Y., Korpelainen, H., Li, C. (2006): Effects of ultraviolet-B radiation on crop growth, development, yield and leaf pigment concentration of tartary buckwheat (*Fagopyrum tataricum*) under field conditions. -European Journal of Agronomy 25: 215-222.

[211] Zaffari, G.R., Peres, L.E.P., Kerbauy, G.B. (1998): Endogenous levels of cytokinins, indoleacetic acid, abscisic acid, and pigments in variegated somaclones of micropropagated banana leaves. -Journal of Plant Growth Regulation 17: 59-61.

[212] Zancan, S., Suglia, I., La Rocca, N., Ghisi, R. (2008): Effects of UV-B radiation on

antioxidant parameters of iron-deficient barley plants. -Environmental and Experimental Botany 63: 71-79.

[213] Zhu, Y.Y., Nomura, T., Xu, Y.H., Zhang, Y.Y., Peng, Y., Mao, B.Z., Hanada, A., Zhou, H.C., Wang, R.X., Li, P.J., Zhu, X.D., Mander, L.N., Kamiya, Y., Yamaguchi, S., He, Z.H. (2006): Elongated uppermost internode encodes a cytochrome P450 monooxygenase that epoxidizes gibberellins in a novel deactivation reaction in rice. -Plant Cell 18: 442-456.

[214] Zu, Y.G., Pang, H.H., Yu, J.H., Li, D.W., Wei, X.X., Gao, Y.X., Tong, L. (2010): Responses in the morphology, physiology and biochemistry of Taxus chinensis var. mairei grown under supplementary UV-B radiation. -Journal of Photochemistry and Photobiology B-Biology 98: 152-158.

[215] Zu, Y.Q., Li, Y., Chen, H.Y., Chen, J.J. (2003): Intraspecific differences in physiological response of 20 soybean cultivars to enhanced ultraviolet-B radiation under field conditions. -Environmental and Experimental Botany 50: 87-97.

MORPHO-CHEMICAL DIVERSITY IN *FESTUCA PRATENSIS* AND *LOLIUM PERENNE* DEPENDING ON CONCENTRATIONS OF *ECKLONIA MAXIMA* EXTRACT

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Abstract. The aim of the research was to determine the relationship between application of various concentrations of *Ecklonia maxima* extract and development of some morphological features, the SAD index as well as selected organic compound content in *Festuca pratensis* and *Lolium perenne*. The experiment was replicated three times and the following concentrations of the extract were used: control, 0.04, 0.4, 4.0 and 40%. 40 days after the extract was sprayed on the plants the following measurements were taken: the number of shoots, the length of leaf blades, the width of the leaf base, the relative chlorophyll index SPAD, and the amount of biomass. The content of protein compounds, simple sugars, lignin, cellulose and hemicellulose were determined after the harvest. The research showed that there was a relationship between a higher concentration of the extract and a higher number of shoots, longer leaf blades and an increase in their width. In consequence of those changes the amount of biomass also increased. The plants sprayed with higher doses of extract had a higher SPAD index, a higher concentration of both protein compounds and simple sugars, and a lower concentration of cellulose, lignin and hemicellulose.

Key words: *seaweed extract, grass, morphology, sugars, protein*

Introduction

According to Christobel (2008), seaweeds are one of the most important underwater resources and they can be used as food, animal feed or fertiliser in agriculture and horticulture. Such seaweeds as *Enteromorpha intestinali, Cladophora dalmatica, Ulva lactuca*, *Corralina mediterranea, Ascophyllum nodosum* and *Ecklonia maxima* are also a raw material to produce an extract stimulating plant growth on an industrial scale (Bai et al., 2007) Research on a reaction of plants to seaweed extract application was only started a few years ago. It has been found, for example, that because of the content of minerals and growth hormones like IAA and IBA or cytokinin, a seaweed extract can increase disease resistance of agricultural plants (Moller and Smith, 1998, 1999; El-Yazied et al., 2012). The extracts contain a variety of chemical compounds and they have a high content of organic calcium, polysaccharides or microelements and a lower content of macro elements: nitrogen, phosphorus and potassium (Crouch and Staden, 1993). Moreover, the extracts have a beneficial impact on plants due to the presence of vitamins and amino acids (Khan et al., 2009; Craigie, 2011). Because of the above advantages *Ecklonia maxima* extract is considered to be a new generation fertiliser (Wajahatullah et al., 2009). In many publications (Temple and Bomie, 1989; Crouch and Staden, 1991; Zodape, 2001; Bai et al., 2007; Zodapea et al., 2009; El-Yazied et al., 2012; Sosnowski et al., 2013a, 2013b; Kociara et al., 2016), the beneficial effect of seaweed extracts on agricultural plants has been confirmed. It has been said that such

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substances make seed germination faster and increase yield of agricultural plants. It has also been stressed (Wajahatullah et al., 2009) that, unlike mineral fertilisers, substances based on seaweeds are biodegradable, nontoxic and harmless to people and animals. Because of all the above reasons products based on seaweeds are becoming more and more popular. According to Christobel (2008) and Galbiatti et al. (2007), some of the effects of using seaweed extracts are improvement of plant vigour, resistance to disease and to adverse conditions in the environment. Additionally, the use of the extract as a growth stimulant has become popular in horticultural practice because of its beneficial effects (Crouch and Staden, 1993). According to Stirk and Staden (1997), the main ingredients of extracts affecting plant growth are cytokinin and auxin, the presence of which in most seaweeds has been confirmed. These hormones trigger many cytological and histological processes in plants and, according to Wierzbowska and Bowszys (2008), they affect the content of macro elements in biomass.

The aim of the research was to determine the relationship between application of various concentrations of *Ecklonia maxima* extract and development of some morphological features, the SAD index as well as selected organic compound content in *Festuca pratensis* and *Lolium perenne*. The following measurements were taken into account in the experiment: the number of shoots, the length and width of leaf blade, the amount of biomass as well as the content of protein, simple sugars, lignin, cellulose and hemicellulose.

Materials and methods

The conditions of experiment establish

The experiment was set up on 12 April 2011 at the experimental station of the University of Natural Sciences and Humanities in Siedlce. 15 plants of *Festuca pratensis* Huds. (Areus variety) and 15 plants of *Lolium perenne* L. (Inka variety) were taken from a long-term experimental field and put into Mitscherlich pots of 9 dm^3 capacity. The pots were put inside into a room with a transparent roof made of plastic. There was a lot of natural light so the plants grew under sunlight with a natural photoperiod. The soil used was loamy medium sand taken from the plough layer. The content of assimilable phosphorus and magnesium in the soil was very high, while potassium and zinc content was high, with an average content of boron, manganese and iron (*Table 1*).

Table 1. *Chemical composition of the soil.*

pH in KCL	Humus [%]	C_{org} [g kg^{-1}]	Dry matter [%]	Humidity [%]
6.1	3.2	16.7	83	12,3
Content of mineral N [mg kg $^{-1}$ DM]		Total content of macro elements [g kg $^{-1}$ DM]		
N-NO_3	N-NH_4	P	K	Mg
1.3	59.9	0.47	0.15	0.11
Total content of microelements [mg kg $^{-1}$ DM]				
B	Mn	Cu	Zn	Fe
2.0	190	8.4	21.2	1567

The mineral fertilisation was as follows: nitrogen (1.5 g N per pot in the form of NH_4NO_3), phosphorus (0.60 g P per pot in the form of KH_2PO_4), potassium (1.0 g K per pot in the form of K_2SO_4) and magnesium (0.5 g Mg per pot in the form of $MgSO_4$). The fertiliser was mixed with the soil and then the pots were filled with it.

All the time during the experiment the soil moisture was 60-70% of total water capacity. On 15 May the grass was mown to the height of 10 cm and the extract from *Ecklonia maxima* (with the brand name of Kelpak SL) was applied in the dose of 3.5 cm^3 pot^{-1}. There were three experimental units: control (with only distilled water added) and units sprayed with the extract of the concentration 0.04, 0.4, 4.0 and 40%, respectively.

According to the producer the chemical composition of the extract was as follows:

- Growth regulators: auxin 11 mg dm^{-3}, cytokinin 0.03 mg dm^{-3},
- Organic master: carbohydrates 35%, agonic acid 10%, total amino acid 6%, manitob 4%,
- Macro elements: N 3.12%, P_2O_5 2.61%, K_2O 4.71%, Ca 0.25%, S 3.56%, Mg 0.58%,
- Micro elements: Fe 150 ppm, Zn 70 ppm, Mn 13 ppm, B 60 ppm, I 30 ppm.

Analysis of morphological and physiological traits

40 days after the spraying the following measurements were taken: the number of shoots [pieces pot^{-1}], leaf blade length [cm], the width of the blade base [cm], the SPAD index and dry matter content [g DM pot^{-1}].

The measurements of leaf blade length and the width of its base were done using 10 leaves taken at random from each unit. Three replicated analyses of the same leaves were done to determine the SPAD index using the SPAD-502 Spektrum Technologies meter. Moreover, taking advantage of the method described by Janowska (2011), the aspect ratio, an abstract entity, was calculated (leaf length: leaf width) and, using the Kluz-Wieloch (2004) publication, the area index was calculated (cm^2).

The methods of chemicals estimation

The plant material was collected, dried and the content of protein compounds, simple sugars, lignin, cellulose and hemicellulose was measured. The airy dry matter was shredded and ground. The obtained material was subjected to chemical analysis to determine dry matter concentration (by determining moisture content) as well as protein compounds and simple sugars concentration. The method of determination was near-infrared spectroscopy (NIRS) using the NIRFlex N-500 spectrometer and ready to use INGOT calibration applications.

Statistical analysis of the results

A statistical analysis was performed using Statistica 6.0 for Windows. Data were calculated by ANOVA. The results were verified statistically using analysis of variance. Tukey's test with $p \leq 0.05$ was applied to find means that were significantly different. Correlation coefficient whose significance was checked using Student's t test.

Results

Plant morphology

This research (*Table 2*) has shown that higher concentrations of the growth regulator (40%), no matter in which species, statistically significantly increased the number of shoots developed by the plants. *Festuca pratensis* developed more shoots, by 12.8% on average, than *Lolium perenne*, no matter what the concentration was.

Table 2. *The number of shoots [pieces pot^{-1}] developed by Festuca pratensis and Lolium perenne in relation to concentration of Ecklonia maxima extract.*

Species	Extract concentration [%]					Mean
	Control	0.04	0.4	4.0	40	
Festuca pratensis	28.6 Ab	30.1 Ab	29.3 Ab	32.5Ab	38.3 Aa	31.8 A
Lolium perenne	25.3 Bb	26.0 Bb	26.1 Bb	27.9Ab	33.0 Ba	27.7 B
Mean	27.0 b	28.1 b	27.7 b	30.2 b	35.7 a	

Mean in line marked with the same small letters don't differentiate significantly, mean in columns marked with the same big letters don't differentiate significantly.

The structure of the above ground part of a plant is determined by the number of shoots as well as the length of the leaf blade and the width of its base, the last two deciding about the size of a leaf blade. As can be seen from *Table 3*, in the case of *Festuca pratensis* the longest leaf blades were noted in plants growing in pots sprayed with the extract of 40% concentration. There was a similar result for *Lolium perenne.* The width of the leaf base was also related to the concentration of the extract. Both of the test species had the widest leaf base after being treated with the 40% extract. Analyzing quality parameters of leaf blades it can be said that the aspect ratio of *Festuca pratensis*leaf blade was the highest for objects sprayed with the 0.4% extract while for *Lolium perenne* plants it was the highest when they grew in the control pots. It means only that in those pots the plants grew leaf blades with a high ratio of their width to the length but, when it comes to an impact on yield, the value of the linear correlation coefficient between the area index and the biomass was more representative (*Table 4*). The value of the linear correlation coefficient between the biomass and the area index was 0.863* for *Festuca pratensis* and 0.793* for *Lolium perenne.* The area index indicates the relationship between the experimental factor used in the experiment and the size of leaf blades of the plant. The value of this parameter for both species (*Festuca pratensis* - 14.5 cm^2, *Lolium perenne* - 13.9 cm^2) was the highest for plants sprayed with 40% concentration of the extract.

Basing on statistical analysis it can be said that the amount of the biomass produced by each plant, *Festuca pratensis* and *Lolium perenne*, irrespective of the concentration of the extract, was the same (*Table 5*), with the average of 54.9 g DM pot^1. There were, however, some differences in the amount of the same plant biomass, depending on various concentrations of the extract. The highest amount of biomass (on average 61.3 g DM pot^{-1}) was produced by the plants growing in pots where plants were sprayed with the 40% extract. Compared to the plants sprayed with the highest extract concentration the biomass was on average 13.2% lower for plants growing in the pots with no extract applied and with the concentration of 0.04, 0.4 and 4% of the extract.

Table 3. *Relationship between the parameters of Festuca pratensis and Lolium perenne leaf blades and the concentration of Ecklonia maxima extract.*

Extract concentration [%]	Features			
	Length of blade [cm]*	Blade width [cm]*	The aspect ratio	Area index [cm^2]
		Festuca pratensis		
control	16.4 C	0.57 C	28.8	9.35
0.04	18.0 B	0.58 C	31.0	10.4
0.4	18.5 B	0.67 C	31.8	12.4
4.0	19.2 AB	0.66 B	29.1	12.7
40	20.1 A	0.72 A	27.9	14.5
		Lolium perenne		
control	16.0 C	0.43 B	37.2	6.88
0.04	16.6 C	0.49 B	33.9	8.13
0.4	16.2 C	0.50 B	32.4	8.10
4.0	17.5 C	0.54 AB	32.4	9.45
40	22.1 A	0.63 A	35.1	13.9

*Mean in columns marked with the same big letters don't differentiate significantly

Table 4. *The value of linear correlation coefficient between dry matter of Festuca pratensis as well as Loluim multiflorum and leaf blade parameters.*

Leaf quality parameters	Biomass [g DM pot^{-1}]
Festuca pratensis	
y. the aspect ratio	0.532*
y. area index	0.863*
Lolium perenne	
y. aspect ratio	0.598*
y. area index	0.793*

*Significant with p≤ 0,05.

Table 5. *Relationship between the amount of the biomass [g DM pot^{-1}] for Festuca pratensis and Lolium perenne and the concentration of Ecklonia maxima extract.*

Species	Extract concentration [%]					Mean
	control	0.04	0.4	4.0	40	
Festuca pratensis	48.8 Ab	53.8 Ab	56.8 Aab	54.3 Ab	60.1 Aa	54.8 A
Lolium perenne	50.9 Ab	52.8 Ab	55.1 Aa	53.7 Ab	62.5 Aa	55.0 A
Mean	49.6 b	53.3 b	56.0 b	54.0 b	61.3 a	

Mean in line marked with the same small letters don't differentiate significantly, mean in columns marked with the same big letters don't differentiate significantly.

The values of the SPAD index of test grass leaf blades varied throughout the experiment depending on the concentration of the growth stimulant. The measurements of the index showed that plants sprayed with the 40 % extract had a bigger value of SPAD (*Table 6*) and the index value for those plants was 30% higher than for plants in the control pots.

Table 6. *Relationship between the Festuca pratensis as well as Lolium perenne SPAD index value and Ecklonia maxima extract concentration.*

Species	Extract concentration [%]					Mean
	control	0.04	0.4	4.0	40	
Festuca pratensis	28.6 Ac	27.8 Ac	31.7 Ab	33.0 Ab	39.3 Aa	37.8 A
Lolium perenne	29.4 Ac	30.0 Ac	34.0 Ab	33.9 Ab	44.0 Ba	34.4,B
Mean	29.0 c	28.9 c	32.9 b	33.5 b	41.7 a	

Mean in line marked with the same small letters don't differentiate significantly, mean in columns marked with the same big letters don't differentiate significantly.

It is worth noting that the use of the 0.4% and 4% also made the SPAD index grow in a statistically significant way, when compared to the control and to the plants sprayed with 0.04% extract. Yet the growth was significantly smaller than for plants sprayed with the highest concentration of the stimulant. Irrespective of the extract concentration, for *Festuca pretensis* the SPAD index was higher on average by 9.9 % while the value of SPAD for leaf blades of the same

Chemical analysis

Apart from affecting the amount and structure of the biomass of the above ground part of plants the *Ecklonia maxima* growth stimulant also increased the amount of protein and simple sugars in the plants (*Table 7*). Irrespective of the species and compared to the control, applying even 0.4% extract increased protein content in the dry matter by 19% on average and simple sugars content by 27%. It is worth noting that applying the extract with a higher concentration of 4 and 40% did not result in a higher protein and sugars content, the value of which did not differ significantly from the content of protein and sugars in plant sprayed with the 0.4% extract. However, spraying grass with the 0.04% extract did not increase the content of those substances in biomass. Apart from that, it was noticed that irrespective of the extract concentration the content of protein and sugars was higher in the dry matter of *Lolium perenne*.

Table 7. *Relationship between protein and simple sugar content [g kg^{-1}DM] in the biomass of the above ground part of Festuca pratensis as well as Lolium perenne and different concentrations of Ecklonia maxima extract.*

Species	Extract concentration [%]					Mean
	Control	0.04	0.4	4.0	40	
Protein compounds						
Festuca pratensis	84.1 Bb	79.9 Bb	98.8 Ba	97.9 Ba	100.1 Ba	92.2 B

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Lolium perenne	92.1 Ab	95.3 Ab	111.2 Aa	108.0 Aa	110.7 Aa	103.5 A
Mean	88.1 b	87.6 b	105.0 a	103.0 a	105.4 a	
Simple sugars						
Festuca pratensis	73.8 Bb	79.0 Bb	98.2 Ba	90.9 Ba	95.6 Ba	87.5 B
Lolium perenne	111.4 Ab	110.8 Ab	138.3 Aa	141.1 Aa	140.8 Aa	128.5 A
Mean	92.6 b	94.9 b	118.3 a	116.0 a	118.2 a	

Mean in line marked with the same small letters don't differentiate significantly, mean in columns marked with the same big letters don't differentiate significantly.

One of the effects of growth stimulant application is a decrease of the amount of cellulose, hemicellulose and lignin in test plants (*Table 8*). Like in the case of protein compounds and simple sugars only 0.4, 4 and 40% extracts were significantly effective in comparison to the control pots. Irrespective of the grass species spraying plants with the extract lowered the concentration of cellulose by 7%, hemicellulose by 9% and lignin by 13% in the biomass of the plants. It is worth noting that out of the two grass species *Festuca pratensis* dry matter had a bigger concentration of cellulose, hemicellulose, irrespective of the extract concentration. For both species lignin concentration was the same, 44.4 g kg^{-1}DM on average, without significant differences between them.

Table 8. *Relationship between Ecklonia maxima concentration [g kg^{-1}s.m.] and cellulose, hemicellulose and lignin content in the above ground biomass of Festuca pratensis and Lolium perenne.*

Species	Extract concntration [%]					Mean
	Control	0.04	0.4	4.0	40	
Cellulose [g kg^{-1}s.m.]						
Festuca pratensis	320.4 Aa	323.8 Aa	304.7 Ab	301.7 Ab	308.1 Ab	311.7 A
Lolium perenne	297.1 Ba	298.1 Ba	267.3 Bb	271.2 Bb	265.9 Bb	279.9 B
Mean	308.8 a	311.0 a	286.0 b	286.5 b	287.0 b	
Hemicellulose [g kg^{-1}s.m.]						
Festuca pratensis	227.1 Aa	230.0 Aa	208.5 Ab	200.8 Ab	210.2 Ab	215.3 A
Lolium perenne	199.5 Bb	198.1 Bb	182.0 Bb	179.7 Bb	187.2 Bb	189.3 B
Mean	213.3 a	214.1 a	195.3 b	190.3 b	198.7 b	
Lignin [g kg^{-1}s.m.]						
Festuca pratensis	49.1 Aa	48.9 Aa	44.7 Ab	43.7 Ab	45.6 Ab	46.4 A
Lolium perenne	46.9 Aa	47.3 Aa	38.2 Ab	39.0 Ab	40.1 Ab	42.3 A
Mean	48.0 a	48.1 a	41.5 b	41.4 b	42.9 b	

Mean in line marked with the same small letters don't differentiate significantly, mean in columns marked with the same big letters don't differentiate significantly.

Discussion

According to many publications (Temple and Bomke, 1989; Zodape, 2001; Sosnowski et al., 2013a, 2013b) growth regulator Kelpak does not do any damage to plants but stimulates their growth and improves their stress resistance. Sultan (2005) says that the effect of substances based on phytohormones depends strongly on their concentration, the way they are applied and on the species and variety of the plant. Many publications (Temple and Bomie, 1989; Zodape, 2001; Fornes et al., 2002; Thirumaran et al., 2009) confirm that *Ecklonia maxima* extract increases the size of some plant organs. There have been experiments with *Festuca pratensis* and *Lolium perenne* which also confirmed the beneficial effect of the extract. Application of the extract resulted in an increase in the number of shoots and leaf blade area of the plants, which contributed to a higher amount of biomass. At the same time the SPAD index value also increased. In another experiment (Bai et al., 2007) foliar treatment of plants with algae extract brought about an increase in shoot length by 35% and roots by 22%, when compared to the control. Thevanathan et al. (2005) present similar results while. Godlewska and Ciepiela (2013) deal with the effects of the Kelpak growth stimulant on plant yield. It has to be stressed, however, that there have been experiments described in literature where no significant effect of the extract on plants was noted (Temple and Bomie, 1989; Nour et al., 2010; Zodape et al., 2010; Sridhar and Rengasamy, 2011). Yet in most cases scientific research has confirmed beneficial effect of Kelpak on crop yield, at the same time showing that the time of the extract application is more important than the dose (Godlewska and Ciepiela, 2013; Sosnowski et al., 2013a, 2013b). It has also been proved (Godlewska and Ciepiela, 2013) that new varieties of the same species may react in a different way to the same regulator. According to Szabo et al. (2011), components in biostimulants such as auxins, gibberellic acid, cytokinins and amino acids increase the physiological activity of plants, for example protein synthesis. There are many reports in the world literature of the positive effect of sea algae extracts on protein content in various plant species (Sivasankari et al., 2006). Joubert and Lefranc (2008) have stated that active substances in sea algae extracts are similar to physiological activators in their mode of action because they may change the chemical composition of plants sprayed with such extracts. The biostimulant applied in the present study was found to influence the concentration of sugars in plants. Kelpak increased monosaccharide concentration in grasses by 37.6%, regardless of the remaining factors investigated in the study. Similar findings have been reported by many authors (Güllüoglu, 2011; Pacholczak et al., 2012) who have stressed that sea algae extracts contribute to increased sugar contents in plants but, as with protein content. Godlewska and Ciepiela (2013) also studied the relationship between 0.5% *Ecklonia maxima* extract and an increase of protein and simple sugar content. They applied the growth stimulant to *Dactylis glomerata* as well as to *Festulolium braunii* and the result was an 11% growth of protein compounds and a 37% growth of simple sugars content in the biomass of the test grass. When both grass species were compared, a higher sugar/protein ratio was calculated for *Festulolium braunii* although Kelpak significantly increased the value of this indicator for both the species. The sugar/protein ratio was also different between harvests. Ratios calculated for consecutive regrowths were significantly lower whereas increasing values were obtained for consecutive cuts when Kelpak had been applied. Analysis of the results showed that carbohydrate/protein ratios in the tested plants decreased in consecutive study years, the differences being statistically significant.

Conclusions

The research has shown that the effects of *Ecklonia maxima* extract application on *Festuca pratensis* and *Lolium perenne* were related to the concentration of the extract applied. The best results, with an increase in biomass of the above part of plants, an increase in the number of shoots as well as the length and width of the leaf blade, were achieved when 40% extract concentration was used. The increase of the SPAD index was statistically significant when even lower doses of the extract were used (0.4 and 4%). Yet the highest increase of the SPAD index was when 40% extract was applied to the plants. Compared to the control, only the 0.04% extract did not affect the content of protein, simple sugars, cellulose or lignin. When the extract of the 0.4, 4 and 40% concentration was applied, then the content of protein and simple sugars increased, whereas the content of polysaccharides in the dry matter decreased.

REFERENCES

[1] Bai, N.R., Banu, N.R.L., Prakash, J.W., Goldi, S.J. (2007): Effects of Asparagopsis taxiformis extract on the growth and yield of *Phaseolus aureus*. - J. Basic. Appl. Biol.1: 6-11.

[2] Christobel, J.G. (2008): Effect of seaweed (*Sargassum wightii* L.) on the germination and growth of green gram (*Phaseolus Aureus* L.). - J. Basic. Appl. Biol. 2: 105-108.

[3] Craigie, J.S. (2011): Seaweed extract stimuli plant science and agriculture. - J. Appl. Phycol . 23: 371-393.

[4] Crouch, I.J., Staden, J. (1991): Evidence for rooting factors in a seaweed concentrate prepared from *Ecklonia maxima*. - J Plant Physiol. 137: 319–322.

[5] Crouch, I.J., Staden, J. (1993): Effect of seaweed concentrate from *Ecklonia maxima* (Osbeck) Papenfuss on Meloidogyne incognita infestation on tomato. - J Appl. Phycol. 5: 37–43.

[6] El-Yazied, A., El-Gizawy, A.M., Ragab, M.I., Hamed, E.S. (2012): Effect of seaweed extract and compost treatments on growth, yield and quality of snap bean. - J Am. Sci. 8: 1-20.

[7] Fornes, F., Sa`nchez-Perales, M., Guadiola, J.L. (2002): Effect of a seaweed extract on the productivity of ‘de Nules’ clementine mandarin and navelina orange. - Bot. Mar. 45: 486–489.

[8] Galbiattia, J.A., Cavalcantea, I.H.L., Ribeiroa, A.G., Pissarraa, T.C.T. (2007): Nitrate and sodium contents on lettuce and drained water as function of fertilizing and irrigation water quality in Brazil. - Int. J Plant Prod. 1: 205-214.

[9] Godlewska, A., Ciepiela, G.A. (2013): The effect of natural growth regulators obtained from *ecklonia maxima* and mineral nitrogen on true protein and simple sugar contents of *Dactylis glomerata* L. and *Festulolium braunii* (K. Richt.) A. Camus. - Turk. J Field Crops. 18: 247-253

[10] Güllüoglu, L. (2011): Effects of growth regulator applications on pod yield and some agronomic characters of peanut in Mediterranean region. - Turk. J Field Crops. 16(2): 210-214.

[11] Janowska, B. (2011): Effect of flurprimidol and benzyladenine on growth and flowering of calla lily (*Zantedeschia spreng.*) cultivated in pots. - Science Nature Technol. 5(1): 1–9.

[12] Joubert, J.M., Lefranc, G. (2008): Seaweed phytostimulants in agriculture: recent studiem on mode of action two types of products from alga:growth and nutrition stimulants and stimulants of plant Demence reactions. Book of abstracts: Biostimulators in modern agriculture. Warsaw, p.16.

[13] Khan, W., Rayirath, U.P., Subramanian, S., Jithesh, M.N., Rayorath, P., Hodges, D.M., Critchley, A.T., Craigie, J.S., Norrie, J., Prithiviraj, B. (2009): Seaweed extracts as biostimulants of plant growth and development. - Plant Growth Regul. 28: 386–399.

[14] Kluza-Wieloch, M. (2004): Variability and heritability of morphological features of stems and leaves in observed types of sunflower (*Helianthus annuus* L.) cultivars. - Rocz. AR Pozn. *CCCLXIII,* Bot. 7: 149–165.

[15] Kocira, A., Świeca, M., Kocira, S., Złotek, U., Jakubczyk A. (2016): Enhancement of yield, nutritional and nutraceutical properties of two common bean cultivars following the application of seaweed extract (*Ecklonia maxima*). - Saudi J. Biol Sci. doi:10.1016/j.sjbs.2016.01.039

[16] Molle, M., Smith, M.L. (1999): The effect of pruning treatments using seaweed suspensions on the water sensitivity of Barley (*Hardeum vulgare* L.) caryopses. - Ann. Appl. Biol. 135: 515-522.

[17] Moller, M., Smith, M.L. (1998): The significance of the mineral component of seaweed suspension on lettuce (*Lactuca sativa* L.) seedling growth. - J Plant Physiol. 153: 658-663.

[18] Nour, K.A.M., Mansour, N.T.S., Abd El-Hakim, W.M. (2010): Influence of foliar spray with seaweed extracts on growth, setting and yield of tomato during summer season. - J Plant Prod. 7: 961-976.

[19] Pacholczak, A., Szydło, W., Jacygard, E., Federowicz, M. (2012): Effect of auxins and the biostimulator algaminoplant on rhizogenesis in stem cuttings of two dogwood cultivars (Cornus alba ‘Aurea’ and ‘Elegantissima’). - Acta Sci. Pol. Hortorum Cultus 11(2):93-103.

[20] Sivasankari, S., Venkatesalu, V., Anantharaj, M., Chandrasekaran, M. (2006): Effect of seaweed extracts on the growth and biochemical constituents of Vigna sinensis. - Bioresour. Technol. 97:1745-1751.

[21] Sosnowski, J., Jankowski, K., Wiśniewska-Kadżajan, B. (2013a): Effect of Kelp SL on the formation of above-ground biomass of *Festulolium braunii* (K. Richt.) A. Camus. - Acta Agrobot. 2: 149-154. DOI:10.5586/aa.2013.031.

[22] Sosnowski, J., Jankowski, K., Wiśniewska-Kadżajan, B., Jankowska, J. (2013b): Effect of different concentrations of Kelpak bioregulator on the formation of above-ground biomass cocksfoot. - J Ecol. Eng. 1: 48−52. DOI:10.5604/208139X.1031536.

[23] Sridhar, S., Rengasamy, R. (2011): Efeect of seaweed liquid fertilizer on the growth, biochemical constituents and yield of *Tagetes erecta,* under field trial. - J Phytology. 6: 61-68

[24] Stirk, W.A., Van Staden, J. (1997): Isolation and identification of cytokinins in a new commercial seaweed product made from *Fucus serratus* L. - J Appl. Phycol. 9: 327-330.

[25] Sultana, V., Ehteshamul-Haque, S., Ara, J., Athar, M. (2005): Comparative efficacy of brown, green and red seaweeds in the control of root infecting fungi and okra. - Int. J Environ. Sci. Tech. 2: 129-132.

[26] Szabo, V., Sarvari, A., Hrotko, K. (2011): Treatment of Stockplants with biostimulators and their effects on cutting propagation of prunus Marianna GF8-1. - Acta Hort. 923:277-282.

[27] Temple, W.D., Bomke, A.A. (1989): Effects of kelp (*Macrocystis integrifolia* and *Ecklonia maxima*) foliar applications on bean crop growth. - Plant Soil 117: 85-92.

[28] Thevanathan, R., Dinamani, D.S., Bhavani, L.G. (2005): Studies on the impact of application of marine algal manure and liquid fertiliser on the linear growth of the seedlings of some pulses. - Seaweed Res. Utili. 27: 125-133.

[29] Thirumaran, G., Arumugam, M., Arumugam, R., Anantharaman, P. (2009): Effect of seaweed liquid fertilizer on growth and pigment concentration of *Cyamopsis tetrogonolaba* (L) Taub. - Am-Euras. J Agron. 2: 50-56.

[30] Wajahatullah, K., Usha, P.R., Sowmyalakshmi, S., Mundaya, N.J., Prasanth, R., Hodges, A., Critchley, T., Craigie, J.S., Norrie, J., Prithiviraj, B. (2009): Seaweed extracts as biostimulants of plant growth and development. - J Plant Growth Regul. 28: 386–399. DOI 10.1007/s00344-009-9103-x

[31] Wierzbowska, J., Bowszyc, T. (2008): Effect of growth regulators applied together with different phosphorus fertilizations levels on the content and accumulation of potassium, magnesium and calcium in spring wheat. - J Elem. 13: 411-422.

[32] Zodape, S.T. (2001): Seaweeds as a biofertilizer. - J Sci. Industrial Res. 60: 378–382.

[33] Zodape, S.T., Mukhopadhyay, S., Eswaran, K., Reddy, M.P., Chikara, J. (2010): Enhanced yield and nutritional quality in green gram *(Phaseoulus radiata L.)* treated with seaweed (*Kappaphycus alvarezii*) extract. - J Sci. Industrial Res. 69: 468-471.

[34] Zodapea, S.T., Mukherjeea, S., Reddya, M.P., Chaudharya, D.R. (2009): Effect of *Kappaphycus alvarezii* (Doty) ex silva. extract on grain quality, yield and some yield components of wheat (*Triticum aestivum* L.). - Int. J Plant Prod. 3: 97-101.

STRUCTURAL AND SOCIAL ENRICHMENT: EFFECTS ON THE MORPHOLOGY OF A TROPICAL HATCHERY FISH

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Abstract. Hatchery tanks offer conditions completely different from the natural environment, with a high degree of environmental homogeneity. As a result, for some species, farmed fish can be morphologically distinct from their wild counterparts and the populations can be morphologically less diverse. To minimize the effects of the homogeneity, the environmental enrichment of tanks can be proposed as a way of resembling natural conditions. In this study we evaluated two types of enrichment: structural, which was performed by adding submerged logs (tree trunks) and artificial aquatic plants to increase environmental complexity; and social, which was accomplished by the simultaneous farming of two species in one pond to increase the degree of ecological interactions. The aim was to test whether social and structural enrichment of hatchery tanks modifies the morphology and anatomical variability of curimba *Prochilodus lineatus*. The results showed that both types of enrichment led to morphological divergence, however social enrichment had a bigger effect, increasing morphological variability and diluting the effect of structural enrichment. Therefore, the simultaneous farming of two or more species can constitute a simple tool for hatchery fish management, to increase population morphological variability and potentially increase the chances for survival after release into the natural environment.

Keywords: *environmental homogeneity; fish stocking; morphological variability; social interactions; structural complexity*

Introduction

Fish stocking consists of breeding fish in captivity (in hatcheries) for subsequent release into aquatic environments to enhance native populations. This management method has been long used worldwide to mitigate the negative effects from habitat loss and environmental disturbances, such as the construction of hydroelectric dams. However, the low fishery yield and the precarious conservation status of native migratory populations in southern and southeastern Brazil indicate that this practice has not been satisfactory (Agostinho et al., 2007). The poor stocking efficiency can be largely explained by low survival rates after release (Maynard et al., 1994), as the released fish are more adapted to captive conditions and consequently ill-suited to live in the wild (Olla et al., 1998; Araki et al., 2008; Belk et al., 2008; Johnsson et al., 2014). Hatchery tanks are quite homogenous and presents high density and less variable population, lacking of predators and prey, but constant food availability by pellets, while in the wild variables are spatially and temporally unpredictable, including turbidity level, water flow and structural complexity provided by gravel, rocks, plants and trees (Johnsson et al., 2014). As a result, farmed fish differ from wild fish in several aspects, like morphology, exhibiting distinct colorations (Maynard et al., 1994), different body, head and fin proportions (Swain et al., 1991; Flemming et al., 1994;

Hard et al., 2000; Solem et al., 2006; Belk et al., 2008) and less morphological variability (Taylor, 1986; Saraiva and Pompeu, 2016).

Thus, the environmental enrichment of hatchery tanks has been proposed as a means of minimizing the effects of captivity on the morphology of farmed fish (Näslund and Johnsson, 2014). The aim of enrichment is to modify captive conditions to improve biological functioning (Newberry, 1995). These modifications can be of various types, including the addition of sensory stimuli, greater complexity and increased ecological interaction (Batzina and Karakatsouli, 2012). Generally, all types of enrichment seek to make the conditions of captivity more similar to those of the natural environment, increasing animal wellbeing and, in the case of fish stocking, increasing the chances of survival after release into the natural environment.

The addition of shelter and substrates to the captive environment is called structural enrichment. Some studies have evaluated the effects of this kind of enrichment on farmed fish behavior (Berejikian et al., 2001, 2000; Brown et al., 2003), growth (Batzina and Karakatsouli, 2012), predation risk (Roberts et al., 2011) and morphology (Garduño-Paz et al., 2010; Saraiva and Pompeu, 2014). The increase of ecological interactions is called social enrichment, which involves the opportunity for hatchery individuals to live together with other conspecifics or individuals of a different species. The coexistence of two or more species can generate interspecific competition, which greatly affects individuals and can reduce their fertility, survival and growth (Begon et al., 2007). Social enrichment has been little studied in fish, but has been evaluated for other animal groups such as mammals (Schapiro et al., 1996; Elliott and Grunberg, 2005; Leonardi et al., 2010).

Fish hatchery tanks are most commonly simple environments lacking shelter, substrates or obstacles, and with a single species. By contrast, natural environments are structurally complex and contain many species sharing the same habitat and competing for food and shelter. In the present study we farmed two tropical characiform fishes, curimba *Prochilodus lineatus* (Valenciennes, 1837) and piapara *Leporinus elongatus* Valenciennes, 1837, simultaneously in structured tanks containing logs and artificial aquatic plants to assess whether social and structural enrichment affect fish morphology.

Curimba and piapara are two migratory fish species native from the Paraná river basin that are commonly used in fish stocking. In the wild, they generally share the same oxbow lake environment during their early development (Agostinho and Júlio-Junior, 1999). In fish hatcheries, these two species, like any others that share a tank, can be considered competitive as they vie for space and food. In structured tanks, they may also compete for the substrate and shelter provided by the structures. Curimba was chosen as the target species of the present study as it is the main species used for stocking enhancement on Fish Station of Volta Grande, on Parana river, due to its easy management in hatcheries.

We assumed that piapara would compete with curimba thereby increasing social complexity, and that the presence of logs and artificial macrophytes in the tanks would increase structural complexity. These two forms of enrichment together would make the hatchery environment more similar to a natural one. Thus, our hypothesis was that curimba farmed in the enriched tanks would exhibit different and more varied morphology than those farmed in conventional tanks.

Material and Methods

Breeding

The experiment was conducted at the fish hatchery station of the Volta Grande hydroelectric dam in Conceição das Alagoas, Minas Gerais State, Brazil. In December 2011, three female and six male curimba and two female and three male piapara, already predisposed for breeding, were selected from the available breeding stocks. Breeding for both species was conducted similarly and simultaneously, although in separate aquariums.

Spawning was induced by the application of three doses of crude catfish pituitary extract (*Ictalurus punctatus* from North America), calculated according to the weight of each individual. The newly fertilized eggs from all the progenitors of each species were pooled, homogenized, and transferred to incubators at equal densities. Three days after hatching, the contents of each incubator were transferred to a tank. The density of the postlarvae that were introduced to each tank was controlled during the egg stage based on the calculated fertilization rate (eggs hatched per milliliter). Using this rate, it was possible to determine the number of viable eggs per milliliter and, therefore, the volume of eggs required to generate the density of postlarvae specified for each tank. Each tank received a density of 150 larvae per square meter. This density is 50% less than the density normally used at Volta Grande Station and was chosen to favor the growth and survival of fish and also to provide at least 50 curimba in each tank at the end of the experiment.

Treatments

Eight fish hatchery tanks (3 m x 2.83 m x 0.75 m) with concrete walls and earthen floors were used. To test social enrichment only curimba were farmed in four tanks, while curimba and piapara were farmed together in the remaining tanks. To test structural enrichment we used two tanks without structures and the other six were structured using three different designs (*Fig. 1*). Each tank represented one of the following treatments:

1. C: control tank with curimba and without structures;
2. L: tank with curimba and logs (tree trunks);
3. M: tank with curimba and artificial macrophytes;
4. B: tank with curimba and both types of structures, logs and macrophytes;
5. P: tank with curimba and piapara, but without structures;
6. PL: tank with curimba, piapara and logs;
7. PM: tank with curimba, piapara and artificial macrophytes;
8. PB: tank with curimba, piapara and both logs and macrophytes.

Logistical problems prevented us to replicate the treatments. However, previous study at the same hatchery and using the same tanks and the same types of structural enrichment have indicated that there was no significant effect of the tank on the morphology of fish (Saraiva and Pompeu, 2014)

In tanks with piapara, to maintain the fish density of 150/m², postlarvae were added at a ratio of 100 curimba to 50 piapara per square meter. All the postlarvae were added to the tanks on a single day (in December 2011), three days after hatching. The tanks were structured one month after adding the fish (in January 2012), when the fish were one month old. During the experiment fish were fed once a day with commercial pellets for tropical fish (52% crude protein) and all tanks received the same amount.

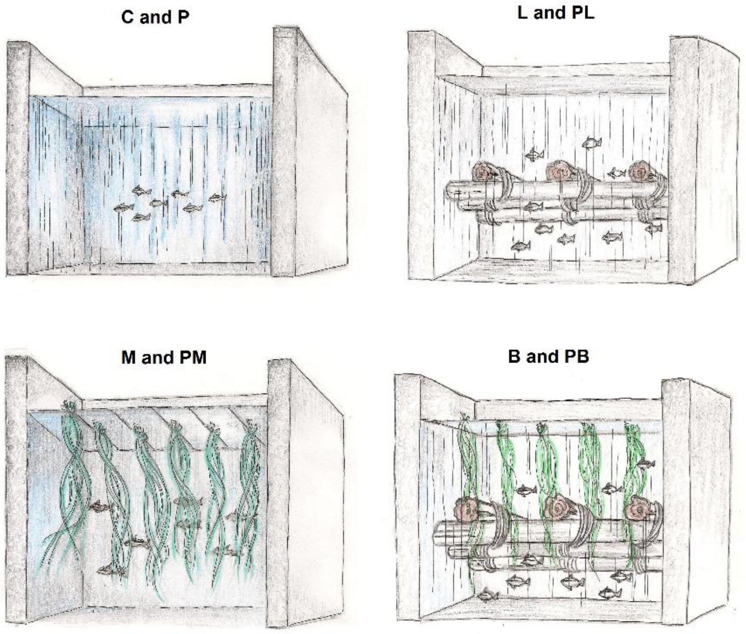

***Figure 1.** Schematic drawing of the four types of tanks used to evaluate the structural enrichment: (C and P) control, the tank did not receive any type of structuring; (L and PL) logs, the tank was structured with six eucalyptus tree logs, set in the middle of the tank so that they were submersed after the tank was filled. Three of the logs were arranged along the tank and the other three were arranged perpendicular; (M and PM) artificial macrophytes, the tank received plastic macrophytes hung on a kind of clothesline placed above the tank, so that the plastic filaments came in contact with the water. Each tank received twenty five plastic macrophytes, arranged in five parallel rows of five plants each; (B and PB) logs and artificial macrophytes, the tank received both types of structures, submersed logs and artificial macrophytes.*

Collection

The collections were made in March of 2012, when the fish were three months old and had been exposed to the treatments for two months. To capture fish, the water level in the tanks was lowered and dip nets were used. All curimba in each tank were captured and the piapara were kept alive in tanks for use by the Volta Grande fish hatchery station. After collection, the curimba were anesthetized with clove oil, fixed in 10% formaldehyde and subsequently preserved in 70% alcohol. The fish voucher specimens were deposited in the Ichthyological Collection of the Federal University of Lavras (CI-UFLA 786-787).

Morphometry

All fish collected were measured and weighed in a laboratory. Twenty-seven morphometric measurements were taken from each of the individuals collected (standard length, SL; body height, BH; body width, BW; mean body height, MH; body area, BA; head length, HeL; head height, HeH; eye height, EH; eye area, EA; length of the caudal peduncle, LCdP; height of the caudal peduncle, HCdP; width of the caudal peduncle, WCdP; length of the dorsal fin, LDsF; height of the dorsal fin, HDsF; area of the dorsal fin, ADsF; length of the caudal fin, LCdF; height of the caudal fin, HCdF; area of the caudal fin, ACdF; length of the anal fin, LAnF; width of the anal fin, WAnF; area of the anal fin, AAnF; length of the pelvic fin, LPlF; width of the pelvic fin, WPlF; area of the pelvic fin, APlF; length of the pectoral fin, LPtF; width of the pectoral fin, WPtF; area of the pectoral fin, APtF). Linear measurements were obtained directly from the body of the fish, always on the left side and by the same person, using a digital caliper accurate to 0.01 mm. Areas were measured by drawing an outline of the body and fins of each fish on paper. The drawings were then scanned and the areas were calculated using the Image J software (U. S. National Institutes of Health, Bethesda, Maryland, USA). From the morphometric measurements, twenty-one ecomorphological traits (compression index, CI = BH/BW; index of ventral flattening, IVF = MH/BH; relative body height, RBH = BH/SL; relative eye position, REP = EH/HeH; relative eye area, REA = $EA/(SL)^2$; relative head length, RHeL = HeL/SL; relative length of the caudal peduncle, RLCdP = LCdP/SL; relative height of the caudal peduncle, RHCdP = HCdP/BH; relative width of the caudal peduncle, RWCdP = WCdP/BW; compression index of the caudal peduncle, CICdP = HCdP/WCdP; relative area of the dorsal fin, RADsF = ADsF/BA; relative area of the caudal fin, RACdF = ACdF/BA; Aspect ratio of the caudal fin, ARCdF = $(HCdF)^2/ACdF$; relative area of the anal fin, RAAnF = $AAnF/(SL)^2$; aspect ratio of the anal fin, ARAnF = $(LAnF)^2/AAnF$; relative length of the pelvic fin, RLPlF = LPlF/SL; relative area of the pelvic fin, RAPlF = APlF/BA; aspect ratio of the pelvic fin, ARPlF = LPlF/WPlF; relative length of the pectoral fin, RLPtF = LPtF/SL; relative area of the pectoral fin, RAPtF = APtF/BA; aspect ratio of the pectoral fin, ARPtF = LPtF/WPtF) were calculated according to Hora (1930); Gosline (1971); Gatz (1979); Watson and Balon (1984); Winemiller (1991); Pouilly et al. (2003); Oliveira & Goulart (2005). Each individual was considered a sampling unit of its related treatment.

Statistical analyses

To evaluate whether tank enrichment affects the growth and nutritional status of fish, we compared the standard lengths and the condition factors of the individuals from different treatments. The standard lengths were compared using ANOVA and Tukey's test. The condition factors (CFs) were calculated using the formula:

$$CF = \frac{W}{(SL)^b} \quad \text{(Eq. 1)}$$

where W is the weight, SL is the standard length, and *b* is the slope of the regression line between the weight and the length. This metric is based on the assumption that individuals of a given length with a greater mass are in better condition than those with a lesser mass (Ricker, 1975). Having established the normality of the data, the condition factors were compared between treatments by ANOVA and Tukey's test.

Principal components analysis (PCA) were used to determine the distribution of individuals in the morphological space. Discriminant analysis (DCA) was performed to assess morphological differences and the Squared Mahalanobis Distances were considered to identify the degree of differentiation among treatments due to social and structural enrichments.

Changes in morphological variability were sought by the distance of each individual from the centroid of its population (CD), which was calculated from distance matrices. The CD metric is one estimate of the relative size of the morphological hypervolume occupied by the population (Winemiller, 1991), which reflects the morphological variability of the population. An ANOVA with Tukey's post hoc test was performed on the calculated CD values to assess possible differences in morphological variability among the populations under each treatment.

Results

The number of curimba at the end of the experiment differed among tanks (C=50, L=51, M=22, B=34, P=48, PL=46, PM=15 and PB=27) and was substantially lower in the tanks enriched with macrophytes (M, B, PM and PB), despite the same post-larvae density introduced at the beginning of the experiment. This indicates a higher mortality rate in tanks with artificial macrophytes, which was even higher than those tanks with piapara. On the other hand, enrichment with logs and/or piapara (i.e. without macrophytes) did not affect curimba survival. The number of piapara left in each tank at the end of the experiment was fairly similar (P=19, PL=20, PM=22, PB= 20).

Average standard length differed between treatments (ANOVA - F(7;284) = 2.696; p = 0.0102). Fish from all enrichment treatments presented higher size averages than the control (C), however Tukey's test revealed that differences were only significant within tanks PL, PM and PB (*Fig. 2a*).

The condition factor (CF) also varied depending on the enrichment type (*Fig. 2b*). Fish with the best condition were from tanks L and B, indicating that the presence of logs in tanks improved the physiological status of fish. However, this effect only occurred when curimba were farmed alone. When piapara were also present, the CF was lower and did not differ from the control (C).

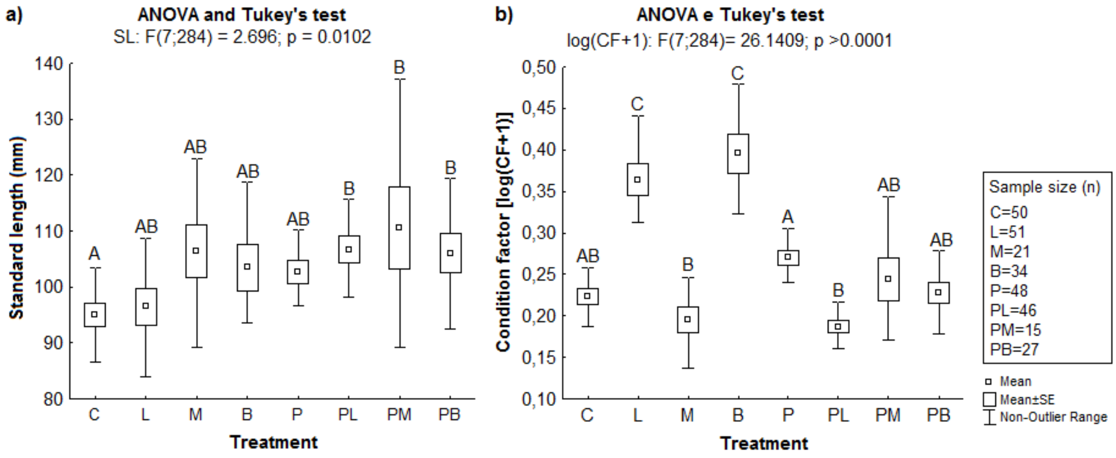

Figure 2. *a) Curimba standard length per treatment. After a significant ANOVA ($P < 0.05$), Tukey's test compared standard length averages among treatments. b) Log of the curimba condition factor plus one [log (CF + 1)] for each treatment. After a significant ANOVA ($p < 0.05$), Tukey's test compared the log (CF + 1) values averages among the treatments (C= control tank, L= tank with logs, M= tank with artificial macrophytes, B= tank with both logs and artificial macrophytes, P= tank with piapara, PL= tank with piapara and logs, PM= tank with piapara and artificial macrophytes and PB= tank with piapara and both logs and artificial macrophytes). Treatments with the same letter above their ranges did not differ significantly.*

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With respect to morphology, the curimba populations of each treatment occupied distinct morphological spaces with little overlap between the tanks with and without piapara, according to PCA (*Fig. 3a*). The first two PCA axes accounted for 39.59% of the variance: the first axis represented 22.76% and the second axis 16.83%. The contributions of the variables to the PCA axes (*Table 1*) showed that RADsF, RACdF, RAAnF and RAPlF positively influenced the first axis, whereas CI negatively influenced the second axis. Analyzing PCA separately for the treatments with and without piapara, stays clear that overlap was bigger when piapara was present (*Figs. 3b-c*). In the absence of piapara the first two PCA axes accounted for 41.83% of the variance: the first axis represented 24.81% and the second axis 17.02%. The traits that most contributed for the differentiation among treatments in the first axis were CI, REA, RHeL (positively) and RHCdP (negatively) and in the second axis were RAPlF and RAPtF (positively). In the presence of piapara the first two PCA axes accounted for 28.58% of the variance: the first axis represented 19.90% and the second axis 8.68%. The traits that most contributed for the differentiation among treatments were CI and RHeL, both positively and in the first axis.

Table 1. *Contributions of ecomorphological traits to the first two axes of the principal component analysis for the treatments without and with pipara, analyzed together and separately. Highlighted in bold are the most important traits for differentiation among treatments in each axis.*

Trait	With and without piapara		Without piapara		With pipara	
	PCA 1	PCA 2	PCA 1	PCA 2	PCA 1	PCA 2
CI	-0,10	**-0,83**	**0,83**	-0,29	**0,81**	-0,10
IVF	0,04	0,31	-0,45	-0,14	0,05	0,01
RBH	-0,22	-0,41	0,26	-0,18	0,52	-0,17
REP	-0,17	-0,43	0,41	-0,11	0,35	0,01
REA	-0,02	-0,34	**0,86**	-0,06	0,11	-0,25
RHeL	0,08	-0,77	**0,87**	-0,03	**0,81**	0,00
RLCdP	0,38	0,31	-0,34	0,19	-0,16	0,14
RHCdP	0,15	0,68	**-0,81**	0,22	-0,70	0,19
RWCdP	-0,20	-0,10	-0,31	-0,16	-0,09	0,58
CICdP	0,15	-0,30	0,65	0,04	0,47	-0,48
RADsF	**0,89**	-0,16	0,37	0,47	0,48	0,47
RACdF	**0,88**	-0,05	0,13	0,61	-0,04	0,31
ARCdF	-0,57	0,15	-0,31	-0,32	0,07	0,06
RAAnF	**0,89**	-0,10	0,18	0,77	0,24	0,47
ARAnF	-0,70	-0,14	-0,04	-0,64	0,10	-0,31
RLPlF	0,00	-0,66	0,62	0,30	0,70	0,18
RAPlF	**0,86**	-0,03	-0,10	**0,88**	0,33	-0,16
ARPlF	0,07	-0,46	0,62	0,05	0,46	0,24
RLPtF	0,08	-0,52	0,35	0,27	0,69	0,14
RAPtF	0,38	0,10	-0,27	**0,81**	0,25	-0,22
ARPtF	0,61	-0,14	0,12	-0,05	0,20	0,52

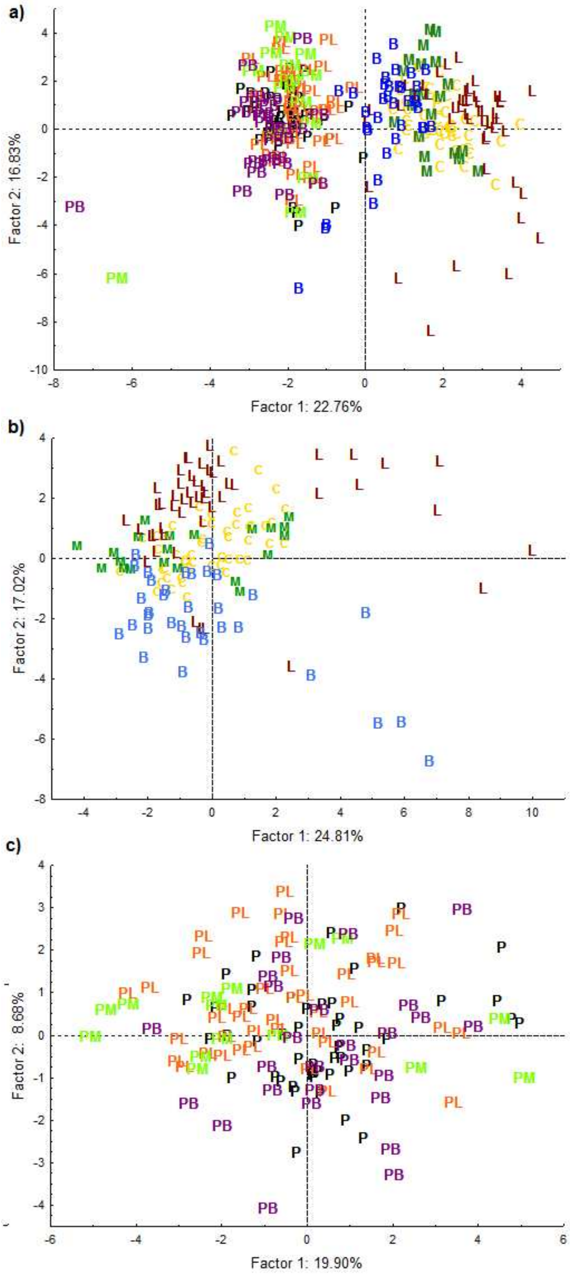

Figure 3. *a) Simultaneous projection of the eight curimba treatments onto the first two axes of the principal components analysis. b) Individual projection of the four curimba treatments without piapara onto the first two axes of the principal components analysis. c) Individual projection of the four curimba treatments with piapara onto the first two axes of the principal components analysis. Each letter or letter combination indicates the space occupied by individual fish from one of the treatments (C= control tank, L= tank with logs, M= tank with artificial macrophytes, B= tank with both logs and artificial macrophytes, P= tank with piapara, PL= tank with piapara and logs, PM= tank with piapara and artificial macrophytes and PB= tank with piapara and both logs and artificial macrophytes). Sample size: C=50, L=51, M=21, B=34, P=48, PL=46, PM=15, PB=27.*

The DCA performed for the eight treatments showed that all treatments differed between themselves ($p < 0.05$) and the squared Mahalanobis distances indicated how different they were from each other (*Table 2*). In relation to the control (C), the distance of P, PL, PM and PB treatments, were much larger than L, M and B distances. Therefore it is evident that the social enrichment effect was much larger than the structural, and when both types of enrichment were used together there was not a big difference related to the P tank. The squared Mahalanobis distances from the control were not related to the number of individuals in the tanks, which clearly shows that density did not affect the morphological traits (*Fig. 4a*). Furthermore, according to the DCA, the morphological traits most affected by treatments varied depending on the presence or absence of piapara (*Table 3*). When piapara were present, the traits most influenced were the relative height of caudal peduncle (RHCdP) and relative width of caudal peduncle (RWCdP). When piapara were absent, the most affected traits were the relative area of dorsal fin (RADsF), aspect ratio of anal fin (ARAnF) and relative area of pectoral fin (RAPtF).

Table 2. *Squared Mahalanobis Distances between treatments. Highlighted in bold are the distances from each treatment compared to control.*

Treatment	C	L	M	B	P	PL	PM	PB
C	0.00001							
L	**9.48614**	0.00001						
M	**10.98950**	6.05425	0.00001					
B	**15.95496**	13.96630	10.86478	0.00001				
P	**63.33056**	57.76256	42.19287	43.62259	0.00001			
PL	**54.22461**	47.92367	36.99225	32.90138	5.64027	0.00001		
PM	**62.38812**	54.43418	42.46321	36.99586	9.51183	3.21852	0.00001	
PB	**60.84078**	58.15736	43.81637	35.84109	7.81064	3.68499	7.64126	0.00001

Table 3. *Discriminant analysis for the two sets of tank treatments with and without Piapara, with Wilks' lambda = 0.07040, F (63, 394) = 8.9793 and p < 0.00001 and Wilks' lambda = 0.20333, F (63, 335) = 3.7513 and p < 0.00001, respectively. Highlighted in bold are the traits that were significant for the discrimination among treatments.*

Trait	Without piapara		With piapara	
	F (3, 132)	P value	F (3, 112)	P value
CI	0.56147	0.641376	**4.509244**	**0.005025**
IVF	1.08495	0.357866	0.221322	0.881416
RBH	**2.85823**	**0.039503**	**9.317058**	**0.000015**
REP	0.45582	0.713624	0.893002	0.447202
REA	1.18988	0.316187	1.691071	0.172998
RHeL	**3.00840**	**0.032617**	**6.279516**	**0.000561**
RLCdP	**3.66715**	**0.014055**	1.129327	0.340394
RHCdP	0.48526	0.693099	**3.779018**	**0.012583**
WCdP	1.09736	0.352691	**3.218605**	**0.025522**
CICdP	0.78022	0.507005	2.622597	0.054132
RADsF	**3.03060**	**0.031705**	1.711437	0.168722
RACdF	**13.58067**	**0.000000**	**3.932137**	**0.010375**
ARCdF	**4.74532**	**0.003553**	**5.905863**	**0.000887**
RAAnF	2.29865	0.080410	0.237166	0.870283
ARAnF	**3.28459**	**0.022920**	0.701614	0.553013
RLPlF	1.16033	0.327458	1.624861	0.187627
RAPlF	2.34158	0.076162	1.558402	0.203494
ARPlF	**8.06223**	**0.000057**	**4.882965**	**0.003149**
RLPtF	1.03137	0.380968	2.618520	0.054411
RAPtF	**16.27473**	**0.000000**	1.946569	0.126179
ARPtF	**3.08515**	**0.029572**	0.615030	0.606661

There was a significant difference between the centroid distances and, therefore, between the morphological variability among treatments. The curimba populations farmed together with piapara showed greater morphological variability than the populations farmed alone (*Fig. 4b*). Relative to both the control (C) and the treatments without piapara, all treatments with piapara generated significantly greater morphological variability, but did not differ among themselves. These results show that social enrichment (as opposed to structural enrichment) increased morphological variability.

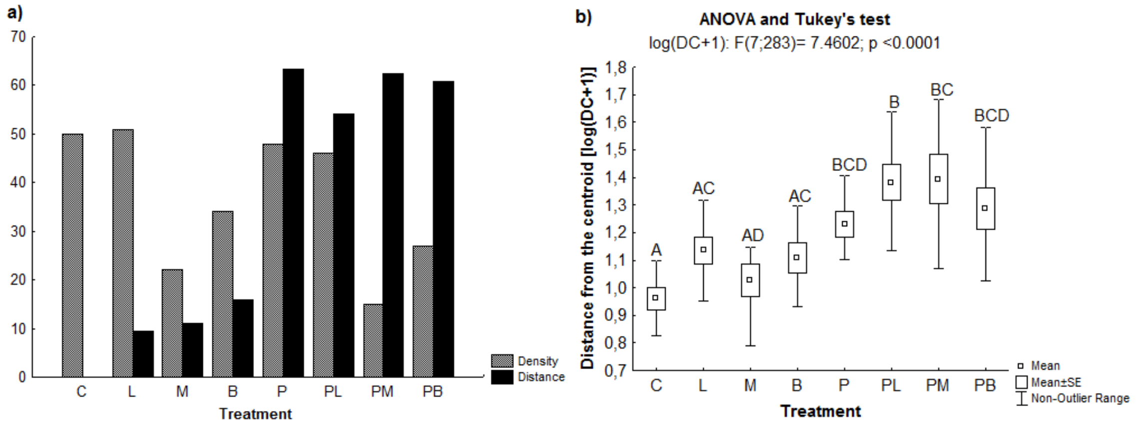

Figure 4. *a) Densities of individuals per tank compared with the squared Mahalanobis distances of each treatment from the control (C). b) Log of the distance from the centroid plus one [log (DC + 1)] for each curimba treatment population. After a significant ANOVA ($P < 0.05$), Tukey's test compared the log (DC + 1) values among the treatments (C= control tank, L= tank with logs, M= tank with artificial macrophytes, B= tank with both logs and artificial macrophytes, P= tank with piapara, PL= tank with piapara and logs, PM= tank with piapara and artificial macrophytes and PB= tank with piapara and both logs and artificial macrophytes). Treatments with the same letter above their ranges did not differ significantly. Sample size: C=50, L=51, M=21, B=34, P=48, PL=46, PM=15, PB=27.*

Discussion

During interspecific competition, individuals from one species suffer a reduction in fertility, growth or survival as a result of resource exploitation or interference by another species (Begon et al, 2007). Intraspecific competition acts in a similar way but on individuals of the same species. In fish hatcheries, individuals compete for the same resources, such as food and space, and in the case of structured tanks, they may also compete for the added substrates. This competition may be intra and/or interspecific, and the two types of competition may interact, since the presence of another species may alter the interactions among individuals of the same species and vice versa (Persson and Greenberg, 1990).

In this study, the presence of piapara in the tanks did not directly affect curimba survival, however enrichment with artificial macrophytes had a negative effect (the number of curimba at the end of the experiment was lower in tanks enriched with macrophytes). A possible explanation is that foraging was impaired by the artificial macrophytes, because the plastic filaments tangled and/or trapped the food particles (personal observation), making them less accessible to fish. Tátrai and Herzig (1995) found similar result in an experiment with razor fish, which showed significantly

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reduced feeding levels in a complex environment with submerged macrophytes. They attribute the lower feeding efficiency to the macrophytes, that obstruct light, restrict predators in their movement and increase the chances for mobile invertebrates to escape from an attack. In another recent study, we found that artificial macrophytes hamper the development of curimba, decreasing the condition factor of individuals (Saraiva and Pompeu, 2014). However, in the present study, the condition factor of curimba in the tanks treated with macrophytes was not significantly affected, but this may be a result of the higher mortality and consequent lower density in these tanks. Curimba mortality was even higher in the tanks with both piapara and artificial macrophytes than in tanks with only artificial macrophytes. This result supports the explanation raised above, indicating an interaction between the effects of the two enrichment types: the tangled artificial macrophytes in addition to intra-specific competition with piapara led to a food resource limitation.

Similar to survival, curimba growth was not directly affected by the presence of piapara, since fish from tank P did not differ in size of those from the control (C). Standard length was significant bigger than the control only when both structural and social enrichment were present in tanks (PL, PM and PB). This result differs from the findings of Berejikian et al. (2001), who found that the growth of steelhead *Oncorhynchus mykiss* varied with the presence of competitors, but not with the structural enrichment of hatchery tanks. However, it is important to note that in PM and PB tanks, the density of curimba was lower, so greater growth was already expected (Maynard et al., 1994). The bigger fish size in these tanks is most likely related to the low density than to the enrichment. In the PL tank, despite the high population density and the presence of piapara, curimba growth rates were higher than those from control, indicating that the presence of logs favored growth. There was a considerable accumulation of periphyton on logs, which can favor fish growth and survival (vam Dam et al., 2002; Azim et al., 2005). However, enrichment with logs alone is not enough to explain the larger growth, since a significant increase in fish size did not occur in L tank. Thus, in some way, the presence of piapara must also have contributed to the higher standard length observed in the PL tank.

Logs also increased the condition factor in L and B tanks in relation to C. This improvement was certainly related to the periphyton on the logs, which worked as a supplementary food resource for curimba (personal observation). However, in the presence of piapara there was no increase, nor decrease, in curimba condition factor in any tank (P, PL, PM and PB). Thus, piapara apparently did not physiologically impair curimba (considering the high growth rates in the PL tank), yet the presence of piapara prevented improvements in curimba condition when logs were present, as occurred in L and B tanks. This finding may be evidence of exploitative competition, which occurs when the levels of a resource (in this case, periphyton on logs) decrease due to the presence and activity of other individuals or species (in this case piapara) (Begon et al., 2007).

Enrichment also affected morphological traits. Morphological divergence caused by competition has been described in some studies (Cussac et al., 1998; Olsson et al., 2006), however, it was not always possible to prove that the competition was the source of the morphological variation detected (Cussac et al., 1998). In the present study, by comparing tanks with and without piapara we were able to detect an effect of this species on the morphology of curimba. By comparing tanks without structures to those with different structure types, we could also demonstrate the structural enrichment effect, in the same way as in a previous study (Saraiva and Pompeu, 2014). Since the same pattern of results was found in both studies we can certify, despite the lack of replications, that the changes in fish morphology were a result of the treatment itself and not of a possible tank effect.

We also have to consider that fishes grow allometrically, so the body shape changes depending on the growth (Klingenberg and Froese, 1991). As fish from PL, PM and PB tanks were bigger than those from control tank, so it was expected that they had suffered the biggest mophological changes. But according to Mahalanobis distances, fish from P tank were as different as PL, PM and PB fish. So, if there is an allometric effect on morphology, it is added to the enrichment effect, which does not nullify it.

As shown above, both the social as the structural enrichment have affected fish morphology, but the Mahalanobis distances indicated that the social enrichment effect was much more stronger than the structural. Contrary to our expectations, the effect of one enrichment type did not add to the effect of the other, increasing fish divergence relative to the control. On the contrary, social enrichment seems to have diluted the effect of structural enrichment, as structuring ceased to be as important for curimba morphological variation when in the presence of piapara. One of the most important results of this study, it was that the social enrichment had increased the morphological variability in the reared curimba populations, approaching them of the wild populations (Taylor, 1986; Saraiva and Pompeu, 2016).

Upon analyzing the morphological traits that most contributed to differentiation among populations in DCA, we note that traits varied depending on the presence or absence of piapara. Relative length of caudal peduncle, relative area of dorsal fin, aspect ratio of anal fin, relative area of pectoral fin and aspect ratio of pectoral fin were only important in tanks without piapara. The first trait is linked to swimming ability (Gatz, 1979;Watson and Balon, 1984) and the others are related to performance of maneuvers (Gosline, 1971; Watson and Balon, 1984; Breda et al., 2005), suggesting that the curimba may have swum and used the structures more when piapara was absent. If so, we can assume that competition for space occurred and piapara dominated the structures at the expense of the curimba. However, in the absence of behavioral data is not possible affirm this.

Despite the logistic limitation of lacking replicates for each treatment, the results obtained indicate that fish raised with an interspecific competitor could present more varied morphology. Wild populations exhibit great variability in shape (Taylor, 1986; Saraiva and Pompeu, 2016). Therefore, simply using two or more species is a potential management tool in hatcheries to obtain fish more similar to wild ones. Structural enrichment could also lead to a distinct morphology, but to a lesser magnitude. Additional studies should be conducted to improve enrichment techniques, by analyzing behavioral data and identifying the most appropriate structures, as the structural enrichment effect will depend on the species, life stage and the enrichment type (Näslund and Johnsson, 2014)

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REFERENCES

[1] Agostinho, A.A., Júlio-Junior, H.F. (1999): Peixes da bacia do Alto Paraná. Pp. 374-400. In: Lowe-McConnell, R.H. (Ed.). Estudos ecológicos de comunidades de peixes tropicais. Universidade de São Paulo, São Paulo.

[2] Agostinho, A.A., Gomes, L.C., Pelicice, F.M. (2007): Ecologia e manejo de recursos pesqueiros em reservatórios do Brasil. Universidade Estadual de Maringá, Maringá.

[3] Araki, H., Berejikian, B.A., Ford, M.J., Blouin, M.S. (2008): Fitness of hatchery-reared salmonids in the wild - Evolutionary Applications 1: 342–355.

[4] Azim, M.E., Verdegem, M.C.J., van Dam, A.A., Beveridge, M.C.M. (2005): Periphyton: ecology, exploitation and management. CABI Publishing, Wallingford.

[5] Batzina, A., Karakatsouli, N. (2012): The presence of substrate as a means of environmental enrichment in intensively reared gilthead seabream *Sparus aurata*: Growth and behavioral effects - Aquaculture 370-371: 54-60.

[6] Begon, M., Townsend, C.R., Harper, J.L. (2007): Ecologia de Indivíduos e Ecossistemas. Artmed, São Paulo.

[7] Belk, M.C., Benson, L.J., Rasmussen, J., Peck, S.L. (2008): Hatchery-induced morphological variation in an endangered fish: a challenge for hatchery-based recovery efforts - Canadian Journal of Fisheries and Aquatic Sciences 65: 401-408.

[8] Berejikian, B.A., Tezak, E.P., Riley, S.C., Larae, A.L. (2001): Competitive ability and social behavior of juvenile steelhead reared in enriched and conventional hatchery tanks and a stream environment - Journal of Fish Biology 59: 1600-1613.

[9] Berejikian, B.A., Tezak, E.P., Larae, A.L., Flagg, T.A., Kummerow, E., Mahnken, C.V.W. (2000): Social dominance, growth and habitat use of age-0 steelhead (*Oncorhynchus mykiss*) grown in enriched and conventional hatchery rearing environments - Canadian Journal of Fisheries and Aquatic Sciences 57: 628–636.

[10] Breda, L., Oliveira, E.F., Goulart, E. (2005): Ecomorfologia de locomoção de peixes com enfoque para espécies neotropicais - Acta Scientarium Biological Sciences 27: 371-381.

[11] Brown, C., Davidson, T., Laland, K. (2003): Environmental enrichment and prior experience of live prey improve foraging behaviour in hatchery-reared Atlantic salmon - Journal of Fish Biology 63: 187-196.

[12] Cussac, V.E., Ruzzante, D., Walde, S., Macchi, P.J., Ojeda, V., Alonso, M.F., Denegri, M.A. (1998): Body shape variation of three species of *Percichthys* in relation to their coexistence in the Limay river basin, in northern Patagonia - Environmental Biology of Fishes 53: 143-153.

[13] Elliott, B.M., Grunberg, N.E. (2005): Effects of social and physical enrichment on open field activity differ in male and female Sprague-Dawley rats - Behavioural Brain Research 165: 187–196.

[14] Fleming, I.A., Jonsson, N.B., Gross, M.R. (1994): Phenotypic divergence of sea-ranched, farmed, and wild salmon - Canadian Journal of Fisheries and Aquatic Sciences 51: 2808-2824.

[15] Garduño-Paz, M.V., Couderc, S., Adams, C. E. (2010): Habitat complexity modulates phenotype expression through developmental plasticity in the threespine stickleback - Biological Journal of the Linnean Society 100: 407-413.

[16] Gatz Junior, A.J. (1979): Ecological morphology of freshwater stream fishes - Tulanne Studies in Zoology Botany 21: 91-124.

[17] Gosline, W.A. (1971): Functional morphology and classification of teleostean fishes. University of Hawaii, Honolulu.

[18] Hard, J.J., Berejikian, B.A., Tezak, E.P., Schroder, S., Knudsen, C.M., Parker, L.T. (2000): Evidence for morphometric differentiation of wild and captively reared adult coho salmon: a geometric analysis - Environmental Biology of Fishes 58: 61-73.

[19] Hora, S.L. (1930): Ecology, bionomics and evolution of the torrential fauna, with special reference to the organs of attachment - Philosophical Transactions of the Royal Society of London 28: 171-282.

[20] Klingenberg, C.P., Froese, R. (1991): A Mulltivariate Comparison of Allometric Growth Patterns - Systematic Zoology 40: 410-419.

[21] Leonardi, R., Buchanan-Smith, H.M., Dufour, V., MacDonald, C., Whiten, A. (2010): Living together: behavior and welfare in single and mixed species groups of capuchin (*Cebus apella*) and squirrel monkeys (*Saimiri sciureus*) - American Journal of Primatology 72: 33-47.

[22] Maynard, D.J., Flagg, T.A., Mahnken, C.V.W. (1994): A Review of Seminatural Culture Strategies for Enhancing the Postrelease Survival of Anadromous Salmonids. National Marine Fisheries Service, Seattle.

[23] Näslund, J., Johnsson, J.I. (2014): Environmental enrichment for fish in captive environments: Effects of physical structures and substrates - Fish and Fisheries 1-30.

[24] Newberry, R.C. (1995): Environmental enrichment : Increasing the biological relevance of captive environments - Applied Animal Behavior Science 44: 229-243.

[25] Olla, B.L., Davis, M.W., Ryer, C.H. (1998): Understanding how the hatchery environment represses or promotes the development of behavioral survival skills - Bulletin of Marine Science 62: 531-550.

[26] Olsson, J., Svanba, R., Eklo, P. (2006): Growth rate constrain morphological divergence when driven by competition - Oikos 1: 15-22.

[27] Persson, L., Greenberg, L. (1990): Interspecific and intraspecific size class competition affecting resource use and growth of perch, *Perca fluviatilis* - Oikos 59: 97–106.

[28] Pouilly, M., Lino, F., Bretenoux, J.G., Rosales, C. (2003): Dietarymorphological relationships in a fish assemblage of the Bolivian Amazonian floodplain - Journal of Fish Biology 62: 1137-1158.

[29] Ricker, W.E. (1975): Computation and interpretation of biological statistics of fish populations. Fisheries and Marine Service, An Arbor.

[30] Roberts, L.J., Taylor, J., Garcia de Leaniz, C. (2011): Environmental enrichment reduces maladaptive risk-taking behavior in salmon reared for conservation - Biological Conservation 144: 1972–1979.

[31] Saraiva, S.O., Pompeu, P.S. (2014): The effect of structural enrichment in hatchery tanks on the morphology of two neotropical fish species - Neotropical Ichthyology 12: 891–901.

[32] Saraiva, S. O., Pompeu, P. S. (2016): Fish hatchering and its effects on the morphology of *Prochilodus lineatus* (Actinopterygii: Prochilodontidae) - Brazilian Journal of Biology 76: 209-217.

[33] Schapiro, S.J., Bloomsmith, M.A., Suarez, S.A., Porter, L.M. (1996). Effects of social and inanimate enrichment on the behavior of yearling rhesus monkeys - American Journal of Primatology 40: 247–260.

[34] Solem, Ø., Berg, O.K., Kjøsnes, A.J. (2006): Inter- and intra-population morphological differences between wild and farmed Atlantic salmon juveniles - Journal of Fish Biology 69: 1466–1481.

[35] Swain, D.P., Riddell, B.E., Murray, C.B. (1991): Morphological differences between hatchery and wild populations of coho salmon (*Oncorhynchus kisutch*): environmental versus genetic origin - Canadian Journal of Fisheries and Aquatic Sciences 48: 1783-1791.

[36] Tátrai, I., Herzig, A. (1995): Effect of habitat structure on the feeding efficiency of young stages of razor fish (*Pelecus cultratus* (L.)): an experimental approach - Hydrobiologia 299: 75-81.

[37] Taylor, E.B. (1986): Differences in morphology between wild and hatchery populations of juvenile Coho Salmon - The Progressive Fish-Culturist 48: 171-176.

[38] van Dam, A.A., Beveridge, M.C.M., Azim, M.E., Verdegem, M.C.J. (2002): The potential of fish production based on periphyton - Reviews in Fish Biology and Fisheries 12: 1–31.

[39] Watson, D.J., Balon, E. (1984): Ecomorphological analysis of taxocenes in rainforest streams of northern Borneo - Journal of Fish Biology 25: 371-384.

[40] Winemiller, K.O. (1991): Ecomorphological diversification in lowland freshwater fish assemblages from five biotic regions - Ecological Monographs 61: 343-365.

BALANCING EFFECT OF LARCH PLANTATIONS (*LARIX KAEMPFERI*) ON UNDERSTORY PLANT DIVERSITY IN A SUB-TROPICAL FOREST ECOSYSTEM, CHINA

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Abstract. We evaluated effects of larch plantations on understory plant diversity by conducting large-area investigations on three types of tree stands, including pure larch plantations (*Larix kaempferi*, an exotic coniferous deciduous tree species), pure liriodendron plantations (*Liriodendron chinense*, a native broad-leaved deciduous tree species) and mixed stands (*L. kaempferi* and *L. chinense*) in a subtropical forest. A total of 19 plantation areas, 684 quardrats, and 10 environmental factors [elevation, slope, ratio of light availability (RLA), soil pH, soil water content (SWC), soil organic matter (SOM), soil total nitrogen (STN), soil total phosphorus (STP), soil total potassium (STK)] were surveyed. Species richness and Shannon's diversity were statistically similar in the three types of tree stands but Shannon's diversity was somewhat higher in mixed plantations. Environmental conditions were similar in the three types of tree stands ($P > 0.05$). Species richness and Shannon's diversity increased with increasing elevation and understory light availability ($P<0.05$). Canonical Correspondence Analysis (CCA) showed that distribution of shrubs was determined by elevation, while herb distribution was related primarily to soil fertility followed by elevation. Based on the unchanged environmental conditions after establishment of larch plantations, we conclude that larch plantations had balancing effect on understory plant diversity.

Keywords: *species richness; Shannon's diversity; environmental factors; larch plantations*

Introduction

In recent decades, fast-growing tree species, such as eucalyptus (Gerber, 2011), rubber (Tan et al., 2011), poplar (Weih et al., 2003), and larch (Yu et al., 2006), have been used widely in forestation or reforestation to meet increasing demand for industrial timber and pulp. These tree species are often exotic and have a wider environmental adaptability than native trees (Turnbull, 1999). Therefore large-scale fast-growing tree plantations have provoked increasing concerns regarding their negative effects on plant diversity (Weih et al., 2003; Morris et al., 2008). Fast-growing tree plantations had lower plant diversity than crop lands (Weih et al., 2003; Britt et al., 2007) but greater species richness than abandoned fields (Lee et al., 2005; Boothroyd-Roberts et al., 2013). Tree plantations appear to have varied effects on understory plant diversity (Boothroyd-Roberts et al., 2013). To date, the extent to which understory plant diversity is affected by fast-growing tree plantations is still unclear.

Environmental change resulting from tree plantation has been recognized as a key factor driving the variation in understory plant diversity (Franklin and Steadman, 2010; Ibáñez and Gazol, 2010; Boothroyd-Roberts et al., 2013; D'Souza and Rodrigues, 2013). Compared to native tree species, fast-growing tree plantations often have lower understory light availability and this might be the main reason for low plant diversity (Meers et al., 2010). Fast-growing tree plantations can result in low soil water content due to higher overstory transpiration rates and leading to low densities of hygrophytes (Boothroyd-Roberts et al., 2013; Li et al., 2014a). Additionally, change in soil fertility after tree plantation also affects understory plant diversity (Černý et al., 2013; Lima and Vieira, 2013). Low concentrations of organic matter and nutrients were the main reasons for low species richness in eucalypt plantations (Bargali et al., 1993). These environmental factors are recognized to be closely correlated with plant diversity. However, unlike in plains ecosystems, topography, such as elevation and slope, are important factors explaining variation of plant diversity in mountainous terrain (Ibáñez and Gazol, 2010). Links between environmental variables and plant diversity are relatively complicated in alpine forest.

Larix kaempferi, a fast-growing coniferous tree species, was introduced in the 1900s, to China from Japan. Due to its higher productivity than native trees, this species was extensively planted in the middle-high mountain areas in southern China. In the past 30 years, larches became the dominant coniferous tree species in forestation or reforestation in Hubei Province, raising concerns regarding the effects on plant diversity (Yu et al., 2006). However, some studies even showed that larches had greater species richness than broad-leaved tree species (Nagaike, 2002). It seems that larch plantations have balancing effect on understory plant diversity. Therefore we conducted a large-area investigation on three types of forest stands, viz. pure larch plantations (*L. kaempferi*), pure liriodendron plantations (*Liriodendron chinense*, a native broad-leaved deciduous tree species) and mixed stands (*L. kaempferi* and *L. chinense*), to test the effects of larch plantations on understory plant diversity.

Materials and methods

Study site

Our study was conducted in western Hubei Province, China. The site is characterized by a northern subtropical monsoon climate, with average annual temperature of 9–11°C, frost-free days of 170–203 d, annual precipitation of 1,400–1,800 mm, and average humidity of 80–85% (Cai, 2000; Lu and Chang, 2003). Since the 1960s, larch (*L. kaempferi*) was planted extensively at this site due to its faster growth than native trees. Before establishment of these larch plantations, the dominant tree in these forests was *Liriodendron chinense*.

Field investigation

In September 2012, three stand types were chosen for this study: two pure stands (*L. kaempferi*, *L. chinense*) and one mixed stand (*L. kaempferi* and *L. chinense*). The number of stands sampled in pure larch, pure liriodendron and mixed stands were 13, 3 and 3, respectively (*Table 1*). In the middle of each stand, three plots (20 × 30 m) were chosen as replications. Stand density, tree height and diameter at breast height (DBH) were measured. Stand age was determined from plantation records. Slope was measured using a gradient meter (XYPD01, China). The latitude, longitude, elevation and aspect were recorded using a global positioning system (GPS). Two illuminometers were used for measuring light availability at 1 m above the ground surface, one inside the plots (L1) and another in an open field (L2). The ratio of light availability (RLA) was calculated as the ratio of L1 to L2. A total of 40 paired-points were measured at each plot.

Table 1. *Investigation areas of tree plantations in the subtropical forest ecosystem.*

Nr.	Location	Elevation (m.a.s.l)	Slope (°)	Aspect	Age (y)	Density (trees hm^{-2})	Height (m)	DBH (cm)	Stand types
1	N31°04′44.3″ E110°54′03.2″	1520	20	Southwest	21	1600.0±23.2	14.8±5.4	14.1±3.6	Pure larch
2	N31°04′42.8″ E110°54′03.3″	1500	5	Southwest	21	1250.0±19.0	14.9±4.4	14.2±2.8	Pure larch
3	N31°04′49.8″ E110°54′07.3″	1490	5	Southwest	21	1250.0±24.2	12.9±4.5	13.5±2.6	Pure larch
4	N31°04′49.6″ E110°54′26.5″	1710	10	Northwest	21	1196.0±21.7	16.0±4.1	15.5±2.6	Pure larch
5	N31°04′51.9″ E110°54′30.0″	1720	25	Northwest	21	937.0±25.3	15.1±3.2	15.2±2.9	Pure larch
6	N31°05′05.4″ E110°54′32.7″	1645	5	Southwest	21	1350.0±13.2	14.7± 4.5	16.5±2.4	Pure larch
7	N30°48′05.6″ E110°02′58.9″	1750	15	Northwest	26	1428.0±17.9	24.2±3.8	22.2±1.7	Pure larch
8	N30°48′04.4″ E110°02′58.9″	1730	15	South	50	1448.0±19.3	38.4±10.0	28.1±3.2	Pure larch
9	N30°48′05.7″ E110°02′51.6″	1770	5	East	24	1750.0±20.3	19.6±2.8	20.0±2.2	Pure larch
10	N30°48′25.5″ E110°04′54.0″	1690	15	Southeast	19	1052.0±15.2	13.3±3.2	12.7±2.0	Pure larch
11	N30°48′37.3″ E110°05′02.4″	1709	8	Southeast	19	2828.0±28.6	14.4±3.8	14.4±2.7	Pure larch
12	N30°42′10.8″ E109°40′56.1″	1870	15	South	27	2941.0±33.5	23.4±3.3	20.6±1.6	Pure larch
13	N30°42′48.6″ E109°40′22.0″	1930	12	East	13	2222.0±28.4	8.3±1.4	9.0±0.9	Pure larch
14	N31°05′04.8″ E110°54′34.0″	1630	30	Northeast	21	1550.0±27.6	10.3± 4.5	10.4±3.8	Pure liriodendron
15	N31°04′57.5″ E110°54′34.2″	1750	8	Southeast	21	1060.0±32.7	23.2±8.1	16.9±2.9	Pure liriodendron
16	N30°42′16.2″ E109°41′18.5″	1790	20	South	24	1428.0±18.6	15.8±4.4	16.0±3.2	Pure liriodendron
17	N30°48′05.7″ E110°03′08.0″	1730	15	North	24	1250.0±21.4	18.5±5.4	15.7±2.1	Mixed stands
18	N30°48′01.9″ E110°02′57.2″	1780	30	North	25	1400.0±23.1	17.2±4.2	17.2±2.3	Mixed stands
19	N30°42′38.8″ E109°40′38.5″	1890	30	West	25	1577.0±19.7	17.5±4.9	17.7±2.0	Mixed stands

A nested sub-plot sampling design described by Avery and Burkhart (1983) was used to form sampling units to determine plant diversity. In each plot, 4 quadrats (2 × 2 m) in upper, middle and lower parts of slope were chosen for investigation. The total number of sampling quadrats in each plot was 12. The species name, number and coverage of each species at each sampling quadrat were recorded. Plant coverage was determined by visual estimate.

Soil fertility analysis

In each plot, soil in the upper 40 cm profile was sampled according to a 5-point sampling method (Pobel et al., 2011) and then immediately transported to laboratory for measurement. Six parameters, including pH, soil water content (SWC), soil organic matter (SOM), soil total nitrogen (STN), soil total phosphorus (STP), and soil total potassium (STK), were quantified. Soil pH was measured in solution with 1: 2.5 (w/v) of soil to distilled water using a Mettler Toledo Delta 320 pH Meter. SWC was measured using the classical method of drying and weighing (Dobriyal et al., 2012). SOM, STN, STP and STK were measured by wet digestion of potassium dichromate oxidation

(Rayment and Higginson, 1992), semi-micro Kjeldahl method (Bao, 1999), NaOH fusion and colorimetric procedures (Olsen and Sommers, 1982), and NaOH melting-flaming luminosity method (Chen et al., 2007), respectively.

Data analysis

Understory plant diversity was quantified using species richness and Shannon's diversity index (H). Species richness was calculated as number of species per sampling quadrat (Magurran, 1998; Weih et al., 2003). H was calculated according to the following equation (Magurran, 1998):

$$H = -\sum_{i=1}^{N} P_i \ln p_i \qquad \text{(Eq.1)}$$

Where, H indicates Shannon's diversity index; N, species number; Pi, the proportion of species *i* relative to the total number of species.

Important Value Index (IV) was determined according to the equation (Misra, 1968):

$$IV_i = (RD_i + RC_i + RF_i) / 3 \qquad \text{(Eq.2)}$$

Where IV_i indicates the importance value index of the *i*th species; RD_i, the relative density; RC_i, relative coverage; RF_i, relative frequency.

General linear model (GLM) of plant diversity, ratio of light availability, and soil fertility among three types of tree stands were analyzed at the 0.05 significance level. Homogeneity of variances was tested using Levene's test, and data were $\log_{10}$-transformed where necessary to reduce the heterogeneity of variances. The relationships between plant diversity (species number, Shannon's diversity) and 9 environmental variables (elevation, slope, RLA, pH, SWC, SOM, STN, STP, and STK) were analyzed by curve regression with R^2 and *P* values. All were performed using SPSS V17.0 (SPSS Inc., USA).

To explore the relationship between environmental variables and two growth forms (shrubs, herbs), a canonical correspondence analysis (CCA) was performed using the software CANOCO 4.5 (Microcomputer Power Ithaca, NY, USA). The vegetations data matrix consisted of the IV of species with frequency >5% and the environmental data matrix consisted of 9 environmental variables.

Results

Species number and Shannon's diversity

Species richness was similar for the three stand types ($P>0.05$, *Table 2*). Shannon's diversity was higher in mixed stands (1.91) than in pure liriodendron (1.44) or pure larch (1.17). It was clear those larches had no significant effects on species richness, but supported a greater Shannon's diversity in mixed stands.

Table 2. *ANOVA of plant diversity, ratio of light availability, and soil fertility (means ± S.E.) in three stand types*

Variables	Pure larch	Pure liriodendron	Mixed stands	*P* value
pH	4.94±0.21	4.93±0.07	5.50±0.29	0.171
Soil water content (%)	5.79±0.35	6.30±0.31	5.86±0.49	0.683
Soil organic matter (g/kg)	53.73±3.08	79.44±5.99	51.52±7.77	0.353
Soil total nitrogenous (g/kg)	2.77±0.22	3.55±0.49	3.15±0.16	0.309
Soil total phosphorous (g/kg)	1.21±0.09	1.55±0.21	1.38±0.08	0.311
Soil total potassium (g/kg)	1.00±0.08	1.29±0.18	1.14±0.06	0.309
Ratio of light availability (%)	25.17±2.13	27.30±1.15	26.03±2.38	0.753
Species number	6.37±0.10	7.53±0.91	9.64±1.31	0.111
Shannon's diversity	1.17±0.04	1.44±0.16	1.91±0.18	**0.029**

Light availability and soil fertility

Understory light availability was similar in the three stand types (*P*>0.05, *Table 2*). Soil fertility (pH, water content, organic matter, total nitrogen, total phosphorus and total potassium) was also similar in the three stand types.

Regression between plant diversity and environmental variables

Species richness and Shannon's diversity increased with increasing elevation and light availability. All other environmental variables were unrelated to both indices (*Fig. 1*).

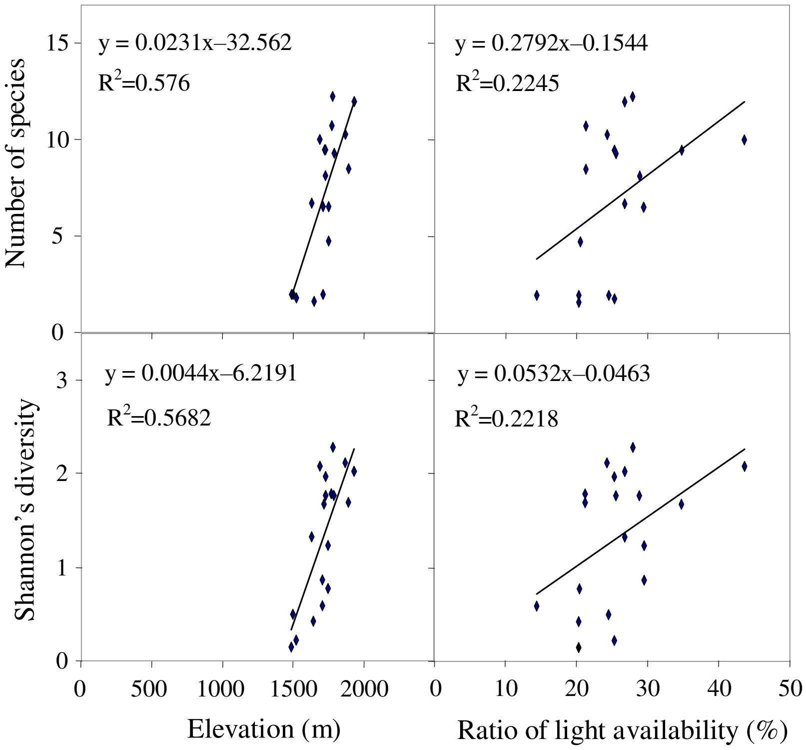

Figure 1. *Relationships between plant diversity and environmental variables. Only significant regressions are shown.*

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Canonical Correspondence Analysis (CCA) between environmental variables and plant species

For shrubs, species–environment correlation coefficient was 0.962 in the first axis and 0.900 in the second axis (*Table 3, Fig. 2*). The first and second axes explained almost 28.2% and 20.7% of the variance of species–environment relationships. The first axis was negatively correlated with elevation ($P<0.001$). The second axis was not significantly correlated with any environmental variables. Species, such as *Weigela japonica* and *Rubus corchorifolius*, distributed at the left of Axis 2, were closely related to higher elevation. Other species, such as *Chimonobambusa quadrangularis* and *Stauntonia chinensis*, distributed at the right of Axis 2, were closely associated with lower elevation.

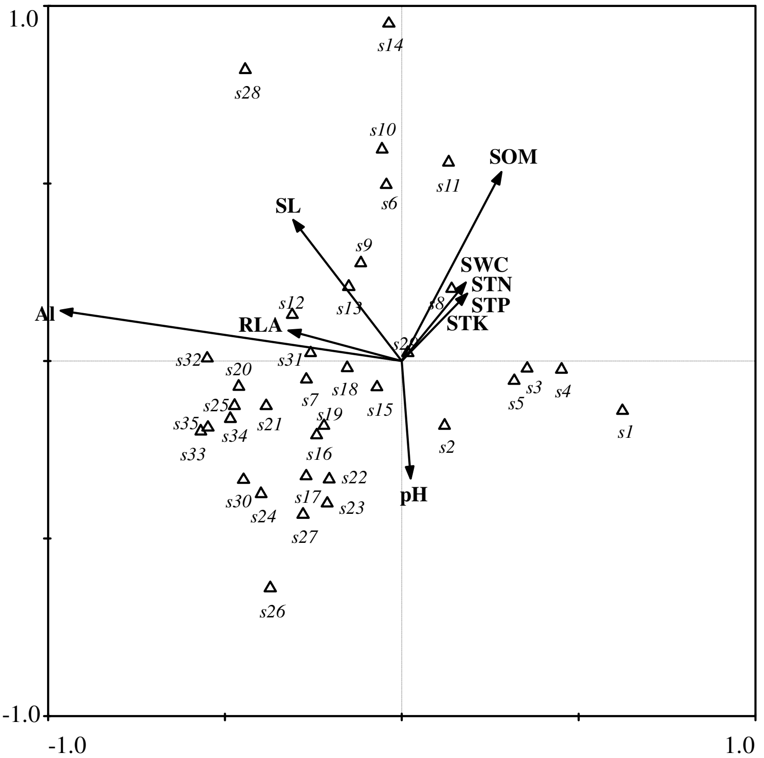

Figure 2. *CCA ordination (Axis 1 by Axis 2) with shrubs and environmental variables. Only shrubs with frequency over 5% are shown. The arrows and points indicate the environmental variables and the plants respectively. S1, Chimonobambusa quadrangularis ; S2, Smilax china; S3, Clerodendrum cyrtophyllum; S4, Stauntonia chinensis; S5, Rosa multiflora; S6, Fargesia spathacea; S7, Rubus corchorifolius; S8, Lonicera japonica; S9, Lindera glauca; S10, Corylopsis sinensis; S11, Ilex pernyi; S12, Euonymus alatus; S13, Lonicera maackii; S14, Callicarpa rubella; S15, Hydrangea macrophylla; S16, Decaisnea insignis; S17, Lonicera trichosantha; S18, Indocalamus tessellates; S19, Smilax glabra; S20, Actinidia chinensis; S21, Spiraea salicifolia; S22, Elaeagnus umbellate; S23, Rubus pinfaensis; S24, Hydrangea longipes; S25, Schisandra chinensis; S26, Viburnum plicatum; S27, Psilopeganum sinensis; S28, Morus australis; S29, Lycium chinense; S30, Cerasus tomentosa; S31, Mahonia fortunei; S32, Lespedeza bicolor; S33, Weigela japonica; S34, Rubus rosaefolius; S35, Rubus corchorifolius.*

For herbs, species–environment correlation coefficient was 0.950 in the first axis and 0.945 in the second axis (*Table 3, Fig. 3*). The first and second axes explained almost

24.5% and 19.9% of the variance of species–environment relationships. The first axis was positively correlated with SOM ($P<0.001$), STN ($P<0.05$), STP ($P<0.05$) and STK ($P<0.05$). The second axis was negatively associated with elevation ($P<0.01$). Species, such as *Trifolium pratense* and *Bupleurum longiradiatum*, distributed at the left of axis 2, were closely related to lower SOM, STN, STP and STK. Other species, such as *Impatiens balsamina* and *Geranium wilfordii*, distributed at the right of Axis 2, were closely correlated with higher SOM, STN, STP and STK. In the top of Axis 1, species, such as *Pilea notata* and *Viola phalacrocarpa*, were associated with lower latitude. However, in the lower of Axis 1, *Polystichum herbaceum* and *Carex pallida*, were correlated with higher latitude.

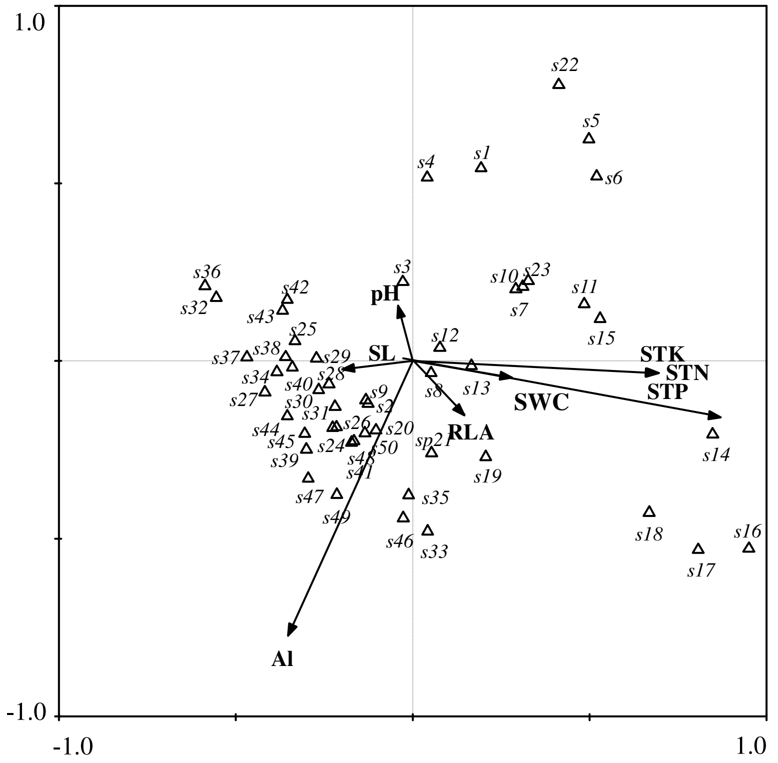

Figure 3. CCA ordination (Axis 1 by Axis 2) with herbs and environmental variables. Only herbs with frequency over 5% are showed. The arrows and points indicate the environmental variables and the plants respectively. S1, Senecio scandens; S2, Viola diffusa; S3, Cyperus stoloniferus; S4, Cyclospermum leptophyllum; S5, Viola phalacrocarpa; S6, Antenoron filiforme; S7, Epimedium sagittatum; S8, Cyperus rotundus; S9, Parathelypteris glanduligera; S10, Asparagus filicinus; S11, Cyclosorus interruptus; S12, Clematis florida; S13, Cyperus iria; S14, Euphorbia pekinensis; S15, Liriope spicata; S16, Impatiens balsamina; S17, Geranium wilfordii; S18, Agrimonia pilosa; S19, Galium spurium; S20, Chrysanthemum indicum; S21, Reineckea carnea; S22, Pilea notata; S23, Athyrium iseanum; S24, Galium bungei; S25, Impatiens davidi; S26, Lysimachia pentapetala; S27, Athyrium otophorum; S28, Selaginella uncinata; S29, Hemsleya sphaerocarpa; S30, Houttuynia cordata; S31, Gentiana leptoclada; S32, Trifolium pratense; S33, Polystichum herbaceum; S34, Astilbe chinensis; S35, Botrychium virginianum; S36, Bupleurum longiradiatum; S37, Carex tristachya; S38, Matteuccia orientalis; S39, Polygonatum odoratum; S40, Phyllostachys heteroclada; S41, Polystichum makinoi; S42, Carex hattoriana; S43, Tiarella polyphylla; S44, Viola thomsonii; S45, Botrychium ternatum; S46, Carex pallida; S47, Osmunda japonica; S48, Clematis repens; S49, Dryopteris chinensis; S50, Potentilla kleiniana.

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Table 3. *Correlations between ordination axes and environmental variables in 19 plantation areas.*

Environmental variables	Shrubs		Herbs	
	Axis-1	Axis-2	Axis-1	Axis-2
Elevation	**-0.9635*****	0.1416	-0.3552	**-0.7733****
Slope	-0.3067	0.3979	-0.1989	-0.0232
pH	0.0245	-0.3315	-0.0416	0.1564
Soil water content	0.1802	0.2204	0.2807	-0.0463
Soil organic matter	0.2808	0.5314	**0.8703*****	-0.1584
Soil total nitrogen	0.1846	0.1898	**0.6931***	-0.0346
Soil total phosphorous	0.1831	0.1919	**0.6920***	-0.0333
Soil total potassium	0.1882	0.1928	**0.6959***	-0.0330
Ratio of light availability	-0.3209	0.0852	0.1463	-0.1529
Species-environment correlations	0.962	0.900	0.950	0.945
Cumulative percentage variance of species-environment relation	28.2	48.9	24.5	44.4

* $P<0.05$; ** $P<0.01$; *** $P<0.001$

Discussion

Species richness and Shannon's diversity were similar in pure larch and pure liriodendron plantations, indicating that the effects of larch plantations on understory plant communities were similar to those of liriodendron plantations. This was different from the most previous results, which showed that fast-growing tree plantations could result in low plant diversity (Elek et al., 2010; Meers et al., 2010). No significant difference in plant diversity between larches and liriodendron was due to the similar understory light availability and soil fertility (Nagaike et al., 2003; Lei et al., 2007; Li et al., 2014).

Environmental conditions are recognized the key factors for understory plant diversity (Aber and Melillo, 2001; Černý et al., 2013). In our study, species richness and Shannon's diversity were positively correlated with the elevation and ratio of light availability. High elevation contributed to a wide range of habitat, especially for temperature and heat (Černý et al., 2013; Khan et al., 2013), which indicated that more ecological niches could be provide for the establishment of plant species. High light availability indicated that low competition among understory plants and more species can survive (Archaux et al., 2010; Boothroyd-Roberts et al., 2013).

Plants distribution was significantly associated with environmental variables (Gazol and Ibáñez, 2010). In this study, elevation was the most important factors for determining vegetation distribution, which was consistent with the previous studies (Aber and Melillo, 2001; Černý et al., 2013). CCA results showed that most shrubs species distributed in the left of Axis 2, such as *Lespedeza bicolor*, and *Rubus rosaefolius*, suggesting that these species survive in habitats with relatively higher elevation. In addition to elevation, soil nutrition also played an important role in plant distribution (Hokkanen, 2006; Cao et al., 2009). Most herbs species located in the left of Axis 2, such as *Carex tristachya*, and *Matteuccia orientalis*, suggesting that these species usually lived in habitat with relatively lower organic matter and total nitrogen. Additionally, the responses of plant distribution to environmental variables were different between two growth forms: shrubs were determined by elevation, while herbs primarily by soil fertility, followed by elevation. Compared to shrubs, herbs distribution was easily affected by local environments (Bhattarai and Vetaas, 2003; Lu et al., 2006).

As a fast-growing tree, larch plantations had balancing effect on understory plant diversity in the subtropical forest ecosystems, China. Studies even showed that larches increased species richness compared to *Quercus crispula* (Nagaike, 2002; Nagaike et al., 2006). Balancing effect of larch plantations on understory plant diversity was due to the unchanged environment after establishment of larch plantations.

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REFERENCE

[1] Aber, J.D., Melillo, J.M. (2001): *Terrestrial Ecosystems*, second ed. - San Diego: Academic Press.

[2] Archaux, F, Chevalier, R., Berthelot, A. (2010): Towards practices favourable to plant diversity in hybrid poplar plantations. - Forest Ecology and Management 259: 2410–2417.

[3] Avery, T.E., Burkhart, H.E. (1983): Forest measurements. New York: McGraw-Hill.

[4] Bao, S.D. (1999): Chemical analysis of agriculture soil. - Beijing: Science Press. (in Chinese)

[5] Bargali, S.S., Singh, R.P., Joshi, M. (1993): Changes in soil characteristics in eucalypt plantations replacing natural broad-leaved forests. Journal of Vegetation Science, 4: 25–28.

[6] Bhattarai, K.R., Vetaas, O.R. (2003): Variation in plant species richness of different life forms along a subtropical elevation gradient in the Himalayas, east Nepal. - Global Ecology and Biogeography 12: 327–340.

[7] Boothroyd-Roberts, K., Gagnon, D., Truax, B. (2013): Can hybrid poplar plantations accelerate the restoration of forest understory attributes on abandoned fields? - Forest Ecology and Management 287: 77–89.

[8] Britt, C.P., Fowbert, J., McMillan, S.D. (2007): The ground flora and invertebrate fauna of hybrid poplar plantations: results of ecological monitoring in the PAMUCEAF project. - Aspects of Applied Biology 82: 83–90.

[9] Cai, S., Liu, X., Zhang, J., Zhou, X., Zhang, X. (2000): Community characteristics of rare trees at Dalaoling of Three-Gorge reservoir area in western Hubei Province. - Chinese Journal of Applied Ecology 2: 165–168.

[10] Cao, J.F., Ma, K.M., Feng, Z.W., Qi, J., Feng, Y. (2009): Coupling effects of elevation and human disturbance on landscape and plant diversity in the vicinity of mountain villages of Beijing, China. - Acta Ecology Sinica 29: 56–61.

[11] Černý, T., Doležal, J., Janeček, Š., Šrůtek, M., Valachovič, M., Petřík, P., Altman, J., Bartoš, M., Song, J.S. (2013): Environmental correlates of plant diversity in Korean temperate forests. - Acta Oecologica 47: 37–45.

[12] Chen, Y.N., Wang, Q., Li, W.H., Ruan, X. (2007): Microbiotic crusts and their interrelations with environmental factors in the Gurbantonggut desert, western China. - Environmental Geology 52: 691–700

[13] D'Souza, J., Rodrigues, B.F. (2013): Biodiversity of Arbuscular Mycorrhizal (AM) fungi in mangroves of Goa in West India. - Journal of Forestry Research 24: 515−523.

[14] Dobriyal, P., Qureshi, A., Badola, R., Hussain, S.A. (2012): A review of the methods available for estimating soil moisture and its implications for water resource management. *Journal of Hydrology*, 458–459: 110–117.

[15] Elek, Z., Dauffy-Richard, E., Gosselin, F. (2010): Carabid species responses to hybrid poplar plantations in floodplains in France. - Forest Ecology and Management 260:

1446–1455.

[16] Franklin, J., Steadman, D.W. (2010). Forest plant and bird communities in the Lau Group, Fiji. - PloS one 5: e15685, 1-14.

[17] Gazol, A., Ibáñez, R. (2010): Variation of plant diversity in a temperate unmanaged forest in northern Spain: behind the environmental and spatial explanation.- Plant Ecology 207: 1–11.

[18] Gerber, J.F. (2011): Conflicts over industrial tree plantations in the South: Who, how and why? - Global Environmental Change 21: 165–176.

[19] Hokkanen, P. (2006): Environmental patterns and gradients in the vascular plants and bryophytes of eastern Fennoscandian herb-rich forests. - Forest Ecology and Management 229: 73–87.

[20] Ibáñez, R., Gazol, A. (2010): Variation of plant diversity in a temperate unmanaged forest in northern Spain: behind the environmental and spatial explanation.- Plant Ecology 207: 1–11.

[21] Khan, N., Shaukat, S.S., Ahmed, M., Siddiqui, M.F. (2013): Vegetation-environment relationships in the forests of Chitral district Hindukush range of Pakistan. - Journal of Forestry Research 24: 205−216.

[22] Lee, E.W.S., Hau, B.C.H., Corlett, R.T. (2005): Natural regeneration in exotic tree plantations in Hong Kong, China. - Forest Ecology and Management 212: 358–366.

[23] Lei, X., Lu, Y., Peng, C., Zhang, X., Chang, J., Hong, L. (2007): Growth and structure development of semi-natural larch-spruce-fir (*Larix olgensis–Picea jezoensis–Abies nephrolepis*) forests in northeast China: 12-year results after thinning. - Forest Ecology and Management 240: 165–177.

[24] Li, Y., Qin, H., Xie, Y., Wang, W., Chen, X., Zhang, C. (2014a): Physiological mechanism for the reduction in soil water in poplar (*Populus deltoides*) plantations in Dongting Lake wetlands. - Wetlands Ecology and Management 22: 25–33.

[25] Li, Y., Chen, X., Xie, Y., Li, X., Li, F., Hou, Z.Y. (2014b): Effects of young poplar plantations on understory plant diversity in the Dongting Lake wetlands, China. - Scientific Reports 4, doi: 10.1038/srep06339.

[26] Lima, T.A., Vieira, G. (2013): High plant species richness in monospecific tree plantations in the Central Amazon. - Forest Ecology and Management 295: 77–86.

[27] Lu, S.P., Chang, S. (2003): Appraising about the niche of the forest resources in Gaoyanzi Forestry Center. - Journal of Hubei Institute for Nationalities (Natural Science Edition) 2: 22–24. (in Chinese)

[28] Lu, T., Ma, K.M., Zhang, W.H., Fu, B.J. (2006): Differential responses of shrubs and herbs present at the Upper Minjiang River basin (Tibetan Plateau) to several soil variables. - Journal of Arid Environments 67: 373–390.

[29] Magurran, A.E. (1998): Ecological diversity and its measurement. - London: Princeton University Press.

[30] Meers, T., Kasel, S., Bell, T.L., Enright, N.J. (2010): Conversion of native forest to exotic Pinus radiata plantation: Response of understory plant composition using a plant functional trait approach. - Forest Ecology and Management 259: 399–409.

[31] Misra, R. (1968): Ecology Work Book. - New Delhi: Oxford and IBH publishing Company.

[32] Morris, T.L., Witkowski, E.T.F., Coetzee, J.A. (2008): Initial response of riparian plant community structure to clearing of invasive alien plants in Kruger National Park, South Africa. - South African Journal of Botany 74: 485–494.

[33] Nagaike, T., Hayashi, A., Abe, M., Arai, N. (2003): Differences in plant species diversity in Larix kaempferi plantations of different ages in central Japan. - Forest Ecology and Management 183: 177–193.

[34] Nagaike, T., Hayashi, A., Kubo, M., Takahashi, K., Abe, M., Arai, N. (2006): Changes in plant species diversity over 5 years in *Larix kaempferi* plantations and abandoned coppice forests in central Japan. - Forest Ecology and Management 236: 278–285.

[35] Nagaike, T. (2002): Differences in plant species diversity between conifer (Larix kaempferi) plantations and broad-leaved (*Quercus crispula*) secondary forests in central Japan. - Forest Ecology and Management 168: 111–123.

[36] Olsen, S.R., Sommers, L.E. (1982): Phosphorus. - In: Page AL, Miller RH, Keene DR (eds), Methods of soil analysis. Madison: Soil Science Society of America, pp 403–448.

[37] Pobel, D., Robin, J., Humbert, J.F. (2011): Influence of sampling strategies on the monitoring of cyanobacteria in shallow lakes: Lessons from a case study in France. - Water Research 45: 1005–1014.

[38] Rayment, G.E., Higginson, F.R. (1992): Australian Laboratory handbook of soil and water chemical methods. - Melbourne, Inkata Press.

[39] Tan, Z.H., Zhang, Y.P., Song, Q.H., Liu, W.J., Deng, X.B., Tang, J.W., Deng, Y., Zhou, W.J., Yang, L.Y., Yu, G.R., Sun, X.M., Liang, N.S. (2011): Rubber plantations act as water pumps in tropical China. - Geophysical Research Letters 38: L24406.

[40] Turnbull, J.W. (1999): Eucalypt plantations. - New Forests 17: 37–52.

[41] Weih, M., Karacic, A., Munkert, H., Verwijst, T., Diekmann, M. (2003): Influence of young poplar stands on floristic diversity in agricultural landscapes (Sweden). - Basic and Applied Ecology 4: 149–156.

[42] Yu, X.D., Luo, T.H., Zhou, H.Z. (2006): Distribution of carabid beetles among regenerating and natural forest types in Southwest China. - Forest Ecology and Management 231: 169–177.

EFFECT OF TEMPORAL LAND USE/LAND COVER CHANGES ON ECOSYSTEM SERVICES VALUE IN COASTAL AREA OF CHINA: THE CASE OF SU-XI-CHANG REGION

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Abstract. Providing goods and services, while maintaining ecosystem service functions and biodiversity that built up their sustainable supply to support human welfare, is one of the great challenges of our time. This study examined the dynamics of land use land cover (LULC) changes, temporal variation of ecosystem service value (ESV), the threatened ecosystem services function in Su-Xi-Chang region, and explore the consequences of unsustainable use of land resources on ESV provision. Remote sensing applications were used to evaluate changes in LULC, and an evaluation method for ESV based on land cover change was applied in 2000, 2005 and 2010. Our study investigated that change in ESV was caused mainly due to the expansion of construction land, which was increased by 88% during the study period. Meanwhile, ESV dropped from 57.1775 x 10^9 CNY in 2000 to 53.7585 x 10^9 CNY in 2010. Ecosystem service function for food provision was the most critically affected followed by climate and gas regulation functions respectively. Waste treatment and water supply were the two largest services functions provided by ecosystems of the area. The expansion of construction land, which have due impact on LULC change, is likely responsible for the overall decline in 3.4190 billion CNY ESV of the region. As they have showed the highest ESV and greatest degradation during the study period, protection of farmland and water bodies should be paid due attention by regional land use planning and policy making offices. As a result, sustainable use of land resources and mitigation of the declining ESV of the region will be realized.

Keywords: *land use land cover changes, ecosystem service value, sustainability, Su-Xi-Chang region*

Introduction

Much importance is attributed to the search of knowledge about anthropogenic impact on our environment as rapid population growth and economic development intensify the stress that human beings place on the biosphere and ecosystems (Omenn, 2006; Long et al., 2009). Under land change science, an interdisciplinary fields seeks to understand the dynamics of LULC as a coupled human environment system to address theory, concepts, models, and applications relevant to environmental and social problems, has emerged as a fundamental component of global environmental change and sustainability research (De-yong et al., 2005; Secretariat, 2005; Turner et al., 2007; Tong et al., 2012; Estoque and Murayama, 2013).

Recently, issues related to LULC change have attracted interest among a wide variety of research, ranging from those who favor modeling spatio-temporal patterns of land conversion to those who try to understand the coupled system causes, impacts, and consequences (Irwin and Geoghegan, 2001; Xie et al., 2003; Long et al., 2009; Polasky et al., 2011; Mendoza-González et al., 2012; Ruishan and Suocheng, 2013; Wu et al., 2013; Long et al., 2014; Han et al., 2015; Ye et al., 2015). These are particularly becoming a hot issue in rapidly urbanizing areas. In connection to rapid socioeconomic development and population growth (Li et al., 2010b; Su et al., 2012) in rapidly urbanizing areas, such as eastern coastal areas of China, the physical and ecological environments have been interrupted by intensive human-transformed land use system. These lead to deterioration and loss of natural and semi-natural environments and their associated ecosystem services value (Li et al., 2010b; Helian et al., 2011).

Since China initiated economic reforms and an open-door policy in 1978, tremendous change in China's development philosophy and regional economics have occurred (Wei and Fan, 2000). As a result, many coastal regions of China such as the Yangtez River Delta region and Pearl River Delta region experienced dramatic economic and spatial restructuring, which resulted in tremendous land use change (Seto and Kaufmann, 2003; Li and Yeh, 2004; Long et al., 2007). Empirical investigations in the coastal areas of China (Long et al., 2009; Liu et al., 2010) have shown that with the continuous growth of China's economy, massive farmland loss for the benefit of market farming and non-agricultural development occur without appropriate planning and management of existing land resources. This is affecting (Seto and Kaufmann, 2003; Liu et al., 2010; Liu et al., 2015) the natural and semi-natural environment and functions of ecosystem of the area, which have been in turn adversely affecting the living condition of the societies. Therefore, in these areas the research of change of ecosystem services under the background of LULC change is very important for the realization of sustainable use of land resources and coordinated development of the economy and environment.

Many studies have been through to consider the dynamics of LULC and its impact on ecosystem services (Guo et al., 2001; Poschlod et al., 2005; Wu et al., 2006; Dewan and Yamaguchi, 2009; Li et al., 2010b; Tianhong et al., 2010; Polasky et al., 2011; Estoque and Murayama, 2012; Long et al., 2014; Ye et al., 2015). Yet, in coastal areas of China, an overall monitoring and assessment of the change in the ecosystem service value, which could provide the needed base line information, is still lacking (Long et al., 2009). Hence, the goal of this study is to contribute to this endeavor by examining the effect of LULC change on the ESVs of these areas.

As one of the main Coastal area of China (Long et al., 2009; Zhou et al., 2014) the characteristics of LULC change in Su-Xi-Chang region is typical and representative as a result of its faster economic and population growth. Thus (Liu et al., 2010) it's a paramount important to assess the effect of dynamics in LULC changes on ESV of this area to attain sustainable ecosystem management and support regional sustainable development policies. In the present study we proposed to analyze change in LULC, and estimate the annual economic value of services provided by the ecosystems of Su-Xi-Chang region and determine its past and current status. The objectives of the study are to: (1) describe the temporal change characteristics of LULC in Su-Xi-Chang region; (2) scrutinize the change in ESV in relation to LULC changes during the study period; (3) explore the most critically affected ecosystem services function in the study area.

Materials and Methods

Study area

Su-Xi-Chang region refers to three municipalities Suzhou, Wuxi, and Changzhou situated in the Southern Jiangsu Province, lies in the middle of Yangtze River Delta Economic zone of east China ($36^0$46’- $32^0$04’N, $119^0$08’-$121^0$15’E). It covers a total area of 17,500 km^2 with an average elevation below 50 meters (*Figure 1*). The area has monsoon climate with an annual average temperature and precipitation of 15.3℃ and 1,092 mm respectively.

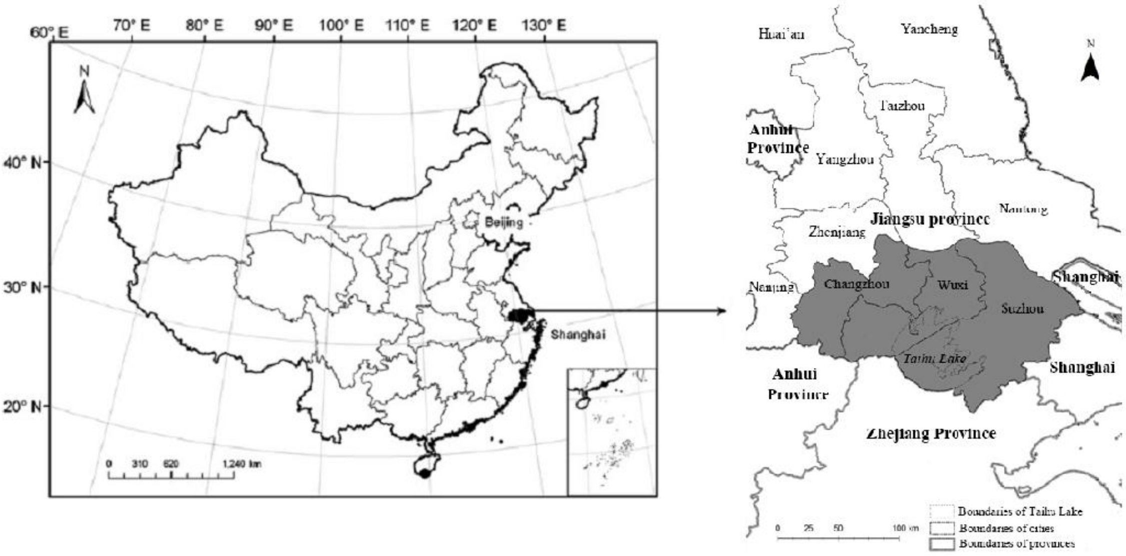

Figure 1. *Location of the study area (gray colour)*

Su-Xi-Chang has a total population of 14.65 million (837/km^2), which was more than 6 times of the total population per km^2 (135.5/km^2) of China in the same year. It is the most densely populated area in the country. The GDP per capita amounted to 123,325 CNY was about three times more than the GDP per capita of China at the same period (NBSC, 2014). However, with the growth of population and economic activities, LULC in Su-Xi-Chang region has changed dramatically as evidenced by the continuous decline of farmland (Long et al., 2007). And also due to loss and neglected protection of the environment the consequences of uncontrolled rural industrialization in this region are particularly serious (Xie et al., 2007). Furthermore (Zhou et al., 2014), rapid change of LULC and the ongoing deterioration of local environment are major concerns in Su-Xi-Chang region, which are triggering significant environmental consequences and causes loss in ESV of the region. These make it an appropriate case study to assess the effect of changes in LULC on ESV of the area to attain sustainable ecosystem service management and provide basic human welfare.

LULC changes detection

The data used in this study included land use maps of 2000, 2005 and 2010, derived from Landsat TM images and DEM data (resolution 30 m) of the Computer Net Work

Information Center, Chinese Academy of Science, and vector data of administrative boundaries at county level, provided by the Data Sharing Infrastructure of Earth system Science, Institute of Geographic Sciences and Natural Resources Research, Chinese Academy of science. The land use types were classified in to six categories: farmland, woodland, grassland, water body, wetland and construction land (*Table 2*).

Model of LULC change

The quantity change of land cover can be represented by the single land cover dynamic index, which denotes the change rate of a certain land cover type for a specific period of time. By analyzing the dynamic of land cover, the extent and rate of land cover change can be compared quantitatively (Yuhai, 1999) (Eq. 1).

$$K = \frac{U_b - U_a}{U_a} \times \frac{1}{T} \times 100\ \% \qquad \text{(Eq. 1)}$$

where K is the single land cover dynamic index; U_a and U_b are the areas of a certain land cover class at time a and time b respectively; T is the time span from time a to time b. When T is in a unit of year, then K is the annual rate of change in area for this land cover type. K ranges from -1 to 1. $K < 0$ means that land cover type is in a state of depletion. The larger the absolute value of K, the more intensively land has been depleted. $K \geqslant 0$ means just the opposite.

Assessment of ecosystem service values (ESVs)

In order to estimate the global ecosystem services value (Costanza et al., 1997) classified the global biosphere into 16 ecosystem types and 17 service functions. Based on Costanza et al. (1997) parameters, (Xie et al., 2003) extracted the equivalent weight factor of ecosystem service per hectare of terrestrial ecosystems in China and modified the value coefficient of Chinese ecosystem. This equivalent weight factor (*Table 1*) can be applied to different regions across China by localizing the average natural food production (Xie et al., 2003). Equivalent weight factors are the potentials of ecosystems to provide ecosystem services. One factor is equal to the economic value of average grain production of crop land per hector per year. Generally, the natural food production is proposed to be 1/7 of the actual food production (Li et al., 2010a; Liu et al., 2012). With Su-Xi-Chang region, the average actual food production of crop was 6,765.33kg/ha from 2000-2010 and the average price for grain was 1.86 CNY/kg in 2010 (NBSC, 2014). Therefore the ESV of one equivalent weight factor for Su-Xi-Chang region is 1,797.65 CNY ha^{-1}.

Table 1. *Equivalent weighting of ecosystem services per hectare of terrestrial ecosystems in China*^{a}

	Farmland	Forest	Grassland	Wetland	Water body	Barren land
Gas regulation	0.50	3.50	0.80	1.80	0	0
Climate regulation	0.89	2.70	0.90	17.10	0.46	0
Water supply	0.60	3.20	0.80	15.50	20.40	0.03

Soil formation and protection	1.46	3.90	1.95	1.71	0.01	0.02
Waste treatment	1.64	1.31	1.31	18.18	18.20	0.01
Biodiversity protection	0.71	3.26	1.09	2.50	2.49	0.34
Food production	1.00	0.10	0.30	0.30	0.10	0.01
Raw material	0.01	2.60	0.05	0.07	0.01	0
Recreation and culture	6.91	1.28	0.04	5.55	4.34	0.01
Total	6.91	21.85	7.24	62.71	46.01	0.42

[a] From Xie et al. (2003).

To obtain the ecosystem services value per unit area for each land use category in Su-Xi-Chang region, each category was compared with the nearest equivalent ecosystem suggested by Xie et al. For example, woodland equates to forestland, and construction land equates to barren land. Although the biome used as proxies for the land use categories are clearly not perfect matches in every case (Kreuter et al., 2001), they are closely related. Estimation of the ESV based on land use data has been applied and has been proven to be feasible in other case studies (Li et al., 2010b; Liu et al., 2012; Wu et al., 2013; Tang et al., 2014).

Estimation of ESVs

Once the ESVs of one unit area for each land use category has been extracted, the total value of ecosystem services in the study area from 2000 to 2010 was obtained as follows (Li et al., 2010a; Liu et al., 2012):

$$\mathrm{ESV} = \sum (\mathrm{A_k} \times \mathrm{VC_k}) \qquad \text{(Eq.2)}$$

where *ESV* is the ecosystem service value, A_k is the area (ha) and VC_k is the value coefficient (CNY ha^{-1}yr^{-1}) for land use category “*k*”.

In addition, we also estimated the impacts of LULC change on nine individual ecosystem functions. The values of services provided by individual ecosystem functions were calculated using the following equation (Li et al., 2010a; Liu et al., 2012):

$$\mathrm{ESV_f} = \sum (\mathrm{A_k} \times \mathrm{VC_{fk}}) \qquad \text{(Eq.3)}$$

were ESV_f is the estimated ESV of the function category “*f*”, A_k is the area (ha) and VC_{fk} is the value coefficient (CNY ha^{-1} yr^{-1}) for land use category “*k*”.

Since the biomes we used as proxies for the land use categories are not perfect matches as mentioned above, and there are uncertainties of the value coefficients, additional sensitivity analysis was used to check the percentage change in ESV for a given percentage change in a value coefficients. For each analysis, we calculated a coefficient of sensitivity (*CS*) using the standard economics concept of elasticity, as follows (Kreuter et al., 2001; Wang et al., 2015):

$$CS = \frac{(ESV_j - ESV_i)/ESV_i}{(VC_{jk} - VC_{ik})/VC_{ik}} \qquad \text{(Eq.4)}$$

were *VC* is the value coefficient, *ESV* is the estimated ecosystem service value, and i and j represent the initial and adjusted values, respectively. If *CS* > 1, then the estimated ecosystem value is elastic with respect to that coefficient and it is important to accurately define *VC* ; but if *VC* < 1, then the estimated ecosystem value is considered to be inelastic and the results of ESV calculation will be reliable even if the *VC* value has relatively low accuracy.

In addition to monitoring the change in ESVs of Su-Xi-Chang region, a human-to-ecosystem service value (H-ESV) ratio index was also determined to assess the relationship between human population and the ESV. This was done by dividing the total ESV of Su-Xi-Chang region at a given period (e.g. 2000) by the population of the region during the said period.

Results

LULC change characteristics

Table 2 summarized the overall change trend of different LULC types over the study period. During this period, areas of woodland, grassland, and construction land increased, while that of farmland, water body and wetland decreased. In 2010, farmland was still the largest land cover category, followed by construction land and water body. Over the past one decade, the construction land has grown by 227,536 ha. In contrast, farm land has decreased by 216, 352 ha, followed by water body and wet land, which decreased by 14,254 ha and 218 ha respectively. The expansion of construction land through the study period was caused mainly by the decrease of farmland and water body. Even though it kept an accelerating rate of decrease through the study period as compared to other land uses, farmland was the largest land use types in the study area.

Table 2. *Su-Xi-Chang region land use patterns in 2000, 2005, and 2010*

	2000		2005		2010	
Land use type	Area (ha)	%	Area (ha)	%	Area (ha)	%
Woodland	117,154	6.72	118,656	6.80	119,831	6.87
Grassland	1,592	0.09	1,875	0.11	2,203	0.13
Farmland	864,338	49.54	776,408	44.50	647,986	37.14
Wetland	3,672	0.21	5,306	0.30	3,454	0.20
Water body	499,309	28.62	489,299	28.05	485,055	27.80
Construction land	258,559	14.82	353,080	20.24	486,095	27.86
Total	1,744,624	100	174,4624	100	1,744,624	100

The quantity change of land cover illustrated by the single land cover dynamic index (*K*) was greatest for construction land, followed by grass land. This indicates that the change rate of construction and grass lands were higher as compared to other. On the contrary, K was smallest for farmland, followed by wetland and water body. In the other way the absolute value of farmland was greater than the values of wetland and water body. This illustrates that farmland was the most reduced land use type in the study region. Overall, farmland, construction land and water body were the primary land cover types in the study area, contributed to about 93% of the total land cover.

Change in ESVs

The ESVs for each land use category and the total value for each study year (2000, 2005, and 2010) were calculated using modified value coefficient and the area covered by each land use category (*Table 3*). The total ESV for Su-Xi-Chang region gradually decreased over the study period. The results revealed a decrease of around 3.42 billion CNY worth of ESV from 2000 to 2010 (*Table 4*). Of the total decrease by 6%, for the past 10 years, about 61% was due to lose of farmland. Although the ESVs of forest and grasslands increased, the increases were too small to compensate the declines.

Table 3. *ESVs (CNY ha^{-1} yr^{-1}) of different land use types in Su-Xi-Chang region*

	Wood land	Grass land	Farm land	Wetland	Water body	Construct-ion land
Gas regulation	6291.5	1438.1	898.8	32335.6	0.00	0.00
Climate regulation	4853.5	1617.8	1599.8	30738.6	826.9	0.00
Water supply	5752.3	1438.1	1078.6	27862.5	36670.6	53.9
Soil formation and retention	7010.6	3505.3	2624.5	3073.9	18.0	36.0
Waste treatment	2354.8	2354.8	2948.0	32680.0	32716.0	18.0
Biodiversity protection	5860.1	1959.4	1276.3	4494.0	4476.0	611.2
Food	179.8	539.3	1797.6	539.3	179.8	18.0
Row material	4673.7	89.9	179.8	125.8	18.0	0.00
Recreational and culture	2300.9	71.9	18.0	9976.6	7801.5	18
Total	39277.2	13014.6	12421.4	141826.3	82706.8	755.1

Because water body has the highest value coefficient (*Table 1*) and covers a larger area (*Table 2*), the ESV of water body was the highest of the six land use types, accounting for about 75% of the total values. Farmland also generates a high service value because of its large area. Water body combined with farmland and woodland accounted for over 98% of the total ESVs produced in the region. This indicated that, the three land use types play major roles in providing ecosystem services. Although the wetland value coefficient was the highest of all land uses (*Table 1*), wetland cover a small area in Su-Xi-Chang region, thus this land use was associated with a very low service value. The ESV for grassland is also low for the same reason.

Table 4. *Total ESV for each land use types in Su-Xi-Chang region in 2000, 2005, and 2010*

Land use type	ESV ($\times 10^9$ CNY yr^{-1})			Change (2000–2010)[a]	
	2000	2005	2010	$\times 10^9$ CNY	%
Woodland	4.6015	4.6605	4.7066	0.1051	2.2840
Grassland	0.0207	0.0244	0.0287	0.008	38.6473
Farmland	10.7363	9.6441	8.0489	-2.6874	-25.0310
Wetland	0.5208	0.7525	0.4899	-0.0309	-5.9332
Water body	41.2962	40.4684	40.1173	-1.1789	-2.8547
Construction land	0.1952	0.2666	0.3671	0.3651	187.0390
Total	57.1775	55.8165	53.7585	-3.4190	-5.9796

[a] Positive and negative values represent increases and decreases, respectively.

Table 5 presented overall trends in the ESV of ecosystem service function types for the study area from 2000 to 2010. Waste treatment and water supply were the dominant ecosystem service function types. They accounted for about 70% of the total ESVs on average, and the relative importance of each category remained the same over the study period. However, it is obvious that the ESVs of all the ecosystem service functions decreased slowly but substantially over time. The substantial decline in total ESV in the study period may be attributed to a loss of natural and semi-natural land cover types, especially farmland and water body, due to an accelerated urban expansion.

Table 5. *Values of ecosystem service functions (ESVf) for Su-Xi-Chang region in 2000, 2005, and 2010*

Ecosystem service function	2000		2005		2010	
	ESV$_f$ ($\times 10^9$ CNY yr^{-1})	%	ESV$_f$ ($\times 10^9$ CNY yr^{-1})	%	ESV$_f$ ($\times 10^9$ CNY yr^{-1})	%
Gas regulation	1.6350	2.85	1.6162	2.90	1.4512	2.70
Climate regulation	2.4798	4.32	2.3887	4.28	2.1288	3.96
Water supply	20.0347	34.93	19.6322	35.16	19.3011	35.90
Soil formation and retention	3.1169	5.43	2.9140	5.22	2.5852	4.81
Waste treatment	19.2878	33.63	18.7605	33.61	18.1885	33.83
Biodiversity protection	4.2020	7.32	4.1287	7.40	4.0172	7.47
Food	1.6722	2.92	1.5153	2.71	1.2854	2.39
Row material	0.7125	1.24	0.7039	1.26	0.6859	1.28
Recreational and culture	4.2219	7.36	4.1637	7.46	4.1151	7.66
Total	57.3709	100	55.8232	100	53.7584	100

Variation of ESVs in response to LULC change and other factors

Figure 2 showed a significant negative linear relationship between expansion of construction land and total ESVs. Construction land increased by a factor of 88%, from 258,559 ha in 2000 to 486,095 ha in 2010. At the same time the total ESVs decreased by about 6%, from 57.1775 billion CNY in 2000 to 53.7585 billion CNY in 2010. Furthermore, Figure 3 showed significant polynomial decline in ESV per capita to GDP per capita from 2000 to 2010. Herein, both *Figure 2* and *3* indicate that both expansion of construction land and rapid economic growth has a substantial negative impact on the regional ecosystem services.

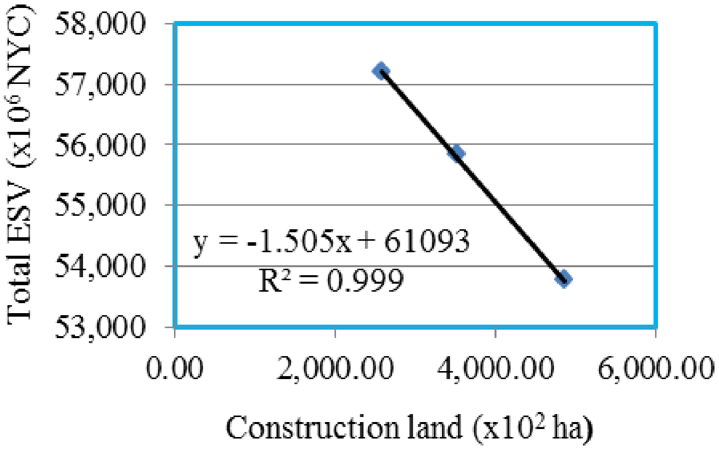

Figure 2. *Relationship between ESV and construction land from 2000 to 2010*

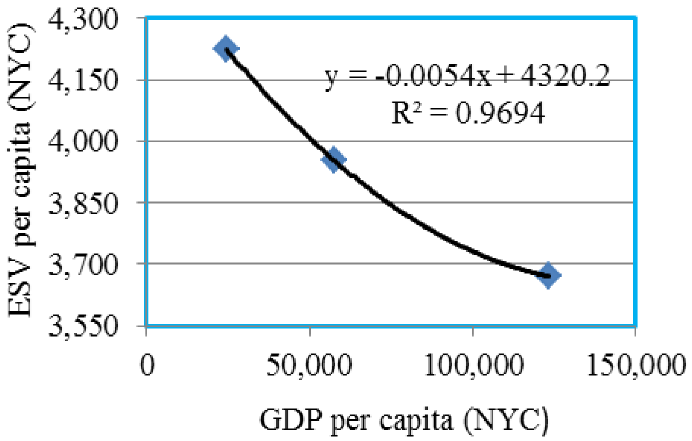

Figure 3. *Relationship between ESV per capita and GDP per capita from 2000 to 2010*

Similarly H-ESV ratio shows a declined trend. The H-ESV ratio in 2000 was about 1: 4,222 (CNY/year), but this has decreased to around 1: 3,953 and 1: 3,670 in 2005 and 2010, respectively. Showing that, the total benefits of human welfare to be obtained from the ecosystem services of the region were decreased by 13% during the study period.

Ecosystem sensitivity analysis

For the results of our analysis to be reliable, the sensitivity of ESV to changes in the value coefficients must be relatively low (*CS* < 1). Using (Eq. 4), the percentage change in the estimated total ESVs and the corresponding coefficient of sensitivity (*CS*) resulting from a 50% adjustment in the value of the service value coefficient, indicated that the total ESVs estimated in this study was relatively inelastic with respect to change in the value coefficients (*Table 6*). *CS* was highest for water body (0.75) because of the large area and high value coefficient for this land use type. Overall, the sensitivity analysis indicated that all the results of *CS* were less than 1, meaning the total value of ecosystem services is not sensitive to the change of *VC*, so the values of *VC* were suitable and the results are credible despite uncertainty in the value coefficient.

Table 6. *Percentage change in estimated total ESV and coefficient of sensitivity (CS) resulting from a 50% adjustment in ecosystem valuation coefficients (VC) in Su-Xi-Chang region*

Change in valuation coefficient	2000		2010	
	%	CS	%	CS
Woodland VC ± 50%	±4.02	0.08	±4.37	0.09
Grassland VC ± 50%	±0.02	0.0003	±0.03	0.0005
Farmland VC ± 50%	±9.39	0.19	±7.49	0.15
Wetland VC ± 50%	±0.46	0.01	±0.46	0.01
Water body VC ± 50%	±36.11	0.72	±37.31	0.75

Discussion

Given the ongoing trend of urbanization and socioeconomic development in the Su-Xi-Chang region an increasing demand for land will speed up LULC change. This change in LULC brought a significant impairment on ecological function that supports the human-dominated ecosystem. During the study period the total ESVs of Su-Xi-Chang region was decreased by 3.4190 billion CNY, which was mainly caused by the decrease in values of farmland and water body. According to the land use conversion matrix, from 2000 to 2010, there was a substantial increase in construction land over the other land use types from 258,559 ha to 486,095 ha. Remarkably, this growth exceeded the amount of land converted from construction land to other land cover types.

From the single land cover dynamic index (*K*) point of view in our study area, farmland, water body and wetland were under the state of depletion, with *K* values < 0. While construction land, woodland and grassland, with *K* value > 0, were under the state of development. The degree of change of construction land, with about 9% of *K* value, was far greater than all other land use categories; showing that it was under fast state of dynamics of development. Its expansion was caused mainly by the decrease of farmland and water body, which have high ESV. Construction land showed significant negative linear relationship (*Figure 2*) with ESV of the region. Even if the ESVs of forest and grasslands improved through the study time, these rises were not adequate to counterbalance the decreases due to construction land expansion. Thus change in LULC, due to increase in construction land with lower ESV, is responsible for a remarkable decline in total ESV of the region.

As proposed by (Costanza et al., 1997) , and modified by (Xie et al., 2003) to account for Chinese conditions, the method we used to estimate ESV derives from multiplying ESV by the area of a given land use category by the corresponding value coefficient (*Table 4*). However, as discussed in (Daily, 1997; Limburg et al., 2002; Turnera et al., 2003; Hein et al., 2006; Liu et al., 2012), estimates produced using this method have low resolution, high variation, and high uncertainty due to the complex, dynamic, and nonlinear properties of ecosystems. In addition, the accuracy of the modified value coefficient (*Table 1*) is doubtful because of ecosystem heterogeneity.

Different studies used various valuation methods to calculate value coefficient (Koschke et al., 2012; Wu et al., 2013) of ESV. Although different valuation methods may lead to different estimated values, leading to criticism of the ecosystem service valuation approach, it is important to remember that accurately calculating coefficients is less critical for time series analyses than for cross-sectional analyses, because these coefficients tend to affect estimates of directional change less than they affect estimates of the magnitude of ecosystem values at specific points in time (Li et al., 2010a; Tianhong et al., 2010; Liu et al., 2012).

Since changes in ESV over time were the focus of our study, the results are reliable, particularly in quantitative terms. In our analysis uncertainties and errors would be reduced or offset by calculating the ESV in 2000, 2005 and 2010 and analyzing changes across this time period. In addition, our sensitivity analysis indicated that the total ESV that we estimated for the study area were relatively inelastic with respect to the value coefficients, and that our estimates were robust despite uncertainties in the value coefficients.

During the study period Su-Xi-Chang region experienced rapid economic development, which often seems to conflict with ecological protection. The ESV change degree index showed an average depletion of 6% of total ESV through the study period. Although the ecological and economical characteristics and the techniques of evaluation varied, the estimated results in Su-Xi-Chang region and the other areas of China consistently showed that ecosystem services and functions tend to decline under current patterns of urban economic development (De-yong et al., 2005; Wu et al., 2006; Liu et al., 2012). From 2000 to 2010 both total ESV versus GDP and ESV per capita versus GDP per capita in Su-Xi-Chang region showed a negative relationship. Although the decline in ESV was relatively low as compared to economic growth, which was still worth attention because the decrease in total ESV, 3.42 billion CNY with an average annual decline of 0.342 billion CNY, was far greater than researches in some other areas of China (Wu et al., 2006; Li et al., 2010a; Helian et al., 2011; Liu et al., 2012; Tang et al., 2014).

In connection to socioeconomic development in the past decade of time there was a change in demographic pattern of Su-Xi-Chang region. The H-ESV ratio index showed a decrease of values of ecosystem services for human welfare by a rate of 1.3 each year. This was mainly due to the unbalance between growth in total population, which was increased by 8.2% of the base year, and less development of ecosystem services of the region. All these are a signal to regional planners that they must focus more on sustainable land management and ecological protection of the region due to its fragile ecological environment, so as to maintain its ESVs in the future.

Conclusion

In this study, LULC changes and their effect on ESVs were analyzed in the Su-Xi-Chang region from 2000 to 2010. The result showed that over the past one decade, the areal extent of construction land was increased by 88%, mainly at the expense of farmland and water body. However, the total ESV was decreased from 57.1775 billion CNY in 2000 to 53.7585 billion CNY in 2010, with a temporal change rate of 6%. Food provision was the most affected ecosystem service function, which was reduced almost by a quarter of its base year value, mainly due to significant loss in farm land. Waste treatment and water supply were the most two ecological functions of the region. Farmland and water body were the ecosystems whose surface reduced the most, and yet, they were the ecosystems with the highest estimated service values.

Overall, our investigation revealed that the expansion of construction land, which have due impact on LULC change has had a direct effect on ecosystem services and their non-market economic values. Therefore, in Su-Xi-Chang region a compromise between expansion in construction land and ecological protection must be reached. To accomplish this, the regional land use planning must emphasize the protection of farmland and water body, for the realization of sustainable use of land resources and coordinated development of the economy and environment. Furthermore, since the ESVs of the region has been decreasing in an accelerating rate it is advisable to conduct research on prediction and modeling of this change in order to discern its rate and extent of dynamics in the upcoming time, to sustain the ecosystem service provision.

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REFERENCES

[1] Costanza, R., dArge, R., deGroot, R., Farber, S., Grasso, M., Hannon, B., Limburg, K., Naeem, S., ONeill, R.V., Paruelo, J., Raskin, R.G., Sutton, P., vandenBelt, M. (1997): The value of the world's ecosystem services and natural capital. - Nature 387: 253-260.

[2] Daily, G. (1997): Nature's services: societal dependence on natural ecosystems. Island Press.

[3] De-yong, Y., Yao-zhong, P., Yan-yan, W., Xin, L., Jing, L., Zhong-hua, L. (2005): Valuation of ecosystem services for Huzhou City, Zhejiang Province from 2001 to 2003 by Remote Sensing data. - Journal of Forestry Research 16: 223-227.

[4] Dewan, A.M., Yamaguchi, Y. (2009): Land use and land cover change in Greater Dhaka, Bangladesh: Using remote sensing to promote sustainable urbanization. - Applied Geography 29: 390-401.

[5] Estoque, R.C., Murayama, Y. (2012): Examining the potential impact of land use/cover changes on the ecosystem services of Baguio city, the Philippines: a scenario-based analysis. - Applied Geography 35: 316-326.

[6] Estoque, R.C., Murayama, Y. (2013): Landscape pattern and ecosystem service value changes: implications for environmental sustainability planning for the rapidly urbanizing summer capital of the Philippines. - Landscape and Urban Planning 116: 60-72.

[7] Guo, Z., Xiao, X., Gan, Y., Zheng, Y. (2001): Ecosystem functions, services and their values–a case study in Xingshan County of China. - Ecological economics 38: 141-154.

[8] Han, H., Yang, C., Song, J. (2015): Scenario Simulation and the Prediction of Land Use and Land Cover Change in Beijing, China. - Sustainability 7: 4260-4279.

[9] Hein, L., Van Koppen, K., De Groot, R.S., Van Ierland, E.C. (2006): Spatial scales, stakeholders and the valuation of ecosystem services. - Ecological economics 57: 209-228.

[10] Helian, L., Shilong, W., Guanglei, J., Ling, Z. (2011): Changes in land use and ecosystem service values in Jinan, China. - Energy Procedia 5: 1109-1115.

[11] Irwin, E.G., Geoghegan, J. (2001): Theory, data, methods: developing spatially explicit economic models of land use change. - Agriculture, Ecosystems & Environment 85: 7-24.

[12] Koschke, L., Fuerst, C., Frank, S., Makeschin, F. (2012): A multi-criteria approach for an integrated land-cover-based assessment of ecosystem services provision to support landscape planning. - Ecological Indicators 21: 54-66.

[13] Kreuter, U.P., Harris, H.G., Matlock, M.D., Lacey, R.E. (2001): Change in ecosystem service values in the San Antonio area, Texas. - Ecological economics 39: 333-346.

[14] Li, J., Wang, W., Hu, G., Wei, Z. (2010a): Changes in ecosystem service values in Zoige Plateau, China. - Agriculture, Ecosystems & Environment 139: 766-770.

[15] Li, X., Yeh, A.G.-O. (2004): Analyzing spatial restructuring of land use patterns in a fast growing region using remote sensing and GIS. - Landscape and Urban Planning 69: 335-354.

[16] Li, Y., Long, H., Liu, Y. (2010b): Industrial development and land use/cover change and their effects on local environment: a case study of Changshu in eastern coastal China. - Frontiers of Environmental Science & Engineering in China 4: 438-448.

[17] Limburg, K.E., O'Neill, R.V., Costanza, R., Farber, S. (2002): Complex systems and valuation. - Ecological economics 41: 409-420.

[18] Liu, X., Li, Z., Liao, C., Wang, Q., Zhu, A., Li, D., Li, Y., Tang, Z. (2015): The development of ecological impact assessment in China. - Environment international 85: 46-53.

[19] Liu, Y., Li, J., Zhang, H. (2012): An ecosystem service valuation of land use change in Taiyuan City, China. - Ecological Modelling 225: 127-132.

[20] Liu, Y., Wang, J., Long, H. (2010): Analysis of arable land loss and its impact on rural sustainability in Southern Jiangsu Province of China. - Journal of Environmental Management 91: 646-653.

[21] Long, H., Liu, Y., Hou, X., Li, T., Li, Y. (2014): Effects of land use transitions due to rapid urbanization on ecosystem services: Implications for urban planning in the new developing area of China. - Habitat International 44: 536-544.

[22] Long, H., Liu, Y., Wu, X., Dong, G. (2009): Spatio-temporal dynamic patterns of farmland and rural settlements in Su–Xi–Chang region: Implications for building a new countryside in coastal China. - Land Use Policy 26: 322-333.

[23] Long, H., Tang, G., Li, X., Heilig, G.K. (2007): Socio-economic driving forces of land-use change in Kunshan, the Yangtze River Delta economic area of China. - Journal of Environmental Management 83: 351-364.

[24] Mendoza-González, G., Martínez, M., Lithgow, D., Pérez-Maqueo, O., Simonin, P. (2012): Land use change and its effects on the value of ecosystem services along the coast of the Gulf of Mexico. - Ecological economics 82: 23-32.

[25] National Bureau of Statistics of China (NBSC) (2014): China statistical yearbook. - China Statistics Press, Beijing.

[26] Omenn, G.S. (2006): Grand challenges and great opportunities in science, technology, and public policy. - Science-New York then Washington- 314: 1696.

[27] Polasky, S., Nelson, E., Pennington, D., Johnson, K.A. (2011): The impact of land-use change on ecosystem services, biodiversity and returns to landowners: A case study in the State of Minnesota. - Environmental and Resource Economics 48: 219-242.

[28] Poschlod, P., Bakker, J., Kahmen, S. (2005): Changing land use and its impact on biodiversity. - Basic and Applied Ecology 6: 93-98.

[29] Ruishan, H., Suocheng, D. (2013): Land use dynamics and landscape patterns in Shanghai, Jiangsu and Zhejiang. - Journal of Resources and Ecology 4: 141-148.

[30] Secretariat, I. (2005): GLP (2005) science plan and implementation strategy. - IGBP Report No 53.

[31] Seto, K.C., Kaufmann, R.K. (2003): Modeling the drivers of urban land use change in the Pearl River Delta, China: integrating remote sensing with socioeconomic data. - Land Economics 79: 106-121.

[32] Su, S., Xiao, R., Jiang, Z., Zhang, Y. (2012): Characterizing landscape pattern and ecosystem service value changes for urbanization impacts at an eco-regional scale. - Applied Geography 34: 295-305.

[33] Tang, Z., Shi, C., Bi, K. (2014): Impacts of land cover change and socioeconomic development on ecosystem service values. - Environmental Engineering and Management Journal 13: 2697-2705.

[34] Tianhong, L., Wenkai, L., Zhenghan, Q. (2010): Variations in ecosystem service value in response to land use changes in Shenzhen. - Ecological economics 69: 1427-1435.

[35] Tong, S., Sun, Y., Yang, Y. (2012): Generating a future land use change scenario with a modified population-coupled Markov Cellular Automata model. - Journal of Environmental Informatics 19: 108-119.

[36] Turner, B.L., Lambin, E.F., Reenberg, A. (2007): The emergence of land change science for global environmental change and sustainability. - Proceedings of the National Academy of Sciences 104: 20666-20671.

[37] Turnera, R.K., Paavolaa, J., Coopera, P., Farberb, S., Jessamy, V., Georgiou, S. (2003): Valuing nature: lessons learned and future research directions. - Ecological economics 46.

[38] Wang, Z., Wang, Z., Zhang, B., Lu, C., Ren, C. (2015): Impact of land use/land cover changes on ecosystem services in the Nenjiang River Basin, Northeast China. - Ecological Processes 4: 1-12.

[39] Wei, Y.D., Fan, C.C. (2000): Regional inequality in China: a case study of Jiangsu province. - The Professional Geographer 52: 455-469.

[40] Wu, H.-I., Wang, X.-L., Ning, L.-M., Lu, Y.-F. (2006): Effects of land use change on ecosystem services value:--A case study in Wuhan City. - Resources and Environment in the Yangtze Basin 15: 186-190.

[41] Wu, K.-y., Ye, X.-y., Qi, Z.-f., Zhang, H. (2013): Impacts of land use/land cover change and socioeconomic development on regional ecosystem services: The case of fast-growing Hangzhou metropolitan area, China. - Cities 31: 276-284.

[42] Xie, G.-D., Lu, C.-X., Leng, Y.-F., Zheng, D., Li, S. (2003): Ecological assets valuation of the Tibetan Plateau. - Journal of natural resources 18: 189-196.

[43] Xie, Y., Batty, M., Zhao, K. (2007): Simulating emergent urban form using agent-based modeling: Desakota in the Suzhou-Wuxian region in China. - Annals of the Association of American Geographers 97: 477-495.

[44] Ye, Y., Zhang, J.e., Chen, L., Ouyang, Y., Parajuli, P. (2015): Dynamics of ecosystem service values in response to landscape pattern changes from 1995 to 2005 in Guangzhou, southern China. - Applied Ecology and Environmental Research 13: 21-36.

[45] Yuhai, W.X.B. (1999): Study on the methods of land use dynamic change research [j]. - Progress in Geography 1.

[46] Zhou, X., Chen, L., Xiang, W. (2014): Quantitative analysis of the built-up area expansion in Su-Xi-Chang region, China. - The journal of applied ecology 25: 1422-1430.

MODELING OF SOIL CO2 EFFLUX DURING WATER TABLE FLUCTUATION BASED ON IN SITU MEASURED DATA FROM A SEDGE-GRASS MARSH

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Abstract. Soils of wetland ecosystems serve as a huge storage of organic carbon. Its decomposition and consequent release of CO_2 into the atmosphere is highly affected by soil hydrology, and this release of CO_2 may severely increase during future climate change. The aim of this study was to describe the immediate response of soil CO_2 efflux to temperature and changes in water level. Soil CO_2 efflux from a marsh, temperature and the water table were continuously measured *in situ* during a gradual decrease of the water table and its consequent rapid increase after heavy rain. CO_2 efflux fluctuated as it followed diurnal changes in temperature. However, it showed an increasing trend as the water table decreased. After the rain, the water table rose above the soil surface and soil CO_2 efflux dropped fast to nearly zero. A simple model based on soil temperature and water table level was created to estimate soil CO_2 efflux. There was far better agreement between this model and measured data than with the widely used model based only on temperature. The results showed the importance of including the soil water conditions in models for estimating soil CO_2 efflux at sites with a high water table level.

Keywords: *Carex acuta, fen, soil chamber, soil respiration, wetland*

Introduction

Carbon dioxide efflux from soil remains the second largest carbon flux in most ecosystems after photosynthesis (Kuzyakov, 2006) and it accounts for ca 60-90 % of total ecosystem respiration (Goulden et al., 1996, Longdoz et al., 2000). Therefore, quantification of soil CO_2 efflux is important for understanding the carbon dynamics of terrestrial ecosystems and predicting possible future scenarios.

Soil respiration consists of autotrophic and heterotrophic components (Kuzyakov, 2006). The first comprises the respiration of plant roots and is a major source of CO_2 loss in plants, while heterotrophic respiration occurs among soil microorganisms.

CO_2 efflux from soil results from autotrophic respiration by live roots and heterotrophic respiration is caused mainly by the decomposition of organic matter by microorganisms (Kuzyakov, 2006). Obtaining accurate estimates of CO_2 efflux from the soil to the atmosphere represents a significant challenge, it is particularly difficult to separate the autotrophic and heterotrophic components without disturbing the soil system (Kutsch et al., 2009).

Both the rate of the CO_2 efflux from the soil and the immediate responses of CO_2 efflux to changing conditions vary in different types of ecosystems. Suitable conditions are necessary for the biogeochemical processes involved in producing CO_2. The crucial factor for these, mostly aerobic, processes is temperature and the availability of water and nutrients. In wetland ecosystems a crucial factor is the level of the water related to the soil surface.

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Generally, the biggest portion of soil respiration takes place in the top soil layers because of root presence, higher soil temperature and oxygen content compared to lower horizons (Sierra and Renault, 1998; Tufekcioglu et al., 1999; Lafleur et al., 2005). At wetlands, moreover, these top horizons get aerated when water table decreases while lower horizons remain often water saturated. The water table and its possible fluctuation determine whether conditions are anoxic or anaerobic and thus they also determine the prevailing biogeochemical processes in wetland soils and in the whole wetland ecosystem (Reddy and DeLaune, 2008). Therefore, the water table level significantly affects CO_2 exchange between the ecosystem and the atmosphere (Dusek et al., 2009; Jimenez et al., 2012), and thus also affects the gross ecosystem production, including the gross radiation use efficiency (Dusek et al., 2012a).

The relationship between CO_2 efflux and temperature is quite well known and has been presented in previous studies (Lloyd and Taylor, 1994; Pavelka et al., 2007; Subke and Bahn, 2010). However, the effect of water table fluctuations on CO_2 efflux has been studied in previous studies. Mostly a decrease of soil respiration was observed with increasing water table (Oechel et al., 1998; Yang et al., 2013) as a result of limited oxygen diffusion in water filled pores (Chamindu Deepagoda and Elberling, 2015). However, the possible synergic effect with other factors (temperature, nutrient availability) causes different responses to unitary water table changes and makes it difficult to understand this phenomena.

CO_2 efflux from the soil or from plant stands can be measured using different methods (Livingston and Hutchinson, 1995; Alm et al., 2007). One of the most commonly used methods for CO_2 flux investigation is the eddy-covariance technique (Baldocchi, 2003; Aubinet et al., 2012). This method measures CO_2 flux over a certain area, footprint, where the size and shape of this footprint depends on the height of measuring system and on the direction and velocity of the prevailing winds (Aubinet et al., 2012). The eddy-covariance technique cannot distinguish between CO_2 fluxes from different parts of the ecosystem and cannot describe flux variation in space. For a detailed focus on CO_2 efflux from the soil it is better to use the chamber method, which is precise enough for soil CO_2 efflux measurement (Livingston and Hutchinson, 1995; Pumpanen et al., 2004; Reth et al., 2005) and is capable of recording the responses to changing conditions (e.g. temperature and water table fluctuations).

The aims of this study were to mathematically describe the immediate response of soil CO_2 efflux to soil temperature and changes in water table level and to compare this model with the model based only on soil temperature. As soil temperature is a driving factor of soil CO_2 efflux only till a certain percentage of soil water content, we aimed to determine what was the level of water table when soil temperature stops affecting soil CO_2 efflux. At these level of water table soil CO_2 efflux also displays a rapid decline. Therefore, determination of this level of water table and of the response of soil CO_2 efflux is crucial for soil CO_2 efflux models at wetland sites.

The data used in this study was measured during a continuous measurement campaign, which was carried out in a sedge-grass marsh under real conditions and without any manipulation of the conditions.

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Materials and methods

Site description

The monitored sedge-grass marsh is a 1.5 ha part of the large "Wet Meadows" wetland complex, situated near the town of Trebon in South Bohemia, Czech Republic, close to an ancient man-made lake called Rozmberk. This ancient lake was created in the Middle Ages for fish production and for agricultural production in the local catchment. Man-made lakes, generally referred to as fishponds, were created in places where originally wet meadows and wetlands occurred. The sedge-grass marsh (location of the meteorological station is 49°01′ 29" N, 14°46′13" E) is a flat area at an altitude of 426.5 m above sea level. The mean annual air temperature and mean annual precipitation, for a 35-year period between 1977 and 2011, were 7.6 °C and 614 mm, respectively (Dusek et al., 2012b). For previous ecological studies that focused on the "Wet Meadows" sedge-grass marsh site see Jenik and Kvet (1983) and Kvet et al. (2002). The water table mostly fluctuates between -0.2 m and 1.0 m throughout the year. Most frequently the water table is situated at about -0.1 m. In some years, spring or summer floods occurred. These floods are a consequence of snow melting or heavy summer rains. The sedge part of the wetland complex was mown once a year until the 1950's. The resulting vegetation was formed mainly by tall sedges (*Carex acuta, Carex vesicaria*) and hygrophytic grasses (especially *Calamagrostis canescens*) (Holubickova, 1959; Gazda, 1983; Prach, 1993; Prach and Soukupova, 2002; Prach, 2008). During the last 50 years, after cessation of mowing, a distinct stand pattern of hummocks and hollows has developed. The hummocks are formed by tussocks of *Carex acuta*.

Soil properties

Soils in the sedge-grass marsh can be classified as histosols (Reddy and DeLaune, 2008) with a high amount of organic matter in the upper soil layers. Formation of these soils is based on sedimentation processes during the post glacial period when lower layers of the soil profile were created. The lower profile consists of sand and clay with accessory organic matter. The upper layer of soil has been formed over the last hundred years as a result of the accumulation of partially decomposed organic matter. These layers contained more organic matter than the lower layers, with slight stripes of sand, clay or gravel. The thickness of the organic layers created by peat vary from 0.5 m to several meters (Jenik et al., 2002). Basic chemical characteristics of the soil are summarized in *Table 1*. The bulk density of the 0 to 0.3 m layer is between 0.30 and 0.40 g cm^{-3} and it contains on average about 21.6% carbon. Due to the constantly high water table levels over the last hundred years, upper organic layers were not decomposed and remained in the site.

Soil CO_2 efflux, soil temperature and water table depth measurements

Measurement of soil CO_2 efflux was carried out using a manual portable and an automated gasometrical systems.

Manual measurements on 30 positions within 40 m around the automated chamber were taken on 21 and 22 September 2010 in the morning hours using portable closed system Li-6200 (Li-Cor, Lincoln, NE, USA). The system consisted of an infrared gas analyzer (Li6250, Li-Cor, USA), a control unit (Li6200, Li-Cor, USA) and an opaque soil chamber developed at the Global Climate Change Institute CAS. On each position CO_2 efflux was measured from collars inserted about 3 cm deep into the soil one week before the first measurement. Next to each collar soil temperature. During each measurement, soil temperature at 1.5 cm (penetrate thermometer, Roth, GE) and soil moisture in the 0–6 cm profile (ThetaProbe ML2x, Delta-T Devices, UK) were measured at three points located 5 cm outside the collar, with the mean values being used for subsequent analysis.

Table 1. *Chemical characteristics of the organic soil in the sedge-grass marsh community of the Wet Meadows at 0-30 cm depth of soil profile. Analyses were performed by the Agro-La Comp. Laboratories at Jindrichuv Hradec.*

Parameter	**mean**	**SD**
pH($CaCl_2$)	4.8	0.1
Potassium [mg/kg]	144.8	37.1
Magnesium [mg/kg]	395.8	32.1
Calcium [mg/kg]	2692.0	227.0
Dry matter [%]	42.3	4.9
Total phosphorus [%]	0.2	0.0
Total nitrogen [%]	0.9	0.1
Ash [%]	71.4	2.5

Automated continuous measurements were done using a system ACSEM (developed at the Global Change Research Institute CAS) between 20 and 26 September 2010. This system consisted of a chamber with an automatic closing function and an EGM-3 analyzer (PP-System, United Kingdom). The chamber was controlled by a DL3000 logger (Delta-T Ltd, UK), which also stored the measured data. The chamber was installed in a hollow (free space between hummocks of *Carex acuta*). The selected hollow was without any vegetation cover. The possibility for aboveground plant organs to contribute to CO_2 efflux was excluded. Soil temperature measurements at depths of 0.5, 1.5, 3, 5 and 10 cm were made near the chamber by PT 1000 platinum thermometers (HIT Uherske Hradiste, Czech Republic). The water table level was continuously measured by a LP 307 hydrostatic pressure sensor (BD Sensors, Czech Republic). CO_2 efflux from the soil and soil temperatures were measured at 15 min intervals, while the water table level was measured at 30 min intervals.

Data analysis and calculations

Soil CO_2 efflux (R_S) was plotted against soil temperature (T_S) and this was fit by an exponential regression curve with the regression equation:

$$R_S = \beta \cdot e^{\alpha \cdot T_S} \qquad \text{(Eq. 1)}$$

where α and β are the regression coefficients. Q_{10} (the proportional change in CO_2 efflux caused by a 10 °C increase in temperature) was calculated as:

$$Q_{10} = e^{10 \cdot \alpha} \qquad \text{(Eq. 2)}$$

where α is the regression coefficient obtained from Equation 1. Then, CO$_2$ efflux was normalized for the temperature of 10 °C (R_{10}) according to equation:

$$R_{10} = \frac{R_S}{Q_{10}^{\frac{T_S - 10}{10}}} \qquad \text{(Eq. 3)}$$

To estimate soil CO$_2$ efflux from measured parameters we used the reversed equation:

$$R_m = \frac{R_{10}}{Q_{10}^{\frac{10 - T_S}{10}}} \qquad \text{(Eq. 4)}$$

For fitting the R_{10} data and the depth of the water table we used the program TableCurve (Systat, USA), the correlation statistics were provided in the analysis software SigmaPlot 11.0 (Systat, USA).

Statistical analyses

The program TableCurve 2D (Systat Software, San Jose, CA, USA) was used to find the relationship between R_{10} and the water table and to fit the curve. The correlation between measured and modelled soil CO$_2$ efflux was tested by using the Pearson Correlation test in SigmaPlot 11.0 (Systat Software, San Jose, CA, USA). Moreover, a Taylor diagram (Taylor, 2001) was applied to compare measured and modelled soil CO$_2$ efflux data. This diagram was implemented in R statistical software (R Development Core Team 2011).

Results

Overall conditions in September

The mean monthly air temperature in September 2010 was 11.2 °C, which was lower than the long-term air temperature for this month (12.2 °C for the period 1977-2010). The air temperature in September 2010 ranged from 7 to 14 °C and decreased in the first half of the month and again in the middle of month (*Figure 1A*). Soil temperature ranged between 10.1°C and 10.8 °C.

The monthly sum of precipitation was 61.6 mm, which was about 13% higher than the long-term mean (53.4 mm for period 1977-2010). During September 2010, there were eight rain events with more than 3 mm of precipitation recorded. The water table fluctuated according to precipitation and high increase in water table were recorded during rainy days (gray area around solid black line in the *Figure 1B*). The water table fluctuated between 8 cm below the soil surface and 5 cm above the soil surface. Precipitation in the middle of the month (12.6 mm) raised the water table daily average from -2 cm below the soil surface to about 4 cm above the soil surface. After that the water table slowly decreased to a minimum daily average value of -8 cm below the soil surface. The water table remained at this minimum for one day and after that it increased rapidly to 5 cm above the soil surface. This increase in water table was caused by heavy rain on 26 September when the daily sum amounted to 19 mm. During the following three days precipitation ranged from between 4 and 8 mm per day and the water table remained stable at 5 cm above the soil surface.

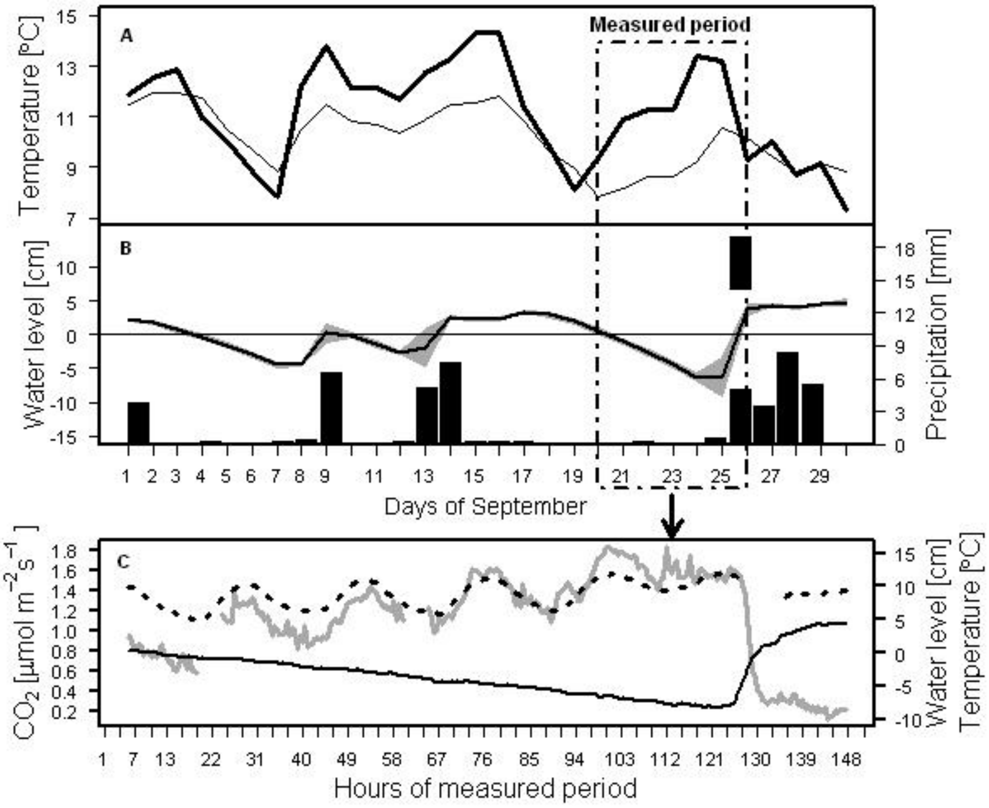

Figure 1. *Daily courses of studied parameters. A – Daily means of air temperatures at 2 m height (thick line); daily means of soil surface temperatures (thin line). B – daily sums of precipitation (bars) in September together with changing water level (black line). Variability of water level, shown as gray area around solid black line. C – Soil CO_2 efflux (gray line); soil temperature (dashed line); water level (solid line). Measured period is marked in the Figures A and B as a dashed box.*

Detailed conditions during experimental period

The experimental period was set in the end of September 2010. During this period there was a controlled discharge of the lake, therefore the water table decreased slowly until 25 September when it started raining (*Figure 1C*). During this period the water table gradually decreased from 0.4 cm above the soil surface to -8.0 cm below the soil surface. After the rain the water table increased up to 4.2 cm above the soil surface and measurements had to be stopped on 26 September due to possible damage to the measuring system.

The highest soil temperature during CO_2 efflux measurement was at a depth of 0 cm with an amplitude of 9.3 °C, the lowest soil temperature was recorded at a depth of 10 cm with an amplitude of only 1.1 °C. In the daytime, the highest temperature was at the soil surface (0 cm) and temperature decreased with soil depth. At night this was reversed. Soil temperature minima and maxima at depths of 1.5, 3, 5 and 10 cm lagged behind the temperature of the soil surface by 0.9, 1.3, 2.2 and 3.2 h for minima, and 1.9, 2.9, 4.1 and

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6.8 h for maxima, respectively. After the rain, fluctuations in of soil temperature were reduced and it was difficult to clearly determine the minimum and maximum.

Soil CO$_2$ efflux

During manual measurements of soil CO$_2$ efflux, mean soil water content at 30 positions reached 88.1±9.7%. Soil CO$_2$ efflux was 1.40±0.92 µmol m^{-2}s^{-1} corresponding to the mean soil temperature 9.2±0.9 °C on 21 September, and 1.47±0.84 µmol m^{-2}s^{-1} corresponding to the mean soil temperature 10.7±10.3 °C on 22 September.

From 20 to 25 September, soil CO$_2$ efflux from the automated chamber followed the diurnal pattern of changes in soil temperature, with maxima in early afternoon and minima at night. However, the relationship between temperature and soil CO$_2$ efflux was not so strong (R^2=0.59). This can be attributed to the gradually decreasing water table accompanied with the increasing trend of CO$_2$ efflux (*Figure 1C*). After heavy rain on 25 September and a rapid increase in the water table, soil CO$_2$ efflux rapidly dropped to about 0.2 µmol m^{-2}s^{-1} despite the temperature remaining at the same level. The most remarkable decrease in CO$_2$ efflux occurred when the water table was between -3 and +3 cm.

The dependence of soil CO$_2$ efflux on soil temperature was the strongest for temperature measured at a depth of 1.5 cm, in accordance with the methodology presented by Pavelka et al. (2007). The Q$_{10}$ value for CO$_2$ efflux normalization using this temperature was 2.2. R$_{10}$ was calculated for every measurement using equitation (Eq. 3). Calculated R$_{10}$ was plotted against the depth of the water table (*Figure 2*). The data were then fitted with a modified exponential curve:

$$y = a \cdot e^{-0.5 \cdot \left| \frac{D_{WT} - (-b)}{c} \right|^{d}} \qquad \text{(Eq. 5)}$$

where a=1.54, b=10.34, c=10.27, d=3.96 and D_{WT} is the depth of the water table. This relationship was incorporated into Equation 4 instead of parameter R$_{10}$. Then, the modeled soil CO$_2$ (R$_m$) was calculated as:

$$R_m = \frac{R_{10}}{Q_{10}^{\frac{10-T_s}{10}}} = \frac{1.54 \cdot e^{-0.5 \cdot \left| \frac{D_{WT} + 10.34}{10.27} \right|^{3.96}}}{Q_{10}^{\frac{10-T_s}{10}}} \qquad \text{(Eq. 6)}$$

When soil CO$_2$ efflux was modeled using only measured temperature (Equation 4), there was not good agreement with measured data. Although the Pearson Correlation test confirmed significant correlation between measured and modelled data ($p < 0.001$), the R^2 value reached only 0.32 (*Figure 3, Figure 4*). Modeled soil CO$_2$ efflux did not show any increasing trend during the decreasing of the water table level. Moreover, this model estimated high CO$_2$ efflux after the rain (about 1.3 µmol m^{-2}s^{-1}) in comparison with measured data.

When the depth of the water table was included into the simple model, modeled soil CO$_2$ efflux was in better agreement with measured data and R^2 increased to 0.95 (*Figure 4, Figure 5*).

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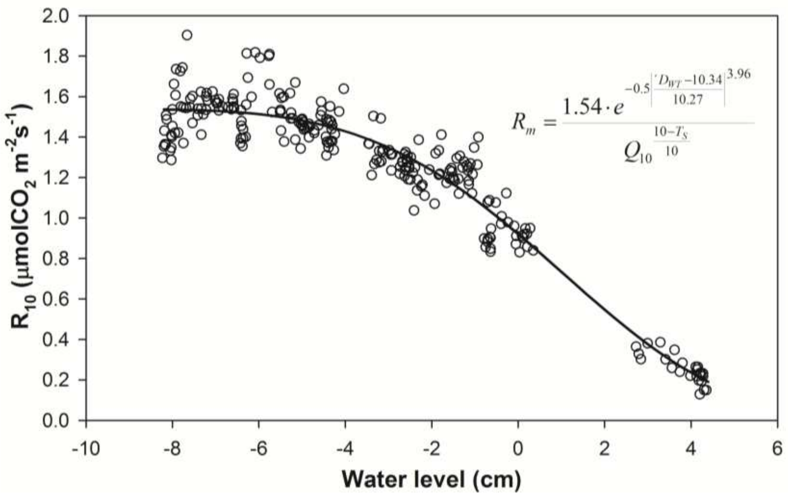

Figure 2. *Dependence of normalized CO$_2$ soil efflux (R_{10}) on water level fluctuations.*

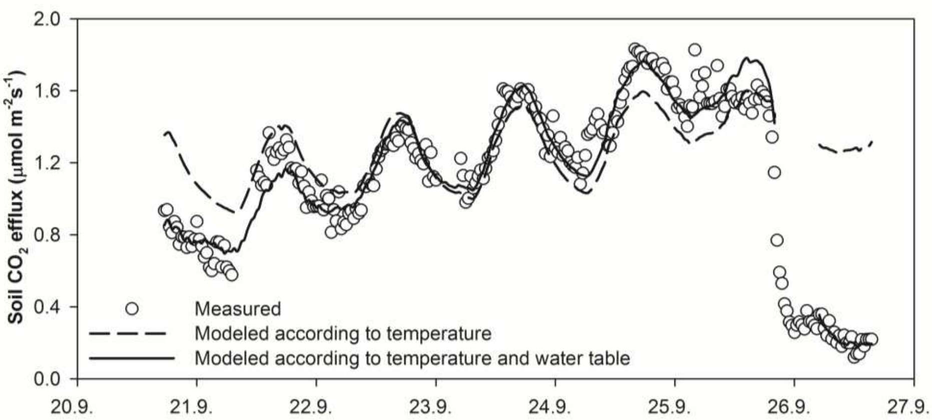

Figure 3. *Soil CO$_2$ efflux measured, modeled according to the soil temperature (Equation 4) and modeled according to the soil temperature and the water level (see Equation 6).*

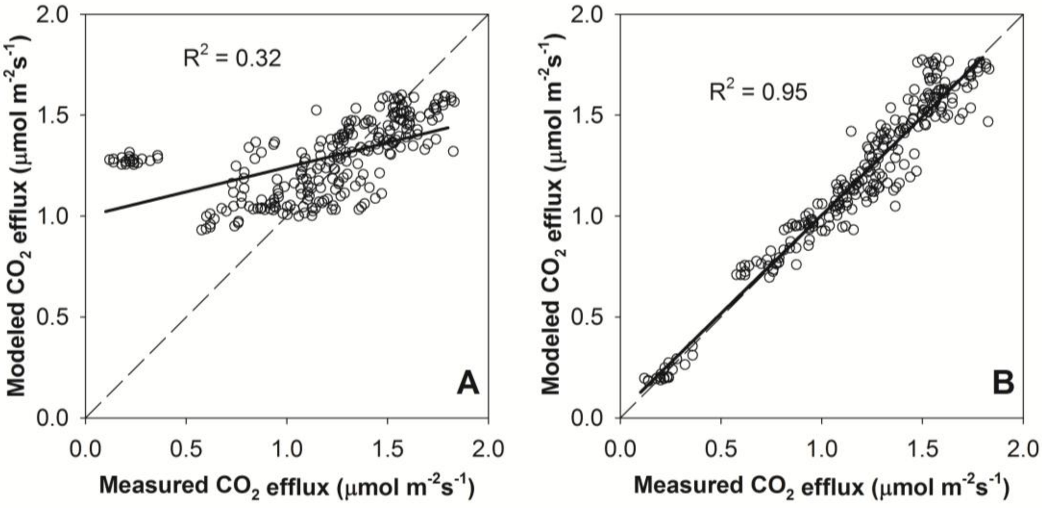

Figure 4. *Relationship between modeled soil CO$_2$ efflux and measured CO$_2$ efflux. A – model based only on soil temperature, B – model based on soil temperature and water level.*

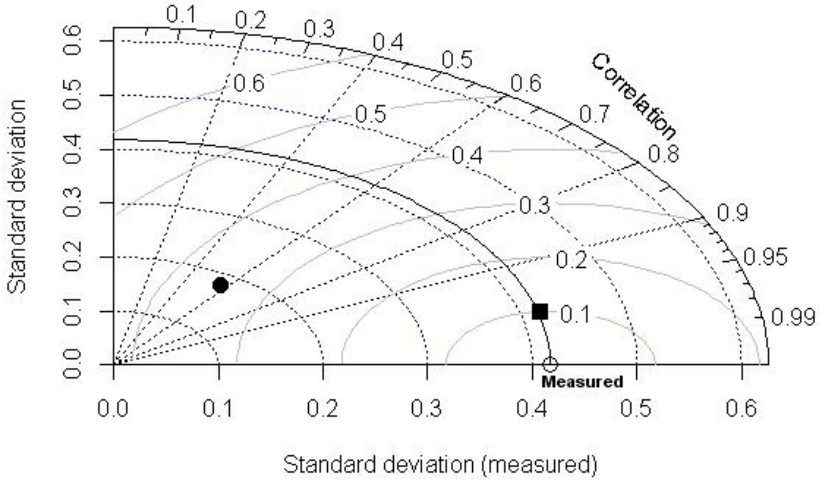

Figure 5. *Taylor diagram comparing the relationships between measured soil CO_2 efflux (empty circle on x axis labeled as "Measured") and soil CO_2 efflux modeled using soil temperature (filled black circle) and modeled using both soil temperature and water table fluctuation (filled black square).*

The similarity between models is presented in the Taylor diagram (*Figure 5*), which is quantified in terms of their correlation, their centered root-mean-square difference (RMS) and the amplitude of their variations (Taylor, 2011). From this graph, differences between two soil CO_2 efflux models and measured data (open circle on the x axis) are evident. The model based only on soil temperature (filled black circle) has a lower variation (0.18), presented as standard deviation, than measured data (0.41). Data variation of the model based on both soil temperature and water table fluctuations is very close to the variation of measured data. The correlation coefficients for the relationships between modeled data and measured data were 0.564 for the model with soil temperature and 0.972 for the model with soil temperature and water table fluctuations, respectively. The model based on both soil temperature and water table fluctuations also had a lower RMS value (0.1) than the model based only on soil temperature.

Discussion

Maxima and minima soil temperatures at depths of 1.5, 3, 5 and 10 cm lagged behind measured temperatures at the soil surface. This time lag increased with increasing depth, while the temperature amplitude decreased. This has been observed in other studies (Pavelka et al., 2007; Phillips et al., 2011). A time lag between soil CO_2 efflux and measured soil temperature has also been described in previous studies (e.g. Reichstein et al., 2005). Riveros-Iregui et al. (2007) or Phillips et al. (2010) investigated the effect of soil moisture on this hysteresis. The authors found that the hysteresis was lower under dry conditions than when the soil was wet. It can be explained by the effect of soil water content on two important soil processes: CO_2 diffusivity and thermal diffusivity, which decrease with increasing soil moisture (Jassal et al., 2005; Wang and Bou-Zeid, 2012). Therefore, soils with high water content get less warm and lower layers do not contribute much to the soil surface CO_2 efflux.

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Soil CO_2 efflux from the automated chamber ranged from 0.6 to 1.8 µmol $m^{-2}s^{-1}$ between 21 and 25 September when water table was below soil surface. Mean values of soil CO_2 efflux measured by the portable system on 30 positions (1.40 and 1.47 0.6 to 1.8 µmol $m^{-2}s^{-1}$) fitted to this range. This supported our assumption that the automated chamber was on a representative position.

Temperature sensitivity of CO_2 efflux (parameter Q_{10}) was estimated based on the temperature measured at the depth of 1.5 cm (according to the methodology of Pavelka et al. (2007)). Q_{10} was equal to 2.2, which is in the range (1.4 – 3.4) of values expected for wetland or peatland soils (Bonnett et al., 2006; Vicca et al., 2009; Inglett et al., 2012). This Q_{10} value was used to normalize soil CO_2 efflux for 10 °C (R_{10}). R_{10} negatively correlated with the depth of the water table. Few studies have described a weak relationship between CO_2 efflux and the water table position (Lafleur et al., 2005; Bubier et al., 1998). However, the majority of previous studies observed a strong relationship (e.g. Jaatinen et al., 2008; Lloyd, 2006; Vicca et al., 2009) similar to the one observed in this investigation.

In our study, the steepest decline in soil CO_2 efflux was observed with water table decrease to 3 cm below the soil surface. Other decrease of water table had much smaller effect on soil CO_2 efflux. This confirm that the biggest portion of CO_2 efflux production is placed in the top soil layer which is in accordance with other studies. *In situ*, the increase in CO_2 emission has mostly been seen only with a lowering to a certain depth, between 10 and 30 cm depending on the study, with no further increase with a further lowering (Silvola et al., 1996; Chimner and Cooper, 2003; Jaatinen et al., 2008). Chimner and Cooper (2003) suggested the lack of easily oxidized labile C in the deeper soil layers as a reason for this pattern. This is supported by the results of Hogg et al. (1992) that in drained samples in vitro the release of CO_2 was about 10 times greater from 0–10 cm peat layer than from 30–40 cm layer, which they attributed to the relatively large pool of non-structural carbohydrates in surface samples, deriving from recently dead plant biomass.

The CO_2 efflux model based on temperature (Equation 4) is a method that is commonly used to estimate the amount of CO_2 released from ecosystems (Davidson et al., 2006). This method is suitable for situations where the soil temperature is the variable driving the seasonal dynamics of CO_2 efflux. However, the influence of temperature can decrease when soil water content is very low or very high (temperature and CO_2 efflux can even become decoupled; Xu et al., 2004). The latter case is common for wetland or peatland ecosystems.

Soil saturation by water results in the decline in activity of the soil processes dependent on the availability of oxygen. Just, the fast submerging of whole soil profile almost immediately switches internal soil conditions from aerobic to anaerobic processes. Therefore, we can observe suppression of aerobic decomposition of organic matter and its alternation with slower anaerobic decomposition (Inglett et al., 2005). This becomes evident as methane (CH_4) is produced and released into the atmosphere (Altor and Mitsch, 2008; Knorr et al., 2008).

Although this switch can have also some time lag as in case of soil temperature changes, submerging of soil profile is accompanied by gradual water table rise up to or above soil surface together with saturation of the soil surface by water from precipitation. The gradual rise of the interstitial water table displaces air in soil pores and usually decreases soil temperature mainly in surfaces layers. Submersion of the whole soil profile changes markedly physical properties of soils. Rates of gas transport in submerged soils are greatly reduced relatively to that in air. The structure of soil reduces the cross-sectional area available for gas flow and increases the tortuosity. Transfer coefficients within soil environments may be 10^2 to 10^5 times lower than in the atmosphere environments (Matson and Harriss, 1995). According to Greenwood (1961) diffusion of gases in water-filled soil pores is in average 10,000 times slower than diffusion in gas-filled soil pores. When a soil is submerged, gas exchange between soil and air is drastically curtails. After submersion the atmospheric gases can enter to the soil profile only by molecular diffusion in the interstitial water (Ponnamperuma, 1972).

The depth of the water table also has an effect on the microbial community. Jaatinen et al. (2008) observed an increase in microbial biomass with the decrease in the water table, accompanied with changes in species level.

Wetlands contain about 12% of the global carbon pool, playing an important role in the global carbon cycle (IPCC 2014). Large areas of hydric soils have been impacted by agricultural conversion, drainage or climate change. Restoring degraded hydric soils and ecosystems and conservation of existing wetlands has a high potential for sequestrating soil carbon. The most important steps for restoration and conservation of the wetlands are the reduction of stressors causes by human activities which can increase the resiliency of habitats and species to the effects of climate change and variability (Erwin, 2009), sustaining convenient species composition, ceasing agriculture and re-establishment of wetland hydrology (Rosenthal, 2003) by raising water table above the crucial when respiration becomes being limited by water saturation of the soil.

Our results shows that it is very important to include soil water conditions in models estimating CO_2 efflux from wetland ecosystems. The steepest decline of soil CO_2 efflux was observed with the increasing water table above the soil depth 3 cm. Therefore, this water table depth is crucial for soil CO_2 efflux models at this site as CO_2 production in the soil profile begins quickly “switching off” due to anaerobic soil conditions.

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REFERENCES

[1] Alm, J., Shurpali, N.J., Tuittila, E.-S., Laurila, T., Maljanen, M., Saarnio, S., Minkkinen, K. (2007): Methods for determining emission factors for the use of peat and peatlands – flux measurements and modelling. - Boreal Environment Research 12: 85–100.

[2] Altor, A.E., Mitsch, W.J. (2008): Methane and carbon dioxide dynamics in wetland mesocosms: Effects of hydrology and soils. - Ecological Applications 18: 1307–1320.

[3] Aubinet, M., Papale, D., Vesala, T. (2012): Eddy Covariance: A Practical Guide to Measurement and Data Analysis. – Springer, Dordrecht.

[4] Baldocchi, D.D. (2003): Assessing the eddy covariance technique for evaluating carbon dioxide exchange rates of ecosystems: past, present and future. Review. - Global Change Biology 9: 479– 492.

[5] Bonnett, S.A.F., Ostle, N., Freeman, C. (2006). Seasonal variations in decomposition processes in a valley-bottom riparian peatland. - Science of the Total Environment 370: 561–573.

[6] Chamindu Deepagoda, T.K.K., Elberling, B. (2015): Characterization of diffusivity-based oxygen transport in Arctic organic soil. - European Journal of Soil Science 66: 983–991.

[7] Chimner, R.A., Cooper, D.J. (2003). Influence of water table levels on CO_2 emissions in a Colorado subalpine fen: an in situ microcosm study. - Soil Biology & Biochemistry 35: 345–351.

[8] Bubier, J.L., Crill, P.M., Moore, T.R., Savage, K., Varner, R.K. (1998): Seasonal patterns and controls on net ecosystem CO_2 exchange in a boreal peatland complex. - Global Biogeochemical Cycles 12: 703–714.

[9] Davidson, E.A., Janssens, I.A., Luo, Y.Q. (2006): On the variability of respiration in terrestrial ecosystems: moving beyond Q_{10}. - Global Change Biology 12: 154–164.

[10] Dusek, J., Cizkova H., Czerny, R. Taufarova, K., Smidova, M., Janous, D. (2009): Influence of summer flood on the net ecosystem exchange of CO2 in a temperate sedge-grass marsh. - Agriculture and Forest Meteorology 149: 1524–1530.

[11] Dusek, J., Cizkova, H., Stellner, S., Czerny, R., Kvet, J. (2012a): Fluctuating water table affects gross ecosystem production and gross radiation use efficiency in a sedge-grass marsh. - Hydrobiologia 692: 57–66.

[12] Dusek, J., Stellner, S., Komarek, A. (2012b): Long-term air temperature changes in a Central European sedge-grass marsh. - Ecohydrology DOI: 10.1002/eco.1256.

[13] Erwin, K L. (2009): Wetlands and global climate change: the role of wetland restoration in a changing world. - Wetlands Ecology and Management 17: 71–84.

[14] Gazda, J. (1983): Vývoj, struktura a primární produkce metlicových luk (Stellario-Deschampsietum Freitag 1957) na Mokrých loukách u Třeboně (Development, structure and primary production of the tufted hair-grass meadows (Stellario-Deschampsietum Freitag 1957) in the “Wet Meadows” near Třeboň). – In: Jeník, J., Květ, J. (eds.) Studie zaplavovaných ekosystémů u Třeboně, Studie ČSAV 1983/4. Academia: Praha: 88–92.

[15] Goulden, M.L., Munger, J.W., Fan, S.M., Daube, B.C., Wofsy, S.C. (1996). Exchange of carbon dioxide by a deciduous forest: response of interannual climate variability. - Science 271: 1576–1578.

[16] Greenwood, D. J. (1961): The effect of oxygen concentration on the decomposition of organic materials in soil. - Plant and Soil 14: 360–376.

[17] Hogg, E.H., Lieffers, V.J., Wein, R.W. (1992): Potential carbon losses from peat profiles - effects of temperature, drought cycles, and fire. - Ecological Applications 2: 298–306.

[18] Holubickova, B. (1959): Příspěvek ke studiu rašeliništní vegetace. I. Mokré louky u Třeboně (A contribution to the study of moorland vegetation. I. Mokré louky near Třeboň). - Sborník Vysoké Školy Zemědělské v Praze: 257–285.

[19] Inglett, P. W., Reddy, K. R., Corstanje R. (2005): Anaerobic Soils. - In: Hillel, D. (ed.) Encyclopedia of Soils in the Environment. Academic Press. 72–78.

[20] Inglett, K.S., Inglett, P.W., Reddy, K.R., Osborne, T.Z. (2012): Temperature sensitivity of greenhouse gas production in wetland soils of different vegetation. -Biogeochemistry 108: 77–90.

[21] IPCC (2014). Climate change 2014: synthesis report. A contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change (Core Writing Team, Pachauri R.K., Meyer L.A., eds.). – IPCC, Geneva, Switzerland, pp. 151.

[22] Jaatinen, K., Laiho, R., Vuorenmaa, A., del Castillo, U., Minkkinen, K., Pennanen, T., Penttilä, T., Fritze, H. (2008): Responses of aerobic microbial communities and soil respiration to water-level drawdown in a northern boreal fen. - Environmental Microbiology 10: 339–353.

[23] Jassal, R., Black, A., Novak, M., Morgenstern, K., Nesic, Z., Gaumont-Guay, D. (2005). Relationship between soil CO_2 concentrations and forest-floor CO_2 effluxes. - Agricultural and Forest Meteorology 130: 176–192.

[24] Jenik, .J, Květ, J. (1983): Studie zaplavovaných ekosystémů u Třeboně (Ecological study of inundated ecosystems near Třeboň, South Bohemia, Czechoslovakia). – Studie ČSAV 1983/4, Academia, Praha.

[25] Jenik, J., Kurka, R., Husak, S. (2002): Wetlands of the Třeboň basin biosphere reserve in the Central European context. – In: Květ, J., Jeník, J., Soukupová, L. (eds.) Freshwater Wetlands and Their Sustainable Future. A Case Study of the Třeboň Basin Biosphere Reserve, UNESCO, Paris and The Parthenon Publishing Group: Boca Rayton: 11–18.

[26] Jimenez, K.L., Starr, G., Staudhammer, C.L., Schedlbauer, J.L., Loescher, H.W., Malone, S.L., Oberbauer, S.F. (2012): Carbon dioxide exchange rates from short- and long-hydroperiod Everglades freshwater marsh. - Journal of Geophysical Research-Biogeosciences 117: DOI: 10.1029/2012JG002117.

[27] Knorr, K.H., Oosterwoud, M.R., Blodau, C. (2008): Experimental drought alters rates of soil respiration and methanogenesis but not carbon exchange in soil of a temperate fen. - Soil Biology & Biochemistry 40: 1781–1791.

[28] Kutsch, W., Bahn, M., Heinemeyer, A. (2009): Soil Carbon Dynamics. Cambridge – University Press, Cambridge.

[29] Kuzyakov, Y. (2006): Sources of CO_2 efflux from soil and review of partioning methods. - Soil Biology & Boichemistry 38: 425–448.

[30] Květ, J., Jeník, J., Soukupová, L. (2002): Freshwater Wetlands and Their Sustainable Future. A Case Study of the Třeboň Basin Biosphere Reserve, Czech Republic. – The Parthenon Publishing Group. Paris.

[31] Lafleur, P.M., Moore, T.R., Roulet, N.T., Frolking, S. (2005): Ecosystem respiration in a cool temperate bog depends on peat temperature but not water table. - Ecosystems 8: 619–629.

[32] Livingston, G.P., Hutchinson, G.L. (1995): Enclosure--based measurement of trace gas exchange: applications and sources of error. - In: Matson P.A., Harriss, R.C. (eds.) Biogenic Trace Gases: Measuring Emissions from Soil and Water. Cambridge, Blackwell Science: 14–50.

[33] Lloyd, C.R. (2006): Annual carbon balance of a managed wetland meadow in the Somerset Levels, UK. - Agricultural and Forest Meteorology 138: 168–179.

[34] Lloyd, J., Taylor, J.A. (1994): On the temperature dependence of soil respiration. - Functional Ecology 8: 315–323.

[35] Longdoz, B., Yernaux, M., Aubinet, M. (2000): Soil CO_2 efflux measurements in a mixed forest: impact of chamber distances, spatial variability and seasonal evolution. - Global Change Biology 6: 907–917.

[36] Matson, P.A., Harriss, R.C. (1995): Biogenic trace gases: measuring emissions from soil and water. – Blackwell Science, Oxford.

[37] Oechel, W.C., Vourlitis, G.L., Hastings, S.J., Ault, R.P., Bryant, P. (1998). The effects of water table manipulation and elevated temperature on the net CO_2 flux of wet sedge tundra ecosystems. - Global Change Biology 4: 77–90.

[38] Pavelka, M., Acosta, M., Marek, M.V., Kutsch, W., Janous, D. (2007): Dependence of the Q_{10} values on the depth of the soil temperature measuring point. - Plant and Soil 292: 171–179.

[39] Phillips, C.L., Nickerson, N., Risk, D., Bond, B.J. (2011): Interpreting diel hysteresis between soil respiration and temperature. - Global Change Biology 17: 515–527.

[40] Phillips, S.C., Varner, R.K., Frolking, S., Munger, J.W., Bubier, J.L., Wofsy, S.C., Crill, P.M. (2010): Interannual, seasonal, and diel variation in soil respiration relative to ecosystem respiration at a wetland to upland slope at Harvard Forest. - Journal of Geophysical Research-Biogeosciences 115.

[41] Ponnamperuma, F.N. (1972): The chemistry of submerged soils. – Academic Press New York.

[42] Prach, K., 1993: Vegetational changes in a wet meadow complex, South-Bohemia, Czech Republic. - Folia Geobotänica and Phytotxonomica 28: 1–13.

[43] Prach, K., Soukupova, L. (2002). Alterations in the Wet Meadows vegetation pattern. – In: Kvet, J., Jenik, J., Soukupova, L. (eds.) Freshwater Wetlands and their sustainable future. A Case Study of the Třeboň Basin Biosphere Reserve, Czech Republic. CRC Press, Boca Raton: 243–254.

[44] Prach, K. (2008): Vegetation Changes in a wet meadow complex during the past half-century. - Folia Geobotanica 43: 119–130.

[45] Pumpanen, J., Kolari, P., Ilvesniemi, H., Minkkinen, K., Vesala, T., Niinisto, S., Lohila, A., Larmola, T., Morero, M., Pihlatie, M., Janssens, I.A., Yuste, J.C., Grunzweig, J.M., Reth, S., Subke, J.A., Savage, K., Kutsch, W., Ostreng, G., Ziegler, W., Anthoni, P.M., Lindroth, A., Hari, P. (2004): Comparison of different chamber techniques for measuring soil CO_2 efflux. - Agriculture and Forest Meteorology 123: 159–176.

[46] R Development Core Team (2011): R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org. ISBN 3–900051–07–0.

[47] Reddy, R.R., DeLaune, R.D. (2008): Biogeochemistry of Wetlands: Science and Applications. – CRC Press: Boca Raton, USA.

[48] Reichstein, M., Falge, E., Baldocchi, D., Papale, D., Aubinet, M., Berbigier, P., Bernhofer, C., Buchmann, N., Gilmanov, T., Granier, A., Grunwald, T., Havrankova, K., Ilvesniemi, H., Janous, D., Knohl, A., Laurila, T., Lohila, A., Loustau, D., Matteucci, G., Meyers, T., Miglietta, F., Ourcival, J.M., Pumpanen, J., Rambal, S., Rotenberg, E., Sanz, M., Tenhunen, J., Seufert, G., Vaccari, F., Vesala, T., Yakir, D., Valentini, R. (2005): On the separation of net ecosystem exchange into assimilation and ecosystem respiration: review and improved algorithm. - Global Change Biology 11: 1424–1439.

[49] Reth, S., Godecke, M., Falge, E. (2005): CO_2 efflux from agricultural soils in eastern Germany – comparison of a closed chamber system with eddy covariance measurements. - Theoretical and Applied Climatology 80: 105–120.

[50] Riveros-Iregui, D.A., Emanuel, R.E., Muth, D.J., McGlynn B.L., Epstein, H.E., Welsch, D.L., Pacific, V.J., Wraith, J.M. (2007): Diurnal hysteresis between soil CO_2 and soil temperature is controlled by soil water content. - Geophysical Research Letters 34.

[51] Rosenthal, G. (2003): Selecting target species to evaluate the success of wet grassland restoration. - Agriculture Ecosystems & Environment 98: 227–246.

[52] Silvola, J., Alm, J., Ahlholm, U., Nykanen, H., Martikainen, P.J. (1996): CO_2 fluxes from peat in boreal mires under varying temperature and moisture conditions. - Journal of Ecology 84: 219–228.

[53] Sierra, J., Renault, P. (1998): Temporal pattern of oxygen concentration in a hydromorphic soil. - Soil Science Society of America Journal 62: 1398–1405.

[54] Subke, J.A., Bahn, M. (2010): On the ‘temperature sensitivity’ of soil respiration: Can we use the immeasurable to predict the unkonwn? – Soil Biology & Biochemistry 42: 1653–1656.

[55] Taylor, K.E. (2001): Summarizing multiple aspects of model performance in a single diagram. - Journal of Geophysical Research 106: 7183–7192.

[56] Tufekcioglu, A., Raich, J.W., Isenhart, T.M., Schultz, R.C. (1998): Fine root dynamics, coarse root biomass, root distribution, and soil respiration in a multispecies riparian buffer in Central Iowa, USA. - Agroforestry Systems 44: 163–174.

[57] Vicca, S., Fivez, L., Kockelbergh, F., Van Pelt, D., Segers, J.J.R., Meire, P., Ceulemans, R., Janssens,I.A. (2009): No signs of thermal acclimation of heterotrophic respiration from peat soils exposed to different water levels. - Soil Biology & Biochemistry 41: 2014–2016.

[58] Wang, Z.H., Bou-Zeid, E. (2012): A novel approach for the estimation of soil ground heat flux. - Agricultural and Forest Meteorology 154: 214–221.
[59] Xu, L.K., Baldocchi, D.D., Tang, J.W. (2004): How soil moisture, rain pulses, and growth alter the response of ecosystem respiration to temperature. - Global Biogeochemical Cycles 18.
[60] Yang, J., Liu, J., Hu, X., Li, X., Wang, Y., Li, H. (2013): Effect of water table level on CO_2, CH_4 and N_2O emissions in a freshwater marsh of Northeast China. - Soil Biology and Biochemistry 61: 52–60.

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DETERMINING THE ECOLOGICAL FOOTPRINT OF VEHICLES IN TEHRAN, IRAN

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Abstract. Inefficient transport system in big cities such as Tehran causes major environmental problems like increasing fuel consumption, which results in the loss of large amounts of non-renewable natural resources. Increased fuel consumption in Tehran is mainly due to lack of an integrated and efficient transport system, which is one of the main causes of the decline in the country's environmental resources. The aim of this paper is to estimate the ecological footprint of energy in the transportation system within city of Tehran and also estimate the ecological footprint for each of the vehicles per passenger. Finally, some suggestions for improving the sustainability of the transportation system in Tehran will be made. To achieve this goal, the amount of fuel consumption in the transportation sector within city of Tehran was prepared and, based on the carbon release from any type of fuels, energy footprint in 2012 was estimated. Also, fuel consumption per passenger of vehicles was calculated and the ecological footprint per passenger for any type of vehicles was calculated using the ecological footprint model. The results showed that the ecological footprint of energy in the transportation sector in Tehran was 4,384,777.5 gha in 2012, while the total forest land in city of Tehran was equal to 7670.5 ha. So, this ecological deficit showed an unsustainable transport system in Tehran. The results also demonstrated that the subway system, with 0.002 m^2 per passenger, had minimum ecological footprint and private cars with 1.612 m^2per passenger had the highest ecological footprint. This means that each passenger of a private vehicle has footprints equal to 806 times of a subway passenger.

Keywords: *ecological footprint, transportation, Tehran, energy footprint, fuel consumption*

Introduction

In recent decades, the rapid increase in the human population with increasing humanitarian needs has imposed heavy pressures on the limited resources of the earth. Increasing the human population and, consequently, increasing urbanization and city expansion demand more features and needs than the past. In these conditions, lack of proper planning in the development of the infrastructure of the city puts the sustainability of town and environment at risk. One of the main problems of cities is transportation system and, in city of Tehran, problems resulting from this sector can be clearly noticed (Faryadi and Samadpoor, 2010). Population growth of Tehran increases daily urban trips. Rise of daily trips has environmental consequences such as increasing fuel consumption, loss of national natural resources, and air pollution, reducing quality of urban environment, and ultimately, threatening health and welfare of citizens. Now, due to the effects of transportation and heavy traffic in Tehran, it is essential to comprehensively assess environmental quality and provide suitable transport methods to reduce the harmful effects. For this purpose, the ecological footprint can be used as a

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quantitative and comprehensive method for determining the quality of urban environment. The concept of the ecological footprint, for the first time, was introduced by William Rees and Mathis Wackernagel in 1996 at University of British Columbia, Canada. The ecological footprint reflects the amount of consumption or demand for natural goods and services. Ecological footprint is equivalent to the amount of land or water surface, which provides the consumption needs of the community or absorbs the produced waste by them. In other words, the ecological footprint reflects the effects that societies put on the nature due to their life style (Peters and Schouten, 2010). Innovation of ecological footprint method is that, unlike traditional methods, environmental impact assessment is not entirely qualitative and, in this method, there is a factual basis for decision-making, which makes the amount of consumption to the "earth" (Sarayiand Zarei, 2009). Therefore, in the ecological footprint method, evaluation and comparison of the consumption amount of environmental resources have been used in cities and urban neighborhoods (Samadpoor and Faryadi, 2008).

Review of Literature

In recent years, ecological footprint has been used as a tool for assessing the urban and environmental resources, in different countries. Including, Philip Woods in 2004, calculated amount of ecological footprint for the north shores of Sydney (Woods, 2005).

Mark Anielski and Jeffrey Wilson in 2005 calculated ecological footprint of Canada and its various cities including Vancouver, Calgary, Edmonton and other cities and then compared with the ecological footprint of other countries. (Wilson and Anielski, 2005).

Footprint of transportation in the United States of America, Considering all kinds of vehicles ranging from private cars and trucks, motorcycles and buses, rail and air transport and passenger boats, and regarding this point that how much CO2 is released to atmosphere for consuming per KWH of each fuel, was estimated at 1.94 global hectares (McIntyre et al., 2007).

In Great Britain in 2003, with regard to the water, air and rail transport , metro, buses, cars, motorbikes and scooters, and regarding infrastructures, ecological footprint of transportation of 0.67 Global hectares was calculated (Barret and Simmons, 2003).

Footprint of transportation in the city of Adelaide in Australia with regard to the use of private vehicles like cars and trucks, motorcycles, buses, rail and air transport and passenger boats was calculated as 0.66 Global hectares per capita (Agrawal et al., 2006).

In the city of Kermanshah in western Iran, considering the amount of diesel and gasoline consumption by public and private vehicles such as buses, minibuses and cars and motorcycles, the ecological footprint of transportation was estimated at 0.32 hectares (Gharakhluo et al., 2013).

In this study, we used the ecological footprint model to calculate the energy footprint in the transportation sector in city of Tehran. Also, we calculated the energy footprint of each of the vehicles per passenger and, finally, based on the results, recommendations were made for improving the sustainability of the transportation system in Tehran.

Materials and Methods

Studied area

The studied area waa city of Tehran, the capital city of Islamic Republic of Iran (*Fig. 1*) and calculations were done based on data related to 2012 (the latest available data). Tehran's population in 2012 was equal to 8.25 million people. In 2012, area of Tehran was equal to 751 km^2 and population density was 112 persons per hectare (Transport and traffic studies Company of Tehran, 2012).

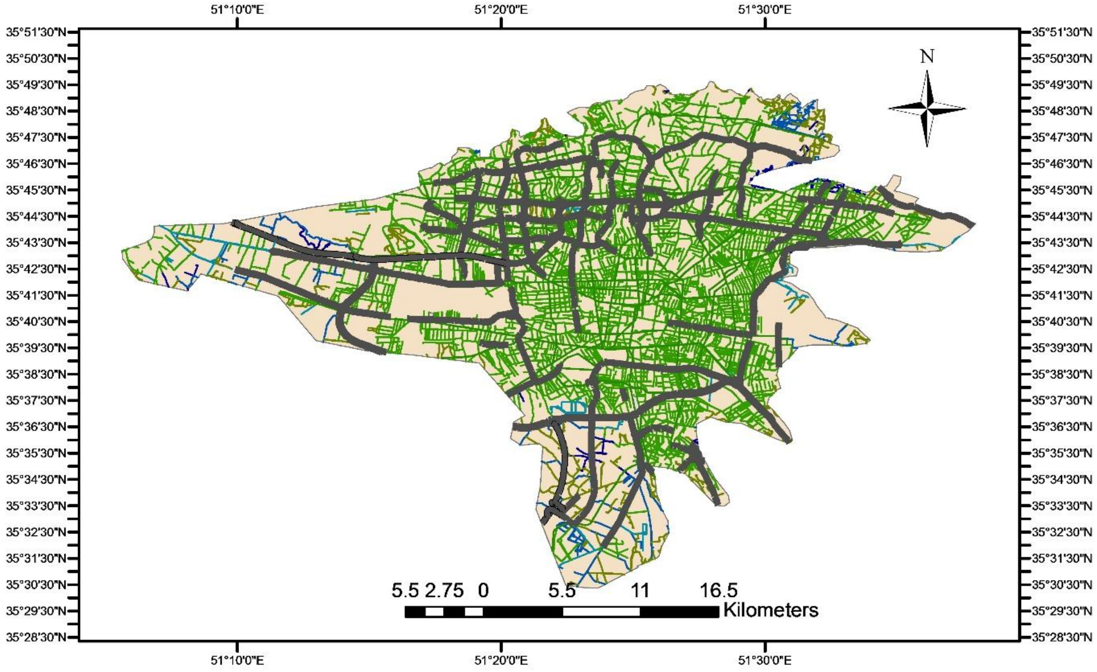

Figure 1. *Studied area, Tehran (Tehran Municipality, 2012)*

In Tehran, along with demographic changes, the transportation situation has changed as well. Changes in the city's population and transport indices, over the period 2010 to 2013, are shown in *Table 1.*

Table 1. *Changes in the city's population and transport indices over the period 2010 to 2013.*

Year	2010	2011	2012	2013
Population (million)	8.1	8.15	8.25	8.6
Number of daily travel (million)	16.6	17	17.4	17.7
Per capita car ownership	0.25	0.36	0.37	0.38
Use of public transport rate (percent)	50.6	54.7	56	56.1
Petrol consumption (million liters)	3577	3538	3641	3736
Diesel consumption (million liters)	950	1093	1337	1411
CNG consumption (million cubic meters)			485	485

(Source: Transportation and Traffic Studies Company of Tehran, 2012)

Transportation and movement in Tehran are done using various vehicles such as buses, minibuses, taxis, subway, private cars, etc. The total number of daily trips in

Tehran in 2012 was equal to 17.4 million daily travel. Distribution of daily trips and the share of each of the vehicles in passenger transportation in Tehran are shown in *Table 2*.

Table 2. *Distribution of daily trips in Tehran in 2012*

System	Type of vehicle	Total daily trips		Movement Coefficient	Daily movement	
		Count (million)	Percentage		Count (million)	Percentage
Private	Motorcycle	1.044	6	1	1.044	4.7
	Private car	6.8556	39.4	1	6.8556	30.95
Public	Subway	2.2272	12.8	1.5	3.3408	15
	Bus	3.0798	17.7	1.5	4.6197	20.85
	Minibus	0.174	1	1.8	0.261	1.3
Semipublic	Taxi	4.0194	23.1	1.6	6.0291	27.2
Total		17.4	100		22.1505	100

(Source: Transportation and Traffic Studies Company of Tehran, 2012)

Also, types of fuels used in the transport sector in Tehran and the amount of each are provided in *Table 3*.

Table 3. *Fuel consumption in the transport sector in Tehran in 2012*

Fuel type	Annual consumption
CNG (cft)	17118
Diesel (gallons)	461868
Petrol (gallons)	353208464
Electricity (GW)	370.9

(Source: Transportation and Traffic Studies Company of Tehran, 2012)

Analysis Method

Estimating ecological footprint of energy in the transport sector

To calculate the ecological footprint of energy (land area required to absorb or sequester CO_2 emitted from burning fossil fuels used in urban transport), the footprint of each fuel is regarded separately because of the differences in the rate of CO_2 emissions per volume unit of each fuel (Ebadi et al, 2015; Shayesteh et al, 2014). In addition to the fuel consumed by vehicles traveling along a network, energy consumed in the process of network construction and annual road maintenance must be also reflected in the total transportation network footprint (Chi and Stone, 2005). Wackernagel and Rees (1996) estimated indirect carbon emissions for road construction and maintenance as equivalent to 45% of the total annual fuel consumed for vehicle travel (Shayesteh et al, 2014). To estimate the energy footprint, the amount of carbon emissions in the construction and maintenance of networks should be also added to the carbon produced by all types of vehicles (Zamba and Hadjibros, 2007). This estimate is multiplied by a carbon equivalence factor to estimate the area of forestland required to absorb the CO_2 emitted from fuel consumption in the studied area (Wiedmann and Lenzen, 2007). The equivalence factor for energy footprint is 1.26 (Ewing et al, 2010).

Footprint is calculated for each of the used fossil fuels and electricity in transport network as follows:

I. Diesel

Diesel fuel will produce approximately 138700 BTU per gallon which finally releases 19.95 ton of carbon per billion BTU (Pezzetta and Drossman, 2005) and each hectare of forestland would absorb 1.8 ton of carbon. So, the ecological footprints of diesel fuel are estimated using Equation 1:

$$EF(d) = \frac{YC \times 138700 \times 19.95 \times 1.26}{1800000000} \quad \text{(Eq. 1)}$$

EF (d) = Diesel EF
YC = Yearly consumption (gallon)

II. Petrol

Petrol would produce approximately 125000 BTU per gallon which finally releases 19.35 ton of carbon per billion BTU (Pezzetta and Drossman, 2005) and each hectare of forestland would absorb 1.8 ton of carbon. So, the ecological footprints of petrol p are estimated using Equation 2:

$$EF(P) = \frac{YC \times 125000 \times 19.35 \times 1.26}{1800000000} \quad \text{(Eq. 2)}$$

EF (g) = Petrol EF
YC = Yearly consumption (gallon)

III. CNG

Per cubic foot of CNG, there is an amount of 0.0532 kg CO_2 (Climate Leaders - EPA, USA, 2012). Each hectare of forestland would absorb 1.8 ton of carbon. So, the ecological footprints of CNG are estimated using Equation 3:

$$EF(CNG) = \frac{YC \times 0.0532 \times 1.26}{1.8} \quad \text{(Eq. 3)}$$

EF (CNG) = CNG EF
YC = Yearly consumption (cft)

IV. Electricity

To calculate CO_2 emissions from electricity consumption, we must first determine the KJ amount in a certain amount of kWh. Then, the amount of coal used to produce kJ electricity consumption should be calculated (amount of coal needed to produce 20 kJ of electricity, equal to 1 g). Finally, admitting the fact that there is 85% carbon in coal, it is possible to estimate the released CO_2 (Gharakhluo et al, 2013).

Estimating per capita ecological footprint of each vehicle

In this study, the ecological footprint methods used in Colorado College (Pezzetta and Drossman, 2005) were modified and adapted to carry out the study. Thus, according to the daily consumption of fuels, the number of daily trips and number of people displaced ecological footprint of vehicles were estimate in m^2. The amount of fuel consumption per vehicle for transporting a passenger is used to calculate the ecological footprint of each vehicles (*Table 4*).

Table 4. *Amount of fuel consumed to transport a passenger-kilometer by a variety of vehicles*

Description	Private		Public and semipublic		
	Car	Motorcycle	Taxi	Minibus	Bus
Amount of fuel consumed to transport a passenger-kilometer(liter)	0.097	0.046	0.083	0.025	0.011

(Source: Transportation and Traffic Studies Company of Tehran, 2012)

Also, based on data obtained from Transportation and Traffic Studies Company of Tehran, distance traveled per trip for a variety of motor vehicles is 4.7 km and, for the subway, it is 12 km.

Therefore, footprint is calculated for each of the vehicles in transport network as follows:

i. Given that several movements take place on each trip, the number of daily trips for each vehicle is multiplied in movement coefficient to obtain the number of movements (*Table 2*), therefore:

$$A = \frac{B}{C} \tag{Eq. 4}$$

A = Share of a passenger of all daily trips
B = Number of daily trips done by each vehicle
C = Number of daily movement done by each vehicle

ii. Now, with regard to the amount of distance traveled by each vehicle per trip, the distance traveled by each passenger per day is calculated according to the following formula:

$$D = A \times F \tag{Eq. 5}$$

D = Distance traveled by each passenger per day
A = Share of a passenger of all daily trips

iii. Amount of fuel consumption per passenger per kilometer is calculated according to the following formula:

$$G = D \times H \tag{Eq. 6}$$

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G = Fuel consumption per passenger per kilometer
D = Distance traveled by each passenger per day
H = Fuel consumption for movement of a passenger with a variety of vehicles

iv. Finally, per capita ecological footprint is calculated for each vehicle.

The methodology of this study is shown in the *Diagram 1*.

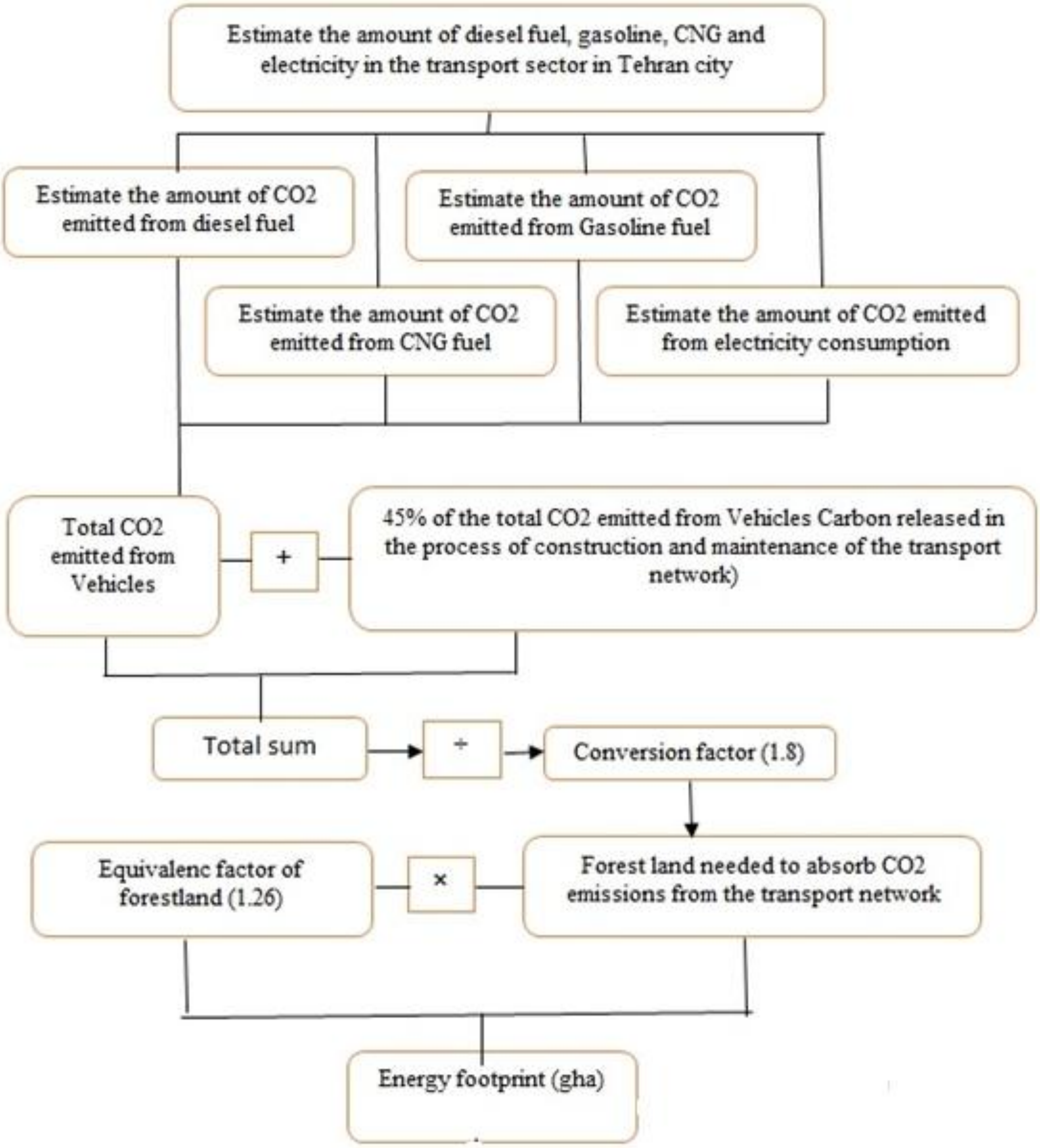

Diagram 1. *The method of calculating the ecological footprint of energy in urban transport system*

Results

Total ecological footprint of energy

To calculate the energy footprint in the transport sector, the ecological footprint was calculated for each type of fuels and then they were combined. Then energy footprint for road construction and maintenance (45% of the carbon released from the consumption of all fuels) was also added.

Ecological footprint of each type of fuel used in transportation sector and energy footprint is provided in *Table 5* and *Figure 1*.

Table 5. *Ecological footprint of each type of fuels used in transportation sector in Tehran in 2012*

Type of fuel	Petrol	Diesel	CNG	Electricity	Fuel used in construction and maintenance of roads	Total
	1628581.122	684145.476	624011	126507.6	1321532.3	4384777.5

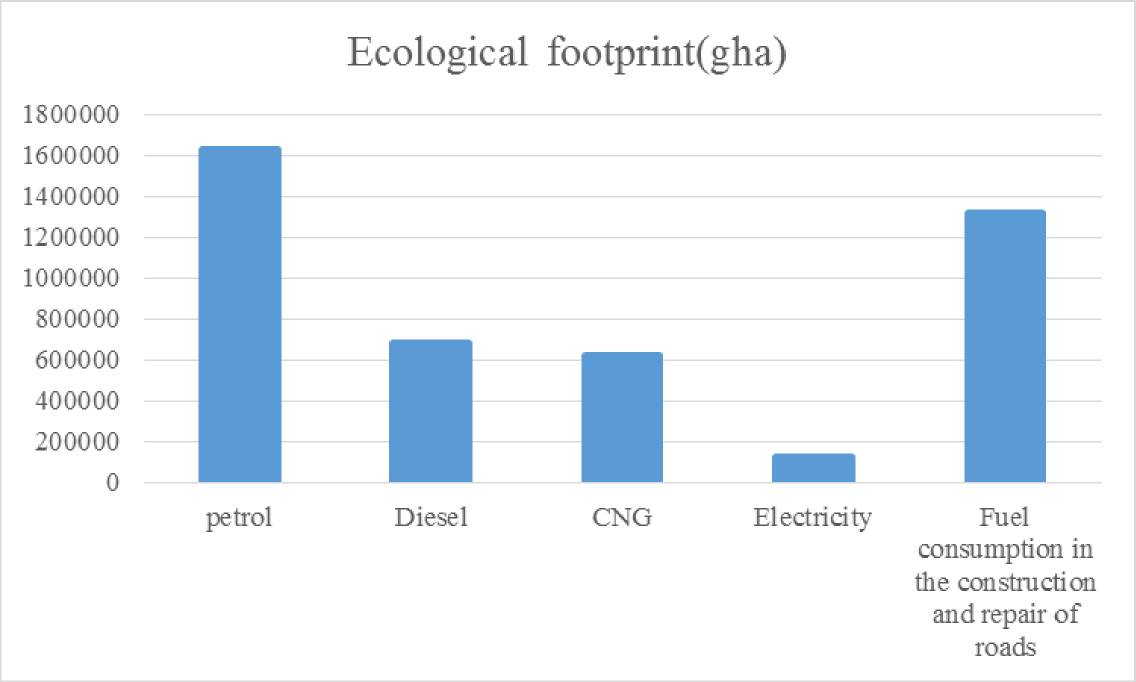

Figure 1. *Ecological footprint of each type of fuels used in transportation sector in Tehran in 2012*

Ecological footprint of energy of each vehicle per passenger

Ecological footprint of energy per passenger to each of the vehicles is provided in *Table 6* and *Figure 2*. It should be noted that the type of fuel consumed by buses and minibuses is diesel and that of taxis, motorcycles, and private cars is petrol. Also, in this section, to calculate the per capita ecological footprint subway, annual electricity consumption was divided by the number of annual movements and per capita annual

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consumption was calculated. Then, ecological footprint was calculated per capita electricity consumption.

Table 6. *Per capita ecological footprint for each vehicle in city of Tehran in 2012*

Type of vehicle	Bus	Minibus	Taxi	Subway	Motorcycle	Private car
A	0.66	0.66	0.66		1	1
D	3.102	3.102	3.102		4.7	4.7
G	0.009 (gallon)	0.020 (gallon)	0.068 (gallon)	0.0008 (kw)	0.057 (gallon	0.12 (gallon)
Per capita ecological footprint (m^2)	0.138(m^2)	0.307(m^2)	0.913(m^2)	0.002(m^2)	0.765(m^2)	1.612(m^2)

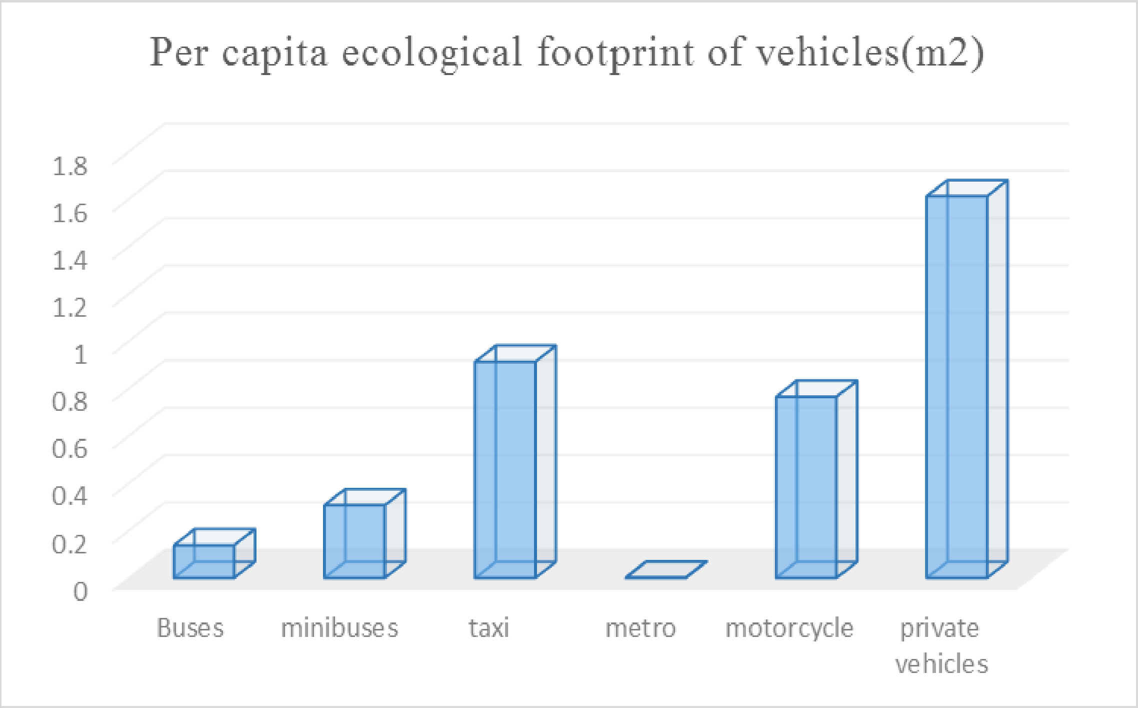

Figure 2. *Per capita ecological footprint for each of the vehicles in city of Tehran in 2012*

Discussion and Conclusion

A prerequisite for sustainable development in transportation system is the creation of green spaces to absorb the carbon dioxide produced in this sector. The results of this study showed that the ecological footprint of energy in transportation system in Tehran was equal to 4,384,777.5 gha. This means that Tehran should has 4,384,777.5 ha forest to absorb carbon dioxide produced in the transportation sector; however, the whole forestland in Tehran is equal to 7670.5 ha. In other words, per capita ecological footprint of energy in the transportation system in Tehran equaled 5315 m^2, but per capita forestland in Tehran was equal to 9.2 m^2, representing the intense unsustainable development in transportation system in Tehran. Thus, Tehran has a deficit of per capita forestland required to absorb CO_2 emissions from transportation sector with the value of 5305.8 m^2 and could not absorb and isolate the released CO_2 in transportation sector. On the other hand, Tehran area equal to 751 square kilometers and forestland needed to absorb carbon dioxide released in the transportation sector is 58 times the size of Tehran. This estimation is comparable to other findings like the corresponding estimation for Oslo which is about 22.5 (Aall and Norland, 2002) and estimation for Isphahan which is about 18 (Shayesteh et al, 2014). Therefore, this pollution will disperse in Tehran and the surrounding areas, which causes many environmental problems.

Also, according to the results, it can be concluded that use of the subway system has minimum damaging effects on environmental resources and use of private cars has the most damaging effects on environmental resources. In other words, in the subway system for the movement of a passenger, 0.0008 kW electricity will be used and, to provide this amount of electricity, 0.002 m^2 natural resources (land) is required. However, when using a private car, for every passenger, 0.12 (gallon) of fuel (petrol) is used and, to supply this amount of petrol, 1.612 m^2 natural resources (land) is required. As a result, use of a private car results in the faster loss of environmental resources. With a look at the results, we can conclude that, if environmental degradation (erosion of resources caused by fuel consumption) caused by a subway passenger is assumed equal to 1 unit, so environmental degradation caused by any one passenger of buses, minibuses, taxis, motorcycles, and private cars will be equal to 69, 185, 456, 382, and 806 unit, respectively.

In a same study, in the city of Urmia in Iran, also private cars had the highest ecological footprint (Habibi et al, 2013) and the results of these two studies support each other.

Based on the above discussion, planning to increase sustainability in transportation sector in Tehran should be a priority for policy-makers.

Suggestions

1) Reducing the use of private cars by applying techniques such as increasing fuel prices, parking cost, area and odd-even traffic control schemes, and expanding culture and education at all levels
2) Increasing satisfactions with using public transportation by techniques such as improving internal and external conditions of the public transport system, reduced latency, reduced waiting time, increased speed and regularity of movement, and creation of special lines by adopting new management practices
3) Using natural gas instead of gasoline and use of hybrid vehicles to optimize fuel consumption
4) Reducing gasoline consumption by gas consuming appliances
5) Restoring and improving urban transport fleet to cope with urban pollution
6) Replacing old cars with a suitable schedule
7) Reducing demand for urban trips with the expansion of the Internet, IT technology, and electronic systems

REFERENCES

[1] Aall, C., Norland, T.I. (2002): The Ecological Footprint of the City of Oslo. - Centre for Development and the Environment & University of Oslo.

[2] Agrawal, M., Boland, J., Filar, J. (2006): The Ecological Footprint of Adelaide City. - Center for industrial and applied mathematics institute of sustainable systems and technologies, University of South Australia Mawson Lakes, 26 p.

[3] Barrett, J., Simmons, C. (2003): An Ecological Footprint of the UK: Providing a Tool to Measure the Sustainability of Local Authorities. - Stockholm Environment Institute – York, 81p.

[4] Chi, G., Stone, B. (2005): Sustainable Transport Planning: Estimating the Ecological Footprint of Vehicle Travel in Future Years. - J. Urban Planning and Development 131: 170-180.

[5] Climate Leaders - EPA, USA. (2012): Cross-Sector Guidance: Direct Emissions from Mobile Combustion Source. Retrieved January 10, 2012, from Center for Corporate Climate, https://www.epa.gov/climateleadership/center-corporate-climate-leadership-greenhouse-gas-inventory-guidance#Cross-Sector

[6] Ebadi, A., Moharram Nejad, N., Rahnamaee, M., Zarandi, S. (2015): Evaluating Sustainable Transportation of Tehran Applying Ecological Footprint Model. – Journal of Pure and Applied Microbiology 9: 495-504.

[7] Ewing, B.D., Moore, S., Goldfinger, A., Oursler, A., Reed, A., Wackernagel, M. (2010): The Ecological Footprint Atlas 2010. - Oakland: Global Footprint Network, 113p.

[8] Faryadi, S.H., Samadpoor, P. (2010): Determining the optimal fit using a variety of transportation methods to reduce the ecological footprint in Tehran. - Journal of Environmental Studies 54: 97-108. (In Persian).

[9] Gharakhluo, M., Hataminejad, H., Baghvand, M., Yalve, M. (2013): Urban Sustainable Development Assessment with Regard to Footprint Ecological Method (Case Study: Kermanshah City). – Journal of Human Geography Research Quarterly 45(2):105-120. (In Persian).

[10] Habibi, K., Rahimi, A., Abdi, M.H. (2013): Evaluation the ecological foot print of urban transportation as a novel approach to sustainable transport planning, Case Study: Urmia. - Journal of Planning Geographic Space 5: 99-116.

[11] McIntyre, S., Peters, H.M., Christensen, M., Emmi, P., Martinson, W., Mielke, M., Senbel, M., Stark, D. (2007): The Ecological Footprint of Utah: A Sustainability Indicators Project of the Utah Population and Environment Coalition. - Utah Vital Signs: Salt Lake City, UT, USA, p. 59.

[12] Peters, P., Schouten, F. (2010): Reducing the Ecological Footprint of Inbound Tourism and Transport to Amsterdam. – Journal of Sustainable Tourism 14: 141-156.

[13] Pezzetta, W.E., Drossman, H. (2005): The Ecological Footprint of the Colorado College: An examination of sustainability; http://www.coloradocollege.edu/sustainability/EcoFootprint.pdf.

[14] Samadpoor, P., Faryadi, S.H. (2008): Determination of the ecological footprint in high density areas of cities (Case Study: Elahiyeh Region of Tehran) - Journal of Environmental Studies 45: 63-72. (In Persian).

[15] Sarayi, M.H., Zarei, F. (2009): Ecological footprint (EF) as an indicator of social sustainability. - Journal of Environmental Studies 50: 15-26. (In Persian).

[16] Shayesteh, K., Melhosseini-Darani, K., Ildoromi, A. (2014): Estimating the Ecological Footprint of Transportation in the City of Isphahan (Iran). – Journal of Current World Environment 9(3): 760-767.

[17] Transport and traffic studies Company of Tehran (2012). Selection of Statistics and Information urban transport of Tehran. - Press of Tehran Municipality, Tehran.

[18] Wackernagel, M., Rees, W. (1996): Urban Ecological Footprints: why cities cannot be sustainable-and why they are a key to sustainability. – Journal of Environmental Impact Assessment Review 16: 223-248.

[19] Wiedmann, T., Lenzen, M. (2007): On the Conversion between Local and Global Hectares in Ecological Footprint Analysis. – Journal of Ecological Economics 60: 673-677.

[20] Wilson, J., Anielski, M., (2005): Ecological Footprints of Canadian Municipalities and Regions. - Federation of Canadian Municipalities.

[21] Woods, P. (2005): Ecological Footprint: North Sydney. Discussion paper. - The University of South Wales and Coastal Council Group Partnership Program.

[22] Zamba, A., Hadjibros, K. (2007): Estimating the Ecological Footprint of Vehicles in the City of Athens. - Proceeding of the 10th International Conference on Environmental Science and Technology Kos Island, Greece 5-7 September 2007. 8p.

VARIABILITY IN MACRONUTRIENT COMPOSITION OF WEED SEEDS

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Abstract. The analysis of the element composition of weed seeds and shoots could provide new data for their biological and ecological assessment. In the present study, seed samples of 30 weed species from different plant families were analysed for macronutrients (K, Ca, Mg, N, P, S) and high variability was found. There was a considerable difference in the N content of weed seeds, with the highest value for *Asclepias syriaca* and the lowest for *Xanthium spinosum*. The macroelement ratio also exhibited a wide range of values, e.g. the N to S ratio varied from 0.2 to 14.2. When crop seed data were compared to those of weed seeds from the same family, the K, Ca, Mg, P and S concentrations in weed seeds were found to be significantly higher in many cases. There were significant differences both in the macroelement concentrations and, in some cases, in the nutrient ratios of weed seeds from different plant families. Significant differences were found for K, Mg, P and S, and for the N/S and Ca/P ratios. This was confirmed by PCA, which illustrated not only differences between weed species, but also the fact that weed plants from the same family formed groups.

Keywords: *weed seeds; macroelement composition; plant families; diversity*

Introduction

In agricultural systems there is generally a prolonged time gap between two cropping cycles, which poses a potential risk of nutrient losses. Weeds, however, may preserve residual moisture and early rains, and could also fulfil the task of conserving nutrients during the fallow period (Promsakha Na Sakonnakhon et al., 2006; Lehoczky et al., 2012). Weeds may not only fix nutrients, but may also contribute to the nutrient supply of crops in the following growing season. The composition of plant residues has a great influence on how they decay and what nutrients are released from them. Palm et al. (2001) showed marked differences in the quality of residues from different plant families, e.g. leguminous plants release accumulated N more rapidly. Hence, the composition of the weed plants may have a great impact on nutrient cycling in arable soils. Although numerous studies have been carried out to determine the N content, amino acids and protein content in weed seeds (Friedman and Levin, 1989; Oderinde and Tairu, 1989; Friedman, 1996), limited information is available on the concentrations and ratios of macroelements (K, Ca, Mg, P and S).

Weeds reduce crop yields by competing for water, nutrients, space and light (Lehoczky et al., 1995; Lehoczky et al., 2005; Wang et al., 2007). Losses in crop yield caused by weeds are well documented in many studies (Akobundu, 1987; Swanton et al., 1993; Oerke and Dehne, 2004; Lehoczky et al., 2009; 2013b), leading to the conclusion that there is a need for effective weed management programmes. To

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establish such programmes, accurate information is needed on the weed flora, including the distribution, abundance and phenology of weed species and the biodiversity of weed communities (Frick and Thomas, 1992; Lehoczky et al., 2014a, b). Additionally, these data may also be valuable for understanding the dynamics of weed communities and for creating higher biodiversity on arable land (Andreasen and Skovgaard, 2009).

The uptake and accumulation of nutrients in plants depend on their genetic properties and thus on the species and the family (Singh et al., 2003; Győri et al., 2014). Consequently, it would be worth clarifying how weed families affect macroelement accumulation and revealing differences in the rates of accumulation between families. A systematic assessment of the nutrient concentrations in weed seeds, based on weed families, has not yet been carried out.

Among cereal crops, winter wheat and maize are most affected by summer weeds in Hungary (Ujvárosi, 1973). The cover percentage of the top ten weed species in Hungary was *Ambrosia artemisiifolia* L. (5.3 %), *Echinochloa crus-galli* (L.) P. B. (4.2 %), *Chenopodium album* L. (3.7 %), *Cirsium arvense* (L.) Scop. (1.8 %), *Setaria pumila* (Poir.) Roem. & Schult. (1.6 %), *Convolvulus arvensis* L. (1.5 %), *Amaranthus retroflexus* L. (1.4 %), *Tripleurospermum inodorum* (L.) Sch. Bip. (1.1 %), *Datura stramonium* L. (1.0 %) and *Panicum miliaceum* L. (1.0 %) during the 2007-2008 period (Novák et al., 2009).

The present study investigated the macronutrient composition of 30 seed samples of 30 weed species collected from sites with similar environmental conditions in Hungary. The objectives were (i) to determine the nutrient (N, P, K, Ca, Mg, S) contents in 30 weed seeds from 12 weed families, (ii) to evaluate the potential of weed seeds to conserve nutrients compared to crops, (iii) to show the influence of the weed family on the element contents, and (iv) to evaluate the diversity of weed species based on nutrient content of seeds using principal component analysis.

Materials and methods

Study area

The survey area was on cultivated land in Keszthely (Zala County, Hungary) with an average annual precipitation of 678±134 mm (Debreczeni and Németh, 2009). The soil type was Eutric Cambisol, according to the FAO classification (FAO 1998). The $pH_{(KCl)}$ of the top 30 cm soil layer was 6.8, with 1.9% organic matter content and 0.4% $CaCO_3$ content. The texture of the soil was loam. The most common crops were wheat, maize, barley, rape and sunflower.

Plant sampling procedure

Weed samples were taken from July to the end of October by sampling mature weed plants. In the laboratory, weed seeds were manually separated and ground. Prior to the digestion and ICP analysis, the seed samples was washed with distilled water. The 30 weed species investigated are listed in *Table 1*.

Laboratory analysis

The soil pH was measured in 1:2.5 soil:1 M KCl suspensions 12 hours after mixing (MSZ-08-0206/2:1978). The organic matter content was determined by the modified Walkley-Black method (Walkley and Black, 1934), which involved digesting the soil organic matter with 5% $K_2Cr_2O_7$ and cc. H_2SO_4 and analysing the colour of the suspension, which was related to the organic matter content of the samples, colorimetrically (MSZ-08-0452:1980). Particle-size distribution was determined by the pipette method. The soil:water suspension was mixed in a sedimentation cylinder, then sampled with a pipette to collect particles of a given size (MSZ-08-0215:1978). The $CaCO_3$ content was measured with a calcimeter; the soil was mixed with diluted HCl solution and the volume of CO_2 released was determined (MSZ-08-0206/2:1978).

***Table 1.** The weed species investigated*

No.	Name	EPPO code	Family	Raunkiær's life form[a]	Ujvárosi's life form[b]
1	*Amaranthus albus* L.	AMAAL	Amaranthaceae	Th	T_4
2	*Amaranthus retroflexus* L.	AMARE	Amaranthaceae	Th	T_4
3	*Asclepias syriaca* L.	ASCSY	Asclepiadaceae	G	G_3
4	*Ambrosia artemisiifolia* L.	AMBEL	Asteraceae	Th	T_4
5	*Cirsium arvense* (L.) Scop.	CIRAR	Asteraceae	G	G_3
6	*Erigeron canadensis* L.	ERICA	Asteraceae	Th	T_4
7	*Galinsoga parviflora* Cav.	GASPA	Asteraceae	Th	T_4
8	*Tripleurospermum inodorum* (L.) Sch.Bip.	MATIN	Asteraceae	TH/H	T_4
9	*Senecio vulgaris* L.	SENVU	Asteraceae	Th	T_1
10	*Xanthium spinosum* L.	XANSP	Asteraceae	Th	T_4
11	*Xanthium strumarium* L.	XANST	Asteraceae	Th	T_4
12	*Sinapis arvensis* L.	SINAR	Brassicaceae	Th	T_3
13	*Stellaria media* (L.) Vill.	STEME	Caryophyllaceae	Th	T_1
14	*Chenopodium album* L.	CHEAL	Chenopodiaceae	Th	T_4
15	*Chenopodium hybridum* L.	CHEHY	Chenopodiaceae	Th	T_4
16	*Calystegia sepium* (L.) R. Br.	CALSE	Convolvulaceae	(G)H	G_1
17	*Convolvulus arvensis* L.	CONAR	Convolvulaceae	G	G_3
18	*Abutilon theophrasti* Medic.	ABUTH	Malvaceae	Th	T_4
19	*Veronica hederifolia* L.	VERHE	Scrophulariaceae	Th	T_1
20	*Cynodon dactylon* (L.) Pers.	CYNDA	Poaceae	G(H)	G_1
21	*Echinochloa crus-galli* (L.) P. Beauv.	ECHCG	Poaceae	Th	T_4
22	*Panicum miliaceum* L.	PANMI	Poaceae	Th	T_4
23	*Sorghum halepense* (L.) Pers.	SORHA	Poaceae	G(H)	G_1
24	*Fallopia convolvulus* (L.) Á. Löve	POLCO	Polygonaceae	Th	T_4
25	*Persicaria lapathifolia* (L.) Delarbre	POLLA	Polygonaceae	H	T_4
26	*Polygonum aviculare* L.	POLAV	Polygonaceae	Th	T_4
27	*Persicaria maculosa* Gray	POLPE	Polygonaceae	Th	T_4
28	*Consolida regalis* Gray	CNSRE	Ranunculaceae	Th	T_2
29	*Datura stramonium* L.	DATST	Solanaceae	Th	T_4
30	*Solanum nigrum* L.	SOLNI	Solanaceae	Th	T_4

[a] Th: therophyta; H: hemikryptophyta; G: kryptophyta; TH: hemitherophyta (Raunkiær, 1934).

[b] G_1: Geophyta, rhizomatous plants; G_3: Geophyta, perennials with reproductive roots; T_1: Therophyta, winter annuals, ripen seeds in spring; T_2: Therophyta, winter annuals, ripen seeds in summer; T_3: Therophyta, summer annuals, emergence in spring, ripen in summer; T_4: Therophyta, summer annuals, emergence in summer, ripen in autumn (Ujvárosi, 1973).

The K, Ca, Mg, P and S concentrations in the weed seeds was determined with the ICP-MS method after microwave Teflon bomb digestion with cc. HNO_3 + H_2O_2 (Kovács et al., 1996). The N content of the weed seeds was analysed with an Elementar VarioMax instrument (Hanau, Germany) based on the Dumas combustion method (Jones, 1992).

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Quality control

The high purity water (18.2 MΩ cm^{-1}) used for the preparation of all blanks, standards and sample solutions was obtained from a Millipore water purification system (Millipore, France). The instrument was calibrated using multi-element standard solutions prepared in 1% nitric acid by mixing and diluting AAS stock solutions of individual elements.

For ICP-MS analysis, each sample was analysed in triplicate, using an external standard (BCR CRM 189 wheat) to assure the accuracy of the whole analytical procedure (*Table 2*).

***Table 2.** Results of analysis on certified samples*

Element	Certified value	Measured value
K (mg kg^{-1})	33800±800[a]	32786±2563
Ca (mg kg^{-1})	6400±100	6384±175
Mg (mg kg^{-1})	1450±40	1601±114.2
P (mg kg^{-1})	2360±70	2440±116.8
S (mg kg^{-1})	3160±40	3308±112

[a] mean±standard deviation

Statistical methods

Analysis of variance (ANOVA) was carried out on the element concentrations in the different weed species and families using SPSS 16.0 software. To evaluate the ranking order of macroelement contents in the plant families, the Duncan pot hoc test was performed at $p<0.05$, using only families represented by more than one species.

Principal component analysis (PCA) was used to evaluate the diversity of weeds with respect to nutrient content. PCA is a multivariate technique in which new variables (called principal components or PCs) are calculated as linear combinations of the original variables (nutrient concentrations of weed seeds). The PCA was performed using varimax rotation and the PCA scores were displayed as a biplot diagram, which attempts to represent both the observations and variables of multivariate data in the same plot.

Results and Discussion

Nutrient concentrations and ratios in weed seeds

Both the whole weed plant and its various parts, including the seeds, can be considered as a nutrient pool for the soil and consequently as a nutrient source for crops grown for human or animal consumption. There is a considerable difference in the N content of weed seeds, with the highest value for *Asclepias syriaca* and the lowest for *Xanthium spinosum*. The S content ranged from 1.3 g kg^{-1} in *Persicaria lapathifolia* to 17.8 g kg^{-1} in *Sinapis arvensis*. High variability was found in the K concentration, with the highest value of 20.5 g kg^{-1} in *Erigeron canadensis* and the lowest value of 2.2 g kg^{-1} in *Panicum miliaceum* (*Table 3*). The Ca content ranged from 17.4 g kg^{-1} in *Polygonum aviculare* to only 0.2 g kg^{-1} in *Panicum miliaceum*. In the case of Mg, there was a moderate variability in the concentrations, from 1.3 to 4.0 g kg^{-1}. Five-fold

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differences were revealed for P, with values of 10.2 g kg^{-1} in *Abutilon theophrasti* and 1.9 g kg^{-1} in *Cynodon dactylon*.

With respect to element ratios, the Ca/P ratio ranged from 0.06 to 7.6 in the weed seeds investigated (*Table 3*), with the highest value for *Polygonum aviculare*. The N/S ratio varied from 0.2 to 14.2, with the highest value for *Abutilon theophrasti*.

Table 3. *Nutrient contents and macroelement ratios in the weed seeds investigated*

Code[a]	Family	N	P	K	Ca	Mg	S	N/S	Ca/P
		g kg^{-1}						*ratio*	
AMAAL	Amaranthaceae	23.0	3.4	3.8	3.6	3.2	2.2	10.4	1.0
AMARE	Amaranthaceae	25.7	5.7	5.3	3.4	3.7	2.4	10.9	0.6
ASCSY	Asclepiadaceae	58.5	7.7	7.9	3.5	4.0	4.7	12.6	0.5
AMBEL	Asteraceae	44.6	7.7	9.5	4.1	3.2	4.1	10.9	0.5
CIRAR	Asteraceae	22.5	6.3	6.7	6.2	2.5	2.5	9.0	1.0
ERICA	Asteraceae	31.8	6.3	20.5	12.3	2.7	4.0	7.9	1.9
GASPA	Asteraceae	16.1	4.0	11.1	6.8	2.1	2.4	6.6	1.7
MATIN	Asteraceae	27.0	6.1	11.3	3.2	2.6	2.9	9.2	0.5
SENVU	Asteraceae	39.0	5.9	8.9	7.2	3.2	3.7	10.4	1.2
XANSP	Asteraceae	9.9	2.7	11.8	2.6	1.7	1.7	5.9	1.0
XANST	Asteraceae	31.0	5.6	12.6	1.9	2.4	2.7	11.5	0.3
SINAR	Brassicaceae	45.2	9.9	6.3	5.2	3.5	17.8	2.5	0.5
STEME	Caryophyllaceae	27.7	4.3	3.9	2.6	2.7	2.5	11.0	0.6
CHEAL	Chenopodiaceae	24.9	4.4	8.8	2.1	2.4	2.2	11.2	0.5
CHEHY	Chenopodiaceae	20.6	3.0	11.3	1.6	2.0	2.0	10.1	0.5
CALSE	Convolvulaceae	33.9	6.5	15.7	1.2	2.9	3.0	11.2	0.2
CONAR	Convolvulaceae	34.9	6.3	16.7	1.4	2.7	2.7	13.0	0.2
ABUTH	Malvaceae	35.1	10.2	13.4	1.9	3.6	2.5	14.2	0.2
CYNDA	Poaceae	18.6	1.9	3.5	4.7	2.6	2.2	8.6	2.4
ECHCG	Poaceae	19.3	5.4	5.8	2.2	3.0	2.0	9.9	0.4
PANMI	Poaceae	20.9	3.5	2.2	0.2	1.2	1.9	10.9	0.1
SORHA	Poaceae	17.1	4.4	2.6	0.7	1.9	2.0	8.5	0.2
POLAV	Polygonaceae	24.0	2.3	5.3	17.4	2.1	1.8	13.0	7.7
POLCO	Polygonaceae	19.2	2.8	11.4	4.7	2.7	2.0	9.6	1.7
POLLA	Polygonaceae	16.3	3.2	2.8	1.0	2.0	1.3	12.5	0.3
POLPE	Polygonaceae	17.4	5.1	7.7	1.2	2.2	1.5	11.9	0.2
CNSRE	Ranunculaceae	32.2	6.6	7.2	11.4	2.9	3.4	9.6	1.7
VERHE	Scrophulariaceae	20.1	4.8	5.5	2.2	1.3	2.1	9.6	0.5
DATST	Solanaceae	29.1	5.9	5.4	1.1	3.1	3.2	9.1	0.2
SOLNI	Solanaceae	26.0	8.8	4.1	0.9	3.9	2.6	9.9	0.1

[a] The full name of the weeds are in *Table 1.*

Comparison of weed data to crop seed concentrations

To evaluate the nutrient conservation potential of the weeds, the nutrient content of crop species was compared to that of weed species from the same family. High nutrient contents were determined in the thirty weed seed samples in comparison with crop seed concentrations, as shown in *Table 4*. Differences in the element concentrations have been reported both for crop seeds (White and Broadley, 2009; Lehoczky et al., 2013a) and for weed seeds, due to a combination of environmental and genetic factors.

In the *Poaceae* family the potassium concentration in weed seeds was found to be higher than in the seeds of small grain crops. In experiments in Keszthely (Győri, 2009) maize and wheat grains were found to have lower values of K than those recorded for weed seeds in the present work, despite the differences in species, genotypes and

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environmental conditions. This was particularly true of the very high K value found in the weed seed *Erigeron canadensis* (20.0 g kg^{-1}). Similar conclusions could be drawn for the *Polygonaceae* family, while the rest of the families had comparable K concentrations in weed and crop seeds.

Table 4. *Comparison of the nutrient contents of weed seeds and crop seeds from the same family*

K	Ca	Mg	P	S	Crop	Family
g kg^{-1}						
5.1	0.46	1.5	3.9	1.3	wheat[1]	Poaceae
3.0	0.4	1.5	3.2	1.5	wheat[2]	Poaceae
2.4-3.2	0.01-0.13	0.8-1.6	2.4-4.6	0.97-1.8	maize[3]	Poaceae
3.5±1.6	*1.9±2.0*	*2.2±0.8*	*3.8±1.5*	*2.0±0.1*	*weeds*	Poaceae
15.6±3.6	6.4±2.4	5.3±2.2	4.6±0.7	3.1±0.5	sunflower[4]	Asteraceae
11.6±4.1	*5.5±3.4*	*2.5±0.5*	*5.5±1.5*	*3.0±0.8*	*weeds*	Asteraceae
2.4	0.30	0.92	1.3	0.47	buckwheat[5]	Polygonaceae
0.80-1.3	0.52-0.74	0.50-0.78	1.6-2.4	-	buckwheat[6]	Polygonaceae
6.8±3.7	*6.1±7.7*	*2.2±0.3*	*3.3±1.2*	*1.6±0.3*	*weeds*	Polygonaceae
2.4-6.9	0.01-0.28	0.11-0.38	0.33-1.3	-	potato[7]	Solanaceae
4.1	0.09	0.21	0.62	-	potato[8]	Solanaceae
47±0.9	*1.0±0.1*	*3.5±0.6*	*7.3±2.0*	*2.9±0.4*	*weeds*	Solanaceae
8.2	3.5	2.7	5.7	-	rapeseed[9]	Brassicaceae
6.3	*5.2*	*3.5*	*9.9*	*17.8*	*weed*	Brassicaceae
7.1-7.7	7.4-9.3	4.6-6.8	5.9-6.5	-	amaranth[10]	Amaranthaceae
4.5±1.1	*3.5±0.1*	*3.5±0.4*	*4.6±1.6*	*2.3±0.1*	*weeds*	Amaranthaceae

[a] Mean±standard deviation;

[1] Győri, 2009; [2] Kádár and Daood, 2001; [3] Menkir, 2008; [4] Kötschau et al., 2014; [5] Vogel-Mikuš et al., 2009; [6] Peng et al., 2014; [7] Burlingame et al., 2009; [8] USDA National Nutrient Database, No: 11365; [9] Kádár, 2002; [10] Haghighi et al., 2012.

The Ca concentrations in weed seeds were found to be an order of magnitude higher than those in crop species from the *Poaceae*, *Polygonaceae* and *Solanaceae* families (*Table 4*). In the *Amaranthaceae, Asteraceae* and *Brassicaceae* families, the Ca content was similar in crop and weed seeds. Some weed species were found to have very high Ca concentrations, e.g. *Erigeron canadensis*, *Consolida regalis* and *Polygonum aviculare* gave values of 12.3, 11.4 and 17.4 g kg^{-1} Ca, respectively.

Very little variation was detected in the Mg concentration of the weed seeds, indicating that no weed species have extremely high or low Mg contents. However, in crop seeds, significant differences were recorded in many cases. For instance, the Mg content of weed seeds from the *Poaceae*, *Polygonaceae* and *Solanaceae* families was higher than in crop seeds from the same family.

The average P concentrations in crop seeds ranged from 0.33 to 6.5 g kg^{-1} (*Table 4*). Among the *Solanaceae* the crop species (potato) was found to have a significantly lower P content than the average P concentration of weed seeds from the same family. Especially high P concentrations were determined for *Abutilon theophrasti* (11.1 g kg^{-1}) and *Sinapis arvensis* (9.9 g kg^{-1}).

Consistently with the well-known fact that plants of the *Brassicaceae* family have high S concentration due to the mustard oil content of the seeds (Björkman et al., 2011;

Li et al., 2012), 17.8 g kg^{-1} S was detected in *Sinapis arvensis*. The concentrations in all the weed seeds ranged from 1.4 to 4.6 g kg^{-1}, which was higher than the values recorded for the crop species.

In general, all the macroelements investigated had in higher or at least comparable concentrations in the weed seeds. The Ca concentration in particular was found to be much higher in weed seeds than in crop seeds.

Differences in the element contents and ratios between weed seeds of different families

Among the macroelements significant differences between weed families were found in the concentrations of P, K, Mg and S (*Fig. 1*).

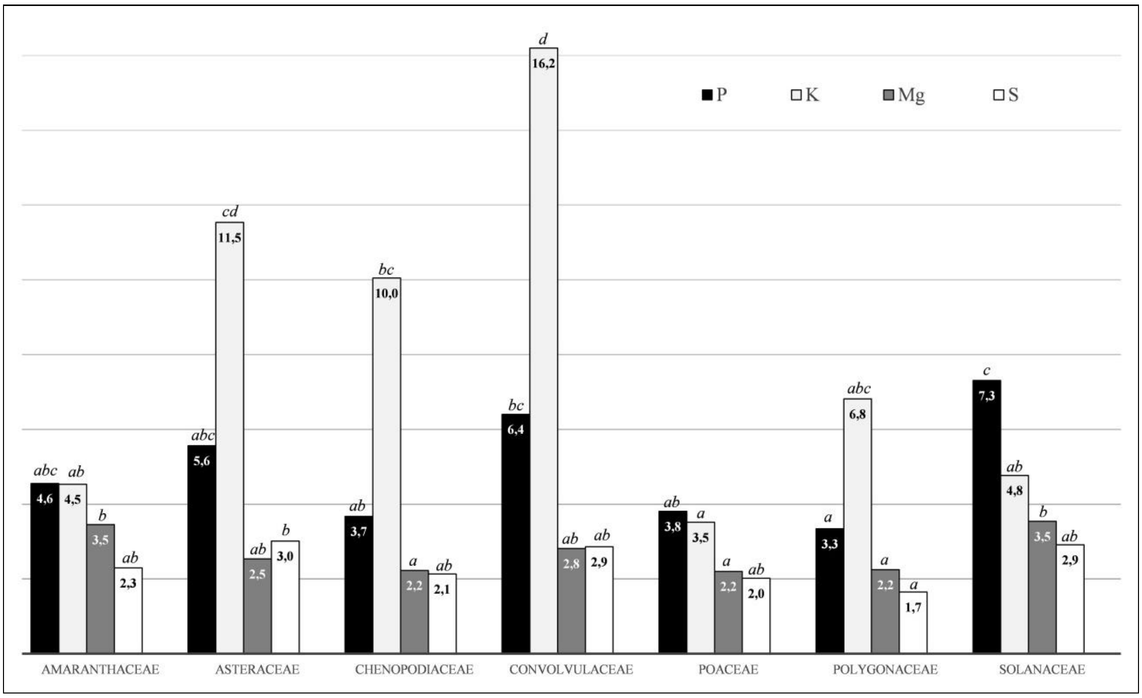

Figure 1. *Macronutrient contents in weed seeds from different families (a–d indicate significant differences within families for the given element at the 5% level of probability according to Duncan's test)*

For K, the following ranking order was found for the weed families from the highest to the lowest K contents: Convolvulaceae > Asteraceae > Chenopodiaceae > Amaranthaceae = Polygonaceae > Poaceae = Solanaceae. Similar orders were found for P and S, with the highest contents in Asteraceae, Convolvulaceae and Solanaceae, and relatively low concentrations in Polygonaceae, Poaceae and Amaranthaceae. For Mg the ranking order was as follows: Solanaceae > Asteraceae = Amaranthaceae = Convolvulaceae > Chenopodiaceae = Poaceae = Polygonaceae.

Other authors investigating the effect of the family on the element concentrations in weed seeds also reported significant differences in most cases (Tanji and Elgharous, 1998). The fact that individual genotypes may differ in their nutrient demands, uptake ability and nutrient transport within the plant is well documented (Ramage and Williams, 2002; Lovkova et al., 2008; White and Brown, 2010).

The main factor affecting the concentrations of different elements in a plant appears to be the nutrient requirements of the plant rather than the nutrient supplies in the soil (Markert, 1989). This was confirmed by surveys showing different concentrations of trace and macro-elements in different plants even if they were grown in the same place (Løbersli and Steinnes, 1988; Willey et al., 2005; Lehoczky et al., 2013b).

Evaluation of diversity based on nutrient content of seeds using PCA

As shown in the previous section, significant variability has been found between weed families with respect to macroelement status. Principal component analysis is another way to reveal the diversity or similarity between weed communities. The factor loadings and score plots obtained using this method are presented for the 30 weed seeds in *Table 5* and *Figures 2A* and *B*.

The first three principal components were found to have eigenvalues > 1 and accounted for 36.6 %, 23.6 % and 17.9 % of total variation, respectively, giving a cumulative variance of 78.1 %.

Table 5. *Factor loadings for the elements analyzed in the weed seed samples*

	PC1	PC2	PC3
N	**0.914**	0.034	0.076
P	**0.858**	-0.260	0.206
K	0.382	0.140	-0.147
Ca	0.117	**0.960**	0.108
Mg	**0.830**	-0.059	0.049
S	0.479	0.020	**0.817**
N/S	0.194	-0.026	**-0.929**
Ca/P	-0.156	**0.952**	-0.070

The first PC (PC1) showed high loadings for N, P and Mg, while PC2 gave high loadings for Ca and the Ca/P ratio and PC3 for S and the N/S ratio (shown in bold in *Table 5*). The high loadings for N and P in PC1 can be attributed to the fact that these elements have similar chemical and consequently biological properties, being non-metal elements. Surprisingly, Mg also made a high contribution to PC1 rather than to PC2, which consists of metal elements, especially Ca.

To visualize the differences and similarities in the nutrient contents of weed species, PC scores were calculated, as displayed on the biplots (*Fig. 2A* and *2B*) illustrating the patterns of nutritional status in weed seeds. The great differences between the weed species are indicated by the scattered dots plotted for the weed plants. The position of the given weed in the factor space shows the nutritional status of the weeds, e.g. the PC3 value is very high for the *Brassiceae* due to the high S content (*Fig. 2B*), while the PC1 score for the *Malvaceae* family is indicative of its high content of N, P and Mg (*Fig. 2A*).

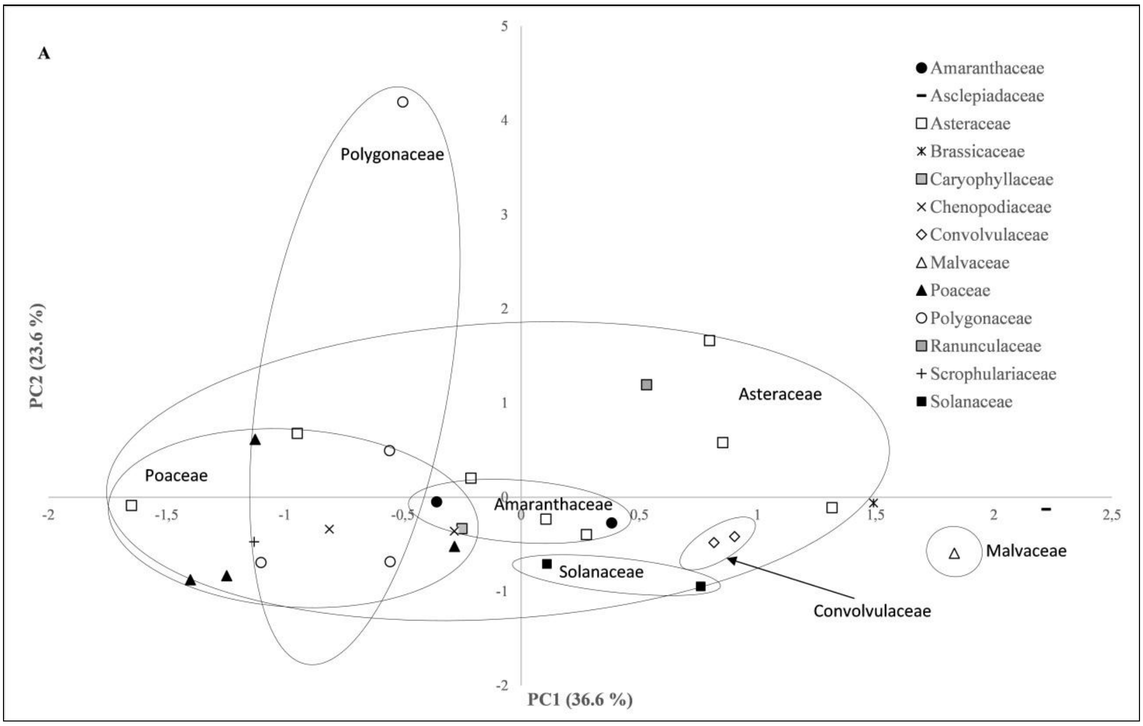

Figure 2A. *PCA ordination of weed species investigated (PC1 vs PC2)*

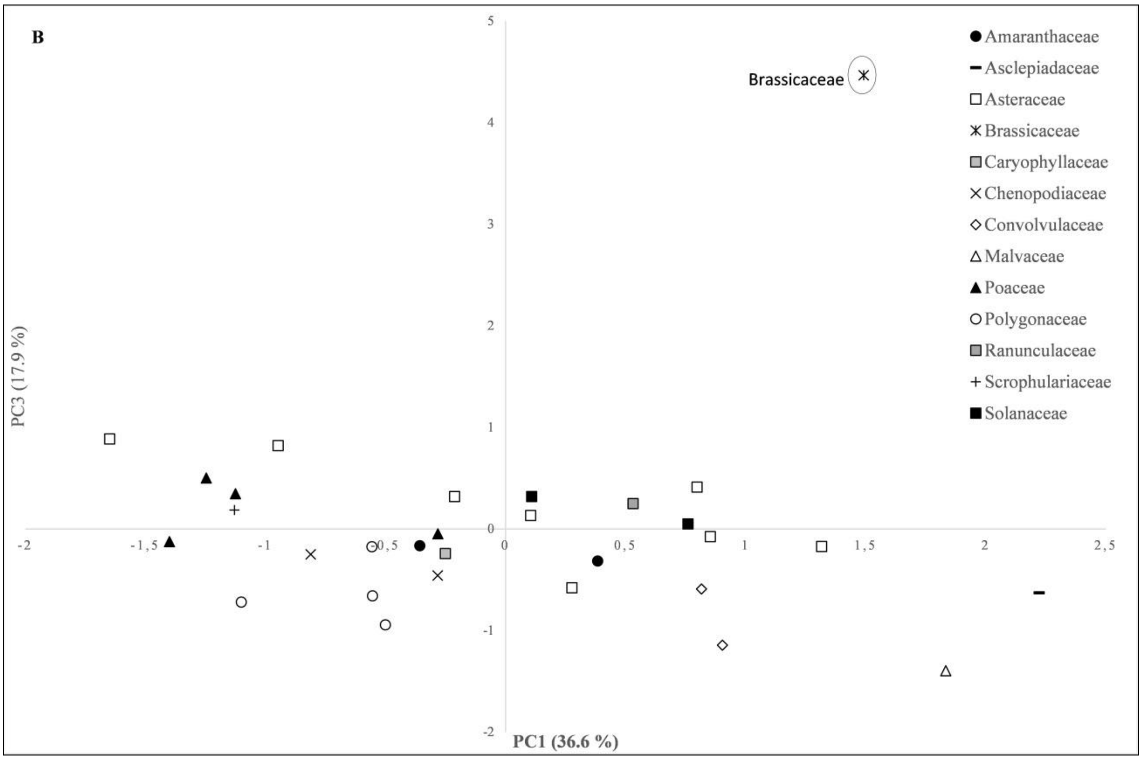

Figure 2B. *PCA ordination of weed species investigated (PC1 vs PC3)*

Although the points representing the weed samples are very scattered, it is possible to detect groups of weeds that belong to the same family. The *Polygonaceae* family, for instance, has similar scores on axis 1 (-0.5 to -1), indicating that the common feature of

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this family is the N, P and Mg content. In the case of the *Asteraceae*, the weed samples had similar PC scores on axis 2, which indicates that the common feature for weeds classified as *Asteraceae* is the Ca content. For the other families, there is no clear orientation, implying that they have a mixed type of features. However, the weed families can be separated by their PC scores: the *Polygonaceae*, *Convolvulaceae* and *Malvaceae* can be separated on the basis of PC1, while, as shown earlier, the *Brassicaceae* differ from the other weed families in terms of PC3.

Conclusions

Although great variability was observed for the nutrient quantities in weed seeds, the N, K, Ca, Mg, P and S concentrations in weed seeds were significantly higher than those in crop seeds from the same family, indicating that they could help to improve nutrient conservation during the fallow period. However, the variability in the nutrient content must be taken into account when determining the beneficial impact of weeds on the nutritional status of soil-plant systems; e.g. weeds from the *Asteraceae* family have more potential to conserve N as they have significantly higher N content than weeds from the *Polygonaceae* family.

Differences between weed families were mainly found in the case of macroelement accumulation, and these differences were mainly caused by the genetic diversity of weed species belonging to different families.

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REFERENCES

[1] Akobundu, I.O. (1987): Weed Science in the Tropics: Principles and Practices. – John Wiley & Sons Ltd, New York.

[2] Andreasen, C., Skovgaard, I.M. (2009): Crop and soil factors of importance for the distribution of plant species on arable fields in Denmark. – Agriculture, Ecosystems & Environment 133: 61–67.

[3] Björkman, M., Klingen, I., Birch, A.N.E., Bones, A.M., Bruce, T.J.A., Johansen, T.J., Meadow, R., Mølmann, J., Seljåsen, R., Smart, L.E., Stewart, D. (2011): Phytochemicals of Brassicaceae in plant protection and human health - influences of climate, environment and agronomic practice. – Phytochemistry 72: 538–556.

[4] Burlingame, B., Mouillé, B., Charrondiére, R. (2009): Nutrients, bioactive non-nutrients and anti-nutrients in potatoes. – Journal of Food Composition and Analysis 22: 494-502.

[5] Debreczeni, B-né, Németh, T. (eds.) (2009): Hungarian National Long-term Fertilization Experiment Network (in Hungarian). – Akadémiai Kiadó, Budapest.

[6] Frick, B., Thomas, A.G. (1992): Weed surveys in different tillage systems in south western Ontario field crops. – Canadian Journal of Plant Science 72: 1337–1347.

[7] Friedman, M. (1996): Nutritional value of proteins from different food sources. A review. – Journal of Agricultural and Food Chemistry 44: 6-29.

[8] Friedman, M., Levin, C.E. (1989): Composition of Jimson weed (*Datura stramonium*) seeds. – Journal of Agricultural and Food Chemistry 37: 998-1005.

[9] Győri, Z., Filep, T., Lehoczky, É. (2014): Trace element content of several weed seeds. – Carpathian Journal of Earth And Environmental Sciences 9(2): 251-257

[10] Győri, Z. (2009): The effect of fertilization on the chemical composition of test plants and quality parameters (in Hungarian). – In: Debreczeni, B-né, Németh, T. (eds.) Hungarian National Long-term Fertilization Experiment Network, Akadémiai Kiadó, Budapest.

[11] Haghighi, M.L., Abbaszadeh, B., Changaei, N. (2012): Variation in macronutrients uptake by two Amaranth varieties planted in two different times. – Annals of Biological Research 3(8): 3949-3951.

[12] Jones, J.B. (1992): Methods of nitrogen determination in soils and plant tissue. – Communications in Soil Science and Plant Analysis 19: 493-505.

[13] Kádár, I. (2002): The evaluation of nutrient status of rapeseed (*Brassica napus* L.) by plant analysis (in Hungarian). – Agrokémia és Talajtan 52(3-4): 395-416.

[14] Kádár, I., Daood, H. (2001): The effect of microelement-charge on wheat on calcareous chernozem soil (in Hungarian). – Agrokémia és Talajtan 50: 353-370.

[15] Kötschau, A., Büchel, G., Einax, J.W., Meißner, R., von Tümpling, W., Merten, D. (2014): Element pattern recognition and classification in sunflower (*Helianthus annuus*) grown on contaminated and non contaminated soil. – Microchemical Journal 114: 164-174.

[16] Kovács, B., Győri, Z., Prokisch, J., Loch, J., Dániel, P. (1996): A study of plant sample preparation and inductively coupled plasma emission spectrometry parameters. – Communications in Soil Science and Plant Analysis 27: 1177-1198.

[17] Lehoczk,y É., Reisinger, P., Kőmíves, T. (2005): Loss of nutrients caused by excessive weediness at the early stage of maize vegetation period. – Communications in Soil Science and Plant Analysis 36(4-6): 415-422.

[18] Lehoczky, É., Busznyák, J., Gólya, G., Pálmai, O. (2012): Green water – *Ambrosia artemisiifolia* L. on winter wheat stubble. – Növénytermelés 61(3): 259-262.

[19] Lehoczky, É., Gólya, G., Radimszky, L., Riczu, P., Tamás, J. (2013a): Study ont he weed flora in maize in connection with nutrient supply. – Növénytermelés 62: 147-150.

[20] Lehoczky, É., Kamuti, M., Mazsu, N., Radimszky, L., Sándor, R. (2014b): Composition, density and dominance of weeds in maize at different nutrient supply levels. – Növénytermelés 63(1): 287-290.

[21] Lehoczky, É., Kamuti, M., Mazsu, N., Tamás, J., Sáringer-Kenyeres, D., Gólya, G. (2014a): Influence of NPK fertilization on weed flora in maize field. – Agrokémia és Talajtan 63(1): 139-148.

[22] Lehoczky, É., Kismányoky, A., Tóth, V., Németh, T. (2009): Weediness and the nutrient uptake by weeds in relation to the soil tillage. – Communications in Soil Science and Plant Analysis 40(1-6): 871-878.

[23] Lehoczky, É., Márton, L., Nagy, P. (2013b): Competition for nutrients between cold-tolerant maize and weeds. – Communications in Soil Science and Plant Analysis 44(1-4): 526-534.

[24] Li, Y., Hussain, N., Zhang, L., Chen, X., Ali, E., Jiang, L. (2013): Correlations between Tocopherol and Fatty Acid Components in Germplasm Collections of Brassica Oilseeds. – Journal of Agricultural and Food Chemistry 61: 34-40.

[25] Løbersli, E.S., Steinnes, E. (1988): Metal uptake in plants from a birch forest area near a copper smelter in Norway. – Water, Air, & Soil Pollution 37: 25–39.

[26] Lovkova, M.Ya, Buzuk, G.N., Sokolova, S.M. (2008): Genetic aspects of the interrelation between alkaloids and chemical elements in *Atropa belladonna* L. and *Glaucium flavum* Crantz. plants. – Prikladnaia biokhimiia i mikrobiologiia 44: 459–462.

[27] Markert, B. (1989): Multi-element analysis in ecosystems: basic conditions for representative sampling of plant materials. – Fresenius' Zeitschrift für Analytische Chemie 335: 562–565.

[28] Menkir, A. (2008): Genetic variation for grain mineral content in tropical-adapted maize inbred lines. – Food Chemistry 110: 454–464.

[29] MSZ-08-0206/2:1978 Evaluation of some chemical properties of the soil. Laboratory tests. (pH value, phenolphtaleine alkalinity expressed in soda, all water soluble salts,

hydrolite (y1-value) and exchanging acidity (y2-value). (In Hungarian) – Hungarian Standard Association, Budapest.

[30] MSZ-08-0215:1978: Determination of the cation adsorption capacity of the soil. Modified Mechlich technique. (In Hungarian) – Hungarian Standard Association, Budapest.

[31] MSZ-08-0452:1980 Use of high-capacity analyser systems for soils analyses. Quantitative determination of the organic carbon content of the soil on Contiflo analyzer system. (In Hungarian) – Hungarian Standard Association, Budapest.

[32] Novák, R., Dancza, I., Szentey, L., Karamán, J. (2009): Weeds of arable lands in Hungary. Fifth weed survey of Hungary (2007-2008) (in Hungarian). – FVM, Budapest.

[33] Oderinde, R.A., Tairu, A.O. (1989): Utilisation of yellow nutsedge tuber for composite flour. – Pakistan Journal of Scientific and Industrial Research 32: 570-573.

[34] Oerke, E.C., Dehne, H.W. (2004): Safeguarding production - losses in major crops and the role of crop protection. – Crop Protection 23: 275-285.

[35] Palm, C.A., Gachego, C., Delve, R., Cadisch, G., Giller, K.E. (2001): Organic inputs for soil fertility management in tropical agroecosystems: application of an organic resource database. – Agriculture, Ecosystems & Environment 83: 27–42.

[36] Peng, L., Huang, Y., Liu, Y., Zhang, Z., Lu, L., Zhao, G. (2014): Evaluation of essential and toxic element concentrations in buckwheat by experimental and chemometric approaches. – Journal of Integrative Agriculture 13(8): 1691-1698.

[37] Promsakha Na Sakonnakhon, S., Cadisch, G., Toomsan, B., Vityakon, P., Limpinuntana, V., Patanothai, A. (2006): Weeds – friend or foe? The role of weed composition on stover nutrient recycling efficiency. – Field Crops Research 97: 239-247.

[38] Ramage, C.M., Williams, R.R. (2002): Mineral nutrition and plant morphogenesis. – In Vitro Cellular & Developmental Biology 38: 116–124.

[39] Raunkiær, C. (1934): The life forms of plants and statistical plant geography. – Calderon Press, Oxford.

[40] Singh, O.V., Labana, S., Pandey, G., Budhiraja, R., Jain, R.K. (2003): Phytoremediation an overview of metallic ion decontamination from soil. – Applied Microbiology and Biotechnology 61: 405–412.

[41] Swanton, C.J., Harker, K.N., Anderson, R.L. (1993): Crop losses due to weeds in Canada. – Weed Technololgy 7: 537–542.

[42] Tanji, A. Elgharous, M. (1998): A survey of mineral composition of weed seeds. – Weed Research 38: 79-86.

[43] Ujvárosi, M. (1973): Weeds. (in Hungarian) – Mezőgazdasági Kiadó, Budapest.

[44] Vogel-Mikuš, K., Pelicon, P., Vavpetic, P., Kreft, I., Regvar, M. (2009): Elemental analysis of edible grains by micro-PIXE: Common buckwheat case study. – Nuclear Instruments and Methods in Physics Research 267: 2884-2889.

[45] Walkley, A., Black, I.A. (1934): An examination of the Degtjareff method for determining organic carbon in soils: Effect of variations in digestion conditions and of inorganic soil constituents. – Soil Science 63: 251-263.

[46] Wang, S., Duan, L., Li, J., Tian, X., Li, Z. (2007): UV-B radiation increases paraquat tolerance of two broad leaved and two grass weeds in relation to changes in herbicide absorption and photosynthesis. – Weed Research 47(2): 122–128.

[47] White, P.J., Broadley, M.R. (2009): Biofortification of crops with seven mineral elements often lacking in human diets - iron, zinc, copper, calcium, magnesium, selenium and iodine. – New Phytologist 182: 49-84.

[48] White, P.J., Brown, P.H. (2010): Plant nutrition for sustainable development and global health. – Annals of Botany 105: 1073-1080.

[49] Willey, N.J., Tang, S., Watt, N.R. (2005): Predicting inter-taxa differences in plant uptake of cesium-134/137. – Journal of Environmental Quality 34: 1478–1489.

CALCULATION OF WETLANDS ECOLOGICAL WATER REQUIREMENT IN CHINA'S WESTERN JILIN PROVINCE BASED ON REGIONALIZATION AND GRADATION TECHNIQUES

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Abstract. In this study a method for measuring the regional hydrological connectivity and water supply requirement of wetlands in Western Jilin Province (WJL) is defined and verified. The ecological water requirements of 216 wetlands, under various guarantee frequencies, were calculated and analyzed. In addition, twelve indicators were used to establish an evaluation index system, to characterize their ecological water requirements. The results showed that 12 hydrologically connected regions were delineated in WJL, and the 216 wetlands can be classified to six grades by AHP (Analytic Hierarchy Process) method. The annual minimum, suitable, and maximum ecological water requirements of these wetlands were found to be 7.72×10^8 m^3, 13.76×10^8 m^3, 26.15×10^8 m^3 respectively, with 50% guarantee frequency. The primary water supply to the lakes and marshes was found to be from regional sources that provided a scientific basis for the allocation of water resources from the interconnected river-lake system network (IRLSN) in WJL.

Keywords: *Western Jilin Province, hydrological connectivity regionalization, AHP, ecological water demand.*

Introduction

Western Jilin Province (WJL) is located in the hinterland of Songnen Plain, an ecological fragile region in the transitional zone from semi-humid to semi-arid climate. The region has the largest, and one of the most important, wetland ecosystems in Songhua River Basin (Pan et al., 2006; Ren et al., 2007). At present, maintaining the ecological health of these wetlands is hampered by insufficient knowledge of their water supply requirements.

Geographically, the WJL region is flat and situated mainly on an alluvial plain, making the discharge of surface waters into the region difficult. In recent decades, the influence of climate change (rising temperatures and declining rainfall) and human activities

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(engineered hydraulic structures, such as reservoir) have weakened and damaged existing river-lake connection networks, causing a sharp decline of streamflows into two major rivers in the region, Huolin River and Tao'er River. As a result, the wetlands are showing clear signs of deteriorating ecological functions and acreage losses (Yue et al., 2008).

The amount of river water flowing across WJL (in particular, Nenjiang River, Songhua River) is still abundant for sustaining agricultural activities and wetland ecosystem, if the water resource is appropriately connected and allocated. A large-scale water management scheme, namely "Interconnected River-Lake System Network" (IRLSN), is being planned in WJL. It aims to coordinate the use of various water resources, to replenish lakes of significant ecological or economic values, provide water storage, and restore some ecologically important natural wetlands, such as Xianghia, Momoge, Chagan Lake Nature Reserve Wetlands (Dong et al., 2014). The IRLSN must be guided by a scientific approach of analyzing water supply-demand relation in hydrologically connected or separated regions, as it would be reckless to link waterways and storages without careful research and design.

Hydrology regime is the driving force behind the formation of wetlands (Zhang et al., 2014). Hydrological connectivity is the basis of water supply and allocation; it should be the first to be analyzed in quantitative studies of how waterways in neighboring regions can be connected in water management. Bracken et al. (2013) classified the research around hydrological connectivity into five broad themes based on: i) soil moisture; ii) flow processes; iii) terrain; iv) models; and v) indices. This classification yielded a definition of hydrological connectivity, and laid a foundation of conceptualization and methodology of different research approaches. Many researchers studied regional water resources based on hydrology, bio-ecology, nutrient migration and material deposited (Freeman et al., 2007; Lexartza-Artza and Wainwright, 2009; Lesschen et al., 2009; Luo et al., 2011; Shore et al., 2013). The boundary of their studies were individual catchments, as it is relatively easier to carry out research on a single catchment compared with studies on a vast region with numerous rivers, scattered lakes and marsh wetlands. In China, studies on hydrological connections need to be based on functional structures of regional water network, with evaluation standards and methods that suit the regional conditions (Zang et al., 2014; Li et al., 2014); such approach is used in this study.

After studying hydrological connectivity, the evaluation of water requirement of wetlands needs to be carried out, for protecting and restoring different wetlands and meeting their ecological water requirement according, especially when managed flood is the main source of water connection. While the method of calculating wetland ecological water requirement is well known (Li et al., 2009), only limited studies have been carried out on the wetland water requirement for sustaining ecological functions. Zhang et al. (2013) used the Analytic Hierarchy Process (AHP) approach to determine weighted indices of different factors that affect wetland ecosystem values, incorporating environmental constraints and socio-economic value in each index (Wang et al., 2003; Herath, 2004). Jiang and Lee (2013) proposed a method to simplify the selection of wetlands for priority management. Four types of evaluation categories were proposed: areas with a high value for the conservation of the ecosystem; areas with a high potential impact on biodiversity; wetland size; and eco-networks. Then, the overall wetland water requirement was classified via an overlay analysis performed using the analytical results for each evaluation indicator. These previously studies divided the wetlands in WJL to Class 1 and 2 wetlands.

The objectives of this study are to: (1) determine the water supply domain of wetlands in WJL through hydrological connection analyses, (2) establish a more refined grading system of wetland water requirements, using AHP method, and (3) calculate the ecological water requirements of wetlands under different guarantee frequencies. In addition, this study aims to contribute to formulating water resource management strategies for wetland eco-system protection and flood management in the IRLSN scheme.

Materials and Methods

Study Area

Located between 43°22′N–46°18′N and 121°36′E–126°12′E, Western Jilin has an area of 55,340 km^2 that consists of 11 counties (cities): Baicheng, Zhenlai, Taonan, Tongyu, Daan, Songyuan, Qianguo, Qian'an, Changling, Fuyu, Nong'an (*Figure 1*). It is the transitional zone between arid climate in inner-Mongolia and semi-humid in Eastern Jilin. Based on data collected from 20 meteorological stations in WJL, the mean annual rainfall during 1975-2010 was 350-500 mm, while the temperature ranged -30 to -2 °C in January and 12 to 33 °C in July (Li et al., 2015).

Major rivers merging in WJL include Tao'er River, Huolin River. Major rivers crossing WJL include Nenjiang River and the Second Songhua River. As illustrated in *Figure 1*, Nenjiang River is located in northern border of WJL, the Second Songhua River is located in the northeast border, Tao'er River in western WJL, and Huolin River (in central WJL) are tributaries of Nenjiang River.

According to survey data, the total area of natural wetlands in WJL decreased 1100 km^2 from 1980 to 2002. The current area of wetlands is only 22% of the original size (Li, 2011). Three major objectives of IRLSN scheme include: (a) effectively utilizing the flood waters of Songhua River, Nenjiang River, Tao'er River and Huolin River to connect the main wetlands, lakes, ponds and reservoirs in the region, (b) building four core ecological plates on Xianghai, Momoge, Chagan and Boluo Lake, and (c) through effective management of flood water, improving the existing, or creating new, ecological communities in Xianghai, Momoge, Chagan and Boluo Lake areas (Jilin Daily, 2015). As such, the existing 216 wetlands were selected as the main research objects in this study.

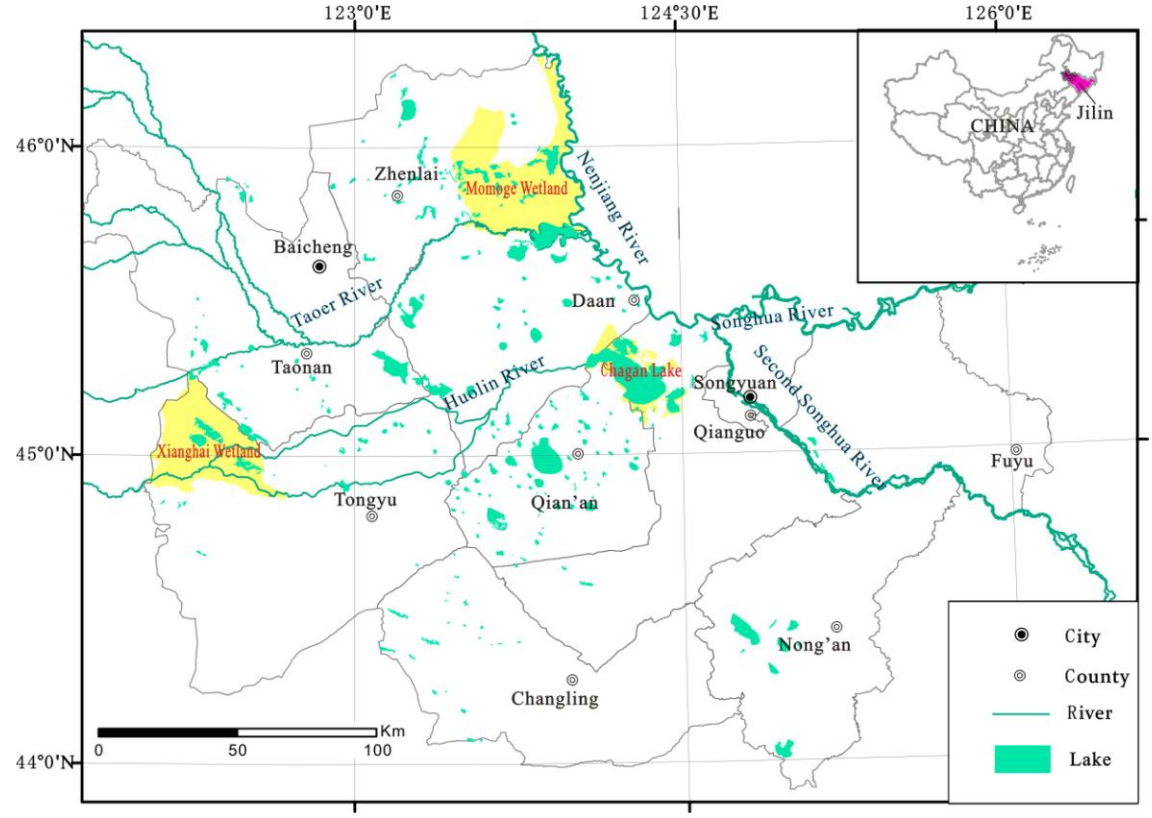

Figure 1. *Distribution of wetlands in Western Jilin province*

Hydrological connectivity analysis

Flood inundation method for analyzing the hydrological connectivity of lakes in WJL

Some flood waters are considered water resources in the IRLSN scheme. A source-flood method (Wang et al., 2010) can be used for flood inundation analysis. In this study, when flood level (H) is given, the flood inundation area has been calculated by seed filling algorithm used in a previous study (Wang et al., 2010), as follows:

a) With known starting grid unit (grid unit with dike break position) and flood level (H), initial a queue structure, add starting grid unit to the queue;

b) Pop first element (grid unit) of the queue, check “up, down, left, right” four nearby grid units in DEM, if grid unit elevation is lower than flood level(H), then add this grid unit to the queue;

c) If the queue is not null, go to step 2, else go to step 4;

d) Output queue, all grid units in queue compose the submerged area.

Watershed delineation method

Watershed delineation is one of the most commonly performed activities in hydrological analyses. Digital elevation models (DEMs) provide good terrain representation, from which watersheds can be derived automatically using GIS technology. In this study 1:50000 scale DEM was used for watershed delineation, followed by merging sub-watersheds in WJL according to different water supply resources; allowing different regional hydrological connectivity to be determined.

Grading of wetland water supply requirements by AHP method

The Analytic Hierarchy Process (AHP) (Saaty, 1980) was used to establish the indexed grading system for wetlands. The procedure of using the AHP method, as described by Wang et al. (2003) , included: (a) establishing the index system; (b) constructing the judgment matrix and single permutation of layer; (c) sequencing the ~~al~~ layers; and (d) drading of single element and comprehensive evaluation. Then the natural breaks classification method in the Arcgis technique was used to assign the grades of water supply requirements of the wetlands.

In WJL, the calculation range of wetland ecological water requirement was the wetland in different hydrological connectivity and grading regions. The sum of ecological water requirement of lake, reservoir, and other open water and water requirement of marsh wetland is shown below:

$$D=\sum A_i（ET_i-P）\times 10^{-3} \qquad \text{(Eq.1)}$$

Where: D represents ecological water requirement (m^3); A_i is the area of each part (m^2); ET_i represents the evapotranspiration of each part (mm); and P represents the precipitation of each part (mm).

Results and Discussion

The hydrological connectivity regionalization

Considering different sources of water supply, and combining flood inundation analysis with subwatershed delineation results, the regions that are hydrologically connected have been identified, as shown in *Figure 2*. Flood waters that generated flow into the 216 wetlands in WJL has also been identified (*Table 1*).

The flood water from Nenjiang River artesian flow connected region, Tao’er River artesian flow connected region, Huolin River artesian flow connected region and Second Songhua River artesian flow connected region were found to be Nenjiang River, Tao’er River, Huolin River and Second Songhua River, respectively. Additionally, the water levels corresponding to each of these water supply sources were 131 m, 141.2 to 221.6 m, 152 m, and 175 m, respectively. In addition to flood waters, water sources to the wetland included water diversion from Nenjiang River to Baicheng, and water diversion from Tao’er River to Xianghai and Hadashan hydro junction. The water diversion project from Nenjiang River to Baicheng was dependant on water lifting, and the water level of the main channel needed to reach 133m, which could artesianly flow into the channel artesian flow connected region. The function of Yangshapao reservoir was to store the water in the project, and the normal impounded water level was 135.2 m. So, 135.2 m was considered as a threshold of artesian flow, and through the flood inundation analysis, the water level for water lifting was 141 m. For water diversion project from Tao'er River to Xianghai, when the water level reached 175.4 m, the water artesianly supplied the channel artesian connected region. Chuangye reservoir, used to store water, is located at high geographical position; its water level of 163m artesianly supplied the lakes in its waterhsed. At Hadashan hydro junction, water was diverted from Tao'er River to Huaaopao Lake, and the normal diversion water level was 141 m. The normal water level of Huaaopao was 139 m, which was also considered as a threshold of artesian flow. When the water lifting level reached 154 m, the water supplied Huaaopao Lake lifting the connected region.

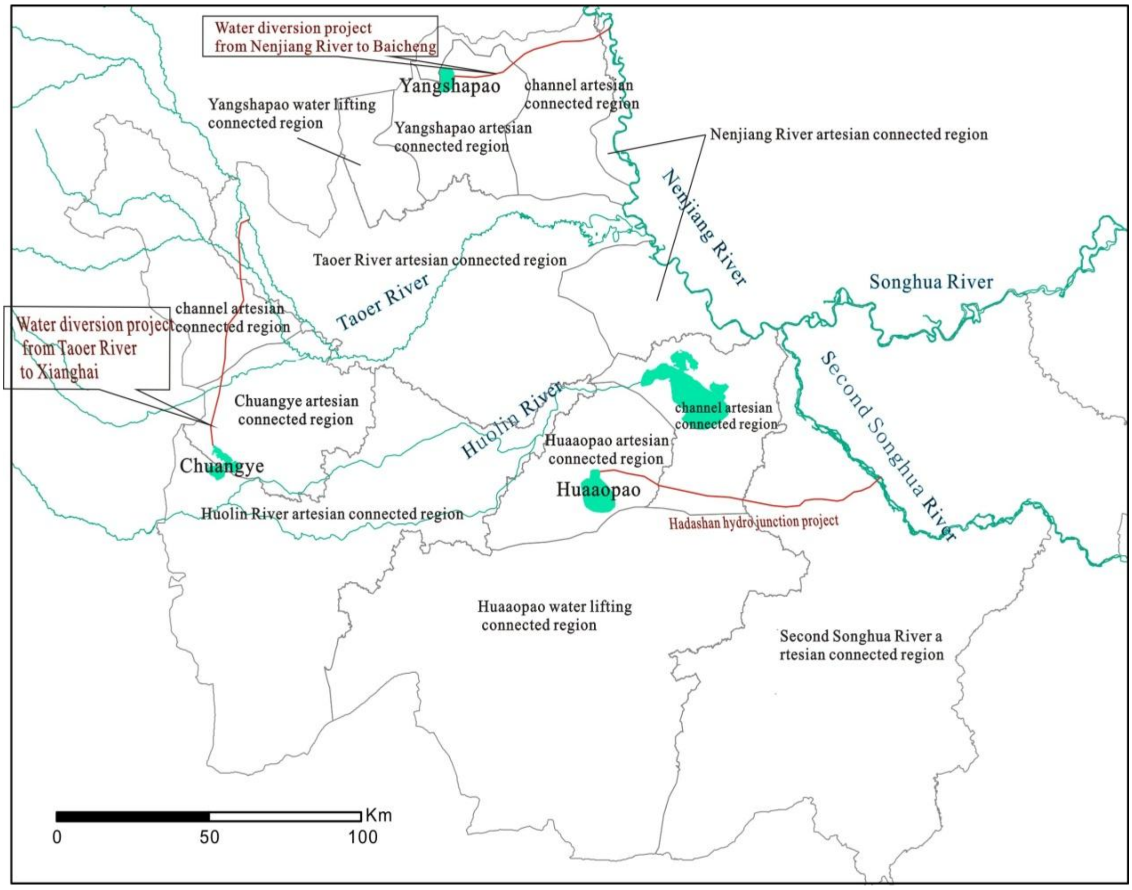

Figure 2. Hydrological connected regions in WJL

Table1. *Water level of different water supply sources for hydrological connected regions*

Name of hydrological connected regions		**Water level elevation as source of supply (m)**
Nenjiang River artesian connected region		131
Tao'er River artesian connected region		downstream,141.2; upsream, 221.6
Huolin River artesian connected region		152
Second Songhua River artesian connected region		175
Water diversion project from Nenjiang River to Baicheng (NB) connected region	NB channel artesian connected region	133
	Yangshapao Reservoir artesian connected region	135.2
	Yangshapao Reservoir water lifting connected region	141
Water diversion project from Tao'er River to Xianghai (TX) connected region	TX channel artesian connected region	175.4
	Chuangye Reservoir artesian connected region	163
Hadashan hydro junction (HD) connected region	HD channel artesian flow connected region	129
	Huaaopao Lake artesian connected region	139
	Huaaopao Lake lifting connected region	154

Grading of water supply requirements by wetlands

Indices for evaluating

The establishment of the evaluation index system was the key of AHP. Combining the hydro-geomorphology, ecological characteristics and function, and influence of human activities, a grading system was derived to signify water supply requirements by the wetlands in WJL (*Table 2*).

Table 2. *Indexes for evaluating water supply grading for wetlands*

Target hierarchy	Criteria hierarchy	Indices hierarchy
Grading of water supply requirements by wetlands in WJL (A)	Hydro-geomorphology (C1)	The type of water source (P1)
		Distance to water source (P2)
		Geomorphic conditions (P3)
		Relative altitude to water source (P4)
	Wetlands' characteristics (C2)	Normal storage capacity (P5)

Wetlands' function (C3)	Normal storage level (P6)
	water surface area (P7)
	regulation capacity of flood (P8)
	Water supply capacity (P9)
	Matter production capacity (P10)
Influence of human activities (C4)	Distance to residential area (P11)
	Distance to farmland (P12)

Scheme of AHP weighting for the indices

After establishing the evaluation index system, the relations among various layers and factors were placed in comparing matrixes that had the ratios of comparative importance of paired factors. Then, the values of the relative weight were calculated. Finally, Matlab was used to calculate the weight of each index according to AHP; resuls shown in *Table 3*.

Table 3. *Weights Scheme for each index*

Weight of Ci	**Weight of Pi**
(C1) 0.564	(P1) 0.3265
	(P2) 0.0897
	(P3) 0.1162
	(P4) 0.0316
(C2) 0.263	(P5) 0.1086
	(P6) 0.0684
	(P7) 0.0860
(C3) 0.118	(P8) 0.0648
	(P9) 0.0284
	(P10) 0.0248
(C4) 0.055	(P11) 0.0275
	(P12) 0.0275

Grading of water supply requirements of wetlands

Using the comprehensive evaluation method, the composite index of each wetland was calculated. The natural breaks classification method in the Arcgis technique was used to finally determine the water supply grading for wetlands; results shown in *Figure 3*.

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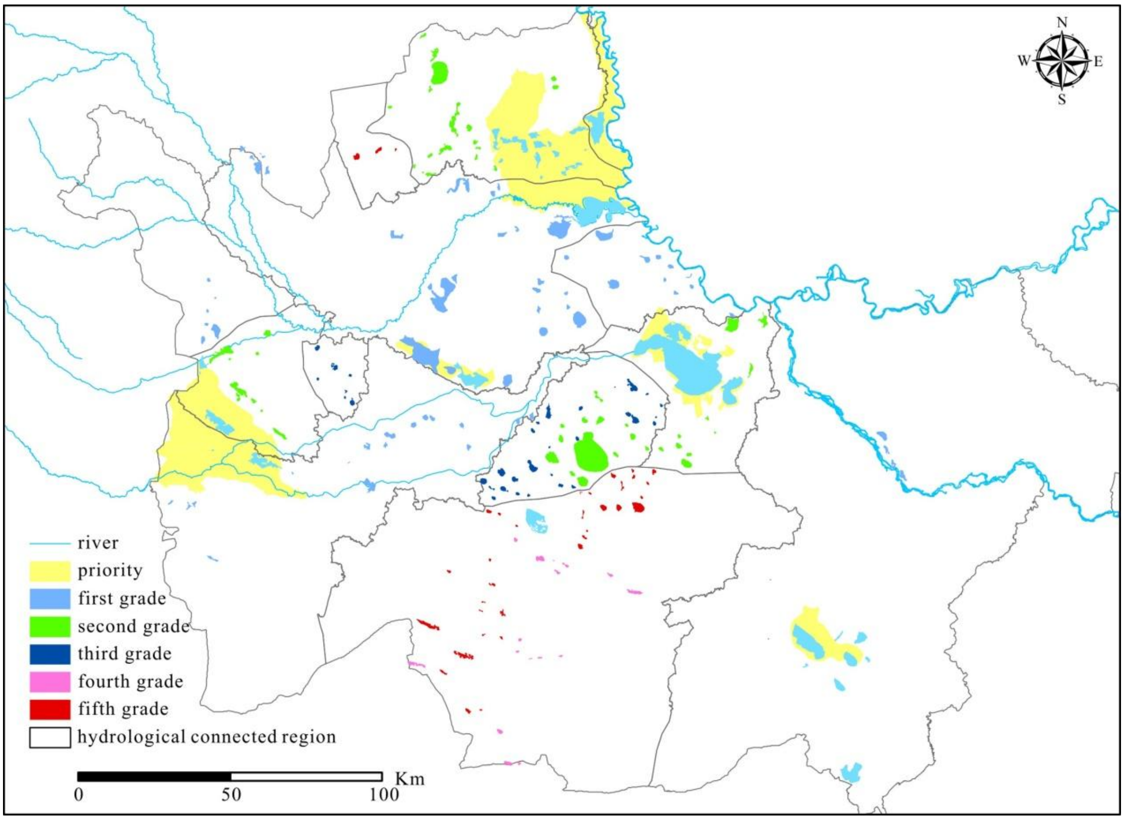

Figure 3. *Water supply grading for wetlands in WJL*

According to the grading results in *Figure 3*, the national or provincial wetland nature reserve (Chagan Wetland, Momoge Wetland, Xianghai Wetland, Niuxintaobao Wetland, Dabusu Wetland and Boluo Wetland) should be prioirities for water supply in the IRLSN scheme. The first grade lakes were mainly near the river. The second and third grade lakes were mostly located near the Water diversion projects. The fourth and fifth grade lakes were at the south of WJL, where the water source was primarily rainfall and surface runoff.

Regionalization and Grade Calculation of Wetlands Ecological Water requirement in WJL

According to the regionalization and grading result, the corresponding ecological water requirement of wetlands was calculated. The ecological water requirements of Chagan Wetland, Momoge Wetland, Xianghai Wetland(national natural wetlands), and Niuxintaobao Wetland, Dabusu Wetland and Boluo Wetland (provincial nature reserves), were found to have the minimum ecological water requirements in WJL. The ecological water requirements of rest open water of lakes, ponds, reservoirs and adjacent marsh wetlands extracted from 2012 landuse map (*Figure 4*) were considered as suitable ecological water requirement in WJL. The maximum ecological water requirements were the sum of suitable ecological water requirements and the ecological water requirements of adjacent marsh wetlands extracted from 1950s landuse map (*Figure 4*), and the adjacent mash wetlands were only the the area of marsh wetlands (in 1950s' landuse map) that were converted to saline-alkali soil lands in 2012 landuse map (*Figure 4*).

Through the statictical analysis of monthly precipitation and evaporation from 1960 to 2014 in 10 counties (cities), the monthly precipitation and evaporation under 25%, 50%, 75%, and 90% guarantee rate in each counties (cities) were determined. Sheng et al. (2007) found that the conversion coefficient between evaporation measured by a small evaporation pan and water surface evaporation was 0.5-0.6. In this study, the conversion coefficient was found to be 0.6. In marsh wetlands, the main vegetation was found to be *Phragmites* that typically grow between April and September in WJL. Thus, the ecological water requirement of marsh wetlands were only calculated between April and September. The evaporation was calculated by the 2.5 times of water surface evaporation from June to August, and 1.3 times in April, May and September (Tang et al., 2005). The ecological water requirement of lakes and marshes in each region and grading calculated in this study are shown in *Tables 4, 5* and *6*.

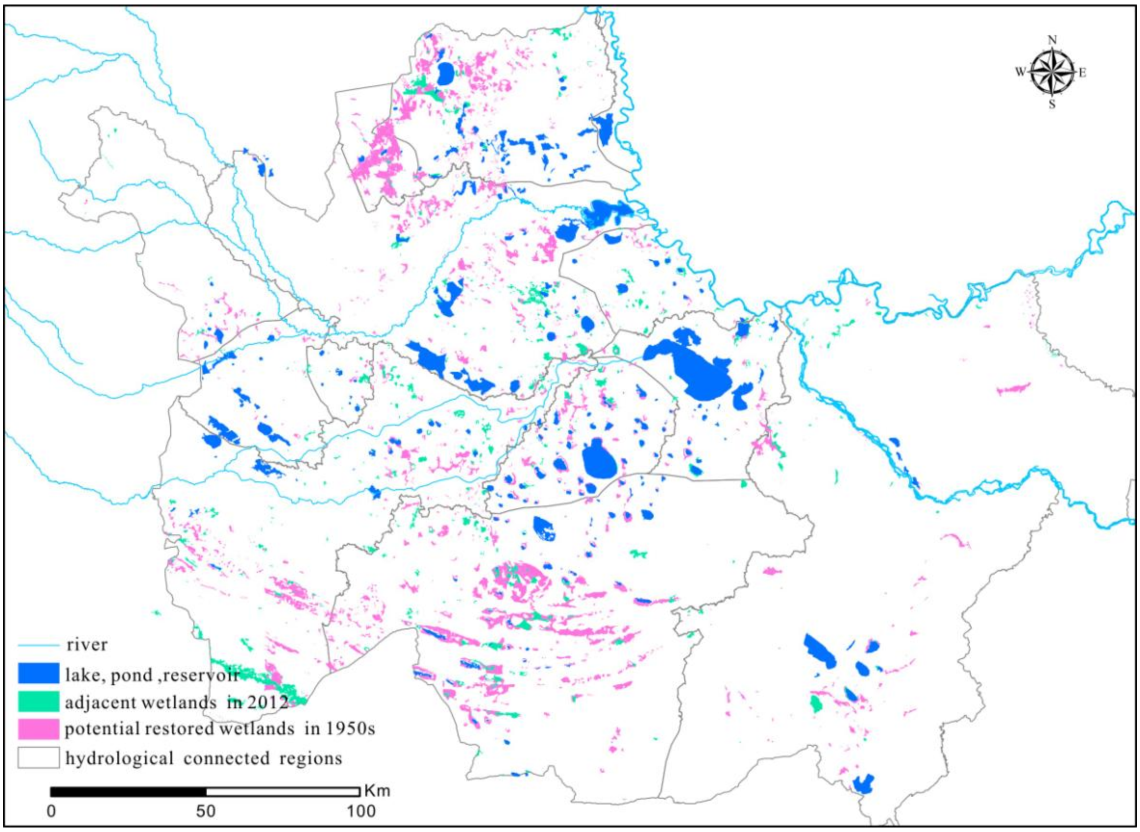

Figure 4. *Distribution of existing and potential restoring wetlands of WJL*

Table 4. *Minimum ecological water requirements in WJL*

Water supply grade	**name**	**Hydrological connected region**	**Ecological water requirement of open water (10^6m^3)**				**Ecological water requirement of marsh wetland（10^6m^3）**			
			25%	**50%**	**75%**	**90%**	**25%**	**50%**	**75%**	**90%**
priority	Chagan wetland	HD channel artesian flow connected region	-34.16	55.28	115.57	168.10	11.57	22.03	29.45	36.08
	Momoge wetland	NB channel artesian connected region	-0.52	74.66	136.72	178.30	156.96	263.77	357.31	424.62
	Xianghai wetland	TX channel artesian connected region	0.42	26.54	40.36	52.11	41.58	71.98	90.42	106.69
	Niuxintaobao wetland	Tao’er River artesian connected region	0.33	10.64	18.37	24.95	9.23	14.31	18.55	22.22
	Boluohu wetland	Second Songhua River artesian connected region	-24.47	82.66	178.80	241.82	-24.49	82.66	178.80	241.82
	Dabusu wetland	Huaaopao Lake lifting connected region	0.72	8.60	13.46	18.23	36.96	58.62	72.78	87.10
subtotal			-57.70	258.38	503.28	683.51	231.83	513.36	747.31	918.54
total			174.13	771.74	1250.60	1602.06				

Table 5. *Suitable ecological water requirement in WJL*

Water supply grade	**Hydrological connected region**	**Ecological water requirement of open water (10^6m^3)**				**Ecological water requirement of marsh wetland （10^6m^3）**			
		25%	**50%**	**75%**	**90%**	**25%**	**50%**	**75%**	**90%**
first	Nenjiang River artesian connected region	0.26	1.19	20.73	28.20	11.43	19.20	26.01	30.92
	Second Songhua River artesian connected region	-6.13	2.46	10.95	16.49	17.70	34.66	47.54	60.03
	Tao’er River artesian connected region	10.73	64.74	104.42	137.49	41.13	66.38	86.36	102.41
	Huolin River artesian connected region	0.20	12.19	18.71	24.25	73.00	126.38	158.74	187.32
second	Yangshapao Reservoir artesian connected region	-0.15	21.79	39. 90	52.03	23.45	39.41	53.39	63.45
	Chuangye Reservoir artesian connected region	2.85	9.41	13.88	17.84	2.56	4.43	5.56	6.56
	Huaaopao Lake artesian connected region	-1.43	19.59	31.07	41.98	14.16	22.46	27.89	33.37
	HD channel artesian flow connected region	-0.89	9.77	16.58	22.97	9.61	18.28	24.44	29.95
third	Huaaopao Lake artesian connected region	-0.95	13.06	20.71	27.99	9.44	14.98	18.59	22.25
	Chuangye Reservoir artesian connected region	1.48	4.76	7.01	9.01	3.92	6.78	8.52	10.08
fourth	Huaaopao Lake lifting connected region	-2.17	16.08	29.49	40.21	24.63	41.16	57.02	68.22
fifth	Huaaopao Lake lifting connected region	-1.17	8.66	15.88	21.65	13.26	22.16	30.70	36.74
	Yangshapao Reservoir water lifting connected region	-0.01	1.85	3.39	4.42	1.23	2.08	2.81	3.34
subtotal		2.62	185.57	332.74	444.53	245.53	418.38	547.60	654.63
total		248.16	603.95	880.34	1099.16				
Minimum ecological water requirement		174.13	771.74	1250.60	1602.06				
Suitable ecological water requirement		422.29	1375.69	2130.94	2701.22				

Table 6. *Maximum ecological water requirement in WJL*

Water supply grade	**Hydrological connected region**	**Ecological water requirement of marsh wetland（10^6m^3）**			
		25%	**50%**	**75%**	**90%**
priority	Chagan wetland	0	0	0	0
	Momoge wetland	47.45	82.08	103.10	121.67
	Xianghai wetland	253.60	0.42	26.54	40.36
	Niuxintaobao wetland	15.59	24.16	31.32	37.52
	Boluohu wetland	2.62	5.88	9.75	12.23
	Dabusu wetland	0	0	0	0
first	Nenjiang River artesian connected region	7.45	12.52	16.96	20.15
	Second Songhua River artesian connected region	32.58	63.82	87.52	110.52
	Tao’er River artesian connected region	97.70	157.65	205.11	243.22
	Huolin River artesian connected region	96.70	167.39	210.26	248.12
second	Yangshapao Reservoir artesian connected region	125.05	210.14	284.67	338.30
	Chuangye Reservoir artesian connected region	7.13	12.35	15.51	18.31
	Huaaopao Lake artesian connected region	34.53	54.75	67.98	81.35
	HD channel artesian flow connected region	6.67	12.70	16.98	20.80
third	Huaaopao Lake artesian connected region	23.02	36.50	45.32	54.23
	Chuangye Reservoir artesian connected region	2.02	3.49	4.39	5.18
fourth	Huaaopao Lake lifting connected region	116.72	195.10	270.26	323.34
fifth	Huaaopao Lake lifting connected region	95.17	159.08	220.37	263.65
	Yangshapao Reservoir water lifting connected region	24.37	40.95	55.47	65.93
total		988.34	1239.00	1671.52	2004.89
Suitable ecological water requirement		422.29	1375.69	2130.94	2701.22
Maximum ecological water requirement		1410.63	2614.69	3802.46	4706.11

Overall, results from this study demonstrate that the minimum ecological water requirement in WJL was 174.13, 771.74, 1250.60, 1602.06 million m^3 under the precipitation guarantee rate of 25%, 50%, 75%, 90%, respectively. The suitable ecological water requirement in WJL was 422.29, 1375.69, 2130.94, 2701.22 million m^3 under the precipitation guarantee rate of 25%, 50%, 75%, 90%, respectively. The maximum ecological water requirement in WJL was 1410.63, 2614.69, 3802.46, 4706.11 million m^3 under the precipitation guarantee rate of 25%, 50%, 75%, 90%, respectively. Among the wetlands being studied, 54% were identified as priorities for water supply , first grade was about 24%. Among the first grade of suitable ecological water requirements, the Tao’er River artesian connected region accounted for about 39%, and Huolin River artesian connected region accounted for about 41%. The critical sources of water supply to most lakes and marsh wetlands were Tao’er River and Huolin River. Currently, water supplies from these two rivers are rapidly decreasing; this poses a significant risk to the ecological function of the wetlands in WJL.

Conclusion

This study proposed and tested a method for determining the hydrological connectivity and grades of water supply requirements by wetlands in WJL. The ecological water requirement of wetlands in WJL can be classified into three categories: minimum, suitable, maximum. In a hydrological connectivity analysis, WJL was found to consist of 12 hydrological connected regions. Twelve indicators were selected to establish the evaluation index system of wetlands water supply grading in WJL, and 6 grades were determined. The minimum ecological water requirement in WJL was 1.74×10^8, 7.72×10^8, 12.51×10^8, 16.02×10^8 m^3 with precipitation guarantee rate of 25%, 50%, 75%, 90%, respectively. The suitable ecological water requirement in WJL was 4.22×10^8, 13.76×10^8, 21.31×10^8, 27.01×10^8 m^3 with precipitation guarantee rate of 25%, 50%, 75%, 90%, respectively. The maximum ecological water requirement in WJL was 14.11×10^8, 26.15×10^8, 38.02×10^8, 47.06×10^8 m^3. Effective utilization of flood water, as a resource, was found to be essential for supplying water to sustain the lakes and marsh wetlands in WJL.

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REFERENCE

[1] Bracken, L.J., Wainwright, J., Ali, G.A., Tetzlaff, D., Smith, M.W., Reaney, S.M., Roy, A.G. (2013): Concepts of hydrological connectivity: Research approaches, pathways and future agendas. - Earth-Science Reviews 119: 17-34.

[2] Dong, J.W., Yang, J.Y., Yang, W.P., Zhang, S.J. (2014): Eco-environmental issues related to river-lake connection in western part of Jilin Province. - China Water Resources 22: 26-30.(in Chinese)

[3] Jilin Daily. (2015): West Jilin Province's "River and Lake Connectivity" Describes a Beautiful Scene of Thriving Fishery and Animal Husbandry and Exuberant Grass and Abundant Grains. http://english.jl.gov.cn/News/GeneralNews/201504/t20150414_ 1974448. html. Accessed 10 April 2015.

[4] Li, F., Zhang, S.W., Bu, K., Yang, J.C., Wang, Q., Chang, L.P. (2015): The relationships between land use change and demographic dynamics in western Jilin province. - Journal of Geographical Sciences 25(5): 617-636.

[5] Li, L.J., Li, J.Y., Liang, L.Q., Liu, Y.M. (2009): Method for calculating ecological water storage and ecological water requirement of marsh. - Journal of Geographical Sciences 19: 427-436.

[6] Freeman, M.C., Pringle, C.M., Jackson, C.R. (2007): Hydrologic connectivity and the contribution of stream headwaters to ecological integrity at regional scales. - JAWRA Journal of the American Water Resources Association 43(1): 5-14.

[7] Herath, G. (2004): Incorporating community objectives in improved wetland management: the use of the analytic hierarchy process. - Journal of Environmental Management 70(3): 263-273.

[8] Jang, H.J., Lee, G.G. (2013): A Study on National Wetland Evaluation for the Selection of Priority Control Target Wetlands in South Korea. - KSCE Journal of Civil Engineering 17(7): 1603-1613.

[9] Lesschen, J.P., Schoor, J.M., Cammeraat, L.H. (2009): Modelling runoff and erosion for a semi-arid catchment using a multi-scale approach based on hydrological connectivity. - Geomorphology 109: 174-183.

[10] Lexartza-Artza, I., Wainwright. J. (2009): Hydrological Connectivity: Linking Concepts with Practical Implications. - Catena 79: 146-152.

[11] Li, Y.Y., Li, Z.L., Huang, H.J., Wang, Z.G., Chen, M. (2014): Analyzing the Evolution of the Interconnected River System Network and Driving Factors. - Resources Science 36(6): 1152-1157.(in Chinese)

[12] Luo, W., Grudzinski, B., Pederson, D. (2011): Estimating hydraulic conductivity for the Martian subsurface based on drainage patterns - A case study in the Mare Tyrrhenum Quadrangle. - Geomorphology 125: 414-420.

[13] Pan, X.L., Zhang, D.Y., Quan L. (2006): Interactive factors leading to dying-off Carex tato in Momoge wetland polluted by crude oil, Western Jilin, China. - Chemosphere 65: 1772-1777.

[14] Ren, C.Y., Zhang, B., Wang, Z.M., Song, K.S., Liu, D.W., Liu, Z.M. (2007): A GIS-based Tupu Analysis of Dynamics of Saline-alkali Land in Western Jilin Province. - Chinese Geographical Science 17(4): 333-340.

[15] Saaty, T.L. (1980): The Analytic Hierarchy Process. - McGraw Hill, New York.

[16] Sheng, Q., Shen, S.H., Gu, Z. (2007): Conversion Coefficient between Small Evaporation Pan and Theoretically Calculated Water Surface Evaporation in China. -Journal of Nanjing Institute of Meteorology 30(4): 561-565.

[17] Shore, M., Murphy, P.N.C., Jordan, P., Mellander, P-E., Kelly-Quinn, M., Cushen, M., Mechan, S., Shine, O., Melland, A.R. (2013): Evaluation of a surface hydrological connectivity index in agricultural catchments. - Environmental Modelling & Software 47: 7-15.

[18] Tang, Y., Wang, H., Yan, D.H. (2005): Wetland Ecological Water Demand in Xianghai Natural Reserve. - Resources Science 27(5): 101-106.

[19] Wang, X.L., Ning, L.M., Hu, W.B. (2003): Application of analytic hierarchy process to assessing the ecological vulnerability of wetlands in the Jianghan plain. - Chinese Geographical Science 13(3): 272-276.

[20] Wang, Y.F., Zhong, Y.S., Cui, W.H., Lv, P. (2010): Flood inundation and disaster prediction based on DEM. - 2010 Second IITA International Conference on Geoscience and Remote Sensing, 54-57.

[21] Wang, Z.Q., Zhang, B., Yu, L., Zhang, S.Q., Wang, Z.M. (2006): Study on LUCC and the ecological security response of wetlands in Western Jilin Province. - Arid Zone Research 23(3): 419-426.

[22] Yue, S.P., Zhang, S.W., Yan, Y.C. (2008): Analysis of wetland landscape pattern change and its driving mechanism in Jilin western part. - China Environmental Science 28(2): 163-167.(in Chinese)

[23] Zang, C., Zou, Q.T., Ma, J.X. (2014): Vulnerability analysis of regional interconnected river system network and its applications. - Water Resources & Power 32(9): 28-31.(in Chinese)

[24] Zhang, G.X., Zhang, L., Fan, W., Feng, X.Q., Dong, L.Q. (2014): Wetland ecohydrology and water resource management.- Beijing: science press.

[25] Zhang, R.Q., Zhang, X.D., Yang, J.Y., Yuan, H. (2013): Wetland ecosystem stability evaluation by using Analytical Hierarchy Process (AHP) approach in Yinchuan Plain, China. - Mathematical and Computer Modelling 57(3–4): 366-374.

IMPACT OF AGRICULTURAL CHEMICALS ON SELECTED HEAVY METALS ACCUMULATION IN HERB PLANTS

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Abstract. The aim of this paper is to determine cadmium, lead and nickel concentration in different herb plants and in the soil collected in the areas close to winter wheat and grain maize fields. The following herbs were analyzed: *Achillea millefolium, Cichorium intybus* L., *Equisetum arvense*, *Polygonum persicaria, Plantago lanceolata L.*, and *Plantago major* L.. They were sampled one and ten meters away from the edge of the fields. The plants, 70 of each species, were collected during the flowering stage from 1 to 20 July 2014. At the same places soil samples were taken. Cadmium, lead and nickel concentration was determined with inductively coupled plasma-atomic emission spectrometry. All the herbs growing near the wheat field, except for *Cichorium intybus* L., had lead concentration above the limit. For other metals the concentration was below the limits set for dried herbs. Herbs plants growing close to the wheat field had a higher concentration of heavy metals than the same plants growing near the grain maize field. The average concentration of cadmium, lead and nickel in the soil around the fields was much lower than the concentration of the same metals in average, non-contaminated soil. In the soil-plant system the highest accumulation coefficient of heavy metals was for cadmium, then for nickel while the lowest was for lead.

Keywords*: cadmium, lead, nickel, herbs, soil*

Introduction

Heavy metals are among the most harmful environmental pollutants and because of that many publications are devoted to their effects on plants and soil in the areas close to main roads and motorways (Van Bohemen and Van de Laak, 2003; Plesničar and Zupančič, 2005; Jankowski et al., 2015; Malinowska et al., 2015). Yet, not much research has been done to study soil and plant contamination around agricultural fields. Due to the fact that the use of agricultural chemicals is growing, such places are exposed to more and more pollutants. Herbs growing in the natural habitat around farm fields are often used for medical purposes and that is why the accumulation of heavy metals and other pollutants in those plants should be monitored so that they will not pile up in living organisms (Sembratowicz et al., 2009; Ulewicz-Magulska et al., 2009; Luginina and Egoshina, 2013). Not only heavy metals but also pesticide residues in herbs growing in the wild should be monitored because in Europe such plants are used to treat many health conditions (Deng et al., 2004; Gracia-Rico et al., 2007; Arslan et al., 2010).

The aim of this paper is to determine cadmium, lead and nickel concentration in different herb plants, in the aspect of their medicinal use, and in the soil around cultivated fields of wheat and grain maize.

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Materials and methods

The research material included the following species sampled both one and ten meters away from the edges of a winter wheat field and grain maize field: *Achillea millefolium* L., *Cichorium intybus* L., *Equisetum arvense* L., *Polygonum persicaria* L., *Plantago lanceolata* L. and *Plantago major* L. The plant material originated from farms located in the Siedlce County, in the east-central part of Poland. The fields were located far away from main roads and were not affected by dust emission. According to recommendations, both mineral and organic fertilizers but also plant protection products were used for growing the crops. The plants, 70 samples of each species, were gathered during the flowering stage from 1 to 20 July 2014. At the same places, from the root zone, soil samples were also collected. Crops growing in the fields were not tested for heavy metals because that will be the subject of further studies.

The plants: leaves, stalks and inflorescence, were dried at the temperature of 105°C. Next the plant material was ground to particles of 0.25 mm in diameter and 1 g of it was weighed and put into a porcelain crucible. Then the crucible was placed in a muffle furnace for 15 hours to oxidize organic matter at the temperature of 450 ^{0}C. Next 10 ml of hydrochloric acid solution (1:1) was added and the mixture was put into a sand bath to evaporate, to decompose carbonates and to remove silica. After adding 5 ml of 10% hydrochloric acid solution, the contents of the crucible were filtered through a hard filter into a 100 ml volumetric flask and water was added up to the mark. Lead, cadmium and nickel concentration was determined using inductively coupled plasma atomic emission spectrometry (ICP-AES). Soil pH, the value of which ranged from 5.05 to 6.30, was measured with the potentiometric method.

All of the data were statistically analyzed and differences between means were assessed using analysis of variance with the Statistica software, Version 10.0 StatSoft, applied. Tukey's test was used to determine $LSD_{0.05}$ for means that were significantly different (StatSoft, Inc. 2011). The intensity of lead, cadmium and nickel accumulation in the herbs was measured by means of the accumulation coefficient (AC), using the formula (Wesołowski and Radecka, 2003) $AC = c_p / c_s$ (c_p – content of the metal in the plant, c_s –content of the metal in the soil):

AC <0,01	- no accumulation;
AC <0,1	- slight accumulation;
AC -1	- medium accumulation;
AC >1	- high accumulation.

Additionally, correlation coefficient values between the content of lead, cadmium and nickel in the soil and the content of the same metals in the plants growing there were calculated.

Results and discussion

Cadmium concentration in the plants was significantly related to the herb species and to the distance from the farming fields (*Table 1*). The highest average amount of this metal was found in *Polygonum persicaria* plants sampled close to both winter wheat and grain maize fields: 0.256 and 0.220 $mg \cdot kg^{-1}$, respectively. In herbs growing close to the winter wheat field the lowest amount of cadmium was found in *Equisetum arvense* (0.165 $mg \cdot kg^{-1}$), while in the area of the grain maize field the lowest cadmium concentration was in *Plantago major* (0.122 $mg \cdot kg^{-1}$). This concentration was

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significantly higher in herb plants collected at the distance of one meter from the fields than in those growing ten meters from the field. Like in the case of cadmium, there was a relationship between the amount of lead and the herb species as well as between the concentration of this metal and the place from which the plant was collected. Thus, the average lead concentration was two times higher in herbs growing close to the winter field than in those sampled close to the grain maize field. It might have been caused by the fact that wheat was treated with agricultural chemicals more often than maize. The highest average concentration of lead (3.01 mg· kg^{-1}) was found in *Equisetum arvense* growing near the winter wheat field, while in plants sampled around the maize field the highest concentration of lead was in *Plantago major* L. (1.29 mg · kg^{-1}). According to Regulation of the Minister of Health (2003), lead concentration in herbs and in dry herbs used in cooking should not exceed 2 mg·kg^{-1}DM while in the case of cadmium this concentration should not be higher than 0.3 mg·kg^{-1}DM. The amount of cadmium did not exceed the limits but lead concentration was too high in all herb plants growing close to the wheat field, with the exception of *Cichorium intybus* L.

Table 1. *Average heavy metal concentration (mg·kg^{-1}) in selected herb species growing around winter wheat and grain maize fields*

Species	**Cd**			**Pb**			**Ni**		
	1 m	10 m	mean	1m	10 m	mean	1 m	10 m	Mean
				Winter wheat field					
Achillea millefolium	0.251	0.150	0.201	2.05	1.99	2.02	0.850	0.901	0.875
***Cichorium intybus* L.**	0.290	0.200	0.245	1.57	1.43	1.50	1.00	1.75	1.38
Polygonum persicaria	0.320	0.192	0.256	2.09	2.00	2.05	1.81	1.25	1.53
***Plantago lanceolata* L.**	0.190	0.170	0.185	2.81	2.45	2.63	1.05	1.39	1.22
***Plantago major* L.**	0.171	0.181	0.176	2.67	2.05	2.36	1.20	1.35	1.28
Equisetum arvense	0.200	0.130	0.165	3.05	2.96	3.01	0.980	1.05	1.02
mean	0.237	0.171	0.205	2.37	2.15	2.26	1.15	1.28	1.22
LSD$_{0.05}$ for: A-species		0.068			0.266			0.167	
B-distance		0.026			0.103			0.064	
A/B- interaction		0.097			0.377			0.237	
B/A –interaction		0.065			0.251			0.158	
				Grain maize field					
Achillea millefolium	0.220	0.190	0.205	1.02	1.24	1.13	0.901	1.05	0.975
***Cichorium intybus* L.**	0.171	0.161	0.165	1.23	1.12	1.18	1.10	0.891	0.996
Polygonum persicaria	0.252	0.190	0.220	0.998	1.02	1.01	0.95	1.14	1.05
***Plantago lanceolata* L.**	0.190	0.111	0.147	1.03	1.09	1.06	1.03	1.18	1.11
***Plantago major* L.**	0.140	0.101	0.122	1.24	1.34	1.29	1.21	1.02	1.11
Equisetum arvense	0.190	0.140	0.163	0.988	1.17	1.08	0.850	0.980	0.915
mean	0.193	0.147	0.170	1.09	1.16	1.12	1.01	1.04	1.03
LSD$_{0.05}$ for: A-species		0.035			0.109			0.181	
B-distance		0.014			0.042			n.s.	
A/B- interaction		0.050			0.154			0.256	
B/A –interaction		0.033			0.103			0.171	

n.s.- not significant difference, confidence interval $p \leq 0.05$

Nickel accumulation was significantly affected by the plant species only. On average the highest concentration of this metal was in *Polygonum persicaria,* while the lowest was in *Equisetum arvense* and *Achillea millefolium* growing close to both wheat and maize fields. The concentration of nickel in all herb plants ranged from 0.850 to 1.81 mg·kg^{-1}DM. Contrary to those results, Wiechuła et al. (2012) found a few times higher concentration of nickel in *Utrica dioica* L. growing in woods in the Lower Silesia Voivodeship. According to Błoniarz et al. (2008) average nickel concentration in plants usually ranges from 0.1 to 5.0 mg·kg^{-1}DM. In another publication Başgel and Erdemoğlu (2006) say that nickel concentration in *Utrica dioica* L. stands at 3.6 mg·kg^{-1}DM. The differences in the amount of heavy metals in plants can be explained by differences in the mobility of the metal and in the soil pH but this amount also varies in different plant species and in different plant parts (Barazani et al., 2004; Ražic et al., 2008; Salahinejad and Aflaki, 2010).

The highest concentration of cadmium (0.238 mg·kg^{-1}DM) and nickel (1.29 mg·kg^{-1}DM) was found in the biomass of *Polygonum persicaria,* and the highest concentration of lead (2.04 mg·kg^{-1}DM) was in *Equisetum arvense* (*Table 2*). The average concentration of all the metals in the experiment did not exceed natural concentration of those metals in plants, which means that the latter can be used in medicine. Average concentration is not always a good indicator and should be used together with other ways of controlling accumulation of heavy metals to reduce their harmful effects on living organisms. In *Plantago lanceolate*, *Plantago major* and *Equisetum arvense* lead concentration was exceeded by 50 % and in *Polygonum persicaria* by 25% (Regulation of the Minister of Health, 2003). Considering heavy metals, the average highest coefficient of variation was for cadmium (13.20%), and the lowest for nickel Ni (6.34%). Thus, the monitoring should be detailed, among other things taking into account the plant species and its distance from agricultural fields.

Table 2. *Heavy metal concentration (mg·kg^{-1}s.m.) in selected plant species growing close to farming fields*

Species	**Cd**				**Pb**				**Ni**			
	Mean	Min	Max	V (%)	Mean	Min	Max	V (%)	Mean	Min	Max	V (%)
Achillea millefolium	0.203	0.178	0.254	5.42	1.58	1.23	2.15	16.27	0.925	0.874	1.09	3.46
***Cichorium intybus* L.**	0.206	0.185	0.296	5.83	1.34	1.03	1.78	15.60	1.19	1.01	1.85	10.59
Polygonum persicaria	0.238	0.201	0.328	6.73	1.53	1.03	2.56	19.22	1.29	1.10	1.98	9.22
***Plantago lanceolata* L.**	0.166	0.116	0.210	18.67	1.71	1.55	2.99	8.30	0.775	0.712	1.49	2.97
***Plantago major* L.**	0.149	0.100	0.198	16.11	1.83	1.56	2.96	6.61	1.20	1.02	1.44	9.75
Equisetum arvense	0.164	0.100	0.230	18.29	2.04	1.20	3.11	13.19	0.965	0.923	1.15	2.07
mean	0.188	0.147	0.252	11.84	1.67	1.27	2.59	13.20	1.06	0.940	1.50	6.34
$LSD_{0.05}$	0.032				0.201				0.158			

V-coefficient of variation, confidence interval $p \leq 0.05$

Similarly to plants, there was a relationship between soil concentration of the metals and the plant species but also between heavy metal concentration in the soil and the distance of the sampling point from the fields (*Table 3*). The average cadmium, lead and nickel concentration in soil sampled from around the fields was much lower than the corresponding values for non-contaminated soil included by Terelak and Piotrowska (1998) and Kabata-Pendias (1999). Natural cadmium concentration in soil around the world ranges from 0.20 to 1.05 mg$\cdot$kg^{-1}, and is usually not higher that 0.5 mg $\cdot$ kg^{-1} (Alloway, 1990; Kabata-Pendias and Pendias, 1999). In Poland it varies between 0.03 and 0.22 mg $\cdot$ kg^{-1} (Kabata-Pendias 2000). In turn, natural nickel concentration in Polish soil ranges from 0.1 to 173 mg$\cdot$kg^{-1} (Terelak et al., 2002). On average heavy metal concentration in the soil around the grain maize field was higher than that concentration in the soil around the winter wheat field. Nickel concentration in the soil sampled one meter from the fields was significantly higher than in the soil sampled ten meters from the fields while for other metals the relationship was more complicated. There was more lead ten meters away from the wheat field than one meter. For the grain maize field it was different, with more of this metal one meter away from the field than ten meters from the field.

Table 3. *Heavy metal concentration in soil sampled close to a winter wheat field and grain maize field*

Species	Cd			Pb			Ni		
	1 m	10 m	mean	1m	10 m	mean	1 m	10 m	Mean
			Winter wheat field						
Achillea millefolium	0.089	0.087	0.088	5.32	5.87	5.60	1.32	1.01	1.16
***Cichorium intybus* L.**	0.071	0.089	0.080	6.87	6.01	6.44	1.02	1.23	1.13
Polygonum persicaria	0.094	0.098	0.096	6.41	6.87	6.64	1.57	1.42	1.50
***Plantago lanceolata* L.**	0.089	0.074	0.082	5.87	5.21	5.54	1.41	1.02	1.22
***Plantago major* L.**	0.087	0.065	0.076	6.21	6.01	6.11	1.63	1.38	1.46
Equisetum arvense	0.084	0.057	0.070	5.01	6.14	5.58	1.56	1.27	1.42
mean	0.085	0.079	0.082	5.95	6.02	5.98	1.40	1.22	1.31
LSD$_{0.05}$ for: A-species		0.009			0.150			0.240	
B-distance		0.004			0.058			0.093	
A/B- interaction		0.013			0.212			0.227	
B/A –interaction		0.009			0.141			0.340	
			Grain maize field						
Achillea millefolium	0.074	0.090	0.082	4.41	4.78	4.59	1.05	1.21	1.13
***Cichorium intybus* L.**	0.085	0.080	0.083	7.01	6.89	6.95	1.31	1.08	1.20
Polygonum persicaria	0.074	0.068	0.071	7.25	6.54	6.90	1.78	1.51	1.65
***Plantago lanceolata* L.**	0.102	0.089	0.096	6.33	5.36	5.85	1.58	1.31	1.45
***Plantago major* L.**	0.097	0.085	0.091	7.84	6.87	7.36	1.62	1.48	1.55
Equisetum arvense	0.111	0.087	0.099	6.58	6.01	6.30	1.41	1.09	1.25
mean	0.090	0.099	0.087	6.57	6.07	6.32	1.46	1.28	1.37
LSD$_{0.05}$ for: A-species		0.013			0.566			0.227	
B-distance		0.005			0.218			0.087	
A/B-interaction		0.018			0.800			0.214	
B/A–interaction		0.012			0.534			0.321	

confidence interval $p \leq 0.05$

In the case of cadmium a higher concentration of this metals was in the sample points one meter from the wheat field and ten meters in the soil around the maize field. It may indicate that some soil processes affect heavy metals mobility and, at the same time, their accumulation by plants. Finally, the value of correlation coefficient between metal concentration in the soil and in the plants did not show any considerable relation.

However, there were significantly different values of the coefficient of heavy metal accumulation in herbs (*Table 4*). The highest accumulation coefficient was for cadmium, then for nickel and the lowest for lead. According to Kloke et al. (1984) and Kabata-Pendias and Pendias (1999) in the plant-soil system each metal has different absorption properties and, at the same time, different accumulation coefficient. According to those publications cadmium has the highest accumulation coefficient (1-10) while for nickel it is much lower (0.1-1), and for lead it was the lowest (0.01-0.1). For cadmium this coefficient ranged from 1.19 to 4.08 and was much higher in herbs sampled one meter away from the field than ten meters from the field.

Table 4. *Cadmium, lead and nickel accumulation coefficient in herb plants*

Species	Cd			Pb			Ni		
	1 m	10 m	mean	1m	10 m	mean	1 m	10 m	Mean
Winter wheat field									
Achillea millefolium	2.82	1.72	2.27	0.385	0.339	0.362	0.644	0.892	0.768
***Cichorium intybus* L.**	4.08	2.82	3.45	0.238	0.238	0.238	0.980	1.42	1.20
Polygonum persicaria	3.40	1.96	2.68	0.326	0.291	0.309	1.15	0.880	1.02
***Plantago lanceolata* L.**	2.13	2.30	2.22	0.479	0.470	0.475	0.745	1.36	1.05
***Plantago major* L.**	1.97	2.78	2.38	0.430	0.341	0.386	0.736	0.978	0.857
Equisetum arvense	2.38	2.28	2.33	0.609	0.482	0.546	0.628	0.827	0.728
mean	2.80	2.31	2.55	0.411	0.360	0.386	0.814	1.06	0.937
Grain maize field									
Achillea millefolium	2.97	2.11	2.54	0.231	0.259	0.245	0.858	0.868	0.863
***Cichorium intybus* L.**	2.01	2.01	2.01	0.175	0.163	0.169	0.840	0.825	0.833
Polygonum persicaria	3.41	2.79	3.10	0.138	0.156	0.147	0.534	0.755	0.645
***Plantago lanceolata* L.**	1.86	1.25	1.56	0.163	0.203	0.183	0.652	0.901	0.777
***Plantago major* L.**	1.44	1.19	1.32	0.158	0.195	0.177	0.747	0.689	0.718
Equisetum arvense	1.71	1.61	1.66	0.150	0.195	0.173	0.603	0.899	0.751
mean	2.23	1.83	2.03	0.169	0.195	0.182	0.706	0.823	0.764

For lead the values of accumulation coefficient in all herb plants was much higher than the limits, up to six times, ranging from 0.138 to 0.609 despite the fact that lead concentration in the soil was low (*Table 3*). In herbs sampled close to the maize field nickel accumulation coefficient was higher than 1 whereas in herbs growing close to the winter wheat field it was often lower than 1. Values of nickel accumulation coefficient higher than the limits were found in *Cichorium intybus* L., *Polygonum persicaria* and *Plantago lanceolata* L.. Those results show that monitoring of herb plants growing around agricultural fields is necessary if they are to be used in medicine.

Conclusion

Such factors as the species and the distance from the field significantly affected cadmium and lead concentration in the plants. In the case of nickel there was a significant relationship between its concentration and the plant species only.

Comparing cadmium, lead and nickel concentration in the biomass of six herb species it can be said that in the plants sampled close to the winter wheat field lead concentration exceeded the limits in all plants, apart from *Cichorium intybus* L. For the rest of metals the concentration in herb plants was below the limits for dry herbs.

Herbs sampled around the winter wheat field had a higher concentration of heavy metals than those sampled around the grain maize field. In herbs sampled one meter from each field cadmium concentration was significantly higher while for nickel the concentration was higher in plants growing ten meters from the fields. Plants growing one meter from the winter wheat field had a higher concentration of lead than those growing further from the field. In herbs sampled ten meters from the maize field lead concentration was higher than in those growing at the distance of one meter.

The average cadmium, lead and nickel concentration in soil sampled close to each field was much lower than concentration of those metals in average, non-contaminated soil.

In the soil-plant system there was diversity in the vales of accumulation coefficient of heavy metals in plants. The highest value was for cadmium then for nickel and the lowest for lead.

Comparing the result it can be concluded that using agricultural chemicals in growing maize and wheat did not cause a considerable rise in cadmium and nickel concentration outside the fields. Therefore it can be assumed that the concertation of those metals did not exceed the limits in crops growing in the fields. However, lead concentration in plants growing close to the winter wheat field was over the limit. Because of that heavy metal concentration in herbs growing in the natural habitat should be monitored in detail, separately for different species because heavy metal accumulation is significantly related to plant species.

REFERENCES

[1] Alloway, B.J. (1990): Heavy metals in soils. - Blackie, John Wiley & Sons Inc., London, Glasgow, New York, pp. 339.

[2] Arslan, H., Güleryüz, G., Leblebici, Z., Kırmızı, S., Aksoy, A. (2010): *Verbascum bombyciferum* Boiss. (Scrophulariaceae) as possible bio-indicator for the assessment of heavy metals in the environment of Bursa, Turkey. - Environmental Monitoring and Assessment 163: 105-113. doi: 10.1007/s10661-009-0820-1

[3] Barazani, O., Sathiyamoorthy, P., Manandhar, U., Vulkan, R., Golan-Goldhirsh, A. (2004): Heavy metal accumulation by *Nicotiana gluaca* Graham in a solid waste disposal site. - Chemosphere 54: 867-872.

[4] Başgel, S., Erdemoğlu, S.B. (2006): Determination of metal and trace elements in some medicinal herbs and their infusions consumed in Turkey. - Science of the Total Environment 359: 82-89.

[5] Błoniarz, J., Zaręba, S., Wójcik, P. (2008): Evaluation of nickel and chromium in herbs and infusions used for the treatment of cardiovascular. - Bromatologia and Toxicological Chemistry 41(3): 349-353. (in Polish)

[6] Deng, H., Ye, Z.H., Wong, M.H. (2004): Accumulation of lead, zinc, copper and cadmium by 12 wetland plants species thriving in metal-contaminated sites in China. - Environmental Pollution 134: 29-40.

[7] Garcia-Rico, L., Leyva-Perez, J., Jara-Marini, M.E. (2007): Content and daily intake of copper, zinc, lead, cadmium and mercury from dietary supplements in Mexico. - Food and Chemical Toxicology 45: 1599-1605.

[8] Jankowski, K., Ciepiela, G.A., Jankowska, J., Szulc, W., Kolczarek, R., Sosnowski, J., Wiśniewska-Kadżajan, B., Malinowska, E., Radzka, E., Czeluściński, W., Deska, J. (2015): Content of lead and cadmium in aboveground plant organs of grasses growing on the areas adjacent to route of big traffic. - Environmental Science and Pollution Research 22(2): 978-87. doi: 10.1007/s11356-014-3634-9.

[9] Kabata-Pendias, A. (2000): Biogeochemistry of zinc. Zinc the environment ecological and analytical problems. - Papers Scientific Committee „Humans and the Environment" PAN Warsaw 26: 17-24. (in Polish)

[10] Kabata-Pendias, A., Pendias, H. (1999): Biogeochemistry of trace elements. PWN, Warsaw pp 398. (in Polish)

[11] Kloke, A., Sauerbeck, D.R., Vetter, H. (1984): The contamination of plants and soils with heavy metals and the transport of metals in the terrestrial food chains. In: Nriagu J.O. (eds.), Changing metal cycles and human health. Springer Verlag, Berlin, Heidelberg, New York, Tokyo, 113-141.

[12] Luginina, E.A., Egoshina, T.L. (2013): The peculiarities of heavy metals accumulation by wild medicinal and fruit plants. Annals of Warsaw University of Life Sciences, Agriculture 61: 97-103.

[13] Malinowska, E., Jankowski, K., Sosnowski, J., Wiśniewska-Kadżajan, B. (2015): Pesticide residues in cereal crop grains in Poland in 2013. - Environmental Monitoring and Assessment 187: 329. doi: 10.1007/s10661-015-4566-7

[14] Plesničar, A., Zupančič, N. (2005): Heavy metal contamination of roadside soils along Lujbljana-Obrežje highway. - RMZ-Materials and Geoenvironment. 52(2): 403-418.

[15] Ražic, S., Đogo, S., Slavković, L. (2008): Investigation on bioavailability of some essential and toxic elements in medicinal herbs. - Journal of Natural Medicines 62: 340-344.

[16] Regulation of the Minister of Health of 13 January 2003 on maximum levels of chemical and biological contaminants that may be present in food, food ingredients, allowed additional substances, substances that help the processing or on food. (Dz.U.Nr 37, poz. 326, zał. 1). (in Polish)

[17] Salahinejad, M., Aflaki, F. (2010): Toxic and essential mineral elements content of black tea leaves and their tea infusions consumed in Iran. - Biological Trace Elements Research 134: 109-117.

[18] Sembratowicz, I., Rusinek, E., Ognik, K., Truchliński, J. (2009): Concentrations of trace elements and heavy metals at selected medicinal plants harvested in two vegetation periods. - Herba Polonica 55(1): 22-28. (in Polish)

[19] StatSoft, Inc. 2011 STATISTICA (data analysis software system), version 10. www.statsoft.com

[20] Terelak, H., Motowicka-Terelak, T., Struczyńska, T., Pietruch, Cz. (2002): Trace elements (Cd, Cu, Ni, Pb, Zn) in soils of the Polish agricultural land. Library Environmental Monitoring, Inspection of Environmental Protection, Warsaw, 69. (in Polish)

[21] Terelak, H., Piotrowska, M. (1998): The lead content in the soils of farmland Polish and selected provinces. Lead the environment ecological and analytical problems. - Papers Scientific Committee „Humans and the Environment" PAN Warsaw 21: 19-24.

[22] Ulewicz-Magulska, B., Baranowska, M., Wesołowski, M. (2009): Assessment of the content of copper, manganese, zinc and iron in herbs and leaves of medicinal plants. - Bromatologia and Toxicological Chemistry 42: 815-821. (in Polish)

[23] Van Bohemen, H.D., Van de Laak, W.H.J. (2003): The influence of road infrastructure and traffic on soil, water, and air quality. - Environmental Management 31(1): 50-68.

[24] Wesołowski, M., Radecka, I. (2003): Medicinal plants. Elemental composition, source of minerals for plants, indicators of environmental contamination by heavy metals. - Polish Pharmacy 59(20): 911-919. (in Polish)

[25] Wiechuła, D., Loska, K., Jonderko, W. (2012): Assessment of pollution nickel stinging nettle (*Urtica dioica* L.) from the Silesian region. - Bromatologia and Toxicological Chemistry 45: 20-25. (in Polish)

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IMPACT OF LARGE SCALE DAM CONSTRUCTION ON MOVEMENT CORRIDORS OF MAMMALS IN ARTVIN, NORTH-EASTERN TURKEY

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Abstract. The long term viability of many animals depends on maintaining connection between their subpopulations. However, man-made infrastructures can severely damage the connection of subpopulations through fragmenting prime habitats. Our goal in this study is to investigate impact of a series of dam constructions on the potential movement corridors of the wild goat (*Capra aegragus*), Eurasian lynx (*Lynx lynx*), and the golden jackal (*Canis aureus*) in northeastern Turkey. We used one of the most common approaches, least cost corridor modeling to determine movement corridors of target species for before and after dam constructions and compared the differences in physical structures, habitat suitability and total cost of movement in corridors. We found that constructions of dams would negatively affect subpopulations of target species and their movement corridors. Some subpopulations are expected to lose suitable habitat to flooding while others to be divided into distant, smaller units once the construction is completed. Moreover, resistance to movement will increase due to a decline in habitat suitability and an increase in total cost of movement. In brief, dams and their reservoirs in northeastern Turkey will likely become serious barriers and considerably constrain movements of target species within and between subpopulations.

Keywords: *animal movement, corridors, dam construction, fragmentation, least cost corridor modeling, Turkey*

Introduction

Habitat loss and fragmentation are among major threats facing biodiversity (Rosenberg et al., 1997; Myers et al., 2000; Macdonald, 2003; Jongman, 2004). Such processes usually lead to isolated populations that have a higher risk of extinction due to higher demographic stochasticity and increased rates of inbreeding (Mills and Smouse, 1994; Riley et al., 2006; Sawyer et al., 2011). Impacts of fragmentation on isolated populations can be mitigated by providing connectivity with corridors (Beier et al., 2006; Crooks and Sanjayan, 2006; Sawyer et al., 2011). Corridors are “natural vegetation strips running between the reserves” (Bentley and Catteral, 1997; Beier and Noss, 1998). They increase colonization (Hale et al., 2001), promote movement and dispersal (Briers, 2002; Beier et al., 2008; LaPoint et al., 2013) and facilitate gene flow (Beier, 2011; LaPoint et al., 2013; Saura et al., 2014).

Although, functions of corridors are well defined within the metapopulation concept of conservation biology, there are some doubts about their efficiency due to limited empirical research (Shkedy and Saltz, 2000; Niemela, 2001; Fagan and Calabrese, 2006; Parks et al., 2013). Early studies about efficiency of corridors were mostly based on comparison of individual densities between connected and unconnected habitat

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patches (Mac Clintock et al., 1977; Merriam and Lanove, 1990; Machtons et al., 1996; Haddad and Baum, 1999). However, later studies do not only rely on observations, but also demonstrate gene flow occurring between isolated populations through corridors (Manel et al., 2003; Proctor et al., 2004; Dixon et al., 2006; Riley et al., 2006). In this context, corridors have become a fundamental component to supply long term viability for populations (Beier, 1993; Fahrig and Merriam, 1994; Noss et al., 1996; Mateo-Sanchez et al., 2014). Therefore, designing and retaining corridors has had high priority in biodiversity conservation in recent years (Kusak et al., 2009).

Conservation organizations and agencies also focus on the significance of corridors for wildlife conservation and corridors are becoming an integral parts of conservation plans (Anderson and Jenkins, 2006; Morrison and Boyce, 2009). Such corridors may be especially critical for large mammals which are highly susceptible to the habitat fragmentation (Dixon et al., 2006). Large mammals are wide ranging animals and they require large home ranges and accomplish long distance dispersals. Hence, they are more likely to use corridors for movement (Harrison and Voller, 1998). However in some cases, human induced developments can irreversibly damage existing movement corridors of animals.

We present here the possible effects of a large scale hydroelectric power project along River Çoruh in northeastern Turkey on wildlife connectivity (*Fig. 1*). A series of dams are either planned, under construction or have been completed along this fast flowing river. These dam constructions may create barriers for wildlife by severing habitat connectivity between a numbers of sites. In this study, our aim is to investigate the possible impacts of this series of constructions on potential movement corridors of the wild or bezoar goat (*Capra aegragus*), Eurasian lynx (*Lynx lynx*), and the golden jackal (*Canis aureus*). We modeled potential movement corridors of these target species as before and after dam construction using least cost modeling. Subsequently, differences in movement corridors for before and after constructions were investigated and finally, a general conclusion was drawn to emphasize major consequences of the impact of dam constructions and some suggestions were made to mitigate these impacts.

Material and methods

Study area

Our study area is in northeastern Turkey and lies in between latitude 41.15 to 42.60 and longitude 40.40 to 41.55 (*Fig. 1a*). The area is about 10,000 km² and encompasses parts of Artvin and northern districts of Erzurum including the River Çoruh valley. The study area has several remarkable geographic features. It is characterized by high mountains, broad plateaus and deep valleys, and has a vast altitude range of roughly between 65 to 3500m (*Fig. 1b*). River Çoruh is approximately 431 km long, of which 410 km lies within Turkey. It flows in a deep valley, flanked by some of the tallest mountains in the country (e.g. Vercenik Peak of 3711 m) and empties into the Black Sea around Batumi, Georgia (*Fig. 1*). This varied topography is covered by humid temperate forest in the north and dry high mountain steppe-meadows in the south (Bilgin et al., 2006; unpublished data). Most common stand forming species are Scots pine (*Pinus sylvestris*), oriental spruce (*Picea orientalis*), Black Sea fir (*Abies nordmannia*), oriental beech (*Fagus orientalis*), alders (*Alnus* spp.) and oaks (*Quercus* spp.) Moreover, Çoruh valley that bisects the area roughly in the middle is dominated by a relict Mediterranean type vegetation including maquis elements such as *Arbutus andrachne*, *Laurus nobilis*,

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Rhus coriaria, *Ruscus aculeatus* and stone pine (*Pinus pinea*) (Eminağaoğlu and Anşin, 2003). These heterogeneous communities provide habitat for many native wildlife species such as brown bear, wild goat, Alpine chamois, Eurasian lynx, grey wolf, golden jackal, red fox, stone marten and possibly even leopard (Ambarlı and Bilgin, 2008; Gundogdu and Ogurlu, 2009; Ambarlı et al., 2010; Gokturk et al., 2011; Ambarlı and Bilgin, 2012; Sekercioglu, 2012).

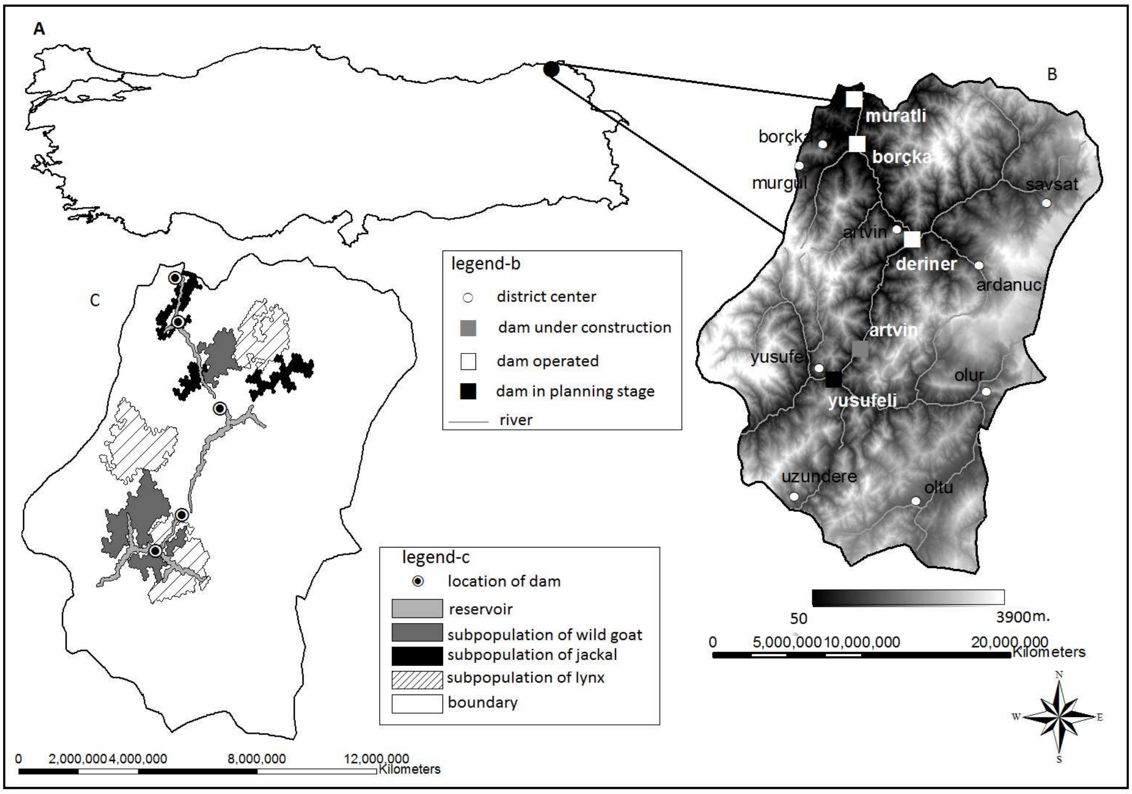

Figure 1. *a. Geographic location of the study area; b. location and condition of dams with digital elevation model of the area; c dam reservoirs and local subpopulations of target species*

Modelled species

For modeling, we selected a diurnal ungulate, a social canid and a solitary felid since they represent different functional groups and/or their occurrences only partially overlap within the elevational gradient of the area. This allows us to evaluate the effects of dams for a broad altitude range and a wider range of ecological requirements, although it may not always be possible to extend findings to other species due to differences in spatial ecology or behavior.

Wild goat is a medium-sized, sexually-dimorphic ungulate that occurs throughout western Asia. It inhabits mountainous areas, where there is a mixture of rocky outcrops and shrubby vegetation or dry conifer woodland (Shackelton, 1997). For most of the year sexes remain separate, with females and their young forming stable groups, except during the mating season in late fall, when males can travel long distances to join receptive females (Turan, 1984; Gundogdu, 2011). In the study area, they range from about 500 m. to 2500 m. (Bilgin et al., 2006). Most ungulates require good visibility on a horizontal plane and a moderate amount of cover for crossing unknown territory

(Kintsch and Cramer, 2011). Cliff-dwelling species, such as ibex, have been observed to use steep terrain while travelling between core zones since they rely on their ability to move fast in such terrain in face of danger (e.g. Shkedy and Saltz, 2000). While passing through overpasses or underpasses, they prefer a natural substrate and clear lines of sight from one end to the other (Kintsch and Cramer, 2011). This suggests wild goats stay away from paved surfaces, especially when there is no escape terrain in close proximity. They probably also avoid constrained spaces such as narrow foot bridges.

Golden Jackal (hereafter "jackal") is a medium-sized canid that occurs in south and west Asia, eastern Africa and southeastern Europe. It is an adaptable, opportunistic and social species that feeds on small mammals, birds or carrion. Jackals can trot long distances in search of food and will venture into human dominated landscapes (Turan, 1984; Jhala and Moehlman, 2004). In the study area, they occur from sea level up to 1200 m. altitude, probably constrained by the presence of wolves at the upper limit of their range (Bilgin et al., 2006; Ambarlı and Bilgin, 2012). Most canids make use of paved roads, including man-made bridges, especially under cover of darkness (Kintsch and Cramer, 2011). For example, coyotes are known to use a variety of structure types, including culverts, underpasses and bridges (Way, 2009). Similarly, Blanco et al. (2005) showed that Spanish wolves regularly used vehicle bridges to cross a highway and a river. Jackals are assumed to behave similarly; in fact, they are commonly found as road casualties on roads and bridges in Turkey (unpublished data). This is supported by the fact that road mortality made up almost half of all recorded deaths in Italy during 1984-2011 (Lapini et al., 2011), and that they are the most common road killed species in India besides dogs (Jhala and Moehlman, 2004).

Eurasian lynx (hereafter "lynx") is a medium-sized felid with a solitary existence, except when females are accompanied by dependent young. It is distributed in suitable habitats over most of northern Eurasia, typically preying on small ungulates or lagomorphs (Nowell and Jackson, 1996). Subadult males can move up to 150 km. during natal dispersal (Schmidt, 1998; Samelius et al., 2011). In our study area, the species is associated with open woodland or scrubland between 800 m. and 2000 m. (Bilgin et al., 2006; Ambarlı et al., 2010). On the other hand, lynx require some sort of cover to cross unfamiliar territory. However, it can easily climb trees and do not hesitate to use logs across streams as bridges.

Habitat suitability models

The habitat suitability models for jackal, lynx and wild goat were originally produced within the scope of another project covering the Turkish Lesser Caucasus (Bilgin et al., 2006). Prior to the corridor analysis, we revised and refined these models by using information obtained from new species records. The suitability models were built using digital layers of elevation, slope, land cover, human population density and settlement patterns. The land cover surface of 20 (twenty) classes, was produced by the classification of Landsat TM images with the aid of terrain layers and digitized forest stand maps. 90-m pixel resolution Shuttle Radar Topographic Mission (SRTM) digital elevation model was used for slope and elevation. The human population density layer was based on the census data and distance to settlements was calculated in order to define settlement patterns.

Using presence records, we identified attributes of the variables affecting the distribution of target species. This information, together with that provided by wildlife experts, was then used in building a fuzzy relationship function describing the association for each environmental variable with habitat suitability for the target species (*Table 1*). Fuzzy suitability layers were then produced, by applying these fuzzy distribution functions. The suitability values in these layers range from 0.00 (least suitable) to 1.00 (most suitable). Wildlife experts then ranked the fuzzy suitability layers according to their importance for the target species, in order to assign a weight for each layer. Ranking is a popular decision support aid (Store and Kangas, 2001; Clevenger et al., 2002), and provides a flexible way for determining relative weights of the variables in habitat models (Kovacs et al., 2004). Our ranking relied on personal experience, ecological requirements of target species (LaRue and Nielsen, 2008) and correlation of modeling variables. Additionally, we generated a constraint layer for each of the target species. We then calculated the weighted linear combination of fuzzy layers and this constraint layer, using the modeling equation:

$$HSM = \sum \mathrm{Wi}(ffsi) * (cl) \qquad \text{(Eq.1)}$$

where *HSM* is the habitat suitability model, W_i is weight of i'th variable (fuzzy suitability layer), ffs_i is the i'th fuzzy suitability layer and *cl* is constraint layer. Values in the resulting layers ranged between 0.00 and 1.00, and indicated the probability of occurrence of each species, limited by the impact of constraints. These habitat suitability layers were used as a basis for the least cost corridor modeling procedure.

Table 1. *Fuzzy suitability functions and weights for environmental variables used to produce of habitat suitability models of target species*

Variable	Fuzzy Functions	Weight
Golden Jackal		
Elevation (dem)	dem=0m, 1; dem>1500m, 0; 0m<dem<1500m, decreasing sigmoid distribution range between 0 and 1	0.20
Slope (slp)	0°≤slp<35°,1; slp=45, 0.5; slp≥35° decreasing J- shaped distribution range between 0 and 1	0.10
Land Cover (ld.c)	snow, water, 0; mixed humid coniferous broad leafed forests (10 classes), 0.7; damaged oak forest, semi-drought oak forest, 1; agriculture, meadow-alpine meadow, open areas, sparse vegetation, decreasing sigmoid distribution, 2 km away from vegetation= 0,	0.30
Distance to Settlements (dis.stt)	dis.stt <250m, 0; dis.stt =1500m, 1; dis.stt ≥250, increasing sigmoid distribution range between 0 and 1	0.20
Population Density (pop)	pop>1000, 0; pop=0, 1; 0≤pop≤1000, decreasing sigmoid distribution range between 0 and 1	0.20
Wild Goat		
Elevation (dem)	dem<500m and dem>3000m, 0; 1000m<dem<2000m, 1; 500m≤dem≤3000m, left skewed normal distribution range between 0 and 1	0.15

Slope (slp)	slp<20°, 0; slp=35°, 1; 20°≤slp≤35°, sigmoid distribution range between 0 and 1	0.30
Land Cover (ld.c)	snow, water, agriculture, mixed humid coniferous broad leafed forests (10 classes), 0; meadow-alpine meadow, open areas, sparse vegetation, 1; damaged oak forest, semi-drought oak forest, oak-juniper forest, 0.5	0.30
Distance to Settlements (dis.stt)	dis.stt <1500m, 0; dis.stt =500m, 1; 1500≤ dis.stt ≤5000, increasing sigmoid distribution range between 0 and 1	0.10
Population Density (pop)	pop>600, 0; pop=0, 1; 0≤pop≤600, decreasing sigmoid distribution range between 0 and 1	0.15
Lynx		
Elevation (dem)	dem>2500, 0; 1000<dem<2000, 1; 0≤dem≤2500, normal distribution range between 0 and 1	0.10
Slope (slp)	25°≤slp≤35°, 1; slp=45° and slp= 10°, 0.5; 0°≤slp≤90°, symmetric J-distribution range between 0 and 1	0.20
Land Cover (ld.c)	snow, water, agriculture, open areas, sparse vegetation, meadow-alpine meadow, 0; , mixed humid coniferous broad leafed forests (13 classes), 1	0.30
Distance to Settlements (dis.stt)	dis.stt <1000m, 0; dis.stt =3500m, 1; dis.stt≥1000, increasing sigmoid distribution range between 0 and 1	0.20
Population Density (pop)	Pop=0, 1; pop=100, 0; pop≥,0 decreasing sigmoid distribution range between 0 and 1	0.20

Local subpopulations

We determined local subpopulation cores for jackal, wild goat and lynx using their respective suitability models, and corroborated the resulting maps with additional presence data. Areas with high habitat suitability values, especially clusters of such suitable areas indicate sites with high densities of target species (WHCWG, 2010; Beier et al., 2011). Based on our knowledge of each species' occurrence in the study area, we defined a suitability value of 0.90, 0.80 and 0.95 as thresholds for jackal, wild goat and lynx, respectively and assigned planning units with an average suitability value higher than these thresholds as core areas for subpopulations. These thresholds resulted in the designation of three local subpopulations for jackal and wild goat each, and four for lynx (*Fig. 1c*). These core areas coincide with the approximate locations of actual local subpopulation cores.

Of the study species, both wild goat and jackal are largely restricted to the large valley formed by River Coruh, although the range of the former extends to southwest and of the latter to northwest. The lynx occurs more widely. Natural movements of all three species in and out of the study area are restricted by very high mountains and/or unfavorable habitats that surround it.

Protected areas

In the study area, there are nine legal protected areas of which four are national parks, two are wildlife reserves, two are strict nature reserves and one is a nature park (*Fig. 2*). Their corresponding layer was included in corridor modeling since protected areas are far from human pressures and more sheltered than unprotected landscape. They act as refuges and provide survival for target species. Moreover, they enable connectivity between habitat patches behaving like stepping stone corridors. Thus, they facilitate animal dispersal and movements. With these functions, protected areas increase habitat suitability for target species and provide priority sites for corridor design.

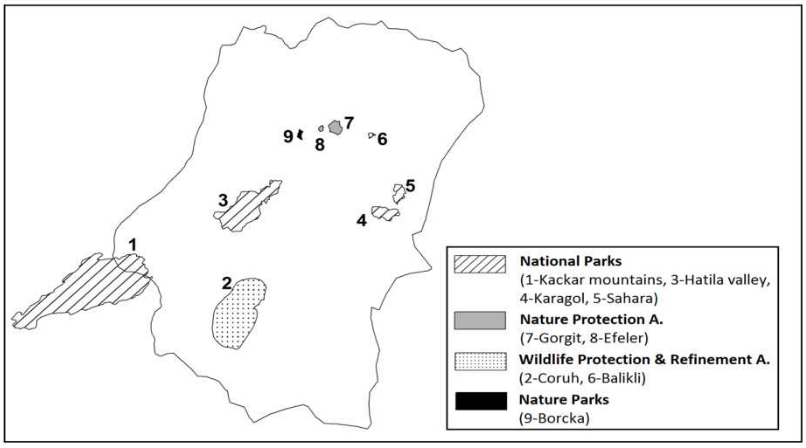

Figure 2. *The map showing distribution and status of legally established protected areas*

We expressed functions related to protected areas using protection coefficients. These coefficients were based on the actual status of protection at those sites. Accordingly, national parks with better protection were assigned 1 for protection coefficient, while a value of 0.66 was assigned for other protected areas. The resulting layer reflected level of protection and functions of protected areas as refuges and dispersal corridors for target species.

Dams and reservoirs

A series of five dams have been planned on River Çoruh (*Fig. 1c*), with constructions starting from the lower reaches and proceeding upstream (Sucu and Dinç, 2008). The first one, Muratli Dam, lies to the north of our study area. Its construction was completed and is operational since 2005 (*Fig. 1b*). Another two, Borçka and Deriner Dams are respectively located around centre of Borçka and 5 km south of the town of Artvin. They were both completed and began to hold water in 2012 (*Fig. 1b*). A fourth dam planned to be built is the Artvin Dam, which is still in the planning stage, and there is no construction activity yet (*Fig. 1b*). Finally, the Yusufeli Dam is under construction since 2013 and is planned to be operational by 2018 (*Fig. 1b*). Although, not all of these dams had begun to hold water, we produced a dam layer assuming their reservoirs filled with water. A digital elevation model was used as the primary input to generate this dam layer and the extent of reservoirs was determined by reclassifying elevation model along the river according to the maximum water level for each dam. The dam layer was included in corridor modeling analysis only for the situation after dam construction and used as an absolute barrier for target species.

Least cost corridor modeling

Movement corridors were designed using least cost modeling before and after dam construction between each local subpopulation of a target species. Least cost modeling is the most widely used approach to design movement corridors or movement paths for wildlife species (LaRue and Nielsen, 2008; Sawyer et al., 2011). This approach delineates the most likely used routes by calculating the lowest cumulative cost of movement between two patches of suitable habitat (Verbeylen et al., 2003; Larkin et al., 2004; Chetkiewicz and Boyce, 2009). Unlike least cost paths, least cost corridors do not limit the width to a single pixel resolution (Verbeylen et al., 2003; Beier et al., 2009); instead, they include the most permeable slice of the area (Sawyer et al., 2011). In other words, they encompass the most suitable habitat to move with least resistance and fewest barriers (Larkin et al., 2004; Larue and Nielsen, 2008; Poor et al., 2012; Zeller et al., 2012). Therefore, least cost corridor modeling is an appropriate approach to determine the best theoretical wildlife corridors for target species both before and after dam constructions.

Analyses were performed using corridor planning tool of Idrisi Andes. The tool applies cost distance procedure and builds least cost corridors on measures of suitability for movement. It firstly constitutes a movement suitability surface. The surface consists of an aggregation of all factors effecting resistance to/ease of movement, such as habitat suitability, presence of barriers or other resistance structures, or protection. This surface is used to calculate the cost of travel from one point to another and a least cost path is delineated. This is the route with least resistance. Afterwards, a cost distance is calculated from the least cost path and the mean relationship between cost distance and spatial distance is determined to assign a cost threshold for designing movement corridors (*Fig. 3*).

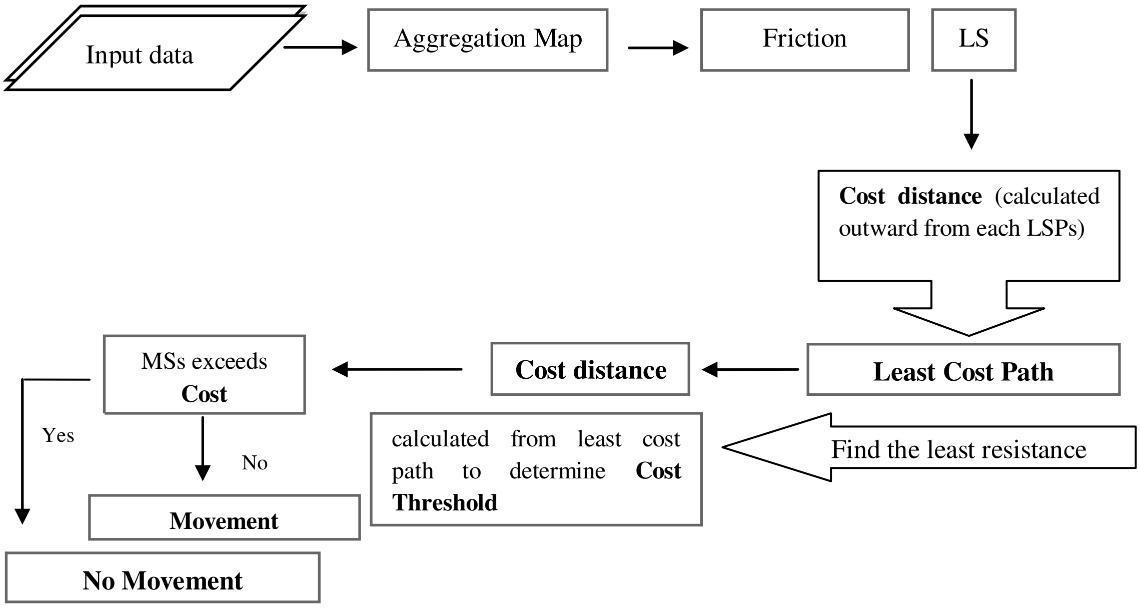

Figure 3. *Flow chart for the procedure of least cost movement corridors, (LSP) local subpopulation and (MS) Movement suitability (Aggregtion map)*

We designed the width of movement corridors to be between 1 and 2.5 km to enable unobstructed movement of target species. Afterwards, length, area and perimeter of corridors were calculated for before and after conditions and changes were evaluated. Moreover, differences in habitat suitability of movement corridors were examined by detecting total suitability of each corridor and cost of moving in corridors were compared for before and after constructions.

Results

Our findings indicate that local subpopulations of target species and their movement corridors are negatively affected from dam constructions. The first prominent adverse effects of dams are that habitats of some local subpopulations are going to be flooded while some others are split into smaller discrete subpopulations (*Fig. 4a, Fig. 5a* and *Fig. 6a*). Almost half of the one local subpopulation of golden jackal (population-1) has already remained under reservoir of the Muratli Dam, and one of the local subpopulation of wild goat (population-3) will be flooded by Yusufeli Dam in the near future (2018) (*Fig. 4d,e,f* and *Fig. 5d,e,f*). Moreover, one golden jackal subpopulation (population-2) was divided into two small discrete subpopulations when Borçka Dam became operational in 2012 (*Fig. 4d, e* and *f*) and one subpopulation of lynx (population-2) will be fragmented as well after Yusufeli Dam is completed (*Fig. 6d,e,f*). Several local subpopulations of target species become smaller after dam construction by either remaining under water or being fragmented with reservoirs.

A further finding is that some movement corridors of target species disappear after dam construction due to reservoirs acting as absolute barriers and preventing formation of movement corridors for target species. This is mostly observed for lynx, for which movement corridors between population 1 and 2, population 1 and 4, and population 3 and 4 could not be generated after completion of the dams. This means that lynx subpopulations remain isolated and thus, movements between subpopulations become impossible (*Fig. 6*). Wild goat sub-populations remain better connected than the lynx since formation of movement corridors was only blocked between populations 1 and 3 (*Fig. 5b,e*). In contrast, corridors for golden jackal are not much affected by dam constructions. The movement corridors formed are of similar configuration, although some now connect even smaller sub-populations formed after fragmentation of original suitable habitat (*Fig. 4d, f*).

Another observed effect of dams on movement corridors is related with the configuration of corridors. Reservoirs lead to changes in the physical parameters of some corridors such as area, length and perimeter (*Table 2*). Such changes are at moderate levels for golden jackal (only to 14.62% decline in corridor area). However, declines in this parameter reach serious levels for wild goat (45.57% decline) and lynx (57.95% decline). Movement corridors for the latter two species are either completely removed or some branches of corridors cannot be constituted following dam constructions – in some cases, a new, completely different corridor is generated (*Fig. 5* and *Fig. 6*). As a result, roughly half of the movement corridors of wild goat and lynx are lost after all dam reservoirs are filled with water. Overall, the total costs of movement increase slightly for jackal, moderately for wild goat, and a great deal for lynx (*Table 3*). Similarly, a decline in habitat suitability of corridors is evident, with losses of relative suitability reaching 26.36%, 41.45% and 112.12% respectively for golden jackal, wild goat and lynx (*Table 3*).

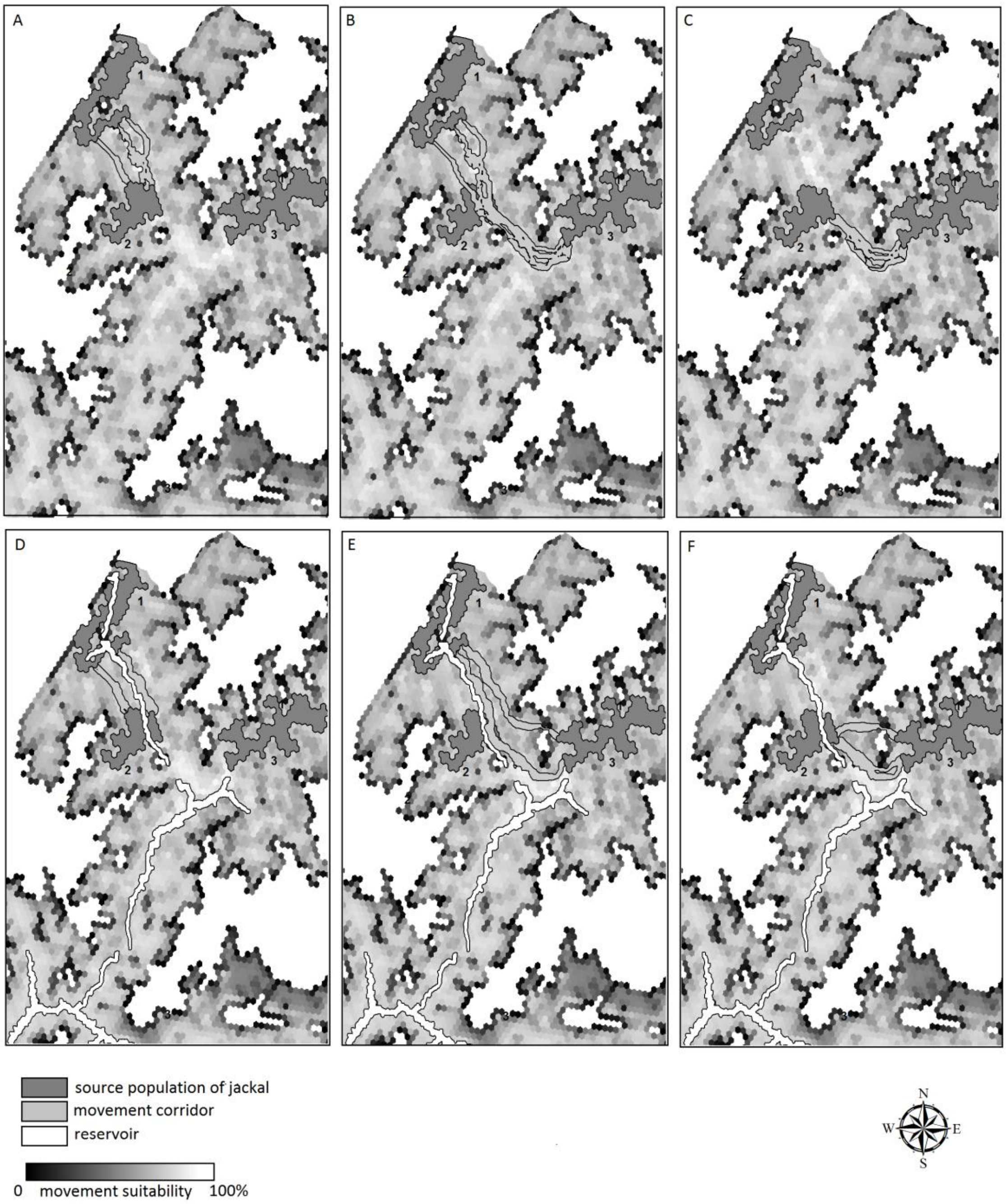

Figure 4. *Movement corridors of golden jackal before (upper row) and after (lower row) dam construction, between populations 1-2 (left column), between populations 1-3 (middle column), and between populations 2-3 (right column) respectively*

Dam constructions will not only change the route and structure of movement corridors, but also remove prime habitats of target species. An estimated 80.58% (137 km²), 65.24% (111 km²) and 28.80% (49 km²) of the built or planned reservoir area is prime habitat of golden jackal, wild goat and lynx respectively, and will disappear after dam constructions. Moreover, the cost of movement (measured as a function of distance traveled and habitat suitability) increases up to 50% for some corridors after dam building (*Table 3*). This means that linkages between subpopulations are expected to become weaker compared to when dams were non-existent.

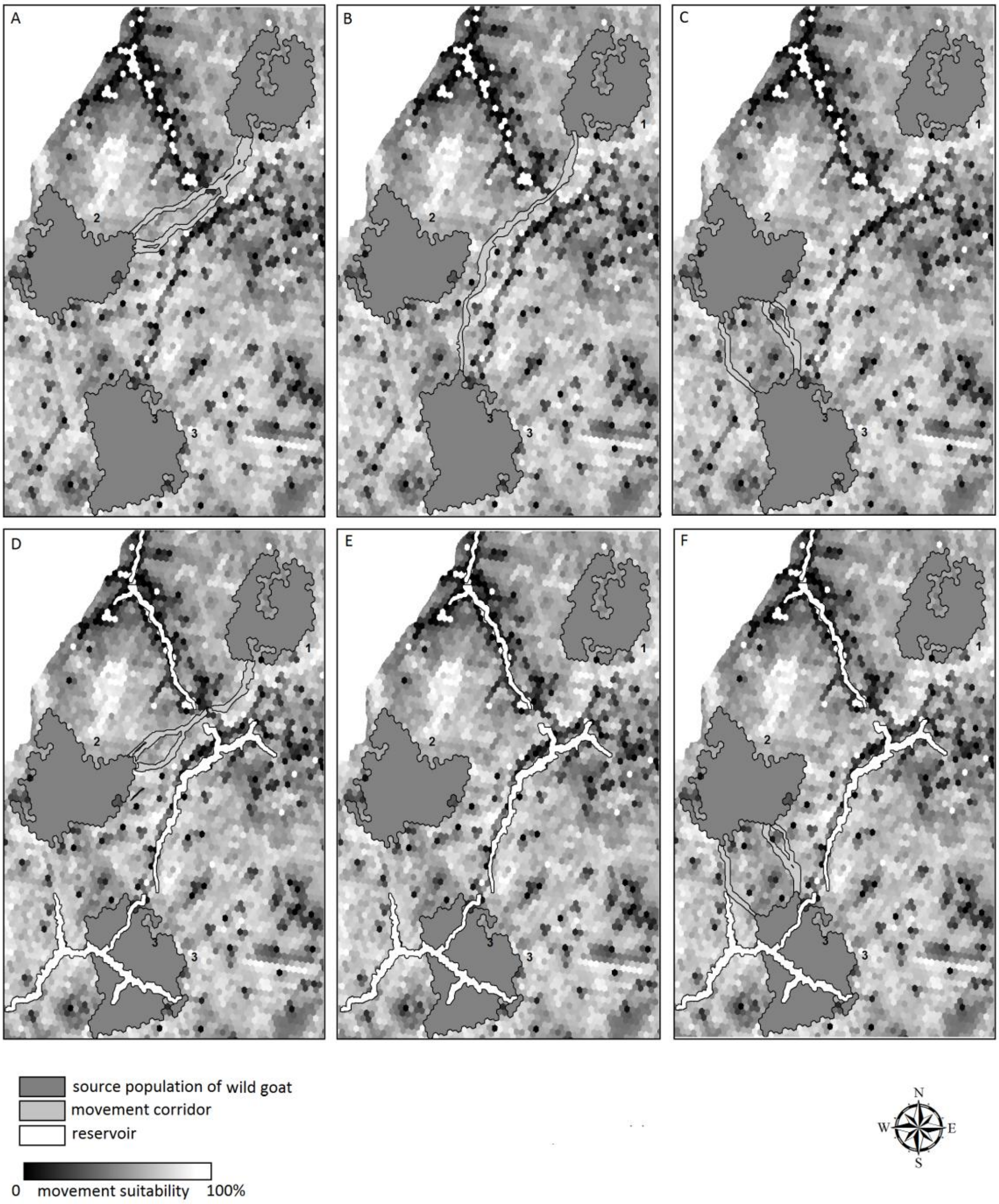

Figure 5. *Movement corridors of wild goat before (upper row) and after (lower row) dam construction, between populations 1-2 (left column), 1-3 (middle column) and 2-3 (right column) respectively*

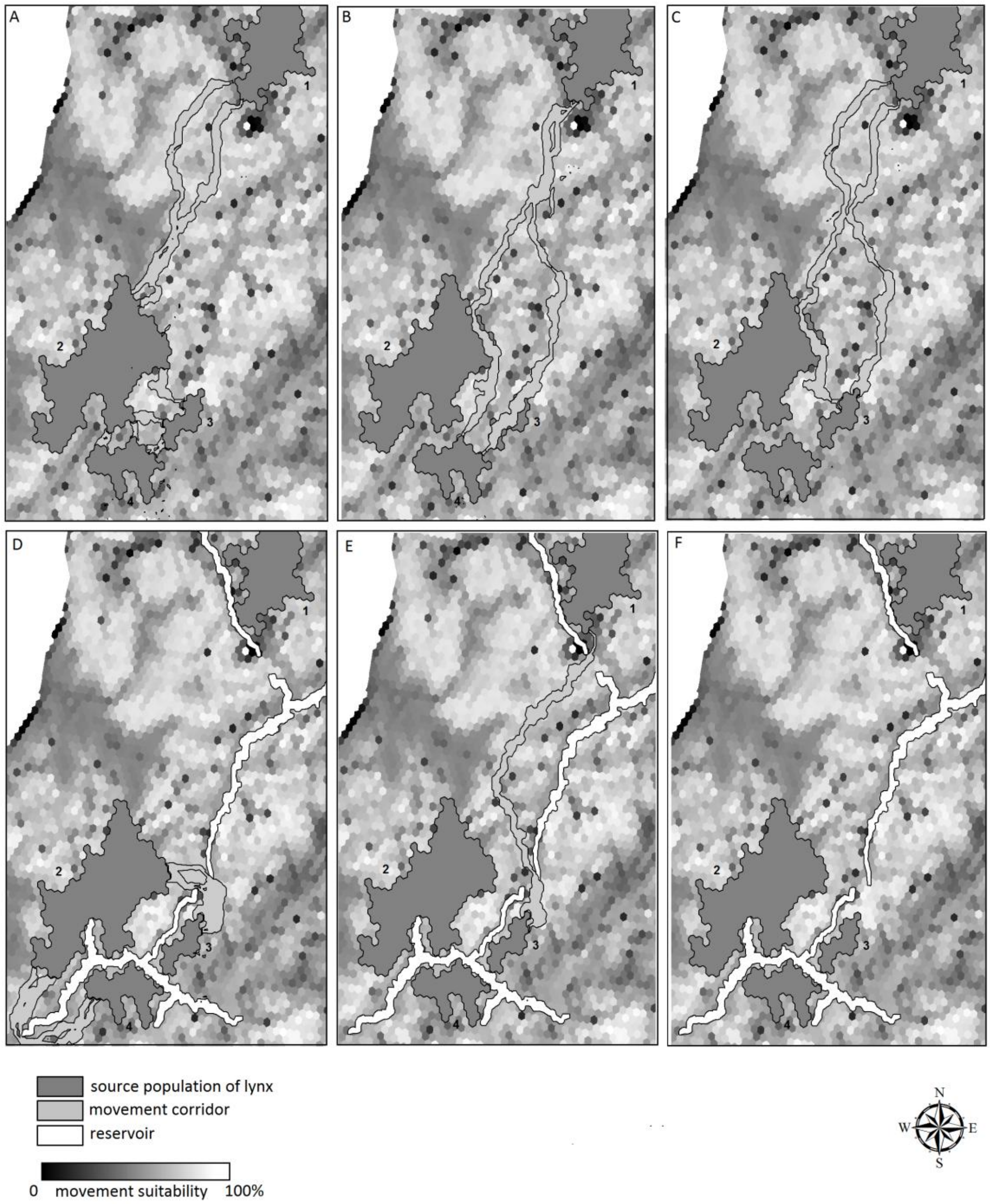

Figure 6. *Movement corridors of lynx before (upper row) and after (lower row) dam construction, between populations 1-2, 2-3, 2-4 and 3-4 (left column), between populations 1-3 (middle column), and between populations 1-4 (right column), respectively*

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Table 2. *Total decrease in the physical structures of movement corridors according to the situations before and after dam constructions*

Corridor between		Area (km^2)	Length (km)	Perimeter (km)
		Golden Jackal		
Before	LSP 1-2 (three branch)	34.18	32.54	108.15
	LSP 1-3 (three branch)	95.89	104.55	272.22
	LSP 2-3 (three branch)	43.78	51.6	120.26
After	LSP 1-2 (three branch)	30.86	29.90	76.40
	LSP 1-3 (two branch)	76.80	57.90	175.90
	LSP 2-3 (three branch)	40.78	99.67	42.04
% decrease in total		14.62	0.65	41.21
		Wild Goat		
Before	LSP 1-2 (three branch)	62.82	66.8	164.92
	LSP 1-3 (one branch)	51.37	131.33	49.5
	LSP 2-3 (three branch)	37.79	116.28	39.7
After	LSP 1-2 (three branch)	44.93	48.6	91.76
	LSP 1-3 (no branch)	0.00	0.00	0.00
	LSP 2-3 (three branch)	37.79	116.28	39.7
% decrease in total		45.57	47.56	48.27
		Lynx		
Before	LSP 1-2 (three branch)	77.71	69.57	191.79
	LSP 1-3 (three branch)	118.14	94.9	310
	LSP 1-4 (three branch)	124.41	118.5	303.19
	LSP 2-3 (two branch)	14.13	9.6	49.75
	LSP 2-4 (three branch)	4.77	6.9	27.55
	LSP 3-4 (two branch)	3.28	6.7	27.52
After	LSP 1-2 (no branch)	0.00	0.00	0.00
	LSP 1-3 (three branch)	61.8	48.5	157.18
	LSP 1-4 (no branch)	0.00	0.00	0.00
	LSP 2-3 (three branch)	34.59	73.18	41.7
	LSP 2-4 (two branch)	47.61	47.4	116.15
	LSP 3-4 (no branch)	0.00	0.00	0.00
% decrease in total		57.95	44.78	63.37

“LSP” is local sub-population

Table 3. *Comparisons of habitat suitability and total cost of movement in movement corridors for before and after dam constructions*

Corridor between	Habitat suitability		Total cost in movement	
Golden Jackal	Before	After	Before	After
LSP 1-2	7731.89	5824.51	339026.84	433973.537
LSP 1-3	22145.22	16045.43	3129723.03	3765873.71
LSP 2-3	9544.19	7161.14	615990.69	764980.66
Total change	21.55 % decrease		26.36 % increase	
Wild Goat				
LSP 1-2	11688.39	9330.2	3649136	3763724.34
LSP 1-3	9087.03	no corridors	3641010.09	no corridors
LSP 2-3	6838.45	6838.45	858730.7	858730.7
Total change	2.54 % decrease		41.45 % decrease	
Lynx				
LSP 1-2	18057.45	no corridors	2666289.87	no corridors
LSP 1-3	28010.14	14339.84	2199091.23	4046865.58
LSP 1-4	29646.78	no corridors	4811656.2	no corridors
LSP 2-3	3360.56	7945.88	318993.85	65737.79
LSP 2-4	3406.95	10565.57	13086.32	845485.34
LSP 3-4	1959.54	no corridors	8887.96	no corridors
Total change	174..40 % decrease		112.12 % increase	

"LSP" is local sub-population

Discussion

The efficacy of corridors in wildlife conservation has been a subject for discussion for a long time (Rosenberg et al., 1997; Niemela, 2001; Dixon et al., 2006). Several studies on mammal dispersal suggest that animal movement usually takes place within available suitable habitats (McLellan and Hovey, 2001; Poole et al., 2001; Maehr et al., 2002; Crooks and Sanjanyan, 2006; Cushman et al., 2013; Mateo-Sanchez et al., 2014). Therefore, degradation, loss or fragmentation of suitable habitats by man-made barriers such as highways, dams and other structures may seriously affect mobility of animals. In our study, models indicated that dams will lead to serious habitat fragmentation (*Fig 4, 5* and *6*), and thereby target species (and perhaps other ecologically related species) will be forced to live in restricted habitats and/or to migrate into new environments. Reductions in the quality of habitat within corridors and/or an increase in distances to travel will act to restrict mobility of target species and weaken linkages between separate patches. This implies that individuals would meet insufficient habitats encounter increased risks while moving between subpopulations (Fletcher et al., 2007). Furthermore, such isolated small populations tend to lose genetic variability and experience increased levels of inbreeding depression (Crnokrak and Roff, 1999).

Moreover, it is highly probable that local subpopulations will become smaller and isolated once the constructions are completed. This would increase the likelihood of rapid fluctuations in abundances and local extinctions in the face of environmental stochasticity. This is of particular concern for wild goat population-3, which largely overlaps with a wildlife reserve but will be divided into three fragments by the reservoir of Yusufeli Dam once it is built. In short, a combination of above mentioned effects may eventually drive target species populations to local extinctions.

Furthermore, our theoretical results indicate that dam constructions will remove prime habitat of some target species. In our case, golden jackals appear to be worst impacted through loss of habitat. However, the fact that jackals are opportunistic mammals that can tolerate human presence may help the species to survive in the future. Yet other species might suffer more from the loss of a considerable portion of their preferred habitat.

Dams on River Coruh were planned to be built sequentially with a maximum of 3 km gap between each. A route connecting two adjacent sub-populations through any of these gaps is on average about 19 km. This is a relatively long distance for any of our target species. More importantly suitable structures for wildlife to use at the crossings do not currently exist at any of the gaps. This problem can be solved by establishing artificial structures at gaps between neighboring reservoirs. Such structures are generally in the form of overhead passes for large animals (Glista et al., 2009). They may need to be supported with fence blocks to protect animals from falling into reservoirs while trying to get across. Unfortunately, such solutions are rather costly, particularly when a number of them are required.

Large dams are known to have an effect on aquatic ecosystems through formation of artificial lakes and changes in hydrology of river systems. It is predicted that food webs and other ecological processes may be negatively affected by reservoirs (Kingsford, 2000). Relatively less known are impacts on the local microclimate following changes in evaporation, humidity and precipitation pattern (UNEP, 2000; Lagadinou, 2003). We demonstrate here that impacts on wildlife, particularly on large and medium mammals, also need to be taken into account whenever large-scale dam construction is planned. Extensive monitoring of populations likely to be impacted, and field research to better understand how animals adapt to their newly formed landscape are urgently needed to develop sound solutions against the irreversible ecological impact of dams on wildlife.

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REFERENCES

[1] Ambarlı, H., Bilgin, C. C. (2008): Human–Brown Bear Conflicts in Artvin, Northeastern Turkey: Encounters, Damage, and Attitudes. - Ursus 19(2):146-153.

[2] Ambarlı, H., Mengüllüoğlu, D., Bilgin, C. C. (2010): First camera trap pictures of Eurasian Lynx from Turkey. - Cat News 52, pp 32.

[3] Ambarlı, H., Bilgin, C. C. (2012): First record of a melanistic golden jackal (*Canis aureus*) from Turkey. - Mammalia 77(2):219–222.

[4] Anderson, A. B., Jenkins, C. N. (2006): Applying nature's design: corridors as a strategy for biodiversity conservation. - Columbia University Press, New York.

[5] Beier, P. (1993): Determining minimum habitat areas and habitat corridors for cougars. - Conservation Biology 7:94-108.

[6] Beier, P., Noss, R. F. (1998): Do habitat corridors provide connectivity? - Conservation Biology 12:1241-1252.

[7] Beier, P., Penrod, K. L., Spencer, W. D., Cabanero, C. (2006): South cost missing linkages: restoring connectivity to wildlands in the largest metropolitan area in the United States. - In: Crooks KR, Sanjayan M, eds. Connectivity conservation, Cambridge University Press, Newyork, pp. 555-586.
[8] Beier, P., Majka, D. R., Spencer, W. (2008): Forks in the road: choices in procedures for designing wildlife linkages. - Conservation Biology 22:836-851.
[9] Beier, P., Majka, D. R., Newell, S. L. (2009): Uncertainty analysis of least-cost modeling for designing wildlife linkages. - Ecological Applications 19:2067-2077.
[10] Beier, P., Spencer, W., Baldwin, R. F., Mcrae, B. H. (2011) Towards best practices for developing regional connectivity maps. - Conservation Biology 25:879-892.
[11] Bentley, J. M., Catteral, C. P. (1997): The use of brushland corridors and linear remnants by birds in southeastern Queensland Australia. - Conservation Biology 68:155-165.
[12] Bilgin, C. C., Zeydanlı, U., Bal, M. (2006): Lesser Caucasus Gap Analysis Project Final Report (unpublished report). Middle East Technical University, Turkey.
[13] Blanco, J. C., Cortes, Y., Virgos, E. (2005): Wolf response to two kinds of barriers in an agricultural habitat in Spain. - Canadian Journal of Zoology 83(2):312-323.
[14] Briers, R. A. (2002): Incorporating connectivity into reserve selection procedures. - Biological Conservation 103:77-83.
[15] Chetkiewicz, C. L. B., Boyce, M. S. (2009): Use of resource selection functions to identify conservation corridors. – Journal of Applied Ecology 46:1036-1047.
[16] Clevenger, A. P., Wierzchowski, J., Chruszcz, B., Gunson, K. (2002): GIS-generated, expert-based model for identifying wildlife habitat linkages and planning mitigation passages. - Conservation Biology 16:503-514.
[17] Crnokrak, P., Roff, D. A. (1999): Inbreeding depression in the wild. - Heredity 83:260–270.
[18] Crooks, K. R., Sanjayan, M. (2006): Connectivity conservation. - Cambridge University Press, Newyork.
[19] Cushman, S. A., McRae, B., Adriansen, F., Beier, P., Shirley, M., Zeller, K. (2001): Biological corridors and connectivity. - In Conservation in theory and practice: 284-404, Mcdonald, D. (Ed.). Oxford: Blackwell.
[20] Dixon, J. D., Oli, M. K., Wooten, M. C., Eason, T. H., McCown, J. W., Paetkau, D. (2006): Effectiveness of a regional corridor in connecting two Florida black bear populations. - Conservation Biology 20:155-162.
[21] Eminağaoğlu, Ö., Anşin, R. (2003): The Flora of Hatila Valley National Park and its Close Environs (Artvin). - Turkish Journal of Botany 27:1-27.
[22] Fagan, W. F., Calabrese, J. M. (2006): Quantifying connectivity: balancing metric performance with data requirements. - In: Crooks KR, Sanjayan M, eds. Connectivity conservation, Cambridge University Press, Newyork, pp. 297-317.
[23] Fahrig, L., Merriam, G. (1994): Conservation of fragmented populations. – Conservation Biology 8:50-59.
[24] Fletcher, R. J., Ries, J. R. L., Battin, J., Chalfoun, A. D. (2007): The role of habitat area and edge in fragmented landscapes: definitively distinct or inevitably intertwined? - Canadian Journal Zoology 85:1017-1030.
[25] Glista, D. J., DeVault, T. L., DeWoody, J. A. (2009): A review of mitigation measures for reducing wildlife mortality on roadways. - Landscape Urban Planning 91:1–7.
[26] Gokturk, T., Bucak, F., Artvinli, T. (2011): Mammalian Fauna of Artvin. - African Journal Agricultural Research 6(6):1418-1425.
[27] Gundogdu, E., Ogurlu, I. (2009): The Distribution of Wild Goat *Capra aegagrus* Erxleben 1877 and Population Characteristics in Isparta, Turkey. - Journal of Animal and Veterinary Advances 8(11):2318-2324.
[28] Gundogdu, E. (2011): Population Size, Structure and Behaviours of Wild Goat in Cehennemdere Wildlife Improvement Area. - Asian Journal of Animal Veterinary Advances 6:555-563.

[29] Haddad, N. M., Baum, K. A. (1999): An experimental test of corridor effects on butterfly densities. - Ecological Applications 9:623-633.
[30] Hale, M. L., Lurz, P. W. W., Shirley, M. D. F., Rushton, S., Fuller, R. M., Wollf, K. (2000): Impact of landscape management on the genetic structure of red squirrel populations. - Science 293:2246-2248.
[31] Harrison, S., Voller, J. (1998): Connectivity, In: Harrison S, Voller J, eds. Conservation biology principles for forested landscapes. - University of British Columbia Press, Vancouver, pp. 76-97.
[32] Jhala, Y. V., Moehlman, P. D. (2004): 6.2. Golden Jackal Canis aureus Linnaeus, 1758. In: Sillero Z, Hoffmnn CM, Macdonald DW (eds): Canids: Foxes, Wolves, Jackals and Dogs. Status, Survey and Conservation Action Plan. - IUCN/SSC Canid Specialist Group, Gland, Switzerland and Cambridge, UK, pp. 156–161.
[33] Jogman, R. (2004). The context and concept of ecological networks. In: Jongman R, Pungetti G, eds. Ecological networks and Greenways Concept, Design and Implementation. - Cambridge University Press, pp. 7-33.
[34] Kingsford, R. T. (2000): Ecological impacts of dams, water diversions and river management on floodplain wetlands in Australia. - Austral Ecolgy 25:109-127.
[35] Kintsch, J., Cramer, P. C. (2011): Permeability of Existing Structures for Terrestrial Wildlife: A Passage Assessment System. - Report to the Washington State Department of Transportation, Research Report No. WA-RD 777.1.
[36] Kovacs, J. M., Malczewski, J., Verdugo, F. F. (2004): Examining local ecological knowledge of hurricane impacts in mangrove forest using analytical hierarchy process (AHP) approach. - Journal of Coastal Research 20:792-800.
[37] Kusak, J., Huber, D., Gomercic, T., Schwaderer, G., Guzvica, G. (2009): The permeability of highway in Gorski Kotar (Croatia) for large mammals. - European Journal of Wildlife Research 55:7-21.
[38] Lapini, L., Conte, D., Zupan, M., Kozlan, L. (2011): Italian Jackals 1984-2011: An updated review (Canis aureus: Carnivore, Canidae). - Boll Mus St Nat Venezia, 62:219-232.
[39] LaPoint, S., Gallery, P., Wikelski, M., Kays, R. (2013): Animal behavior, cost based corridor models and real corridors. - Landscape Ecology 28(8):1615-1630.
[40] Lagadinou, E. (2003): Assessment of the impact of artificial reservoirs to the microclimate. - Postgraduate Thesis, Department of Water Resources, Hydraulic and Maritime Engineering, National Technical University of Athens, Athens.
[41] Larkin, J. L., Maehr, D. S., Hoctor, T. S., Orlando, M. A., Whitney, K. (2004): Landscape linkages and conservation planning for black bear in the west central Florida. Animal Conservation 7:23-34.
[42] Laure, M. A., Nielsen, C. K. (2008): Modeling potential dispersal corridors for cougars in Midwestern North America using least- cost path methods. - Ecological Modeling 212: 372-381.
[43] MacClintock, L., Whitcomb, R. F., Whitcomb, B. L. (1977). Evidence for the value of corridors and minimization of isolation in preservation of biotic diversity. - American Birds 31:6-12.
[44] Macdonald, M. A. (2003): The role of corridors in biodiversity conservation in production forest landscapes: a literature review. - Tasforests 14:41-52.
[45] Machtans, C. S., Villard, M. A., Hannon, S. J. (1996): Use of riparian buffer strips as movement corridors by forest birds. - Conservation Biology 10:1366-1379.
[46] Maehr, D. S., Land, E. D., Shindle, D. B., Bass, O. L., Hoctor, T. S. (2002): Florida panther dispersal and conservation. - Biological Conservation 106:187-197.
[47] Manel, S., Schwartz, M.K., Luikart, G., Taberlet, P. (2003): Landscape genetics: combining landscape ecology and population genetics. - Trends in Ecology and Evolution 18:189-197.

[48] Mateo-Sanchez, M. C., Cushman, S. A., Saura, S. (2014): Connecting endangered brown bear subpopulations in the Cantabrian Range (north-western Spain). - Animal Conservation 17:430-440.

[49] McLellan, B. N., Hovey, F. W. (2001): Natal dispersal of grizzly bears. Canadian Journal of Zoology 79:838-844.

[50] Merriam, G., Lenoue, A. (1990): Corridor use by small mammals: field measurements for three experimental types of *Peromyscus leucopus*. - Landscape Ecology 4:123-131.

[51] Mills, L. S., Smouse, P. E. (1994): Demographic consequences of inbreeding in remnant populations. - American Naturalist 144:412-431.

[52] Morrison, S. A., Boyce, W. M. (2008): Conserving connectivity: some lessons from mountain lions in southern California. - Conservation Biology 23:275-285.

[53] Myers, N., Mittermeier, R. A., Mittermeier, C. G., Da Fonseca, G. A. B., Kent, J. (2000): Biodiversity hotspots for conservation priorities. - Nature 403:853-858.

[54] Niemela, J. (2001): The utility of movement corridors in forested landscapes. - Scandinavian Journal of Forest Research 3:70-78.

[55] Noss, R. F., Quigley, H. B., Hornocker, M. G., Merrill, T., Paquet, P. (1996): Conservation biology and carnivore conservation. - Conserv Biol 10:949-963.

[56] Nowel, K., Jackson, P. (1996): IUCN/SSC Cat Specialist Group. Wild cats: status survey and conservation action plan. - Gland (Switzerland), IUCN.

[57] Parks, S. A., Mckelvey, K. S., Schwartz, M. K. (2013): Effects of weighting schemes on the identification of wildlife corridors generated with least-cost methods. - Conservation Biology 27(1):145-154.

[58] Poole, K. G., Mowat, G., Fear, D. A. (2001): DNA-based population estimation for grizzly bears *Ursus arctos* in northeastern British Columbia, Canada. - Wildlife Biology 7: 105-115.

[59] Poor, E. E., Loucks, C., Jakes, A., Urban, D. L. (2012): Comparing habitat suitability and connectivity modeling methods for conserving pronghorn migrations. - PLoS One 7:e49390.

[60] Proctor, M. F., McLellans, B. N., Strobeck, C., Barclay, R. M. R. (2004): Gender-specific dispersal distances of grizzly bears estimated by genetic analysis. - Canadian Journal of Zoology 82:1108-1118.

[61] Riley, S. P. D., Pollinger, J. P., Sauvajot, R. M., York, E. C., Bromley, C., Fuller, T. K., Wayne, R. K. (2006): A southern California freeway is a physical and social barrier to gene flow in carnivores. - Molecular Ecology 15:1733-1741.

[62] Rosenberg, D. K., Noon, B. R., Meslow, C. (1997): Biological Corridors: Form, Function, and Efficacy. - Bioscience 47(10):677-687.

[63] Samlius, G., Andren, H., Liberg, O., Linnell, J. D. C., Odden, J., Ahlqvist, P., Segerström, P., Sköld, K. (2011): Spatial and temporal variation in natal dispersal by Eurasian lynx in Scandinavia. - Journal of Zoology 286(2):120-130.

[64] Saura, S., Bodin, Ö., Fortin, M. J. (2014): Stepping stones are crucial for species long distance dispersal and range expansion through habitat networks. - Journal of Applied Ecology 51:171-182.

[65] Sawyer, S. C., Epps, C. W., Brashares, J. S. (2011): Placing linkages among fragmented habitats: do least-cost models reflect how animals use landscapes? - Journal of Applied Ecology 48:668-678.

[66] Schmidt, K. (1998): Maternal behavior and juvenile dispersal in the Eurasian Lynx. - Acta Theriologica 43(4):391-408.

[67] Sekercioglu, C. (2012): Turkey's First Wildlife Corridor Links Bear, Wolf and Lynx Populations to the Caucasus Forest. - http://voices.nationalgeographic.com /2012/02/13/turkeys-first-wildlife-corridor-links-bear-wolf-and-lynx-populations-to-the-caucasus-forests/.

[68] Shackleton, D. M. (1997): Wild Sheep and Goats and their Relatives: Status Survey and Conservation Action Plan for Caprinae. - IUCN, Gland, Switzerland.

[69] Shkedy, Y., Saltz, D. (2000): Characterizing core and corridor use by Nubian Ibex in the Negev desert, Israel. - Conservation Biology 14:200-206.

[70] Store, R., Kangas, J. (2001): Integrating spatial multi-criteria evaluation and expert knowledge for GIS-based habitat suitability modeling. – Landscape and Urban Planning 55:79-93.

[71] Sucu, S., Dinç, T. (2008): Çoruh Basin Projects. - Downloaded from http://www.imo.org.tr/resimler/ekutuphane/pdf/10912.pdf on 8 May 2015 (in Turkish).

[72] Turan, N. (1984): Game and Wild Animals-Mammals of Turkey. - Ministry of Forest Publications, Ankara, 87 pp. (in Turkish).

[73] United Nations Environment Programme (UNDP), (2000): CLIMATE CHANGE AND DAMS: An Analysis of the linkages between the UNFCCC Legal Regime and Dams. - In cooperation with The World Commission on Dams.

[74] Verbeylen, G., De Bruyn, L., Adriaensen, F., Matthysen, E. (2003): Does matrix resistance influence red squirrel (*Sciurus vulgaris L.* 1758) distribution in an urban landscape? - Landscape Ecology 18:791-805.

[75] Washington Habitat Connectivity Work Group (WHCWG), (2010): Connected landscapes project. - WHCWG Available from http://www.waconnected.org/.

[76] Way, J. G. (2009): Observations of Coywolves, *Canis latrans* × *lycaon*, Crossing Bridges and Using Human Structures on Cape Cod, Massachusetts. - Canadian Field Naturalist 123(3):206-209.

[77] Zeller, K. A., McGarigal, K., Whiteley, A.R. (2012): Estimating landscape resistance to movement: a review. - Landscape Ecology 27:777–797.

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THE DIVERSITY OF SULFUR-OXIDIZING BACTERIAL POPULATIONS AT AN IRANIAN COPPER MINE AND THE SURROUNDING AGRICULTURAL SOILS

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Abstract. Sulphur-oxidizing bacteria (SOB) are one of the most important metabolic types of bacteria living in very different soil environments. Mine environments with high concentrations of metals and extreme conditions have led to habitat-specific genera and operational taxonomic units (OTUs) of bacteria. In this study, we compared the diversity of SOB and non-SOB at the Sarcheshmeh Copper Mine Complex (Iran) with the surrounding agricultural soil. Thirty-six mine and agricultural sites were sampled aseptically and assessed by culture-dependent (full-length 16S rRNA cloning) and independent (Denaturing Gradient Gel Electrophoresis (DGGE)) approaches. Results indicated that soil samples from the copper mine region contained diverse OTUs affiliated with of SOB genera including *Acidithiobacillus sp.* and *Sulfobacillus sp.* and non-SOB including *Pseudomonas sp.*, *Bacillus sp.*, *Sphingobium sp.*, *Sphingomonas sp.*, *Acinetobacter sp.*, *Halospirulina sp.*, and *Propionibacterium sp.* In addition, the soil samples from the surrounding agricultural sites contained representatives of SOB genera as *Thiobacillus sp.*, *Starkeya sp.* and non-SOB as *Pseudomonas sp.*, *Acinetobacter sp.*, *Bacillus sp.*, *Sphingobium sp.*, *Streptomyces sp.*, and *Propionibacterium sp.* These bacterial communities were significantly affected by sulphur in the mine area, and by pH and calcium concentrations in the agricultural soils.

Keywords: *Acidithiobacillus, Thiobacillus, bacterial diversity*

Abbreviations: *SOB, sulphur-oxidizing bacteria; OTU, operational taxonomic unit; DGGE, Denaturing Gradient Gel Electrophoresis; ANOSIM, analysis of similarities; N-MDS, non-metric multidimensional scaling; UPGMA, un-weighted pair group method with arithmetic mean; AT, Acidithiobacillus; Th, Thiobacillus; AC, Acinetobacter.*

Introduction

Sulphur-oxidizing bacteria (SOB) are one of the metabolic types of bacteria, which, according to environmental conditions, could be classified into the following groups: mesophilic, thermophilic, acidophilic, neutrophilic, alkalophilic, autotrophic, chemolithotrophic, and heterotrophic (Friedrich et al., 2001; Kelly and Wood, 2000). SOB use sulphur and its ionic derivatives (e.g. thiosulfate) as an electron donor for energy requirements. Most SOB are investigated for their importance in biology, agriculture, economics, and other scientific fields. Heterotrophic SOB could degrade toxic organic matter and play an important role in detoxifying their environments (Johnson, 2008). In addition, soil SOB increase the availability of sulphate (SO_4^{-2}) for plant absorption and improve plant growth and agricultural production (Smith et al., 2000). SOB belong to different genera as *Thiobacillus*, *Thiospherea*, *Thiomicrospira*,

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Thiospira, *Acidiphilum*, *Thiobacterium*, *Macromonas*, and *Acidiphilum*. *Thiobacillus*-like are the most important and frequently occurring SOB, and Kelly and Wood (2000) have classified them into three genera: *Acidithiobacillus*, *Thermithiobacillus*, and *Hallothiobacillus* (Kelly and Wood, 2000).

The bacterial diversity and its dynamism in soil environments have a direct relationship with soil characteristics, *i.e.* kind of minerals, organic materials, water content, heavy metals, salinity, and pH (Rodrigues et al., 2014). In most geographical regions with mining activities, high concentrations of sulphur and its subsequent chemical reactions cause decreasing pH levels into acidic conditions, limiting the colonization of bacterial communities, and imposed selection pressure on specific bacterial populations, such as SOB. SOB present in these mines are considered extremophiles, and account with different species of genera as A*cidiphilum sp.*, *Leptospirillium sp.* (Robertson and Kuenen, 2006), *Acidocella sp.*, *Acidithiobacillus sp.* (Johnson, 2008), *Ferroplasma sp.*, *Sulfobacillus sp.* (Johnson, 2008), *Ferrimicrobium sp.*, *Ferribacter sp.* (Havlin et al., 2005), *Thiobacillus sp.*, and *Hallothiobacillus sp.* (Brito et al., 2013). Halophilic SOB species that belong to *Hallothiobacillus sp.* and *Thiomicrospira sp.* were found in soils with high salinity concentrations (Sorokin et al., 2006). Soils with high concentrations of chromium heavy metal have shown a colonization of *Thiobacillus* SOB (Brito et al., 2013). In addition, some indirect factors, such as mine topography characteristics (e.g. slope, direction, and soil texture), affect soil properties and subsequent SOB diversity. A copper mine environment with a slope has indicated specific genera of *Acidobacteria*, *Chloroflexi*, *Firmicutes*, and *Gammaproteobacteria*, which correspond to their differences in soil organic matter and water percentage (Rodrigues et al., 2014).

Mine by-product processing, transportation, drainage, and tailings could alter the property of surrounding soils with different applications, *i.e.* agriculture and forestry (Castilla and Nealler, 1978; Mendez et al., 2008; Ying et al., 2008; Yun-Guo et al., 2006).

The Iranian copper mine complex of Sarcheshmeh is one of the largest open-cast copper mines in the world and it is located in a region with a high density of pistachio cultivation. Copper mining has been performed through conventional pyrometallurgy and hydrometallurgy with sulphide minerals. Mechanical and biochemical manipulation and processing of minerals has resulted in a large quantity of dust and polluted drainage and tailings that could alter the surrounding environment, and, hence, the soil properties and bacterial populations. No previous studies have investigated the effect of the Sarcheshmeh copper mine on the surrounding soil SOB diversity and frequency. Therefore, in this study we used both culture-dependent and- independent methods of isolate 16S rRNA sequencing and Denaturing Gradient Gel Electrophoresis (DGGE), respectively, to compare the diversity of SOB present in soil samples from the copper mine and the adjacent agricultural soils.

Material and methods

Samples

Sampling was performed on two kinds of soil: 22 soil samples from the copper mine region (Sarcheshmeh Copper Mine Complex, Kerman, Iran) and 14 soil samples from the surrounding agricultural areas, as shown in *Figure 1*. The sampling region had a mean annual temperature of 25°C, rainfall of 160 mm, and a clay-loam soil texture. The soils were classified as *Typic Haplosalids* according to soil taxonomy (Soil Survey Staff, 2010).

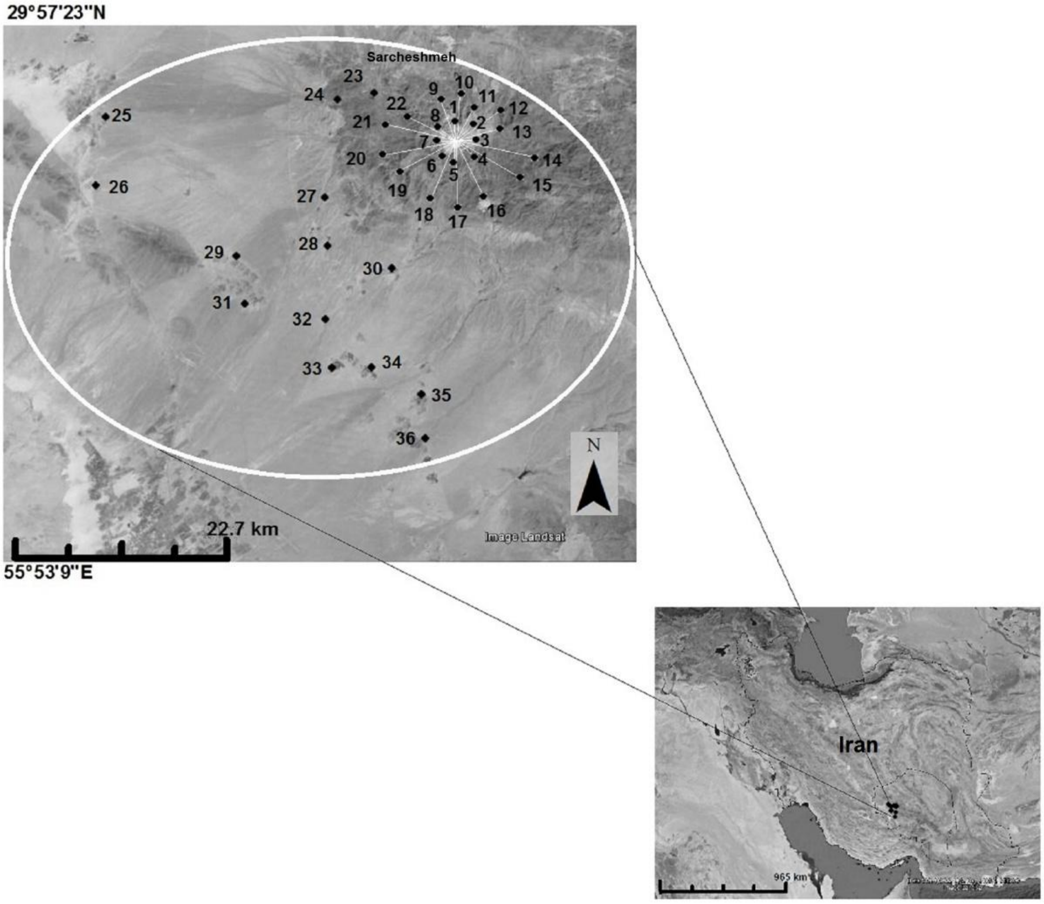

Figure 1. *Location of samples selected in this study*

Mining has occurred at the copper mine complex since 1972 and the extraction of copper has since increased from 15,000 tonnes to 70,000 tonnes (http://nicico.com). The surrounding agricultural region cultivated with pistachio contains saline, calcareous, and low organic material soils. Samples were taken from the soil surface using a random compound sampling strategy, which employed square method/stratification of sampling. Each studied sample comprised a mixture of five subsamples of each site from the vertices and centre of a 2.5 m square (Baker and Banfield, 2003; Garrity et al., 2005). Samples were preserved in sterile tubes (50 ml) in an aseptic condition, immediately transported to the laboratory, and stored at 5°C and -20°C in two series for dependent and independent culturing methods respectively. Complete data on soil samples are included in *Table 1*. Chemical characteristics of the soil samples, including pH, electrical conductivity (EC, dS m^{-1}), and organic carbon (OC, %) (Walkley and Black, 1934), were determined. The percentage of major chemical elements (aluminium (Al), arsenic (As), calcium (Ca), copper (Cu), magnesium (Mg), manganese (Mn), lead (Pb), zinc (Zn), molybdenum (Mo), sulphur (S), and iron (Fe)) were measured by atomic absorption spectrometry analysis (Schultz, 1964).

Table 1. *Chemical composition of copper mine soil samples (elements concentration in unit of %, EC in unit of dS/m)*

Elements	**Samples**																						**Mean**
	S1	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**S10**	**S11**	**S12**	**S13**	**S14**	**S15**	**S16**	**S17**	**S18**	**S19**	**S20**	**S21**	**S22**	
Al	3.01	2.17	2.46	3.01	2.31	2.82	2.23	2.38	2.15	2.61	2.12	2.01	2.44	2.18	2.31	2.16	2.22	2.17	3.02	2.04	2.13	2.51	**2.38**
As	0.01	0.02	0.02	0.01	0.01	0.02	0.01	0.01	0.00	0.00	0.01	0.01	0.01	0.01	0.01	0.00	0.00	0.01	0.01	0.01	0.01	0.01	0.01
Ca	0.10	0.10	0.13	0.10	0.13	0.10	0.13	0.12	0.13	0.10	0.12	0.10	0.12	0.12	0.11	0.14	0.13	0.10	0.12	0.13	0.10	0.11	0.12
Cu	0.10	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.10	0.20	0.20	0.20	0.30	0.30	0.20	0.20	0.20	0.10	0.20	0.20	0.20	0.20	0.20
Mg	0.04	0.01	0.02	0.03	0.02	0.05	0.05	0.03	0.03	0.03	0.03	0.02	0.03	0.03	0.02	0.03	0.03	0.03	0.02	0.04	0.04	0.04	0.03
Mn	0.06	0.03	0.06	0.06	0.06	0.04	0.03	0.06	0.07	0.07	0.06	0.07	0.06	0.07	0.05	0.05	0.06	0.06	0.06	0.07	0.05	0.05	0.06
Pb	0.02	0.02	0.02	0.01	0.02	0.02	0.03	0.01	0.02	0.02	0.03	0.02	0.02	0.02	0.01	0.02	0.02	0.02	0.03	0.01	0.02	0.02	0.02
Zn	0.05	0.05	0.05	0.05	0.05	0.03	0.06	0.05	0.04	0.05	0.05	0.05	0.06	0.04	0.04	0.05	0.05	0.03	0.06	0.05	0.05	0.05	**0.05**
Fe	6.28	6.31	6.38	6.27	6.28	6.21	6.37	6.25	6.27	6.27	6.25	6.27	6.21	6.27	6.27	6.24	6.27	6.27	6.27	6.28	6.21	6.27	6.27
S	4.10	4.50	4.45	4.01	4.50	4.43	4.43	4.41	4.03	4.10	4.01	4.01	4.23	4.06	4.06	4.21	4.00	3.98	4.01	4.01	4.04	4.14	**4.03**
Mo	0.009	0.007	0.007	0.007	0.008	0.008	0.006	0.009	0.007	0.007	0.007	0.007	0.006	0.008	0.007	0.007	0.007	0.007	0.006	0.008	0.007	0.007	**0.007**
pH	4.56	4.53	4.31	4.43	4.69	4.69	4.59	5.01	4.51	4.6	4.52	4.4	4.56	4.46	4.56	4.58	4.56	4.57	4.54	4.56	4.56	4.56	4.56
EC	2.75	2.76	2.76	2.75	2.75	2.75	2.77	2.71	2.75	2.75	2.71	2.74	2.73	2.75	2.75	2.75	2.75	2.69	2.75	2.74	2.81	2.75	2.75
OC	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Continued Table 1. *Chemical composition of agricultural soil samples*

Elements	**Samples**														**Mean**
	S23	**S24**	**S25**	**S26**	**S27**	**S28**	**S29**	**S30**	**S31**	**S32**	**S33**	**S34**	**S35**	**S36**	
Al	2.16	2.16	2.16	2.01	2.24	2.21	2.16	2.16	2.16	2.18	2.14	2.16	2.18	2.14	2.16
As	0.03	0.02	0.02	0.02	0.02	0.03	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	**0.02**
Ca	2.16	2.12	2.12	2.16	2.11	2.00	2.00	2.12	2.14	2.13	2.14	2.11	2.11	2.11	**2.11**
Cu	0.30	0.32	0.32	0.31	0.31	0.31	0.32	0.30	0.31	0.31	0.31	0.31	0.30	0.30	**0.31**
Mg	0.05	0.07	0.04	0.05	0.05	0.05	0.05	0.04	0.06	0.05	0.05	0.04	0.06	0.04	**0.05**
Mn	0.14	0.10	0.13	0.12	0.12	0.10	0.13	0.12	0.11	0.10	0.13	0.12	0.10	0.11	**0.12**
Pb	0.02	0.02	0.01	0.03	0.04	0.01	0.01	0.02	0.02	0.03	0.02	0.02	0.02	0.02	0.02
Zn	0.04	0.03	0.04	0.04	0.04	0.03	0.02	0.05	0.04	0.05	0.04	0.03	0.04	0.04	0.04
Fe	6.91	6.87	6.91	6.94	6.88	6.91	6.91	6.91	6.91	6.96	6.98	6.91	6.92	6.82	**6.91**
S	0.57	0.58	0.53	0.57	0.59	0.59	0.54	0.56	0.56	0.57	0.58	0.58	0.54	0.57	0.57
Mo	0.006	0.004	0.008	0.006	0.006	0.007	0.005	0.006	0.006	0.007	0.006	0.008	0.007	0.008	0.006
pH	7.89	7.84	7.84	7.89	7.89	7.86	7.89	7.89	7.89	7.97	7.99	7.92	7.87	7.89	**7.89**
EC	3.18	3.19	3.19	3.21	3.18	3.19	3.19	3.18	3.19	3.10	3.19	3.22	3.10	3.20	**3.18**
OC	0.70	0.70	0.60	0.50	0.50	0.60	0.70	0.80	0.70	0.70	0.70	0.60	0.60	0.70	**0.70**

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Cultivation-dependent SOB identification

Selective bacterial cultivation

SOB isolation procedures were carried out according to Marsh and Norris (1983) with some modifications (Marsh and Norris, 1983). For SOB population screening in a culture medium, 0.2 mg of each soil sample was used for culturing in 9K medium (Sigma-Aldrich, USA)(for the achievement of a more comprehensive diversity of sulfur oxidizing bacteria) with a pH of 3.5 or 6.5 to allow the acidophilic and neutrophilic SOB to growth (Schoenborn et al., 2004; Widawati et al., 2005). The samples were incubated at 120 rpm, 30°C or 45°C for 14 days, and finally cultured on agar plates to separate the single colonies for morpho-physiological confirmation. To isolate new bacteria, the culture medium was monitored for six months with two-week intervals. Morphological characteristics of colonies such as size, colour, surface, and colony boundary (Holt et al., 1994) were determined. Also, physiological characteristics such as sulphate concentration, pH/Eh, cellular growth, source of energy, aerobic/anaerobic, and temperature were measured (Bielefeldt et al., 2009). Confirmed SOB colonies were transferred to -20°C for DNA extraction and subsequent applications.

DNA extraction and 16S rRNA amplification

The soil bacterial DNA was extracted using a QIAamp® Mini Kit (Qiagen, Germany) according to manual instructions. Extracted DNA quality was measured by 1% agarose gel electrophoresis and NanoDrop 2000c 260/280 nm spectrophotometry (Thermo Scientific, USA). Polymerase chain reaction (PCR) amplification of the full-length 16S rRNA gene was performed using 27f (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492r (5′-CGGTTACCTTGTTACGACTT-3′) primers. For identification of uncultured bacteria according to DGGE, the primers used were 357f (5′-CGCCCGCCGCGCGCGGCGGGC GGGGCGGGGGCACGGGGGGACTCCTACGGGAGGCAGCAG-3′) and 517r (5′-ATT ACC GCG GCT GCT GG-3′). PCR reactions were carried out in a gradient PEQLAB thermocycler (PEQLAB Biotechnologie, Germany) in a final volume of 50 µl containing 20 µM of each primer, 0.2 units/µl Taq DNA polymerase (Ampliqon, Denmark), PCR buffer 1x (Tris-HCl pH 8.5, $(NH_4)_2S0_4$, 0.2% Tween20), 0.5 mM dNTPs, 1.5 mM $MgCl_2$, and 20-50 ng gDNA. The PCR amplification program was as follows: initial denaturation in 95°C for 5 min, 35 cycles of denaturation in 94°C for 30 s, primer annealing in 59°C for 50 s, extension in 72°C for 2 min, and final extension in 72°C for 10 min. PCR products of the full-length 16S rRNA gene were cloned in a TA cloning vector using InsTAclone PCR Cloning Kit (Fermentas, USA) and sequenced bi-directionally using an ABI 3730 capillary sequencer. DGGE analysis of uncultured bacterial populations was performed in a D-code DGGE system (Muyzer and Smalla, 1998) with 8% polyacrylamide gel and 40%–75% gradient of formamide at a 55°C buffer temperature. Electrophoresis ran at a constant electrical power of 70 v for 17 h and gels were then stained in florescent safe stain (0.5 µg/ml), which were finally visualized in a G-box system (Syngene, UK). Each identified DGGE band was re-amplified according to the above conditions and sequenced in triplicate.

Data analysis

Obtained sequences of the 16S rRNA gene from culture-dependent and -independent methods were checked in Chromas 2.01 (Technelysium Pty Ltd) and reviewed manually for any sequencing errors. Similar analysis of samples was performed in BLASTn software implemented on the National Center for Biotechnology Information homepage (http://www.ncbi.nlm.nih.gov/) and Ribosomal Database Project II and aligned using the clustalW program (Cole et al., 2005). Sequences with a similarity percentage greater than 97% were selected as a species and an OTU (Stackebrandt et al., 2002). All identified sequences have been deposited in the GenBank data server with the following accession numbers: KR020042-KR020052 and KR065700-KR065720.

The diversity indices of copper mine and agricultural soil samples (S1-S36) and every OTU including the number of taxa (S), Shannon index (H), and Berger-Parker index were calculated in the statistical program PAST (http://folk.uio.no/ohammer/past/). In addition, frequency of each OTU in all samples and the comparison between the two sample environments (mine and agriculture) was investigated using analysis of similarities (ANOSIM). The pattern of bacterial distribution and inter-relationships with environmental characteristics (physicochemical properties) were measured using non-metric multidimensional scaling (N-MDS), according to the Bray-Curtis distance matrix (Clarke, 1993). A similarity percentage (SIMPER) calculation was used to find the most frequent OTU in each mine and agricultural sample. Cluster analysis of samples was processed with the un-weighted pair group method with arithmetic mean (UPGMA) based on Bray-Curtis dissimilarities and 1000 bootstrap replications.

Results

Soil chemical analysis

The results of chemical analyses of soil samples from the copper mine and its surroundings are shown in *Table 1*. Chemical comparison of samples indicated a significant difference between the two sites according to pH level, organic carbon, and calcium concentrations ($p < 0.05$). Agricultural soil samples were neutral to slightly alkaline with a pH of 7.9, but copper mine soil samples were acidic with a pH of 4.56. Organic carbon content in the agricultural soil was 0.7%, but it was not detected in the mine soil samples. EC values of copper mine and agricultural soil samples differed significantly ($P < 0.05$) but the value was similar among all mine samples (almost 2 dS/m) ($P > 0.05$). Except for Pb, concentrations of other elements (Ca, As, Al, Zn, S, Cu, Mg, Mn, Mo, and Fe) differed in agricultural and mine soil samples. Thus, agricultural soil in contrast to copper mine soil had higher levels of organic matter and salinity and was measured as neutrophilic.

Bacterial OTU identification

According to *Table 2 (2.1 and 2.2)*, different strains of SOB were isolated from mine soil sample using culture-dependent approach (after enrichment). *Acidithiobacillus thiooxidans* was isolated from samples S1 and S8 while from samples S4, S5, S8, S9, S12, S15, S16, S17, S19, S20 ,S22 and S2, S7 , S14 *Acidithiobacillus ferridurans* and *Sulfobacillus acidophilus* were respectively isolated. In agricultural soil, different species of genus *Thiobacillus* include *thiophilus*, *thioparus*, *aquaesulis* respectively from samples S23, S32 and S24, S36 and S25, S26 and S27, S30, S28 and the *Starkeya novella* from S32 sample were isolated.

Results indicated that 19 main bacterial OTUs were identified. The culture-dependent 16S rRNA almost full-length sequencing identified seven OTUs including four specific OTUs in agricultural soils and three specific OTUs in copper mine soils (*Table 3*). Bacterial isolates of agricultural soils belonged to *Alphaproteobacteria* (*Starkya novella*) and *Betaproteobacteria* (*Thiobacillus thiophilus*, *Thiobacillus thioparus*, *Thiobacillus aquasulis*), and in copper mine soils belonged to *Gammaproteobacteria* (*Acidithiobacillus thiooxidans*, *Acidithiobacillus ferridurans*) and *Firmicutes* (*Sulfobacillus acidophilus*).

Table 2.1. *Identification of SOB in samples at culture-dependent approach*

Closest relative	**Mine soil sample**																						∑ Isolate	**Relative abundance (%)**
	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16	S17	S18	S19	S20	S21	S22		
Acidithiobacillus thiooxidans	*							*															2	7.69
Acidithiobacillus ferridurans				*	*			*	*			*			*	*	*		*	*		*	11	42.31
Sulfobacillus acidophilus		*					*							*									3	11.54
∑ Isolate	1	1		1	1		1	2	1			1		1	1	1	1		1	1		1	16	61.54
Relative abundance (%)	3.85	3.85		3.85	3.85		3.85	7.69	3.85			3.85		3.85	3.85	3.85	3.85		3.85	3.85		3.85		

Table 2.2. *Identification of SOB in samples at culture-dependent approach*

Closest relative	**Agricultural soil sample**														∑ Isolate	**Relative abundance (%)**
	S23	S24	S25	S26	S27	S28	S29	S30	S31	S32	S33	S34	S35	S36		
Thiobacillus thiophilus	*									*					2	7.69
Thiobacillus thioparus		*												*	2	7.69
Thiobacillus aquaesulis			*	*											2	7.69
Starkeya novella					*	*		*		*					4	15.38
∑ Isolate	1	1	1	1	1	1		1		2				1	10	38.46
Relative abundance (%)	3.85	3.85	3.85	3.85	3.85	3.85		3.85		7.69				3.85		

Table 3. *List of identified bacterial species and OTUs in agricultural and mine soil samples*

Method	Sample	SOB/non-SOB	Putative Division	Genus	Closest relative (Species,OUT)	Accesion No	Similarity (%)
Non-cultured (DGGE)	**Mine**	SOB	*Gammaproteobacteria*	*Acidithiobacillus sp.*	*Acidithiobacillus ferrooxidans*	KM 527215	99.9
					Acidithiobacillus ferridurans	NR 117036	99.9
					Acidithiobacillus caldus	NR 102970	99.9
		non- SOB	*Firmicutes*	*Bacillus sp.*	*Bacillus megaterium*	KP 017584	99.9
					Bacillus subtilis	GQ 267463	99.5
		non- SOB	*Cyanobacteria*	*Halospirulina sp.*	*Halospirulina sp.*	AB 900936	99.8
		non- SOB	*Alphaproteobacteria*	*Sphingobium sp.*	*Sphingobium yanoikuyae*	KP 313542	99.9
		non- SOB	*Gammaproteobacteria*	*Pseudomonas sp.*	*Pseudomonas syringae*	KP 09980	99.2
					Pseudomonas sp.	KJ 413318	99.9
		non- SOB	*Actinobacteria*	*Propionibacterium sp.*	*Propionibacterium sp.*	GU 332269	99.8
		non- SOB	*Gammaproteobacteria*	*Acinetobacter sp.*	*Acinetobacter johnsonii*	LN 774358	99.9
		non- SOB	*Alphaproteobacteria*	*Sphingomonas sp.*	*Sphingomonas sp.*	DQ 923856	99.9
	Agri	non- SOB	*Firmicutes*	*Bacillus sp.*	*Bacillus megaterium*	KP 017584	99.9
					Bacillus subtilis	GQ 267463	99.5
		non- SOB	*Alphaproteobacteria*	*Sphingobium sp.*	*Sphingobium yanoikuyae*	KP 313542	99.9
		non- SOB	*Gammaproteobacteria*	*Pseudomonas sp.*	*Pseudomonas syringae*	KP 09980	99.2

					Pseudomonas sp.	KJ 413318	99.9
		non- SOB	*Actinobacteria*	*Propionibacterium sp.*	*Propionibacterium sp.*	GU 332269	99.8
		non- SOB	*Actinobacteria*	*Streptomyces sp.*	*Streptomyces sp.*	LN 649246	99.8
		non- SOB	*Gammaproteobacteria*	*Acinetobacter sp.*	*Acinetobacter johnsonii*	LN 774358	99.9
Cultured	**Mine**	SOB	*Gammaproteobacteria*	*Acidithiobacillus sp.*	*Acidithiobacillus thiooxidans*	NR 115265	95.0
					Acidithiobacillus ferridurans	NR 117036	95.0
		SOB	*Firmicutes*	*Sulfobacillus sp.*	*Sulfobacillus acidophilus*	NR 074758	94.6
	Agri	SOB	*Betaproteobacteria*	*Thiobacillus sp.*	*Thiobacillus thiophilus*	NR 044555	93.9
					Thiobacillus thioparus	NR 117864	93.9
					Thiobacillus aquaesulis	NR 117675	99.9
		SOB	*Alphaproteobacteria*	*Starkeya sp.*	*Starkeya novella*	NR 074219	95.3

Culture-independent approach

Table 4 (4.1, 4.2 and 4.3) shows the identification of bands in samples in the DGGE banding patterns (shown in *Figure 2a, b and c*). Based on *Table 4 (4.1, 4.2, 4.3)* 156 bands were detected from all samples. The samples were heterogeneous in terms of the number of bands in DGGE pattern. Samples exhibiting just 1 band (S31, S26, S24, S23, S35 and S36) belonged to agricultural samples while samples containing the highest number of bands belonged to mine samples (The samples S1, S2, S3, and S5 7 bands; S16, 9 bands; S12, and S4, 10 bands). This ceases can be concerned to role of climate. Since the climate of the study area was dry, and in the terms of the year was associated with dryness, in such conditions, growth of bacteria can be difficult and it was resulted in a lower band number was found in agricultural soil. While in mining copper, because of copper bioleaching processes and copper mining, medium had the water and thus provides the conditions for the growth of bacteria and as a result, more bands were observed in mine samples.

Both mine and agricultural samples containing 2, 3, 4, 5, 6 bands belonged to sample groups (S25, S10, S33, S34), (S22), (S13, S14, S15, S32), (S28, S7, S18, S19),(S20), (S6, S29, S30, S8) and (S27, S9, S11, S17, S21) respectively. Therefore, minimum number of bands appeared in agricultural soil samples and the maximum number of bands appeared in mine samples. On the other hand heterogeneity among mine samples was higher than for the agricultural soil samples.

As shown in *Table 4.1*, S1 sample exhibited 7 bands in where 2 belonged to *Acidithiobacillus sp.,* (coincident among samples S2, S3, S4, S5 and S6), 2 bands belonged to *Bacillus sp.,* (these bands were coincident among samples S2, S3, S4, S5, S6, S27, S28, S29 and S30) and 3 bands belonged to *Pseudomonas sp.* (coincident among samples S1, S2, S3, S4, S5, S6, S27, S28, S29, S30 and S31). Sample S4 contain 10 bands which is visible coincident among S1 sample, except one band that belongs to *Halospirulina sp.* Also, S5 sample has 7 bands which in 6 bands were resemble to S4 sample and one band that belong to *Sphingobium sp* was different. Similar result observed only in samples S2, S3, S4 and S30.

As shown on *Table 4.2*, the samples S9 and S11 exhibited 6 bands, 3 bands belonging to *Acidithiobacillus sp*., (coincident among samples S7, S8 and S10), 1 band belonging to *Propionibacterium sp*., (coincident among samples S23, S7, S25, S8, S26, S10), 1 band belong to *Acinetobacter sp* (coincident among samples S7, S25, S8) and finally, 1 band belonged to *Bacillus sp*. S24 sample exhibited only 1 band that belonged to *Streptomyces sp.* Coincident among samples (S17, S18, S21 and S22), 1 band belong to *Halospirulina sp.* (this band was coincident among samples S12, S17, S22), 3 bands belonging to *Pseudomonas sp.* (coincident among samples S12, S13, S14, S15, S17, S18, S19, S32, S20, S33, S21, S34, S35), 1 band belonging to *Sphingobium sp.* (coincident among samples S18, S22 and S36) and 1 band belonging to *Acinetobacter sp.* (coincident among samples S12, S20, S21). The samples 17 and 18 included only one band that belonged to *Sphingomonas sp.*

The most important difference between mine and agricultural samples were related to the occurrence of the genera *Acidithiobacillus sp*, *Streptomyces sp* and *Acidithiobacillus sp.,* which were exclusively found in the mine soil samples. While in agricultural samples only *Streptomyces sp* was dominant.

The result of DGGE of the V3 region of the 16S rRNA gene indicated 12 bacterial OTUs in copper mine soils and 8 OTUs in agricultural soils (*Table 3*). Mine bacterial populations included *Alphaproteobacteria*, *Gammaproteobacteria*, *Firmicutes*, *Cyanobacteria*, and *Actinobacteria,* of which only the species *Gammaproteobacteria* belonged to SOB. In agricultural soil samples, all previously mentioned mine bacterial species, except cyanobacteria, were identified as non-SOB.

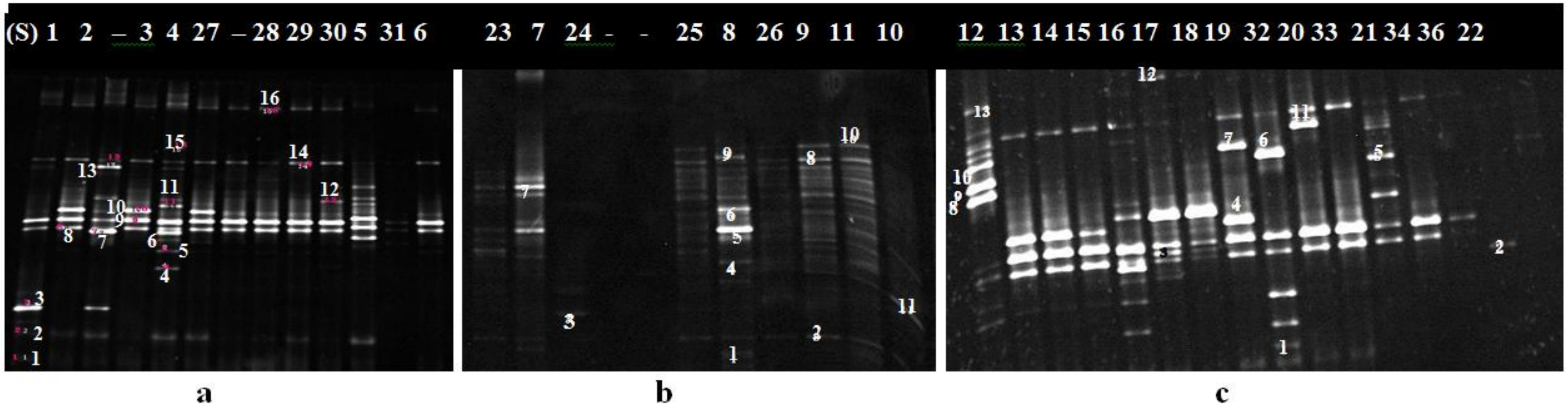

Figure 2. *DGGE profiles of PCR amplicons of 16S rDNA*

The position of bands is indicated by numbers that correspond to species of bacterial.

a, *1: Acidithiobacillus ferrooxidans; 2: Bacillus sp.; 3: Acidithiobacillus ferridurans; 4: Sphingobium sp.; 5: not detected; 6: Halospirulina sp.; 7: Acidithiobacillus ferridurans; 8: not detected; 9: Pseudomonas sp.; 10: Pseudomonas sp.; 11: Sphingobium sp.; 12: Sphingobium sp.; 13: Acinetobacter sp.;14: Pseudomonas sp.; 15: Bacillus sp.; 16: Pseudomonas sp.*

b, *1: Acidithiobacillus ferrooxidans; 2: Propionibacterium sp.; 3: Streptomyces sp.; 4: not detected; 5: Acidithiobacillus ferridurans; 6: Acidithiobacillus ferridurans; 7: Acinetobacter johnsonii; 8: not detected; 9: not detected; 10: Bacillus sp.; 11: Acidithiobacillus caldus.*

c, *1: not detected; 2: Halospirulina sp.; 3: Sphingomonas sp.; 4: Bacillus sp.; 5: Bacillus sp.; 6: Pseudomonas sp; 7: Pseudomonas sp; 8: Pseudomonas sp; 9: Pseudomonas sp; 10: Pseudomonas sp; 11: Pseudomonas sp; 12: Sphingobium sp.; 13: Pseudomonas sp.*

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Table 4.1. *Identification of bands in samples at DGGE banding patterns based on Figure 2 (a)*

Closest relative	**Sample**											**∑ bands**
	S1	**S2**	**S3**	**S4**	**S27**	**S28**	**S29**	**S30**	**S5**	**S31**	**S6**	
Acidithiobacillus ferrooxidans	*										*	2
Acidithiobacillus ferridurans				*					*			2
Acidithiobacillus ferridurans	*											1
Acidithiobacillus caldus		*	*	*					*			4
Bacillus megaterium	*			*	*		*		*			5
Bacillus subtilis	*	*	*	*	*	*	*	*			*	9
Halospirulina sp.				*					*			2
Sphingobium sp.				*				*				2
Sphingobium sp.		*	*	*					*			4
Pseudomonas syringae	*	*	*	*	*	*	*	*			*	9
Pseudomonas syringae	*	*	*	*	*	*	*	*			*	9
Pseudomonas sp		*	*		*				*			4
Pseudomonas syringae	*	*	*	*	*	*	*	*	*	*	*	11
∑ *bands*	7	7	7	10	6	4	5	5	7	1	5	64

Arrangement of bands in *Table 5 (5.1, 5.2, 5.3)* according to *Figure 2 (a, b, c)*, respectively.

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Table 4.2. *Identification of bands in samples at DGGE banding patterns based on Figure 2 (b)*

	Sample									∑ bands
Closest relative	**S23**	**S7**	**S24**	**S25**	**S8**	**S26**	**S9**	**S11**	**S10**	
Acidithiobacillus ferrooxidans					*					1
Acidithiobacillus ferridurans		*			*		*	*		4
					*		*	*		3
Acidithiobacillus caldus		*					*	*	*	4
Propionibacterium sp.	*	*		*	*	*	*	*	*	8
Streptomyces sp.			*							1
Acinetobacter johnsonii		*		*	*		*	*		5
Bacillus subtilis							*	*		2
∑ bands	1	4	1	2	5	1	6	6	2	28

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Table 4.3. *Identification of bands in samples at DGGE banding patterns based on Figure 2 (c)*

	Sample																∑ bands
Closest relative	**S12**	**S13**	**S14**	**S15**	**S16**	**S17**	**S18**	**S19**	**S32**	**S20**	**S33**	**S21**	**S34**	**S35**	**S36**	**S22**	
Acidithiobacillus ferrooxidans					*											*	2
Acidithiobacillus ferrooxidans					*	*											2
Acidithiobacillus ferrooxidans					*	*	*					*					4
Halospirulina sp.	*				*	*										*	4
Sphingomonas sp.						*	*										2
Bacillus subtilis		*	*	*				*									4
Bacillus subtilis												*					1
Pseudomonas sp									*								1
Pseudomonas sp	*							*				*					3
Pseudomonas sp	*																1
Pseudomonas syringae	*																1
Pseudomonas sp	*																1
Pseudomonas sp	*				*					*		*					4
Pseudomonas sp	*																1
Pseudomonas syringae	*	*	*	*	*	*		*	*	*	*		*				11
Pseudomonas syringae	*	*	*	*	*	*	*	*	*	*	*	*	*	*			14
Sphingobium yanoikuyae							*								*	*	3
Sphingobium yanoikuyae					*												1
Acinetobacter johnsonii	*				*					*		*					4
∑ bands	10	3	3	3	9	6	4	4	3	4	2	6	2	1	1	3	64

Table 5. *Mean abundance of bacterial populations of copper mine and surrounding agricultural soil samples*

Sub Class	**Relative Abundance,%**		**Taxon**	**Av. Dissim**	**Contrib, %**	**Relative Abundance,%**	
	Mine	**Ag**				**Mine**	**Ag**
Gammaproteobacteria	63.3	38.8	*Acidithiobacillus ferridurans*	7.99	10.65	12.87	0.00
			Acidithiobacillus thiooxidans	0.63	0.84	0.99	0.00
			Pseudomonas sp.	7.42	9.89	11.87	10.81
			Pseudomonas syringae	6.34	8.44	14.84	23.94
			Acinetobacter johnsonii	5.39	7.18	7.92	3.63
			Acidithiobacillus ferrooxidans	4.47	5.96	6.92	0.00
			Acidithiobacillus caldus	5.00	6.66	7.92	0.00
Alphaproteobacteria	8.9	19.1	*Sphingobium yanoikuyae*	4.92	6.56	6.92	5.76
			Sphingomonas sp.	1.19	1.58	1.98	0.00
			Starkeya novella	4.18	5.57	0.00	13.43
Cyanobacteria	5.9	0	*Halospirulina sp.*	3.66	4.87	5.95	0.00
Betaproteobacteria	0	16	*Thiobacillus thiophilus*	1.77	2.36	0.00	5.26
			Thiobacillus thioparus	2.19	2.91	0.00	5.56
			Thiobacillus aquaesulis	2.04	2.71	0.00	5.26
Firmicutes	16.8	15.7	*Sulfobacillus acidophilus*	1.96	2.61	2.97	0.00
			Bacillus subtilis	7.48	9.97	11.87	10.51
			Bacillus megaterium	2.34	3.12	1.98	5.26
Actinobacteria	4.9	10.5	*Streptomyces sp.*	1.09	1.46	0.00	2.63
			Propionibacterium sp.	5.00	6.66	4.95	7.87

The frequency and relative frequency (abundance) of identified bacterial OTUs in mine and agriculture environments as well as each sample are indicated in *Figures 3 and 4*, respectively. Although *Pseudomonas syringae* in both environments had the highest frequency and relative frequency (14.84% and 23.64%, respectively), significant differences in *Alpha-* and *Betaproteobacteria* distribution were observed (*Table 5*). The dominant taxa of copper mine soil samples were *Pseudomonas*, *Acidithiobacillus* (*At. thiooxidans*, *At. ferridiurans* and *At. caldus*), and *Bacillus* bacteria, while in the agricultural environment it was *Thiobacillus* bacteria (*Th. thiophilus*, *Th. thioparus*, *Th. aquasulis*). SOB including *Acidithiobacillus ferrooxidans*, *Acidithiobacillus ferridurans, Acidithiobacillus thiooxidans*, and *Acidithiobacillus caldus* had relative frequency of 6%, 13%, 1%, and, 7% respectively. The major OTUs that helped differentiate between the two environments were *At. ferridurans*, *Bacillus subtilis*, *Pseudomonas sp.*, and *Pseudomonas syringae* (contribution of 10.65, 9.97, 9.89, and 8.44, respectively).

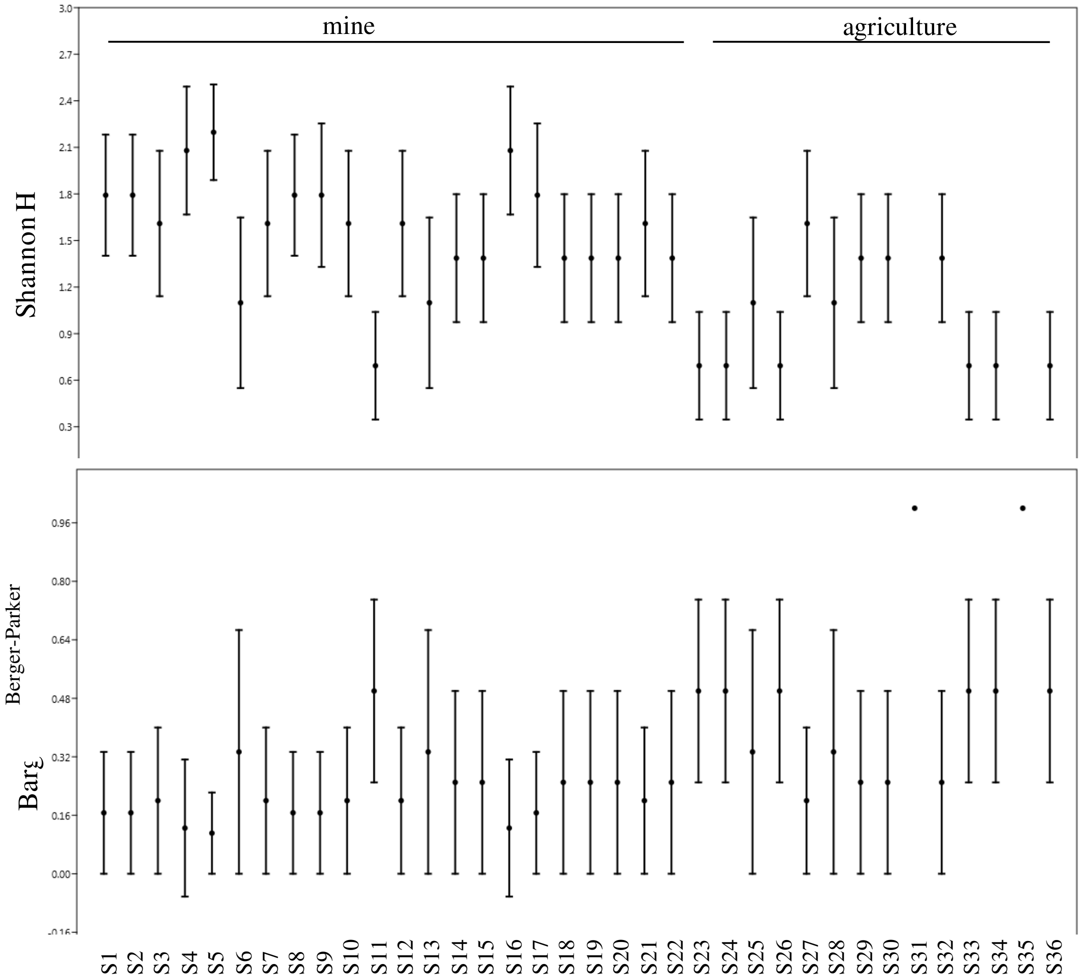

Figure 3. *Estimates of bacterial diversity indices in agricultural and mine samples*

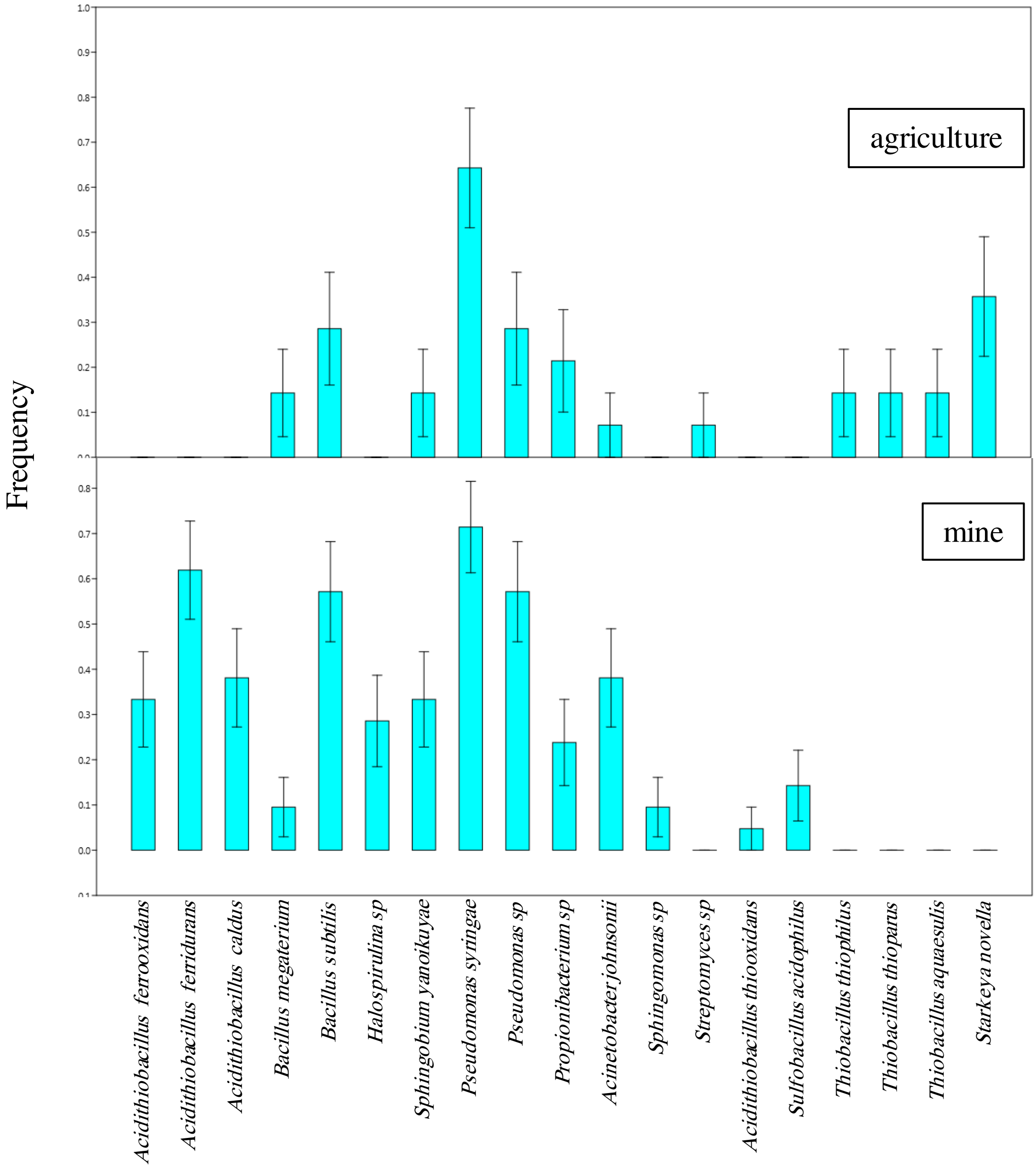

Figure 4. *Frequency of identified bacterial populations in mine and agricultural environments*

Results of diversity indices of different mine and agricultural soils are indicated in *Figure 5*. A comparison of resultant Shannon H values revealed that the bacterial diversity of the copper mine area was greater than the agricultural soils (mean H value: 1.57 and 0.87, respectively). According to these results, the samples from the southward area of the copper mine (S4, S5, and S16 samples) had the greatest bacterial diversity and samples from the northward area (S11 and S13 samples) had the lowest bacterial diversity. In addition, agricultural soil samples near the southward area of the mine (S27, S28, and S30) had more bacterial diversity in comparison with the other samples. In contrast, the Berger-Parker index indicated that agricultural soil samples had more dominant OTUs than mine soil samples (d = 0.47 *vs.* 0.22). In addition, the reciprocal

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value of the Berger-Parker index confirmed more diversity of bacterial populations in mine soil samples (1/d = 4.52 *vs.* 2.11).

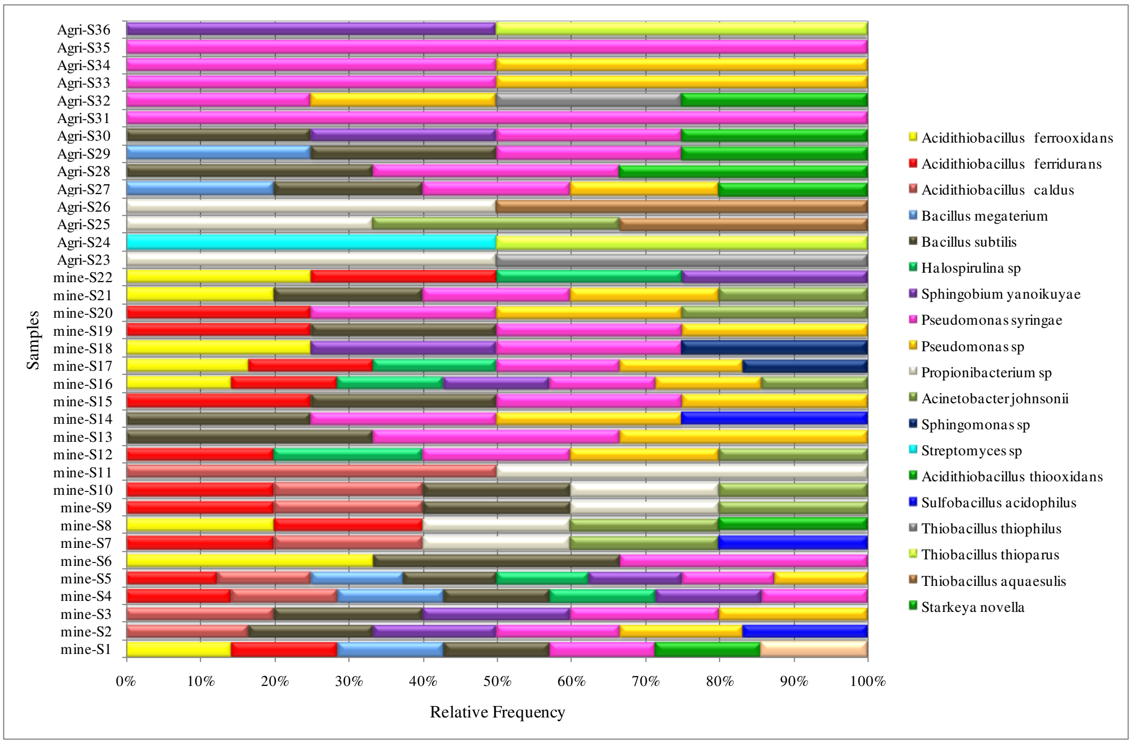

Figure 5. *Relative frequency of identified bacteria of mine and agricultural soil samples (S1-S36)*

Analysis of the similarities between mine and agricultural samples and identified OTUs revealed a significant difference related to environmental conditions (R = 0.2565, P = 0.001). UPGMA cluster analysis indicated the coordinate result with the ANOSIM method with two separated clusters that belonged to each environmental type (*Figure 6*). The result of non-metric multidimensional scaling (N-MDS) suggested a large dissimilarity between bacterial population structures in the mine and surrounding agricultural soils with a stress value of 0.1864, as shown in *Figure 7*. The pattern of environmental distribution in the N-MDS plot indicated the importance of sulphur and, to a smaller extent, Al, Zn, and Mo in determining bacterial communities in the copper mine environment. The bacterial communities of the agricultural soil samples had an association with a mixture of environmental parameters such as Ca, OC, pH, and other elements (Fe, As, Mg, Mn, and cobalt (Co)). Contributions of these elements and environmental factors in determining bacterial communities in the two types of soils was revealed by principal components analysis (PCA), as shown in *Figure 8*. Sulphur and calcium were the main environmental elements that could influence the pH of the soil and prepare conditions for acidophilic, neutrophilic, and alkalophilic bacterial populations. Therefore, the copper mine soil samples had a high concentration of sulphur (4.16 ± 0.04%) and a low pH value (pH = 4.5) which caused the colonization of acidophilic bacterial species such as *Acidithiobacillus sp*. and *Sulfobacillus sp*. with greater abundance than other neutrophilic bacteria. On the other hand, agricultural soil samples had a low concentration of sulphur (0.56 ± 0.004%), a high concentration of calcium (2.10 ± 0.013%), and a higher pH value (pH = 7.8), which eliminated the growth of the aforementioned acidophilic bacteria and induced the proliferation of neutrophilic bacterial populations such as *Thiobacillus sp*. and *Starkeya sp*.

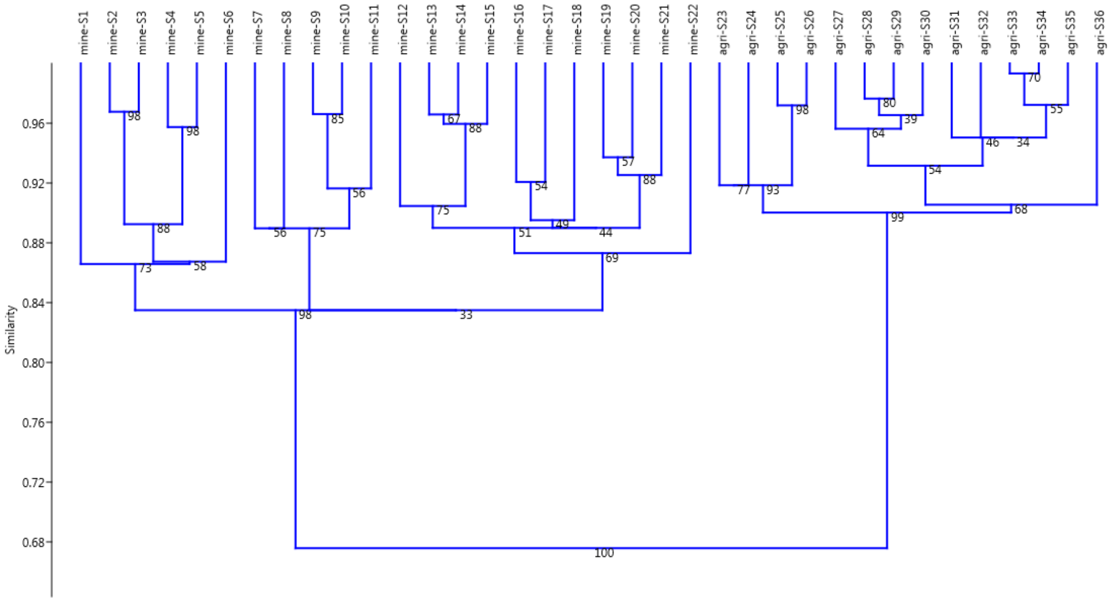

Figure 6. UPGMA cluster analysis of mine and agricultural soil samples

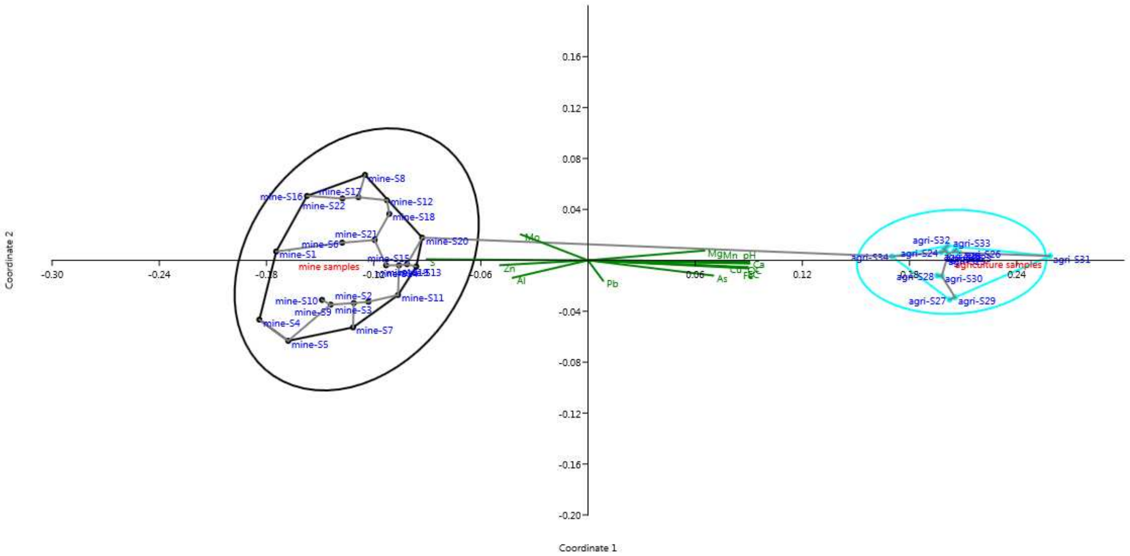

Figure 7. Non-metric multidimensional scaling (N-MDS) of dissimilarities between samples and physicochemical parameters. Mine soil samples (black), agricultural soil samples (blue), and physicochemical parameters (Al, As, Ca, Cu, Mg, Mn, Pb, Zn, Mo, S, Fe, and pH) (green)

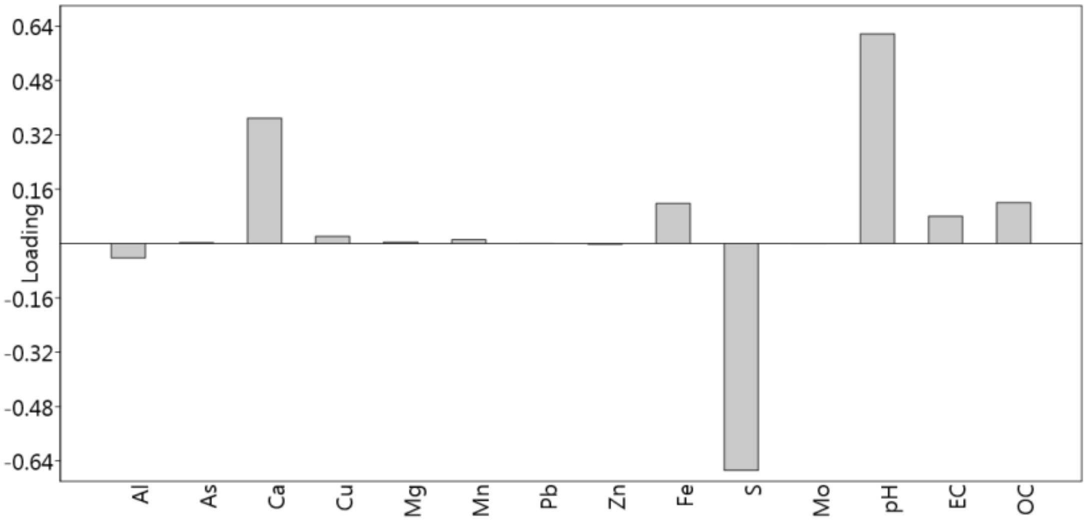

Figure 8. *Principal components analysis (PCA) of environmental factors on bacterial communities in mine and agricultural samples*

Discussion

DNA-based bacterial identification techniques (full-length cloning and DGGE screening of the 16S rRNA gene) provided successful methods to elucidate the diversity of bacterial populations of the copper mine and the surrounding agricultural area, similar to other studies (Bielefeldt et al., 2009; Blaud et al., 2014; Brito et al., 2013; Gelsomino et al., 1999). The noticeable environmental conditions of the mine and agricultural sites strongly influenced the production of habitat-specific communities of *Acidithiobacillus sp.* and *Sulfobacillus sp.* bacteria in the mine area and communities of *Thiobacillus sp.* and *Starkeya sp.* in the agricultural area. Detection of facultative sulfur oxidizing bacteria in agriculture soil samples could be due to root exudates of plants (Widawati et al., 2005).

The *Gammaproteobacteria* class was the main bacterial community in the mine environment, with 63% abundance at the copper mine and 39% in the surrounding agricultural area. As is clear from most reported data of copper mine environments (Hao et al., 2007; Heinzel et al., 2009; Rodrigues et al., 2014), bacterial species which belong to *Gammaproteobacteria* had higher frequency than other bacterial species. *Gammaproteobacteria* species of *At. ferroxidans, At. ferridurans, At. thiooxidans*, and *At. caldus* convert insoluble sulphide to soluble minerals which are frequent in acidic mine soils (Friedrich et al., 2001). In this study these *Gammaproteobacteria* comprised 7%, 13%, 1%, and 9% relative abundance of bacterial communities of the mine environment, respectively, and were not identified in agricultural soil. This could be due to the acidophilic characteristic of these bacterial species, in concordance with Norris (2007), Rojas-Avelizapa et al. (2013), and Rodrigues et al. (2014) (Norris, 2007; Rodrigues et al., 2014; Rojas-Avelizapa et al., 2013).

Heterotrophic *Gammaproteobacteria*, including *Pseudomonas sp.*, *Pseudomonas syringae*, and *Acinetobacter johnsonii*, were isolated from both the mine and agricultural environments with 12%, 15%, and 8% at the mine and 11%, 24%, and 4% in agricultural soils, respectively. Since these bacterial species use organic matter as a carbon resource in soil for metabolism (Okibe and Johnson, 2004), colonization conditions are sustained in both mine and agricultural environments. *Ac. johnsonii* OTU as a heterotroph phosphate reducing bacteria in mine soils had a relative frequency twice that of agricultural soil (8% and 4%, respectively). This could be due to the impact of the overgrowth of other bacterial communities that consequently decreased its relative abundance.

In contrast to the mine soil where *Gammaproteobacteria* was dominant, in agricultural soil a high abundance of *Betaproteobacteria* class was detected while none was detected in mine soil samples. Neutrophilic characteristics of these bacteria differentiate them for proliferation in agricultural soil with a pH of 7 (Chapman, 1990; Widawati et al., 2005). There have been no reports of the existence of *Betaproteobacteria* in studies of copper mine environments until this study (Norris, 2007; Rodrigues et al., 2014; Sánchez-Andrea et al., 2011).

The *Actinobacteria* genus relative frequency in agricultural soils was greater than in mine soils, at 10% and 5%, respectively. This pattern was discovered in studies of Jiang et al. (1991) and Dhanasekaran et al. (2013) that implied a high coordination between organic matter of agricultural soil and the frequency of *Actinobacteria* (Dhanasekaran et al., 2013; Jiang and Xu, 1990). The *Halospirulina sp.* OTU as an autotroph and extremophile bacteria was identified solely in mine soil (6% relative abundance), similar to studies of Rodrigues et al. (2014), Díez et al. (2007), and Hao et al. (2007) (Díez et al., 2007; Hao et al., 2007; Rodrigues et al., 2014).

Clustering analysis of all the above bacterial combinations in mine and agricultural soil samples separates the two sites completely in a UPGMA tree (*Figure 5*). Significant differences in element concentrations, soil manipulation, water, and organic matter content of mine and surrounding agricultural soils over the long term produce habitat-specific bacterial communities. According to the results of N-MDS analysis, chemical parameters of sulphur, pH, and Ca have the most effect on a bacterial community's proportion in different soil samples (*Figure 7*). Rodrigues et al. (2014) addressed the water and organic matter content of soil in a copper mine area as the main parameter of bacterial differentiation between slope and drainage sites (Rodrigues et al., 2014).

Conclusion

Bacterial communities of a copper mine and the surrounding soil are dependent on different environmental conditions such as element concentration (S, Ca, Al, Mg, Zn, Mn, etc.), pH, organic matter, mineralogy, and other climatic properties (temperature, precipitation, evaporation, etc.) and produce habitat-specific communities over a long time period. In this study, relative frequency of SOB in two different soils using culture-dependent and independent approach has been investigated. Result indicated that soil samples from a mine site at the Sarcheshmeh Copper Complex contained these diverse genera of sulphur-oxidizing bacteria, including *Acidithiobacillus sp.* and *Sulfobacillus sp.* and non-sulphur oxidizing bacteria, including *Pseudomonas sp.*, *Bacillus sp.*, *Sphingobium sp.*, *Sphingomonas sp.*, *Acinetobacter sp.*, *Halospirulina sp.*, and *Propionibacterium sp.* which were derived from the significant effect of sulphur concentrations. In addition, in soil samples from agricultural land surrounding the mine, genera of SOB included *Thiobacillus sp.*, *Starkeya sp.*, and non-SOB genera included

Pseudomonas sp., *Acinetobacter sp.*, *Bacillus sp.*, *Sphingobium sp.*, *Streptomyces sp.*, and *Propionibacterium sp.*, the presence of which was owing to the significant effect of pH and calcium concentrations.

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REFERENCES

[1] Bielefeldt, A., Gutierrez-Padilla, M.G.D., Ovtchinnikov, S., Silverstein, J., Hernandez, M. (2009): Bacterial kinetics of sulfur oxidizing bacteria and their biodeterioration rates of concrete sewer pipe samples. - Journal of Environmental Engineering 136: 731-738.

[2] Blaud, A., Chevallier, T., Virto, I., Pablo, A.-L., Chenu, C., Brauman, A. (2014): Bacterial community structure in soil microaggregates and on particulate organic matter fractions located outside or inside soil macroaggregates. - Pedobiologia 57: 191-194.

[3] Bond, P.L., Smriga, S.P., Banfield, J.F. (2000): Phylogeny of microorganisms populating a thick, subaerial, predominantly lithotrophic biofilm at an extreme acid mine drainage site. - Applied and Environmental Microbiology 66: 3842-3849.

[4] Brito, E.M., Piñón-Castillo, H.A., Guyoneaud, R., Caretta, C.A., Gutiérrez-Corona, J.F., Duran, R., Reyna-López, G.E., Nevárez-Moorillón, G.V., Fahy, A., Goñi-Urriza, M. (2013): Bacterial biodiversity from anthropogenic extreme environments: a hyper-alkaline and hyper-saline industrial residue contaminated by chromium and iron. - Applied microbiology and biotechnology 97: 369-378.

[5] Brofft, J.E. (2002): Molecular analysis of bacterial communities associated with a forested wetland impacted by reject coal. Diss. University of Georgia.

[6] Castilla, J., Nealler, E. (1978): Marine environmental impact due to mining activities of El Salvador copper mine, Chile. - Marine Pollution Bulletin 9: 67-70.

[7] Chapman, S. (1990): Thiobacillus populations in some agricultural soils. - Soil Biology and Biochemistry 22: 479-482.

[8] Clarke, K.R. (1993): Non-parametric multivariate analyses of changes in community structure. - Australian journal of ecology 18: 117-117.

[9] Cole, J.R., Chai, B., Farris, R.J., Wang, Q., Kulam, S., McGarrell, D.M., Garrity, G.M., Tiedje, J.M. (2005): The Ribosomal Database Project (RDP-II): sequences and tools for high-throughput rRNA analysis. - Nucleic acids research 33: 294-296.

[10] Dhanasekaran, D., Thajuddin, N., Panneerselvam, A. (2013): Distribution and Ecobiology of Antagonistic Streptomyces from Agriculture and Coastal Soil in Tamil Nadu, India.

[11] Díez, B., Bauer, K., Bergman, B. (2007): Epilithic cyanobacterial communities of a marine tropical beach rock (Heron Island, Great Barrier Reef): diversity and diazotrophy. - Applied and environmental microbiology 73: 3656-3668.

[12] Friedrich, C.G., Rother, D., Bardischewsky, F., Quentmeier, A., Fischer, J. (2001): Oxidation of reduced inorganic sulfur compounds by bacteria: emergence of a common mechanism? - Applied and Environmental Microbiology 67: 2873-2882.

[13] Gelsomino, A., Keijzer-Wolters, A.C., Cacco, G., van Elsas, J.D. (1999): Assessment of bacterial community structure in soil by polymerase chain reaction and denaturing gradient gel electrophoresis. - Journal of Microbiological Methods 38: 1-15.

[14] Grundmann, L., Gourbiere, F. (1999): A micro-sampling approach to improve the inventory of bacterial diversity in soil. - Applied Soil Ecology 13: 123-126.

[15] Hao, C.-b., Zhang, H.-x., Bai, Z.-h., Qing, H., Zhang, B.G. (2007): A novel acidophile community populating waste ore deposits at an acid mine drainage site. - Journal of Environmental Sciences 19: 444-450.

[16] Heinzel, E., Hedrich, S., Janneck, E., Glombitza, F., Seifert, J., Schlömann, M. (2009): Bacterial diversity in a mine water treatment plant. - Applied and environmental microbiology 75: 858-861.

[17] Holt, J., Krieg, N., Sneath, P., Staley, J., Williams, S. (1994): Bergey's manual of determinative microbiology. Williams and Wilkins, Maryland.

[18] Jiang, C., Xu, L. (1990): Characteristics of the populations of soil actinomycetes in Yunnan. - Actinomycetes 1: 67-74.

[19] Johnson, D. (2008): Biodiversity and interactions of acidophiles: Key to understanding and optimizing microbial processing of ores and concentrates. - Transactions of Nonferrous Metals Society of China 18: 1367-1373.

[20] Kelly, D.P., Wood, A.P. (2000): Reclassification of some species of Thiobacillus to the newly designated genera Acidithiobacillus gen. nov., Halothiobacillus gen. nov. and Thermithiobacillus gen. nov. - International Journal of Systematic and Evolutionary Microbiology 50(2): 511-516.

[21] Marsh, R.M., Norris, P.R. (1983): The isolation of some thermophilic, autotrophic, iron- and sulfur-oxidizing bacteria. - FEMS Microbiology Letters 17: 311-315.

[22] Mendez, M.O., Neilson, J.W., Maier, R.M. (2008): Characterization of a bacterial community in an abandoned semiarid lead-zinc mine tailing site. - Applied and environmental microbiology 74: 3899-3907.

[23] Muyzer, G., Smalla, K. (1998): Application of denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE) in microbial ecology. - Antonie van Leeuwenhoek 73: 127-141.

[24] Norris, P.R. (2007): Acidophile diversity in mineral sulfide oxidation. - Biomining. Springer 199-216.

[25] Okibe, N., Johnson, D.B. (2004): Biooxidation of pyrite by defined mixed cultures of moderately thermophilic acidophiles in pH-controlled bioreactors: Significance of microbial interactions. - Biotechnology and bioengineering 87: 574-583.

[26] Ranjard, L., Lejon, D.P., Mougel, C., Schehrer, L., Merdinoglu, D., Chaussod, R. (2003): Sampling strategy in molecular microbial ecology: influence of soil sample size on DNA fingerprinting analysis of fungal and bacterial communities. - Environmental Microbiology 5: 1111-1120.

[27] Rodrigues, V.D., Torres, T.T., Ottoboni, L.M. (2014): Bacterial diversity assessment in soil of an active Brazilian copper mine using high-throughput sequencing of 16S rDNA amplicons. - Antonie van Leeuwenhoek 106: 879-890.

[28] Rojas-Avelizapa, N., Gómez-Ramírez, M., Hernández-Gama, R., Aburto, J., García de León, R. (2013): Isolation and selection of sulfur-oxidizing bacteria for the treatment of sulfur-containing hazardous wastes. - Chemical and Biochemical Engineering Quarterly 27: 109-117.

[29] Sánchez-Andrea, I., Rodríguez, N., Amils, R., Sanz, J.L. (2011): Microbial diversity in anaerobic sediments at Rio Tinto, a naturally acidic environment with a high heavy metal content. - Applied and environmental microbiology 77: 6085-6093.

[30] Schoenborn, L., Yates, P.S., Grinton, B.E., Hugenholtz, P., Janssen, P.H. (2004): Liquid serial dilution is inferior to solid media for isolation of cultures representative of the phylum-level diversity of soil bacteria. - Applied and environmental microbiology 70: 4363-4366.

[31] Schultz, L.G. (1964): Quantitative interpretation of mineralogical composition from X-ray and chemical data for the Pierre Shale - United States GovernmentPrinting Office, Washington

DOI: http://dx.doi.org/10.15666/aeer/1403_509533

[32] Smith, F.W., Rae, A.L., Hawkesford, M.J. (2000): Molecular mechanisms of phosphate and sulphate transport in plants. Biochimica et Biophysica Acta (BBA). - Biomembranes 1465: 236-245.

[33] Soil Survey Staff (2010): Keys to Soil Taxonomy, 11th ed. USDA-Natural Resources Conservation Service, Washington, DC

[34] Sorokin, D.Y., Tourova, T.P., Lysenko, A.M., Muyzer, G. (2006): Diversity of culturable halophilic sulfur-oxidizing bacteria in hypersaline habitats. - Microbiology 152: 3013-3023.

[35] Stackebrandt, E., Frederiksen, W., Garrity, G.M., Grimont, P.A., Kämpfer, P., Maiden, M.C., Nesme, X., Rosselló-Mora, R., Swings, J., Trüper, H.G. (2002): Report of the ad hoc committee for the re-evaluation of the species definition in bacteriology. - International journal of systematic and evolutionary microbiology 52: 1043-1047.

[36] Walkley, A., Black, I.A. (1934): An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. - Soil science 37: 29-38.

[37] Widawati, S., Suliasih, H.L., Sugiharto, A. (2005): Biodiversity of Soil Microbes from Rhizosphere at Wamena Biological Garden (WBiG), Jayawijaya, Papua. - Biodiversitas 6: 6-11.

[38] Ying, T., Yong-Ming, L., HUANG, C.-Y., Jian, L., Zhen-Gao, L., Christie, P. (2008): Tolerance of grasses to heavy metals and microbial functional diversity in soils contaminated with copper mine tailings. - Pedosphere 18: 363-370.

[39] Yun-Guo, L., Zhang, H.-Z., Guang-Ming, Z., Huang, B.-R., Xin, L. (2006): Heavy metal accumulation in plants on Mn mine tailings. - Pedosphere 16: 131-136.

LIFE CYCLE ASSESSMENT (LCA) OF WINTER WHEAT IN AN INTENSIVE CROP PRODUCTION SYSTEM IN WIELKOPOLSKA REGION (POLAND)

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Abstract. This study aimed to assess the environmental impact of winter wheat production in an intensive crop production system. In view of environmental concerns linked to modern practices of crop production, it is necessary to identify the ecological profile of highly productive winter wheat. The life cycle assessment (LCA) of this crop was performed in two large-scale farms, located in the Wielkopolska region (Poland), in the years 2011-2013. Results obtained indicated that the core processes related to wheat production on the farm had the biggest influence on acidification, eutrophication and global warming potentials. The possibility of photochemical ozone creation and depletion of abiotic resources, such as minerals and fossil fuels, were most dependent on the upstream processes. The mineral fertilization process was identified as the most important source of environmental threats. The grain transport stage had the lowest share in all analyzed impact category indicators. Normalization showed that acidification attributed to wheat production had the greatest environmental impact on the overall environmental profile. This indicates that the priority direction in mitigation of environmental impacts associated with intensive wheat production should be lowering adverse effect on soil acidification, followed by global warming and eutrophication.

Keywords: *agriculture, environment, impact category indicators , life cycle stages, grain crop*

Introduction

Cereal production is one of the most important agricultural activities in Poland. The share of cereals in the total cropping patterns is around 73% (CSO, 2014). In cereal cultivation, wheat is a dominant crop. Its acreage decreased by 19% over the years 2000-2013 and occupied 2138 thous. ha in 2013 (LDB CSO, 2015). Despite this drop, an upward trend in its production output has been observed. This was caused by an increase in yield by nearly 38%, up to 44.4 dt per hectare. Yield increases are still below the wheat yielding potential, which in favourable edaphic and climatic conditions as well as in modern cultivation technology can reach the level of 120 dt per hectare in Poland (Weber at al., 2011). Utilization of modern agricultural machinery, mineral fertilizers and pesticides contributed to the intensification of production and higher yields. However, the result of this process may have an adverse impact on the environment. The use of large amounts of industrial means of production in farming contributes largely to the depletion of non-renewable resources, an increase in greenhouse gas (GHG) emissions, and pollution from chemical substances that can lead to soil acidification and eutrophication (Bieńkowski et al., 2014; Nemecek et al., 2011).

Striving for lower emissions of gaseous pollutants and improvements in the use of natural resources and energy has been emphasized by strategies for sustainable development and use of renewable energy. It caused an increased interest in methods

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for environmental impact assessments in the EU, such as the LCA (Life Cycle Assessment) (Hontelez and Buitenkamp, 2006; EC, 2013; European Council Conclusions, 2014). This method allows for an analysis of the potential environmental risks throughout the life cycle of a product, i.e. from the extraction of raw materials, their processing and transport, through the production, distribution and utilization, until the final waste management. It was created primarily for the needs of the industry, but it has also become a useful tool in the agricultural sector (Caffrey and Veal, 2013). The results obtained at the production stage of primarily raw materials are essential for the future analysis of the final products, manufactured in the agri-food industry (Roy et al., 2009). In order to achieve reliable and accurate results using the life cycle analysis, research at the regional and national level should be conducted. Using data of global significance does not allow for an objective assessment due to the differentiation of production conditions across world (Biswas and John, 2009).

Application of the LCA method in the assessment of environmental effects of cereals production in Poland will allow to identify the most hazardous parts of the production cycle. It will create the basis for improving technology of production process, for the environment and farmers' benefits. The aim of the study was to determine the environmental impact of winter wheat grown in an intensive production system, considered to be the most representative production type of this crop in the Wielkopolska region.

Materials and methods

The materials used for the analysis are data from two agricultural farms: Trzebiny (Farm 1) and Długie Stare (Farm 2), located in the south-western part of the Wielkopolska region (Poland), during the period 2011-2013. The region is distinguished by high technical efficiencies in plant and livestock production, as well as by a large agricultural commodity output compared to other ones (Bieńkowski and Jankowiak, 2006). Farms are part of the Długie Stare Agricultural Company Ltd., a subsidiary of the State Treasury, which belongs to a small group of strategic state-owned companies. The company focuses on crop production including cereals, oilseed rape, sugar beet and in livestock farming – dairy cows and beef cattle. It achieves good production results in both crop and animal farming. It is very well organized in terms of balance between the main branches of production.

Each of the studied farms has area of about 500 hectares of agricultural land (AL) (*Table 1*). They run intensive agricultural production, as evidenced by high levels of mineral fertilizers (NPK), compared to lower fertilization in Poland with an average of 129.3 kg ha^{-1} AL (CSO, 2014). Farm 1 had markedly higher variability of NPK fertilization. The reason for that was a drop in the use of phosphorus and potassium fertilizers during the research period after determined build up of soil phosphorus and potassium reserves. Total cereal yields in the analyzed period in both farms were higher than the average yield in the country (by 54.8% in Farm 1 and by 80.6% in Farm 2). Livestock density was more than 2-fold lower than the upper limit of 1.5 DJP ha^{-1} AL, which is recommended for environmental reasons by a code of good agricultural practices (Duer et al., 2004). The share of cereals in cropping patterns ranged from 51.9 to 61.1%. Industrial plants, including root and oil crops, accounted for 16.5% in Farm 1 and 27.0% in Farm 2. High overall intensity of farm management and the importance of livestock processes have also been reflected by the presence of the annual and the perennial fodder crops (in total 18.1% of AL in the Farm 1 and 21.1% of AL in the Farm 2).

Table 1. *Characterization of studied farms (averages for the years 2011-2013 ± standard deviation)*

Specification	Farm 1	Farm 2
Area AL (ha)	492.29	516.24
Soil valuation index (points)	0.67	1.21
NPK ferilization (kg ha^{-1} AL)	245.94 ±43.61	269.80 ±14.71
Livestock density (AU ha^{-1})	0.66 ±0.01	0.72 ±0.03
Cereal yield (dt ha^{-1})	56.4 ±5.1	65.8 ±2.3
Cropping pattern (%)		
- cereals	61.1 ±8.2	51.9 ±5.5
- root crops	7.9 ±1.2	12.0 ±4.4
- oil plants	12.9 ±10.7	15.0 ±8.1
- annual fodder crops	13.4 ±4.6	10.6 ±6.9
- perennial fodder crops	4.7 ±4.1	10.5 ±0

Data for the analysis came from the source documents of the farms. They were collected in specially prepared registration forms and supplemented with interviews among the farms’ managers. Additional sources of information were the accounting documents and the technical documentation of agricultural machines and vehicles. The collected data included all production inputs, such as seeds, fertilizers, pesticides, fuel, engine oil, grease, electricity, plus a detailed characterization of tractors and machines. Data on type and duration of technological operations were collected systematically during the development of the field works.

The LCA method used in this study was composed of four phases: 1) goal and scope definition, 2) inventory analysis, 3) impact assessment and 4) interpretation (Brentrup et al., 2004a). In the first phase, the following elements were defined: the purpose, system boundaries, assumptions, limitations and functional unit. The analysis of a set of inputs and outputs, i.e. life cycle inventory (LCI), is featured by collecting quality and quantity data of used materials and energy, production results and estimated emissions. They constitute the basis for the life cycle impact assessment (LCIA), in which after the selection of category and category indicators, the classification and characterization procedures are performed. In the last phase, conclusions are made, according to the pre-determined purpose of the study (interpretation).

The study was carried out from "cradle-to-farm gate", i.e. from manufacturing of means of agricultural production, through the process of field cultivation, harvesting and grain transport to the customer (in this case, it was the company that exports cereals), without the stage of use and waste management. Two functional units were used: 1.0 hectare - expressing the intensity of the wheat production system, and 1.0 ton of grain, which is a measure of its efficiency, quantitatively characterizing the product with reference flow specifically estimated for this unit. The CML methodology based on midpoint approach was applied in the LCIA (phase 3) (Guinée et al., 2002). The impact assessment included the following environmental categories indicators: global warming potential (GWP_{100}), eutrophication potential (EP), acidification potential (AP), photochemical ozone creation potential (POCP), abiotic resources depletion potential for minerals (ADP minerals) and for fossil fuels (ADP fossil fuel), as well as land use and pesticide use.

Impacts for the categories of climate change, soil and water acidification, eutrophication, the formation of photochemical oxidants and depletion of abiotic resources were calculated by using the following formula (Guinée et al., 2002):

$$I_{cat} = \sum_i (m_i \cdot CF_{cat,i}) \qquad \text{(Eq.1)}$$

where I_{cat} is an impact category indicator, m_i - the amount of the i-th substance used or emitted, $CF_{cat,\,i}$ – an impact category characterization factor of the substance.

The use of land was expressed by the indicator calculated as the ratio of surface unit and the yield obtained.

To widen interpretation of the results a normalization procedure was carried out. It is an optional step of the LCIA phase and consists in the presentation of the participation of the specific impact in the general environmental problem. Normalized impact category indicators (NI_{cat}) were calculated as the ratio of the product of the average indicator value for farms (I_{cat}) and the size of the winter wheat production in Europe (P), to the value of the impact category reference indicator in Europe for the year 2005 (IR_{cat}) (Sleeswijk et al., 2008), as shown in the equation below:

$$NI_{cat} = \frac{I_{cat} \cdot P}{IR_{cat}} \qquad \text{(Eq.2)}$$

Within the system, there were distinguished three stages of the life cycle: upstream, core and downstream processes (*Fig. 1*). Production and distribution of the means of agricultural production, including: energy, agricultural machinery, agrochemicals and seeds were analyzed within the upstream processes. The core processes included wheat production on the farm and consisted of the following processes: cultivation, seeding, fertilization, pest management, harvesting and internal transport. Analysis of the downstream processes focused on the grain transport for sale. Environmental effects of each process were investigated on the basis of materials and energy inputs, as well as emissions of mineral substances to the soil environment and gases into the atmosphere. The impact category indicators for the upstream processes were calculated using information provided by the manufacturer, literature sources (Jayasundara et al., 2014; Lillywhite et al., 2007) and AGRIBALYSE® database (Colomb et al., 2013). Agricultural production processes were evaluated on the basis of detailed data from the farms. Methods for calculating direct and indirect emissions of N_2O from fertilizers were adopted in accordance with IPCC (2006) and the Emission Inventory Guidebook (EMEP/EEA, 2013). Emissions from combustion of fuel during field and transport operations were estimated, according to the amount of fuel consumed and emission factors given by the EMEP/EEA (2013). Data for wheat production in Poland and Europe were collected from LDB CSO (2015) and EUROSTAT (2015) databases.

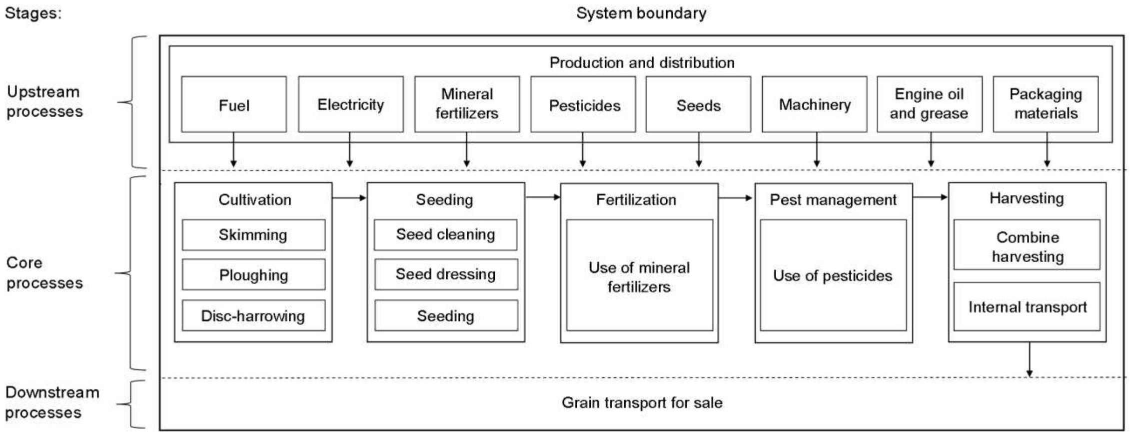

Figure 1. *Schematic diagram of processes according to life cycle stages within the analyzed wheat production system*

Results and discussion

Inventory databases of production processes were created on the basis of information collected from the farms. Quantities of utilized materials and energy were used as the input data for the LCI phase. Farm 2 used higher amounts of inputs per 1.0 hectare of crop area than Farm 1 (*Table 2*). This has been reflected by the calculated impact category indicators, which are presented in *Table 3*. Higher values in relation to the functional units of 1.0 hectare of area and 1.0 ton of grain were noted in the Farm 2. It can be assumed that this resulted mainly from the differences in the amount of applied nutrient between farms. The average value of GWP_{100} indicator per 1 ha amounted to 2457.5 kg CO_2 eq., and in terms of 1 t of grain - 364.1 kg CO_2 eq. It is comparable with results obtained by Charles et al. (2006). While AP and EP indicators for wheat in these farms were two and three times higher than the data from this literature source indicated. The type of nitrogen fertilizers consumed was presumably most responsible for the estimated potentials of acidification and eutrophication. Around 78% of nitrogen applied to winter wheat came from the nitrogen solution (data not presented). According to data reported in literature, 8% of nitrogen in this fertilizer is subject to volatilization loss in the form of ammonia (EMEP/EEA, 2013). A high ammonia emission during the fertilization process makes the most important contribution to these impact categories. While planning to introduce improvements in fertilizer management, the most important issue would be displacing liquid nitrogen fertilizer with another one with low on-field nitrogen emission. In the case of analysis of the environmental impact of wheat production in Chile, the impact indicators for 1.0 ton of grain were even higher: GWP_{100} - 792.76 kg CO_2 eq., AP - 15.28 kg SO_2 eq. and EP - 4.83 kg PO_4^{-3} eq. (Huerta et al., 2012). Considering the land use by both farms, it can be assumed that the lower value of this indicator was due to poorer soil quality in Farm 1 than in Farm 2 (*Table 1*). Hence, the differences in productivity of soils which are directly linked with land use could also be explained by the influence of the same factor.

Application of large amounts of pesticides causes the risk of their leakage into surface water and groundwater, air and food products, as well as the reduction of biodiversity in agricultural landscape (Wattiez and Wiliamson, 2003). The average amount of pesticides used in wheat protection treatments for the farms was 3.2 kg a.i.

ha^{-1}. The application rate was higher than in Poland and Europe, where the estimated levels of plant protection product used were 1.9 and 2.3 kg a.i. ha^{-1}, respectively (Syp et al., 2012).

Table 2. *Inventory data of agricultural inputs by 1 ha of winter wheat in the analyzed farms (averages for the years 2011-2013 ± standard deviation)*

Type of input	Unit	Farm 1	Farm 2
Seeds	kg	182.0 ±5.5	168.9 ±6.0
Fertilizers			
- nitrogen (N)	kg	159.6 ±8.1	229.8 ±6.6
- phosphorous (P_2O_5)	kg	8.1 ±4.2	17.7 ±2.5
- potassium (K_2O)	kg	29.2 ±11.6	58.7 ±7.6
Pesticides			
- herbicides (a.i.)	kg	1.5 ±0.6	2.1 ±2.2
- fungicides (a.i.)	kg	0.8 ±0.1	0.7 ±0.4
- insecticides (a.i.)	kg	0.2 ±0.1	0.0 ±0.0
- plant growth regulators (a.i.)	kg	0.5 ±0.0	0.5 ±0.0
Tractors and mobile machinery	kg	10.8 ±3.5	7.6 ±3.3
Machines and equipment	kg	10.7 ±2.1	9.2 ±2.1
Spare parts and materials for the repair	kg	6.7 ±1.7	5.3 ±1.5
Electricity	kWh	2.9 ±0.5	2.8 ±0.5
Diesel oil	l	98.0 ±4.4	109.4 ±5.8
Gear oil	l	0.6 ±0.1	1.0 ±0.2
Engine oil	l	1.0 ±0.1	0.7 ±0.1
Liquid refrigerant and others	l	0.4 ±0.0	0.4 ±0.0
Polyethylene mesh	m^2	176.8 ±0.9	217.7 ±1.3
500 kg polypropylene woven bag	kg	0.8 ±0.2	0.9 ±0.2

Table 3. *Impact category indicators of the winter wheat production per functional units in analyzed farms (averages for the years 2011-2013)*

Impact category indicators	Farm 1		Farm 2		Mean	
	Functional unit					
	1 ha	1 t	1 ha	1 t	1 ha	1 t
GWP_{100}, kg CO_2 eq.	2173.1	323.9	2742.0	404.4	2457.5	364.1
AP, kg SO_2 eq.	30.8	4.6	44.5	6.6	37.6	5.6
EP, kg PO_4^{-3} eq.	8.8	1.3	12.0	1.8	10.4	1.5
POCP, kg C_2H_4 eq.	0.5	0.1	0.6	0.1	0.6	0.1
ADP minerals, kg Sb eq.	0.013	0.002	0.020	0.003	0.016	0.002
ADP fossil fuel, kg Sb eq.	3.3	0.5	4.2	0.6	3.7	0.6
Land use, ha t^{-1} yr^{-1}	-	0.149	-	0.147	-	0.148
Use of pesticide, kg a.i.	3.0	0.4	3.3	0.5	3.15	0.45

The results showed that among all the technological processes of the analyzed system, the largest influence on the formation of potential environmental impacts had mineral fertilization (*Fig. 2*). Cultivation, sowing and harvesting affected environment to a lesser extent. Plant protection and grain transport had a relatively minor impact. In New Zealand, 52% of the overall amount of GHG emissions from wheat cultivation

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were caused by fertilization, including 48% by nitrogen fertilizer (Safa and Samarasinghe, 2012). Biswas et al. (2010) comparing two phases of wheat production in Australia: the upstream and core processes found that their share in the total GHG emissions amounted to 2.6% and 97.4%, respectively, out of which 59% originated from N_2O emissions from the soil.

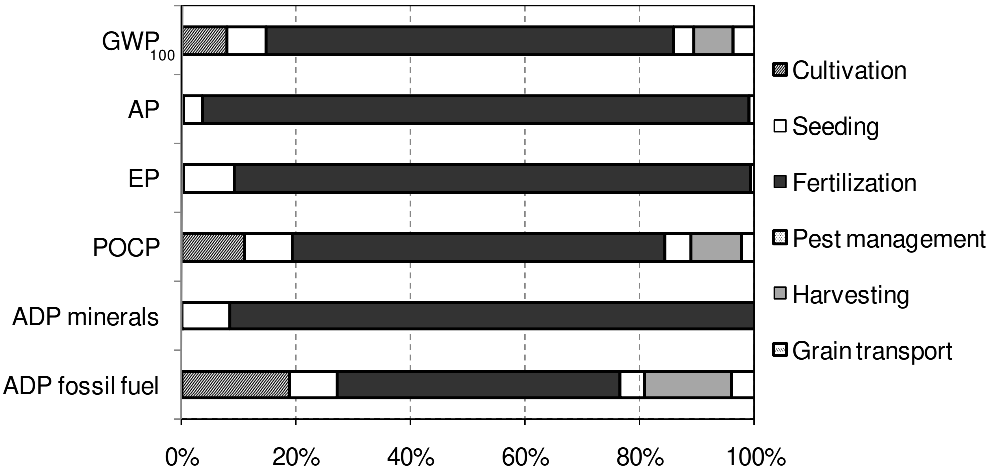

Figure 2. *Relative contribution of technological processes in wheat production to the impact category indicators (averages for the farms and the years 2011-2013)*

Roer et al. (2012) noted that emissions from fields had a remarkable contribution in the indicators of POCP and soil AP, of above 60%. Their studies, indicated that manufacturing and utilization of production means had approximately 50% share in the total value of the ADP mineral, 30% of which was generated by the production of nitrogen fertilizers. In turn, the phosphate fertilizers production was quite important for the caused environmental impact associated with eutrophication, contributing above 30% to the indicator of EP.

The indicators of the GWP_{100}, AP and EP depended mainly on the core processes in the wheat life cycle, which included transport of production means to the farm, cultivation and harvest (*Fig. 3*). At the same time, POCP and ADP mineral were related largely to the production of agricultural means in the upstream processes. The stage of downstream processes had a small part in the formation of the analyzed environmental impacts. In this stage, emissions from fuel combustion and the production of tractors and machines used in grain transport had a significant effect only for GWP_{100}, ADP and POCP. The main factor determining the impact of the processes in this stage is the distance of transport (Meisterling et al., 2009).

An average indicator of land use in farms per 1 t of produced wheat grain had a value of 0.15 ha $year^{-1}$ and was lower, compared to the same indicators calculated for Poland (37.5%) and the European Union (16.7%) (*Fig. 4*). Brentrup et al. (2004b) demonstrated that the utilization of land resources for this purpose improves with increasing intensity of fertilization. It should be noted that the observed differences in the results of this indicator may be due not only to the type of used technology but also to the natural environmental conditions, such as soil quality, climate, terrain relief and water conditions, which determine the potential productivity of crop plants. Schenck et al.

(2008) confirmed this by showing dependence of the yield and land use indicator on the influence of the sum of natural rainfall.

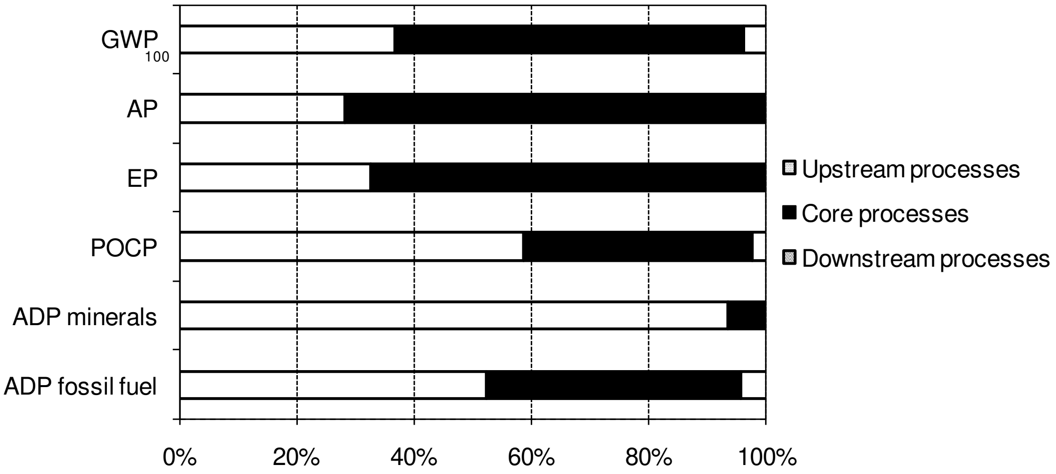

Figure 3. *Share of life cycle stages in wheat production for the analyzed environmental impacts (averages for the farms and the years 2011-2013)*

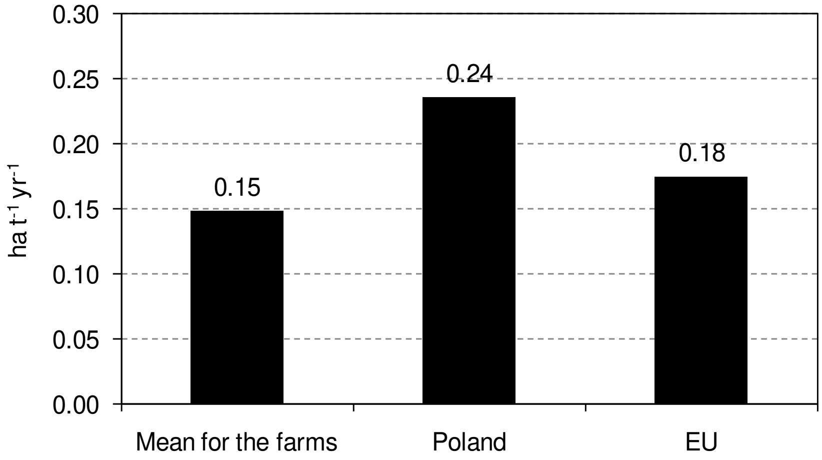

Figure 4. *Land use indicator of winter wheat production in the analyzed farms, Poland and EU (averages for the years 2011-2013)*

Considering results of normalization, it can be concluded that the biggest environmental risk linked to the intensive wheat production system, in the overall environmental profile, is associated with soil acidification (*Fig. 5*). The analyzed production system showed also a relatively large impact on the greenhouse effect and eutrophication. Similar results were also obtained by Huerta et al. (2012) after normalization data with world values.

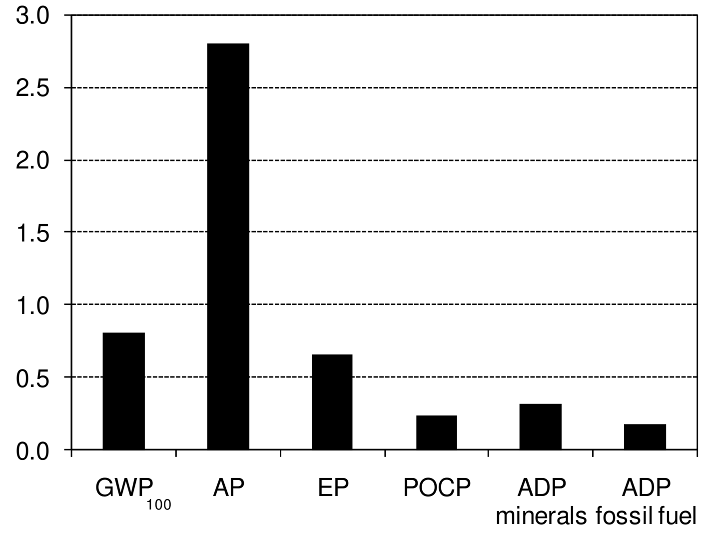

***Figure 5.** Normalized environmental effects of winter wheat production in the analyzed farms (averages for the years 2011-2013)*

Conclusions

Different streams of materials and energy were used in the upstream, core and downstream processes of wheat production. The negative effect of these processes are environmentally harmful emissions. In view of the EU policy requirements in the field of environmental protection, there is necessity to look for new solutions limiting the formation of environmental threats associated with an intensive wheat production. LCA indicates environmental hotspots of the product chain of wheat. This allows to indentify the places and to determine ways to reduce the environmental impact.

The analysis of winter wheat production impact on the environment showed that in the development of more environmentally friendly technologies, emission risks related to the use of mineral fertilizers have to be taken into account. The precise application technology and optimization of fertilization could be very important advances for reducing emissions from the fields and lowering the consumption of raw materials in the fertilizer manufacturing. Implementing reduced tillage systems in grain production in the Wielkopolska region may also reduce the use of fossil fuels and GHG emissions from fuel. In addition to technological solutions on farms, we should also seek the opportunities to increase technical efficiency at the stage of manufacturing the agricultural inputs in industrial processes. Taking into account the results of normalization, the most important impact category of wheat production, in terms of the overall environmental profile, was the acidification, followed by greenhouse effect and eutrophication.

Life cycle analysis of winter wheat cultivation in intensive production system provides relevant inventory data for the national area that can be used in the food-processing sector. They are source basis for the environmental assessment of products in the country for which wheat is the raw material in industrial processing.

REFERENCES

[1] Bieńkowski, J., Jankowiak, J. (2006): Sustainable farming in the Wielkopolska Region – economic and environmental perspective. p. 130–141. - In: Kundzewicz, Z. W., Hattermann, F. F. (eds.) Natural Systems and Global Change. Research Centre for Agricultural and Forest Environment of the Polish Academy of Sciences, Potsdam Institute for Climate Impact Research, Poznan, Poland, Potsdam, Germany.

[2] Bieńkowski, J., Jankowiak, J., Dąbrowicz, R., Holka, M. (2014): Regional differentiation of greenhouse gas emissions from agriculture in Poland. Abstract 425-26. - In: Pepó, P., Csajbók, J. (eds.) Book of Abstracts, ESA XIIIth Congress, Debrecen, Hungary. 25-29 August. University of Debrecen, Debrecen, Hungary.

[3] Biswas, W. K., Graham, J., Kelly, K., John, M. B. (2010): Global warming contributions from wheat, sheep meat and wool production in Victoria, Australia - a life cycle assessment - Journal of Cleaner Production 18: 1386-1392.

[4] Biswas, W., John, M. (2009): Review of life-cycle assessment research for the Australian grain industries - CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources 4(16): 1-7.

[5] Brentrup, F., Küsters, J., Kuhlmann, H., Lammel, J. (2004a): Environmental impact assessment of agricultural production systems using the life cycle assessment methodology I. Theoretical concept of a LCA method tailored to crop production - European Journal of Agronomy 20: 247-264.

[6] Brentrup, F., Küsters, J., Lammel, J., Barraclough, P., Kuhlmann, H. (2004b): Environmental impact assessment of agricultural production systems using the life cycle assessment (LCA) methodology. II. The application to N fertilizers use in winter wheat production systems - European Journal of Agronomy 20: 265-279.

[7] Caffrey, K. R., Veal, M. W. (2013): Conducting an Agricultural Life Cycle Assessment: Challenges and Perspectives - The Scientific World Journal: 1-13. Available at: http://dx.doi.org/10.1155/2013/472431 (accessed March 2015).

[8] Charles, R., Jolliet, O., Gaillard, G., Pellet, D. (2006): Environmental analysis of intensity level in wheat crop production using life cycle assessment - Agriculture, Ecosystems and Environment 113: 216-225.

[9] Colomb, V., Aït-Amar, S., Basset-Mens, C., Dollé, J. B., Gac, A., Gaillard, G., Koch, P., Lellahi, A., Mousset, J., Salou, T., Tailleur, A., van der Werf, H. (2013): AGRIBALYSE®: Assessment and lessons for the future, Version 1.0. ADEME, Angers, France.

[10] CSO (2014): Statistical Yearbook of the Republic of Poland 2014. Warsaw.

[11] Duer, I., Fotyma, M., Madej, A. (2004): Code of Good Agricultural Practice. - Ministry of Agriculture and Rural Development, Ministry of the Environment, Warsaw, Poland. [In Polish]

[12] EC (2013): Commission Recommendation of 9 April 2013 on the use of common methods to measure and communicate the life cycle environmental performance of products and organizations. Official Journal of the European Union L124: 1-210.

[13] EMEP/EEA (2013): Air Pollutant Emission Inventory Guidebook. Technical guidance to prepare national emission inventories. EEA technical report no 12/2013. EEA, Copenhagen, Denmark.

[14] European Council Conclusions (2014): 2030 Climate and energy policy framework. Conclusions – 23/24 October 2014, EUCO 169/14. Available at http://www.consilium.europa.eu/uedocs/cms_data/docs/pressdata/en/ec/145397.pdf (accessed March 2015).

[15] EUROSTAT (2015): Agriculture and Fisheries Database. Available at http://ec.europa.eu/eurostat/web/agriculture/data/database (accessed March 2015).

[16] Guinée, J. B., Gorrée, M., Heijungs, R., Huppes, G., Kleijn, R., de Koning, A., van Oers, L., Wegener Sleeswijk, A., Suh, S., Udo de Haes, H. A., de Bruijn, H., van Duin, R., Huijbregts, M. A. J. (2002): Handbook on life cycle assessment. Operational guide to the

ISO standards. I: LCA in perspective. IIa: Guide. IIb: Operational annex. III: Scientific background. Kluwer Academic Publishers, Dordrecht, Netherlands.

[17] Hontelez, J., Buitenkamp, M. (2006): EU sustainable development strategy. From theory to delivery. EEB, Brussels, Belgium.

[18] Huerta, J. H., Alvear, E. M., Navarro, R. M. (2012): Evaluation of two production methods of Chilean wheat by life cycle assessment (LCA) - IDESIA 30(2): 101-110.

[19] IPCC (2006): 2006 IPCC Guidelines for National Greenhouse Gas Inventories. Volume 4 Agriculture, Forestry and Other Land Use, http://www.ipcc-nggip. iges.or.jp /public/2006gl/vol4.html (accessed March 2015).

[20] Jayasundara, S., Wagner-Riddle, C., Dias, G., Kariyapperuma, K. A. (2014): Energy and greenhouse gas intensity of corn (*Zea mays* L.) production in Ontario: A regional assessment - Canadian Journal of Soil Science 94: 77-95.

[21] LDB CSO (2015): Local Data Bank, Central Statistical Office of Poland, Warsaw. Available at http://stat.gov.pl/bdl (accessed March 2015).

[22] Lillywhite, R., Chandler, D., Grant, W., Lewis, K., Firth, C., Schmutz, U., Halpin, D. (2007): Environmental footprint and sustainability of horticulture (including potatoes) – a comparison with other agricultural sectors. - University of Warwick, Warwick, Defra, London, United Kingdom.

[23] Meisterling, K., Samaras, C., Schweizer, V. (2009): Decisions to reduce greenhouse gases from agriculture and product transport: LCA case study of organic and conventional wheat - Journal of Cleaner Production 17: 222–230.

[24] Nemecek, T., Huguenin-Elie, O., Dubois, D., Gaillard, G., Schaller, B., Chervet, A. (2011): Life cycle assessment of Swiss farming systems: II. Extensive and intensive production - Agricultural Systems 104: 233–245.

[25] Roer, A.-G., Korsaeth, A., Henriksen, T. M., Michelsen, O., Strømman, A. H. (2012): The influence of system boundaries on life cycle assessment of grain production in central southeast Norway - Agricultural Systems 111: 75–84.

[26] Roy, P., Nei, D., Orikasa, T., Xu, Q., Okadome, H., Nakamur, N., Shiina, T. 2009. A review of life cycle assessment (LCA) on some food products - Journal of Food Engineering 90: 1-10.

[27] Safa, M., Samarasinghe, S. (2012): CO_2 emissions from farm inputs Case study of wheat production in Canterbury, New Zealand - Environmental Pollution 171: 126-132.

[28] Schenck, R. C., Ostrom, M., Granatstein, D., Painter, K., Kruger, C. (2008): Life cycle assessment of wheat grown in Washington State. Abstract 248-253. - In: Nemecek, T., Gaillard, G. (eds.) Proceedings of the 6th International Conference on LCA in the Agri-Food Sector, Zurich, November 12–14.

[29] Sleeswijk, A. W., van Oers, L. F. C. M., Guinée, J. B., Struijs, J., Huijbregts, M. A. J. (2008): Normalisation in product life cycle assessment: An LCA of the global and European economic systems in the year 2000 - Science of the Total Environment 390: 227-240.

[30] Syp, A., Jarosz, Z., Faber, A., Borzęcka-Walker, M., Pudełko, R. (2012): Greenhouse gas emissions from winter wheat cultivation for bioethanol production in Poland - Journal of Food, Agriculture and Environment 10(3-4): 1169-1172.

[31] Wattiez, C, Williamson, S. (2003): Pesticide use reduction in working: An assessment of national reduction strategies in Denmark, Sweden, the Netherlands and Norway. - Pesticides Action Network Europe.

[32] Weber, R., Bujak, H., Kaczmarek, J., Gacek, E. (2011): Analysis of yield variability in winter wheat cultivars in South-Western Poland. - Bulletin of Plant Breeding and Acclimatization Institute 260/261: 121-133.

EFFECT OF BIOFERTILIZERS AND PLANT GROWTH PROMOTING BACTERIA ON THE GROWTH CHARACTERISTICS OF THE HERB *ASPARAGUS OFFICINALIS*

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Abstract. The purpose of this research was to investigate the effects of applying vermicompost, Nitroxin and cow manure on the growth characteristics of *Asparagus officinalis* L. Significant differences in germination percentage were observed between fertilizer treatments with control. Vermicompost was more efficient at lower concentrations and in non-combined treatment, but higher effects were resulted from the mixture of cow manure with Nitroxin and also higher concentrations. The maximum means of germination time and germination rate were related to the treatments of 15% and 30% vermicompost, and cow manure mixed with Nitroxin. The higher effects and significant differences of root and shoot length were related to treatment of 15% vermicompost, however the comparison of the combined and non-combined treatments of vermicompost showed higher effect by the mixture of 30%, moreover, these treatments enhanced the length of root and shoot. Significant differences and higher effects on root fresh weight were induced by treatment with 15% vermicompost, but the dry weight of root and fresh and dry weight of shoot showed significant differences and higher effects by the treatment with 30% vermicompost mixed with Nitroxin. All treatments have affected positively the dry weight of root and fresh and dry weight of shoot.

Keywords: *cow manure, germination percentage, growth characteristics, Nitroxin, vermicompost*

Introduction

Intensive farming practices, that warrant high yield and quality, require the extensive use of chemical fertilizers, which are costly and create environmental problems. Therefore, more recently there has been a resurgence of interest in environmental friendly, sustainable and organic agricultural practices (Esitken et al., 2005). Organic agriculture is a production system, which avoids or largely excludes the use of synthetically compounded fertilizers, and, as far as possible, organic agricultural systems rely upon bio-fertilization, however yield reduction is an important problem in organic production system (Lind et al., 2003). Using biofertilizers containing beneficial microorganisms instead of synthetic chemical improve plant growth through supplying plant nutrients and may help to sustain environmental health and soil productivity (O'Connell, 1992). So far considerable number of bacterial species, mostly associated with the plant rhizosphere, were tested and found to be beneficial for plant growth, yield and crop quality. They have been called 'plant growth promoting rhizobacteria (PGPR)'

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including the strains in the genera Azospirillium, Azotobacter, (Rodriguez and Fraga, 1999; Sturz and Nowak, 2000; Sudhakar., et al., 2000). PGPR participates in many key ecosystem processes, such as those involved in the biological control of plant pathogens, N fixation, solubilisation of nutrients and phytohormone synthesis (Vessey, 2003). Vermicomposts, which are stabilized organic materials produced by interactions between earthworms and microorganisms in a non-thermophilic process, have been reported to enhance plant germination growth and yields in greenhouse crops (Edwards and Burrows, 1988; Buckerfield et al., 1999; Atiyeh et al., 2000, 2001; Edwards et al., 2004). Such increasing productivity of crops, in response to vermicomposts amendments, have been attributed to the greater availability of mineral nutrients, than in commercial plant growth media containing only inorganic nutrients (Edwards and Burrows, 1988; Werner and Cuevas, 1996), as well as their rich microbial populations (Edwards, 1983; Tomati et al., 1987). The presence of plant growth-influencing substances, such as plant growth hormones and humic acids in vermicomposts has also been suggested as a possible factor contributing to increased plant growth and yields (Arancon et al., 2003). Gupta et al. (2014) showed that the addition of vermicompost, in appropriate quantities, to potting media had significantly positive effects on growth and flowering of marigold seedlings including plant biomass, plant height, the number of buds and flowers. Subler et al. (1998) reported that optimum plant growth responses occurred when 10–20% vermicompost was amended with potting media which may be due to enhanced micronutrient availability, the presence of plant growth regulators, or the activity of beneficial microorganisms in the vermicompost, however when the vermicompost concentration was >40% in the potting media then the number and diameter of the flowers reduced than control. These antagonistic effects at higher vermicomposts dosage may be due to reduction in aeration and porosity and increased salt concentrations (Tucker, 2005). Yadav and Garg (2015) stated that the seed germination is an important and decisive phase in growth cycle of a plant since it determines plant establishment and yield, while the several authors have reported moderate or no effect of vermicompost on seed germination (Singh et al., 2013; Roy et al., 2010; Alves and Passoni, 1997). In contrast, Ievinsh (2011) has reported that vermicompost addition inhibits seed germination depending on crop species and cultivar tested even at moderate concentrations.

The objectives of this research were the comparison of the effects of fertilizers such as vermicompost, cow manure and Nitroxin on the growing characteristics of asparagus and the effects of Nitroxin, 15% and 30% cow manure, 15% and 30% vermicompost, the combination of 15% and 30% vermicompost with Nitroxin and the combination of 15% and 30% cow manure with Nitroxin on the characteristics of germination percentage, length of root and shoot, and fresh and dry weight of root and shoot, and the mean time of germination and germination rate.

Methods

Experimental site and material

This experiment was done on 2013-2014 crops by randomized complete block design with 10 treatments and four replications conducted in pots with the mouth diameter of 15 and height of 19 cm in the greenhouse of Isfahan (Khorasgan) Branch, Azad University located in Isfahan, Iran. The maximum temperature of day and night were 35°C and 10°C and the average temperature of day and night were 14°C and 25°C, respectively. When the experiment was being conducted in the greenhouse with the conditions listed above, the effects of vermicompost, Nitroxin and cow manure on the herb *asparagus* (*Asparagus officinalis* L.) were evaluated. *Asparagus officinalis* L. is a perennial monocotyledon, a member of the Liliaceae family. It grows in temperate climates and under subtropical conditions. Asparagus requires well-drained loam or sandy-loam soils, or heavier soils where there is good drainage. The soil used in potting media was collected from the agricultural research fields of University of Azad Islamic Khorasgan, Isfahan. The soil was sandy-loam in nature.

Experimental treatments

At first 40 pots were used for 10 treatments (4 pots per each treatment). The pots 1 to 4 were filled with the sand-soil mixture as control sample (T1), 5 to 8 with 15% vermicompost and 85% sand and soil(T2), 9 to 12 with 15% vermicompost mixed with Nitroxin and 85% sand and soil (T3), 13 to 16 with 30% vermicompost and 70% sand and soil(T4), 17 to 20 with 30% vermicompost mixed with Nitroxin and 70% sand and soi(T5), 21 to 24 with Nitroxin and sand and soil(T6), 25 to 28 with 15% cow manure and 85% sand and soil (T7), 29 to 32 with 15% cow manure mixed with Nitroxin and sand and soil (T8), 33 to 36 with 30% cow manure and 70% sand and soil(T9), 37 to 40 with 30% cow manure mixed with Nitroxin and 85% sand and soil(T10). A certain proportion of sand-soil mixture was used in all substrates. The seeds of asparagus have purchased from Pakan seed Company in Isfahan. The biofertilizer, comprising *Azospirillum* and *Azotobacter*, was developed by the soil and water Research Institute (SWRI) in Tehran and now is manufactured under the brand name "Nitroxin" by the private company in Semnan, Iran. The Nitroxin solution was purchased from Agricultural Products Distribution Company. The half of seeds has placed in Nitroxin solution. After filling the pots in the mentioned order, 25 seeds of asparagus were planted in each pot and covered with 1.5cm soil. The seeds which were in Nitroxin solution have infused on the soil of all pots. The vermicompost used in potting media were prepared in the research institute of organic waste recycling in the Islamic Azad University of Khorasgan, Isfahan using (a) cow dung and (b) agriculture residue spiked cow dung. The cow manure used in the study was obtained from a local cowshed located about 10 km North West of the Islamic Azad University of Khorasgan, Isfahan. The Nitroxin was sprayed on the verrmicompost, cow manure and soil of the T3, T5, T6, T8 and T10.

Measuring growth

Germinated seeds were counted from day 8 in the intervals of 4 day until the end of the experiment (lasted 90 days). At the end, the shoots and roots length (cm) were measured using a transparent ruler. To determine the dry weight, roots and shoots of samples were placed in the oven at 72 °C for 48 hours, and then the dry weight was measured by a digital scale with a precision of one ten-thousandth of a gram.

Germination percentage was obtained from the ratio of the number of seeds germinated (after 14 days) to the total number of seeds planted in each pot. Germination rate and the mean time to germination were calculated based on Equations 1 and 2, respectively.

$$RS = \sum_{i=1}^{n} \frac{Si}{Di} \tag{Eq.1}$$

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RS is the germination rate, Si is the number of germinated seeds of each Counted; Di is days to the counting the n-th and n is the number of the counting.

Germination percentage (GP) and mean germination time (MGT) for each species and each treatment was calculated according to Equations 2 and 3:

$$GP\ (\%) = \sum \frac{n_i}{N \times 100} \qquad \text{(Eq.2)}$$

$$MGT\ (days) = \frac{\sum (t_i \times n_i)}{\sum n_i} \qquad \text{(Eq.3)}$$

N_i is the number of germinated seeds, N is the total number of seeds per Petri dish and ti is the number of days since the start of the experiment (Eliss and Robert, 1981).

Data analyses

The effect of different treatments on the characteristics of germination percentage, length of root and shoot, and fresh and dry weight of root and shoot, and the mean time of germination and germination rate were measured in all substrates (in a biological reaction). The data set was obtained from four replicates in ten treatments. ANOVA was used to evaluate the effect of different treatments on the germination percentage and mean germination time. The means of measured data set were compared by Duncan test. All statistical analyses and the drawing of charts were performed by software SPSS 19. The outcomes were presented as tables and figures.

Results and discussion

Germination percentage

The results of all treatments showed significant differences in germination percentage compared with the control one. The highest and lowest effects on the germination were related to the control sample and 15% cow manure, respectively. Therefore, the used organic fertilizers and biofertilizers inhibited the seed germination of the *Asparagus officinalis* L. This is in agreement with the findings of Ievinsh (2011). Organic substances needed for seed germination have been isolated from various sources of animal waste composts and identified as phenolic acids (Garraway and Ramirez, 1982; Marambe and Ando, 1992). Marambe et al. (1993), isolated and identified some long-chain fatty acids as strong inhibitory substances for sorghum seed germination in animal-waste compost and reported the mode of action. The germination percentage significantly differed in 15% and 30% vermicompost treatments but there was no significant difference when the mixture of vermicompost with Nitroxin was used, however both of them had higher effects on the level of 15%. In this regard, Warman and AngLopez (2010) showed that the percentage of germination increased with the maturation of vermicompost but the addition of vermicompost to the soil or water often resulted in poorer germination compared with the soil or water (only) controls. Nitroxin treatment showed no significant difference compared with treatments by 30% cow manure and 30% vermicompost mixed with Nitroxin howbeit, the improved effectiveness of the mixture. Significant differences were observed in the treatments of cow manure and its mix with Nitroxin and greater effect found when the cow manure was mixed with Nitroxin, moreover germination at higher concentrations showed an increase (*Fig. 1*). Zaller (2007), also showed a stimulatory effect on germination when the growth medium was composed of varying proportions of vermicompost mixed with soil. Several studies have related the immaturity of composts to phytotoxic reactions which inhibit or retard seed germination and plant growth (Jimenez and Garcia, 1989; Baca et al., 1990; Warman, 1999b). Warman and AngLopez (2002) reported although little or no work has described such toxic components in vermicompost but surface crusts that were observed on soils containing the greatest amounts of vermicompost could explain the consistently poor germination of all three test species in these soils. The crusting may have limited water infiltration and created anaerobic conditions in the growth mix, which can result in the production of phytotoxic substances. They hypothesize that the organic substances in the vermicomposts may have contributed to phytotoxicity rather than soluble salts in addition; they showed the germination percent reduction of all three test plants with all the vermicomposts in % 25 V compared to %12.5 V signified greater sensitivity to phytotoxic substances in the vermicompost.

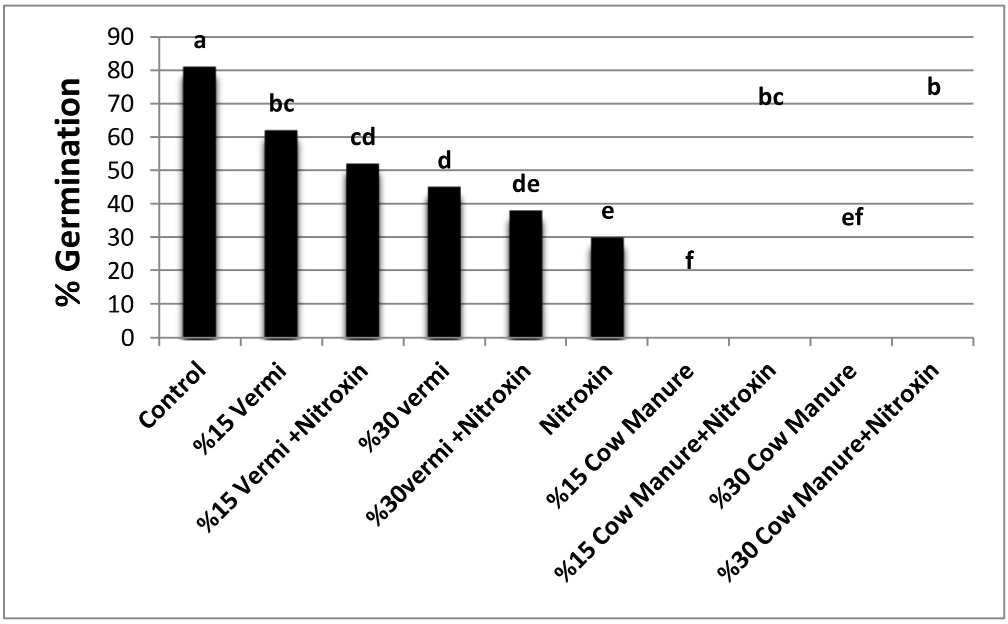

Figure 1. *The average of Germination percentage in four replications*
NOTE: Control =T1, 15% Vermi=T2, 15% Vermi. Nitroxin=T3, 30% Vermi=T4, 30% Vermi. Nitroxin=T5, Nitroxin=T6, 15% Cow Manure=T7, 15% Cow Manure. Nitroxin=T8, 30% Cow Manure=T9, 30% Cow Manure. Nitroxin=T10.

The average germination time

The average germination time showed significant differences between the treatment of 15% vermicompost and the mixture of 15% vermicompost with Nitroxin, 15% cow manure with Nitroxin, and 30% cow manure. The comparison of treatments showed that only 15% vermicompost and mixture of 30% cow manure with Nitroxin treatments increased the mean germination time compared with the control (*Fig. 2*). This occurred due to the gradual reduction of germination barrier and the release of the germination-promoting substances.

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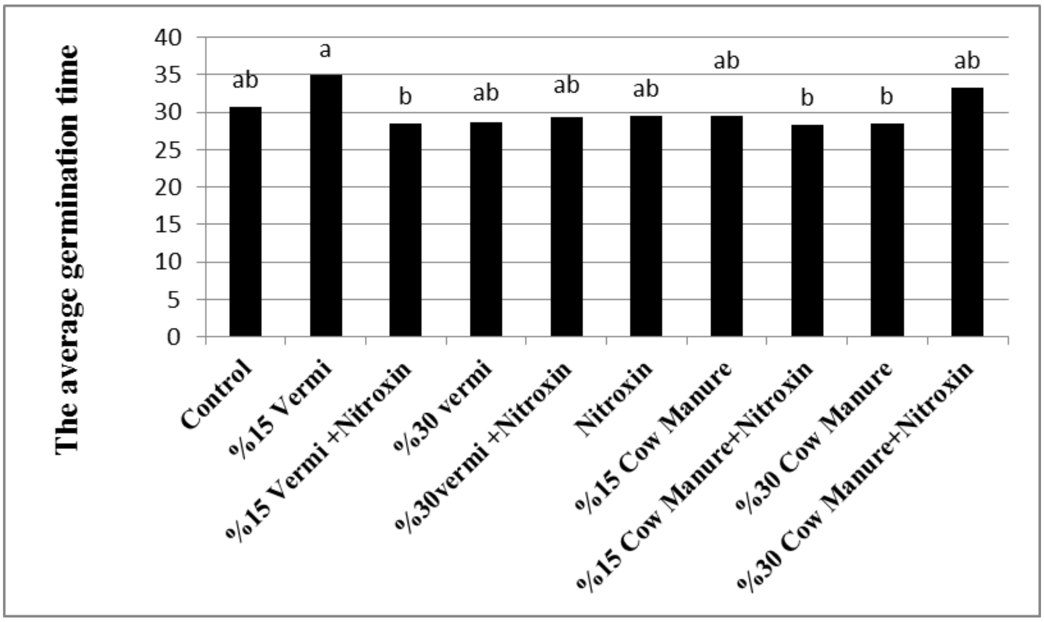

Figure 2. *The average of Germination time*
NOTE: Control =T1, 15% Vermi=T2, 15% Vermi. Nitroxin=T3, 30% Vermi=T4, 30% Vermi. Nitroxin=T5, Nitroxin=T6, 15% Cow Manure=T7, 15% Cow Manure. Nitroxin=T8, 30% Cow Manure=T9, 30% Cow Manure. Nitroxin=T10.

The rate of germination

The germination rate of fertilizer treatments (*Fig. 3*) showed that 30% cow manure mixed with Nitroxin in all time periods had the highest rate of germination and the lowest rate of germination was related to treatment of 15% cow manure.

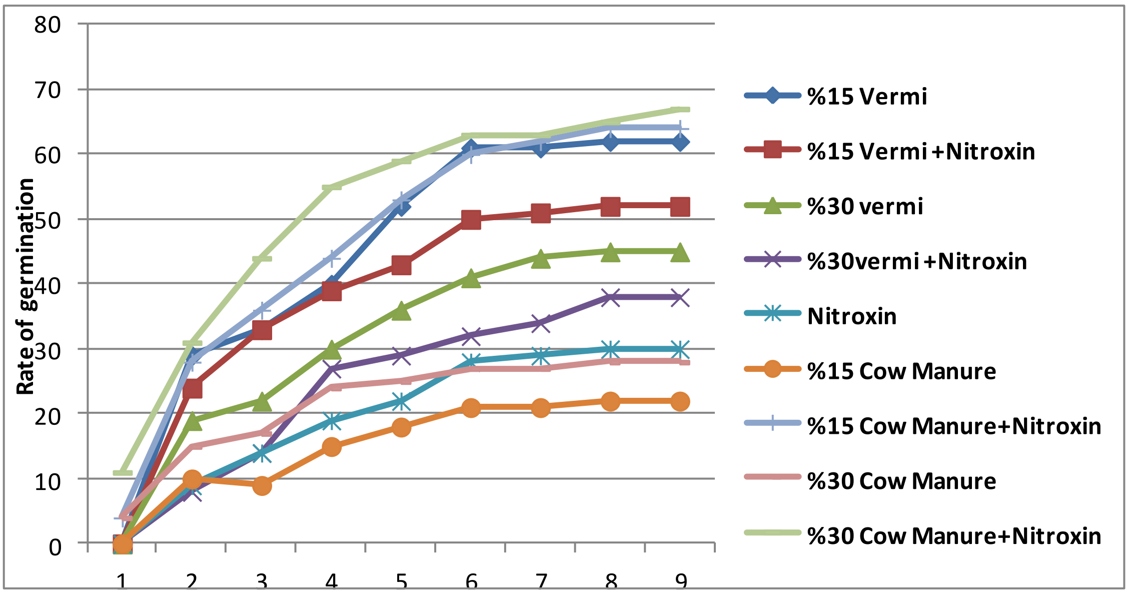

Figure 3. *The comparison of Germination rate in different fertilizer treatments*
NOTE: Control =T1, 15% Vermi=T2, 15%Vermi. Nitroxin=T3, 30% Vermi=T4, 30%Vermi. Nitroxin=T5, Nitroxin=T6, 15% Cow Manure=T7, 15% Cow Manure. Nitroxin=T8, 30%Cow Manure=T9, 30%Cow Manure. Nitroxin=T10.

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Root and stem length (cm)

Root length in treatments with 15% vermicompost and two levels of the 30% and 15% vermicompost mixed with Nitroxin made a significant difference to the control and other treatements. 15% vermicompost and control treatments showed highest and lowest effect on root elongation, respectively. Significant differences were found between using 15% and 30%vermicompost as well as between each level with the same level that had been mixed with Nitroxin. The greatest effect on root elongation caused by 15% vermicompost but its combination with Nitroxin reduced the length of the root while at 30% level the effect was stronger by mixing. The treatments of Nitroxin, cow manure and cow manure mixed with Nitroxin although were not significantly different compared with the control, but their impact were improved compared with the control (*Fig. 4*). The results showed significant difference in shoot length between the plants treated with 15% vermicompost and 30% vermicompost. The plants treated with 15% vermicompost and the mixture of 30% vermicompost with Nitroxin showed significant difference in shoot length with the Control sample. Nitroxin had the lowest effect on shoot length among the other fertilizers and control. The shoot length in the plants treated with Nitroxin showed significant difference with the plants treated with 15% and 30% cow manure, cow manure mixed with Nitroxin, 15% vermicompost, 15% and 30% vermicompost mixed with Nitroxin. The stem length increased in the treatments with the 15% and 30% vermicompost. While 15% vermicompost mixed with Nitroxin caused the negative effect on stem elongation compared with non-mixed at this level, at level of the 30% higher impact was observed by mixed treatment. There was no significant difference between the Nitroxin and control, although Nitroxin made reduction in stem length. Cow manure and its combination with Nitroxin showed more effect compared with the control (*Fig. 5*).

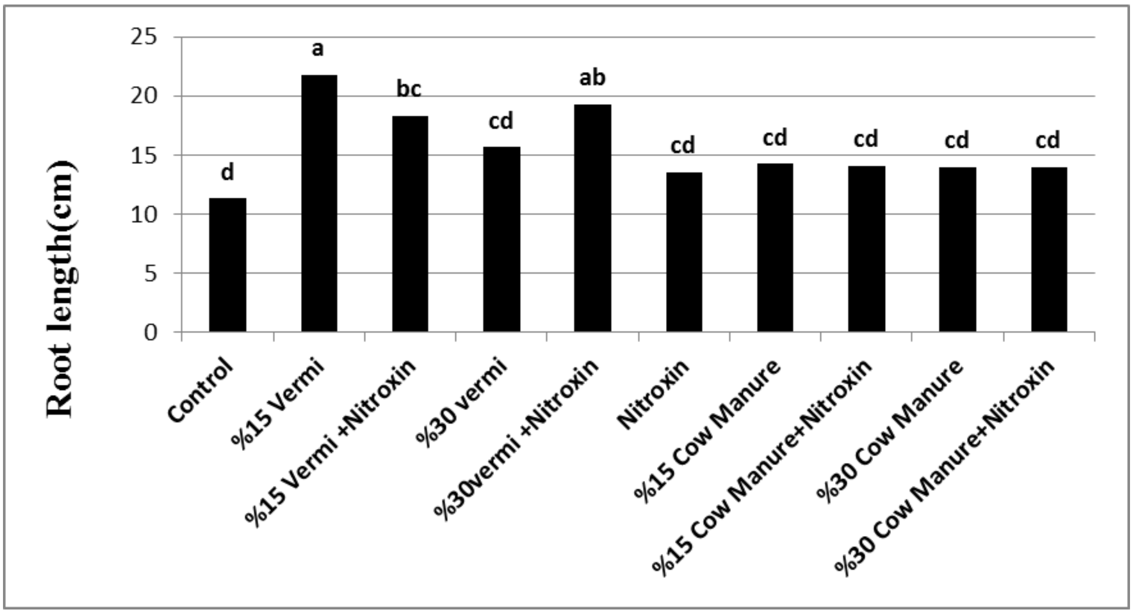

Figure 4. *The average of root elongation in four replications*
NOTE: Control =T1, 15% Vermi=T2, 15%Vermi. Nitroxin=T3, 30% Vermi=T4, 30%Vermi. Nitroxin=T5, Nitroxin=T6, 15% Cow Manure=T7, 15% Cow Manure. Nitroxin=T8, 30%Cow Manure=T9, 30%Cow Manure. Nitroxin=T10.

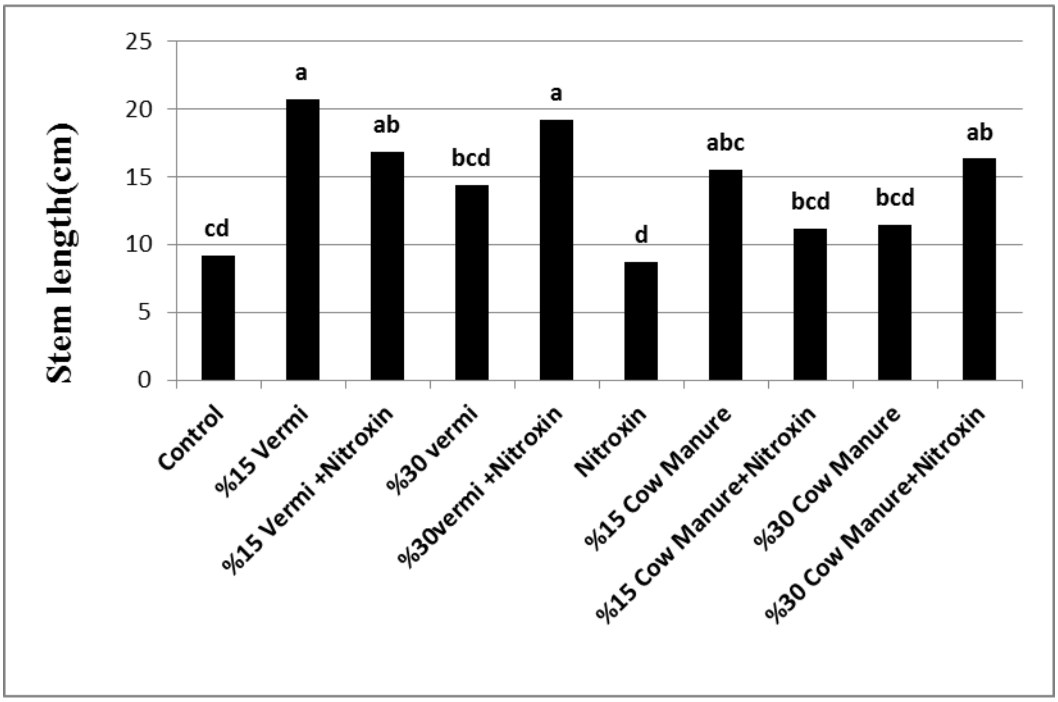

***Figure 5.** The average of stem elongation in four replications*
NOTE: Control =T1, 15% Vermi=T2, 15%Vermi. Nitroxin=T3, 30% Vermi=T4, 30%Vermi. Nitroxin=T5, Nitroxin=T6, 15% Cow Manure=T7, 15% Cow Manure. Nitroxin=T8, 30%Cow Manure=T9, 30%Cow Manure. Nitroxin=T10.

Martens et al. (1992) and Hendrix et al. (1994) revealed that the higher yields in plants may be due to the fact that vermicompost supplies direct available nutrients such as nitrogen for the plants and improves the proportion of water table of the soil. Channabasanagowda et al. (2008), have also shown that the differential action of vermicompost may be because of the fact that the vermicompost has the slow release of nitrogen due to slow mineralization which helps in availability of nutrients to the plants throughout the growth of the plant and thus resulting in higher yields. Ismail (1995) reported higher yield in bhendi, chillies, watermelon and paddy and number of canes per hill, internodes distance, stem length, shoot length, cane yield and quality were observed when they were exposed to different concentration of vermicompost than the farm yard manure. Arancon et al. (2004) reported that the differential response of plants to different doses of vermicompost might be due to the production of growth-promoting substances in lesser quantity by lower doses of vermicompost than higher doses. Singh et al. (2008), showed that increasing in vermicompost dose from 2.5 to 7.5 t ha^{-1} increased all growth characteristics significantly, but vermicompost dose beyond 7.5 t ha^{-1} could not influence these characteristics significantly. However, the growth of plants with vermicompost at 7.5 t ha^{-1} was significantly at par with that of 10 t ha^{-1} indicating that this dose of VC (7.5 t ha^{-1}) was enough for supplying the desirable amount of growth-promoting substances for higher growth and fruit yield of strawberry.

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The fresh and dry weight of root and shoot (gr)

The comparison of the root and shoot mean weight using the Duncan test at 5% (*Table 1*) indicated that the fresh weight of roots in the treatments with 15% vermicompost and two levels of the 30 and 15% vermicompost mixed with Nitroxin made a significant difference to the control and other treatments, so that these treatments caused the greatest positive impact on the fresh weight of roots. The comparison of treatments showed significant difference in root dry weight just by the treatment of 30% vermicompost mixed with Nitroxin compared to all other treatments. It was also observed positive effect on the root dry weight by all treatments compared to the control. Moreover, the treatment of 30% vermicompost mixed with Nitroxin showed the greatest positive impact. Significant difference was observed for shoot fresh weight by the treatment of 30% vermicompost mixed with Nitroxin with all other treatments. It showed that the most positive effects were related to this treatment.

Table 1. *The effect of fertilizer treatments on the growth characteristics of Asparagus*

Treatment	fresh weight(gr)	Root dry weight(gr)	Stem fresh weight(gr)	Stem dry weight(gr)
Control	0.37b	0.24c	0.02bc	0.03c
15% Vermi	4.42a	1.002bc	1.4b	0.12ab
15%Vermi. Nitroxin	3.2a	0.82bc	0.25bc	0.11ab
30% Vermi	1.035b	0.8c	0.7bc	0.09bc
30%Vermi. Nitroxin	4.2a	1.025a	2.7a	0.17a
Nitroxin	0.39b	0.31bc	0.42bc	0.03c
15% Cow Manure	0.15b	0.52bc	0.8 bc	0.07bc
15% Cow Manure. Nitroxin	0.16b	0.39b	0.6 bc	0.07 bc
30%Cow Manure	0.23b	0.23bc	0.6 bc	0.08 bc
30%Cow Manure. Nitroxin	0.55b	0.69c	0.17 bc	0.1 bc

Means within the column with the same letter are not significantly different by Duncan multiple range test at $p \leq 0.05$.

NOTE: Control =T1, 15% Vermi=T2, 15%Vermi. Nitroxin=T3, 30% Vermi=T4, 30%Vermi. Nitroxin=T5, Nitroxin=T6, 15% Cow Manure=T7, 15% Cow Manure. Nitroxin=T8, 30%Cow Manure=T9, 30%Cow Manure. Nitroxin=T10.

Shoot dry weight showed similar results to shoot fresh weight. Rivera-Cruz et al. (2008) showed that the shoot and root biomass of banana significantly increased by the preparation of bio-fertilizers, using either poultry manure (PM) or banana waste (BW) as the carrier for the bacterial (Azospirillum, Azotobacter) inoculant. They observed positive effects on the growth of banana by the application of both biofertilizers. It seemed to be due to the supply of nutrients to the crop and both biofertilizers can be considered potentially useful as inoculant carriers of PGPR but the usefulness of BW appears to be restricted to moderate doses of application. In this study, the seed germination were not stopped in the organic fertilizer and biofertilizer treatments, however a reduction was observed when these compared with the control. Nevertheless, the vermicompost could improve the vegetable features of root and stem from a few number seedlings. This may be due to a highly fertile, finely divided peat-like material with high porosity, aeration, water-holding capacity and low C:N ratios. In fact, the properties listed lead to the better water absorption and consequently the absorption and transport of nutrients in the root zone. Therefore, the significant differences were observed between some treatments of vermicompost regarding the elongation and root and shoot weight. These results can be supported by the finding of Edwards and Burrows (1988), Werner and Cuevas (1996), Gupta et al. (2014), Subler et al. (1998) and Tucker (2005).

Conclusions

There were significant differences between control treatment and the other fertilizer treatments. The germination percentage was higher in control treatment compared with the others. The highest germination percentage was related to the mixture of 30% cow manure with Nitroxin and the lowest one was related to the 15% cow manure. The effect of 15% vermicompost on the germination percentage was higher than 30% vermicompost. Significant differences in mean germination time in comparison with the control treatment were related to the fact that 15% vermicompost had the highest mean germination time and the highest rate observed in the control of seed germination of the asparagus. The comparison of fertilizer treatments showed 30% cow manure mixed with Nitroxin treatment has the highest rate of germination and 15% cow manure resulted in lower germination rate than other treatments. Significant differences of root length in relation to other treatments were produced by treatments of 15% vermicompost and 30% vermicompost mixed with Nitroxin that caused the greatest impact on root length also the effect was higher by 15% vermicompost treatment alone but at the level of 30% was higher the effect of vermicompost mixed with Nitroxin. However, all fertilizer treatments caused increasing the shoot length but the highest impact and significant differences compared with the control have observed by treatments with 15%vermicompost, 30 and 15%vermicompost mixed with Nitroxin and 30% of cow manure mixed with Nitroxin. At the level of 15% vermicompost and cow manure treatments alone but at level of 30% the treatments of cow manure and vermicompost mixed with Nitroxin showed the impact of higher elongation of stems. Significant differences were observed in root fresh weight by the treatments of 15% vermicompost, mixture of 30 and 15% vermicompost and Nitroxin than control and other treatments and while fresh weight of roots was reduced by mixed treatment of 15% vermicompost with Nitroxin, but at the level of 30% the mixed treatment showed higher impact. Positive effect and significant difference was not observed for root fresh weight between treatments of Nitroxin and cow dung alone or in combination, however, these treatments caused greater influence on root dry weight than control. Significant differences were observed at root dry weight by combined treatments of 30% vermicompost also 15% cow manure with Nitroxin in general. Root dry weight increased by fertilizer treatments similarly in the fresh and dry weight of shoot and the maximum effect was obtained from 30% vermicompost mixed with Nitroxin and at the level of 15% non-mixed treatment but at the level of 30% combined treatment a greater impact was observed.

REFERENCES

[1] Alves, W.L., Passoni, A.A.(1997): Compost and vermicompost of urban solid waste in Licania tomentosa (Benth) seedlings production to arborization. – Pesqui Agropecu Bras 32:1053-1058.

[2] Arancon, N .Q., Lee, S., Edwards, C. A., Atiyeh, R. M.(2003): Effects of humic acids and aqueous extracts derived from cattle, food and paper-waste vermicomposts on growth of greenhouse plants. – Pedobiologia 47: 741-744.

[3] Arancon, N.Q., Edwards, C.A., Bierman, P., Welch, C., Metzer, J.D.(2004): Influence of vermicomposts on field strawberries: effect on growth and yields. – Bioresource Technology 93:145-153.

[4] Atiyeh , R. M., Dominguez, J., Subler, S., Edwards, C. A. (2000); Biochemical changes in cow manure processed by earthworms (Eisenia andreii) and their effects on plant-growth. – Pedobiologia 44,709-724.

[5] Atiyeh, R. M., Arancon, N .Q., Edwards, C .A., Metzger, J .D. (2001): The influence of earthworm-processed pig manure on the growth and productivity of marigolds. – Bioresource Technology 81:103-108.

[6] Baca, M.T., Delgado I C, Sanchez-Raya A J, Gallardo-Lara F. (1990): Comparative use of cress seed germination and physiological parameters of Helianthus annuus L. to assess compost maturation. – Biol. Wastes 33:251-261.

[7] Buckerfield, J. C., Flavel ,T., Lee, K .E., Webster, K. A. (1999): Vermicomposts in solid and liquid form as plant-growth promoter. – Pedobiologia 43: 753-759.

[8] Channabasanagowda, N. K., Patil ,B. N., Patil, J. S., Awaknavar, B. T., Ninganur, R.H. (2008): Effect of Organic Manure on Growth, Seed Yield and Quality of Wheat. – Karnataka Journal of Agricultural Sciences 21:366-368.

[9] Edwards, C. A. (1983): Utilization of earthworm composts as plant growth media. – In: Tomati, U., Grappelli, A. (Eds.) International Symposium on Agricultural and Environmental Prospects in Earthworm, Rome, Italy, pp. 57-62.

[10] Edwards,C .A., Burrows, I. (1988): The potential of earthworm composts as plant growth media. – In: Edwards, C.A., Neuhauser, E.F. (Eds.) Earthworms in Environmental and Waste Management. SPB Academic Publ. b.v, The Netherlands, pp. 211-220.

[11] Edwards, C .A., Dominguez, J., Arancon, N. Q.(2004): The influence of vermicomposts on plant growth and pest incidence. – In: Mikhail, W.Z.A, Shakir, S.H. (Eds.) Soil Animals and Sustainable Development, pp. 397-420.

[12] Ellis, R. H., Roberts, E. H. (1981): The quantification of ageing and survival in orthodox seeds. – Seed Science and Technology 9: 377-409.

[13] Esitken, A.,Ercisli, S., Karlidag, H., Sahin, F. (2005): Potential use of plant growth promoting rhizobacteria (PGPR) in organic apricot production. – In: Proceedings of the International Scientific Conference of Environmentally Friendly Fruit Growing, Tartu-Estonia, 7-9 September, pp. 90-97.

[14] Garraway, J .L., Ramirez, AME. (1982): Phenolic acids in pig slurry subjected to various treatment processes. – Journal of the Science of Food and Agriculture 33,697-705 Gowing DP and Leeper.

[15] Gupta, R., Yadav, A., Garg, V. K.(2014): Influence of vermicompost application in potting media on growth and flowering of marigold crop. – International Journal of Recycling of Organic Waste in Agriculture 3- 47.

[16] Hendrix, P. F., Callaham, M. A., James, S. W.(1994): Ecology of nearetic earthworms in the Southern USA-I. Characteristics of diplocardia longa surface casts in grass, hardwood and pine micro habitats on the lower pied mount of Georgia. – Megdrilogica 5: 45-51.

[17] Ievinsh, G. (2011): Vermicompost treatment differentially affects seed germination, seedling growth and physiological status of vegetable crop species. – Plant Growth Regulation 65:169-181.

[18] Ismail, S. A. (2005): The Earthworm Book. – Other India Press, Mapusa, Goa, India. pp. 73-74.

[19] Jimenez E I, Garcia, V.P. (1989): Evaluation of city refuse compost maturity: a review. – Biological Wastes 27: 114-115.

[20] Lind, K., Lafer,G., Schloffer, K., Innerhoffer, G., Meister, H.(2003): Organic Fruit Growing. – CABI Publishing, Wallingford, UK.

[21] Marambe, B., Ando, T. (1992): Phenolic acids as potential seed germination inhibitors in animal-waste composts. – Soil Science and Plant Nutrition 38:723-733.

[22] Marambe, B., Nagaoka, T., Ando, T. (1993): Identification and biological activity of germination-inhibiting long-chain fatty acids in animal-waste composts. – Plant Cell Physiology 34:605-612.

[23] Martens, D.A., Johanson, J. B., Frankenberger, W. T.(1992): Production and persistence of soil enzymes with repeated addition of organic residues. – Soil Science 153: 53-61.

[24] O'Connell, P. F. (1992): Sustainable agriculture-a valid alternative. – Outlook Agriculture 21: 5-12.

[25] Rivera-Cruz, M .D .C., Narcı´a, A .T., Ballona, G. C., Kohler, J., Caravaca, F., Rolda´n, A . (2008): Poultry manure and banana waste are effective biofertilizer carriers for promoting plant growth and soil sustainability in banana crops. – Soil Biology and Biochemistry 40: 3092-3095.

[26] Rodriguez, H., Fraga, R.(1999): Phosphate solubilizing bacteria and their role in plant growth promotion. – Biotechnology Advances 17: 319-339.

[27] Roy, S., Arunachalam, K., Dutta, B.K., Arunachalam, A.(2010): Effect of organic amendments of soil on growth and productivity of three common crops viz. Zea mays, Phaseolus vulgaris and Abelmoschus esculentus. – Applied Soil Ecology 45:78-84

[28] Singh, A., Jain, A., Sarma, B.K., Abhilash, P.C., Singh, H.B.(2013): Solid waste management of temple floral offerings by vermicomposting using Eisenia fetida. – Waste Management 33:1113-1118

[29] Singh, R., Sharma, R.R., Kumar, S., Gupta, R. K, Patil, R. T. (2008): Vermicompost substitution influences growth, physiological disorders, fruit yield and quality of strawberry (Fragaria x ananassa Duch). – Bioresource Technology 99: 8507-8511.

[30] Sturz, A .V., Nowak, J. (2000): Endophytic communities of rhizobacteria and the strategies required to create yield enhancing associations with crops. – Applied Soil Ecology 15: 183-190.

[31] Subler, S., Edwards, C.A., Metzger, J. (1998): Comparing vermicomposts and composts. – Biocycle 39:63-66

[32] Sudhakar, P., Chattopadhyay, G. N., Gangwar, S. K., Ghosh, J.K. (2000): Effect of foliar application of Azotobacter, Azospirillum and Beijerinckia on leaf yield and quality of mulberry (Morus alba). – Journal of Agricultural Science 134: 227-234.

[33] Tucker, P. (2005): Co-composting paper mill sludges with fruit and vegetable wastes. Dissertation. University of Paisley, Paisley

[34] Vessey,J .K. (2003): Plant growth promoting rhizobacteria as biofertilizers. – Plant and Soil 255: 571-586.

[35] Tomati, U., Grappelli, A., Galli, E. (1987): The presence of growth regulators in earthworm-worked wastes. – In: Bonvicini Paglioi, A.M., Omodeo, P. (Eds.) On Earthworms. Proceedings of International Symposium on Earthworms. Selected Symposia and Monographs, Unione Zoologica Italiana, 2, Mucchi, Modena, pp. 423-435.

[36] Warman, P. R. (1999b) Evaluation of seed germination and growth tests for assessing compost maturity. – Compost Science and Utilization 7 (3):33-37.

[37] Warman,,P.R., AngLopez, M. J. (2010):Vermicompost derived from different feedstocks as a plant growth medium. – Bioresource Technology 101:4479-4483.

[38] Werner, M., Cuevas, R.(1996): Vermiculture in Cuba Biocycle. vol. 37(6). – JG Press, Emmaus, PA, pp. 61-62

[39] Yadav, A., Garg, V. K.(2015): Influence of vermi-fortification on chickpea (Cicer arietinum L.) growth and photosynthetic pigments. – International Journal of Recycling of Organic Waste in Agriculture 4:299-305

[40] Zaller, J.G. (2007): Vermicompost in seedling potting media can affect germination, biomass allocation, yields and fruit quality of three tomato varieties. – European Journal of Soil Biology 43:332-336.

ENVIRONMENTALLY RESPONSIBLE PURCHASING – ANALYSIS OF THE EX-YUGOSLAVIAN REPUBLICS

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Abstract. The aim of this study is to compare the environmentally-conscious behavior of buyers between the ex-Yugoslavian republics. The starting points are H0: there are differences in the environmentally-conscious behavior of buyers in the ex-Yugoslavian republics and H1: there are demographic and socio-economic differences in environmentally-conscious behavior of buyers between the ex-Yugoslavian republics. The survey was conducted on a sample of 1550 respondents from all the ex-Yugoslavian republics. The obtained results show that there are differences between Montenegro and Macedonia, Montenegro and Croatia, and Montenegro and Slovenia. Respondents from Montenegro purchase environmentally friendly products less often than respondents from Macedonia, Croatia, and Slovenia. The results also indicate that there are differences in the level of education and monthly income by household when purchasing environmentally friendly products between respondents from the ex-Yugoslavian republics. Through adequate implementation of obtained data, behavior of buyers becomes a tool of environmental management. Increasing the purchase of environmentally friendly products will affect environmental protection.

Keywords: *environmentally responsible purchasing, ex-Yugoslavian republics, environmental management*

Introduction

Environmentally-conscious behavior of buyers is the purchasing of products that are characterized as ecological (Roozen and Pelsmacker, 2000).

In 2006 households all over the world spent more than $24 trillion on goods and services while in 1960 for the same purposes they spent $4.8 trillion (UNED, 2008: 14). The increased level of purchases affects the increase in spending natural resources which as a consequence has the depletion of non-renewable resources, while many renewable resources are extremely degraded. Ecological, social and economic costs of today's unsustainable purchases are: natural disasters, biodiversity loss, poverty, epidemic disease, etc. (UNED, 2008: 14). Current patterns of purchase are unsustainable. Because of that, management of environmentally-conscious behavior of buyers is a global task.

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Numerous studies in different countries have been conducted with the aim of determining patterns of environmentally-conscious behavior of buyers. They have been studied in: the UK (Samdahl and Robertson, 1989; Gilg et al., 2005), Singapore (Shamdasani et al., 1993), the USA (Roberts, 1996), China (Chan, 1999), Portugal (De Paço and Raposo, 2010), India (Jain and Kaur, 2006), Italy (Pedrini and Ferri, 2014), and Germany (Pagiaslis and Krontalis, 2014; Pinto et al., 2014).

However, there are not enough cross-cultural studies on the topic of environmentally-conscious purchasing: the USA-France (Arbuthnot and Lingg, 1975), the USA-Germany (Sriram and Forman, 1993), Sweden-Baltic countries (Gooch, 1995), Canada-France (Laroche et al., 1996) and Belgium-Poland (Roozen and Pelsmacker, 2000). Environmental purchasing is a global problem, which is why the cross-cultural studies are very important because they provide information that will provide a more complete picture for efficient management of consumer purchasing than studies in individual countries.

In researching cross-cultural studies on the topic of environmentally-conscious purchasing (Arbuthnot and Lingg, 1975; Sriram and Forman, 1993; Gooch, 1995; Laroche et al., 1996; Roozen and Pelsmacker, 2000), the authors have concluded that there is no relevant research paper published with reference to the ex-Yugoslavian republics.

The aim of the study is to compare the environmentally-conscious behavior of buyers between ex-Yugoslavian republics. The ex-Yugoslavian republics are: the Republic of Serbia (Serbia), the Republic of Croatia (Croatia), the Republic of Slovenia (Slovenia), the Former Yugoslav Republic of Macedonia (FYR Macedonia), the Federation of Bosnia and Herzegovina (Bosnia and Herzegovina) and Montenegro. The ex-Yugoslavian republics had 73 years of shared history which certainly influenced similarities in consumer behavior. Today all the ex-Yugoslavian republics are independent countries with different macro, structural, demographic, socio-economic, etc. characteristics (State Statistical Office of Macedonia, 2014; Institute for Statistics of Serbia, 2014; Statistical Office of the Slovenia, 2014; Statistical Office of the Montenegro, 2014; Croatian Bureau of Statistics, 2014; Federal Office of Statistics of Bosnia and Herzegovina, 2014). According to the author's knowledge this will be the first cross-cultural study of the ex-Yugoslavian republics on the topic of environmentally-conscious purchasing.

Differences in Environmentally Responsible Purchasing Between Countries

Purchase of environmentally friendly products is different in different countries, according to the results of previous empirical comparative cross-cultural researches (Arbuthnot and Lingg, 1975; Sriram and Forman, 1993; Gooch, 1995; Laroche et al., 1996; Roozen and Pelsmacker, 2000). The purchase of environmentally friendly products was more often in the USA than in France (Arbuthnot and Lingg, 1975). Swedes were buying environmentally friendly products more than buyers from Baltic countries (Gooch, 1995). Canadians were purchasing environmentally friendly products more than the Frenchmen (Laroche et al., 1996). Also, Belgians were purchasing environmentally friendly products more than Poles (Roozen and Pelsmacker, 2000). However, one of the studies suggests that there were no differences in the purchase of environmentally friendly products among buyers from the USA and Germany (Sriram and Forman, 1993).

Considering the results of earlier cross-cultural research (Arbuthnot and Lingg, 1975; Sriram and Forman, 1993; Gooch, 1995; Laroche et al., 1996; Roozen and Pelsmacker, 2000) it can be assumed that:

H1: There are differences in environmentally-conscious behavior of buyers from different the ex-Yugoslavian republics.

The most common demographic characteristics represented in previous researches of environmentally responsible purchasing, as in this study, are: gender, age, degree of education and monthly income by household.

If we look at the link between environmentally responsible purchasing of consumer and gender, most of the research, from: Singapore (Shamdasani, et al, 1993), UK (Gilg et al., 2005), Portugal (De Paco and Raposo, 2010) and Italy (Pedrini and Ferri, 2014) shows that women and men are equal in environmentally responsible purchasing. However, some researches from India (Jain and Kaur, 2006) and Germany (Pinto et al., 2014) have come to the result that women are more environmentally responsible consumers than men related to purchasing environmentally friendly products.

If we look at the link between environmentally responsible purchasing and age, most of the research, from: the USA (Roberts, 1996), the UK (Samdahl and Robertson, 1989; Gilg et al., 2005), China (Chan, 1999), Portugal (De Paco and Raposo, 2010), Italy (Pedrini and Ferri, 2014) and Germany (Pagiaslis and Krontalis, 2014) has reached the conclusion that older consumers purchase environmentally friendly products more than the younger ones. One study conducted in India (Jain and Kaur, 2006) resulted in data that young consumers purchase environmentally friendly products more than the older ones. Part of the research, from the Singapore (Shamdasani et al., 1993), China (Chang, 1999) and Germany (Pinto et al., 2014) suggests that environmentally responsible purchasing does not depend on the age of the consumer.

If we look at the link between environmentally responsible purchasing and consumer education level, most of the studies, from: the USA (Roberts, 1996), China (Chan, 1996), India (Jain and Kaur, 2006), Portugal (De Paco and Raposo, 2010), Italy (Pedrin and Ferri, 2014) and Germany (Pinto et al., 2014; Pagiaslis and Krontalis, 2014) confirm that consumers which are more educated are more environmentally responsible when purchasing. However, one study in the UK (Samdahl and Robertson, 1989) has come to the result that lower educated consumers purchase environmentally friendly products more. Finally, the result of a study from Singapore (Shamdasani et al., 1993) shows that the level of educational attainment is not associated with environmentally responsible purchasing.

If we look at the link between environmentally responsible purchasing and monthly income by household, most of the studies from: China (Chan, 1999), India (Jain and Kaur, 2006), Portugal (De Paco and Raposo, 2010), Italy (Pedrini and Ferri, 2014) and Germany (Pinto et al., 2014; Pagiaslis and Krontalis, 2014) have reached the conclusion that consumers with higher incomes by household purchase environmentally friendly products more. One study from the UK (Samdahl and Robertson, 1989) resulted in data suggesting that consumers with lower incomes by household purchase environmentally friendly products more. Still, one study from Singapore (Shamdasani et al., 1993) found no association between monthly income by household and environmental responsibility purchasing.

According to the data presented above, it can be concluded that the studies from different countries have resulted in different data. It can be observed that the different results come from the same country. This happens because the studies were carried out

in the same country with a different sample, with different methods and in different time periods. From the results of previous researches (Samdahl and Robertson, 1989; Shamdasani et al, 1993; Roberts, 1996; Chan, 1999; Gilg et al., 2005; Jain and Kaur, 2006; De Paco and Raposo, 2010; Pedrini and Ferri, 2014; Pinto et al., 2014; Pagiaslis and Krontalis, 2014) it can be assumed that:
H2: Differences in environmental responsibility between consumers of the ex-Yugoslavian republics are caused by demographic characteristics.

Materials and Methods

Sample

The study included 1,550 respondents from six ex-Yugoslavian republics: 276 participants from Serbia, 250 participants from Croatia, 265 participants from Bosnia and Herzegovina, 250 participants from Montenegro, 253 participants from Macedonia and 256 participants from Slovenia (*Table 1*).

Table 1. *Gender structure of the sample*

Country	Male	Female	Total
Serbia	114	162	276
Croatia	78	172	250
Bosnia and Herzegovina	111	154	265
Montenegro	109	141	250
FYR Macedonia	93	160	253
Slovenia	70	186	256
Total	575	975	1550

Table 2 shows the age structure of the sample by country. Almost half of the sample consists of younger respondents, aged 18 to 30. The next category consists of respondents aged 31 to 40. The fewest respondents are in the category aged 71 to 80. Only seven respondents did not provide information about their age.

Table 2. *Age structure of the sample (N = 1543)*

	Age categories (in years)					
Country	18-30	31-40	41-50	51-60	61-70	71-80
Serbia	118	86	22	32	14	2
Croatia	137	48	34	24	6	1
Bosnia and Herzegovina	146	66	36	15	1	0
Montenegro	197	34	19	2	1	0
FYR Macedonia	105	68	28	24	14	8
Slovenia	84	89	55	22	5	0
Total	787	391	194	119	41	11

Table 3 shows the structure of the sample by level of education by country. The smallest number of respondents have completed only primary school (N = 15). The

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same number of respondents have completed high school and college/university (N = 617). A somewhat smaller number of respondents have completed post-graduate studies (N = 297).

Table 3. *Sample structure by education level (N = 1546)*

Country	Education level Primary school	 High school	 College, university	 MSc/ PhD
Serbia	5	126	93	50
Croatia	2	121	95	32
Bosnia and Herzegovina	0	134	83	48
Montenegro	0	123	89	41
FYR Macedonia	6	69	104	70
Slovenia	2	44	153	56
Total	15	617	617	297

Table 4 shows the structure of the sample by the average monthly income by household by country. Most of the respondents (N = 758) have estimated that they have average income by household, while fewer respondents have estimated their income at below average. The fewest number of respondents have estimated to have an above average income by household.

Table 4. *Sample structure according to monthly income by household (N = 1548)*

Country	The amount of monthly income by household Below average	 Average	 Above average
Serbia	70	111	94
Croatia	42	174	34
Bosnia and Herzegovina	21	193	51
Montenegro	57	120	76
FYR Macedonia	28	76	146
Slovenia	50	84	121
Total	522	758	268

Disparities in age and professional qualifications of the participants in the sample (age categories: 61-70 and 71-80; and education level: elementary) could be considered as limitations of this research. It should be noted, however, that it is really difficult to create the same pattern of demographics and socio-economic characteristics across all categories in several different countries (Raaij, 1978). The other cross-cultural researches also had disparities in sample (Arbuthnot and Lingg, 1975; Gooch, 1995; Roozen and Pelsmacker, 2000). Disparities exist because younger people and people who have higher education are more interested in taking part in studies such as this one, compared to older and less educated people (Chan, 2001).

The sampling method was stratified sample. The total population from the territory of the ex-Yugoslavian republics, which makes about 20 million people, is divided into stratums, now independent countries: Serbia, Croatia, Bosnia and Herzegovina, Montenegro, FYR Macedonia, and Slovenia. After that, method simple random sample was used for each stratum. For example, the researcher asked her friend who lives in Slovenia to participate in research and to distribute the questionnaire to his friends and colleagues from Slovenia.

Questionnaire

For the purposes of this study, and following the example of previously conducted research (Soutar et al., 1994; Barr and Gilg, 2005; Gilg et al., 2006; De Paco and Raposo, 2010; Young et al., 2010) a questionnaire was composed to test the difference between environmentally conscious behavior of buyers in the ex-Yugoslavian republics. The items in the questionnaire were adapted to the national and the international environment of the countries in which the research was conducted.

The questionnaire is composed of three parts *(Appendix 1)*. The first part of the questionnaire is the respondent's consent for doing the questionnaire. Completing the questionnaire was anonymous. The second part refers to the demographic and socio-economic characteristics of respondents: country, gender, age, level of education, and monthly income by household. In order to adapt the questionnaire to this study, the question about income had to be standardized. Data about income were differently shown in the Statistical Yearbooks of each country for 2013 (State Statistical Office of Macedonia, 2014; Institute for Statistics of Serbia, 2014; Statistical Office of the Montenegro, 2014; Statistical Office of the Slovenia, 2014). Data for incomes were presented at the annual level for household in the Statistical Yearbook of Macedonia (State Statistical Office of Macedonia, 2014), at the monthly level for one member of household in the Statistical Yearbook of Serbia (Institute for Statistics of Serbia, 2014) and at the monthly level for household in the Statistical Yearbooks of Montenegro (Statistical Office of the Montenegro, 2014) and Slovenia (Statistical Office of the Slovenia, 2014). Unfortunately, data for income does not exist for Bosnia and Herzegovina and Croatia. Standardization meant equalization data of income at the same instance of time and to the same dimension to whom income refers. Author opted for monthly income by household, because such data existed in two of the four Statistical Yearbooks of the countries. In addition, the question for monthly income by household was asked in the currency of the country where the questionnaire was implemented, so that it would be easier to understand and answer it. The third part of the questionnaire refers to environmentally-conscious behavior of buyers. It has 7 items relating to the behavior of buyers of which each item refers to another group of products: 1) food and beverage, 2) chemicals and pharmacy products, 3) clothing, 4) furniture, 5) electrical appliances, 6) means of transport and 7) office supplies (*Table 5*). Answers to the questionnaire were measured by Likert scale, 1-strongly disagree, 2-mostly disagree, 3-undefined, 4-mostly agree, and 5- completely agree. The aim of this construction of questionnaire was to get a more precise picture of environmentally-conscious behavior of buyers.

Table 5. *Descriptive statistics of items of the total sample*

Items	M	SD
I buy organically grown products.	3.11	1.10
I buy home care products that threaten the environment less.	3.11	1.19
In the flat/house I also have furniture that I bought at a marketplace or in a second-hand store.	2.10	1.58
I buy technical appliances that save energy.	2.09	1.38
I also buy clothes in second hand stores.	3.56	1.25
I would rather buy a bicycle than a car.	3.69	1.41
I buy toilet paper, books, notebooks, etc. made of recycled paper.	2.99	1.23

The questionnaires were translated into Slovenian, Macedonian, Croatian, and Serbian. Respondents from Serbia, Bosnia and Herzegovina, and Montenegro filled in questionnaires in Serbian considering that they can be classified in the same language area.

The questionnaire was distributed personally and through various Internet platforms (Google Drive, FB, email, etc.). It was sent to the addresses of various entities: private individuals, non-governmental organizations, higher education institutions, businesses, statistical bureaus of the countries, etc.

Variables

Independent variables in this study are: country, gender, age, professional qualification and monthly income by household. There is one dependent variable - environmentally responsible purchasing.

Data Analysis

For data processing we used the software package SPSS:20 (SPSS, 2008).

Preparing the data for the main analysis included the replacement of missing values by EM method and the treatment of extreme values that resulted in not showing even one outlier.

After preparing the data, exploratory factor analysis (EFA) was conducted. The results of EFA suggested that there is a stable factor structure of the questionnaire, with three different dimensions. Next, the obtained factor solution was confirmed by confirmatory factor analysis (CFA) in statistical packages "lavaan" (Rosseel, 2012) and "semTools" (Pornprasertmanit et al., 2013), written for the R environment.

One-way ANOVA with factor: country (6 levels) and Scheffe post hoc test were used as the methods for determining the differences in environmentally responsible purchasing between the ex-Yugoslavian countries. Also, two-way ANOVA and Scheffe post hoc test were used to test the differences in demographic and socio-economic characteristics of participants related to environmentally responsible purchasing between the ex-Yugoslavian republics, with the following factors: country (6 levels) and gender (2 levels), country (6 levels) and age of respondents (6 levels), country (level 6) and degree of education (4 levels), and country (6 levels) and monthly income by household (3 levels).

Results

Descriptive statistics

It may be noted that the distribution of subscales purchasing does not deviate significantly from normal values (values Skunis and Kurtosis are within the permissible values), despite the significant value of K-S statistics (*Table 6*). Reliability coefficients of the subscales do not show quite adequate in the case of half the countries and in the case of total of subscales, with respect to their values, which are lower than .70 (*Table 6*). Cronbach's alpha level can be explained by a small number of items for each subscale. The author decided on a small number of items due to the nature of this cross-cultural study.

Table 6. *Descriptive statistics and reliability coefficients of questionnaire*

Country	M	SD	Skunis	Kurtosis	K-S	α
Serbia	20.38	5.18	-.116	-.268	.065*	.624
Croatia	20.65	5.47	-.011	-.060	.066*	.724
Bosnia and Herzegovina	20.39	5.51	-.010	-.448	.061*	.704
Montenegro	18.91	5.07	.029	-.179	.053	.652
Macedonia	21.79	4.59	.401	.211	.081*	.628
Slovenia	21.73	5.12	.140	-.258	.071*	.742
Total	20.64	5.25	.020	-.131	.046*	.682

Note: *p<.01.

Differences in the environmentally responsible purchasing between the ex-Yugoslavian republics

The results of one-way ANOVA with the factor: the state (level 6) and the dependent variable buying organic products indicate that among respondents from different countries there is a statistically significant difference when it comes to eco-buying, F (5, 1544) = 10.64, p <.01, ηp2 = .03. Post hoc tests (Scheffe) have shown that there are *statistically significant differences between respondents from Montenegro (M = 18.92, SD = 5.07) and FYR Macedonia (M = 21.79, SD = 4.59, p <.01), Montenegro and Croatia (M = 20.65, SD = 5.47, p <.01), and Montenegro and Slovenia (M = 21.73, SD = 5.12, p <.01).* In all three cases, it was shown that respondents from Montenegro achieve significantly lower scores in environmentally-conscious purchase when compared to respondents from aforementioned countries.

Demographic and socio-economic differences in the environmentally responsible purchasing between the ex-Yugoslavian republics

The results of two-way ANOVA with the factors: the country (level 6) and the gender (2 levels) indicate a statistically significant main effect of the gender, F (1, 1537) = 7.73, p <.01, , ηp2 = .005 and a statistically significant main effect of the country, F (5, 1537) = 9.42, p <.01, ηp2 = .03, while the interaction between country*gender is not statistically significant, F (5, 1537) = 1.36, ns. Respondents from Montenegro (M = 18.91, SD = 5.07) differ when compared to respondents from Croatia (M = 20.65, SD =

5.47), Slovenia (M = 21.73, SD = 5.12) and respondents from FYR Macedonia (M = 21.79, SD = 4.59) in purchasing environmentally friendly products (p <.01). Also, results indicate that, regardless of the country, female buyers (M = 96, SD = 15.63) buy environmentally friendly products more than male buyers (M = 93.11, SD = 14.81).

The results of two-way ANOVA with the factors: the country (level 6) and the age (level 6) indicate a statistically significant main effect of the age, F (4, 1510) = 15.82, p <.01, ηp2 = .04, and a statistically significant main effect of the country, F (5, 1510) = 11.23, p <.01, ηp2 = .03, while the interaction of country*age is not shown as a statistically significant, F (22, 1510) = 1, ns. Respondents from Montenegro (M = 18.91, SD = 5.07) differ when compared to respondents from Croatia (M = 20.65, SD = 5.47), Slovenia (M = 21.73, SD = 5.12) and respondents from FYR Macedonia (M = 21.79, SD = 4.59) in purchasing environmentally friendly products (p <.01). Also, results indicate that, regardless of the country, older respondents spend more money on ecological products (age 18-30: M= 19.38, SD= 5.12; age 31-40: M = 21.39, SD = 5.20; age 41-50: M = 22.56, SD = 4.07; age 51-60: M= 22.06, SD = 5,24; age 61-70 M= 23.42, SD = 4.65; age 71-80 M= 22.71, SD = 4.27).

The results of two-way ANOVA with the factors: the country (level 6) and the level of education (4 level) show *statistically significant interaction between country*level of education F (13, 1524) = 2.58, p <.01, ηp2 = .02*, and a statistically significant main effect of the country F (3, 1524) = 3.43, p <.01, ηp2 = .006. The main effect of educational level, however, is not shown as statistically significant, F (3, 1524) = <1 ns. Post hoc tests (Scheffe) indicated that the respondents from Montenegro with secondary education (M = 17.98, SD = 5.08) differ from respondents in Slovenia with completed university/college education (M = 21.66, SD = 5.26, p <.01), and participants from FYR Macedonia (M = 21.19, SD = 5.11) with secondary education. Participants from Montenegro have significantly lower scores on scale of environmentally-conscious purchase in relation to the aforementioned participants from FYR Macedonia and Slovenia.

Finally, the results of two-way ANOVA with the factors: the country (level 6) and the monthly household income (3 levels) indicate *statistically significant interaction between factors country * monthly household income, F (10, 1530) = 2.65, p <.01, ηp2 = .02,* and statistically significant main effects of monthly household income, F (2, 1530) = 6.51, p <.01, ηp2 = .008, and of the country, F (5, 1530) = 9.44, p <.01, ηp2 =. 03. Post hoc test (Scheffe), however, did not show statistically significant differences between countries and categories when it comes to environmentally-conscious purchase (p> .05).

Discussion

The study results show that there are differences in environmentally-conscious behavior of buyers between: Montenegro and FYR Macedonia, Montenegro and Croatia and Montenegro and Slovenia. Respondents from Montenegro buy environmentally friendly products less when compared to respondents from FYR Macedonia, Croatia and Slovenian. *The results partially confirm H1,* there are differences in environmentally-conscious behavior of buyers from different ex-Yugoslavian countries. Other cross-cultural researches confirmed that differences in purchasing environmental friendly products have existed between other countries (Arbuthnot and Lingg, 1975; Gooch, 1995; Laroche et al., 1996; Roozen and Pelsmacker, 2000).

In all ex-Yugoslavian republics women buy environmentally friendly products more than men, which is also confirmed by previous research results (Jain and Kaur, 2006; Pinto et al., 2014). In addition, in all ex-Yugoslavian republics older customers buy environmentally friendly products more than younger consumers, which is consistent with the results of previous studies (Roberts, 1996; Samdahl and Robertson, 1989; Gilg et al., 2005; Chan, 1999; De Paco and Raposo, 2010; Pedrini and Ferri, 2014; Pagiaslis and Krontalis, 2014). That consumers who have higher level of education are more likely to buy environmentally friendly products than less-educated consumers (respondents with secondary education from Montenegro buy environmentally friendly products less than respondents with university/college education from Slovenia) also confirmed by previous research results (Roberts, 1996; Chan, 1996; Jain and Kaur, 2006; De Paco and Raposo, 2010; Pedrin and Ferri, 2014; Pinto et al., 2014; Pagiaslis and Krontalis, 2014). Results related to the differences in monthly income by household between countries show that there are differences, but the post hoc tests (Scheffe) did not show which countries and which groups are the ones that differ. Differences in monthly income by household between countries related to purchasing environmentally friendly products are also confirmed by previous studies (Samdahl and Robertson, 1989; Chan, 1999; Jain and Kaur, 2006; De Paco and Raposo, 2010; Pedrini and Ferri, 2014; Pinto et al., 2014; Pagiaslis and Krontalis, 2014). Based on all of the above, it can be concluded that there are demographic differences in environmentally-conscious behavior of buyers in ex-Yugoslavian countries, which *partially confirms H2*.

Limitations of research

Although this cross-cultural research was done with caution, it has certain limitations. The first limitation can be that the questionnaire was designed for research purposes and it has not been used elsewhere, and cannot be characterized as multiply successful. The second limitation can be the number of items in the questionnaire, seven items. That can be considered as a small number of items which is why maybe the structure of the questionnaire is not the best. Disparities in age and professional qualifications in the sample are also a limitation. Respondents answered on the basis of their past behavior and potential future behavior, which is considered the fourth limitation of the study. Environmentally-conscious purchasing is socially desirable behavior and it is assumed that the respondents were subjective and that they were giving socially desirable answers, which is also considered a limitation of the study.

Conclusion

This study is one of the first cross-cultural research of the environmentally responsible purchasing in the regon of ex-Yugoslavian republics as such as provides valuable information.

The results indicate that there are differences in environmentally-conscious behavior of buyers between respondents from: Montenegro and FYR Macedonia, Montenegro and Croatia and Montenegro and Slovenia.

The data obtained by this research indicates different segments of environmentally-conscious buyers in republics of the ex-Yugoslavian which can be used to create instruments for increasing environmentally-conscious purchases. For example, if a market subject (international institutions, government bodies, NGOs, businesses, etc.) wants to increase the purchase of environmentally friendly products in FYR Macedonia, Croatia and Montenegro they will apply the same instrument for FYR Macedonia and Croatia and a different one for Montenegro. The instruments directed towards Montenegro should be more stimulating considering that the purchase of environmentally friendly products is the lowest in Montenegro. Or, if a market subject wants to increase the purchase of environmentally friendly products in Serbia, Bosnia and Herzegovina, and FYR Macedonia, they can apply one strategy for all countries, since the countries do not differ according to environmentally-conscious behavior of buyers. Also, the data indicate that the strategies of increasing environmentally-conscious purchases of consumers aimed at different former Yugoslavian countries should take into account the differences in the level of education and monthly income by household. The data obtained can serve as a starting point for entering into horizontal and vertical partnerships between the various market entities at national and international level with the aim of environment protection through increased purchases of environmentally friendly products. Through adequate implementation of obtained data, the behavior of buyers can become a tool of environmental management and improve the quality of life in the region. Increasing the purchase of environmentally friendly products will reduce the degradation of renewable natural resources and the use of non-renewable natural resources.

From a theoretical perspective, the data obtained is important because it provides an empirical basis for other studies through creation of an environmental profile of responsible consumers. Also, the research was conducted with a newly created questionnaire which can be used for other research, and for create another questionnaire on the same topic.

Authors suggest that future research should be carried out in the same sample of countries five years later using the same instrument. Such research would provide information on the change in behavior in the countries of the ex-Yugoslavian republics. The future research should involve other variables, for example, religion variables or types of products. Such research would provide a more complex picture of environmentally-conscious behavior of buyers in the ex-Yugoslavian republics. The use of qualitative methods would be very significant because it would provide data about reasons that cause certain behaviors, or in this case, the similarities and differences between environmentally-conscious purchasing from the ex-Yugoslavian republics. Finally, the authors suggest that more studies should be carried out on the topic of environmental responsibility of buyers because it is the only way to analyze the limits and motivators of environmentally responsible behavior of buyers.

REFERENCES

[1] Arbuthnot, J., Lingg, S. (1975): A comparison of French and American environmental behaviors, knowledge and attitude. - International Journal of Psychology 10 (4): 275-281.
[2] Chan, R.Y.K. (1999): Environmental Attitudes and Behavior of Consumers in China. - Journal of International Consumer Marketing 11 (3): 25-52.
[3] Chan, R. (2001): Determinants of Chinese consumers' green purchasing behavior. - Psychology and Marketing 18: 389-413.
[4] Croatian Bureau of Statistics (2014): Statistical Yearbook. - Croatian Bureau of Statistics. Retrieved 15 October 2014, from: http://www.dzs.hr/Hrv_Eng/ljetopis/2014/sljh2014.pdf
[5] De Paco, A.M.F., Raposo, M.L.B. (2010): Green consumer market segmentation: empirical findings from Portugal. - International Journal of consumer studies 34 (4): 429–436.

[6] Federal Office of Statistics of Bosnia and Herzegovina (2014): Statistical Yearbook. - Federal Office of Statistics of Bosnia and Herzegovina. Retrieved 15 October 2014, from: http://www.fzs.ba/SG2014.pdf.

[7] Gilg, A., Barr, S., Ford, N. (2005): Green consumption or sustainable lifestyles? Identifying the sustainable consumer. - Futures 37 (6): 481–504.

[8] Barr, S., Gilg, A. (2006): Sustainable lifestyles: framing environmental action in and around the home. - Geoforum 37: 906–920.

[9] Gooch, G. (1995): Environmental beliefs and attitudes in Sweden and the Baltic states. - Environment and Behavior 27 (4): 513–539.

[10] Institute for Statistics of Republic Serbia (2014): Statistical Yearbook. - Institute for Statistics of Republic Serbia. Retrieved 13 October 2014, from: http://webrzs.stat.gov.rs/WebSite/repository/documents/00/01/57/22/Potrosnja_stanovnistva_2014.pdf

[11] Jain, S., Kaur, G. (2006): Role of socio-demographics in segmenting and profiling green consumers: an exploratory study of consumers in India. - Journal of International Consumer Marketing 18 (3): 107–117.

[12] Laroche, M., Toffoli, I.R., Kim, C., & Muller, T.E. (1996): The influence of culture on pro-environmental knowledge, attitudes, and behaviors: a Canadian perspective. - Advances in Consumer Research 23:196-202.

[13] Marx, A.M., De Paula, I.C., & Sum,

[14] Pagiaslis, A., Krontalis, A.K. (2014): Green Consumption Behavior Antecedents: Environmental Concern, Knowledge, and Beliefs. - Psychology and Marketing 31 (5): 335–348.

[15] Pedrini, M., Feffi, M.L. (2014): Socio-demographical antecedents of responsible consumerism propensity. - International journal of consumer studies 38 (2): 127–138.

[16] Pinto, D.C, Herter, M.M., Rossi, P., Borges, A. (2014): Going green for self or for others? Gender and identity salience effects on sustainable consumption. - International journal of consumer studies 38 (5): 540–549.

[17] Pornprasertmanit, S., Miller, P., Schoemann, A., Rosseel, Y. (2013): semTools: Useful tools for structural equation modeling. - R Package available version 0.4-0.on CRAN. http://CRAN.R-project.org/package=semTools.

[18] Raaij, V.W.F, (1978): Cross-cultural resource methodology as a case of construct validity. - Advances in Consumer Research 5: 693-701.

[19] Roberts, J.A. (1996): Green consumers in the 1990s: profile and implications for advertising. - Journal of Business Research 36 (3): 217–231.

[20] Roozen, I.T.M., De Pelsmacker, P. (2000): Polish and Belgian consumers' perception of environmentally friendly behavior. - Journal of consumer studies and home economics 24 (1): 9–21.

[21] Rosseel, Y. (2012): Lavaan: An R package for structural equation modeling. - Journal of Statistical Software 48(2):1-36.

[22] Samdahl, D., Robertskon, R. (1989): Social determinants of environmental concern: Specification and test of the model. - Environment and Behavior 21 (1): 57–81.

[23] Shamadasini, P., Chon-Lin, G.O., Richmond, D. (1993): Exploring green consumers in an oriental culture: role of personal and marketing mix factors. - Advances in Consumer Research 20 (1): 488-493.

[24] Souter, G.N., Ramaseshan, B., Molseter, C.M. (1994): Determinants of pro-environmental consumer purchase behavior: some Australian evidence. - Asia Pacific Advances in Consumer Research 1 (2): 28-35.

[25] Sriram, V. Forman, A.M. (1993): The relative importance of products' environmental attributes: a cross-cultural comparison. - International Marketing Review 10 (3): 51–70.

[26] State Statistical Office of the Macedonia (2014): Statistical Yearbook. - State Statistical Office of the Macedonia. Retrieved 14 October 2014, from:

http://www.stat.gov.mk/Publikacii/PDFSG2014/08-PrihodiPotrosCeni-IncomeExpPrices.pdf
[27] Statistical Office of the Montenegro (2014): Statistical Yearbook. - Statistical Office of the Montenegro. Retrieved 13 October 2014, from: http://www.monstat.org/userfiles/file/publikacije/godisnjak%202014/potrosnja%20stanovnistva.pdf
[28] Statistical Office of Slovenia (2014): Statistical Yearbook. - Statistical Office of Slovenia. Retrieved 15 October 2014, from: http://www.stat.si/StatWeb/Common/PrikaziDokument.ashx?IdDatoteke=5651
[29] United Nations Environment Programme (UNEP) (2008): Planning for change-Guidelines for National Programs on Sustainable Consumption and Production. - UNEP, Paris
[30] Young, W., Hwang, K., Mcdonald, S., Oates, C.J. (2010): Sustainable consumption: green consumer behavior when purchasing products. - Sustainable development 18 (1): 20–31.

APPENDIX

***Appendix 1.** Questionnaire used in this study*

“Dear Sir/Madam,

Bearing in mind that this is one of rare cross cultural studies in our region, and that the results will provide valuable information on similarities and differences between our cultures, we kindly ask you to devote 10 minutes of your time, which will be enough to fill out all of the questionnaires.

This survey is entirely ANONYMOUS, which means that nobody will be able to associate you with your results. Survey data will be processed in groups and will be used for scientific research purpose only.

We kindly ask you to answer ALL of the questions and check carefully whether you have completed the ENTIRE questionnaire.

You are free to send all of your questions and doubts related to this research via e-mail: rsasaca@hotmail.com.

Filling out these questionnaires is voluntary and requires your consent. If you agree to participate in this survey, circle YES.

I AGREE TO PARTICIPATE IN THIS SURVEY:

YES NO

THANK YOU IN ADVANCE FOR YOUR COOPERATION!

Circle the number in front of the statement that applies to you.

Country:

1. Serbia
2. Croatia
3. Bosnia and Herzegovina
4. Slovenia
5. FRY Macedonia
6. Montenegro

Gender:
1. Male 2. Female

Age (write how old you are) _________

Education:
1. Primary school 2. High school 3. College/University 4. MSc/MA/Mr/PhD

*According to the Statistical Office of the Republic of Serbia, average monthly income per household in the Republic of Serbia is 56.073, 00 dinars in 2013. Is your average monthly income:
1. above average 2. average 3. below average

In the table below there are several groups of statements describing different behaviors and attitudes which relate to ecological responsibility of consumers. Please, carefully read each of the statements and answer by circling the number and expressing to what degree you agree with each of the statements. Numbers have the following meaning:

1 - I strongly disagree, 2 - I disagree, 3 - Undecided, 4 - I agree, 5 - I strongly agree

	Items	Answers				
1	I buy organically grown products.	1	2	3	4	5
2	I buy home care products that threaten the environment less.	1	2	3	4	5
3	In the flat/house I also have furniture that I bought at a marketplace or in a second-hand store.	1	2	3	4	5
4	I buy technical appliances that save energy.	1	2	3	4	5
5	I also buy clothes in second hand stores.	1	2	3	4	5
6	I would rather buy a bicycle than a car.	1	2	3	4	5
7	I buy toilet paper, books, notebooks, etc. made of recycled paper.	1	2	3	4	5

*Note: This research was conducted with six questionnaires, where the questionnaires differ in the matter relating to the average monthly income per household. Therefore, Appendix 1 should have six pieces. However, the authors believe that it is unnecessary. For a better understanding of the questionnaires the authors explain that the question refers to the average monthly income per household adjusted for each country in which the research was conducted. For every country were used data relating to the average monthly income per household exactly of that country, from Statistical Yearbooks for 2013. years (State Statistical Office of Macedonia, 2014, 296; Institute for Statistics of Serbia, 2014, 123; Statistical Office of the Montenegro, 2014, 68; Statistical Office of the Slovenia, 2014, 27). Unfortunately, data for income did not exist for Bosnia and Herzegovina and Croatia."

THE ASSESSMENT OF LANDSCAPE AND ENVIRONMENTAL RISKS AND HAZARDS CAUSED BY LANDSLIDES IN MOUNTAIN AREAS

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Abstract. The landslide is a kind of natural disaster responsible for creation of risks and hazards in relation to population and its economic activity. Each year the occurrence of landslides causes killing of people and economic losses, as well as leads to destruction and deterioration of pastures, arable lands, forests and highways. The southeastern slope of Greater Caucasus is one of the most active landslide zones in Azerbaijan. As a result of the occurrence of landslide in the Mughanli area in 2011, 168 houses out of 279 ones became unusable while the village of the same name was moved to a new residential area. Moreover, the 3 km-long part of the Ismayilli-Mughanli-Shamakhi highway near the village became unusable repeatedly. In 2014, USD 52 million was spent in order to eliminate the consequences of landslides. Topographic maps of scale of 1:10000 and also aerospace images were used to assess the landscape- and environmental risks and hazards caused by landslides. Maps of various factors of the same scales, compiled in ArcGIS program were analysed through GIS technologies, which allowed compilation of a map of relevant landscape- and environmental risks and hazards. The carried out studies will enable to estimate natural disasters and reduce the related economic losses.

Keywords: *environmental, landslide, morphodynamic, natural disasters, economic losses*

Introduction

In recent years, mountain geosystems were under the influence of considerable anthropogenic load, driven by such factors as the rapid development of new settlements, the laying of new asphalted motorways, the construction of industrial facilities as well as the development of mining industry. In this regard, the investigation of landscape- and geomorphological processes, posing a hazard to the sustainable development of the natural-economic systems in the mountainous areas, as well as the forecasting and the prevention of this processes are regarded as topical issues. High seismicity in the southeastern part of the Greater Caucasus favourably affects the occurrence of morphodynamic processes, including the landslides. The preliminary assessment of hazards and risks posed by landslides in the mountain areas through the application of up-date methods may prevent or reduce damage.

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The carried out studies regarding the grade of landslide-related risks and hazards reveal that the growth of factors increases the level of accuracy and reliability of results (Lee, 2005; Wang et al., 2009).

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Material and methods

Study Area

The study area covers the intensive areas of Azerbaijan in terms of occurrence and spread of landslide, namely the southeastern slope of Greater Caucasus Mountains where Mughanli village and part of Nuran village, as well as the municipalities of Shirvanzadeh and Mirikend are located. Population and the economic activities in the mentioned settlements suffer from landslides periodically. The study area is situated on the 41°53' north latitude and 49°33' east longitude. The territory area makes up 1057,5 hectares. The area typically has low relief and hilly terrains with the maximum height of 1214 m and the lowest point at 326 m. This mountainous territory is densely populated compared to its surrounding areas *(Fig. 1)*.

Source: Esri, DigitalGlobe, GeoEye, i-cubed, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AEX, Getmapping, Aerogrid, IGN, IGP, swisstopo, and the GIS User Community

Figure 1. *Location of the study area*

Methodological approaches

In order to increase the extent of accuracy and reliability of the determination of level of landslide-related risk and hazard, ArcGIS/ArcMap programs and GIS were applied. Landslide risk model based on GIS (Lee, 2005; Wang et al., 2009; Mora et al., 1994; Varner, 1984; Griffiths et al., 2008) as well as related investigations (Kumtepe et al., 2011) were used in the work. The assessment and modelling of level of landslide risk and hazard along with the compiling of relevant maps will allow to increase the efficiency of mastering of territory and apply the landscape planning properly. Such maps will contribute to minimize and eliminate losses caused by landslides that may occur in the future.

The GIS-based landslide risk model of Mora et al. (1994) was used. The reasons of occurrence of landslides are classified in the above mentioned studies. The first group of reasons includes are the main reasons of while the second group takes are responsible for the activation of landslides. The morphometric parameters of relief along with the lithological composition of rocks and the soil moisture index are listed as the main reasons of landslides, while the average perennial precipitation and seismicity were considered as activating factors. The factors were analysed through GIS in accordance with the following formula:

$$A(fet)_{landslide} = (S_d \times S_{lc} \times S_{sm}) \times (T_{al} + T_{sr}) \qquad \text{(Eq. 1)}$$

where S_d is the degree of inclination of slopes; S_{lc} is the lithological composition of rocks; S_{sm} is soil moisture index; T_{al} is the average monthly amount of precipitation in multiyear period; and T_{sr} is the seismicity rate.

Kumpete et al. (2011) used eight indicators to assess the extent of landslide-related hazard: inclination of a slope, direction of a slope, curvature of a slope, index of plant cover, lithological composition of rocks, tectonic fracture, farness in relation to rivers and roads. Landscape- and environmental risks of landslides were identified with taking into account the specific characteristics of the studied southeastern slope of Greater Caucasus as well as such factors as anthropogenic loading, land use, relief, vertical and horizontal fragmentation, indices of landscape's ecomorphological tensity and factors of landscape- and environmental assessment. Relatedly, the following formula was used:

$$S_i = \frac{\sum S}{\sum S_{fic}} : N \qquad \text{(Eq. 2)}$$

where S_i is the landslide intensity index; S is the area affected by landslides; S_{fic} is the total area of factors, involved to the comparison; and N is the number of measurements.

The figures of landslide intensity index obtained due to calculations are ranked as follows: <0,1 –very weak; 0,1-0,3 – weak; 0,3-0,5 – middle; 0,6-0,8 – high; >0,8 very high. Researchers note that the correlation between the indicator of landslide intensity index and the level of landslide-related risk and hazard is available (Varner, 1984). The landslide intensity index was identified by each factor responsible for the activation of landslide. Then the gained data was analysed through GIS. The last phase of was carried out based on the following formula:

$$S_i = \frac{S_a + S_l + S_{vt} + S_i + S_e + S_{si} + S_{sd} + S_{vc} + S_{lc} + S_{tf} + S_{dr} + S_{eb}}{S_{fic}} \qquad \text{(Eq. 3)}$$

where S_a is the anthropogenic loading; S_l is the use of lands; S_{vf} is the vertical fragmentation of relief; S_e is the eco-geomorphological tension; S_{si} is the inclination of slope; S_{sd} is the direction of slope; S_{sc} is the curvature of slope; S_{vc} is the vegetation cover; S_{lc} is the lithological composition of the rocks; S_{tf} is the tectonic fracture; S_{dr} is the distance from rivers; S_{dr} is the distance from roads; and S_{eb} is the environmental balance.

Results

Tectonic fault

The purpose is to determine the relation between soil use, coefficient of vegetation cover and also environmental and landscape balance (I) and the intensity coefficient of landslide as well as the level of risk and hazard (II). Active landslides in the study area are mapped, and are grouped by five levels in accordance with the risk and hazard, on the basis of which, the conducted assessment is expressed by five points (*Fig. 2*).

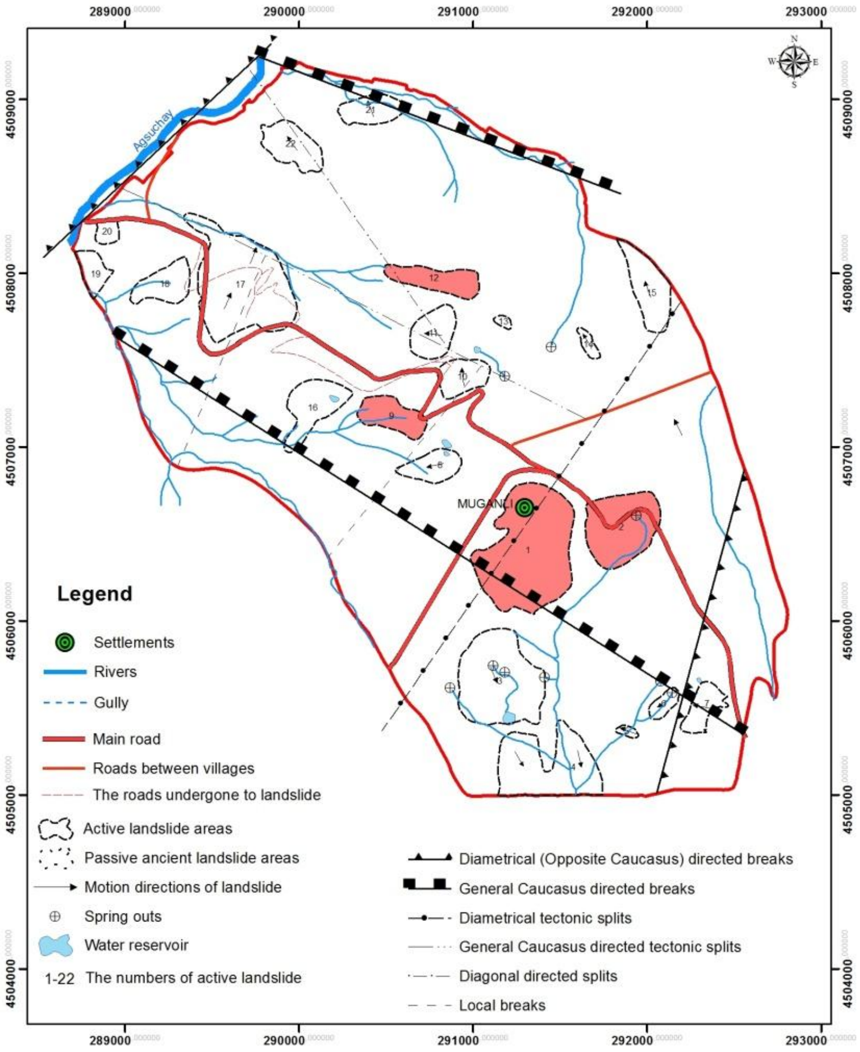

Figure 2. *Prevalence of active landslide areas and tectonic faults*

The assessment of the impact of soil use on the risk level and dynamic of landslides

The use of land as one of forms of anthropogenic activity is regarded as an important factor of assessment of risk and hazard posed by landslide (Ismayilov et al., 2012). Researches show that landslides may happen at very different extent depending on agricultural activities, perennial plant-growing, gardening, pasturing, settlements' impact, the availability of roads and communications, etc. *(Fig. 3)*.

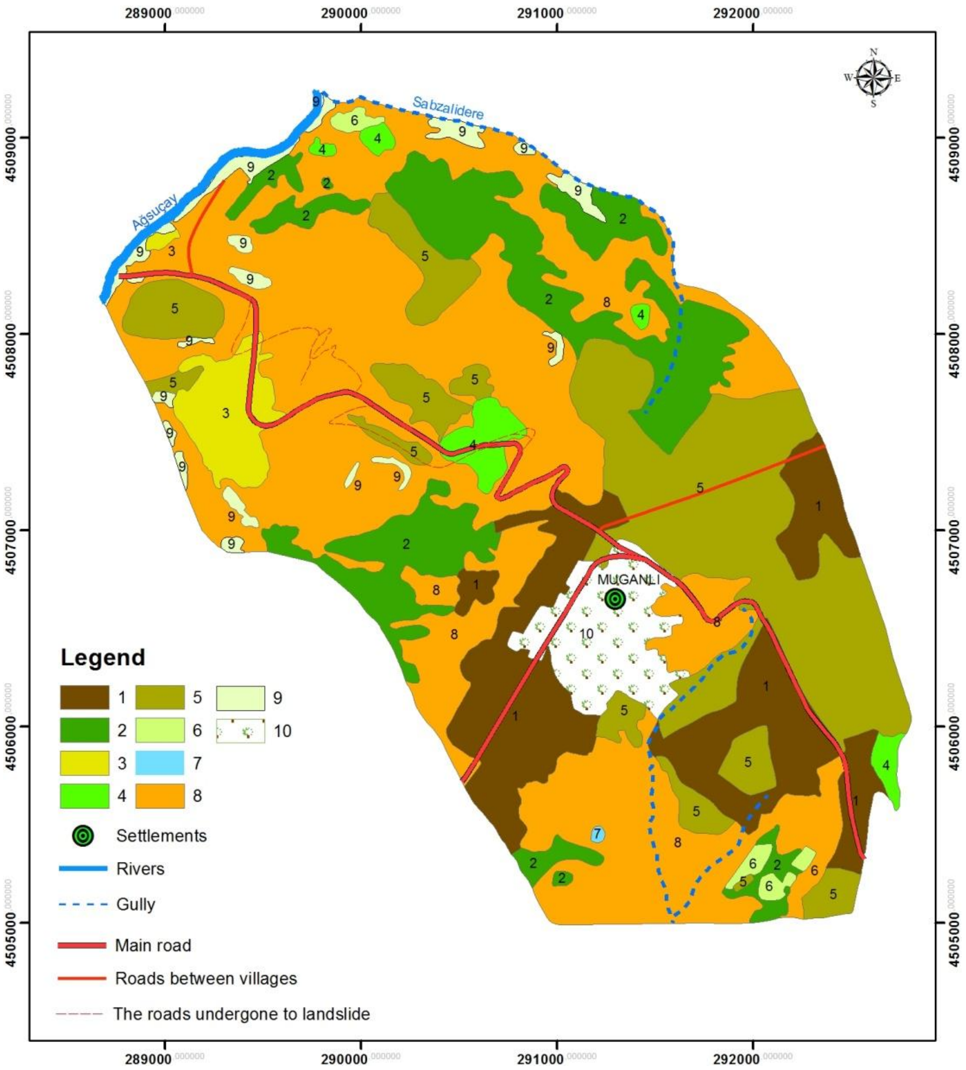

Figure 3. *Impact of soil use on the landslides.*

The map legend reflects the impact of soil use on the occurrence of landslides in the municipality of Mughanli and surrounding areas: 1. Plantations of perennial plant-growing – medium hazard of landslide. 2. Rare trees and shrubs used in pasturing – medium hazard of landslide. 3. Shrubberies and meadows composed of small bushes and used as pastures and hayfields – very high hazard of landslide. 4. Woodlands, partially used in pasturing and anthropogenically degraded – low hazard of landslide. 5. Arable or fallow lands of low-incline watershed areas – very weak hazard of landslide. 6. Fruit gardens, composed of perennial plants – weak hazard of landslide. 7. Artificial water reservoirs – very high hazard of landslide. 8. Pastures and hayfields on low-incline slopes – high hazard of landslide. 9. Cliffs and sandy areas, not usable in agriculture – very low hazard of landslide. 10. Rebuilt settlements, courtyards and roads – very high hazard of landslide.

The analysis of electronically compiled maps (1:10000), reflecting the land use in the Mughanli municipality and surrounding areas shows that the most part of the lands (42,1%) is occupied by pastures and hayfields of low-incline slopes, while 20,4% is composed of ploughed arable and fallow lands; 13,0% is composed of rare trees and shrubberies used in pasturing; 12,9% is composed of perennial plants and plantations; 4,4% is composed of rebuilt settlements, courtyards and roads; 2,7% is composed of shrubberies and meadows with small bushes usable in pasturing; 2,1% is composed of cliffs and sandy areas, unusable in agriculture; and 1,8% is composed of woodlands, weakly degraded due to anthropogenic factors. Fruit gardens, composed of perennial plants as well as artificial water reservoirs occupy very small area.

In order to increase the extent of accuracy and confidence of spread of landslides, ArcGIS program was applied. Furthermore, the relations between the land use and the intensity of landslides as well as related risks and hazards were studied and given on *Table 1.*

Table 1. *The relations between land use and risks, hazards and intensity of landslides in the study area*

Land use form	Contours	Area, m^2	Area, %	Areas affected by landslide, m^2	Intensity index of landslide	Risk of landslide by point
1	5	1363317,38	12,89333	408995,15	0,3-0,5	3
2	9	1372456,849	12,97977	411737,04	0,3-0,5	3
3	2	282397,8519	2,670728	169438,74	0,6-0,8	4
4	5	185242,5323	1,751898	18524,25	0,1-0,3	1
5	12	2155254,285	20,38294	215525,43	0,1 and lesser	1
6	4	71388,13235	0,675141	7138,813	0,1 and lesser	2
7	1	4891,499597	0,046261	3913,1996	0,8 and higher	5
8	5	4455169,254	42,13398	356135,41	0,6-0,8	5
9	17	223933,4774	2,117812	11196,74	0,3-0,5	4
10	1	459764,8675	4,348146	367811,97	0,8 and higher	5

The analysis of the *Table 1* shows that the pastures and hayfields (4 and 5 scores) as well as the areas of rebuilt settlements, courtyards and roads along with the areas near the artificial reservoirs have high (5 scores) and very high landslide risks. Such approach reveals that 51,4% of the study area has high or very high risk of landslide.

The assessment of impact of vegetation cover indexes on the dynamics of landslides and the risk level.

The compiling of maps of vegetation cover of the study area has been justified by some researchers engaged in the study of landslide dynamics and the related risk level (Kumtepe et al., 2011). The analysis of old topographic and vegetation maps and also aerospace photos, taken in different years along with the surveys held among the elder population contributed to the conduction of researches on reasons and results of relations between the intensity of landslides and the vegetation cover during the last 100-110 years. The researches revealed that in the past the vegetation of this territory was presented by arid and semi-arid forests and shrubs. The forests were composed mostly of drought-resistant Georgian oak (Quercus L.), elm (Ulmus L.), the hornbeam (Carpinis L.), juniper (Yuniperus L.), pears (Prus), Caucasian olive (Elaeagnus L.), Iberian tree (Aser L.), hawthorn etc. In the following periods, these forests faced heavy adverse impact and were converted to various vineyards, orchards and pastures. The deep root system of drought-resistant trees and shrubs of those arid forests were increasing the resistance of natural complexes in regard to exogenous geomorphological processes. The transformation of arid forests into anthropogenic complexes in the 80s of 20th century as a result of the implementation of government programs has entailed the intensification of landslides. Eventually, in 1982, about 20 houses of the village of Mughanli were almost destrcuted by this natural disaster. The second phase of activation of landslides in Mughanli and surrounding areas was observed in 2010 and following years. This happened due to the massive deforestation, conducted in 1992-2008 years, and was driven by the demand for firewood in the villages. More than 100 houses became unusable during the second phase of activation of the Mughanli landslide (*Fig. 4*).

The analysis of contemporary vegetation map (1:10000) indicates that 10,2% of the study area consists of anthropogenically violated derivative forests, while out of the remaining, 6,4% is mixture of forests and bushes, 37,3% are shrubby meadows, 24,2% are cereals and other cultivated plants, and 8,9% are courtyard areas consisting of cultivated plants (*Fig. 3*). The analysis of the maps of vegetation and landslide intensity in ArcGIS (*Fig. 3* and *Table 2*) reveals that 58,6% of the study area is high or very high hazardous in terms of occurrence of landslides, while 6,4% is the area is of medium hazard, 10,2% of low hazard and 24,7% of lesser hazard.

Table 2. *Distribution of vegetation coverage according to risk and hazard intensity indexes of landslide areas*

Number of plant group on the map	Quantity of contours	Area, m^2	Areas affected by landslide, m^2	Area, %	Intensity index of landslide	Risk of landslide by point
1	10	1082116,302	324634,79	10,179	0,1-0,3	2
2	5	2582968,086	258296,8086	24,29686	0,1 and lesser	1
3	3	45969,51975	4596,951975	0,432415	0,1 and lesser	1
4	6	3963562,311	2378137,3866	37,28351	0,6-0,8	4
5	7	681243,8121	204373,14363	6,408166	0,3-0,5	3
6	12	997977,0763	598786,24578	9,387539	0,6-0,8	4
7	2	177842,3607	142273,88856	1,672887	0,8 and higher	5
8	11	151173,1914	120938,55312	1,422021	0,8 and higher	5
9	2	948017,9514	568810,77084	8,917596	0,6-0,8	4

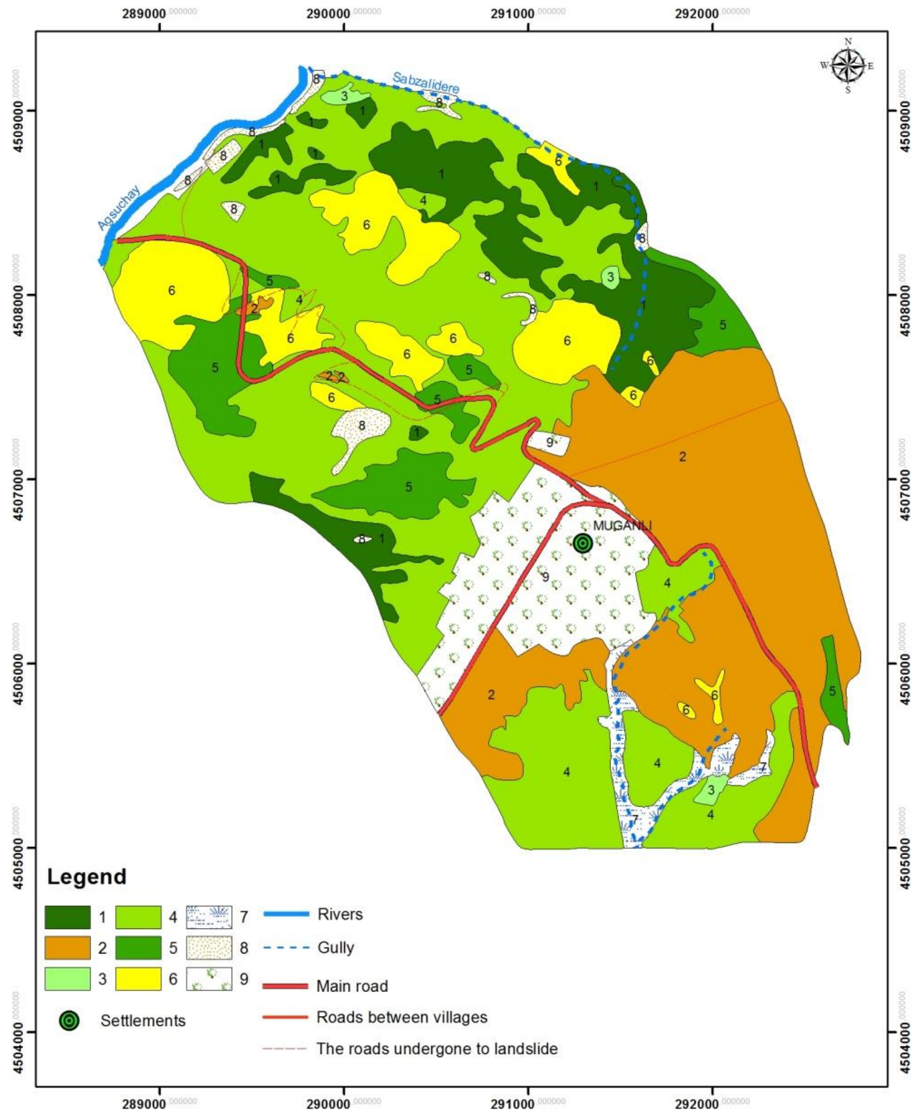

Figure 4. *The impact of the extent of vegetation cover on landslides.*
The legend of the map, reflecting the impact of vegetation cover on landslides includes: 1. Forests damaged due to anthropogenic impacts and composed of trees of lesser height (2-4 m) and low density such as Oriental beech (Fagus L), Georgian oak (Querkus L.), Caucasian hornbeam (Carpinus L.), elm (Ulmus L), hawthorn (Crataequs L.), medlar (Mespilus L.), etc. 2. Crops, like wheat, oats, barley, corn, grapes, etc. 3. Fruit gardens and perennially growing shrubberies. 4. Shrubberies and grasslands, composed of blackthorn (Paliurus spina Christi), buckthorn (rhamnus L), etc. 5. Forests and shrubberies, reduced in density due to anthropogenic impact. 6. Meadow- and steppe vegetation, composed of thorny plants (spiana, asantha, murex), cereals (Gramieak) and various mountain steppes (Valliherbetum). 7. Reedy plants; wetland meadows and pastures; sparse and low trees and bushes. 8. Sandy, rocky, stony areas and steep slopes without vegetation. 9. Courtyards, consisting of cultivated plants.

Assessment of landscape- and environmental risks and hazards caused by the landscape landslides

In order to assess landscape- and environmental risks and hazards, posed by landslides in the study area, the existing areals of landslides were compared with the maps of land use (I), extent of vegetation cover (II) and also landscape and environment (III) of large scale (1:10000). Based on this comparison and the above-mentioned formula, the intensity indexes of spread of active landslides through ArcGIS program were measured. This enabled compile the map of landscape-and environmental risks and hazards caused by landslides in the study area (*Fig. 5*).

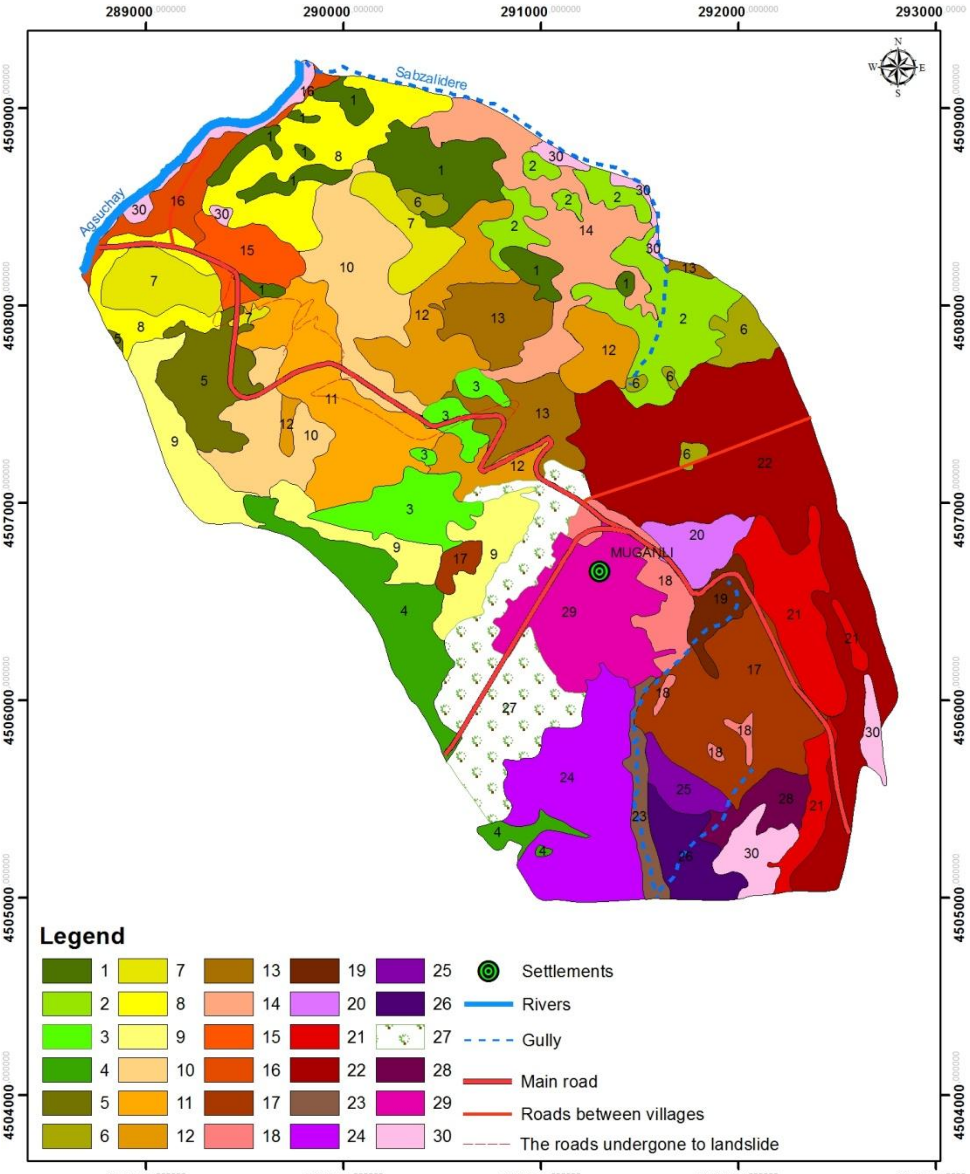

Figure 5. *Landscape and environmental risks and hazards caused by landslides. The legend of map of landscape- and environmental risks and hazards caused by landslides:*
I. Landscapes of mountainous forests in low mountain areas. 1. Secondary forests, composed of oak and hornbeam and widespread over brown forest soils of highly shattered slopes of medium hazard of sliding; and shrubs grown under forests (25º-35º of inclination). 2. Secondary sparse arid forests, composed of hornbeam, elm, oak, hawthorn, hips, buckthorn, etc. and spread over brown mountain soils on slopes, shattered at medium extent, with medium hazard of sliding (20º-25º of inclination). 3. Trees and shrubs like elm, hornbeam, hawthorn, blackthorn, etc. widespread on brown forest soils of slopes with wavy surface, shattered at medium extent, with very high hazard of sliding (25º-35º of inclination).
II. Landscapes of secondary forest and bush in low mountain areas: 4. Secondary meadows, bushes, pastures and hayfields, covering brown forest soils of extremely shattered and wavy south-western slopes with medium hazard of sliding (25º-35º of inclination). 5. Forest bushes, covering light brown and partially carbonate soils on hilly and highly shattered slopes of medium hazard of sliding (20º-35º of inclination).

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III. Landscapes of shrubby meadows and steppes in low mountain areas. 6. Meadow bushes, separate trees and pastures, spread over light brown, blackish and carbonate forest soils of slightly shattered south-western and north-western slopes of with high hazard of sliding (20°-25° of inclination). 7. Complexes of field and garden, composed of sparse blackthorn, blackberry, grasses etc. on slightly carbonate brown soils of smooth and weakly shattered watershed areas with less hazard of sliding (5-10° of inclination). 8. Cereals, clovers, sparse shrubs, growing over brown carbonate mountain forest soils of highly shattered and eroded steep slopes with very high hazard of sliding (30-35 of inclination). 9. Arid forests and shrubs of blackthorn, buckthorn, sparse oak, elm, etc. spread over dark-brown mountain forest soils of lower mountain slopes, shattered at medium extent; with high hazard of sliding. 10. Post-forest steppe plants, pastures and hayfields on brown forest soils of slopes, shattered at medium extent and affected by ancient landslides; with medium hazard of sliding. 11. Post-forest shrubberies, spread on mountain forest soils of western slopes; shattered at medium extent; with high hazard of sliding. 12. Mogpog, oat, various shrubs, etc., spread on brown mountain forest soils of weakly shattered slopes with medium hazard of sliding. 13. Hawthorn, blackberry, blackthorn and various kinds of grasses, covering slightly carbonate brown soils of averagely shattered mountain slopes, violated due to anthropogenic impact; with very high hazard of sliding. 14. Cereals and shrubberies, spread on brown and also dark chestnut soils of western and southwestern slopes with wavy surface shaped in post-forest areas and shattered considerably; with high hazard of sliding (25° of inclination). 15. Various grasses and sparse shrubs spread on brown soils of weakly shattered slopes of dry river valleys, with very high hazard of sliding. 16. Anthropogenically violated grassland shrubberies, spread on meadow soils of accumulative and weakly shattered river terraces, safe in terms of sliding. 17. Various grasses, cereals and partially vineyards, spread on dark-chestnut and blackish soils of weakly shattered, smooth and a little wavy watershed areas of lesser hazard of sliding. 18. Grassland and steppe vegetation, pastures and hayfields on brown- and meadow soils of eastern and southeastern slopes of medium hazard of sliding. 19. Post-forest mountain steppes covering brown- and meadow soils of valleys with waterlogged bottom and of high hazard of sliding. 20. Various herbs, clovers and bushes, spread on brown soils of low-incline and wavy mountain slopes of very high hazard of sliding. 21. Pastures, widespread in weakly shattered and less-incline smooth watersheds of low hazard of sliding. 22. Sparse shrubberies, pastures and hayfields, spread on brown mountain soils of slopes of high hazard of sliding (20-25° of inclination). 23. Vegetation, presented by poplar, elm, blackberry and etc. on brown meadow soils of dry river valleys. 24. Grasslands and steppes with sparse shrubberies covering eroded brown soils of ancient southern, southeastern and south-western slopes of very high hazard of sliding. 25. Various herbs, cereals and mostly pastures covering blackish and light chestnut soils of slopes of smooth watershed with less hazard of sliding. 26. Post-forest shrubberies, meadows and steppes, spread on brown mountain soils of weakly shattered watershed with smooth and less-incline surface (5-6°). 27. Orchards and vineyards covering brown forest soils in weakly fragmented southwestern watershed with smooth surface; with hazard of sliding at medium extent. 28. Reeds, shrubberies and tall grasses covering brown meadow soils of expanded dry valleys. 29. Sandy areas with poor soil cover in accumulative river valleys. 30. Landscapes of residential areas of low-incline and smooth watersheds of active hazard of sliding.

Results of environmental risks and hazards are grouped as reflected in *Table 3*. Analysis of this table shows that 55,9% of the territory is of high and very high hazard of sliding. Based on the map, natural complexes were grouped in 5 classes. 1. Landscapes of very high hazards. 2. Landscapes of high hazard. 3. Hazardous landscapes. 4. Landscapes of low hazard. 5. Landscapes of very low hazard.

As *Figure 3* reflects, the landscapes of high and very high hazard of sliding are covering landscape-and environmental facies, numbered as 3, 6, 8, 9, 11, 12, 15, 19, 20 and 30, the area of which makes up 55,9% the overall territory of the study area. These areals consist of mostly ellevial and delluvial sediments, clayey, allevrolit and limestone rocks, and encompass mainly the western, south-western, north-western and northern slopes. The vegetation coefficient is less than 1 and is represented by complexes of very rare forests and bushes, shrubberies and shrubby meadows, violated due to anthropogenic impact. These areas are used mostly as pastures and hayfields, and partially as planting areas, residential houses, courtyards and networks of highway and communication. The seismicity reaches 8-9 points. The implementation of serious measures against landslides is needed in this landscape-environmental facies. Namely, the grazing norm should be followed properly, whereas the planting of trees and shrubs adapted to local environmental conditions is needed as well. Beside with this, the regulation of surface waters, as well as the improvement of regime of underground waters through the establishment of vertical and horizontal system of drainage should be managed. Anthropogenic loading must comply with environmental norms.

29,1% of the study area is composed of the landscapes of medium hazard of sliding. As the analysis of environmental risks and hazards indicates, contours numbered as 1, 2, 5, 10, 12, 14, 18 and 27 (*Fig. 4*) are listed in this class. Slopes, typically of 10-15º and over of inclination in some cases are very complicated due to the influence of ancient landslides. Inclination of some slopes equates 5-6º. Rocks of high hazard of sliding like clay, shale, marl, sand, sandstone, lime are widely found. The vegetation cover coefficient of the area is about 1, while the flora is represented by derivative forests, consisting of oak, hornbeam and other trees, density of which is at low or medium extent, as well as woodland bushes and steppe xerophytes. The relief is vertically and horizontally is shattered at medium or high extent. This part of the study area is widespread especially on the south-east slopes and low-incline smoothed watershed, and is used in pasturing, planting, gardening, forestry, cutting, ground roads and motorways, etc. The above-mentioned measures are needed to reduce the risk of landslide.

Landscapes of low and much lesser hazard of sliding are small in territory (less than 15% of total study area). The map in *Fig. 4* shows that 8 contours (No. 7, 16, 17, 21, 23, 25, 28, 29) represent the areas of low or very low risk of hazard of sliding. Although the risk of landside is lessened due to the spread of low-incline slopes, the seismicity reaches up to 8 points, while rocks are of high hazard of sliding. In this regard, the area is vulnerable to erosion of river, ravine and gorges origin *(Table 3).*

Table 3. *Areas, measured based on the map of landscape- and environmental risks and hazards*

Landscape- and environmental types	Number of contours	Area, m^2	Area, %	Area, %	Intensity of landslides	Risk of landslide in points
1	9	403721,9626	121116,588	3,808719	0,3-0,5	3
2	5	447114,5066	134134,35	4,218084	0,3-0,5	3
3	4	275531,5717	220425,257	2,599369	0,8 <	5
4	3	366759,1383	293407,310	3,46001	0,8 <	5
5	2	228524,2451	685572,73	2,1559	0,3-0,5	3
6	5	128459,8277	770758,96	1,211891	0,6-0,8	4
7	3	294656,6776	88397,003	2,779795	0,1-0,3	2
8	2	502674,8198	402139,855	4,742241	0,8 <	5
9	3	458754,2391	275252,543	4,327893	0,6-0,8	4
10	2	600683,7308	180205,119	5,666858	0,3-0,5	3
11	1	460718,6534	276431,192	4,346426	0,6-0,8	4
12	4	512405,2509	307443,150	4,834038	0,6-0,8	4
13	3	379231,5856	303385,268	3,577676	0,8 <	5
14	1	425235,2653	255141,159	4,011675	0,6-0,8	4

15	1	150574,6216	1204596,97	1,420523	0,8 >	5
16	2	148934,4501	148934,45	1,40505	0,1	1
17	2	557686,7885	446149,43	5,261224	0,1-0,8	2
18	4	186174,8853	558524,65	1,756376	0,3-0,5	3
19	1	99553,24803	597319,488	0,939187	0,6-0,8	4
20	1	116724,6676	700348,00	1,101182	0,6-0,8	4
21	3	383304,8363	114991,450	3,616102	0,1-0,3	2
22	1	1202574,679	721544,80	11,34511	0,6-0,8	4
23	1	89312,68918	8931,268918	0,842577	0,1<	1
24	1	545600,6958	436480,55664	5,147204	0,8 <	5
25	1	80973,59801	24292,079	0,763906	0,1-0,3	2
26	1	149856,3331	44956,899	1,413746	0,1-0,3	2

Conclusion

1. The research model, used during this study will allow modelling and assess landscape- and environmental risks and hazards, posed by landslides in young seismic mountain areas of Azerbaijan as well as other young mountain geosystems, shaped during the Alpine folding period. The used model enables to carry out this research as well as similar works in a shorter time based on decipherment of satellite images.
2. In recent years, the study of the sensitivity of landslide areas was widely reported by various aspects in scientific works of researchers (Gritzer et al., 2005; Gupta et al., 1990; Kumtepe et al., 2011; Moreiras, 2005) from European countries, North America and Central America. These researchers have taken into account four or eight factors at best. Unlike these studies, our research proposes new relevant formula and research model. Beside with this, the landscape- and environmental balance of the area was studied. Over related 16 factors were involved in the study. As a result of this, the extent of accuracy and reliability, as well as the possibility of application of the carried out research were increased.
3. The obtained results allow to assess risks and hazards, posed by landslides in the Greater and Lesser Caucasus, as well as Talysh Mountains and mountain areas of Nakhchivan. These results can be used in the implementation of the future regional development programs, landscape planning and also the organization of transport infrastructure in the southeastern part of the Greater Caucasus.

REFERENCES

[1] Lee, S. (2005): Application of logistic recreation model and its validation for landslide susceptibility mapping using GIS and remote sensing data. - International Journal of remote sensing 26 (7): 1477-1991.

[2] Wang, B.L., Paudel, B., Li, H.Q. (2009): Retrogression characteristics of landslides in fine-grained permafrost soils, Mackenzie Valley, Canada. - Landslides 6(2):121–127.

[3] Mora, S., Yahrson, W. (1994): Macrozonation methodology for landslide hazard determination. - Bulletin of the association of engineering Geology 31(1): 49-58.

[4] Varner, J. D. (1984): Landslide hazard zonation: a review of principles and practice. - United Nations Educational Scientific and Cultural Organization (UNESCO) Press, Paris

[5] Griffiths, J. S., Mather, A. E., Hart, A. B. (2008): Landslide susceptibility in the Rio Aguas catchment. - Quarterly Journal of Engineering Geology and Hydrogeology 35: 9-17.

[6] Gritzer, L., Andrev, M., Appinal, R. (2005): Assessing landslide Potential Using GIS, Soil Wetness Modeling and Topographic Attributes, Poyette River, Idaho. - Geomorphology 37: 149-165.

[7] Gupta, R. P., Joshi, B. C. (1990): Landslide hazard zoning using the GIS approach; A case study from the Ramganga Catchment, Himalayas. - Engineering Geology 28:119-131.

[8] Kumtepe, P., Nurlu, J.,Cengiz, T., Sutcu, E. (2011): Heylan Diyarlılık haritalarının hazırlanmasında Cografi bilgi Sistemlerinin kullanımı. HKM Geodezi. - Geoinformasyon ve Arazi Yönetim Dergisi 3: 41-46.

[9] Moreiras, S. M. (2005): Landslide susceptibility zonation in the Rio Mendoza Valley, Argentina. - Geomorphology 66: 344-358.

[10] Parise, M. (2001): Landslide mapping techniques and their use in the assessment of the landslide hazard. - Physics and chemistry of the Earth 26(9): 698-703.

[11] Siagian, J. O., Nitihargo, S., Sugalang and Hermovan (2000): Landslide susceptibility Analysis using remote sensing and GIS of the south Cehanjir area, West Java, Indonesia. - Report of International research and Development Cooperation ITT project, 11-28, Japan: 114-121.

[12] Uromeiny, A., Mahdavifar, M. R. (2000): Landslide hazard zonation of the Khorshrostam area, Iran. - The bulletin of Engineering geology and the Environment 58: 207-213.

[13] Saha, A. K., Gupta, R. P., Arora, M. K. (2002): GIS-based landslide hazard zonation in the Bhagirathi (Ganga) Valley, Himalayas. - International Journal of remote sensing 23(2): 357-369.

[14] Carrura, A., Cardinaly, M., Guzetty, F., Reichenbach, P. (1995): GIS technology in mapping landslide hazarde. - GIS system in assessing natural hazards 12: 135-175.

[15] Shulz, W. H. (2007): Landslide susceptibility revealed by LIDAR imagery and historical records, Seattle, Washington. - Engineering Geology 89: 67-87.

[16] Shuster, R. L., Fleming, R.W. (1986): Economic losses and fatalities due to landslides. - Environmental & Engineering Geoscience 23(1): 11-28.

[17] Yalchin, A. (2008): GIS- based landslide susceptibility mapping using analytical hierarchy process and bivariate statistic in Ardesen (Turkey). - Catena 72: 1-12.

[18] Guzrtti, F., Reichenbach, P., Cardinally, M., Gally, M., Ardizzone, F. (2005): Probabilistic landslide hazard assessment at the basin scale. - Geomorphology 72: 272-299.

[19] Dai, F. C., Lee, C. F., Li, J., Xu, Z. W. (2001): Assessment of landslide susceptibility on the natural terrain of Lantauisland, Hong Kong. - Environmental Geology 43(3):381-390.

[20] Cevik, E., Topal, T. (2003): GIS-based landslide susceptibility mapping for a problematic segment of the natural gas pipeline, Hendek, Turkey. - Environmental Geology 44: 949-962.

[21] Ismayilov, M. J., Mustafayev, N. M. (2012): Features of the landslide development on the southeastern slope of Greater Caucasus. - Transactions of Azerbaijan Geographical Society 12: 95-103.

[22] Khazaradze, G., Klotz, J. (2003): Short and long-term effect GPS-measured crustal deformation rates along the South-Central Andes. - Journal of Geographical research 108(134): 1-13.

[23] Akgun, A., Bulut, F. (2007): GIS based landslide susceptibility for Arsin-Yomra (Trabzon, North Turkey) region. - Environ. Geology 51: 1377-1387.

[24] Ayalev, L., Yamagichi, H. (2005): The application of GIS-based logistic regression for landslide susceptibility mapping the Kakuda-Jahikomountains, Central Japan. - Geomorphology 65: 15-31.

[25] Oh, H. J., Pradhan, B. (2011): Application of neuro-fuzzy model to landslide-susceptibility mapping for shallow-landslides in a tropical hilly area. - Comput. Geosci-UK 37(9):1264-1276.

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EFFECT OF WATER DEFICIENCY ON PHYSIOLOGICAL AND CHEMICAL PROPERTIES OF PEPPER GROWN IN GREENHOUSE

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Abstract. The cultivar Jalapeno pepper (*Capsicum annuum var.* annuum) was used in this research. The experimental design was split plot with five replications and four water restriction levels (control, 0%, 25%, 50%) were used. The research was made in a cold plastic greenhouse at Namik Kemal University, Agricultural Faculty, Department of Horticulture. After planting, plants were grown in normal growing conditions (hoeing, watering and fertilization) for a month and water restriction treatments were applied from the second month onwards. The plants development and other parameters were recorded. During the experiment leaf water potential (MPa), leaf relative water content (%), membrane damage index (%), total chlorophyll content (SPAD) and leaf temperature (°C) were measured. Artificial draught stresses made by water restrictions affected the growth and development of plants badly. The normal growth was observed in control (100% water). The plants watered with 50% were affected less than 25% and 0% water treatments. The treatment (25% water) gave noticeable stress symptoms, leaf wilting, reduced growth and development and yield reduction. The treatment (0% water) caused reduced growth, small leaf, wilting, drying, reduced yield and gradually the death of plants. As a result, leaf water potential, leaf relative water content and total chlorophyll content were the highest in 100% water treatment. The lowest leaf damage, membrane damage and leaf temperature were determined in 100% water treatment as well.

Keywords: *water stress, leaf water potential, relative water content, total chlorophyll content, membrane damage index*

Introduction

The problem of water deficit is becoming more serious and United Nation Organization is ringing the bell of danger from the looming crisis of drought. Water is one of the principle environmental limiting factors for crop production and distribution throughout the world. Water deficit, which is a consequence of either intermittent or terminal period of drought, causes significant yield reduction on presently cultivated lands. It was found that in fertile soil, plant growth and yield are reduced more often by water deficits than any other cause. These restrictions on yield potential are of great concern in terms of meeting food demand for accelerated increasing world population (Mohd et al., 2004).

In order to produce sufficient food for the world's growing population, control of these problems, increasing productivity of water use in agriculture and also finding new ways to utilize the extensive sodic and saline soils and water resources are vital and urgent. (Pessarakli and Szabolcs, 1999).

Especially drought stress during early growth stages might result in reduced plant number of blossoms and fruits (Rylski and Spigelman, 1982). However, despite that water management in pepper is extremely important at all stages of plant development

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due to its influence on stand establishment, fruit set and quality, there are very limited data on mechanisms of drought avoidance or defence in pepper (Sziderics et al., 2010)

Pepper (*Capsicum spp.*) is a member of the Solanaceae family (Parson et al. 2013). Capsicum annuum is one of the most diverse species among the Solanaceae, and continues to be the primary subject of selection to this day, mainly focused on increasing fruit yield and quality (Paran and Van der Knaap, 2007).

Pepper cultivation is confined to warm and semi-arid countries where water is often a limiting factor for production. This necessitates optimisation of water management (Dorji et al., 2005). Pepper is cultivated nearly all over the world and 29601 Mt produced from 1837704 ha area in 2011. Turkey is the third of largest pepper producer (1975 Mt) after China (15520 Mt) and Mexico (2131 Mt) (Unlukara et al., 2015).

The motherland of Jalapeno pepper which has an important place in global industrial pepper production is Mexico and it is not prevalently cultivated in Turkey (Oguz et al., 2012).

Jalapeno cultivation is especially made in moist areas. However there is not enough information about its cultivation in highlands and especially in dry areas. For this reason cultivation of this species in dry areas and determination of its resistance against drought will be very useful for cultivators.

In our research the aim was to determine the physiological and chemical changes occurring in pepper due to various water deficits.

Materials And Methods

Plant material and experimental design

The research was carried out in the polyethylene greenhouse condition of the Horticulture Department, Faculty of Agriculture, Namik Kemal University, Tekirdag, Turkey (40°59' N, 27°29' E and 4 m altitude) in the summer season of 2012.

The experiment was conducted on clayey loam soil. Some of the physical and chemical properties of soil determined in the laboratory are given in *Tables 1* and *2*.

In this study, Mexican origin Jalapeno species pepper (Lee et al., 2006) which is grown was used as material (*Capsicum annuum* var. *Annuum*). The seedlings were transplanted into the greenhouse on the 30th May 2012. Some of the climate data results of experiment area are shown in *Table 3*. Seedlings were planted with 100x40 cm distance in greenhouse borders. It was grown in normal maintenance and watering conditions (Crosby, 2008). The plants were irrigated through pressure compensated drip irrigation system. Dripper spacing was 40 cm and having 2 Lh^{-1}.

The plants, normal water requirements of which was fulfilled by drip irrigation until flowering period (first month), were later subject to artificial drought stress. Criteria measurements initiated starting from flowering.

The experiment was carried out according to split plot design with 5 replications and 4 different irrigation applications (100% irrigation (control), 50% irrigation, 25% irrigation and 0% irrigation). In all the experiments; 20 parcels in total and 20 plants for each parcel were used. In order to find out differences between treatment, LSD multiple comparison test was made using the SPSS 18.0 for Windows statistical software (SPSS Inc., Chicago, USA) and the difference degrees were stated at 1% probability. No analysis of variance was made for leaf water potential and measurement of leaf temperature. The results were given as observations and for additional information.

Four different irrigation programs were tested in polyethylene greenhouse:

- *100% irrigation (control)*: Irrigation was applied in 7 days intervals starting from flowering period (2nd July 2012). Climate data of Tekirdag city were examined for long years and the average of the sum of 7 days long evaporation was taken as a basis in determination of irrigation water amounts.
- *50% irrigation*: 50% of the control application.
- *25% irrigation*: 25% of the control application.
- *0% irrigation*: No irrigation was applied.

Table 1. Some physical properties of soil*

Soil depth (cm)	Texture class	EC (dS m^{-1})	Field capacity		Wilting point		Bulk density (gcm^{-3})	Usable water retention capacity (mm)
			%	mm	%	mm		
0-30	CL	0.6	28	157.1	16	89.8	1.87	67.2
30-60	SCL	0.5	26	134.9	17	88.2	1.73	46.7
60-90	SC	0.4	27	137.7	17	86.7	1.70	51.0

**Source: Agricultural soil analysis report of T.R. Tekirdag commercial exchange*

Table 2. *Some chemical properties of soil**

Parameter	Unit	Result	Method
pH		7.64	Saturation
Salt	(%)	0.06	Saturation
Lime	(%)	2.46	Calcimetry
Structure	(%)	59	Saturation
Organic matter	(%)	1.06	Walkey-Black
Total nitrogen (N)	(%)	0.05	Kjeldahl
Phosphorus (P)	(ppm)	73.9	Olsen-ICP
Potassium (K)	(ppm)	290.36	A. acetate-ICP
Calcium (Ca)	(ppm)	5.194.97	A. acetate-ICP
Magnesium (Mg)	(ppm)	432.07	A. acetate-ICP
Iron (Fe)	(ppm)	8.05	DTPA-ICP
Copper (Cu)	(ppm)	1.45	DTPA-ICP
Zinc (Zn)	(ppm)	1.33	DTPA-ICP
Manganese (Mn)	(ppm)	4.05	DTPA-ICP

**Source: Agricultural soil analysis report of T.R. Tekirdag commercial exchange.*

Table 3. *2012 climate data of experiment area**

Months	Average temperature (°C)	Max. temperature (°C)	Min. temperature (°C)	Average relative humidity (%)
May	20.1	27.2	10.1	91.2
June	26.2	31.4	19.1	78.2
July	30.4	36.7	24.4	68.7
August	29.0	35.0	23.7	62.7
September	24.6	29.6	16.8	73.6

**Source: Tekirdag directorate of meteorology (2012)*

Leaf water potential measurement (MPa)

Leaf water potential was measured by Scholander Pressure Chamber. The measurements were made 2 hours before (Ψ_{pd}: Pre-dawn leaf water potential) and 6 hours after (Ψ_{md}: Midday leaf water potential) sun rise respectively. The measurements were made on most developed leaves of plant (Scholander et al., 1965). The measurements were repeated with 1 week intervals after flowering.

Leaf relative water content (RWC) (%)

In trials related with resistance to drought. Studies of researchers working on various plants about leaf relative water content (RWC) (Sanchez et al., 2004). At the end of stress fresh weights of the plants (FW) were taken in order to determine the relative water contents. After that the leaves were kept in pure water for 4 hours and during this period their turgid weights (TUW) were calculated. The weight samples weights of which were determined were dried in stove at 65 °C for 48 hours and then dry weight (DW) was measured in grams. The ratios of obtained fresh and dry weights were calculated by below Equation (1) in order to find leaf relative water contents (%).

$$\mathrm{RCW} = \frac{100\,(\mathrm{FW-DW})}{\mathrm{TW-DW}} \qquad \text{(Eq.1)}$$

Membrane damage index (%)

Membrane damage index (MDI) was calculated by measuring the electrolyte released from the cell (Fan and Blake. 1994). In each vegetation period disks with diameter of 17 mm were taken from leaves of stress and control plants were kept in ionized water for 5 hours and then their electricity conductivities (EC) were measured. Same disks were kept in autoclave at 100 °C for 10 minutes and then the EC value of the solution was measured again. From the obtained value the membrane damage in leaf cells (%) was calculated with the help of the below equation (2).

$$\mathrm{MDI} = \frac{100(\mathrm{Lt-Lc})}{1-\mathrm{Lc}} \qquad \text{(Eq.2)}$$

Lt: EC before autoclaving / EC after autoclaving of the leaf which is under drought stress
Lc: EC before autoclaving / EC after autoclaving of the control leaf.

Determination of total chlorophyll content (SPAD)

In the research the total chlorophyll content of the pepper leaves was measured by “Konica Minolta SPAD-502” portable chlorophyll-meter. In each period same readings was made from two regions of the leaf (close to midrib) and from five plants in each parcel (Geravandi et al., 2011).

Measurement of Leaf Temperature (°C)

Infrared thermometer technique which is based on measuring plant surface temperature allows faster and more precise measurement without touching the plant. The mentioned technique is based on transpiration which decreases the leaf surface temperature. In the measurements infrared thermometer having filters which detects rays at a wavelength of 7-18 nm (IRT) (Raynger ST8 model) were used (Erdem et al., 2010).

Results And Discussion

Leaf water potential (MPa)

In leaf water potential examination the effect of different water deficits on pepper at pre-dawn (Ψ_{pd}) and in the midday (Ψ_{md}) leaf water potential were shown in *Table 4* and *Figure 1*. The measurements were made in 6 different dates and significant differences occurred between 0% application and control applications in both pre-dawn and midday measurements. At the same time as vegetation periods advanced plants could not get over the stress.

One week after starting first water restriction application, leaf water potential measurements were initiated. After this period each week measurements were made in pre-dawn and in the midday. As harvest period was long, measurements were continued during fruiting period. First measurements were made one week after water restriction and the measurements were -1.20 MPa in 0% and -0.25 MPa in 100% application pre-dawn while the measurements were -2.20 MPa in 0% and -0.90 MPa in 100% application in the midday. Kaya and Dasgan (2013) stated that the mean and % of in plants increased compared to control as a result of salt and drought stress.

Leaf relative water content (%)

The effects of different water restrictions on leaf relative water content can be observed in *Table 5*. There is a direct proportion between irrigation increase applied to plants and leaf proportional water content and greatest decrease in leaf proportional water content was found to be 43.4% in 0% application. Due to increase in irrigation ratios of 55.6% was reached in 25% application and 65.2% in 50% application. Highest increase was observed in the control plant in which 100% irrigation was made (95.2%). Witting occurred in leaves with 0% and 25% applications due to low leaf proportional water content.

As the osmotic pressure increases in salty soils the water intake of the plant becomes more difficult and accordingly the leaf proportional water content also decreases (Topaloglu, 2010).

Table 4. *Effect of different water deficiency levels on average pre-dawn (ψpd) and midday (ψmd) leaf water potentials* (MPa)*

Leaf water potentials	**Water deficiency**	**The number of weeks after flowering**					
		1 (27.07.2012)	**2** (04.08.2012)	**3** (11.08.2012)	**4** (18.08.2012)	**5** (25.08.2012)	**6** (01.09.2012)
Pre-dawn leaf water potential (Ψ_{pd})	0%	-1.20	-1.35	-1.65	-1.75	-1.88	-2.05
	25%	-0.80	-0.95	-1.38	-1.42	-1.48	-1.54
	50%	-0.35	-0.40	-0.55	-0.74	-0.85	-1.05
	100% (control)	-0.25	-0.30	-0.36	-0.43	-0.55	-0.63
Midday leaf water potential (Ψ_{md})	0%	-2.20	-2.35	-2.46	-2.53	-2.62	-2.85
	25%	-1.71	-1.90	-1.99	-2.15	-2.27	-2.40
	50%	-0.98	-1.04	-1.26	-1.36	-1.42	-1.45
	100% (control)	-0.90	-0.94	-1.02	-1.06	-1.15	-1.31

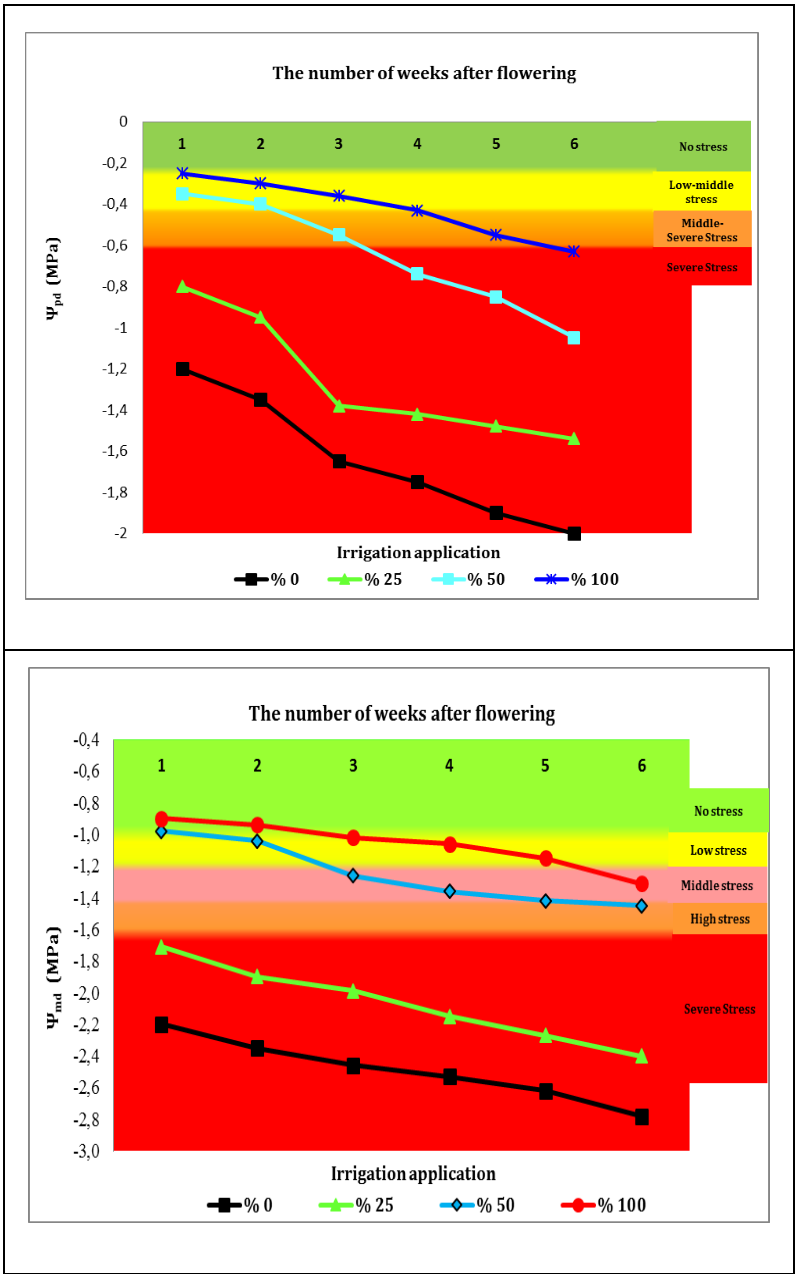

Figure 1. *Effect of different water deficiency levels on average pre-dawn (ψ_{pd}) and midday (ψ_{md}) leaf water potentials (MPa).*

Membrane damage index (%)

According to the measurements made and statistical analysis results the membrane damage occurred in Jalapeno pepper leaves are shown in *Table 5*. According to statistical results in terms of membrane damage in leaf cells the effect of irrigation applications remained within the 1% statistical error limits.

The values in *Table 5* varies between 6.9% and 67.3% and lowest value was in control application (6.9%) and highest value in no irrigation (67.3%). 58.4% value was obtained from 25% application while 47% value was obtained from 50% application. Based on this result we can say that there is an inverse relation between increase of

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irrigation ratio and membrane damage. Stress was very obvious in no irrigation, many vital functions stopped and this situation resulted with the death of the plant. As a result of the damage occurred in the call membranes of the plants which were subject to stress, it was emphasized that the materials dissolved in water in the cell leaked to the gaps between cells and accordingly the tissue electrical conductivity increased. In other words it was determined that there is an inverse relation between tissue electrical conductivity and membrane unity (Houimli et al., 2010).

Total chlorophyll content (SPAD)

Averages of total chlorophyll amount according to water restriction in drought stress were measured as shown in *Table 5* the chlorophyll amount averages remained within 1% statistical error limits among all applications as seen in *Table 5*.

When we emphasize the effect of water stress applications in terms of total chlorophyll amount it was observed that averages varied between 46.8 and 72.1 the lowest chlorophyll amount average was measured in 0% application (46.8) and the from control application (72.1). While total chlorophyll average was measured as 59.8 in 25% application this average was 67.4 in 50% applications. It was determined that chlorophyll amount decreased by increasing water stress.

Oliveira Neto et al., (2009), stated that drought stress negatively affected chlorophyll content and that photosynthetic pigments were damaged as a result of drought stress and chlorophyll decreased in all plants. Mishra et al., (2002) proved (by chlorophyll level) in a study they conducted in tomato that tolerant species decreased the negative effects of water restriction.

Table 5. *Effect of different water deficiency levels on average leaf relative water content (%). membrane damage index (%) and total chlorophyll content (SPAD) and LSD groups**

	0%	**25%**	**50%**	**100% (control)**
Leaf relative water content[1] (%)	43.41 d	55.63 c	65.28 b	95.24 a
Membrane damage index[2] (%)	67.31 a	58.45 b	47.02 c	6.92 d
Total chlorophyll content[3] (SPAD)	46.88 d	59.86 c	67.46 b	72.10 a

**There is no difference in the level of 0.01 among averages that have the same letter ($p<0.01$).*
$LSD^1 = 2.421385$, $LSD^2 = 3.233776$, $LSD^3 = 3.30512$

Measurement of Leaf Temperature (°C)

Mean leaf temperature (°C) was measured in pre-dawn and midday by infrared thermometer before each irrigation application without touching the leaves from beginning of water limitation till harvest period were shown in *Table 6* and *Figure 2*.

As seen in *Table 6* while highest value in leaf temperatures were measured in 0% application both in sun rise and middle of the day measurements, lowest value was measured in both periods in control application.

In measurements made in 6 different dates in pre-dawn and midday, control application leaf temperatures had values lower than the inner temperature of greenhouse while in other applications where water limitation was applied, temperature was measured to be higher than inner greenhouse temperature. This is an indication that plants are affected by stress. When we look at the drought and salinity study values of Suyum (2011) in water melon, it was stated that leaf temperature increases in salty and drought conditions.

Table 6. *Effect of different water deficiency on pre-dawn and midday leaf temperature (°C) of pepper plant*

		The number of weeks after flowering					
	Water deficiency	**1** (27.07.2012)	**2** (04.08.2012)	**3** (11.08.2012)	**4** (18.08.2012)	**5** (25.08.2012)	**6** (01.09.2012)
Pre-dawn leaf temperature (°C)	0%	27.4	25.4	26.9	24.1	21.1	23.3
	25%	27.1	25	26.3	23.5	20.1	22.9
	50%	26.9	24.9	26.0	22.8	19.6	21.8
	100%	25.8	23.2	24.4	21.9	18.7	20.6
	Greenhouse temp.	26.3	24.2	25.2	22.5	19.6	21.2
Midday leaf temperature (°C)	0%	33.9	34.2	36.8	34.0	34.5	30.3
	25%	33.4	33.6	36.0	33.1	31.3	28.9
	50%	33.2	34.0	35.3	32.2	30.3	28.1
	100%	32.7	32.9	34.4	31.1	28.8	26.8
	Greenhouse temp.	33.1	33.8	35.0	31.8	29.8	27.6

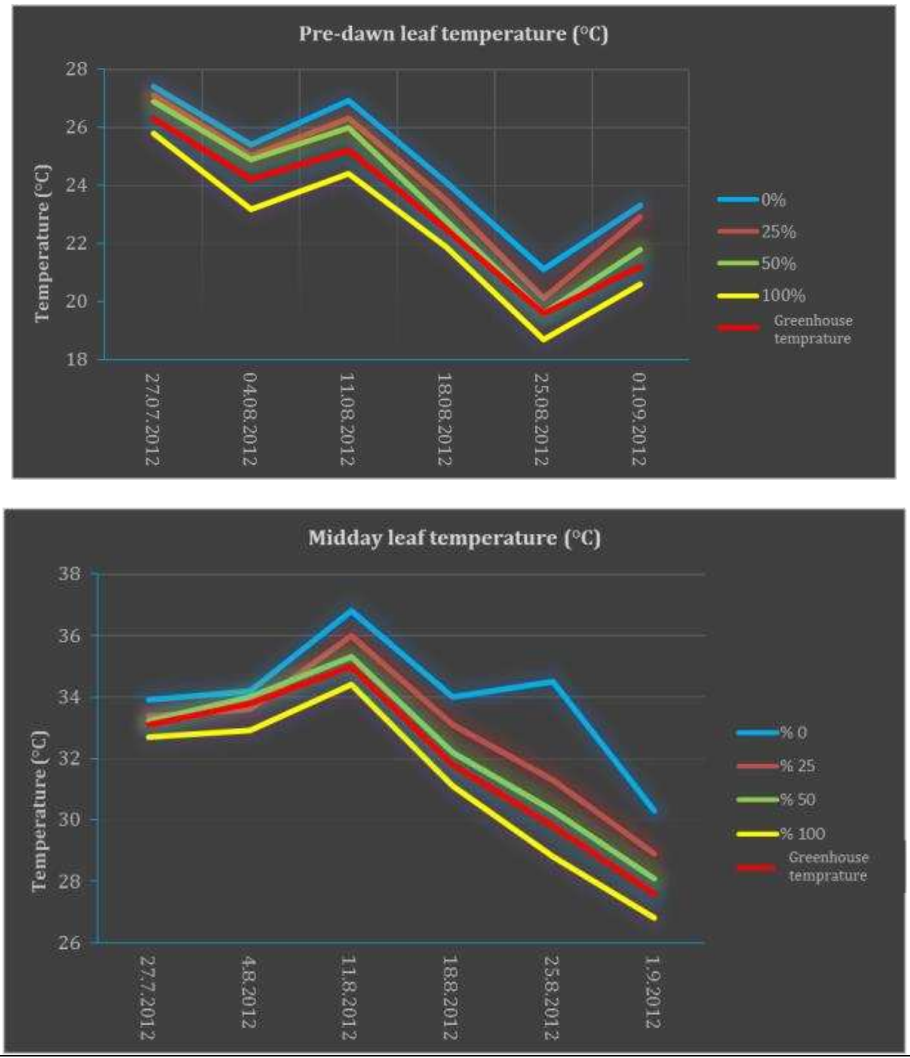

Figure 2. *Effect of different water deficiency on pre-dawn and midday leaf temperature (°C) of pepper plant.*

Conclusions

As a result, in case of water restriction averages decreased in criteria such as leaf proportional water content, total chlorophyll, leaf water potential, while in criteria such as membrane damage index and leaf temperature, the averages increased. In pepper cultivation in order to prevent yield and quality losses, the following results should be provided as in 100% irrigations:

- Pre-dawn leaf water potential should not decrease below -0.6 MPa after flowering and also.
- It should not decrease below -0.25 MPa in the first 15 days after flowering.
- It should not decrease below -0.40 MPa during harvest and maintain this value.
- Midday leaf water potential should not generally decrease below -1.6Mpa.
- It should not decrease below -1MPa in the first 15 days after flowering.
- It should remain over -1.3MPa during harvest.
- RWC should not decrease below 95%.
- Leaf temperatures should not exceed 35°C and these criteria should practically be taken into consideration in the irrigation activities to be conducted.

REFERENCES

[1] Crosby, K. M. (2008): Pepper vegetables II, pp.221–248.

[2] Dorji, K., Behboudian, M. H., Zegbe-Domínguez, J. A. (2005): Water relations, growth, yield, and fruit quality of hot pepper under deficit irrigation and partial rootzone drying. - Scientia Horticulturae 104 (2): 137–149.

[3] Erdem, Y., Arin, L., Erdem, T., Polat, S., Deveci, M., Okursoy, H., Gultas, H. T. (2010): Crop water stress index for assessing irrigation scheduling of drip irrigated broccoli (*Brassica oleracea* L. var. *italica*). - Agricultural Water Management 98 (1): 148–156.

[4] Fan, S., Blake, T.J. (1994): Abscisic acid induced electrolyte leakage in woody species with contrasting ecological requirements. - Physiologia Plantarum 90 (2): 414–419.

[5] Geravandi, M., Farshadfar, E., Kahrizi, D. (2011): Evaluation of some physiological traits as indicators of drought tolerance in bread wheat genotypes. - Russian Journal of Plant Physiology 58 (1): 69–75.

[6] Houimli, S. I. M., Denden, M., Mouhandes, B. D. (2010): Effects of 24-epibrassinolide on growth, chlorophyll, electrolyte leakage and proline by pepper plants under NaCl-stress. - EJOBIOS, pp. 96–104.

[7] Kaya, E., Dasgan, H. Y. (2013): Screening of the bean genotypes for their tolerance to salinity and drought stresses at the early plant growth phase. - Cukurova University, Journal of Science and Engineering 29 (2): 39-48.

[8] Lee, J.-H., Kiyota, N., Ikeda, T., Nohara, T. (2006): Acyclic diterpene glycosides, capsianosides VIII, IX, X, XIII, XV and XVI from the fruits of paprika *Capsicum annuum* L. var. *grossum* bailey and jalapeño *Capsicum annuum* L. var. *annuum*. - Chem. Pharm. Bull. 54 (10): 1365–1369.

[9] Mishra, S. K. (2002): In the complex family of heat stress transcription factors, HsfA1 has a unique role as master regulator of thermo tolerance in tomato. - Genes & Development 16 (12): 1555–1567.

[10] Mohd, R. I., Mohd, K. Y., Marziah, M. (2004): Growth, water relations, stomatal conductance and proline concentration in water stressed banana (Musa Spp.) plants. - Asian Journal of Plant Sciences 3 (6): 709–713.

[11] Oguz, I., Noyan, O. F., Karaman, M. R., Kocyigit, R., Ozen, M. (2012): The effect of different organic and inorganic materials on soil properties and crop yield in jalapeno pepper farming. - University of Sakarya, Journal of Science Literary (1): 393-403.

[12] Oliveira Neto, C. F., Silva Lobato, A. K., Goncalves-Vidigal, M. C., Lobo da Costa, R. C., Santos Filho, B. G., Ruffeil Alves, G. A., Mello e Silva Maia, W. J., Rodrigues Cruz, F. J., Borges Veves, H. K., Santos Lopes, M. J. (2009): Carbon compounds and chlorophyll contents in sorghum submitted to water deficit during three growth stages. - Journal of Food, Agriculture & Environment 7 (3-4): 588- 593.

[13] Paran, I. and Van der Knaap, E. (2007). Genetic and molecular regulation of fruit and plant domestication traits in tomato and pepper. - Journal of Experimental Botany 58 (14), 3841–3852.

[14] Pessarakli, M., Szabolcs, I. (1999): Soil salinity and sodicity as particular plant/crop stress factors. - Handbook of Plant and Crop Stress, Second Edition, pp.1–15.

[15] Rylski, I., Spigelman, M. (1982): Effects of different diurnal temperature combinations on fruit set of sweet pepper. - Scientia Horticulturae 17 (2): 101–106.

[16] Sánchez, F., de Andrés, E., Tenorio, J., Ayerbe, L. (2004): Growth of epicotyls, turgor maintenance and osmotic adjustment in pea plants (*Pisum sativum* L.) subjected to water stress. - Field Crops Research 86 (1): 81–90.

[17] Scholander, P. F., Bradstreet, E. D., Hemmingsen, E. A., Hammel, H. T. (1965): Sap pressure in vascular plants: negative hydrostatic pressure can be measured in plants. - Science 148 (3668): 339–346.

[18] Suyum, K. (2011): Determination of tolerance level of watermelon genetic resources to salinity and drought.MSc Thesis. 145 pages. - Cukurova University Institute of Natural and Applied Sciences Department of Horticulture, Adana, Turkey.

[19] Sziderics, A. H., Oufir, M., Trognitz, F., Kopecky, D., Matušíková, I., Hausman, J.-F., Wilhelm, E. (2010): Organ-specific defence strategies of pepper (*Capsicum annuum* L.) during early phase of water deficit. - Plant Cell Reports 29(3): 295–305.

[20] Topaloglu, K., (2010): Changes in pigments and capsaicinoids of chile peppers under salt stress and relation with peroxidase activity. MSc Thesis. 131 pages. - Cukurova University Institute of Natural and Applied Sciences Department of Horticulture, Adana, Turkey.

[21] Unlukara, A., Kurunc, A., Cemek,. B. (2015): Green long pepper growth under different saline and water regime conditions and usability of water consumption in plant salt tolerance. - Journal of Agricultural Sciences 21: 167- 176.

EFFECTS OF ELEVATED CO_2 ON ABOVEGROUND GROWTH IN SEEDLINGS OF FOUR DOMINANT *QUERCUS* SPECIES

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Abstract. Seedlings of four *Quercus* species dominant in Korea (*Quercus mongolica*, *Quercus serrata*, *Quercus acutissima*, and *Quercus variabilis*) were grown at different CO_2 levels (ambient; 380 ppmv and enriched; 800 ppmv) to determine growth responses under elevated CO_2. Three weeks after germination, seedlings were transplanted into chambers and grown over a period of 105 days. Aboveground plant parts were harvested at the end of the experiment to measure dry weight, leaf area, specific leaf area (SLA), leaf area ratio (LAR), leaf weight ratio (LWR), and leaf quality (carbon, nitrogen). *Q. mongolica* exhibited increased growth, *Q. serrata* and *Q. acutissima* did not respond to enrichment, and the growth of *Q. variabilis* was diminished with elevated CO_2. Total aboveground biomass of each seedling increased by 31% for *Q. mongolica*, but for *Q. variabilis*, it decreased significantly by 39% under CO_2-enriched conditions ($p < 0.05$). SLA and LAR decreased, and LWR was unchanged or decreased slightly in the elevated CO_2 treatment for all species, with the exception of *Q. acutissima*. In addition, the elevated CO_2 treatment was correlated with a decrease in total N concentrations, and an increase in the C/N ratio of the leaves of *Q. mongolica* and *Q. acutissima*. In conclusion, plant growth responses to elevated CO_2 were species-specific, and they showed large interspecific variation.

Keywords: *CO_2 enrichment, Quercus, tree growth, nitrogen, C/N ratio*

Introduction

Since the industrial revolution, anthropogenic activities, such as fossil fuel use and deforestation, have caused a dramatic increase in the atmospheric CO_2 concentration. As a result, global climate change is accelerating and is a major concern worldwide.

In the preindustrial age, atmospheric CO_2 concentrations were approximately 280 ppm, increasing over time to reach the current level of 400 ppm (IPCC, 2014). This increase in atmospheric CO_2 is likely to continue due to anthropogenic activities and is expected to reach about 700 ppm by the end of the 21st century (Houghton et al., 2001; IPCC, 2007). As a result, extreme weather, desertification expansion, melting polar ice, rising sea levels, ecosystem destruction, and reduced species diversity are rapidly progressing, and the main cause is the increasing atmospheric CO_2 concentration (IPCC, 2007). According to IPCC Fourth Assessment Report, continuous greenhouse gas emissions at or above the current rates will cause further warming and serious changes in the global climate system in the 21st century.

Natural terrestrial and ocean ecosystems are absorbing more than half of the total anthropogenic CO_2 emissions (Tans et al., 1990; Fan et al., 1998; Canadell et al., 2007; Lal, 2008). Among them, the forest ecosystem is a major C sink in terrestrial ecosystems (Ceulemans et al., 1999), stores more than 50% of total terrestrial C, and

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has a high potential for sequestration of atmospheric CO_2 (Dixon et al., 1994; Lorenz and Lal, 2010). In particular, temperate forests cover only 8% of the global land surface, but are known to account for about 40% of total terrestrial C uptake and play an important role for C sequestration of atmospheric CO_2 (Martin et al., 2001; Wamelink et al., 2009; Tyrrell et al., 2012). Many studies have focused on aboveground tree responses and have shown that increasing concentrations of atmospheric CO_2 may lead to changes in forest ecosystem structure and function through the direct effects of elevated CO_2 on tree physiology, development, and growth (Bazzaz, 1990; Jarvis, 1998; Ceulemans, 1999).

Forest comprise 64% of the total land area in Korea (Korea Forest Service, 2012), and tree species in the genus *Quercus* are dominant (Yim, 1977; Yim, 1995; Yang, 2001), covering about 27% of total forested area (Sim and Han, 2003). However, the effects of elevated CO_2 on the growth of dominant tree species and, relatedly, understanding C sequestration by terrestrial forest ecosystems are still not sufficiently studied in Korea. Accordingly, this study selected dominant deciduous tree species that occupied large areas and seem to be migrating to northern latitudes in response to warming caused by elevated CO_2 on the Korean peninsula. The seeds of *Q. mongolica, Q. variabilis, Q. serrata,* and *Q. acutissima* were germinated, and the seedlings were raised in ambient (380 ppm) and elevated CO_2 conditions (800 ppm). We harvested the plant at leaf falling season and analysed plant yield, growth parameters, and C and N concentrations in growing leaves because the C/N ratio is a measure of litter quality and is correlated with decomposition rates.

Materials and Methods

Study species

Quercus tree species are common on the Korea peninsula and distributed in most areas of the country, including the foothills and mountainous areas (Yim, 1977; Kim, 1990; Yim, 1995; Yang, 2001). Yang (2001) estimated that *Q. mongolica* occupied about 10% of total forested area, and the four species of oaks, *Q. mongolica, Q. variabilis, Q. serrata, and Q. acutissima*, occupied 72% of total deciduous forest area in South Korea. These oaks also determine ecosystem structure and functioning in temperate deciduous forest ecosystems on the Korean peninsula, and are situated in areas impacted by global warming caused by elevated CO_2.

Experimental design and treatments

The experiment was carried out in two growth chambers (2.4 m length × 1.2 m width × 1 m height) that controlled temperature, soil moisture, and CO_2 concentration in a green house. The control chamber was maintained at ambient CO_2 levels (380 ppmv) and the elevated CO_2 chamber was treated at twice the concentration of ambient CO_2 levels (approximately 800 ppmv). The CO_2 concentration in the chambers was regulated and monitored by an infrared gas analyzer (LI-840, LI-COR), and the plants were grown from April to September. Air temperatures in the chambers were maintained near ambient temperatures at 25 ± 3.1°C during the day and 19 ± 1.6°C during night. Seeds of the study species were provided by the Korean Forest Research Institute.

Seeds of the four species were sown in plastic trays and germinated at 25°C in an incubator. Three weeks after germination, seedlings of each species were transplanted

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into a pot (1.2 m length × 0.6 m width × 0.3 m height) with an artificial soil mixture of peat moss and vermiculite (2:1, v/v). Transplanted seedlings were uniform in size; those that did not meet this requirement were excluded. Thirty individual seedlings of each species were transplanted from each pot into four individual pots filled with artificial soil mixture, which were placed in the chamber (in all, 240 seedlings in eight pots). Each pot was fertilized once before transplanting seedlings (N: 330 mg L^{-1}, P: 220 mg L^{-1}, K: 400 mg L^{-1}). The seedlings were watered twice a week to prevent water stress.

Plant growth analysis

Aboveground plant parts were harvested at 105 days from transplantation in the ambient and CO_2 elevated treatment chambers. Harvested plants were separated into stems and leaves, and then stems of individual plants were measured for height, and leaves were scanned to determine leaf area. Scanned leaf images were used to determine leaf area using an image-editing program (ImageJ Version 1.48; http://rsb.info.nih.gov/ij/). Separated plant parts were oven-dried for 4 days at 80°C, and then weighed.

The dry weight of each plant part and leaf area were used to calculate the following parameters:

Specific leaf area (SLA): leaf area / leaf dry weight ($cm^2\ g^{-1}$)
Leaf area ratio (LAR): total leaf area / total plant dry weight ($cm^2\ g^{-1}$)
Leaf weight ratio (LWR): total leaf weight / total plant dry weight ($g\ g^{-1}$)

The effects of elevated CO_2 on plant growth are presented as the percent change in biomass in the elevated CO_2 treatment compared to the ambient air treatment.

C and N analysis

The fresh leaves were sampled twice, at 40 and 80 days after transplantation, in both treatments to compare the impact of elevated CO_2 on leaf quality and C/N ratio. The plant samples were oven-dried for 4 days at 80°C and ground in a ball-mill. C and N concentrations were then determined by an automatic element analyzer (Flash EA 1112 series, Thermo Fisher Scientific).

Statistical analysis

Statistically significant differences between treatments were determined using *t*-tests with SPSS (ver. 12.0.1). The *t*-tests were used to identify significant differences ($p < 0.05$) in the morphological characteristics and growth parameters of the four species, and the chemical content (carbon, nitrogen and C/N ratio) of two of these species (*Q. mongolica* and *Q. acutissima*). Principal component analysis (PCA) was performed to study the effects of elevated CO_2 on morphological characteristics and growth of the four *Quercus* species using R v.2.15.3 (R Development Core Team, 2012). Data for the PCA analysis were converted to percent change in elevated CO_2 values relative to ambient values.

Results

Plant growth

Plant growth results for each oak species in ambient and elevated CO_2 conditions for one growing season are summarized in *Table 1*.

Table 1. *Comparison of morphological characteristics and growth of four species (Q. mongolica, Q. variabilis, Q. serrata, and Q. acutissima) grown in ambient and elevated CO_2 chambers*

	Q. moglica			*Q. variabilis*			*Q. serrata*			*Q. acutissima*		
	Ambient	Elevated CO_2	*p*	Ambient	Elevated CO_2	*p*	Ambient	Elevated CO_2	*p*	Ambient	Elevated CO_2	*p*
Stem length (cm)	18.79 ±0.83	22.42 ±0.98	***	31.84 ±3.10	25.09 ±2.21	*	23.89 ±1.84	22.81 ±1.71	*NS*	50.57 ±1.52	48.19 ±1.81	*NS*
Leaf size (cm^2 $leaf^{-1}$)	12.59 ±0.75	14.12 ±0.82	*NS*	17.22 ±1.28	11.60 ±0.82	***	7.86 ±0.39	6.98 ±0.55	*NS*	18.77 ±0.81	18.24 ±0.80	*NS*
Leaf area (cm^2 $capita^{-1}$)	149.1 ±12.3	185.8 ±9.1	**	297.3 ±42.5	149.9 ±15.7	***	105.8 ±9.1	100.8 ±10.8	*NS*	461.3 ±20.7	426.2 ±27.7	*NS*
Leaf weight (g $leaf^{-1}$)	0.068 ±0.004	0.080 ±0.005	*	0.091 ±0.008	0.077 ±0.006	*NS*	0.036 ±0.002	0.033 ±0.003	*NS*	0.091 ±0.004	0.093 ±0.004	*NS*
Stem biomass (g $capita^{-1}$)	0.499 ±0.047	0.638 ±0.036	**	0.977 ±0.197	0.581 ±0.086	*	0.283 ±0.031	0.316 ±0.040	*NS*	1.720 ±0.114	1.473 ±0.125	*NS*
Leaves biomass (g $capita^{-1}$)	0.796 ±0.066	1.058 ±0.060	***	1.625 ±0.257	0.999 ±0.115	**	0.482 ±0.042	0.472 ±0.055	*NS*	2.256 ±0.124	2.179 ±0.148	*NS*
Total aboveground biomass (g $capita^{-1}$)	1.295 ±0.108	1.696 ±0.081	***	2.602 ±0.445	1.580 ±0.194	**	0.765 ±0.071	0.788 ±0.087	*NS*	3.976 ±0.229	3.651 ±0.263	*NS*

Difference of parameters within individual species has been tested by t-test. *: $p < 0.1$; **: $p < 0.05$; ***: $p < 0.01$; *NS*: not significant (mean ± standard deviation)

Q. acutissima showed the largest total aboveground biomass, *Q. mongolica* and *Q. variabilis* had intermediate levels, and *Q. serrata* exhibited the smallest amount of aboveground biomass at both CO_2 levels. The other growth characteristics of stems and leaves were different depending on the tree species.

Only *Q. mongolica* showed increased growth in the elevated CO_2 treatment compared with ambient (*Fig. 1*). Stem length and leaf area were significantly larger at elevated CO_2 compared to ambient conditions ($p < 0.05$). The mean size and weight of a single leaf showed an increase of approximately 12% and 18%, respectively, in the elevated CO_2 treatment, but this was not statistically significant. Stems, leaves, and total aboveground biomass of individual seedlings increased by 28%, 33%, and 31%, respectively relative to the ambient treatment ($p < 0.05$).

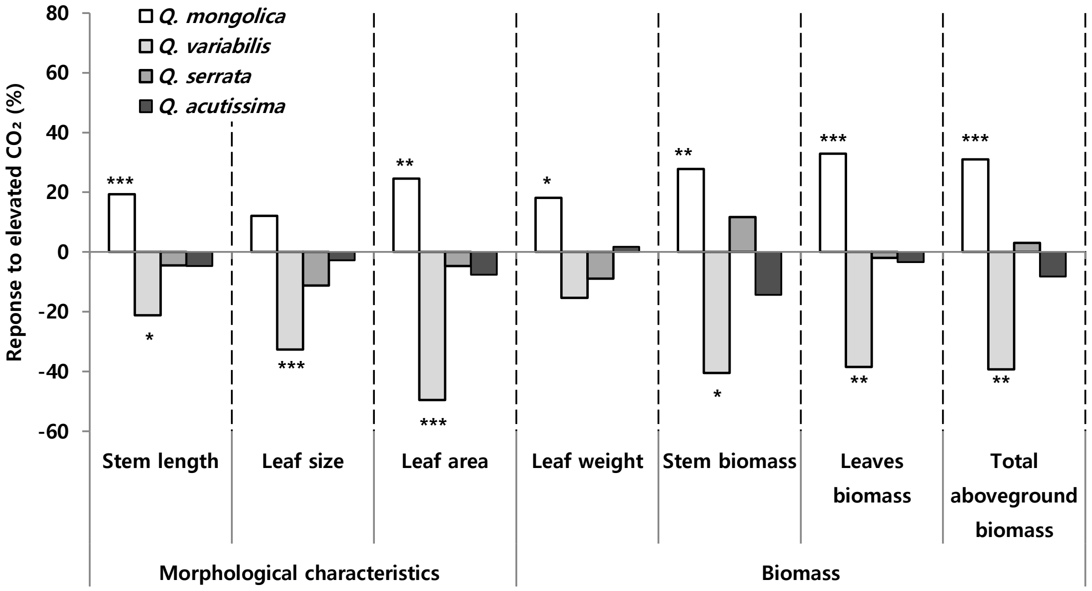

Figure 1. *Percent response of morphological characteristics and growth of four species (Q. mongolica, Q. variabilis, Q. serrata, and Q. acutissima) to elevated CO_2. Species differences were determined by t-tests. *: $p < 0.1$; **: $p < 0.05$; ***: $p < 0.01$*

Seedlings of *Q. serrata* and *Q. acutissima* did not respond to the elevated CO_2 treatment, and the growth responses were not statistically significant. However, there were patterns of decreases in morphological traits and growth in the elevated CO_2 treatment. Stem height and total aboveground biomass of *Q. serrata* increased by 11.7% and 3.1%, respectively, and leaf weight of *Q. acutissima* increased by 1.7% relative to ambient, which was not statistically significant.

On the other hand, the growth of *Q. variabilis* seedlings significantly decreased in the elevated CO_2 treatment (*Fig. 1* and *Table 1*). Plant height and single leaf size significantly decreased by 21% and 33%, respectively, in the CO_2 enriched conditions ($p < 0.05$). Leaf area per capita decreased to 50% and the leaf weight of a single leaf also decreased, but there was no statistical significance. Stems, leaves, and aboveground biomass significantly decreased by 41%, 39%, and 39%, respectively, under CO_2 enriched conditions relative to ambient ($p < 0.05$; except for stem biomass: $p = 0.071$).

Biomass allocation patterns

The SLA of all species decreased in the elevated CO_2 treatment, and *Q. mongolica*, *Q. serrata*, and *Q. variabilis* showed significant differences between ambient and CO_2 enrichment conditions (*Fig. 2*). The LAR of all species also decreased in the elevated CO_2 treatment, and *Q. variabilis* and *Q. serrata* showed significant differences between ambient and elevated CO_2 treatments ($p < 0.05$). In addition, the LWR was also unchanged or decreased slightly in all species with no significant differences between the two CO_2 treatments, except for *Q. acutissima*, which showed increased LWR ($p < 0.05$). These results indicate that elevated CO_2 reduced the leaf area. However, there were trends of increased weight or mass of leaves by CO_2 enrichment.

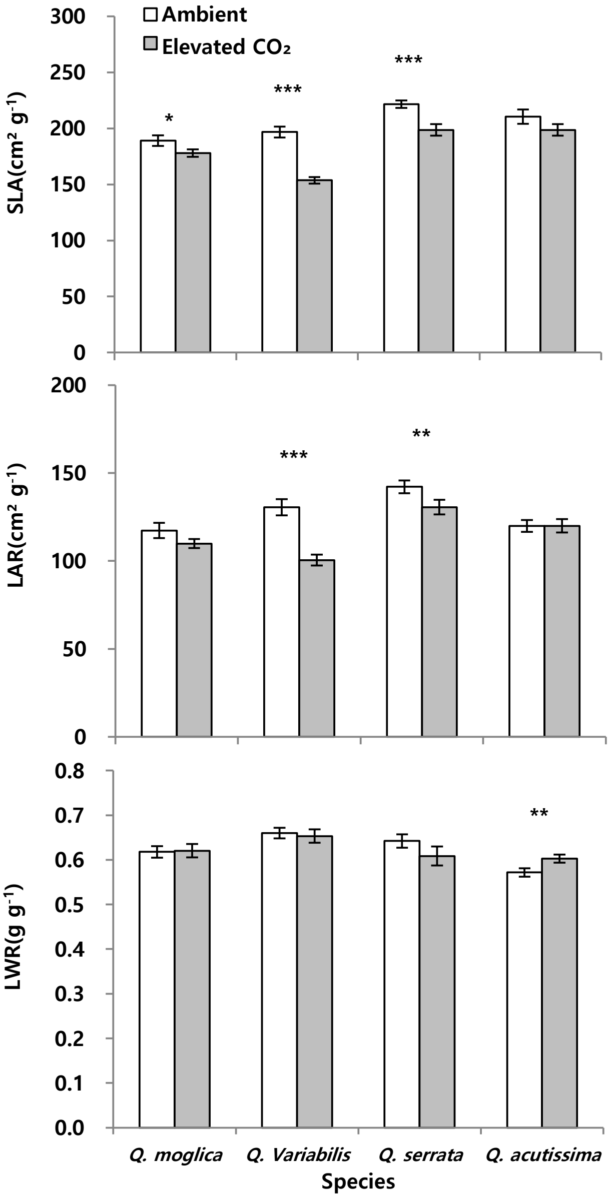

***Figure 2.** Specific leaf area (SLA), leaf area ratio (LAR), and leaf weight ratio (LWR) for four species (Q. mongolica, Q. variabilis, Q. serrata, and Q. acutissima) grown in ambient and elevated CO_2 chambers. Species differences were determined by t-tests. *: $p < 0.1$; **: $p < 0.05$; ***: $p < 0.01$*

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C and N concentrations

The changes in leaf C and N concentrations during plant growth are shown in *Table 2*. There were no significant differences in the C concentrations in all leaves between ambient and elevated CO_2, and ranged between 44% and 45% during the growing period. The total N concentration of *Q. mongolica* was 2.42% and 2.49% in the ambient, and 2.35% and 2.26% in the elevated CO_2 conditions at 40 days and 80 days, respectively. In *Q. mongolica*, the elevated CO_2 treatment reduced total N concentrations and increased the C/N ratio, however this was not statistically significant.

Table 2. *C and N concentrations and C/N ratio of Q. mongolica and Q. acutissima leaves at 40 and 80 days during growth periods*

	40 day			80 day		
	Ambient	Elevated CO_2	*p*	Ambient	Elevated CO_2	*p*
Q. mongolica						
C (%)	44.92±0.62	45.48±0.80	*NS*	44.62±0.30	44.32±0.68	*NS*
N (%)	2.42±0.20	2.35±0.43	*NS*	2.49±0.33	2.26±0.49	*NS*
C/N	18.60±1.42	19.87±4.00	*NS*	18.17±2.55	20.26±3.96	*NS*
Q. acutissima						
C (%)	45.85±0.23	45.66±0.81	*NS*	45.90±0.31	45.66±0.88	*NS*
N (%)	2.72±0.39	2.24±0.23	*	3.04±0.19	1.85±0.29	***
C/N	17.10±2.46	20.59±2.10	*	15.17±0.96	25.18±3.80	***

Difference of parameters within individual species has been tested by t-test. *: $p < 0.1$; **: $p < 0.05$; ***: $p < 0.01$; *NS*: not significant (mean ± standard deviation)

In *Q. acutissima*, total N concentrations were 2.72% and 3.04% in ambient, and 2.24% and 1.85% in elevated CO_2 at 40 days and 80 days, respectively, and the elevated CO_2 treatment significantly reduced total N concentrations in leaves. Therefore, the C/N ratio was increased by elevated CO_2 ($p < 0.01$), and the difference in the C/N ratio between the ambient and elevated CO_2 treatments increased with time.

PCA analysis

The results of the PCA to identify the relative effects of elevated CO_2 on each aboveground part and the morphological parameters of the four *Quercus* species are shown in *Fig. 3*. The horizontal PC1 axis elucidated 56.8% of the variation in all the factors, including factors related to growth in seedling height, growth in the mass of each plant part, and leaf area and weight. The vertical PC2 axis explained 21.9% of the variation in all the factors, and leaf area and leaf weight parameters (SLA, LAR, and LWR) are shown.

Q. mongolica formed a cluster to right side of the PC1 axis, which demonstrates that all aboveground growth components positively responded to elevated CO_2 conditions.

However, *Q. variabilis* clustered on the left side of the PC1 axis, and the growth components responded negatively to elevated CO_2 treatment. *Q. serrata* and *Q. acutissima* were widely distributed in each quadrant of the central axis, and growth was not affected by elevated CO_2. In particular, these patterns show very different responses to elevated CO_2 between each species studied.

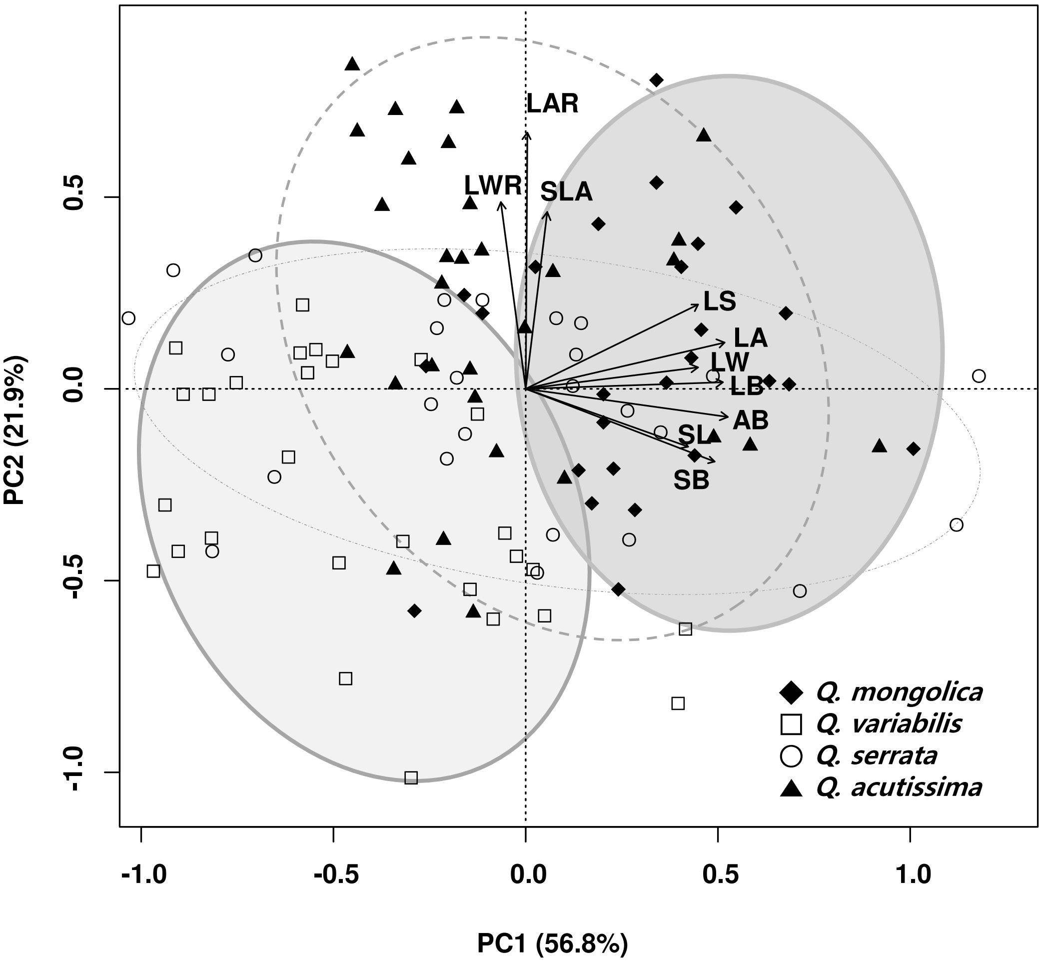

Figure 3. *Principal component analysis on morphological characteristics and growth parameters of the four species (Q. mongolica, Q. variabilis, Q. serrata, and Q. acutissima) grown in the ambient and elevated CO_2 chambers. AB: aboveground biomass; LA: leaf area; LB: leaf biomass; LS: leaf size; LW: leaf weight; SB: stem biomass; SL: stem length*

Discussion

Climate change due to increased atmospheric CO_2 is the biggest environmental issue facing humanity. Several problems are associated with this issue, including changes in primary production and C dynamics of natural ecosystems. To understand and recognize the changes in production and C dynamics, it is necessary to examine whether and how changes in growth and the chemical composition of plant species affect biogeochemical cycling caused by CO_2 elevation and climate change.

In the present study, oak species from the same genus exhibited different growth responses to elevated CO_2 (*Fig. 1* and *3*). Total aboveground biomass for *Q. mongolica* increased by 31% relative to ambient CO_2 in response to elevated CO_2. However, the growth of aboveground biomass of *Q. variabilis* markedly decreased, and *Q. serrata* and *Q. acutissima* exhibited no significant response to elevated CO_2. Joel et al. (2001) suggested that the stimulation of plant growth by elevated CO_2 depends in part on the growth potential of individual plants or species. Each species responds very differently to elevated CO_2 for unknown reasons (Norby, 1996; Tangley, 2001; Craine et al., 2003; Körner, 2005), including aboveground growth responses (Smith et al., 2013). The responses of plants to elevated CO_2 vary greatly depending on other environmental factors such as light, water, nutrients, and resource availability (Bazzaz and Carlson, 1984; Field et al., 1992; Hungate et al., 1997a, b). Moreover, elevated CO_2 has consistently shown positive effects on plants with mild temperature increases, but plant growth responses to elevated CO_2 vary under different temperature regimes, and can diminish with heat stress (Wang et al., 2012).

Although root responses to elevated CO_2 were not investigated in this study, many studies have reported that elevated CO_2 stimulates root growth (El Kohen and Mousseau, 1994; Crookshanks et al., 1998; De Graaff et al., 2006), and that the root/shoot ratio is significantly higher under elevated CO_2 than under ambient treatments (Luo et al., 2006; Nie et al., 2013). In a meta-analysis, root biomass exhibited larger increases than shoot biomass with elevated CO_2; therefore increasing root biomass with elevated CO_2 may enhance the potential to store C (De Graaff et al., 2006; Luo et al., 2006; Nie et al., 2013). In addition, these effects are expected to cause increased amounts of C input into the soil (De Graaff et al., 2006).

In addition, the increased atmospheric CO_2 concentrations caused significant leaf morphology changes (*Fig. 1* and *Table 1*). In the total area of leaves per capita and single leaf size, leaf biomass and dry weight showed variable responses to elevated CO_2 depending on the species. The results for *Q. mongolica*, which exhibited increased leaf area with elevated CO_2, were consistent with the findings of many studies (EL Kohen and Mousseau, 1994; Ceulemans et al., 1995; Oksanen et al., 2001), and Gielen et al. (2001) even found that *Phyllostachys nigra* had a 225% increase in leaf area index. In contrast, *Q. serrata* and *Q. acutissima* exhibited no response to elevated CO_2, which is comparable to the findings of other studies (El Kohen and Mousseau, 1994; Gielen et al., 2001). Moreover, leaf area was found to be either reduced or unchanged with elevated CO_2 in a few studies (Mousseau and Enoch, 1989a, b), which is analogous to the negative response of *Q. variabilis*.

However, different patterns were observed with leaf growth and morphological characteristics. SLA and LAR decreased, and LWR was unchanged by elevated CO_2 in the present study (*Fig. 2*), which is consistent with the findings of other studies, although the effects are not always significant. In a meta-analysis of previous studies, plants exposed to elevated CO_2 exhibited a decrease in SLA of 6% (Ainsworth and Long, 2005) or 13% (Poorter and Navas, 2003), although this trend varied with plant functional group and species. Moreover, LAR decreased by an average of 13% in a meta-analysis of 130 studies (Poorter and Navas, 2003). In many C_3 plants, LWR remained unchanged (Poorter et al., 1996; Poorter and Navas, 2003) or decreased under elevated CO_2 conditions (Ishikawa, 2008). The reduction in SLA by elevated CO_2 is due to increases in leaf mass rather than leaf size (Yin, 2002), resulting in increased leaf density (Roumet et al., 1999). These results suggest that increases in leaf thickness and decreases in leaf size are consistent responses to elevated CO_2. Therefore, plants will potentially produce a smaller and thicker leaf owing to elevated CO_2.

Generally, plants, including trees, respond to elevated atmospheric CO_2 by enhanced net photosynthesis, growth, and dry mass production (Lemon, 1983; Strain and Cure, 1985; Jarvis et al., 1989), because CO_2 is used as a source for photosynthesis. Moreover, elevated CO_2 enhances growth rates to a greater extent in young seedlings than in mature trees (Tolley and Strain, 1985; Bazzaz et al., 1989). However, in our study, plant responses to elevated CO_2 were species-specific, and in some cases, there was no impact, or even a negative impact on growth. Therefore, the response of plants to elevated CO_2 is not consistent among species. The response of plants to elevated CO_2 depends largely on the spatial and temporal availability of other resources, in particular water and nutrients (Field et al., 1992).

The genus *Quercus* is distributed throughout South Korea, existing in different microclimates and has locally different distribution depending on species. In other studies, *Q. mongolica,* which exhibits increased growth with elevated CO_2, is the most shade-tolerant species among the oak species (Sim and Han, 2003). The growth of *Q. serrata* and *Q. acutissima* are unaffected by elevated CO_2 and grow well in higher light and moisture conditions than other *Quercus* species (Jeong et al., 2009; Lim et al., 2012). A meta-analysis of literature data revealed a greater growth response to elevated CO_2 in shade-tolerant species than in shade-intolerant species (Kerstiens, 2001). In contrast, *Q. variabilis*, which exhibited a negative response to elevated CO_2, reduced in growth rate with high levels of moisture, and preferred dry environmental conditions (Jeong et al., 2009). Cho et al. (2013) found that with elevated CO_2 levels, *Q. variabilis* has a reduced ability to cope with environmental changes. In this view, the negative response of *Q. variabilis* appears to be due to the reduction in the tolerance to environmental changes caused by elevated CO_2. The response to CO_2 enrichment might depend strongly on the response of specific species under varying patterns of environmental factors, such as precipitation and warming (Xu et al., 2014). Additionally, individual species and multifactor dependencies must be considered in a projection of terrestrial ecosystem response to climatic change (Xu et al., 2014).

On the other hand, increased CO_2 resulted in significantly lower N concentrations in leaf tissue, which led to high C/N ratios, whereas there was no effect of CO_2 on C concentrations (Billings et al., 2003). In a meta-analysis, leaf N concentrations decreased to 5.2–16.5% with elevated CO_2, regardless of temperature treatments (Curtis and Wang, 1998; Norby et al., 1999; Billings et al., 2003; Wang et al., 2012), and Cotrufo et al. (1998) found a 14% reduction in N concentrations in plant tissue with elevated CO_2. Reductions in litter N concentrations are expected to result in decreased litter decomposition rates (Cotrufo et al., 1995). In addition, the increased C/N ratio was strongly influenced by decreased N concentrations, because C concentration was unchanged with elevated CO_2 (*Table 2*). In many studies, increased C/N ratios have been observed in elevated CO_2 experiments (Johnson et al., 1997; Norby et al., 2001; Billings et al., 2003). Consequently, these leaf chemical changes, such as N concentrations and C/N ratio, are considered highly important factors because they are good predictors of decomposition rates (Melillo et al., 1982; Berg and Ekbohm, 1991; Gallardo and Merino, 1993; Bargali, 1996; Ganjegunte et al., 2004; Ge et al., 2013).

These results might indicate an increase in annual litter fall (Finzi and Schlesinger, 2002; Norby et al., 2002) and a decrease in decomposition on the forest floor caused by lower initial litter N concentrations with elevated CO_2 (Cotrufo et al., 2005) in temperate deciduous forests. Thus, elevated CO_2 levels in the atmosphere might increase the leaf litter layer, because leaves are not easily decomposed on the forest floor (Cotrufo et al., 2005). These mechanisms might lead to a reduction in atmospheric CO_2 concentrations by the accumulation of C on the forest floor (Schlesinger and Lichter, 2001). Moreover, Norby and Cotrufo (1998) suggested that there is a potential for increased C sequestration in the terrestrial biosphere. However, Norby and Zak (2011) demonstrated that increased net primary production with elevated CO_2 does not necessarily increase ecosystem C storage. Therefore, future studies should focus on reductions in atmospheric CO_2 by organic matter accumulation on the forest floor in temperate deciduous oak trees, which are the dominant species on the Korean peninsula. To this end, well-thought-out research on the C balance between soil organic matter and decomposition of organic matter under the various environmental conditions of elevated atmospheric CO_2 is required.

Conclusions

The present study demonstrated that *Q. mongolica* exhibited a pattern of increasing growth under elevated CO_2 conditions, whereas *Q. serrata* and *Q. acutissima* did not exhibit a distinct pattern of change in growth with elevated CO_2, owing to great variation in responses to CO_2 enrichment. However, *Q. variabilis* exhibited a dramatic reduction in aboveground biomass to 40% in enriched CO_2 as compared to that in ambient CO_2. These results suggest a potential for future changes in the distribution areas of dominant species caused by climate change on the Korean peninsula. Consequently, there is a requirement for more research, particularly long-term research, on the effects of elevated CO_2 levels and other environmental changes on oaks, as this study was conducted in limited conditions with respect to elevated CO_2.

REFERENCES

[1] Ainsworth, E.A., Long, S.P. (2005): What have we learned from 15 years of free-air CO_2 enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO_2. - New Phytologist 165: 351-372.

[2] Bargali, S.S. (1996): Weight loss and N release in decomposing wood litter in a eucalypt plantation age series. - Soil Biology and Biochemistry 28: 699-702.

[3] Bazzaz, F.A. (1990): The response of natural ecosystems to the rising global CO_2 levels. - Annual Review of Ecology and Systematics 21: 167-196.

[4] Bazzaz, F.A., Carlson, R.W. (1984): The response of plants to elevated CO_2. - Oecologia 62: 196-198.

[5] Bazzaz, F.A., Garbutt, K., Reekie, E.G., Williams, W.E. (1989): Using growth analysis to interpret competition between a C_3 and a C_4 annual under ambient and elevated CO_2. - Oecologia 79: 223-235.

[6] Berg, B., Ekbohm, G. (1991): Litter mass-loss rates and decomposition patterns in some needle and leaf litter types. Long-term decomposition in a Scots pine forest. VII. - Canadian Journal of Botany 69: 1449-1456.

[7] Billings, S.A., Zitzer, S.F., Weatherly, H., Schaeffer, S.M., Charlet, T., Arnone, J.A., Evans, R.D. (2003): Effects of elevated carbon dioxide on green leaf tissue and leaf litter quality in an intact Mojave Desert ecosystem. - Global Change Biology 9: 729-735.

[8] Canadell, J.G., Le Quéré, C., Raupach, M.R., Field, C.B., Buitenhuis, E.T., Ciais, P., Conway, T.J., Gillett, N.P., Houghton, R.A., Marland, G. (2007): Contributions to accelerating atmospheric CO_2 growth from economic activity, carbon intensity, and

efficiency of natural sinks. - Proceedings of the national academy of sciences 104: 18866-18870.

[9] Ceulemans, R., Janssens, I.A., Jach, M.E. (1999): Effects of CO_2 enrichment on trees and forests: Lessons to be learned in view of future ecosystem studies. - Annals of Botany 84: 577-590.

[10] Ceulemans, R., Jiang, X.N., Shao, B.Y. (1995): Growth and physiology of one-year old poplar (*Populus*) under elevated atmospheric CO_2 levels. - Annals of Botany 75: 609-617.

[11] Cho, K.T., Jang, R.H., Lee, S.H., Han, Y.S., You, Y.H. (2013): Effects of global warming and environmental factors of light, soil moisture, and nutrient level on ecological niche of *Quercus acutissima* and *Quercus variabilis*. - Korean Journal of Ecology and Environment 46(3): 429-439.

[12] Cotrufo, M.F., de Angelis, P., Polle, A. (2005): Leaf litter production and decomposition in a poplar short-rotation coppice exposed to free air CO_2 enrichment (POPFACE). - Global Change Biology 11: 971-982.

[13] Cotrufo, M.F., Ineson, P., Roberts, J.D. (1995): Decomposition of birch leaf litters with varying C-to-N ratios. - Soil Biology and Biochemistry 27: 1219-1221.

[14] Cotrufo, M.F., Ineson, P., Scott, A. (1998): Elevated CO_2 reduces the nitrogen concentration of plant tissues. - Global Change Biology 4: 43-54.

[15] Craine, J.M., Reich, P.B., Tilman, G.D., Ellsworth, D., Fargione, J., Knops, J., Naeem, S. (2003): The role of plant species in biomass production and response to elevated CO_2 and N. - Ecology Letters 6: 623-625.

[16] Crookshanks, M., Taylor, G., Broadmeadow, M. (1998): Elevated CO_2 and tree root growth: contrasting responses in *Fraxinus excelsior*, *Quercus petraea* and *Pinus sylvestris*. - New Phytologist 138: 241-250.

[17] Curtis, P.S., Wang, X. (1998): A meta-analysis of elevated CO_2 effects on woody plant mass, form, and physiology. - Oecologia 113: 299-313.

[18] De Graaff, M.A., Van Groenigen, K.J., Six, J., Hungate, B., Van Kessel, C. (2006): Interactions between plant growth and soil nutrient cycling under elevated CO_2: a meta-analysis. - Global Change Biology 12: 2077-2091.

[19] Dixon, R.K., Solomon, A.M., Brown, S., Houghton, R.A., Trexier, M.C., Wisniewski, J. (1994): Carbon pools and flux of global forest ecosystems. - Science 263: 185-190.

[20] El Kohen, A., Mousseau, M. (1994): Interactive effects of elevated CO_2 and mineral nutrition on growth and CO_2 exchange of sweet chestnut seedlings (*Castanea sativa*). - Tree Physiology 14: 679-690.

[21] Fan, S., Gloor, M., Mahlman, J., Pacala, S., Sarmiento, J., Takahashi, T., Tans, P. (1998): A large terrestrial carbon sink in North America implied by atmospheric and oceanic carbon dioxide data and models. - Science 282: 442-446.

[22] Field, C.B., Chapin, F.S., Matson, P.A., Mooney, H.A. (1992): Responses of terrestrial ecosystems to the changing atmosphere: a resource-based approach. - Annual Review of Ecology and Systematics 23: 201-235.

[23] Finzi, A.C., Schlesinger, W.H. (2002): Species control variation in litter decomposition in a pine forest exposed to elevated CO_2. - Global Change Biology 8: 1217-1229.

[24] Gallardo, A., Merino, J. (1993): Leaf decomposition in two Mediterranean ecosystems of southwest Spain: Influence of substrate quality. - Ecology 74: 152-161.

[25] Ganjegunte, G.K., Condron, L.M., Clinton, P.W., Davis, M.R., Mahieu, N. (2004): Decomposition and nutrient release from radiata pine (*Pinus radiata*) coarse woody debris. - Forest Ecology and Management 187: 197-211.

[26] Ge, X., Zeng, L., Xiao, W., Huang, Z., Geng, X., Tan, B. (2013): Effect of litter substrate quality and soil nutrients on forest litter decomposition: A review. - Acta Ecologica Sinica 33: 102-108.

[27] Gielen, B., Calfapietra, C., Sabatti, M., Ceulemans, R. (2001): Leaf area dynamics in a closed poplar plantation under free-air carbon dioxide enrichment. - Tree Physiology 21: 1245-1255.

[28] Houghton, J.T., Ding, Y., Griggs, D.J., Noguer, M., van der Linden, P.J., Dai, X., Maskell, K., Johnson, C.A. (2001): Climate change 2001: the scientific basis. - Cambridge University Press, Cambridge, UK

[29] Hungate, B., Chapin Iii, F., Zhong, H., Holland, E., Field, C. (1997a): Stimulation of grassland nitrogen cycling under carbon dioxide enrichment. - Oecologia 109: 149-153.

[30] Hungate, B.A., Lund, C.P., Pearson, H.L., CHAPIN III, F.S. (1997b): Elevated CO_2 and nutrient addition after soil N cycling and N trace gas fluxes with early season wet-up in a California annual grassland. - Biogeochemistry 37: 89-109.

[31] IPCC (2007): Climate change 2007-the physical science basis - In: Solomon, S., Qin, D., Manning, M., chen, Z., Marquis, M., Averyt, K.B., Tignor, M., Miller, H.L. (ed.) Working group I contribution to the fourth assessment report of the IPCC. Cambridge University Press, cambridge, UK,

[32] IPCC. (2014): Climate change 2014-Impacts, adaptation, and vulnerability. Part A: Global and sectoral aspect - In: Field, C.B., Barros, V.R., Dokken, D.J., Mach, K.J., Mastrandrea, M.D., Bilir, T.E., Chatterjee, M., Ebi, K.L., Estrada, Y.O., Genova, R.C., Girma, B., Kissel, E.S., Levy, A.N., MacCracken, S., Mastrandrea, P.R., White, L.L. (ed.) Contribution of Working Group II to the fifth assessment report of the intergovernmental panel on climate change. Cambridge University Press, cambridge, UK,

[33] Ishikawa, S.I. (2008): Growth and Photosynthetic Responses of One C_3 and Two C_4 Chenopodiaceae Plants to Three CO_2 Concentration Conditions. - Journal of Ecology and Environment 31: 261-267.

[34] Jarvis, P.G. (1998): European forests and global change: the likely impacts of rising CO_2 and temperature. - Cambridge University Press, Cambridge, UK

[35] Jarvis, P.G., Morison, J.I.L., Chaloner, W.G., Cannell, M.G.R., Roberts, J., Jones, H.G., Amtmann, R. (1989): Atmospheric carbon dioxide and forests. - Philosophical Transactions of the Royal Society of London B, Biological Sciences 324: 369-392.

[36] Jeong, H.M., Kim, H.R., You, Y.H. (2009): Growth difference among saplings of *Quercus acutissima*, *Q. variabilis* and *Q. mongolica* under the environmental gradients treatment. - Korean Journal of Environment and Ecology 27(1): 82-87.

[37] Joel, G., Chapin, F.S., Chiariello, N.R., Thayer, S.S., Field, C.B. (2001): Species-specific responses of plant communities to altered carbon and nutrient availability. - Global Change Biology 7: 435-450.

[38] Johnson, D.W., Ball, J., T, Walker, R.F. (1997): Effects of CO_2 and nitrogen fertilization on vegetation and soil nutrient content in juvenile ponderosa pine. - Plant and Soil 190: 29-40.

[39] Kerstiens, G. (2001): Meta-analysis of the interaction between shade-tolerance, light environment and growth response of woody species to elevated CO_2. - Acta Oecologica 22(1): 61-69.

[40] Kim, J.W. (1990): A syntaxonomic scheme for the deciduous oak forests of South Korea. - Abstracta Botanica 14: 51-81.

[41] Korea Forest Sevice (2012): Forestry statistics at a glance. - Korea Forest Sevice, Daejeon, Korea

[42] Körner, C. (2005): An Introduction to the Functional Diversity of Temperate Forest Trees - In: Scherer-Lorenzen, M., Körner, C., Schulze, E.D. (ed.) Forest Diversity and Function: Temperate and Boreal systems. Springer, Berlin, Germany, 13-37

[43] Lal, R. (2008): Carbon sequestration. - Philosophical Transactions of the Royal Society B: Biological Sciences 363: 815-830.

[44] Lemon, E.R. (1983): CO_2 and plants. The response of plants to rising levels of atmospheric carbon dioxide. - Westview Press, Inc., Boulder, Colorado, USA

[45] Lim, H., Kim, H.R., You, Y.H. (2012): Growth difference between the seedlings of *Quercus serrata* and *Q. aliena* under light, moisture and nutrient gradients. - Journal of Wetlands Research 14(2): 237-242.

[46] Lorenz, K., Lal, R. (2010): Carbon sequestration in forest ecosystems. - Springer, New York

[47] Luo, Y., Hui, D., Zhang, D. (2006): Elevated CO_2 stimulates net accumulations of carbon and nitrogen in land ecosystems: a meta-analysis. - Ecology 87: 53-63.

[48] Martin, P.H., Nabuurs, G.J., Aubinet, M., Karjalainen, T., Vine, E.L., Kinsman, J., Heath, L.S. (2001): Carbon Sinks in Temperate Forests 1. - Annual Review of Energy and the Environment 26: 435-465.

[49] Melillo, J.M., Aber, J.D., Muratore, J.F. (1982): Nitrogen and lignin control of hardwood leaf litter decomposition dynamics. - Ecology 63: 621-626.

[50] Mousseau, M. (1993): Effects of elevated CO_2 on growth, photosynthesis and respiration of sweet chestnut (*Castanea sativa* Mill.). - Vegetatio 104/105: 413-419.

[51] Mousseau, M., Enoch, H.Z. (1989a): Carbon dioxide enrichment reduces shoot growth in sweet chestnut seedlings (*Castanea sativa* Mill). - Plant, Cell and Environment 12: 927-934.

[52] Mousseau, M., Enoch, H.Z. (1989b): Effect of doubling atmospheric CO_2 concentration on growth, dry matter distribution and CO_2 exchange of 2 yr old sweet chestnut trees (*Castanea sativa* Mill). - Annals of forest Science 46: 506-508.

[53] Nie, M., Lu, M., Bell, J., Raut, S., Pendall, E. (2013): Altered root traits due to elevated CO_2: a meta-analysis. - Global Ecology and Biogeography 22: 1095-1105.

[54] Norby, R.J. (1996): Oaks in a high-CO_2 world. - Annales des sciences forestieres 53: 413-429.

[55] Norby, R.J., Cotrufo, M.F. (1998): Global change: a question of litter quality. - Nature 396: 17-18.

[56] Norby, R.J., Cotrufo, M.F., Ineson, P., O'Neill, E.G., Canadell, J.G. (2001): Elevated CO_2, litter chemistry, and decomposition: a synthesis. - Oecologia 127: 153-165.

[57] Norby, R.J., Hanson, P.J., O'Neill, E.G., Tschaplinski, T.J., Weltzin, J.F., Hansen, R.A., Cheng, W., Wullschleger, S.D., Gunderson, C.A., Edwards, N.T. (2002): Net primary productivity of a CO_2-enriched deciduous forest and the implications for carbon storage. - Ecological Applications 12: 1261-1266.

[58] Norby, R.J., Wullschleger, S.D., Gunderson, C.A., Johnson, D.W., Ceulemans, R. (1999): Tree responses to rising CO_2 in field experiments: implications for the future forest. - Plant, Cell & Environment 22: 683-714.

[59] Norby, R.J., Zak, D.R. (2011): Ecological lessons from free-air CO_2 enrichment (FACE) experiments. - Annual review of ecology, evolution, and systematics 42: 181-203.

[60] Oksanen, E., Sober, J., Karnosky, D.F. (2001): Impacts of elevated CO_2 and/or O_3 on leaf ultrastructure of aspen (*Populus tremuloides*) and birch (*Betula papyrifera*) in the Aspen FACE experiment. - Environmental Pollution 115: 437-446.

[61] Poorter, H., Navas, M.L. (2003): Plant growth and competition at elevated CO_2: on winners, losers and functional groups. - New Phytologist 157: 175-198.

[62] Poorter, H., Roumet, C., Campbell, B.D. (1996): Interspecific variation in the growth response of plants to elevated CO_2: a search for functional types - In: Körner, C., Bazzaz, F.A. (ed.) Carbon dioxide, Populations and Communities. Academic Press, New York, 375-412

[63] Roumet, C., Laurent, G., Roy, J. (1999): Leaf structure and chemical composition as affected by elevated CO_2: genotypic responses of two perennial grasses. - New Phytologist 143: 73-81.

[64] Schlesinger, W.H., Lichter, J. (2001): Limited carbon storage in soil and litter of experimental forest plots under increased atmospheric CO_2. - Nature 411: 466-469.

[65] Sim, J.S., Han, S.S. (2003): Ecophysiological characteristics of deciduous oak species (III) - Photosynthetic responses of leaves to change of light intensity. - Journal of Korean Forestry Society 93: 208-204.

[66] Smith, A.R., Lukac, M., Hood, R., Healey, J.R., Miglietta, F., Godbold, D.L. (2013): Elevated CO_2 enrichment induces a differential biomass response in a mixed species temperate forest plantation. - New Phytologist 198: 156-168.

[67] Strain, B.R., Cure, J.D. (1985): Direct effects of increasing carbon dioxide on vegetation. - United States Department of Energy, National Technical Information Service, Springfield, Virginia, USA

[68] Tangley, L. (2001): High CO_2 Levels May Give Fast-Growing Trees an Edge. - Science 292: 36-37.

[69] Tans, P.P., Fung, I.Y., Takahashi, T. (1990): Observational contrains on the global atmospheric CO_2 budget. - Science 247: 1431-1438.

[70] Tolley, L.C., Strain, B.R. (1985): Effects of CO_2 enrichment and water stress on gas exchange of *Liquidambar styraciflua* and *Pinus taeda* seedlings grown under different irradiance levels. - Oecologia 65: 166-172.

[71] Tyrrell, M.L., Ross, J., Kelty, M. (2012): Carbon dynamics in the temperate forest - In: Ashton, M.S., Tyrrell, M.L., Spalding, D., Gentry, B. (ed.) Managing Forest Carbon in a Changing Climate. Springer, New York, 77-107

[72] Wamelink, G.W.W., Wieggers, H.J.J., Reinds, G.J., Kros, J., Mol-Dijkstra, J.P., Van Oijen, M., De Vries, W. (2009): Modelling impacts of changes in carbon dioxide concentration, climate and nitrogen deposition on carbon sequestration by European forests and forest soils. - Forest Ecology and Management 258: 1794-1805.

[73] Wang, D., Heckathorn, S.A., Wang, X., Philpott, S.M. (2012): A meta-analysis of plant physiological and growth responses to temperature and elevated CO_2. - Oecologia 169: 1-13.

[74] Xu, Z., Shimizu, H., Ito, S., Yagasaki, Y., Zou, C., Zhou, G., Zheng, Y. (2014): Effects of elevated CO_2, warming and precipitation change on plant growth, photosynthesis and peroxidation in dominant species from North China grassland. - Planta 239(2): 421-435.

[75] Yang, K.C. (2001): Classification of major habitats based on the climate conditions and topographic features in Korea. - Chung-Ang University, Seoul, Korea

[76] Yim, Y.J. (1977): Distribution of forest vegetation and climate in the Korean peninsula. IV: Zonal distribution of forest vegetation in relation to thermal climate. - Japanese Journal of Ecology 27: 269-278.

[77] Yim, Y.J. (1995): Composition and distribution of deciduous broad-leaved forests in Korea - In: Box, E.O., Peet, R.K., Masuzawa, T., Yamada, I., Fujiwara, K., Maycock, P.F. (ed.) Vegetation Science in Forestry. Kluwer Academic Publishers, Dordrecht, Netherlands, 273-298

[78] Yin, X. (2002): Responses of leaf nitrogen concentration and specific leaf area to atmospheric CO_2 enrichment: a retrospective synthesis across 62 species. - Global Change Biology 8: 631-642.

CLIMATE WARMING: DOES NORTHWEST CHINA FACE A STARK FOOD SECURITY CHALLENGE?

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Abstract. China's economy may be vulnerable to climate change, and Northwest China is an area sensitive to climate change. This review paper summarizes the impacts of climate warming on crop production in typical arid and semi-arid areas of Northwest China. Over the last 50 years, the climate has undergone a series of changes, including higher temperatures, increased drought and warmer winters. The future climate will become warmer and drought will become more severe. This will result in accelerated crop growth, more damage by crop pests, degraded soil, a decline in rain resource utilization, serious effects on the crop growing environment, weakening of agricultural ecological system stability, increased fluctuation in grain yield, difficulty in adjusting crop planting structure, threatened food nutrition and security, increased grain production costs, and risk and uncertainty in grain security. Taking scientific measures to actively keep pace with climate change will be a key strategy for reducing grain security risks in Northwest China.

Keywords: climate warming; crop; yield; quality; food security; semi-arid regions

Introduction

Climate change is predicted to increase both drought frequency and duration (Dai, 2011; Ponce-Campos et al., 2013). Arid and semi-arid regions account for 45% of the global land area, support 38% of the global population and are among the regions with the most fragile ecological and water resource systems (Ye et al., 2015). In the past 100 years, the mean temperature increase of global arid regions was 0.94°C, which is higher than the global mean temperature increase of 0.74°C. Temperature increases in arid regions of each continent, except South America, were above the global mean temperature increase. In the past 100 years, abrupt changes in rainfall have been mostly in arid and semi-arid regions globally (Tollefson, 2015). Since the 1960s, rainfall in semi-arid regions of West Africa has decreased, with rainfall in the Sahel decreasing by 20–40%. About 10–20% of land in global arid and semi-arid regions have been seriously degraded, the area of extremely arid regions has doubled and the area of arid regions is close to 30%. Large-scale droughts have recently occurred in North America, Africa,

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Europe, South America and Australia, resulting in major changes to terrestrial ecosystems, carbon balance and food security (Saleska et al., 2007;Piao et al., 2010).

The continuous aridification in the last 30 years in the semi-arid region of Northwest China has led to a serious environmental aggravation and lack of water resources, and has seriously restricted sustainable regional development. Northwest China, covering the Qinghai–Tibet Plateau and the Loess Plateau over Shaanxi, Gansu, Qinghai, Xinjiang, Ningxia and part of Inner Mongolia, consists of arid and semi-arid areas, semi-wet dry areas and dank and cold areas, and is sensitive to climate change (*Fig. 1*). Agriculture, comprising rain-fed and irrigated farming, dominates the national economy. However, grain production in this area has long been seriously restricted by drought (Chang et al., 2015). There is a narrow and long belt of frequent droughts in the semi-arid areas of north Shaanxi, mid-south Ningxia and mid-east Gansu, and there have been low, if any spring wheat yields over the past 10 years. It is questionable whether spring wheat is still suitable for growing in this area given the background of global warming.

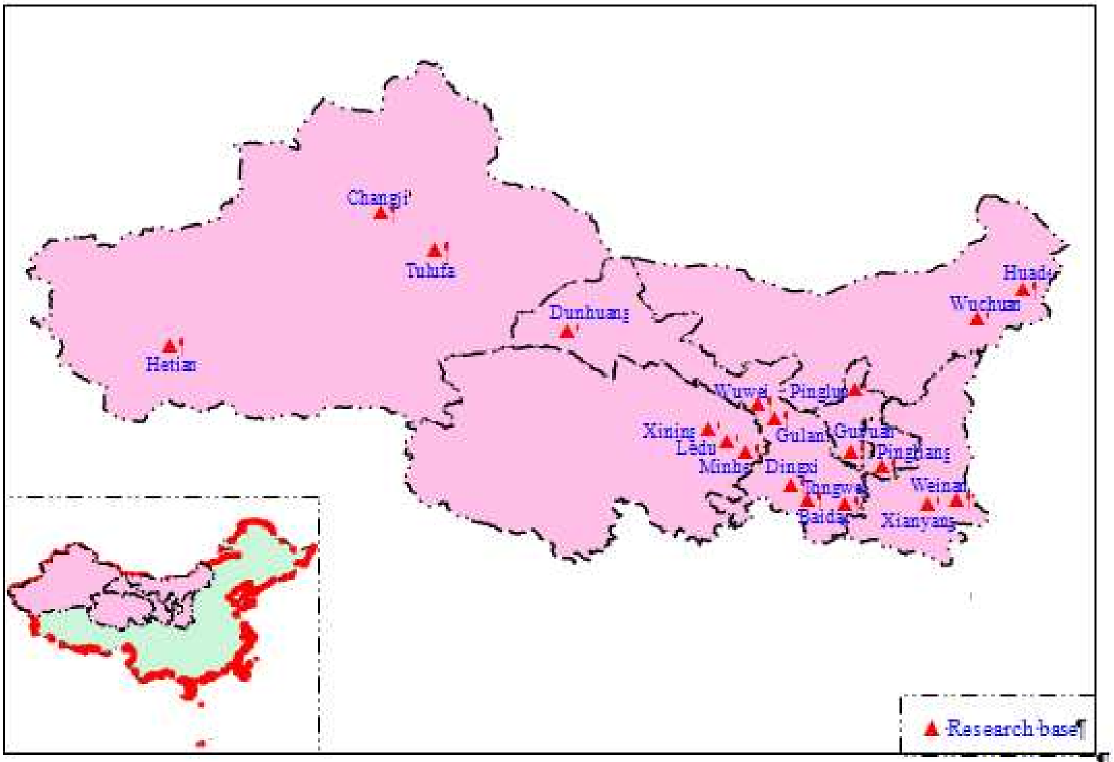

Figure 1. *Map of northwest China. Over the past 10 years, researchers from Lanzhou Arid Meteorology Institute of the China Meteorological Administration and Ningxia University selected some experimental bases in Dingxi, Guyuan and Zhangye in typical arid and semi-arid areas of Northwest China to study the impacts of climate warming on crop production and provide important scientific information that can be used to improve grain security.*

This paper aims to review climate observations and agricultural production trends in Northwest China and to use these observations to predict likely future changes. We

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highlight the main areas of vulnerability and sources of uncertainty based on recent literature and published data. The first section deals with the impact of recent climate change on food production, with particular attention to drought. Severe drought and rapid plant maturation are seriously threatening rain-fed crops in semi-arid regions. Increasing soil salinization and increases in plant diseases and insect pests are seriously threatening irrigated crops in arid regions. The second section deals with the main problems in agriculture caused by climate change, which has led to a decrease in agricultural ecological system stability, increased crop structure adjustment difficulties, threatened food nutrition, sharpened fluctuations in crop yield and an increase in the costs and investment needed for agricultural production. The last section integrates the measures taken against climate change, which mainly include selecting anti-drought species, adjusting crop structure, generalizing water saving techniques, developing cloud water resources, improving forecasting ability and developing low carbon agriculture.

Grain production is influenced by multiple factors

Over the past 50 years, the climate in Northwest China has experienced a series of changes, including higher temperatures, increased drought and warm winters. The future climate will continue to warm and droughts will become more severe.

Drought

The overall drought levels in Northwest China have become more severe. Extremely high and low temperature events have occurred and the frequency and duration of serious drought events have increased significantly (Lei et al., 2016). Over the past 50 years in Northwest China, spring drought has occurred in 43, summer drought in 37 and autumn drought in 28 of these years. Continuous spring and summer droughts have occurred in 22, continuous summer and autumn droughts in 17 and continuous spring, summer and autumn droughts in 14 of the past 50 years. In the 1990s, serious drought disasters occurred at a much higher frequency than previous years. In 1995, the drought disaster area in Gansu Province was 2,087,000 ha, the direct disaster area was 1,708,000 ha and 150.0×10^4 t of grain yield was lost (Gao and Zhang, 2016). In 2000, the drought disaster area in Gansu Province was 1,622,000 ha, the direct disaster area was 566,000 ha and 114.2×10^4 t of grain yield was lost (Lin et al., 2010). Based on the Fourth IPCC evaluation, by 2050 the temperature in Northwest China will rise by 1.93–2.77°C; precipitation in mid-east Gansu, mid-south Ningxia and mid-north Shaanxi will decrease by 0.01–4.34%; drought disasters will be much more frequent, the dry hot wind effect will worsen and drought restrictions on grain production will be much greater (Zhang et al., 2010; Deng et al., 2010).

Rapid plant maturation

It is well known that crop photosynthesis is influenced by excessively high temperatures. Some studies have shown that if temperature increases by 1.0–2.5°C, then photosynthesis in spring wheat at the trefoil stage significantly declines, ear separation and formation is seriously affected and the number of ear grains significantly decreases. As temperature rises, the photosynthesis rate at booting stage will also significantly decrease, dry matter accumulation will be seriously affected, thousand-grain weight will significantly decline and yield of spring wheat will decline (Xiao et al., 2010). In the semi-arid rain-fed farming areas, the net photosynthesis rate and stomatal conductance of spring wheat at the milk filling and milk ripe stages declines as temperature rises, the transpiration rate increases and dry matter accumulation decreases (Zhao et al., 2007a). The warming climate accelerates crop growth, shortens the growth duration and seriously decreases crop yield. Studies have shown that if temperature rises 0.6–2.2°C, the full growth period of peas in semi-arid rain-fed farming areas will be shortened by 3–17 d (Xiao et al., 2009) and the full growth period of corn in the semi-arid irrigated area will be shortened by 4–17 d (Wang et al., 2004). If temperature rises 0.5–2.0°C, the full growth duration of the pea–spring wheat–potato rotation system in semi-arid rain-fed areas will be shortened by 11–42 d (*Table 1*).

Crop pests and diseases

Because of the warming climate, pest breeding periods will be prolonged, the growth of bacteria and pests will accelerate, pest lifecycles will be shortened and there will be more pest generations. Warm winters in Northwest China favor pest breeding and enable them to live through winter, thus the crop pest problem will increase as winters get warmer. Warm springs favor pest breeding because the dry and rainless weather in spring creates favorable conditions for wheat aphids and wheat spider mite (Deng et al., 2012). The occurrence of wheat sharp eyespot, powdery mildew and wheat spider mite are correlated with climate change (Zhao et al., 2005). The incidence of stripe rust (Liu et al., 2009), corn bollworm and red spider mite (Zheng et al., 2001), pea root rot (Xiao et al., 2009) and potato late blight in Northwest China has increased as temperature has risen, and the area affected by diseases and pests has risen by 3.5% annually (Yao et al., 2010).

Accelerated soil degradation

The rate at which loess becomes soil in the semi-arid areas is correlated with temperature and rainfall. Climate warming accelerates decomposition of soil organic matter, which leads to a decline in soil fertility; and ion exchange in soil increases and soil pollution becomes worse (Li et al., 2009). Climate warming also accelerates the upward movement of salts as the soil water content drops, which leads to an increase in salinity of the farming layer (*Fig. 2*). If the temperature rises by 0.5–2.5°C in winter, the effective nitrogen in soil will decrease by 2.45–4.66 g/kg (Xiao et al., 2012) and soil salinity will increase by 0.39–0.50 g/kg. It is estimated that the irrigation water needed to neutralize the increase in salinity caused by climate warming will be 129,000,000–140,000,000 m^3 in the Ningxia Yellow River Irrigation Area. After winter warming, the water content in soil in arid areas will significantly decrease. If the temperature rises by 0.5–2.5°C in winter, then the water content in the crop growing layer of the soil will decrease from 18.5% to 16.2–12.5% (Xiao et al., 2010).

Table 1. *Influence of climate change on crop growth in Northwest China*

	Meteorological data/simulation experiment	**Trend in climate change**	**Crop growth**	**Yield and quality change**	**Reference**
Spring wheat	Data from positioned observations, encrypted observations and corresponding parallel meteorological observations for spring wheat growing during 1986–2009. Experimental base: Dingxi.	Annual temperature significantly increased, the temperature change rate was 0.362°C/10 years; the annual rainfall decreased and the rate of rainfall change was –15.796 mm/10 years.	Growth of spring wheat was influenced by climate warming. The period of milky ripeness–maturity was shortened by 2–3 d/10 years, and the full growing period was shortened by 4–5 d/10 years. Climate warming accelerated the growth of spring wheat and shortened the full growing period.	Annual yield of spring wheat decreased by –34.584 g/m^2/10 years. Due to climate warming, the influence of meteorological conditions on the growth and yield of spring wheat increased and was affected by more indeterminate factors.	Yao et al. (2011c)
	Meteorological data and positioned observation data for the growth of spring wheat during 1986–2004. Experimental base: Dingxi, Tongwei.	Annual increase in temperature was 0.081°C/year. Annual rainfall decrease was 0.833 mm/year.	Growth period of spring wheat was advanced and shortened. Growth period was shortened by about 1.7 d while temperature rose by 1°C.	Spring wheat yields decreased.	Zhao et al. (2007a)
	Meteorological data and positioned observation data for the growth of spring wheat during 1986–2005. Experimental base: Dingxi.	Daily mean annual temperature increased by 0.0786°C/year; annual rainfall decreased by 1.876 mm/year.	Growth period of spring wheat advanced by 0.195 d/year.	Annual yield of spring wheat declined by 5.5 g/m^2; the yield fell by 1.2 g/m^2 and the annual rainfall declined by 1 mm. The main meteorological factor influencing the growing days and yield of spring wheat in the semi-arid rain-fed farming area was the change in rainfall.	Zhao et al. (2007b)
	Meteorological data and positioned observation data for the growth of spring wheat during 1955–2005. Experimental base: Huade, Wuchuan.	Annual mean temperature increased by 0.40°C/year, but the change in annual rainfall was not significant.	Growth period of spring wheat was significantly shortened by 24 d, the seeding period was significantly delayed by 17 d, the emergence period was postponed, the maturing period was advanced and the whole growing period was shortened.	Yield of spring wheat decreased significantly. Planted area of spring wheat declined year on year in the warm–cool semi-arid area.	Hou, et al. (2009)
	Meteorological data and positioned observation data for the growth of irrigated spring wheat during 1981–2005. Experimental base: Wuwei.	Annual increase in daily mean temperature was 0.0374°C/year and rainfall increased by 0.19 mm.	Seeding, elongation, blooming and maturing periods of spring wheat were advanced by −0.345, –0.962, –1.997 and –0.136 d/year, respectively.	Yield of spring wheat increased by 8.8 g/m^2/year. Accumulated temperature rose by 1°C. The main meteorological factor influencing the full growing days and yield of irrigated spring wheat in the arid area was the ≥ °C accumulated temperature and sunshine hours. The influence of rainfall was not significant.	Cao et al. (2008)

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	Meteorological data and positioned observation data for the growth of irrigated spring wheat during 1981–2005. Experimental base: Dunhuang.	Annual increase in daily mean temperature was 0.0687°C/year. The annual rainfall change was –0.319 mm/year.	Seeding period of spring wheat was delayed by 0.02 d/year. The elongation, blooming, maturing and full growing periods were advanced by –0.188, –0.413, –0.341 and −0.370 d/year, respectively.	Yield of spring wheat decreased by 0.3 g/m^2/year. The main meteorological factors influencing the full growing days of irrigated spring wheat in the arid area were daily mean temperature, rainfall and sunshine hours.	Cao et al. (2008)
	Meteorological data and positioned observation data for the growth of spring wheat during 1980–2007. Experimental base: Minhe.	Annual mean temperature and the highest and lowest temperatures significantly increased.	Sowing period was delayed, maturing period was advanced and growing period was shortened by 11.7 d if the daily mean temperature increased by 1°C during the growing period.	There was a large fluctuation in yields. Climate warming accelerated the growing of spring wheat in the irrigated farming areas in the Qinghai plateau, but decreased the yield.	Wang et al. (2011b)
	Infrared radiator farm warming simulation experiment. Experimental base: Pingluo.	Temperature increased by 2.0–2.5°C.	Full growing period of spring wheat was shortened by 18–22 d.	Yield of spring wheat was reduced by 16.5–18.5%. Photosynthesis rate of irrigated spring wheat in the arid area during the trefoil and booting stages was decreased by warming, and so the yield was reduced by the decrease in the number of grains and the thousand-grain weight.	Xiao et al. (2011a)
	Simulated sunshine greenhouse warming experiment. Experimental base: Dingxi.	Temperature increased by 0.6–2.2°C.	Full growing period was shortened by warming.	Contents of Cd and Zn in the grain of spring wheat exceeded the standard values by 490 and 27%, respectively. The quality of spring wheat was influenced by climate warming.	Li et al. (2012)
Winter wheat	Data from positioned observations, encrypted observations and corresponding parallel meteorological observations for winter wheat during 1980–2010. Experimental base: Dingxi.	There was a rising trend in annual temperature change. The rate of temperature change was 0.325°C/10 years.	Seeding period of winter spring was delayed by 2–3 d/10 years, Greening period was advanced by 4–5 d/10 years and Blooming and maturing periods were advanced by 5–6 d/10 years. Wintering period of winter spring was shortened by 5–6 d/10 years and Full growing period was shortened by 7–8 d/10 years.	Temperature change during the seeding–wintering–elongating–blooming periods of winter wheat in the semi-arid area of the Loess Plateau influenced yield. The rainfall change during the seeding–elongation period influenced yield.	Yao et al. (2011)
	Meteorological data and position observation data for the growth of winter wheat during 1981–2004. Experimental base: Guyuan.	Annual mean temperature increased, but the annual rainfall decreased.	Shortening of the wintering–greening period was significantly correlated with the extremely low temperature. Shortening of the milky ripeness–maturing period was significantly correlated with extremely high temperature, and prolonging of the greening–elongating and blooming–milky ripeness periods was significantly correlated with extremely low temperature.	Yield of winter wheat in the semi-arid area of the Loess Plateau was significantly and positively correlated with the lowest temperature in the wintering period; however, the highest temperature in the greening–booting and milky ripeness–maturing periods was significantly and negatively correlated with yield.	Zhang et al. (2008)

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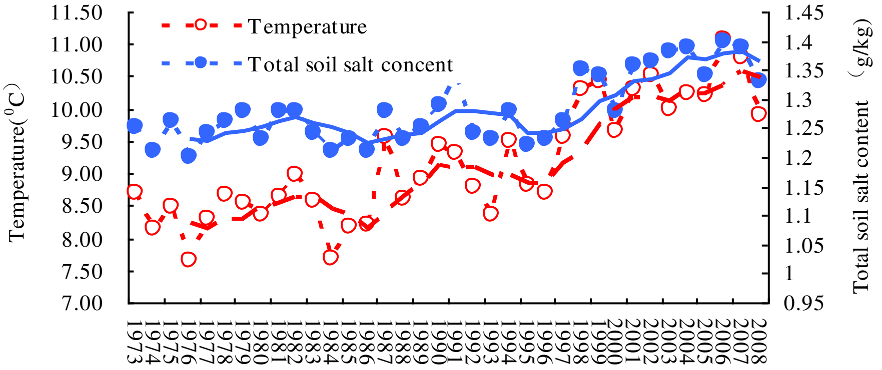
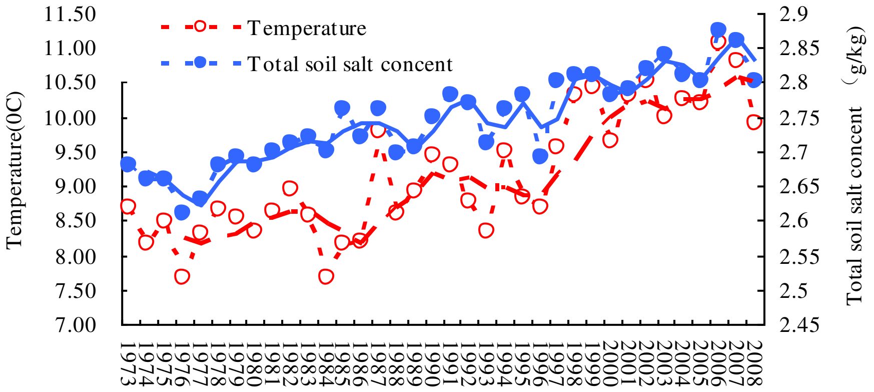
	Meteorological data and positioned observation data for the growth of spring wheat during 1981–2005. Experimental base: Tongwei.	Annual temperature significantly increased and annual rainfall decreased.	Growing period of winter wheat was shortened by 0.6–1.3 d/year.	Temperature rise and rainfall decrease increased the annual yield of winter wheat by 43.2–91.6 kg/ha. It was estimated that the yield will increase 3.1–4.0% by 2030 in the semi-wet dry area.	Xiao et al. (2008)
	Meteorological data and positioned observation data for the growth of spring wheat during 1960–2010. Experimental base: Xianxiang and Weinan.	Annual mean temperature increased significantly, with a large increase in temperature in spring and winter. However, annual rainfall decreased, significantly in spring and autumn.	Greening period of winter wheat advanced by 9 d and the elongating period advanced by 7 d during 2000–2007 compared to 1989–1999.	Because of the excessively high temperature, the wintering period of winter wheat was shortened, the soil moisture fell faster and the wheat seedlings grew more rapidly. In the dry plateau of Weibei, the growing of winter wheat was significantly influenced by climate warming.	Yang et al. (2010); Gao et al. (2012)
	Meteorological data and positioned observation data for the growth of spring wheat during 1981–2004. Experimental base: Hetian, Changji.	Both temperature and rainfall increased.	Sowing period for winter wheat was delayed by 2–6 d/10 years, the greening period was delayed by 4.7 d/10 years, the maturing period was advanced by 7.7 d/10 years and the number of days in the greening–maturing period was shortened by about 16–19 d.	Yield of winter wheat was significantly influenced by rising temperature. The growth of winter wheat after greening was accelerated, the growing period was shortened, the maturing period was generally advanced and the climate conditions were not favorable for the production of local winter wheat.	Du et al. (2011)
	Infrared radiator farm warming simulation experiment. Experimental base: Tongwei.	Temperature increased by 0.6–2.5°C.	An increase in the mean daily temperature of 1.4–2.2°C led to a decrease in duration of the growth stages by 11–21 d.	An increase in temperature will improve the winter wheat yield. It was predicted that climatic changes may lead to the increase of 2.6–6.0% in annual wheat yields.	Xiao et al. (2010)
Pea	Infrared radiator farm warming simulation experiment. Experimental base: Tongwei.	Temperature increased by 0.6–2.5°C.	Growing period for pea was shortened by 3–17 d.	Pea yields were reduced by 6.3–17.5%. Over the last 20 years, pea has disappeared from the semi-arid rain-fed farming area of 34°55′–35°26′N.	Xiao et al. (2009)
Crop rotation	Infrared radiator farm warming simulation experiment. Experimental base: Guyuan.	Temperature increased by 0.5–2.0°C.	Growing period of the crop rotation system of pea–spring wheat–potato was shortened by 11–42 d.	Yield of the crop rotation system: pea–spring wheat–potato, was reduced by 3.2–9.4%. In the semi-arid area of the Loess Plateau, the crop rotation system was significantly influenced by climate warming over the long term.	Xiao et al. (2007b)

A Light saline soil



B Moderate saline soil



C. Severe saline soil

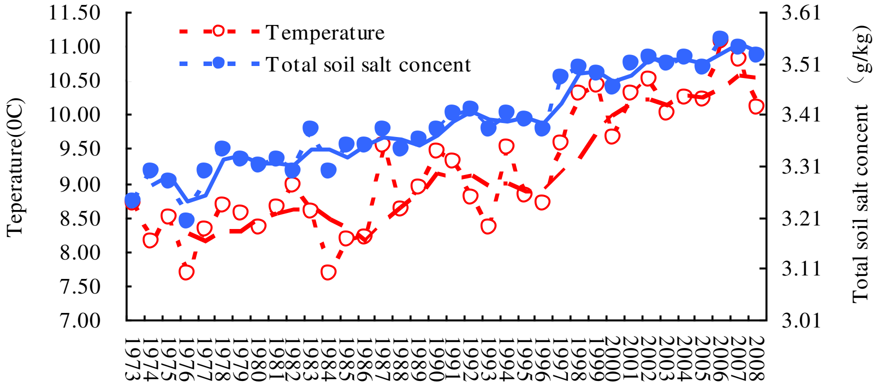


Figure 2. *The change trends for annual average temperature and total salt content in saline soil during 1973–2008.*

Climatic warming has intensified soil moisture evaporation and has led to soil salinity moving upward, resulting in increases in the harm caused by salt and alkali in soil. This figure shows observational, soil salinity and meteorological data over the past 35 years and the influence of climatic warming on soil salinity change in the Yellow River Irrigation Area in Ningxia Hui Autonomous Region. The total soil salt content has an increasing trend because of global climatic warming over the past 35 years, and the total salt content of light, moderate and severe saline soils has increased by 0.08, 0.13 and 0.19 g/kg, respectively (Xiao et al, 2010).

Decreased rain resource utilization ratio

Climate warming directly influences crop photosynthesis and transpiration, accelerates soil moisture evaporation and influences the effective utilization of water. Over the past 50 years, the temperature in semi-arid rain-fed farming areas has risen by 0.6–1.6°C, rainfall has decreased from 105.6 to 67.7 mm and the water utilization rate of spring wheat has decreased by 0.3–0.5 kg/hm^2. In the future, if temperature rises by 0.5–2.5°C, the pea water utilization rate will decrease by 4.3–33.3%, and the water utilization rate of the spring wheat–pea–potato rotation system will decrease by 3.0–12.4%. If the temperature rise is < 1.5°C and annual rainfall is > 310.0 mm, then the water utilization rate of potato will still increase. However, if the temperature rise is > 1.5°C and the rainfall is < 310.0 mm, then the water utilization rate of potato will decrease. The global climate change over the next 50 years will negatively influence the general water utilization of crops in Northwest China (*Table 2*).

Decreased utilization of trace elements

Climate warming will decrease biological utilization and increase the concentration of trace elements by influencing soil microorganism and cell enzyme activities. Studies have shown that if the temperature rises by 1°C, the concentrations of cadmium (Cd), lead (Pb), copper (Cu), zinc (Zn) and manganese (Mn) will increase by 5.8, 2.8, 4.4, 90.6 and 3.6%, respectively (Li et al., 2011). If warming continues, it is estimated that by 2050, the contents of Cd and Zn in spring wheat kernels in the semi-arid rain-fed farming area of Northwest China will exceed acceptable values by 490 and 27%, respectively, and iron (Fe) and Zn will transfer from soil to potato stem tubers and leaves at an increased rate (*Fig. 3*). Climate change will decrease the utilization rate of trace elements, reduce protein and reducing sugar contents and significantly degrade crop quality.

Food security will be challenged by many problems

Climate warming negatively affects the agricultural ecosystem in Northwest China, increases grain yield fluctuations, modifies crop structure, threatens grain nutritional security, increases grain production investment and increases grain security risk and uncertainty.

Decreased agricultural ecosystem stability

Different species have different responses to global climate change and the composition of agricultural ecosystems will significantly change with global climate change. Warming raises the respiratory activity of agricultural ecosystems, accelerates the mineralization of soil organic carbon and reduces carbon storage in the ecosystem. Climate warming accelerates the decomposition of organic matter in the soil, promotes soil ion change and increases soil pollution. It accelerates soil water evaporation and increases saline and alkali damage to the soil farming layer. Climate warming can modify the solubility of trace elements in the soil by influencing soil microorganism activity, crop growth rates, photosynthesis rates and cell enzymatic activity (Li et al., 2013). This means that climate change will modify agricultural ecosystem composition, structure, function and biodiversity and reduce ecosystem stability.

Increased crop structural adjustment

Climate warming considerably improves the effective heat reserve, prolongs the frost-free period and substantially modifies crop structure (Leng et al., 2015). Over the past 50 years, the growth period starting date of suitable crops in Northwest China has advanced, the termination date postponed and the crop growth period prolonged. Compared with 1951–1980, the northern planting border for winter wheat in Shaanxi, Inner Mongolia and Qinghai during 1981–2007 moved northwards and westwards. In the 1970s, the northern planting border for winter wheat in Ningxia was 36°N, but now it has extended to 39°N in the Yellow River Irrigation Area. However, the most-suitable areas and suitable areas for potato growing have been reduced by 35 and 3%, respectively; the less suitable areas and plantable areas have increased by 18.5 and 6.6%, respectively, and the unsuitable area has been reduced by 2.0%. The potato and corn planting areas have extended from south to north and from low to high altitude and have gradually got larger over the years (Yao et al., 2010a). Over the past 30 years, the altitude of suitable planting areas for spring wheat, pea and potato in the Qilianshan mountainous area have risen by 100–200 m, suitable planting areas for potato in mid-east Gansu have risen by 100–200 m and suitable planting areas for corn in the Gansu Corridor have risen by about 150 m (*Table 3*).

Contribution to the shortage of water resources

Climate change has led to the spatial and temporal variations of temperature and precipitation, which may result in hydrological drought and water shortage on the Hexi Corridor in the semi-arid climate zone of Northwest China (Gao and Zhang, 2016). The Weihe River, the largest tributary of the Yellow River, experienced runoff declines as large as 35% in the last century. The results showed that precipitation and runoff have decreased since the baseline decade. We further estimated the relative contributions of human activity and climate change to the hydrological response of the Weihe River Basin and determined that human activity has a greater impact on basin runoff than climate change factors. The percentages in change of runoff due to climate change were 36, 28, 53 and 10% in the 1970s, 1980s 1990s and 2000s, respectively (Chang et al., 2015). The seasonal changes in temperature and rainfall may have positive impacts on water quality. However, an extremely cold spring and high wind speed are likely to affect the self-stabilizing equilibrium states of the reservoir, which requires attention in the future. This study provides useful information regarding the potential effects of climate change on water quality in China (Zhang et al., 2015a).

Table 2. *Influence of climate change on soil and crop moisture utilization*

Area	Meteorological data analysis/ simulation experiment	Climate change	Crop moisture utilization/soil moisture change	Reference
Semi-arid rain-fed farming area	Soil moisture data and corn yield data during 1990–2007.	Temperature increased, rainfall decreased, soil evaporation increased and drought occurred more frequently.	Soil moisture storage was most significantly correlated with corn yield in the elongating–heading period. In the elongating–heading period from 0–50 cm, the yield increased by 180–210 kg/hm^2 if the soil moisture storage increased by 10 mm.	Wang et al. (2009)
	Rainfall, soil temperature and soil moisture data during 1970–2005.	Soil temperature at 0–10 and 10–20 cm depth increased by 0.9 and 0.5°C, respectively.	Soil moisture storage was low during May–June and July–August. Soil moisture storage was insufficient during May–August and corn growth was affected during September–October. Moisture and temperature were suitable for soil moisture storage, but not suitable for corn maturing during the later period.	Yao et al. (2010b)
	Annual mean temperature, highest temperature, lowest temperature, relative humidity and rainfall data during 1961–2008.	Temperature increased, rainfall decreased and the climate became warmer and drier.	During 1961–2008, the annual mean maximum possible moisture evaporated amount was 400–800 mm in the Loess Plateau and was 650–750 mm in most areas. The maximum possible moisture evaporated amount in the northwest region of the Loess Plateau increased significantly.	Yao et al. (2011d)
	Rainfall, soil temperature and soil moisture data during 1981–2000.	Annual mean temperature increased year on year by 0.078°C/year.	Farm soil moisture storage was negatively correlated with temperature, i.e. farm soil moisture storage decreased as temperature rose. Because of climate warming, the farm soil moisture storage in the Loess Plateau of east Gansu decreased year on year.	Ma et al. (2009)
	Rainfall and soil moisture data during 1981–2000.	Annual mean temperature increased year on year by 0.078°C/year.	Farm moisture evaporated transpiration decreased year on year. Annual farm moisture evaporated and transpiration decreased by 157 mm (7.86%).	Wang et al. (2011a)
	Meteorological and soil moisture data during 1981–2000.	Annual mean temperature increased and rainfall decreased year on year.	Soil moisture change was highly correlated with the crop growing period in the semi-arid area of the Loess Plateau. The soil moisture in the crop growing period decreased.	Wang et al. (2005)
	Positioned observation and corresponding parallel meteorological data for winter wheat grown during 1981–2010.	Annual change in rainfall fluctuated in years 3 and 8. The annual temperature increased by 0.325°C/year.	Winter and spring temperatures in the semi-wet area of the Loess Plateau increased significantly, the winter death rate of winter wheat decreased and the moisture utilization rate increased.	Yao et al. (2011b)
	Rainfall and temperature data for each month during 1961–2000.	Temperature increased, rainfall decreased and drought was more common.	Soil drought was highly correlated with climate productivity. Years of soil drought were also years of low climate productivity, but years of high rainfall were not always years of	Wang et al. (2004)

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			high climate productivity because climate productivity was not only correlated with moisture but also with temperature and other factors.	
	Infrared radiator farm warming simulation experiment.	Temperature increased by 0.6–2.5°C.	Moisture utilization rate of pea decreased by 4.3–33.3%.	Xiao et al. (2009)
	Infrared radiator farm warming and rainfall simulation experiment.	Rainfall higher than 310.0 mm when warming was lower than 1.5°C.	Moisture utilization rate of potato increased.	Xiao et al. (2013)
		Rainfall lower than 310.0 mm when warming was higher than 1.5°C.	Moisture utilization rate of potato decreased.	
	Infrared radiator farm warming simulation experiment.	Temperature increased by 0.5–2.0°C.	Moisture utilization rate of a spring wheat–pea–potato crop rotation system decreased by 3.0–12.4%.	Xiao et al. (2008)
Arid irrigated area	Positioned observation data and meteorological data for soil salt during 1973–2008.	Temperature increased by 0.5~3.0°C.	Soil salt content increased by 0.03–0.32 g/kg. Irrigation for scrubbing the increased salt in the soil was 614–685.3 m^3/hm^2 and the volume of irrigation water needed increased by 8.2–9.1%.	Xiao et al. (2010)
	Crop evapotranspiration volume and meteorological data during 1959–2007.	Annual mean temperature rose at a linear rate of 0.326°C/10 years, and the temperature increased by 1.6°C/year over 49 years. Rainfall did not change significantly.	During 1959–2007, evapotranspiration volume from the reference crop decreased by 61 mm/10 years compared to 1959, and decreased by 299.1 mm over the past 49 years.	Zhang et al. (2009)
	All data concerning corn were collected during 1981–2002 from Wuwei Agricultural Experimental Station, Gansu, 1ocated in the eastern part of the Hexi Corridor.	Mean temperature during the corn growth period was less than the critical temperature.	Corn moisture utilization rate decreased because of climate warming.	Wang et al. (2004)
	Rainfall, temperature, crop growing period, yield and soil moisture data during 1960–2009.	During 2000–2009, compared with 1960–1969, the annual mean temperature increased by 1.6°C, and the annual mean rainfall decreased by 105.6 mm.	Moisture utilization rate of spring wheat decreased by 0.3–0.5 kg/hm^2 mm.	Xiao et al. (2011b)
	England Hadley Model.	Temperature increased by 1–4°C during the crop growing period.	Demand for moisture by corn increased by 1.90–11.49% and by spring wheat by 1.80–10.03%. Climate warming increased the sharp conflict between water demand and supply in the green land of the Hexi Corridor.	Wang et al. (2011b)
	Infrared radiator farm warming simulation experiment.	Winter temperature increased by 0.5–2.5°C.	Moisture in the soil farming layer decreased from 18.5% to 16.2–12.5%.	Xiao et al. (2012)

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Table 3. *Influence of climate change on crop structure in northwest China*

	Meteorological data/ simulating experiment	**Climate change**	**Yield and quality**	**Crop structure**	**Reference**
Wheat	Meteorological data during 1961–2003. Degree of change represented by data for 1987–2003 and 1961–1986. The mean value was calculated from 1971–2000 data.	During 1987–2003, the annual mean temperature rose significantly as climate warming increased.	Area planted with spring and winter wheat in the southwest semi-arid area decreased.	The northern cropping border for winter wheat in Shaanxi, Inner Mongolia, Ningxia, Gansu and Qinghai during 1981–2007 moved northwards and westwards compared with 1951–1980.	Liu et al. (2007)
	Meteorological data and positioned observation data for winter wheat growth during 1981–2004. Experimental base: Guyuan.	Annual mean temperature increased and annual rainfall decreased.	Winter wheat yield was significantly and positively correlated with the lowest temperature during the winter period.	In the 1970s, the northern border of the winter wheat rain-fed farming area in Ningxia was at 36°N, but has now moved to 39°N in the Yellow River Irrigation Area.	Zhang et al. (2008)
	Meteorological data and positioned observation data for spring wheat and winter wheat growth during 1981–2005. Experimental base: Tongwei.	Annual temperature significantly increased and annual rainfall decreased.	The harvest ears of spring wheat were highly reduced, and many seedlings died during the elongation period.	Spring wheat has been replaced by winter wheat in the semi-arid rain-fed farming areas within 34°55′–35°26′N.	Xiao et al. (2007b); Liu et al. (2008a)
Potato	Meteorological data and potato growing data from 71 stations in Gansu Province during 1981–2000. Experimental bases: Gulang, Dingxi, Beidao and Pingliang.	Annual mean temperature increased and annual rainfall decreased.	Potato yields decreased as temperature rose and increased as rainfall rose.	Potato growing areas have moved from south to north or from low to high altitude, and the area has increased year on year. The highest altitude suitable for potato in middle Gansu increased by 100–200 m on average.	Yao et al. (2006)
	Meteorological data for 1961–2000. Positioned observation data for potato growth.	Annual mean temperature increased by 0.026°C/year. Rainfall decreased.	The main meteorological factors influencing potato yield were temperature in the tuber expansion period (mean temperature in July–August) and rainfall over the whole growing period (May–September). There was not much demand for moisture during the early and later periods of potato growth. Demand for water was greatest in the blooming period and this period coincided with the main rainfall period in the Loess Plateau.	Climate warming in the Loess Plateau is both advantageous and disadvantageous for potato production.	Song (2008)
	Meteorological data for 1961–2000. Potato yield data for 1971–2000.	Over the past 40 years in the Ningnan mountainous area, annual mean temperature increased and		Over the past 40 years, the climate in Ningxia has become warmer and drier. Climate factors influencing	Sun et al. (2008)

		rainfall decreased.		the potato yield varied during the different growing periods and climate change has been disadvantageous to potato production.	
	Positioned observation, encrypted observation and corresponding parallel meteorological observation data for potato growth during 1989–2007. Meteorological observation data and potato growth and planting suitability dynamic climatic research program data during 1961–2008.	The annual rainfall decreased by –8.239 mm/10 years. Annual temperature increased by 0.144°C/10 years.	Temperature increase negatively influenced the yield of potato, except during the harvest period. The stem expansion period was also quite sensitive to temperature change. Rainfall negatively influenced yield, except during the seedling and harvest periods. The branching–blooming period was also quite sensitive to rainfall change.	In the warm–cool semi-wet areas in the northwest, warming was advantageous for potato production. The most-suitable areas and suitable areas for potato planting were reduced in size by 35 and 3%, respectively. The less suitable areas and plantable areas increased by 18.5 and 6.6%, respectively.	Yao et al. (2010b),
	Positioned observation data for potato growing during 1988–2008, encrypted observation data during 2007–2008, and ground meteorological observation data during 1957–2008.	Annual rainfall decreased by –13.359 m/10 years. Annual mean temperature increased by 0.239°C/10 years.	In the semi-arid area of the Loess Plateau, climate change seriously influenced potato production.	The main influence of climate change on potato yield was warming.	Yao et al. (2012)
	Infrared radiator farm warming simulation experiment. Experimental base: Guyuan.	Temperature increased by 0.5–2.5°C.	If temperature rose by 1.5–2.5°C, the dry matter, starch and vitamin C contents in potato increased, but the protein and reducing sugar contents significantly decreased.	Warming seriously influenced potato quality in the semi-arid area of the Loess Plateau.	Xiao et al. (2013)
Corn	Meteorological observation data and corn climate data during 1981–2008.	Since 1971, the temperature and sunshine suitability for corn during the whole growing period increased, while the rainfall suitability decreased.	The amount of sunshine during the seedling–elongating, elongating–heading and heading–milky ripeness periods was significantly correlated with yield. Temperature during the sowing–seedling and heading–milky ripeness periods was significantly correlated with yield. Rainfall in the elongating–heading and heading–milky ripeness periods was also significantly correlated with yield.	Temperature suitability for corn in the northwest arid farming area became more favorable, so climate change was favorable for corn growing. The corn planting area can be enlarged.	Yao et al. (2011a)
	Temperature and corn yield data in the Hexi Corridor green land irrigated area for 1981–2005.	Accumulated temperature in different areas of the Hexi irrigation area significantly increased with global climate warming.	The active accumulated temperature of ≥ 10°C during the corn growing period was most significantly correlated with yield, and was the key meteorological factor influencing the local corn yield. Corn yield improved as the accumulated temperature ≥ 10°C	Climate warming and heat resource increases improved corn yields. The irrigated corn planting area in the green land of the Hexi Corridor can be further enlarged and corn planting bases can be	Cao et al. (2008)

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			increased.	constructed. The corn planting area expanded from south to north.	
	Degree of change represented by data during 1987–2003 and 1961–1986. Mean value during 1971–2000.	Annual mean temperatures rose sharply as climate warming increased.	The corn planting area significantly rose because of climate warming, temperature rises and heat resource increases. The structure of summer and autumn crops changed significantly.	Corn planting area in the southwest arid area had sharply increased.	Liu et al. (2009)
	All the data concerning corn were collected during 1981–2002 from Wuwei Agricultural Experimental Station in Gansu, 1ocated in the eastern part of the Hexi Corridor.	Mean temperature during the corn growth period was less than the critical temperature.	Corn yield in the semi-arid rain-fed farming area decreased by 3% for every 1°C temperature rise.	Future increases in temperature may cause the mean temperature during the corn growth period to exceed the critical temperature, thus leading to a lengthening of the growth period.	Wang et al. (2004)
	Meteorological data and corn climate data during 1961–2009.	Annual mean temperature increased significantly by 0.37°C/10 years. The annual rainfall decreased by 3.70 mm/10 years.	Warming directly influenced the photosynthesis rate and respiration rate, which affected the accumulation of dry matter and reduced crop quality.	In the plateau area of Qinghai (Xining and Ledu), the climate became warmer, the heat resource significantly increased and the crop plant structure and planting area changed significantly.	Liu et al. (2007)
	Change in mean temperature, highest temperature, lowest temperature and effective temperature accumulated at ≥ 10°C in the corn growing period during the 2020s, 2050s and 2080s.	Future highest temperatures will sharply increase in south Ningxia; the mean temperature, lowest temperature and effective temperature accumulated at ≥ 10°C will sharply increase in north Ningxia.	Future temperature rises will promote corn production in Ningxia, and the extra effective temperature accumulated at ≥ 10°C will provide more heat.	Altitude of suitable planting areas for corn in the south mountainous area of Ningxia will rise by 150 m.	Gou (2012)
	Analysis of temperatures at 10 observation stations in the Ningxia Yellow River Irrigation Area for the corn growing period, starting in the last 10 d of April and ending in the middle 10 d of September, during 1981–2004.	Climate in the Ningxia Yellow River Irrigation Area during the corn growing period became significantly warmer. A sharp change in daily mean temperature during May–September happened in 1993, and temperature increased by 0.7°C.		Climate warming has meant that high yielding varieties can be planted, the variation in corn yield per unit area decreased and ensured stable high yields.	Liu et al. (2008b)

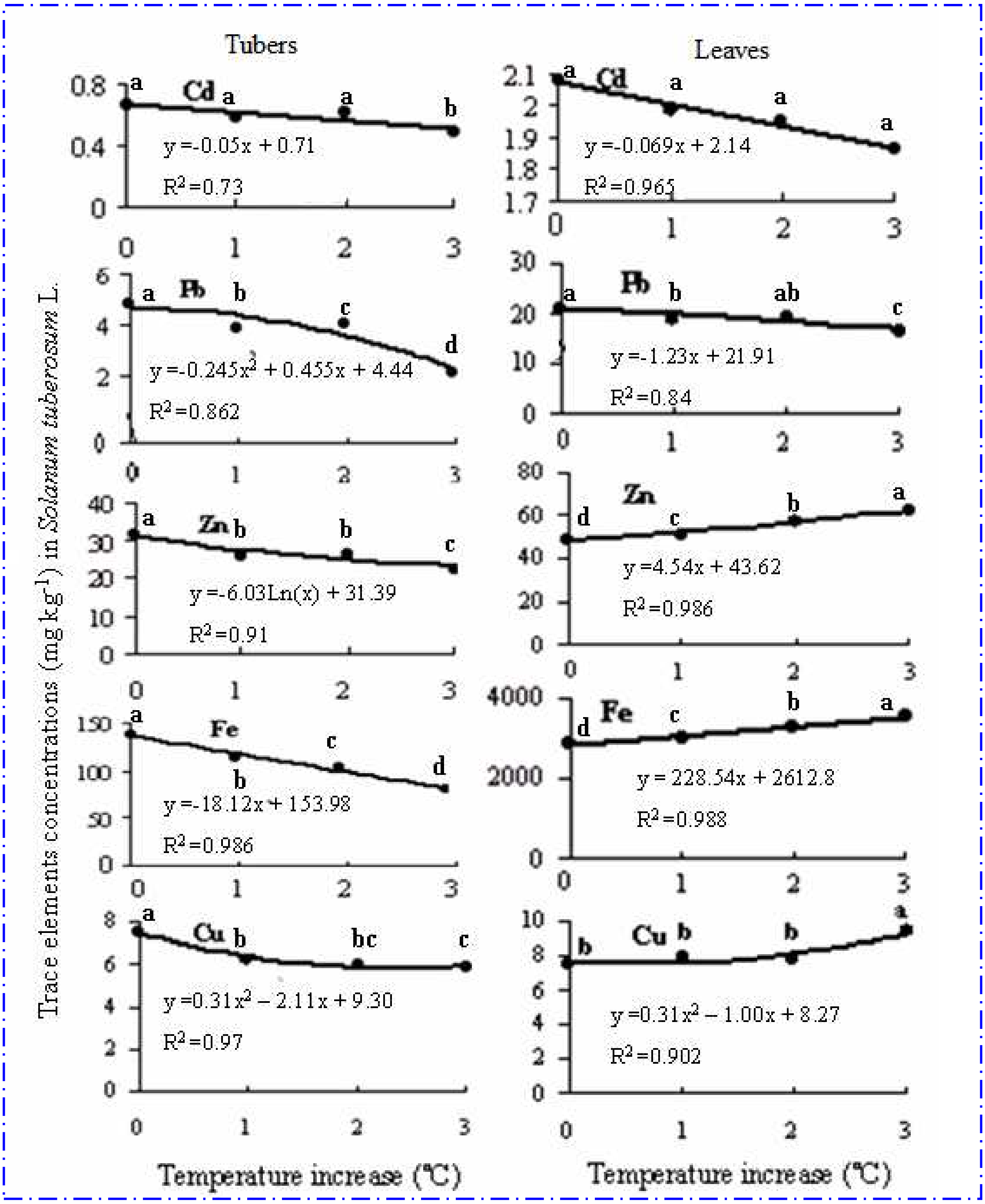

***Figure 3.** Relationship between trace element concentrations (mg/kg) in potato and temperature increase in two different tissues (tubers and leaves).*

Different letters indicate significant differences between treatments ($p < 0.05$, post-hoc Bonferroni–Dunn test, ANOVA). A simulated warming experiment was conducted to evaluate the effects of temperature increase on Cd, Pb, Cu, iron (Fe) and Zn accumulation in potato at Dingxi, Gansu, in semi-arid Northwest China. The aim of this study was to determine if temperature increases significantly changed trace element concentrations and contents in the different ecosystem compartments by affecting soil content, soil solubility and plant capture. The results revealed that an increase in temperature led to a significant change in trace element concentrations and contents in potato. The strongest effects of a 3°C temperature

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increase were a rise in Cu, Zn and Fe leaf concentrations of 25, 27 and 24%, respectively; but decreases in Cd, Pb, Fe, Zn and Cu tuber concentrations of 27, 55, 41, 29 and 23%, respectively. The increasing concentrations of some trace elements in potato leaves were related to greater retranslocation, photosynthetic capacity and growth. Warming decreased the concentrations of some trace elements in tubers, implying that tuber growth rate exceeded its metal uptake rate at higher temperatures. It is expected that by the year 2050, the increased temperatures will have caused a decrease in the concentrations of Cd, Pb, Cu, Fe and Zn of 9.1, 11.5, 18.5, 16.8 and 15.8%, respectively, in tubers in the study area. In addition, the results indicated that a 1–3°C increase in temperature will improve the availability of selected trace elements and the transfer potential of these elements from soil to potato (Li et al, 2012).

Threat to food nutrition

Changes in temperature, drought and rainfall modify the inputs of chemical fertilizer and pesticides, degrade soil quality and increase crop pest problems (Zhang et al., 2015a). Climate warming also modifies the absorption of trace and heavy metal elements and causes a lack of nutrients and excessive uptake of toxic elements, such as aluminum and Mn. This decreases the distributed proportions of photosynthetic products in stems, leaves and fruits and directly reduces food nutritional value (Lu et al., 2015). It is estimated that if temperature rises by 1 °C, the starch content in spring wheat in Northwest China will decrease by 1.6% and protein content will increase by 0.8% (Li et al., 2011). If the temperature rises by 1.5–2.5 °C, the dry matter, starch and vitamin C contents in potato will increase, but the content of protein and reducing sugars will significantly decrease (*Fig. 4*). High temperatures and dry hot winds will increase the spread of pests and threaten food security.

Increased crop yield fluctuations

Climate warming will modify the physiology and ecology of crops in Northwest China, accelerate crop growth and seriously affect crop yield (Chen et al., 2015). During 1980–2010, the yield of spring wheat in Northwest China was generally reduced by climate warming and the size of the yield reduction was larger in the rain-fed than the irrigated farming area. If the temperature increases by 1 °C, the yield of spring wheat in semi-arid rain-fed farming areas will fall by 15–30% and the yield of corn will fall by 3% (Wang, 2009). In the semi-arid rain-fed area of Tongwei and Dingxi, within 34°55′–35°26′, spring wheat has been replaced by winter wheat in recent years and pea is no longer grown (Xiao et al., 2008). If temperature rises by 0.5–2.5°C, the yield of spring wheat will decrease by 16.5–18.5%. If temperature rises by 0.6–2.2°C, the yield of pea in the rain-fed farming area will decrease by 6.3–17.5% and if the rise is 0.5–2.0°C, the yield of pea–spring wheat–potato rotation crops will decrease by 3.2–9.4% (Xiao et al., 2009). It is estimated that over the next 50 years, the threat to grain security in the arid and semi-arid areas of Northwest China will significantly increase.

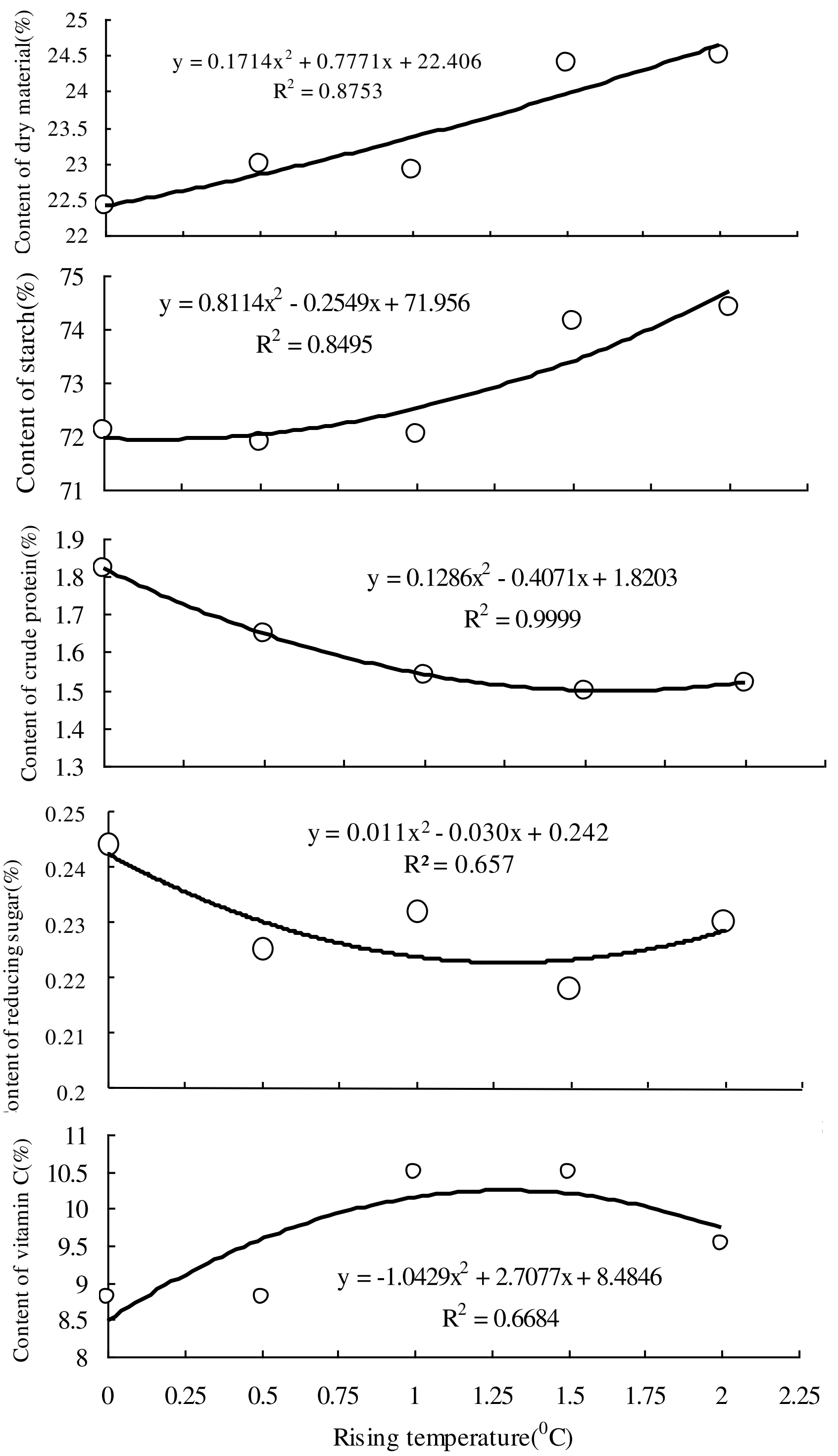

Figure 4. *Impact of rising temperature on the main component content of the potato blocks.*

This experimental study on simulated farm warming using infrared radiators showed that as temperature increased, the dry material and starch content in potato tubers rose significantly, but crude protein and reducing sugar content significantly decreased. Vitamin C first increased and then declined. When temperature increased by 0.5–2.0°C, the dry material content in potato increased from 22.4 to 24.5%, and *starch content increased from 72.1 to 74.4%, suggesting that warming favored the accumulation of dry material and starch in potato. When temperature increased by 0.5–2.0°C, the crude protein content in potato decreased from 1.82 to 1.52% and the reducing sugar content decreased from 0.24 to 0.22%, showing that warming negative affected crude protein and reducing sugar contents. When temperature increased by < 1.5°C, the vitamin C content in potato increased; however, when temperature increased by > 1.5°C, it decreased. Thus, warming also had an unfavorable influence on vitamin C content (Xiao et al, 2013).*

Increased investment in grain production

Climate warming decreases soil fertility, and so demand for fertile land for growing crops will increase. Climate warming will increase soil moisture evaporation and plant transpiration and so a greater water resources will be needed (Liu and Cui, 2011). It will also increase salinity and high pH damage to the soil farming layer and create difficulties when trying to improve saline and high pH soils. Finally, climate warming will greatly increase the risk index of pests during the crop growing period, especially in spring. All these factors mean that climate warming will significantly increase the cost of grain production (Zhang et al., 2015b).

Conclusion and outlook

The development of high thermal requirements, high yield potential and heat-tolerant crop varieties, together with improvements in agronomic management, should be encouraged to meet the challenges of climate change and increasing food demand in future. Taking scientific measures to actively keep pace with climate change will be a key strategy for reducing the risk to grain security in Northwest China. To this end, these regions need to implement crop planting structure strategies, such as ‘Winter Wheat Northward’, ‘Compression of Summer Crop Planting and Expansion of Autumn Crop Planting’ and ‘Multiple Cropping’ to promote the adjustment of agricultural structure and cropping systems needed to enhance the level of food production. There is a need to actively promote study of the impact of climate change on food security to be able to provide an accurate scientific basis for responding to food security threats. Priority needs to be given to water since the stability of ecosystems will be reduced by global climate change and this will exacerbate the complexity of water resource problems (Xiao et al., 2007a; Zhang et al., 2015c). Drought disaster events in northwestern regions have occurred more frequently and the severity of hail, thunderstorms, violent floods and dry, hot winds has increased. It is essential to establish prediction and warning technology for extreme weather events in order to enhance the ability to respond to such events and reduce the impact of future natural disasters (Yang et al., 2015).

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REFERENCES

[1] Cao, L., Deng, Z., Dou, Y., Yang, X. (2008): Influence of Climate Warming on Corn Output in Hexi Corridor Oasis Irrigation Area and Its Countermeasure Research. - Acta Bot. Boreal. Occident. Sin. 28(5): 1043-1048.

[2] Chang, J. X., Wang, Y. M., Istanbulluoglu, E., Bai, T., Huang, Q., Yang, D. W., Huang, S. Z. (2015): Impact of climate change and human activities on runoff in the Weihe River Basin, China. - Quaternary International 380: 169-179.

[3] Chen, C., Zhou, G. S., Pang, Y. M. (2015): Impacts of climate change on maize and winter wheat yields in China from 1961 to 2010 based on provincial data. - Journal of Agricultural Science 153(5): 825-836.

[4] Dai, A. (2011): Drought under global warming: areview. - Climatic Change 2:45-65.

[5] Deng, Z., Zhang, Q., Wang, Q., Wang, R., Wang, J., Wang, H., Xu, J. (2010): Effects of soil water storage capacity and consumption on winter wheat water use efficiency in dry land areas of the Loess Plateau in western China. - Acta Ecologica Sinica 30(4): 3672-3678.

[6] Deng, Z., Zhang, Q., Wang, R., Zhao, H., Xu, J., Liu, M., Zhao, H.,Yao, Y. (2012): The Response of plant diseases and pests to climatic warmer-drying and its adaptive technique in the Northwest China. - Advances in Earth Science 27(1): 1281-1287.

[7] Du, L., Li, Y., Wang, Z. (2011): Analysis on Response of the Winter Wheat Growth to Global Climate Change in Northwest China. - Journal of Shanxi Agricultural Sciences 39(8): 872- 876.

[8] Gao, L., Zhang, Y. N. (2016): Spatio-temporal variation of hydrological drought under climate change during the period 1960-2013 in the Hexi Corridor, China. - Journal of Arid Land 8(2): 157-171.

[9] Gao, M., Fan, J., Wu, Q., Jing, Y. (2012): The change of climate and climate productivity in Weibei plateau under global climate change. - Agricultural Research in the Arid Areas 30(4): 178-183.

[10] Gou, S., Zhang, Y., Xu, Y. (2012): Analysis of climate resource changes during maize growth period in Ningxia under SRES A1B scenario. - Chinese Journal of Eco-Agriculture 20(10):1394-1403.

[11] Ponce-Campos, G. E., Moran, M. S., Huete, A., Zhang, Y., Bresloff, C., Huxman, T. E., Eamus, D., Bosch, D. D., Buda, A. R., Gunter, S. A., Scalley, T. H., Kitchen, S. G., McClaran, M. P., McNab, W. H., Montoya, D. S., Morgan, J. A., Peters, D. P. C., Sadler, E. J., Seyfried, M. S., Starks, P. J. (2013): Ecosystem resilience despite large-scale altered hydroclimatic conditions. - Nature 470(1):1-4.

[12] Hou, Q., Guo, R., Yang, L. (2009): Climate Change and Its Impact on Main Crops in Inner Mongolia. - Chinese Journal of Agrometeorology 30(4): 560-564

[13] Lei, Y. D., Zhang, H. L., Chen, F., Zhang, L. B. (2016): How rural land use management facilitates drought risk adaptation in a changing climate, A case study in arid northern China. - Science of the total Environment 550: 192-199.

[14] Leng, G. Y., Tang, Q. H., Rayburg, S. (2015): Climate change impacts on meteorological, agricultural and hydrological droughts in China. - Global and Planetary Change 126: 23-34.

[15] Li, J-P., Yang, K., Cao, N., Han, Y-J., Zhang, X-Y. (2009): Simulation of Changes of Potato Yields under Different Climate Change Scenarios in Ningxia. - Chinese Journal of Agrometeorology 30(3): 407-412.

[16] Li, Y., Li, L., Zhang, Q., Yang, Y., Wang, H., Wang, R., Zhang, J. (2013): Influence of temperature on the heavy metals accumulation of five vegetable species in semiarid area of northwest China. - Chemistry and Ecology 29(4): 353-365.

[17] Li, Y., Zhang, Q., Wang, R., Gou, X., Wang, H., Wang, S. (2012): Temperature changes the dynamics of trace element accumulation in Solanum tuberosum L. - Change 112(3): 655-672.

[18] Li, Y., Zhang, Q., Wang, R., Liu, N., Xiao, G., Gou, X., Ma, Z. (2011): Influence of climatic warming on accumulation of trace elements in spring wheat. - Transactions of the CSAE 27(12): 96-104.

[19] Liu, D., Dong, A., Liang, D., Ning, H. (2007): Affect of Climate Warming on Crops Planting Structure in Arid Zone of Northwestern China. - Journal of Desert Research 27(5): 831-83.。

[20] Liu, M., Jiang, J., Shi, Z. (2009): Meteorological impact factors and forecast of wheat aphid population dynamic changes. - Journal of Agrometeorology 30(3):440-444.

[21] Liu, Q. A., Cui, B. S. (2011): Impacts of climate change/variability on the streamflow in the Yellow River Basin, China. - Ecological Modelling 222(2): 268-274.

[22] Liu, Y., Zhang, X., Liu, J., Kang, Y. (2008a): Impact of Climate Change on Spring Wheat Yield in Yellow River Irrigation Region of Ningxia. - Advances in Climate Chang Research 4(2):90-94.。

[23] Liu, Y., Zhang, X., Liu, J., Kang, Y. (2008b): Impact of Climate Warming on Maize Yield In Irrigation Area of Ningxia. - Journal of Maize Sciences 16(2):147-149.。

[24] Lu, Y. L., Chadwick, D., Nors, D., Powlsond, D., Shi, W. M. (2015): Sustainable intensification of China's agriculture: the key role of nutrient management and climate change mitigation and adaptation. - Agriculture, Ecosystems & Environment 209(SI): 1-4.

[25] Saleska, S. R., Didan, K., Huete, A. R., da Rocha, H. R. (2007): Amazon forests green-up during 2005 drought. - Science 318: 612.

[26] Piao, S., Ciais, P., Huang, Y., Shen, Z., Peng, S., Li, J., Zhou, L., Liu, H., Ma, Y., Ding, Y., Friedlingstein, P., Liu, C., Tan, K., Yu, Y., Zhang, T., Fang, J. (2010): The impacts of climate change on water resources and agriculture in China. - Nature 467(2): 42-51.

[27] Song, Q., Qian, H., Lai, C. (2008): Research progress on the vulnerability evaluation of agricultural climate changes. - Journal of Anhui Agricultural Sciences 36(24): 9646-9649.

[28] Sun, F., Lin, E.,Wu, Y. (2008): Climate Change and Its Impacts on Potato Production in Ningxia. - Chinese Agricultural Science Bulletin 24(4):465-471.

[29] Tollefson, J. (2015): Climate change China backs cap-and-trade. - Nature 526(7571): 13-14.

[30] Wang, H. L., Gan, Y. T., Wang, R. Y. (2008): Phonological trends in winter wheat and spring cotton in response to climate changes in northwest China. - Agricultural and Forest Meteorology 148: 1242-1251.

[31] Wang, H., Niu, J., Wang, R., Lu, X. (2011a): Impact of climate change on water requirement of main crops in irrigated oasis of Hexi corridor. - Acta Prataculturae Sinica

20(5): 245-251.

[32] Wang, L., Li, F., Xu, W., Li, X., Su, W. (2011b): Climate Warming Impacts on Spring Wheat Growth at Different Altitude Regions in Qinghai Plateau. - Adv. Clim. Change Res. 7(5): 324-329.

[33] Wang, N., Zhang, M., Huang, B., Ma, C. (2009): Relationship Discussion between Soil Moisture Change with Maize Yield in East of Gansu Province. - Chinese Agricultural Science Bulletin 25(21): 320-323.

[34] Wang, R., Zhang, Q., Wang, Y. (2004): Response of Corn to Climate Warming in Arid Areas in Northwest China. - Acta Botanica Sinica 46 (12):1387-1392.

[35] Wang, Y-R., Yao, Y-B. (2005): Dynamics of Soil Moisture in Gansu Loess. - Chinese Journal o f Soil Science 36(3):850-855.

[36] Xiao, G., Zhang, Q., Zhang, F., Luo, C., Wang, R. (2011a): The impact of rising temperature on spring wheat production in the Yellow River irrigation region of Ningxia. - Acta Ecologica Sinica 31(21): 6588-6593.

[37] Xiao, G., Zhang, F., Qiu, Z., Yao, Y., Wang, R., Huang, J. (2013): Response to climate change for potato water use efficiency insemi-arid areas of China. - Agricultural Water Management 127: 119-123.

[38] Xiao, G., Zhang, Q., Bi, J., Zhang, F., Luo, C. (2012): The Relationship Between Winter Temperature Rise and Soil Fertility Properties. - Air, Soil and Water Research 5: 15–22.

[39] Xiao, G., Zhang, Q., Li, Y., Zhang, F., Wang, R., Luo, C. (2010): Impact of climatic warming on soil salinity and irrigation amount of Yellow River irrigation areas in Ningxia Hui Autonomous Region. - Transactions of the CSAE 26(6): 7-13.

[40] Xiao, G., Zhang, Q., Li, Yu (2011b): Effect of winter warming on soil moisture and salinization. - Transactions of the CSAE 27(8): 46-51.

[41] Xiao, G., Zhang, Q., Wang, R., Yao, Y., Zhao, H., Bai, H., Xiong, Y. (2009): Effects of temperature increase on pea production in a semiarid region of China. - Air, Soil and Water Research 2: 31-39.

[42] Xiao, G., Zhang, Q., Xiong, Y., Lin, M., Wang, J. (2007a): Integrating rainwater harvesting with supplemental irrigation into rain-fed spring wheat farming. - Soil & Tillage Research 93: 429-437.

[43] Xiao, G., Zhang, Q., Xiong, Y., Yang, X., Yang, Q., Sun, Z. (2007b): Effects of temperature increase on water use and total yields of crops in a pea–spring wheat–potato rotation system in semiarid regions of China. - Agricultural Water Management 91: 86-91.

[44] Xiao, G., Zhang, Q., Yao, Y., Zhao, H., Wang, R., Bai, H., Zhang, F. (2008): Impacts of recent climatic change on the yields of winter wheat at different altitudes above sea level in semi-arid northwestern China. - Agriculture, Ecosystems & Environment 127: 37-42.

[45] Yang, Q., Chen, X., Xu, Y., Lin, E., Xiong, W. (2009): Response Simulation of Spring Wheat in Ningxia Region to Climate Change Scenario. - Journal of Triticeae Crops 29(3):491-496.。

[46] Yang, S., Mu, W., Xiao, G. (2010): The Temperature Change and Its Influences on Agricultural Production in Weibei Dry land. - Chinese Agricultural Science Bulletin 26(15): 109-114.

[47] Yang, X. G., Chen, F., Lin, X. M., Liu, Z. J., Zhang, H. L., Zhao, J., Li, K. N., Ye, Q., Li, Y., Lv, S. (2015): Potential benefits of climate change for crop productivity in China. - Agricultural and Forest Meteorology 208: 76-84.

[48] Yao, X., Pu, J., Yao, R., Jia, H., Ma, J. (2011a): Variation of Climate Suitability of Maize

in Arid Area in Gansu under the Condition of Climate Dry-warming. - Acta Geographica Sinica 66(1):59-70.

[49] Yao, Y. B., Wang, R. Y., Yang, J. H., Xiao, G. J., Zhang, X. Y. (2011b): Impacts of climatic change on spring wheat growth in a semi-arid region of the Loess Plateau: a case study in Dingxi, Gansu Province. - Acta Ecologica Sinica 31(15): 4225-4234.

[50] Yao, Y., Deng, Z., Wang, R., Wang, Y., Pu, J., Zhang, X. (2006): The influence of climate changes on potato growth in Gansu province. - Agricultural Research in the Arid Areas 24(3): 16-24.

[51] Yao, Y., Wang, R., Deng, Z., Han, S., Xing, T. (2010a): Effects of climate change on potato growth in semi-arid region of Loess Plateau, China. - Chinese Journal of Applied Ecology 21(2):287-295.

[52] Yao, Y., Wang, R.,Yang, J., Xiao, G., Zhang, X. (2011c): Impacts of climatic change on spring wheat growth in a semi-arid region of the Loess Plateau: a case study in Dingxi, Gansu Province. - Acta Ecologica Sinica 31(15):4225-4234.

[53] Yao, Y., Wang, R.,Yang, J., Yue, P., Lu, D., Xiao, G., Wang, Y., Liu, L. (2011c): Change of terrestrial surface potential evapotranspiration in Loess Plateau. - Ecology and Environment 20(8-9): 1189-1195.

[54] Yao, Y., Zhang, X., Wang, R., Deng, Z., Lu, H. (2010b): Impacts of climatic change on potato growth in the semi-humid region over extra-tropical and cool Northwest of China: a case study in Minxian County of Gansu Province. - Acta Ecologica Sinica 30(1):0100-0108.

[55] Yao, Y., Wang, R., Yang, J., Zhang, M., Yue, P., Xiao, G. (2012): Impacts of climate change on growth and yield of winter wheat in the semi-humid region of the Loess Plateau. - Acta Ecologica Sinica 32(16):5154-5163.

[56] Yao, Y., Zhang, X., Lu, H., Han, H., Li, X., Zhang, S. (2009): Influence of Meteorological Conditions on Potato Tuber Formation and Yield in Temperate and Cooi Semi-humid Regions of Northwest China. - Chinese Journal of Agrometeorology 30(2):208-211.

[57] Ye, J-B., Xiao, Z-L., Li, C-H., Wang, F-S., Liao, J-C., Fu, J-Z., Zhang, Z-B. (2015): Past climate change and recent anthropogenic activities affect genetic structure and population demography of the greater long-tailed hamster in northern China. - Integrative Zoology 10(5): 482-496.

[58] Zhang, C., Lai, S-Y., Gao, X- P., Xu, L- P. (2015a): Potential impacts of climate change on water quality in a shallow reservoir in China. - Environmental Science and Pollution Research 22(19): 14971-14982.

[59] Zhang, H-L., Zhao, X., Yin, X-G., Liu, S-L., Xue, J-F., Wang, M., Pu, C., Lal, R., Chen, F. (2015b): Challenges and adaptations of farming to climate change in the North China Plain. - Climatic Change 129: 213-224.

[60] Zhang, J., Li, Y. (2001): Three climatic stages of cotton bollworm living through winter in Xinjiang. - Journal of Agrometeorology 22(1):43-46.

[61] Zhang, Q., Deng, Z-Y., Zhao, Y-D., Qiao, J. (2008): The impacts of global climatic change on the agriculture in northwest China. - Acta Ecologica Sinica 28(3): 1210-1218.。

[62] Zhang, Q., Zhang, C., Bai, H., Li, L., Sun, L., Liu, D., Wang, J., Zhao, H. (2010): New Development of Climate Change in Northwest China and Its Impact on Arid Environment. - Journal of AridMeteorology 28-1:1-7.

[63] Zhang, S., Pu, Z., Song, L., Zhou, H., Song, S. (2009): Effect of Climate Change on Potential Evapotranspiration in Turpan Region. - Chinese Journal of Agrometeorology 30

(4): 532 – 537.

[64] Zhang, Y-Y., Fu, G-B., Sun, B-Y., Zhang, S- F., Men, B-H. (2015c): Simulation and classification of the impacts of projected climate change on flow regimes in the arid Hexi Corridor of Northwest China. - Journal of Geophysical Research-Atmospheres 120(15):7429-7453.

[65] Zhang, Z., Lin, L., Liang, P. (2008): Climate Change and its Impacts on Agricultural Production in Ningxia. - Chinese Journal of Agrometeorology 29(4):402-405.

[66] Zhao, H., Wang, R-Y., Wang, H., Yang, Q., Deng, Z-Y., Xiao, G. (2007a): Regional Diversity in Response of Spring Wheat Growth on Climate Change in Arid or Semiarid Areas. - Advances in Earth Science 22(6):336-641.

[67] Zhao, H., Xiao, G., Wang, R., Deng, Z., Wang, H., Yang, Q. (2007b): Impact of Climate Change on Spring Wheat Growth in Semi-Arid Rain Feed Region. - Advances in Earth Science 22(3):322-327.

[68] Zhao, H., Sun, G., Wang, R. (2005): An analysis of the relation between occurrence of wheat aphid and climatically dynamics in Hedong over 20 years. - Advances in Earth Science 20:95-99.

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FACTORS GOVERNING THE NATURALIZATION-TO-INVASION TRANSITION OF EXOTIC PLANTS IN SHENZHEN, CHINA

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Abstract. Understanding factors increasing the probability of exotic plants becoming invasive is crucial for designing appropriate management strategies to mitigate the detrimental effects of invasive plants on rapidly urbanizing areas. However, no study to date has attempted to determine how various factors may affect the naturalization-to-invasion transition of exotic plants in these areas. Here, we took Shenzhen, one of the largest and most rapidly urbanizing cities of the world, as a case study to explore how various factors may affect the naturalization-to-invasion transition of exotic plants in rapidly urbanizing areas, with an emphasis on exotic plant invasion in urban forests. Our results showed that 33.5% of the 349 naturalized exotic plant species in Shenzhen have become invasive. We found strong evidence that plant attributes and environmental factors have considerable effects on the naturalization-to-invasion transition of exotic plant species in Shenzhen. In contrast, there was little evidence that any socio-economic factor promotes the naturalization-to-invasion transition of exotic plants in Shenzhen. Our findings suggested that although human activities facilitated the initial establishment of exotic plant species in rapidly urbanizing areas, the naturalization-to-invasion transition of exotic plants in these areas appeared to be governed largely by biotic and environmental factors.

Keywords: *exotic plant invasion, determinants of naturalization-invasion transition, invasive plant, naturalized plant, urban forests*

Introduction

Biological invasions by exotic species are a significant component of human-caused global environmental change (Vitousek et al., 1997). More than 2,000 exotic plant species have been recorded in the USA (Vitousek et al., 1997); this figure reaches 5,700 in Europe (Vilà et al., 2010). Furthermore, biological invasions can not only cause disastrous damage to native biodiversity and ecosystem functions (Vilà et al., 2011) but also seriously affect the development of economies and societies (Perrings et al., 2002). In China, biological invasions have been estimated to cause US$ 14 billion worth of economic losses per year (Xu et al., 2006); a similar estimate for the USA reaches US$ 120 billion (Pimentel et al., 2005). Thus, there is an urgent need to understand which

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combinations of species attributes and habitat characteristics most effectively promote or prevent exotic plant invasion (Kolar and Lodge, 2001; Romanuk et al., 2009).

Urbanization, a major global trend (United Nations Population Division, 2006), is always associated with the establishment of a large number of exotic plant species in urban habitats (Pauchard and Alaback, 2004), which can ultimately decrease the ecological, social and economic sustainability of cities. Given that over 50% of the world's population now lives in cities (United Nations Population Division, 2016), a better understanding of patterns and drivers of exotic plant invasion in urban habitats is critical for the sustainability of not only cities but also the human population. Unfortunately, we have a relatively poor understanding of these aspects, largely because most previous research on exotic plant invasion has focused instead on natural or semi-natural habitats (McKinney, 2002; Pauchard et al., 2006; Spear et al., 2013).

Only a few studies have been performed to address the issue of exotic plant invasion in urban habitats (Celesti-Grapow et al., 2006; Dolan et al., 2011; Leung et al., 2009; Lososová et al., 2012; Pyšek, 1998; Ricotta et al., 2009; Ricotta et al., 2010; Wang et al., 2011). The invasiveness of exotic plant species in urban habitats was found to be related to their phylogenetic similarity to native plant species (Ricotta et al., 2010), and the species richness of exotic plants in cities was significantly affected by city size, habitat type and climate (Celesti-Grapow et al., 2006; Lososová et al., 2012; Pyšek, 1998). In addition, the number of naturalized and invasive plant species in a given district of a rapidly urbanizing city was found to be highly predicted by the economic growth of that district (Wang et al., 2011). These findings provide useful insights, but they are inadequate for developing appropriate management strategies to mitigate the detrimental effects of exotic plant invasions in cities experiencing rapid urbanization because none of the previous studies was focused on urban forest despite the fact that urban forest is a major type of habitat in cities. According to the definition proposed by Escobedo and colleagues, urban forests are the sum of all urban trees, shrubs, lawns, and pervious soils. Urban forests are generally considered as non-natural habitats, because they are located in highly altered ecosystems where humans are the main factors determining their types, amounts, and distribution (Escobedo et al., 2011). Unlike other urban habitats, such as roadsides, private gardens and parks (which can serve as important ways of exotic species introductions), urban forests are part of land use in cities (Escobedo et al., 2011; Dobbs et al., 2011). For example, urban forest currently accounts for 35.1% (Nowak et al., 2010) and 39.9% (Chen and Wang, 2013) of land use in cities in the USA and China, respectively. However, there is emerging evidence that urban forests do not escape exotic plant invasion (Singh et al., 2015).

The transition from naturalization to invasion has been considered the most important step for exotic plant species becoming serious ecological, economic and social problems (Phillips et al., 2010; Williamson and Fitter, 1996), although the exotic plant invasion process is always an introduction-naturalization-invasion continuum (Richardson et al., 2000; Richardson and Pyšek, 2006). In this sense, a promising approach to improve our understanding of exotic plant invasion in urban habitats is to explore the transition from naturalization to invasion of exotic plants in urban forests and its driving factors. However, to date, no study has attempted to directly address this issue, which constitutes a critical gap in research on exotic plant invasion in urban habitats. According to the Tens Rule proposed by Williamson and Fitter (Williamson and Fitter, 1996), approximately 10% of the exotic plant species naturalized in urban habitats will become invasive. However, we would expect the real invasive-to-naturalized plant species richness ratios observed in urban habitats to be greater than 10% because urban habitats are generally exposed to higher propagule pressure from exotic plants, stronger human disturbances, and larger and more frequent resource fluctuations than those more natural habitats on which the Tens Rule was based (Lososová et al., 2012).

In this study, we took Shenzhen as a case study to explore how various factors may affect the naturalization-to-invasion transition of exotic plants in cities experiencing rapid urbanization, with an emphasis on plant invasion in urban forests. To this end, we first generated a comprehensive database of all naturalized and invasive exotic plant species in Shenzhen and then used it to assess the effects of the plants' attributes on their transition from naturalization to invasion. Additionally, we conducted a series of ecological field surveys on 19 major urban forests in Shenzhen to determine the effects of environmental and socio-economic factors on the naturalization-to-invasion transition of the exotic plant species found in urban forests. We considered Shenzhen to be fairly representative of other areas experiencing rapid urbanization, as it is one of the largest cities in the world and has been experiencing rapid urbanization over the past 30 years (McKinsey Global Institute, 2012).

Materials and Methods

Study site

Located in South China, Shenzhen is a natural port city and functions as a bridge between Hong Kong and mainland China (*Fig. 1*). As China's first special economic zone, it has experienced rapid urbanization over the past 30 years, and its gross domestic product (GDP) has been growing at an average annual rate higher than 20% over that period (Liu et al., 2007). In 2013, Shenzhen's GDP reached US$ 234 billion, and its total population reached 10.5 million (Statistics Bureau of Shenzhen Municipality, 2013). Shenzhen also plays an important role in China's foreign trade, with a total import and export volume reaching US$ 537 billion in 2013 (Statistics Bureau of Shenzhen Municipality, 2013). Although this rapid urbanization has increased people's wealth and improved their quality of life, it has also had significant environmental impacts (Liu et al., 2007). For example, Shenzhen has been considered one of the areas at highest risk of biological invasions in China (Wu et al., 2006), as indicated by the fact that the number of exotic species intercepted by the Shenzhen Entry-Exit Inspection and Quarantine Bureau in 2010 was as high as 981 (SEEIQB, 2010). Indeed, the number of exotic species in Shenzhen had reached 102 approximately ten years ago (Yan et al., 2004).

Compilation of a database for naturalized and invasive plant species in Shenzhen

In this study, the term 'naturalized species' was used according to the definition of Richardson et al. (Richardson et al., 2000), whereas the term 'invasive species' was used in the sense of McNeely et al. (McNeely et al., 2001). In brief, here, invasive plant species are a subset of naturalized exotic plant species that cause damage to species, habitats, or to the economy. The published literature addressing naturalized and invasive species in Shenzhen or in China (Yan et al., 2004; Feng and Zhu, 2010; Jiang et al., 2011; Xu and Qiang, 2011; Shao et al., 2006; Wan et al., 2012; Weber et al., 2008) was reviewed to generate a comprehensive database of the naturalized and invasive exotic plant species in Shenzhen. The major attributes (including taxonomic group, geographic origin and life form) of the exotic plant species were documented largely according to *Flora of China* (2013) and *Flora of Shenzhen* (Li and Li, 2010).

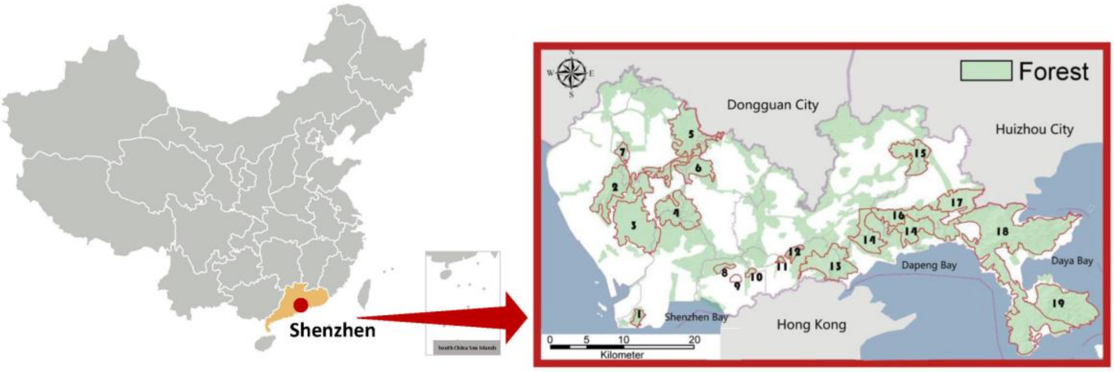

Figure 1. *Map of the study sites. The bold Arabic numbers indicate 19 major urban forests, which were investigated to explore the effects of environmental and socio-economic factors on the naturalization-to-invasion transition of exotic plants in Shenzhen. Red curves show the edges of the studied forests. See Tables S1, S2 and S3 for more details. This figure was drawn based on two maps that are open to the public: the maps of China and Shenzhen can be accessed on http://www.sbsm.gov.cn/article/ zxbs/dtfw/ and http://www.szgeoinfo.com/szgeoinfo/default.htm, respectively.*

Ecological field surveys on 19 major urban forests of Shenzhen

Forest is one of the most important types of urban land cover in Shenzhen (*Fig. 1*). Specifically, Shenzhen's urban forests cover 77,720.9 hectares, which account for 39.9% of Shenzhen Municipality's total area (Shenzhen statistics yearbook, 2013). To determine the effects of environmental and socio-economic factors on the naturalization-to-invasion transition of the exotic plants in urban forests, 19 important urban forests within Shenzhen were selected for surveying (*Fig. 1*). These forests are distributed across the city (*Fig. 1*) and their summed area accounts for 64.4% of the total forest area in the city (*Table 1*). During the ecological field surveys, data were collected from 217 plots, each having an area of 600 m^2 (30 m × 20 m). The number of plots within each urban forest ranged from 4 to 19, depending largely on their area. Replicate plots for each forest were located largely along a specific altitudinal gradient, which was set according to the altitude of individual forests. Plots were typically established on a relatively gentle slope to facilitate access. In each plot, the identity of the plants was recorded and 5 soil samples were collected. The plant species list resulting from the field surveys was used to assess the number of naturalized and invasive exotic plant species in the individual forests. The soil samples were air-dried and used to determine the following physico-chemical properties (Schinner et al., 1996): pH, density, porosity, moisture content, organic matter content, total nitrogen and available phosphorus (*Table 1* and *Table S1* in Appendix).

Table 1. *Descriptive statistics of the environmental and socio-economic factors under consideration in this study.*

Factors*	Unit	Mean	Min	Max
Environmental factors				
Area	ha	2,634	87.3	14,457
Average altitude	m	159	57.4	326
Mean January temperature	ºC	13.6	9.8	14.4
Mean July temperature	ºC	28.4	23.9	29.5
Annual average precipitation	mm	1,617	1,311	1,901
Density of native plant species	species/ha	2.39	0.76	3.78
Soil density	g/cm^3	1.32	0.82	1.86
Soil porosity	%	48.2	40.0	58.7
Soil moisture	%	11.0	1.32	24.3
Soil pH		5.05	4.14	6.39
Soil organic matter content	g/kg	14.3	5.51	38.0
Soil total nitrogen content	g/kg	0.64	0.15	1.59
Soil available phosphorus content	g/kg	3.52	0.39	9.30
Socio-economic factors				
Population density within a 1,000-m radius	person/ha	91.7	2.23	319
Road density within a 1,000-m radius	m/ha	49.6	8.58	125
Road area ratio within a 1,000-m radius	%	5.47	0.55	17.4
Residential land area ratio within a 1,000-m radius	%	11.4	0.96	38.3
Industrial land area ratio within a 1,000-m radius	%	5.06	0.00	13.4

*The relationships between these factors and the invasive/naturalized ratios of 19 major urban forests of Shenzhen were examined (unless otherwise stated).

Information on the environmental and socio-economic factors of 19 major urban forests of Shenzhen

Beyond the soil physico-chemical properties mentioned above, information on six additional environmental factors of the 19 major urban forests were collected (*Tables 1* and *S2*): area, average altitude, mean January temperature, mean July temperature, annual average precipitation, and the density of native plant species (i.e., the number of native plant species per hectare). These factors were selected because they are generally expected to have a marked influence on the richness of exotic plants (Wu et al., 2006; Zhang et al., 2004; Cutway and Ehrenfeld, 2009; Peacock et al., 2006). The data on mean January temperature, mean July temperature, and annual average precipitation were means for the period from 2007 to 2012, which were provided by the Meteorological Bureau of Shenzhen Municipality.

Data on the following five socio-economic factors were obtained from the Development Research Center of the Urban Planning, Land and Resources Commission of Shenzhen Municipality (*Tables 1* and *S3*): population density, road density, road area ratio, residential land area ratio and industrial land area ratio. These values were calculated for a buffer zone within a radius of 1,000 m from the edge of individual urban forests under study. These factors were selected because they are suitable indicators of disturbance resulting from socio-economic development and often have strong associations with exotic plant invasions (Pauchard and Alaback, 2004; Christen and Matlack, 2006; Pyšek et al., 2010; Santos et al., 2011). GDP was not included because the GDPs in the different districts of Shenzhen in the past five years were highly correlated with district population (R > 0.667, P < 0.01). The distance of 1,000 m to the edge of individual forests was selected because the propagule pressure of exotic plant species tends to be strongest within a few hundred meters of the source and declines rapidly with increasing distance (Vilà and Pujadas, 2011).

Statistical analysis

Tracking and characterizing the naturalization-to-invasion transition of many exotic plant species in a given area (e.g., a city) appears to be impractical, if not impossible. Therefore, we tried to circumvent this perceived difficulty by calculating the ratio of the number of invasive exotic plant species belonging to a given subset to the number of naturalized exotic plant species belonging to the subset (hereafter referred to as 'invasive/naturalized') and using it as a surrogate measure of the probability that the naturalized exotic plant species belonging to the subset will succeed in the naturalization-invasion transition. To assess the effects of plant attributes on the transition from naturalization to invasion of the exotic plants in Shenzhen, we calculated invasive/naturalized for subsets of the exotic plant species in Shenzhen with respect to their taxonomic group, geographic origin and life form. To explore the effects of environmental and socio-economic factors on the transition, we calculated invasive/naturalized for each of the 19 major urban forests under study and tested the correlations between these ratios and the environmental and socio-economic factors of the forests and their surroundings, respectively, using linear regression analysis. Note however that Tiantou Mountain was excluded in the analysis of relationships between these ratios and environmental factors, because the soil moisture and organic matter content of the forest deviated from the normal ranges (*Table S2*). All of the statistical analyses were performed using the statistical software package SPSS (Statistical Package for the Social Sciences) for Windows Version 17.0 (SPSS Inc., Chicago, IL, USA).

Results

An overview of naturalized and invasive exotic plant species in Shenzhen

We found 349 naturalized plant species in Shenzhen, including 343 angiosperm species, 5 pteridophyte species and 1 gymnosperm species (*Table S4*). They belong to 241 genera and 75 families, accounting for approximately 12.2% of the total number of plant species in Shenzhen. A total of 117 invasive plant species were identified in Shenzhen (*Table S5*), indicating that 33.5% of the exotic plants naturalized in Shenzhen have become invasive. The invasive plants were all angiosperms, belonging to 88 genera and 33 families (*Table S5*).

The effects of plant attributes on the naturalization-to-invasion transition of exotic plant species in Shenzhen

The invasive plant species in Shenzhen were highly unevenly distributed across taxonomic groups, geographic origins and life forms. The five families that contained the most invasive plant species were Asteraceae (also called Compositae), Fabaceae (Leguminosae), Poaceae (Gramineae), Amaranthaceae and Solanaceae (*Fig. 2*). Specifically, there were 35, 13, 7, 7 and 7 invasive species in these families, respectively (*Fig. 2a*), which accounted for 62.5%, 32.5%, 26.9%, 43.8% and 43.8% of the total number of naturalized exotic species in the corresponding families (*Fig. 2b*). The genera having the most invasive plant species were *Amaranthus* and *Ipomoea*, followed by *Senna*, *Solanum* and *Euphorbia* (*Fig. 3*). There were 5, 5, 3, 3 and 2 invasive species in each of these genera, respectively (*Fig. 3a*), which accounted for 83.3%, 45.4%, 42.9%, 33.3% and 60.0% of the total number of naturalized species in the corresponding genera (*Fig. 3b*). As for geographical origin, the majority of the invasive plants were native to the Americas, followed by Asia, Africa, Europe, the Mediterranean and Oceania (*Fig. 4a*). These areas contributed 90, 8, 8, 6, 2 and 2 invasive species, respectively (*Fig. 4a*), which accounted for 47.4%, 12.7%, 30.8%, 26.1%, 25.0% and 40.0% of the total number of naturalized species that had originated from the corresponding areas (*Fig. 4b*). Regarding life form, the invasive plants were mainly herbs (*Fig. 5a*). Specifically, more than 96 invasive plant species were herbs, accounting for 35.0% of the total number of herbaceous naturalized plant species in Shenzhen (*Fig. 5b*).

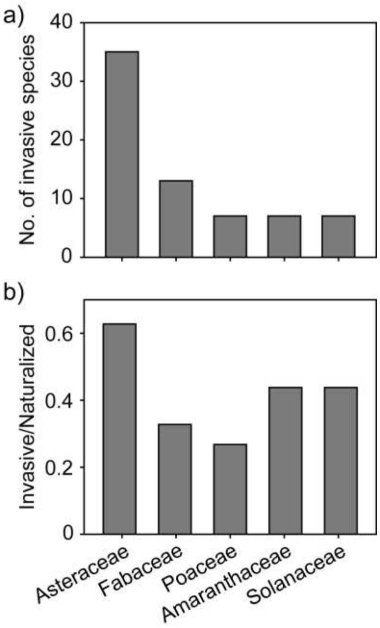

Figure 2. *The most successful families of exotic plant species in Shenzhen. The five families containing the most invasive plant species (a) and the ratios of invasive to naturalized species in these families (b).*

The effects of environmental and socio-economic factors on the naturalization-to-invasion transition of exotic plants in 19 major urban forests of Shenzhen

In the field surveys, we identified 316 naturalized plant species and 111 invasive plant species in the 19 major urban forests, which accounted for 90.5% and 94.9% of the total number of naturalized and invasive plant species in Shenzhen, respectively. The number of invasive plant species recorded in urban forests ranged from 19 to 69. In contrast, invasive/naturalized for these urban forests exhibited considerably less variation, ranging from 0.35 to 0.49. However, invasive/naturalized was significantly ($P < 0.05$) correlated with four environmental factors considered (*Fig. 6*). Specifically, the density of native plant species and soil density were negatively correlated with invasive/naturalized (*Figs. 6a* and *b*), whereas soil moisture and soil organic matter were positively correlated with invasive/naturalized (*Figs. 6c* and *d*). There was no significant relationship between any of the five socio-economic factors and invasive/naturalized.

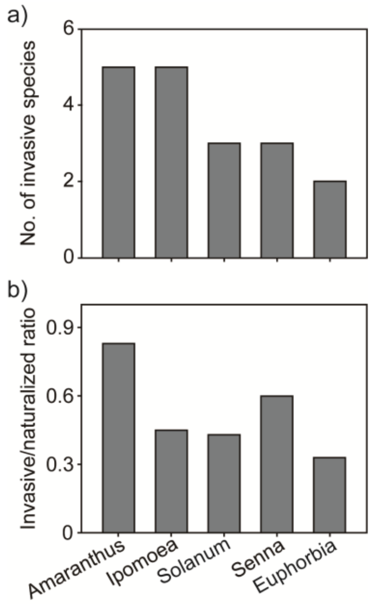

Figure 3. *The most successful genera of exotic plant species in Shenzhen. The five genera containing the most invasive plant species (a) and the ratios of invasive to naturalized species in these genera (b).*

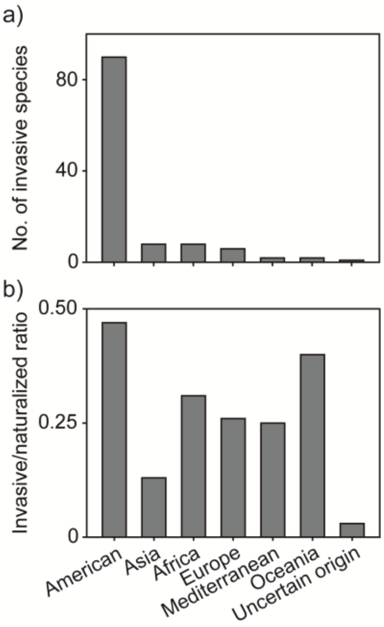

Figure 4. *Effects of geographic origin on the success of exotic plant species in Shenzhen. Effects of geographic origin on the number of invasive species (a) and the naturalization-to-invasion transition of exotic plant species (b) in Shenzhen.*

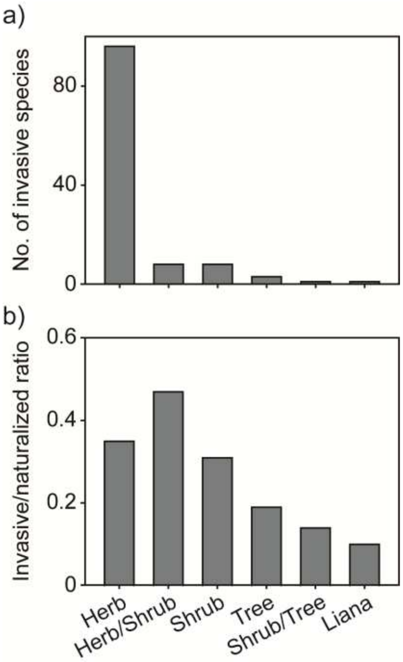

Figure 5. *Effects of life form on the success of exotic plant species in Shenzhen. Effects of life form on the number of invasive species (a) and the naturalization-to-invasion transition of exotic plant species in Shenzhen (b).*

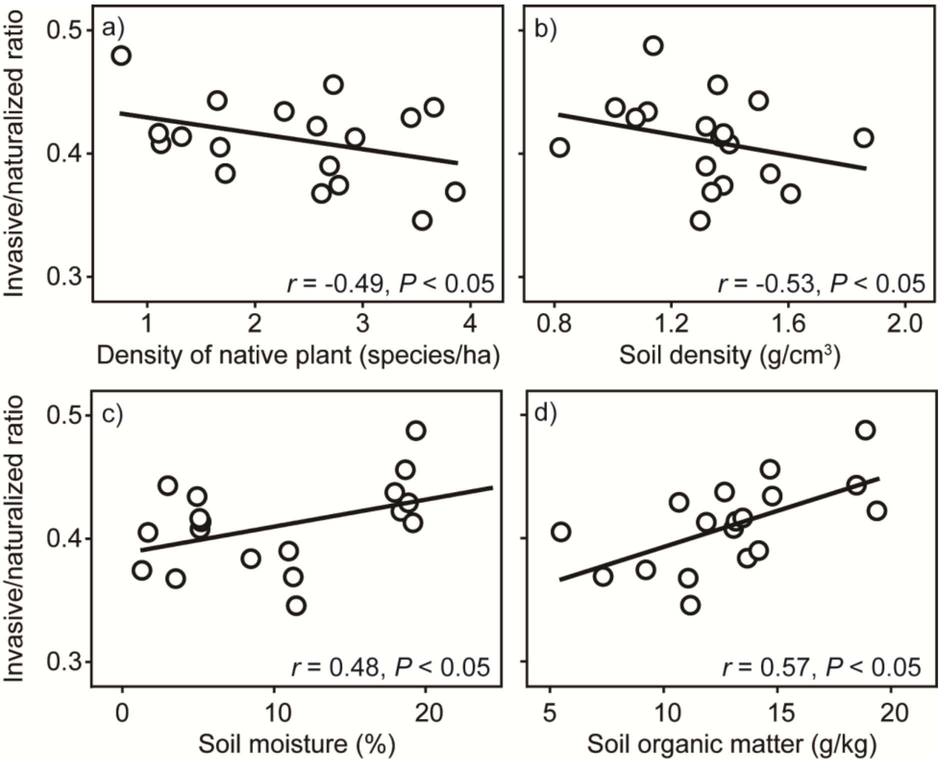

Figure 6. *Effects of environmental factors on the success of exotic plant species in Shenzhen. Effects of density of native plants (a), soil density (b), soil moisture (c) and soil organic matter (d) on the naturalization-to-invasion transition of exotic plant species in 18 major urban forests in Shenzhen. Each data point represents one forest.*

Discussion

In this study, 349 naturalized plant species were found in Shenzhen, representing 40.5% of all 861 naturalized plant species in China (Jiang et al., 2011). Moreover, the number of naturalized plant species in Shenzhen was considerably higher than that (112) in Beijing, another rapidly urbanizing city in China (Wang et al., 2011). The presence of this high number of naturalized plant species in Shenzhen made it a suitable city for studying the naturalization-to-invasion transition of exotic plant species in rapidly urbanizing areas. Indeed, 43.3% of all 270 invasive plant species in China (Weber et al., 2008) were present in Shenzhen.

As expected, the invasive/naturalized ratios recorded in this study far exceeded 0.1. This finding cannot be considered novel, given that Williamson and Fitter (Williamson and Fitter, 1996) had long noted that approximately 20% of exotic crop plant species naturalized in Canada became invasive. However, the naturalization-to-invasion transition of exotic plants in urban forests of rapidly urbanizing cities deserves more explicit attention. The invasive/naturalized ratios for urban forests (ranging from 0.35 to 0.49) observed in this study were comparable to those (with an average of 0.43) obtained in urban roadsides, parks and gardens in Beijing (Wang et al., 2011), possibly indicating that urban forests are not necessarily more resistant to exotic plant invasion than other urban habitats. Here, we have attempted to elucidate the reasons for these extreme invasive/naturalized values by investigating the effects of plant attributes and environmental and socio-economic factors on the naturalization-to-invasion transition of exotic plants in Shenzhen, although further studies are needed to test whether our finding is applicable to other rapidly urbanizing cities around the world.

The effects of plant attributes on the naturalization-to-invasion transition of exotic plant species in Shenzhen were mirrored not only by the highly uneven distribution of invasive species across taxonomic groups, geographic origins and life forms but also by the associated invasive/naturalized ratios. First, the Asteraceae family contained the highest number of invasive plant species (34), which accounted for 29.1% of all invasive plant species in Shenzhen and 12.6% of all invasive plant species in China (Weber et al., 2008). Moreover, invasive/naturalized in this family was 0.61, possibly suggesting that the naturalized members of this family are highly successful in the naturalization-to-invasion transition. Similarly, the Asteraceae family accounted for 31.3% of the total number of invasive plant species in Beijing, associated with an invasive/naturalized value of 0.75 (Wang et al., 2011). This performance may be partially attributed to the plant traits that contribute to the strong dispersal and establishment abilities of this family, such as a large number of small and light seeds, rapid germination and a high germination rate (Pyšek, 1997). At the genus level, *Amaranthus* was found to contain the highest number of invasive species (5) and show a high invasive/naturalized value of 0.83, which corresponds with the fact that many species in this genus have been reported as invasive species in other parts of the world and tend to germinate quickly, grow rapidly and produce more leaf area (Horak and Loughin, 2000; Steckel, 2007; Steckel et al., 2004). Second, 76.9% of the invasive species found in Shenzhen originated from the Americas, a higher percentage than that (70.8%) of Beijing (Wang et al., 2011). However, the percentages for these two Chinese cities were greater than that (68%) reported for China as a whole (Liu et al., 2006). Moreover, the invasive/naturalized ratio (0.47) for American plants was higher than those for other geographic origins. In fact, Wang et al. reported similar patterns in Beijing (Wang et al., 2011). Similar environmental conditions and the high volume of commerce between the invaded and native ranges were considered as possible explanations for the numerical predominance of American invasive plants and their high invasive/naturalized ratios, following the suggestions of previous work (Liu et al., 2006). Our subsequent analyses showed that the former was more likely to be a major reason (see the next paragraph for more details). Third, herbaceous species accounted for 82.1% of the total invasive species in Shenzhen, which exhibited an invasive/naturalized value (0.35) greater than expected from the Tens Rule (Williamson and Fitter, 1996). A similar pattern was observed in Beijing (Wang et al., 2011). Additionally, approximately 88% of all of the invasive plant species in China were herbaceous (Liu et al., 2006). These findings may be partially explained by the fact that many herbaceous invasive species are characterized by their rapid growth and reproduction (Sakai et al., 2001), which may facilitate the rapid evolutionary adaptation of these invasive plants to novel environments (Prentis et al., 2008).

We assessed the effects of environmental and socio-economic factors on the naturalization-to-invasion transition of exotic plants in urban forests of Shenzhen by identifying significant relationships between the selected factors and the invasive/naturalized ratios (Weber et al., 2008; Liu et al., 2006). The negative relationship between the density of native plant species and the invasive/naturalized ratio may indicate that native biodiversity represses the transition, which would be consistent with the widely accepted notion that biodiversity is a barrier to ecological invasion (Kennedy et al., 2002). Although there is currently no report addressing the effects of soil density on plant invasion, the negative relationship between soil density and invasive/naturalized may be attributed to the inhibitory effects of elevated soil density on the germination, growth and reproduction of plants (Ehrenfeld et al., 2005). In contrast, soil moisture and organic matter content were positively correlated with the invasive/naturalized ratio, likely indicating that wetter and richer soils tended to be beneficial to the transition. In agreement with these results, previous studies have shown that wetter and richer soils were associated with higher germination and survival rate of invasive plants, as well as a greater number of invader seeds (Ehrenfeld et al., 2001; Warren et al., 2013). The lack of significant associations between the socio-economic factors and the invasive/naturalized ratio was initially surprising. This unexpected finding, in combination with other results of the present study, instead suggests that although human activities facilitate the initial establishment of exotic plants, biotic and environmental factors are more likely to be the major drivers of their further spread and impacts (Stohlgren et al., 2005). However, the effects of human activities on plant invasion may vary greatly at different spatial scales. Indeed, there is evidence that socio-economic factors could promote plant invasion at a provincial scale in China (Lin et al., 2007; Liu et al., 2005).

There are a few potential caveats with this study. First, the invasive/naturalized ratio may be a good measure to assess the overall success of the naturalization-invasion transition of exotic plants in a given area, but it does not represent the long-term ecological processes associated with the transition. Second, as noted by Vilà and Pujadas (Vilà and Pujadas, 2011), correlations between variables do not necessarily imply causation. Third, some variables not under consideration in this study could have an effect on the transition (Phillips et al., 2010; Milbau and Stout, 2008). However, despite these caveats, this study provided new insights into the factors driving the naturalization-to-invasion transition of exotic plants in a rapidly urbanizing area, particularly for urban forests. Nonetheless, if the findings of this study prove to be applicable to other rapidly urbanizing areas, they will be useful in developing a more objective and accurate approach for managing and predicting plant invasions in these areas.

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REFERENCES

[1] Celesti-Grapow, L., Pyšek, P., Jarošík, V. and Blasi, C. (2006): Determinants of native and alien species richness in the urban flora of Rome. - Diversity and Distribution 12: 490-501.
[2] Chen, W.Y. and Wang, D.T. (2013): Urban forest development in China: natural endowment or socioeconomic product? – Cities 35: 62-68.
[3] Christen, D. and Matlack, G. (2006): The role of roadsides in plant invasions: a demographic approach. – Conservation Biology 20: 385-391.
[4] Cutway, H.B. and Ehrenfeld, J.G. (2009): Exotic plant invasions in forested wetlands: effects of adjacent urban land use type. – Urban Ecosystems 12: 371-390.
[5] Dobbs, C., Francisco, J.E. and Wayne, C.Z. (2011) A framework for developing urban forest ecosystem services and goods indicators. - Landscape and Urban Planning 99: 196-206.
[6] Dolan, R.W., Moore, M.E. and Stephens, J.D. (2011): Documenting effects of urbanization on flora using herbarium records. - Journal of Ecology 99(4): 1055-1062.
[7] Ehrenfeld, J.G., Kourtev, P. and Huang W. (2001): Changes in soil functions following invasions of exotic understory plants in deciduous forests. - Ecological Applications 11(5): 1287-1300.
[8] Ehrenfeld, J.G., Ravit, B. and Elgersma, K. (2005): Feedback in the plant-soil system. - Annual Review of Environment and Resources 30(1): 75-115.
[9] Escobedo, F.J., Kroeger, T. and Wagner, J.E. (2011): Urban forests and pollution mitigation: analyzing ecosystem services and disservices. - Environmental Pollution 159: 2078-2087.
[10] Feng, J. and Zhu, Y. (2010): Alien invasive plants in China: risk assessment and spatial patterns. – Biodiversity and Conservation 19(12): 3489-3497.
[11] Flora of China. 2013: Available: http://foc.eflora.cn/ (accessed on 30th June 2016)
[12] Horak, M.J. and Loughin, T.M. (2000): Growth analysis of four Amaranthus species. – Weed Science 48(3): 347-355.
[13] Jiang, H., Fan, Q., Li, J.T., Shi, S., Li, S.P., Liao, W.B. and Shu, W.S. (2011): Naturalization of alien plants in China. – Biodiversity and Conservation 20(7): 1545-1556.
[14] Kennedy, T.A., Naeem, S., Howe, K.M., Knops, J.M., Tilman, D. and Reich, P. (2002): Biodiversity as a barrier to ecological invasion. – Nature 417(6889): 636-638.
[15] Kolar, C.S. and Lodge, D.M. (2001). Progress in invasion biology: predicting invaders. - Trends in Ecology & Evolution 16(4): 199-204.
[16] Leung, G.P.C., Hau, B.C.H. and Corlett, R.T. (2009): Exotic plant invasion in the highly degraded upland landscape of Hong Kong, China. - Biodiversity and Conservation 18: 191-202.
[17] Li, P.Q., Li, Y. (2010): Flora of Shenzhen. Chinese Forestry Publishing House, Beijing, China.

[18] Lin, W., Zhou, G.F., Cheng, X.Y. and Xu, R.M. (2007): Fast economic development accelerates biological invasions in China. - PLoS One 2(11):e1208.

[19] Liu, J., Dong, M., Miao, S.L., Li, Z.Y., Song, M.H. and Wang, R.Q. (2006): Invasive alien plants in China: role of clonality and geographical origin. – Biological Invasions 8: 1461-1470.

[20] Liu, J., Liang, S.C., Liu, F.H., Wang, R.Q. and Dong, M. (2005): Invasive alien plant species in China: regional distribution patterns. - Diversity and Distributions 11: 341-347.

[21] Liu, X.Z., Heilig, G.K., Chen, J.M. and Heino, M. (2007): Interactions between economic growth and environmental quality in Shenzhen, China's first special economic zone. – Ecological Economics 62(3-4): 559-570.

[22] Lososová, Z., Chytrý, M., Tichý, L., Danihelka, J., Fajmon, K. and Hájek, O. (2012): Native and alien floras in urban habitats: a comparison across 32 cities of central Europe. - Global Ecology and Biogeography 21(5): 545-555.

[23] McKinney, M.L. (2002): Influence of settlement time, human population, park shape and age, visitation and roads on the number of alien plant species in protected areas in the USA. - Diversity Distribution 8: 311-318.

[24] McKinsey Global Institute (MGI). (2012): Urban world: cities and the rise of the consuming class. Available: http://www.mckinsey.com/global-themes/urbanization/urban-world-cities-and-the-rise-of-the-consuming-class (accessed on 30th June 2016).

[25] McNeely, J.A., Mooney, H.A., Neville, L.E., Schei, P. and Waage, J.K. (2001): A Global strategy on invasive alien species. Gland, Switzerland: IUCN.

[26] Milbau, A. and Stout, J.C. (2008): Factors associated with alien plants transitioning from casual, to naturalized, to invasive. - Conservation Biology 22(2): 308-317.

[27] Nowak, D.J., Stein, S.M., Randler, P.B., Greenfield, E.J., Comas, S.J., Carr, M.A. and Alig, R.J. (2010): Sustaining America's urban trees and forests. - General technical report NRS- 62. Newton Square, PA: USDA Forest Service, Northern Research Station.

[28] Pauchard, A., Aguayo, M., Peña, E. and Urrutia, R. (2006): Multiple effects of urbanization on the biodiversity of developing countries: the case of a fast-growing metropolitan area (Concepción, Chile). - Biological Conservation 127(3): 272-281.

[29] Pauchard. A. and Alaback, P.B. (2004): Influence of elevation, land use, and landscape context on patterns of alien plant invasions along roadsides in protected areas of south-central Chile. - Conservation Biology 18(1): 238-248.

[30] Peacock, L., Worner, S.P. and Sedcole, R. (2006): Climate variables and their role in site discrimination of invasive insect species distributions. – Environmental Entomology 35(4): 958-963.

[31] Perrings, C., Williamson, M., Barbier, E.B., Delfino, D., Dalmazzone, S., Shogren, J., Simmons, P. and Watkinson, A. (2002): Biological invasion risks and the public good: an economic perspective. - Conservation Ecology 6(1): 1.

[32] Phillips, M.L., Murray, B.R., Leishman, M.R. and Ingram, R. (2010): The naturalization to invasion transition: are there introduction-history correlates of invasiveness in exotic plants of Australia? – Australian Ecology 35(6): 695-703.

[33] Pimentel, D., Zuniga, R. and Morrison, D. (2005): Update on the environmental and economic costs associated with alien-invasive species in the United States. - Ecological Economy 52(3): 273-288.

[34] Prentis, P.J., Wilson, J.R.U., Dormontt, E.E., Richardson, D.M. and Lowe, A.J. (2008): Adaptive evolution in invasive species. – Trends in Plant Science 13(6): 288-294.

[35] Pyšek, P. (1997): Compositae as invaders - better than the others? – Preslia. 69: 9-22.

[36] Pyšek, P. (1998): Alien and native species in Central European urban floras: a quantitative comparison. - Journal of Biogeography 25(1): 155-163.

[37] Pyšek, P., Jarošík, V., Hulme, P.E., Kühn, I., Wild, J., Arianoutsou, M., Bacher, S., Chiron, F., Didziulis, V., Essl, F., Genovesi, P., Gherardi, F., Hejda, M., Kark, S., Lambdon, P.W., Desprez-Loustau, M.L., Nentwig, W., Pergl, J., Poboljsaj, K., Rabitsch. W., Roques, A., Roy, D.B., Shirley, S., Solarz, W., Vilà, M. and Winter, M. (2010):

Disentangling the role of environmental and human pressures on biological invasions across Europe. – Proceedings of the National Academy of Sciences of the United States of America 107(27): 12157-12162.

[38] Richardson, D.M. and Pyšek, P. (2006): Plant invasions: merging the concepts of species invasiveness and community invisibility. – Progress in Physical Geography 30: 409-431.

[39] Richardson, D.M., Pyšek, P., Rejmánek, M., Barbour, M.G., Panetta, D.F. and West, C.J. (2000): Naturalization and invasion of alien plants: concepts and definitions. – Diversity and Distributions 6(2):93-107.

[40] Ricotta, C., Godefroid, S. and Rocchini, D. (2010): Invasiveness of alien plants in Brussels is related to their phylogenetic similarity to native species. - Diversity and Distribution 16: 655-662.

[41] Ricotta, C., La, Sorte, F.A., Pyšek, P., Rapson, G.L., Celesti-Grapow, L. and Thompson, K. (2009). Phyloecology of urban alien floras. - Journal of Ecology 97(6): 1243-1251.

[42] Romanuk, T.N., Zhou, Y., Brose, U., Berlow, E.L., Williams, R.J. and Martinez, N.D. (2009): Predicting invasion success in complex ecological networks. - Philosophical Transaction of the Royal Society B: Biological Sciences 364(1524): 1743-1754.

[43] Sakai, A.K., Allendorf, F.W., Holt, J.S., Lodge, D.M., Molofsky, J., With, K.A., Baughman, S., Cabin, R.J., Cohen, J.E., Ellstrand, N.C., McCauley, D.E. , O'Neil, P., Parker, I.M. , Thompson, J.N. and Weller, S.G. (2001): The population biology of invasive species. – Annual Review of Ecology and Systematics 32(1): 305-332.

[44] Santos, M., Freitas, R., Crespí, A.L., Hughes, S.J. and Cabral, J.A. (2011): Predicting trends of invasive plants richness using local socio-economic data: an application in North Portugal. – Environmental Research 111(7): 960-966.

[45] Schinner, F., Öhlinger, R., Kandeler, E. and Margesin, R. (1996): Methods in soil biology. - Springer, Berlin, Germany.

[46] Shao, Z.F., Zhao, H.B., Qiu, S.S., Yang, Y.B., Peng, S.L., Lu, C.F.and Chen, Z.Q. (2006): Study on the most harmful exotic plant in Shenzhen city. – Ecological Environment 15(3): 587-593.

[47] Shenzhen Entry-Exit Inspection and Quarantine Bureau (SEEIQB). (2010): An annual report of SEEIQB in 2010. – SEEIQB, Shenzhen, China.

[48] Singh, K.K., Davis, A.J. and Meentemeyer, R.K. (2015): Detecting understory plant invasion in urban forests using LiDAR. – International Journal of Applied Earth Observation of Geoinformation 38: 267-279.

[49] Spear, D., Foxcroft, L.C., Bezuidenhout, H. and McGeoch, M.A. (2013): Human population density explains alien species richness in protected areas. - Biological Conservation 159: 137-147.

[50] Statistics Bureau of Shenzhen Municipality (SBSM). (2013): Shenzhen Statistics Yearbook 2013. – SBSM, Shenzhen, China.

[51] Stecke, L.E., Sprague, C.L., Stoller, E.W. and Wax, L.M. (2004): Temperature effects on germination of nine Amaranthus species. – Weed Science 52: 217-221.

[52] Steckel, L.E. (2007): The dioecious Amaranthus spp.: here to stay. – Weed Technology 21: 567-570.

[53] Stohlgren, T.J., Barnett, D., Flather, C., Kartesz, J. and Peterjohn, B. (2005): Plant species invasions along the latitudinal gradient in the United States. - Ecology 86(9): 2298-2309.

[54] United Nations Population Division (UNPD). (2006): World population prospects: the 2005 revision. - UN, New York, U.S.A.

[55] United Nations Population Division (UNPD). (2016): World urbanization prospects: the 2015 revision. - UNPD, New York; U.S.A.

[56] Vilà, M. and Pujadas, J. (2011): Land-use and socio-economic correlates of plant invasions in European and North African countries. – Biological Conservation 100: 397-401.

[57] Vilà, M., Basnou, C., Pyšek, P., Josefsson, M., Genovesi, P., Gollasch, S., Nentwig, W., Olenin, S., Roques, A., Roy, D., Hulme, P.E., and DAISIE partners (2010): How well do

we understand the impacts of alien species on ecosystem services? A pan-european, cross-taxa assessment. - Frontiers in Ecology and the Environment 8(3): 135-144.

[58] Vilà, M., Espinar, J.L., Hejda, M., Hulme, P.E., Jarošík, V., Maron, J.L., Jan Pergl, J., Schaffner, U., Sun, Y. and Pyšek, P. (2011): Ecological impacts of invasive alien plants: a meta-analysis of their effects on species, communities and ecosystems. - Ecology Letters 14(7): 702-708.

[59] Vitousek, P.M., D'Antonio, C.M., Loope, L.L., Rejmánek, M. and Westbrooks, R. (1997): Introduced species: a significant component of human-based global change. - New Zealand Journal of Ecology 21(1): 1-16.

[60] Wan, F.H., Liu, Q.R., Xie, M. (2012): Biological invasions: color illustrations of invasive alien plants in China. - Science Press, Beijing, China.

[61] Wang, H.F., López-Pujol, J., Meyerson, L.A., Qiu, J.X., Wang, X.K., Ouyang, Z.Y. (2011): Biological invasions in rapidly urbanizing areas: a case study of Beijing, China. - Biodiversity and Conservation 20(11): 2483-2509.

[62] Warren II, R.J., Bahn, V., Bradford, M.A. (2013): Decoupling litter barrier and soil moisture influences on the establishment of an invasive grass. - Plant and Soil 367(1): 339-346.

[63] Weber, E, Sun, S.G., Li, B. (2008): Invasive alien plants in China: diversity and ecological insights. – Biological Invasions 10(8): 1411-1429.

[64] Williamson, M.H., Fitter, A. (1996): The varying success of invaders. – Ecology 77(6): 1661-1666.

[65] Wu, X.W., Luo, J., Chen, J.K., Li, B. (2006): Spatial patterns of invasive alien plants in China and its relationship with environmental and anthropological factors. – Journal of Plant Ecology 30(4): 576-584.

[66] Xu, H., Ding, H., Li, M., Qiang, S., Guo, J., Han, Z., Huang, Z., Sun, H., He, S., Wu, H., Wan, F. (2006): The distribution and economic losses of alien species invasion to China. - Biological Invasion 8(7): 1495-1500.

[67] Xu, H.G., Qiang S. (2011): China's invasive alien species. - Science Press, Beijing, China.

[68] Yan, Y.H., Xing, F.W., Huang, X.X., Fu, Q., Qin, X.S. and Chen, H.F. (2004): Exotic plants in Shenzhen, China. – Guihaia 24(3): 232-238.

[69] Zhang, L.Y., Ye, W.H., Cao, H.L., Feng, H.L. (2004): Mikania micrantha H.B.K in China - an overview. – Weed Research 44: 42-49.

APPENDIX

***Table S1**. Environmental factors of 19 major urban forests of Shenzhen**

Code	Forest ecosystem	Area (ha)	Average altitude (m)	Mean January temperature (ºC)	Mean July temperature (ºC)	Annual average precipitation (mm)	Density of native plant species (species/ha)
1	Nanshan	351	143	14.2	28.3	1311	1.70
2	Fenghuang Mountain	243	126	13.6	28.7	1799	2.57
3	Tiegang	5401	68.0	13.7	28.6	1705	1.62
4	Yangtai Mountain	2843	219	13.8	28.1	1574	2.23
5	Guangming	2063	129	13.7	29.1	1565	1.12
6	Guanlan	2211	120	14.1	29.5	1606	1.30
7	Wuzhipa	485	112	14.4	29.5	1485	1.65
8	Meilin	118	82.0	13.9	28.7	1572	2.72
9	Lianhua Mountain	180	57.4	14.4	28.5	1901	3.78
10	Bijia Mountain	142	86.0	14.4	28.6	1653	3.48
11	Weiling	87.2	107	13.8	28.7	1807	2.52
12	Buxin Mountain	348	119	14.0	28.9	1772	0.76
13	Wutong Mountain	2890	294	9.80	23.9	1548	3.58
14	Sanzhoutian	3483	326	12.3	26.3	1387	2.67
15	Songzikeng	1804	83.0	13.5	29.0	1583	1.09
16	Maluan Mountain	3172	218	13.5	28.8	1509	2.64
17	Tiantou Mountain	3003	229	13.2	29.2	1360	3.66
18	Dapeng Peninsula	14500	208	14.2	28.8	1882	3.37
19	Qiniang Mountain	4642	297	14.3	28.2	1705	2.87

* Excluding soil physico-chemical properties.

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***Table S2**. Soil physico-chemical properties of 19 major urban forests of Shenzhen. Data are presented as means (n = 20-95).*

Code	Forest ecosystem	Density (g/cm^3)	Porosity (%)	Moisture (%)	pH	OM* (g/kg)	TN* (g/kg)	AP* (mg/kg)
1	Nanshan	1.54	47.3	8.51	4.95	13.7	0.57	3.11
2	Fenghuang Mountain	1.61	40.0	3.55	6.39	11.1	0.72	1.53
3	Tiegang	1.50	44.6	3.03	4.55	18.5	0.71	2.51
4	Yangtai Mountain	1.12	45.0	4.96	5.20	14.8	0.20	2.40
5	Guangming	1.40	48.6	5.15	5.10	13.1	0.78	9.30
6	Guanlan	1.37	47.9	5.23	5.51	13.2	0.90	4.60
7	Wuzhipa	0.82	46.6	1.72	4.14	5.51	0.47	0.39
8	Meilin	1.38	48.0	1.32	5.25	9.24	0.27	6.83
9	Lianhua Mountain	1.34	51.4	11.3	5.56	7.36	0.15	4.48
10	Bijia Mountain	1.30	45.4	11.5	5.40	11.2	1.01	2.32
11	Weiling	1.32	55.4	18.4	5.02	19.4	0.54	0.92
12	Buxin Mountain	1.14	52.4	19.4	5.27	18.9	0.44	0.80
13	Wutong Mountain	1.01	55.3	18.0	5.15	12.7	0.30	7.80
14	Sanzhoutian	1.36	50.6	18.7	4.56	14.7	0.72	1.41
15	Songzikeng	1.38	47.2	5.13	5.03	13.5	0.55	1.07
16	Maluan Mountain	1.32	58.7	11.0	4.77	14.2	0.52	2.24
17	Tiantou Mountain	1.20	54.6	24.3	4.23	38.0	1.59	4.81
18	Dapeng Peninsula	1.08	34.2	18.9	4.64	10.7	0.67	3.95
19	Qiniang Mountain	1.86	42.5	19.2	5.30	11.9	1.11	6.33

*: OM, organic matter content; TN, total nitrogen content; AP, available phosphorus content.

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***Table S3**. Socio-economic factors of 19 major urban forests of Shenzhen.*

Code	Forest ecosystem	Population density within a 1,000-m radius (person/ha)	Road density within a 1,000-m radius (m/ha)	Road area ratio within a 1,000-m radius area (%)	Residential land area ratio within a 1,000-m radius (%)	Industrial land area ratio within a 1,000-m radius (%)
1	Nanshan	74.9	72.3	0.91	23.0	5.39
2	Fenghuang Mountain	73.5	57.1	8.46	8.23	6.99
3	Tiegang	107	44.8	6.43	5.53	6.57
4	Yangtai Mountain	80.0	51.5	6.14	6.17	4.91
5	Guangming	68.9	27.4	3.13	5.46	12.8
6	Guanlan	28.2	20.8	2.22	2.85	4.92
7	Wuzhipa	32.6	51.4	7.97	2.06	13.4
8	Meilin	144	59.0	5.31	16.7	0.47
9	Lianhua Mountain	221	125	17.4	32.6	6.29
10	Bijia Mountain	272	101	12.0	25.4	6.53
11	Weiling	319	79.5	7.40	38.3	3.53
12	Buxin Mountain	154	52.7	5.03	21.4	3.77
13	Wutong Mountain	59.2	43.5	5.43	7.42	1.09
14	Sanzhoutian	17.3	25.6	2.61	2.99	2.25
15	Songzikeng	30.3	59.2	7.00	8.06	8.40
16	Maluan Mountain	15.3	23.8	2.16	3.65	2.93
17	Tiantou Mountain	32.9	20.5	2.08	1.56	5.17
18	Dapeng Peninsula	9.37	19.0	1.75	3.43	0.88
19	Qiniang Mountain	2.23	8.58	0.55	0.96	0.00

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Table S4. Checklist of naturalized plant species in Shenzhen.

Code	Species	Family	Genus	Geographic origin	Life form
		Acanthaceae			
1	*Adhatoda vasica*	Acanthaceae	Adhatoda	America	Shrub
2	*Andrographis paniculata*	Acanthaceae	Andrographis	South Asia, India, Australia	Herb
3	*Barleria cristata*	Acanthaceae	Barleria	Asia	Shrub
4	*Ruellia brittonina*	Acanthaceae	Ruellia	Mexico	Herb
5	*Thunbergia grandiflora*	Acanthaceae	Thunbergia	Asia	Liana
		Adiantaceae			
6	*Adiantum capillus-veneris*	Adiantaceae	Adiantum	pantropical	Herb
		Agavaceae			
7	*Agave americana*	Agavaceae	Agave	America	Herb
8	*Agave sisalana*	Agavaceae	Agave	North America	Herb
		Aizoaceae			
9	*Tetragonia tetragonioides*	Aizoaceae	Tetragonia	Australia, Asia, South America	Herb
10	*Trianthema portulacastrum*	Aizoaceae	Trianthema	Tropical Africa and Asia	Herb
		Amaranthaceae			
11	*Achyranthes aspera*	Amaranthaceae	Achyranthes	India, Vietnam, Philippines	Herb
12	*Alternanthera bettzickiana*	Amaranthaceae	Alternanthera	America	Herb
13	*Alternanthera paronychioides*	Amaranthaceae	Alternanthera	South America	Herb
14	*Alternanthera philoxeroides*	Amaranthaceae	Alternanthera	South America	Herb
15	*Alternanthera sessilis*	Amaranthaceae	Alternanthera	Vietnam, Malaysia, Philippines	Herb
16	*Amaranthus hybridus*	Amaranthaceae	Amaranthus	America	Herb
17	*Amaranthus lividus*	Amaranthaceae	Amaranthus	America	Herb

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18	*Amaranthus paniculatus*	Amaranthaceae	Amaranthus	America	Herb
19	*Amaranthus spinosus*	Amaranthaceae	Amaranthus	America	Herb
20	*Amaranthus tricolor*	Amaranthaceae	Amaranthus	Asia	Herb
21	*Amaranthus viridis*	Amaranthaceae	Amaranthus	Africa	Herb
22	*Celosia argentea*	Amaranthaceae	Celosia	America	Herb
23	*Celosia cristata*	Amaranthaceae	Celosia	Asia	Herb
24	*Gomphrena celosioides*	Amaranthaceae	Gomphrena	America	Herb
25	*Gomphrena globosa*	Amaranthaceae	Gomphrena	America	Herb
26	*Iresine herbstii*	Amaranthaceae	Iresine	Brazil	Herb
		Amaryllidaceae			
27	*Hippeastrum rutilum*	Amaryllidaceae	Hippeastrum	South America	Herb
28	*Hippeastrum vittatum*	Amaryllidaceae	Hippeastrum	South America	Herb
29	*Narcissus tazetta*	Amaryllidaceae	Narcissus	Middle Europe, Mediterranean, West Asia	Herb
30	*Zephyranthes candida*	Amaryllidaceae	Zephyranthes	North America	Herb
31	*Zephyranthes grandiflora*	Amaryllidaceae	Zephyranthes	North America	Herb
		Anacardiaceae			
32	*Mangifera indica*	Anacardiaceae	Mangifera	India	Tree
		Annonaceae			
33	*Annona glabra*	Annonaceae	Annona	Tropical America	Tree
34	*Annona squamosa*	Annonaceae	Annona	Tropical America	Shrub, Tree
		Apiaceae			
35	*Coriandrum sativum*	Apiaceae	Coriandrum	Mediterranean	Herb
36	*Daucus carota*	Apiaceae	Daucus	Asia	Herb
37	*Daucus carota var. sativa*	Apiaceae	Daucus	Europe, North Africa, Asia	Herb
38	*Eryngium foetidum*	Apiaceae	Eryngium	America	Herb

39	*Foeniculum vulgare*	Apiaceae	Foeniculum	Mediterranean	Herb
		Apocynaceae			
40	*Catharanthus roseus*	Apocynaceae	Catharanthus	Africa	Herb
41	*Plumeria rubra*	Apocynaceae	Plumeria	South America	Tree
42	*Rauvolfia tetraphylla*	Apocynaceae	Rauvolfia	Tropical America, Tropical Africa	Shrub
43	*Thevetia peruviana*	Apocynaceae	Thevetia	Tropical America	Tree
		Araceae			
44	*Caladium bicolor*	Araceae	Caladium	South America	Herb
45	*Dieffenbachia picta*	Araceae	Dieffenbachia	South America	Herb
46	*Pistia stratiotes*	Araceae	Pistia	Brazil	Herb
		Asclepiadaceae			
47	*Asclepias curassavica*	Asclepiadaceae	Asclepias	Latin America	Herb
		Asteraceae			
48	*Ageratum conyzoides*	Asteraceae	Ageratum	Central and South America	Herb
49	*Ageratum houstonianum*	Asteraceae	Ageratum	North America	Herb
50	*Ambrosia artemisiifolia*	Asteraceae	Ambrosia	North America	Herb
51	*Artemisia annua*	Asteraceae	Artemisia	America	Herb
52	*Artemisia verlotiorum*	Asteraceae	Artemisia	NorthernEurasia	Herb
53	*Aster subulatus*	Asteraceae	Aster	North America	Herb
54	*Bidens bipinnata*	Asteraceae	Bidens	East Asia	Herb
55	*Bidens pilosa*	Asteraceae	Bidens	America	Herb
56	*Bidens pilosa var. radiata*	Asteraceae	Bidens	North America	Herb
57	*Centipeda minima*	Asteraceae	Centipeda	Pantropical	Herb
58	*Chrysanthemum coronarium*	Asteraceae	Chrysanthemum	Mediterranean	Herb
59	*Conyza bonariensis*	Asteraceae	Conyza	America	Herb

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60	*Conyza canadensis*	Asteraceae	Conyza	North America	Herb
61	*Conyza sumatrensis*	Asteraceae	Conyza	South America	Herb
62	*Coreopsis lanceolata*	Asteraceae	Coreopsis	North America	Herb
63	*Coreopsis tinctoria*	Asteraceae	Coreopsis	North America	Herb
64	*Cosmos bipinnata*	Asteraceae	Cosmos	North America	Herb
65	*Cosmos sulphureus*	Asteraceae	Cosmos	North America	Herb
66	*Crassocephalum crepidioides*	Asteraceae	Crassocephalum	Africa	Herb
67	*Crossostephium Chinensis*	Asteraceae	Crossostephium	America	Herb, Shrub
68	*Dahlia pinnata*	Asteraceae	Dahlia	Mexico	Herb
69	*Elephantopus scaber*	Asteraceae	Elephantopus	America	Herb
70	*Elephantopus tomentosus*	Asteraceae	Elephantopus	America	Herb
71	*Emilia sonchifolia*	Asteraceae	Emilia	Pantropical	Herb
72	*Erechthites hieracifolia*	Asteraceae	Erechthites	North America	Herb
73	*Erechthites valerianaefolia*	Asteraceae	Erechthites	South America	Herb
74	*Erigeron annuus*	Asteraceae	Erigeron	North America	Herb
75	*Erigeron karvinskianus*	Asteraceae	Erigeron	Mexico, Panama	Herb
76	*Eupatorium odoratum*	Asteraceae	Eupatorium	Tropical America	Herb
77	*Eupatorium catarium*	Asteraceae	Eupatorium	South America	Herb
78	*Galinsoga parviflora*	Asteraceae	Galinsoga	South America	Herb
79	*Gnaphalium pensylvanicum*	Asteraceae	Gnaphalium	Warm America	Herb
80	*Gnaphalium polycaulon*	Asteraceae	Gnaphalium	Pantropical	Herb
81	*Helianthus annuus*	Asteraceae	Helianthus	America	Herb
82	*Helianthus tuberosus*	Asteraceae	Helianthus	North America	Herb
83	*Lactuca sativa*	Asteraceae	Lactuca	Mediterranean	Herb
84	*Leucanthemum vulgare*	Asteraceae	Leucanthemum	Europe	Herb
85	*Mikania cordata*	Asteraceae	Mikania	Indonesia, LALaos, Vietnam	Herb/Vine
86	*Mikania micrantha*	Asteraceae	Mikania	Central and South America	Herb/Vine
87	*Parthenium hysterophorus*	Asteraceae	Parthenium	America	Herb
88	*Silybum marianum*	Asteraceae	Silybum	Europe, Asia, Africa	Herb

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89	*Solidago canadensis*	Asteraceae	Solidago	North America	Herb
90	*Soliva anthemifolia*	Asteraceae	Soliva	Oceania	Herb
91	*Sonchus arvensis*	Asteraceae	Sonchus	Europe	Herb
92	*Sonchus asper*	Asteraceae	Sonchus	Europe	Herb
93	*Sonchus oleraceus*	Asteraceae	Sonchus	Europe	Herb
94	*Spilanthes paniculata*	Asteraceae	Spilanthes	Pantropical	Herb
95	*Synedrella nodiflora*	Asteraceae	Synedrella	America	Herb
96	*Tagetes erecta*	Asteraceae	Tagetes	North America（Mexico）	Herb
97	*Tagetes patula*	Asteraceae	Tagetes	Mexico	Herb
98	*Tithonia diversifolia*	Asteraceae	Tithonia	Central and North America	Herb
99	*Tridax procumbens*	Asteraceae	Tridax	America	Herb
100	*Vernonia cinerea*	Asteraceae	Vernonia	Pantropical	Herb
101	*Wedelia trilobata*	Asteraceae	Wedelia	America	Herb
102	*Xanthium strumarium*	Asteraceae	Xanthium	Eurasia	Herb
103	*Zinnia elegans*	Asteraceae	Zinnia	Mexico	Herb
		Balsaminaceae			
104	*Impatiens balsamina*	Balsaminaceae	Impatiens	Asia	Herb
105	*Impatiens walleriana*	Balsaminaceae	Impatiens	Africa	Herb
		Basellaceae			
106	*Basella alba*	Basellaceae	Basella	Tropical Asia	Herb
		Begoniaceae			
107	*Begonia cucullata*	Begoniaceae	Begonia	South America	Herb
108	*Begonia semperflorens*	Begoniaceae	Begonia	Brazil	Herb
		Bignoniaceae			
109	*Macfadyena unguis-cati*	Bignoniaceae	Macfadyena	America	Liana
110	*Pyrostegia venusta*	Bignoniaceae	Pyrostegia	America	Liana

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		Bixaceae			
111	*Bixa orellana*	Bixaceae	Bixa	tropical America	Tree
		Boraginaceae			
112	*Heliotropium indicum*	Boraginaceae	Heliotropium	Thailand, Southeast Asia	Herb
		Brassicaceae			
113	*Brassica juncea*	Brassicaceae	Brassica	Asia	Herb
114	*Brassica oleracea var. botrytis*	Brassicaceae	Brassica	Europe	Herb
115	*Brassica oleracea var. capitata*	Brassicaceae	Brassica	Europe	Herb
116	*Brassica rapa var. oleifera*	Brassicaceae	Brassica	Europe	Herb
117	*Capsella bursa-pastoris*	Brassicaceae	Capsella	Europe	Herb
118	*Cardamine flexuosa*	Brassicaceae	Cardamine	Northern Eurasia	Herb
119	*Lepidium virginicum*	Brassicaceae	Lepidium	North America	Herb
120	*Lobularia maritima*	Brassicaceae	Lobularia	Mediterranean	Herb
121	*Nasturtium officinale*	Brassicaceae	Nasturtium	Europe	Herb
122	*Raphanus sativus*	Brassicaceae	Raphanus	Mediterranean	Herb
123	*Rorippa heterophylla*	Brassicaceae	Rorippa	Asia	Herb
124	*Sisymbrium altissimum*	Brassicaceae	Sisymbrium	Europe	Herb
		Cactaceae			
125	*Epiphyllum oxypetalum*	Cactaceae	Epiphyllum	Mexico	Shrub
126	*Hylocereus undatus*	Cactaceae	Hylocereus	America	Liana
127	*Opuntia dillenii*	Cactaceae	Opuntia	Tropical America	Herb
128	*Pereskia aculeata*	Cactaceae	Pereskia	America	Liana
		Cannaceae			
129	*Canna indica*	Cannaceae	Canna	tropical America	Herb

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		Capparidaceae			
130	*Cleome rutidosoerma*	Capparidaceae	Cleome	Africa	Herb
		Caricaceae			
131	*Carica papaya*	Caricaceae	Carica	Tropical America	Tree
		Caryophyllaceae			
132	*Arenaria serpyllifolia*	Caryophyllaceae	Arenaria	Europe	Herb
133	*Stellaria media*	Caryophyllaceae	Stellaria	Cosmopolitan	Herb
134	*Stellaria uliginosa*	Caryophyllaceae	Stellaria	Northern Eurasia	Herb
		Casuarinaceae			
135	*Casuarina equisetifolia*	Casuarinaceae	Casuarina	Australia	Tree
		Chenopodiaceae			
136	*Chenopodium album*	Chenopodiaceae	Chenopodium	Northern Eurasia	Herb
137	*Chenopodium ambrosioides*	Chenopodiaceae	Chenopodium	America	Herb
		Combretaceae			
138	*Quisqualis indica*	Combretaceae	Quisqualis	Europe, Asia	Shrub
		Commelinaceae			
139	*Zebrina pendula*	Commelinaceae	Zebrina	Mexico	Herb
		Convolvulaceae			
140	*Argyreia acuta*	Convolvulaceae	Argyreia	Asia	Liana
141	*Argyreia nervosa*	Convolvulaceae	Argyreia	India	Liana
142	*Cuscuta japonica*	Convolvulaceae	Cuscuta	Asia	Herb/Vine
143	*Ipomoea alba*	Convolvulaceae	Ipomoea	America	Herb/Vine
144	*Ipomoea aquatica*	Convolvulaceae	Ipomoea	America	Herb/Vine

145	*Ipomoea batatas*	Convolvulaceae	Ipomoea	America	Herb
146	*Ipomoea cairica*	Convolvulaceae	Ipomoea	Europe	Herb/Vine
147	*Ipomoea carnea scbsp. fistulosa*	Convolvulaceae	Ipomoea	Tropical America	Shrub
148	*Ipomoea indica*	Convolvulaceae	Ipomoea	South America	Herb/Vine
149	*Ipomoea mauritiana*	Convolvulaceae	Ipomoea	America	Herb/Vine
150	*Ipomoea nil*	Convolvulaceae	Ipomoea	America	Herb/Vine
151	*Ipomoea purpurea*	Convolvulaceae	Ipomoea	Tropical America	Herb/Vine
152	*Ipomoea quamoclit*	Convolvulaceae	Ipomoea	Tropical America	Herb/Vine
153	*Ipomoea triloba*	Convolvulaceae	Ipomoea	Tropical America	Herb/Vine
		Crassulaceae			
154	*Bryophyllum pinnatum*	Crassulaceae	Bryophyllum	Africa	Herb
155	*Kalanchoe tubiflora*	Crassulaceae	Kalanchoe	Africa	Herb
156	*Kalanchoe verticillata*	Crassulaceae	Kalanchoe	Africa	Herb
157	*Sedum mexicanum*	Crassulaceae	Sedum	Mexico, America	Herb
		Cucurbitaceae			
158	*Momordica charantia*	Cucurbitaceae	Momordica	Palaeotropics	Herb/Vine
159	*Sechium edule*	Cucurbitaceae	Sechium	America	Herb/Vine
		Cyatheaceae			
160	*Sphaeropteris lepifera*	Cyatheaceae	Sphaeropteris	Southeast Asia	Tree
		Cyperaceae			
161	*Cyperus alternifolius subsp. flabelliformis*	Cyperaceae	Cyperus	Africa	Herb
162	*Cyperus rotundus*	Cyperaceae	Cyperus	Asia	Herb
163	*Schoenoplectus mucronatus*	Cyperaceae	Schoenoplectus	cosmopolitan	Herb
		Elatinaceae			

164	*Elatine americana*	Elatinaceae	Elatine	America	Herb
		Equisetaceae			
165	*Equisetum arvense*	Equisetaceae	Equisetum	North temperate Zone	Herb
166	*Equisetum ramosissimum*	Equisetaceae	Equisetum	Asia	Herb
		Euphorbiaceae			
167	*Codiaeum variegatum*	Euphorbiaceae	Codiaeum	Malay Peninsula To Oceania	Shrub, Tree
168	*Euphorbia cyathophora*	Euphorbiaceae	Euphorbia	Central America, South America	Herb
169	*Euphorbia graminea*	Euphorbiaceae	Euphorbia	South Mexico, Central and South America	Herb
170	*Euphorbia hirta*	Euphorbiaceae	Euphorbia	Tropical	Herb
171	*Euphorbia marginata*	Euphorbiaceae	Euphorbia	North America	Herb
172	*Euphorbia milii*	Euphorbiaceae	Euphorbia	Africa	Liana
173	*Euphorbia prostrata*	Euphorbiaceae	Euphorbia	Tropical America	Herb
174	*Excoecaria tirucalli*	Euphorbiaceae	Excoecaria	Africa	Herb
175	*Jatropha curcas*	Euphorbiaceae	Jatropha	America	Shrub, Tree
176	*Manihot esculenta*	Euphorbiaceae	Manihot	South America	Shrub
177	*Pedilanthu tithymaloides*	Euphorbiaceae	Pedilanthu	Tropical America	Herb, Shrub
178	*Phyllanthus niruri*	Euphorbiaceae	Phyllanthus	America	Herb
179	*Phyllanthus tenellus*	Euphorbiaceae	Phyllanthus	America	Herb
180	*Ricinus communis*	Euphorbiaceae	Ricinus	Africa	Herb
		Fabaceae			
181	*Acacia farnesiana*	Fabaceae	Acacia	America	Shrub
182	*Aeschynomene indica*	Fabaceae	Aeschynomene	Aisa	Herb
183	*Arachis duranensis*	Fabaceae	Arachis	America	Herb
184	*Arachis hypogaea*	Fabaceae	Arachis	South America	Herb
185	*Caesalpinia pulcherrima*	Fabaceae	Caesalpinia	America	Shrub, Tree
186	*Cajanus cajan*	Fabaceae	Cajanus	Asia	Shrub
187	*Canavalia gladiata*	Fabaceae	Canavalia	America	Herb/Vine

188	*Cassia bicapsularis*	Fabaceae	Cassia	America	Shrub
189	*Cassia surattensis*	Fabaceae	Cassia	America	Shrub
190	*Centrosema pubescens*	Fabaceae	Centrosema	America	Herb/Vine
191	*Chamaecrista nictitans subsp. patellaria var. glabrata*	Fabaceae	Chamaecrista	Tropical America	Herb
192	*Chamaecrista mimosoides*	Fabaceae	Chamaecrista	America	Herb, Shrub
193	*Clitoria ternatea*	Fabaceae	Clitoria	India	Herb
194	*Crotalaria pallida*	Fabaceae	Crotalaria	Ethiopia	Herb
195	*Crotalaria zanzibarica*	Fabaceae	Crotalaria	South America	Herb, Shrub
196	*Derris elliptica*	Fabaceae	Derris	Tropical Asia, India	Liana
197	*Desmodium tortuosum*	Fabaceae	Desmodium	America	Herb
198	*Dolichos lablab*	Fabaceae	Dolichos	Asia	Herb/Vine
199	*Erythrina corallodendron*	Fabaceae	Erythrina	South America	Shrub
200	*Indigofera suffruticosa*	Fabaceae	Indigofera	America	Herb, Shrub
201	*Leucaena leucocephala*	Fabaceae	Leucaena	America	Shrub
202	*Lotus corniculatus*	Fabaceae	Lotus	Europe, Asia, Africa	Herb
203	*Medicago sativa*	Fabaceae	Medicago	Asia	Herb
204	*Mimosa bimucronata*	Fabaceae	Mimosa	America	Shrub
205	*Mimosa invisa*	Fabaceae	Mimosa	America	Herb
206	*Mimosa pudica*	Fabaceae	Mimosa	America	Herb, Shrub
207	*Pachyrhizus erosus*	Fabaceae	Pachyrhizus	America	Herb/Vine
208	*Pithecellobium dulce*	Fabaceae	Pithecellobium	Central America	Tree
209	*Pueraria phaseoloides*	Fabaceae	Pueraria	Indonesia,Malaysia	Herb/Vine
210	*Senna alata*	Fabaceae	Senna	America	Shrub
211	*Senna occidentalis*	Fabaceae	Senna	America	Herb, Shrub
212	*Senna siamea*	Fabaceae	Senna	Burma to Malaysia	Tree
213	*Senna occidentalis var. sophera*	Fabaceae	Senna	Aisa	Shrub
214	*Senna tora*	Fabaceae	Senna	Asia（India）	Herb
215	*Sesbania cannabina*	Fabaceae	Sesbania	Asia（India）	Herb
216	*Stylosanthes guianensis*	Fabaceae	Stylosanthes	South America	Herb, Shrub

217	*Tamarindus indica*	Fabaceae	Tamarindus	Africa	Tree
218	*Tephrosia candida*	Fabaceae	Tephrosia	Asia	Herb, Shrub
219	*Trifolium repens*	Fabaceae	Trifolium	Europe, North Africa	Herb
220	*Zornia gibbosa*	Fabaceae	Zornia	America	Herb
		Geraniaceae			
221	*Geranium carolinianum*	Geraniaceae	Geranium	America	Herb
		Hemionitidaceae			
222	*Pityrogramma calomelanos*	Hemionitidaceae	Pityrogramma	America	Herb
		Lamiaceae			
223	*Hyptis brevipes*	Lamiaceae	Hyptis	America	Herb
224	*Hyptis rhomboidea*	Lamiaceae	Hyptis	America	Herb
225	*Hyptis suaveolens*	Lamiaceae	Hyptis	America	Herb
226	*Mentha haplocalyx*	Lamiaceae	Mentha	Asia	Herb
227	*Mentha spicata*	Lamiaceae	Mentha	Europe	Herb
228	*Ocimum basilicum*	Lamiaceae	Ocimum	Africa, Asia	Herb
229	*Perilla frutescens*	Lamiaceae	Perilla	Asia	Herb
230	*Salvia coccinea*	Lamiaceae	Salvia	America	Herb
		Liliaceae			
231	*Aloe vera var.chinese*	Liliaceae	Aloe	Southern Africa	Herb, Shrub
232	*Asparagus densiflorus cv. densiflorus*	Liliaceae	Asparagus	South Africa	Herb/Vine
233	*Asparagus setaceus*	Liliaceae	Asparagus	Africa	Shrub
234	*Cordyline fruticosa*	Liliaceae	Cordyline	uncertain	Herb
		Iridaceae			
235	*Gladiolus gandavensis*	Iridaceae	Gladiolus	Mediteeranean, Tropical Africa,	Herb

				Southwest and Central Asia	
		Lythraceae			
236	*Cuphea balsamona*	Lythraceae	Cuphea	America	Herb
237	*Lythrum salicaria*	Lythraceae	Lythrum	Europe	Herb
		Malvaceae			
238	*Abelmoschus moschatus*	Malvaceae	Abelmoschus	South Asia	Herb
239	*Bombax malabarica*	Malvaceae	Bombax	South Africa	Tree
240	*Corchorus capsularis*	Malvaceae	Corchorus	Subtropics	Herb
241	*Malva verticillata*	Malvaceae	Malva	Asia	Herb
242	*Malvastrum coromandelianum*	Malvaceae	Malvastrum	America	Herb, Shrub
243	*Sida acuta*	Malvaceae	Sida	Asia	Herb
244	*Sida cordata*	Malvaceae	Sida	Pantropic	Herb, Shrub
245	*Sida rhombifolia*	Malvaceae	Sida	Pantropic	Herb
246	*Urena lobata*	Malvaceae	Urena	Pantropic	Herb, Shrub
247	*Urena procumbens*	Malvaceae	Urena	Pantropic	Herb, Shrub
248	*Waltheria indica*	Malvaceae	Waltheria	America	Herb, Shrub
		Molluginaceae			
249	*Mollugo verticillata*	Molluginaceae	Mollugo	Tropical America	Herb
		Musaceae			
250	*Musa basjoo*	Musaceae	Musa	Ryukyu Islands	Herb
		Myrtaceae			
251	*Eucalyptus robusta*	Myrtaceae	Eucalyptus	Australia	Tree
252	*Psidium guajava*	Myrtaceae	Psidium	America	Shrub, Tree
253	*Syzygium jambos*	Myrtaceae	Syzygium	Southest Asia	Tree

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		Nyctaginaceae			
254	*Bougainvillea glabra*	Nyctaginaceae	Bougainvillea	Brazil	Shrub
255	*Bougainvillea spectabilis*	Nyctaginaceae	Bougainvillea	Brazil	Shrub
256	*Mirabilis jalapa*	Nyctaginaceae	Mirabilis	South America	Herb
		Nymphaeaceae			
257	*Nymphaea alba*	Nymphaeaceae	Nymphaea	North Afica, Eurasia	Herb
		Oleaceae			
258	*Jasminum sambac*	Oleaceae	Jasminum	India	Herb
		Onagraceae			
259	*Ludwigia hyssopifolia*	Onagraceae	Ludwigia	America	Herb
260	*Oenothera drummondii*	Onagraceae	Oenothera	America	Herb
		Oxalidaceae			
261	*Oxalis corniculata*	Oxalidaceae	Oxalis	South America, Afica	Herb
262	*Oxalis corymbosa*	Oxalidaceae	Oxalis	America	Herb
		Palmaceae			
263	*Elaeis guineensis*	Palmaceae	Elaeis	Tropical Africa	Shrub
		Passifloraceae			
264	*Passiflora caerulea*	Passifloraceae	Passiflora	South America	Herb/Vine
265	*Passiflora edulis*	Passifloraceae	Passiflora	Brazil and Netherlands antilles	Herb/Vine
266	*Passiflora foetida*	Passifloraceae	Passiflora	Latin America	Herb/Vine
		Phytolaccaceae			
267	*Phytolacca americana*	Phytolaccaceae	Phytolacca	North America	Herb

		Piperaceae			
268	*Peperomia pellucida*	Piperaceae	Peperomia	America	Herb
269	*Piper betle*	Piperaceae	Piper	Tropical Asia, Africa	Herb/Vine
		Pittosporaceae			
270	*Pittosporum tobira*	Pittosporaceae	Pittosporum	America	Shrub, Tree
		Plantaginaceae			
271	*Plantago major*	Plantaginaceae	Plantago	Cosmopolitan	Herb
		Poaceae			
272	*Alopecurus aequalis*	Poaceae	Alopecurus	North America	Herb
273	*Alopecurus japonicus*	Poaceae	Alopecurus	Asia	Herb
274	*Arundo donax*	Poaceae	Arundo	Mediterranean	Herb
275	*Axonopus compressus*	Poaceae	Axonopus	America	Herb
276	*Brachiaria eruciformis*	Poaceae	Brachiaria	Pantropics	Herb
277	*Cenchrus echinatus*	Poaceae	Cenchrus	America	Herb
278	*Chloris barbata*	Poaceae	Chloris	Tropical America	Herb
279	*Coix lacryma-jobi*	Poaceae	Coix	Tropical Asia	Herb
280	*Digitaria ciliaris*	Poaceae	Digitaria	Tropics, Subtropics	Herb
281	*Digitaria sanguinalis*	Poaceae	Digitaria	Europe	Herb
282	*Echinochloa crusgalli*	Poaceae	Echinochloa	Europe，Asia	Herb
283	*Eleusine indica*	Poaceae	Eleusine	Asia	Herb
284	*Eragrostis cillaris*	Poaceae	Eragrostis	Paleotropics	Herb
285	*Eragrostis perennans*	Poaceae	Eragrostis	Cosmopolitan	Herb
286	*Melinis repens*	Poaceae	Melinis	Africa	Herb
287	*Panicum maximum*	Poaceae	Panicum	Africa	Herb
288	*Panicum repens*	Poaceae	Panicum	Brazil	Herb

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289	*Paspalum conjugatum*	Poaceae	Paspalum	America	Herb
290	*Paspalum distichum*	Poaceae	Paspalum	Tropical America	Herb
291	*Paspalum urvillei*	Poaceae	Paspalum	Tropical America	Herb
292	*Pennisetum purpureum*	Poaceae	Pennisetum	Africa	Herb
293	*Poa annua*	Poaceae	Poa	Europe	Herb
294	*Setaria geniculata*	Poaceae	Setaria	Asia, Europe	Herb
295	*Setaria glauca*	Poaceae	Setaria	Eurasia, America, Australia	Herb
296	*Setaria pallidifusca*	Poaceae	Setaria	Southern Asia, Australia	Herb
297	*Setaria palmifolia*	Poaceae	Setaria	Africa	Herb
298	*Setaria viridis*	Poaceae	Setaria	Eurasia	Herb
		Polygonaceae			
299	*Antigonon leptopus*	Polygonaceae	Antigonon	Central America	Liana
300	*Homalocladium platycladum*	Polygonaceae	Homalocladium	Oceania	Shrub
301	*Polygonum aviculare*	Polygonaceae	Polygonum	Asia	Herb
302	*Rumex acetosella*	Polygonaceae	Rumex	Eurasia	Herb
303	*Rumex crispus*	Polygonaceae	Rumex	Eurasia	Herb
		Pontederiaceae			
304	*Eichhornia crassipes*	Pontederiaceae	Eichhornia	America	Herb
		Portulacaceae			
305	*Portulaca grandiflora*	Portulacaceae	Portulaca	America	Herb
306	*Portulaca oleracea*	Portulacaceae	Portulaca	Cosmopolitan	Herb
307	*Portulaca pilosa*	Portulacaceae	Portulaca	Tropical America	Herb
308	*Talinum paniculatum*	Portulacaceae	Talinum	America	Herb
		Punicaceae			
309	*Punica granatum*	Punicaceae	Punica	Asia	Shrub, Tree

		Ranunculaceae			
310	*Ranunculus japonicus*	Ranunculaceae	Ranunculus	Aisa	Herb
		Rubiaceae			
311	*Borreria articularis*	Rubiaceae	Borreria	India	Herb/Vine
312	*Borreria latifolia*	Rubiaceae	Borreria	South America	Herb
313	*Galium aparine*	Rubiaceae	Galium	Northern Eurasia	Herb
		Rutaceae			
314	*Citrus aurantifolia*	Rutaceae	Citrus	Uncertain	Tree
		Sapindaceae			
315	*Cardiospermum halicacabum*	Sapindaceae	Cardiospermum	America	Herb/Vine
		Scrophulariaceae			
316	*Scoparia dulcis*	Scrophulariaceae	Scoparia	America	Herb
317	*Striga asiatica*	Scrophulariaceae	Striga	Asia	Herb
318	*Torenia fournieri*	Scrophulariaceae	Torenia	Vietnam	Herb
319	*Veronica peregrina*	Scrophulariaceae	Veronica	North America	Herb
320	*Veronica persica*	Scrophulariaceae	Veronica	West Asia, Europe	Herb
321	*Veronica polita*	Scrophulariaceae	Veronica	Asia	Herb
		Solanaceae			
322	*Capsicum annuum*	Solanaceae	Capsicum	Mexico, South America	Herb
323	*Capsicum annuum var. conoides*	Solanaceae	Capsicum	Mexico to Columbia	Herb
324	*Datura metel*	Solanaceae	Datura	America	Herb
325	*Datura stramonium*	Solanaceae	Datura	North America	Herb
326	*Lycopersicon esculentum*	Solanaceae	Lycopersicon	South America	Herb
327	*Nicandra physaloides*	Solanaceae	Nicandra	South America	Herb
328	*Petunia hybrida*	Solanaceae	Petunia	Argentina	Herb

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329	*Physalis angulata*	Solanaceae	Physalis	America	Herb
330	*Physalis minima*	Solanaceae	Physalis	Tropical Asia	Herb
331	*Solanum americanum*	Solanaceae	Solanum	South America	Herb
332	*Solanum capsicoides*	Solanaceae	Solanum	Brazil	Herb, Shrub
333	*Solanum erianthum*	Solanaceae	Solanum	America	Tree
334	*Solanum melongena*	Solanaceae	Solanum	South America	Herb
335	*Solanum pseudocapsicum*	Solanaceae	Solanum	Brazil	Herb
336	*Solanum surattense*	Solanaceae	Solanum	Brazil	Herb, Shrub
337	*Solanum torvum*	Solanaceae	Solanum	America	Shrub
		Tropaeolaceae			
338	*Tropaeolum majus*	Tropaeolaceae	Tropaeolum	South America	Herb
		Urticaceae			
339	*Pilea microphylla*	Urticaceae	Pilea	South America	Herb
		Verbenaceae			
340	*Clerodendrum philippinum*	Verbenaceae	Clerodendrum	Asia	Shrub
341	*Duranta erecta*	Verbenaceae	Duranta	Latin America	Shrub
342	*Lantana camara*	Verbenaceae	Lantana	America	Shrub
343	*Lantana montevidensis*	Verbenaceae	Lantana	Latin America	Shrub
344	*Phyla nodiflora*	Verbenaceae	Phyla	America	Herb
345	*Stachytarpheta jamaicensis*	Verbenaceae	Stachytarpheta	Central and South America	Herb
		Zingiberaceae			
346	*Alpinia officinarum*	Zingiberaceae	Alpinia	South Asia	Tree
347	*Curcuma domestica*	Zingiberaceae	Curcuma	India	Herb
348	*Hedychium coronarium*	Zingiberaceae	Hedychium	Himalaya	Herb
349	*Zingiber officinale*	Zingiberaceae	Zingiber	Tropical Asia	Herb

***Table S5**. Checklist of invasive plant species in Shenzhen.*

Code	Species	Family	Genus	Geographic origin	Life form
		Amaranthaceae			
1	*Alternanthera philoxeroides*	Amaranthaceae	Alternanthera	South America	Herb
2	*Amaranthus hybridus*	Amaranthaceae	Amaranthus	America	Herb
3	*Amaranthus paniculatus*	Amaranthaceae	Amaranthus	America	Herb
4	*Amaranthus spinosus*	Amaranthaceae	Amaranthus	America	Herb
5	*Amaranthus tricolor*	Amaranthaceae	Amaranthus	Asia	Herb
6	*Amaranthus viridis*	Amaranthaceae	Amaranthus	Africa	Herb
7	*Gomphrena celosioides*	Amaranthaceae	Gomphrena	America	Herb
		Apiaceae			
8	*Coriandrum sativum*	Apiaceae	Coriandrum	Mediterranean	Herb
9	*Daucus carota*	Apiaceae	Daucus	Asia	Herb
10	*Eryngium foetidum*	Apiaceae	Eryngium	America	Herb
		Apocynaceae			
11	*Catharanthus roseus*	Apocynaceae	Catharanthus	Africa	Herb
		Araceae			
12	*Pistia stratiotes*	Araceae	Pistia	Brazil	Herb
		Asclepiadaceae			
13	*Asclepias curassavica*	Asclepiadaceae	Asclepias	Latin America	Herb
		Asteraceae			
14	*Ageratum conyzoides*	Asteraceae	Ageratum	Central and South America	
15	*Ageratum houstonianum*	Asteraceae	Ageratum	North America	Liana
16	*Ambrosia artemisiifolia*	Asteraceae	Ambrosia	North America	
17	*Aster subulatus*	Asteraceae	Aster	North America	

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18	*Bidens bipinnata*	Asteraceae	Bidens	East Asia	Herb
19	*Chrysanthemum coronarium*	Asteraceae	Chrysanthemum	Mediterranean	
20	*Conyza bonariensis*	Asteraceae	Conyza	America	
21	*Conyza canadensis*	Asteraceae	Conyza	North America	Herb
22	*Conyza sumatrensis*	Asteraceae	Conyza	South America	
23	*Coreopsis lanceolata*	Asteraceae	Coreopsis	North America	
24	*Coreopsis tinctoria*	Asteraceae	Coreopsis	North America	Herb
25	*Cosmos bipinnata*	Asteraceae	Cosmos	North America	Herb
26	*Crassocephalum crepidioides*	Asteraceae	Crassocephalum	Africa	Herb
27	*Crossostephium Chinensis*	Asteraceae	Crossostephium	America	Herb
28	*Erechthites hieracifolia*	Asteraceae	Erechthites	North America	Herb
29	*Erechthites valerianaefolia*	Asteraceae	Erechthites	South America	Herb
30	*Erigeron annuus*	Asteraceae	Erigeron	North America	Herb
31	*Eupatorium odoratum*	Asteraceae	Eupatorium	Tropical America	Herb
32	*Galinsoga parviflora*	Asteraceae	Galinsoga	South America	Herb
33	*Gnaphalium pensylvanicum*	Asteraceae	Gnaphalium	Warm America	Herb
34	*Helianthus tuberosus*	Asteraceae	Helianthus	Europe	Herb
35	*Leucanthemum vulgare*	Asteraceae	Leucanthemum	North America	Herb
36	*Mikania micrantha*	Asteraceae	Mikania	America	Herb
37	*Parthenium hysterophorus*	Asteraceae	Parthenium	Central and South America	Herb, Shrub
38	*Silybum marianum*	Asteraceae	Silybum	Europe, Asia, Africa	Herb
39	*Solidago canadensis*	Asteraceae	Solidago	North America	Herb
40	*Soliva anthemifolia*	Asteraceae	Soliva	Oceania	Herb
41	*Sonchus asper*	Asteraceae	Sonchus	Europe	Herb
42	*Sonchus oleraceus*	Asteraceae	Sonchus	Europe	Herb
43	*Synedrella nodiflora*	Asteraceae	Synedrella	America	Herb
44	*Tagetes erecta*	Asteraceae	Tagetes	North America（Mexico）	Herb
45	*Tagetes patula*	Asteraceae	Tagetes	Mexico	Herb/Vine
46	*Tithonia diversifolia*	Asteraceae	Tithonia	Central and North America	Herb
47	*Tridax procumbens*	Asteraceae	Tridax	America	Herb

48	*Wedelia trilobata*	Asteraceae	Wedelia	America	Herb
					Herb
		Bignoniaceae			
49	*Macfadyena unguis-cati*	Bignoniaceae	Macfadyena	America	Liana
		Brassicaceae			
50	*Lepidium virginicum*	Brassicaceae	Lepidium	North America	Herb
		Cactaceae			
51	*Opuntia dillenii*	Cactaceae	Opuntia	Tropical America	Herb
		Chenopodiaceae			
52	*Chenopodium ambrosioides*	Chenopodiaceae	Chenopodium	America	Herb
		Convolvulaceae			
53	*Ipomoea cairica*	Convolvulaceae	Ipomoea	Europe	Herb/Vine
54	*Ipomoea indica*	Convolvulaceae	Ipomoea	South America	Herb/Vine
55	*Ipomoea nil*	Convolvulaceae	Ipomoea	America	Herb/Vine
56	*Ipomoea purpurea*	Convolvulaceae	Ipomoea	Tropical America	Herb/Vine
57	*Ipomoea triloba*	Convolvulaceae	Ipomoea	Tropical America	Herb/Vine
		Crassulaceae			
58	*Bryophyllum pinnatum*	Crassulaceae	Bryophyllum	Africa	Herb
		Euphorbiaceae			
59	*Euphorbia hirta*	Euphorbiaceae	Euphorbia	Central America, South America	Herb
60	*Euphorbia marginata*	Euphorbiaceae	Euphorbia	North America	Herb
61	*Jatropha curcas*	Euphorbiaceae	Jatropha	America	Shrub, Tree
62	*Ricinus communis*	Euphorbiaceae	Ricinus	Africa	Herb
		Fabaceae			
63	*Acacia farnesiana*	Fabaceae	Acacia	America	Shrub
64	*Chamaecrista mimosoides*	Fabaceae	Chamaecrista	America	Herb, Shrub

65	*Crotalaria zanzibarica*	Fabaceae	Crotalaria	South America	Herb, Shrub
66	*Indigofera suffruticosa*	Fabaceae	Indigofera	America	Herb, Shrub
67	*Leucaena leucocephala*	Fabaceae	Leucaena	America	Shrub
68	*Medicago sativa*	Fabaceae	Medicago	Asia	Herb
69	*Mimosa bimucronata*	Fabaceae	Mimosa	America	Shrub
70	*Mimosa invisa*	Fabaceae	Mimosa	America	Herb
71	*Mimosa pudica*	Fabaceae	Mimosa	America	Herb, Shrub
72	*Senna alata*	Fabaceae	Senna	America	Shrub
73	*Senna occidentalis*	Fabaceae	Senna	America	Herb, Shrub
74	*Senna tora*	Fabaceae	Senna	Burma to Malaysia	Tree
75	*Trifolium repens*	Fabaceae	Trifolium	Europe, North Africa	Herb
		Geraniaceae			
76	*Geranium carolinianum*	Geraniaceae	Geranium	America	Herb
		Labitae			
77	*Hyptis brevipes*	Lamiaceae	Hyptis	America	Herb
78	*Hyptis rhomboidea*	Lamiaceae	Hyptis	America	Herb
79	*Hyptis suaveolens*	Lamiaceae	Hyptis	America	Herb
		Lythraceae			
80	*Cuphea balsamona*	Lythraceae	Cuphea	America	Herb
		Malvaceae			
81	*Malvastrum coromandelianum*	Malvaceae	Malvastrum	America	Herb, Shrub
82	*Waltheria indica*	Malvaceae	Waltheria	America	Herb, Shrub
		Myrtaceae			
83	*Eucalyptus robusta*	Myrtaceae	Eucalyptus	Australia	Tree
84	*Syzygium jambos*	Myrtaceae	Syzygium	Southest Asia	Tree

		Nyctaginaceae			
85	*Mirabilis jalapa*	Nyctaginaceae	Mirabilis	South America	Herb
		Onagraceae			
86	*Oenothera drummondii*	Onagraceae	Oenothera	America	Herb
		Oxalidaceae			
87	*Oxalis corymbosa*	Oxalidaceae	Oxalis	America	Herb
		Passifloraceae			
88	*Passiflora foetida*	Passifloraceae	Passiflora	Latin America	Herb/Vine
		Phytolaccaceae			
89	*Phytolacca americana*	Phytolaccaceae	Phytolacca	North America	Herb
		Piperaceae			
90	*Peperomia pellucida*	Piperaceae	Peperomia	America	Herb
		Poaceae			
91	*Axonopus compressus*	Poaceae	Axonopus	America	Herb
92	*Cenchrus echinatus*	Poaceae	Cenchrus	America	Herb
93	*Melinis repens*	Poaceae	Melinis	Africa	Herb
94	*Panicum maximum*	Poaceae	Panicum	Africa	Herb
95	*Panicum repens*	Poaceae	Panicum	Brazil	Herb
96	*Paspalum conjugatum*	Poaceae	Paspalum	America	Herb
97	*Pennisetum purpureum*	Poaceae	Pennisetum	Africa	Herb
		Pontederiaceae			
98	*Eichhornia crassipes*	Pontederiaceae	Eichhornia	America	Herb

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		Portulacaceae			
99	*Portulaca pilosa*	Portulacaceae	Portulaca	Tropical America	Herb
100	*Talinum paniculatum*	Portulacaceae	Talinum	America	Herb
		Rubiaceae			
101	*Borreria latifolia*	Rubiaceae	Borreria	South America	Herb
		Scrophulariaceae		North America	Herb
102	*Scoparia dulcis*	Scrophulariaceae	Scoparia	America	Herb
103	*Veronica peregrina*	Scrophulariaceae	Veronica	Asia	Herb
104	*Veronica persica*	Scrophulariaceae	Veronica	West Asia, Europe	Herb
105	*Veronica polita*	Scrophulariaceae	Veronica	America	Herb
		Solanaceae			
106	*Datura metel*	Solanaceae	Datura	America	Herb
107	*Datura stramonium*	Solanaceae	Datura	North America	Herb
108	*Nicandra physaloides*	Solanaceae	Nicandra	South America	Herb
109	*Physalis angulata*	Solanaceae	Physalis	America	Herb, Shrub
110	*Solanum erianthum*	Solanaceae	Solanum	America	Tree
111	*Solanum surattense*	Solanaceae	Solanum	Brazil	Herb, Shrub
112	*Solanum torvum*	Solanaceae	Solanum	America	Shrub
		Urticaceae			
113	*Pilea microphylla*	Urticaceae	Pilea	South America	Herb
		Verbenaceae			
114	*Duranta erecta*	Verbenaceae	Duranta	Latin America	Shrub
115	*Lantana camara*	Verbenaceae	Lantana	America	Shrub
116	*Lantana montevidensis*	Verbenaceae	Lantana	Latin America	Shrub
117	*Stachytarpheta jamaicensis*	Verbenaceae	Stachytarpheta	Central and South America	Herb

SIMILARITY ASSESSMENT OF NATURAL LANDSCAPES BASED ON TAXONOMIC DISTANCE

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Abstract. The degree of similarity/dissimilarity between landscapes is important information for landscape classification, potential assessment and evaluation. In geographical assessments, similarity in landscape functioning and visual attributes is commonly analysed by using landscape factors (e.g., relief, water balance and soil fertility), land use and built-up area. In this study the similarity and dissimilarity of Hungarian landscape units were investigated to reveal the appropriateness and uncertainty of the current delimitation methods. Nine indicators for 230 landscape units, delineated by the traditional methods, were integrated into a data matrix. Dissimilarity coefficients were calculated for each unit to determine the Euclidean distance between all indicators. Dissimilarity analysis was performed by multidimensional scaling using Kruskal's stress test and hierarchical clustering. The applied method enabled a more objective determination of taxonomic distance. Based on the results, the similarity and dissimilarity of landscapes could be evaluated by more accurate and quantitative datasets. Several neighbouring landscape units should require increased attention in spatial planning because these landscapes differ from each other: they belong to different clusters based on the investigated indicators, though they are adjacent.

Keywords: *similarity analysis, multidimensional scaling, taxonomic distance, ecology*

Introduction

Several approaches for landscape interpretation exist (e.g., based on functionality and spatial patterns) that significantly influence the primary determining factors of the landscape and their relations (Bastian and Schreiber, 1999). An obvious approach is to consider landscapes as relatively homogenous units. In many countries, natural factors (e.g., lithology, relief, soil and hydrology) were evaluated, and the results were used later as landscape units (Mosimann, 1984; Naveh and Liebermann, 1984; Haase, 1989). Landscape units are determined by natural factors that are influenced by human impact, contributing to a complex landscape system (Mezősi et al., 2013).

The integrated regional units of landscapes can be evaluated according to two main approaches:

- In the first approach, homogenous landscape units are the basis of the evaluation, and the complex units can be created using clustering or segmentation methods. For example, homogenous vegetation, pedological or relief units can be separated and integrated (Bölöni et al., 2011; Miklos, 2012; Divíšek et al., 2014; Bata et al., 2014). A similar method is based on overlaying (e.g., multiplying) separate homogenous units (e.g., soil, climate and relief), and the resulting spatial units are considered "homogenous" units (e.g., LANMAP – Wascher, 2005; Mücher et al., 2010). The resulting spatial units cannot be considered integrative; however, they can reflect landscape system features. Their acceptance as integrated units depends on the interpretation of the landscape definition.

- According to the second approach, landscape units are considered inherently integrated units (with all their hierarchical levels), but there are significant differences in the involvement of natural and human parameters. According to this interpretation, ecotopes, considering mostly natural factors and higher-level hierarchical elements, are similar; however, the degree of similarity decreases with increasing scale (Mosimann, 1984; Naveh and Liebermann, 1984; Haase, 1989; Blaschke and Strobl, 2003; Jongman, 2003; Bastian et al., 2006).

The degree of similarity between landscapes is important data for landscape classification, landscape potential assessment and landscape evaluation. Similarity is dependent on the scale of the regional units and the applied hierarchical level because the level of complexity and the resulting problems in the assessments increase with decreasing scale.

This study addresses the following questions of the landscape classification problem:

- Is the landscape unit delimitation that is currently used appropriate? Do the landscape units differ from each other? Which units would belong to other hierarchical units or cluster groups based on dissimilarity?
- According to the theoretical approach, what are the effectiveness and accuracy of the different methods for the determination of spatial units?

According to the mathematical definition of similarity, two objects are similar if one can be transformed into the other by mathematical operations (e.g., rotations, zooming or reflections). The rate of similarity can be determined by several methods (e.g., cosine similarity and correlation-based similarity) depending on the attribute structure of the given database. The parameters in this study are primarily nominal, and the classical mathematical operations are not appropriate. Thus, taxonomic distances were used for the evaluation of the relationships between landscape units (*Fig. 1*). This method has been successfully used for similarity analysis in other fields of physical geography, e.g., in the determination, relationship assessment and mapping of pedological classes based on environmental variables (WRB, 2006; Minasny and McBratney, 2007) or in the clustering of tropical dry forests based on environmental parameters (Muenchow et al., 2013). The application of multidimensional scaling (MDS) in geosciences has a 40-year history, mostly in the field of human geography (Rushton and Golledge, 1972).

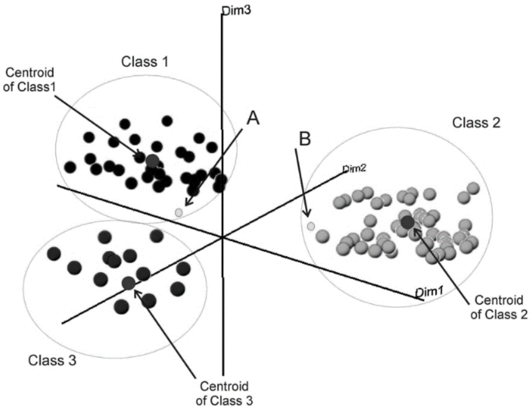

Figure 1. *A 3D visualisation of the landscape units forming three classes/clusters, based on the taxonomic distance method. Points A and B refer to outlier landscape units (in edge position), belonging to another class/cluster*

Material and Methods

Taxonomic distance and the applied data

This study is based on the taxonomic distance between the landscapes in an n-dimensional space. The number of dimensions depends on how many independent parameters describe the similarity of the landscapes (Kruskal and Wish, 1978; Hastie et al., 2001). The application of this method in landscape similarity assessments is difficult because landscape units are described primarily by nominal or interval data; furthermore, several parameters are considered for the determination of similarity. Taxonomic distance measurement using the Euclidean distance method requires numeric datasets, and the high number of applied parameters results in a multidimensional environment that is difficult to interpret. Thus, the initial datasets were converted to numeric type, and using the multidimensional scaling method (MDS), the resulting datasets were reduced to a 3D environment.

For the similarity analysis of the landscape units, the features of the landscape units, described in the Inventory of Landscape units in Hungary, were applied (Marosi and Somogyi, 1990, and the second, improved edition by Dövényi, 2010), in which the landscape units were considered integrated units. Their delineation was primarily based on natural environmental factors (geomorphology, geology, climate, hydrology, vegetation and soil), but social factors (land use, geographical situation, cultivated crops, special landscape character and other socio-economic parameters in the second edition) were also considered (*Fig. 2*).

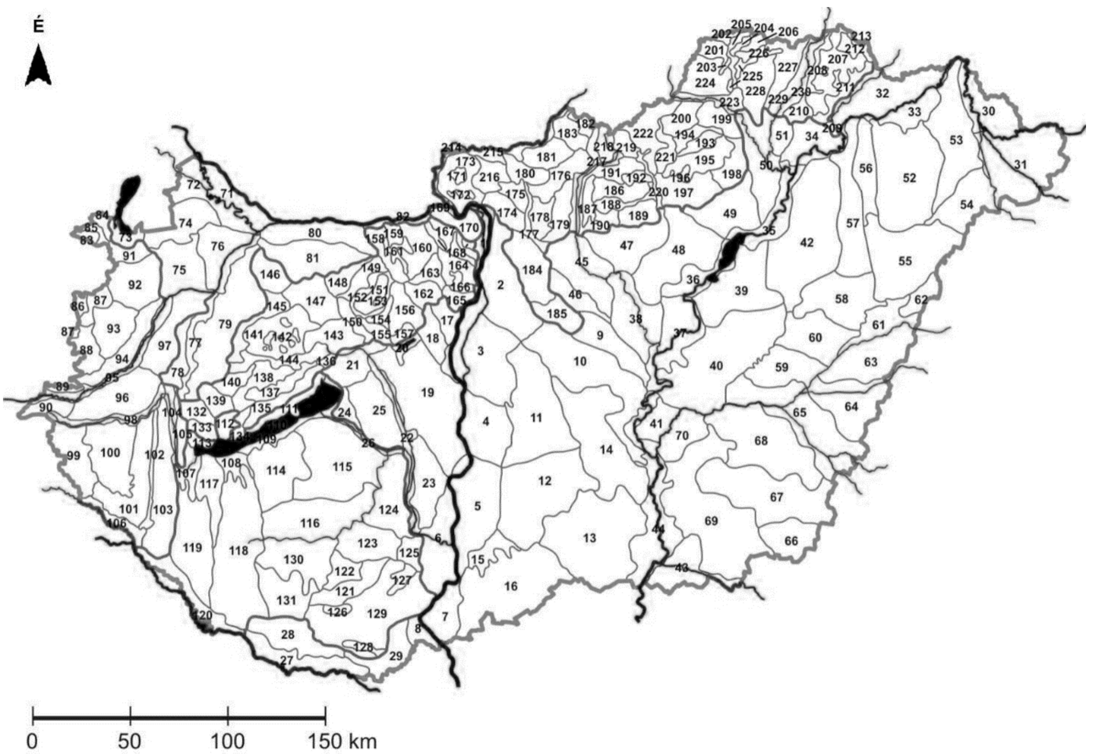

Figure 2. *Landscape units in Hungary based on Marosi and Somogyi (1990) and the second, improved edition by Dövényi (2010). The list of landscape units, indicated by the numbers, is available online at http://www.geo.u-szeged.hu/english/sites/www.geo.u-szeged.hu.english/files/kistajlista.docx*

Nine parameters for 230 landscape units (primarily 100-500 km^2 in area) were investigated by calculating the average values for each unit. The detailed description of the applied parameters can be found in *Table 1*. Several data sources have more recent versions available (e.g., CORINE Landcover Database), but temporal consistency was considered important during the analyses.

Table 1.*The parameters applied during the analysis*

Parameter	Data source	Scale	Calculation of the parameters
relief	SRTM (2000)	90 x 90 m	area weighted average of the elevation (m) for each landscape unit
soil	AGROTOPO (1991)	1:100 000	area weighted average of the topsoil thickness (cm) for each landscape unit
climate	meteorological dataset (OMSZ 1961-1990)	100 x 100 m	1961-1990 average PaDI (Pálfai 2004, Pálfai és Herceg 2011) area weighted average of PaDI for each landscape unit $\mathrm{PaDI} = [\sum_{i=\mathrm{Apr}}^{\mathrm{Aug}} \mathrm{Ti} \ /5*100]/\sum_{i=\mathrm{Oct}}^{\mathrm{Sept}} \mathrm{Pi}*\mathrm{wi}$ Ti – monthly average temperature, °C Pi – monthly precipitation, mm wi – weighting factor
ratio of built-up areas	CORINE (1990)	1:100 000	ratio of built-up areas (%) (CLC111, CLC112, CLC121, CLC122, CLC123, CLC124, CLC131, CLC132, CLC133, CLC141, and CLC142) for each landscape unit
ratio of forest cover	CORINE (1990)	1:100 000	ratio of forest cover (%) (CLC311, CLC312, and CLC313) for each landscape unit
ratio of vineyards	CORINE (1990)	1:100 000	ratio of vineyards (%) (CLC221) for each landscape unit
vegetation	MODIS EVI	250 x 250 m	area weighted average of the Enhanced Vegetation Index (EVI) in July between 2000 and 2010 for each landscape unit
fragmentation	road and railway network 1990-ben (OTAB 1990)	1:100 000	effective mesh size for each landscape unit (Jaeger 2000) $EffectiveMesh = \frac{A_t}{S} = \frac{1}{A_t}\sum_{i=1}^{n} A_i^2$ n – number of patches A_i – size of the patch i A_t – size of the entire area
hemeroby	CORINE (1990)	1:100 000	area weighted average of the hemeroby index (Csorba and Szabó 2009) for each landscape unit

Methods

In the assessment, a combined methodology was used (*Fig. 3*) to allocate the outlier landscape units in the existing classes (see *Fig. 1*), which thus require a reconsideration of their class membership. Using the dataset of the parameters correlation analysis, principal component analysis and multidimensional scaling (MDS) were performed to quantify the similarity of the landscape units. MATLAB was used for the calculations.

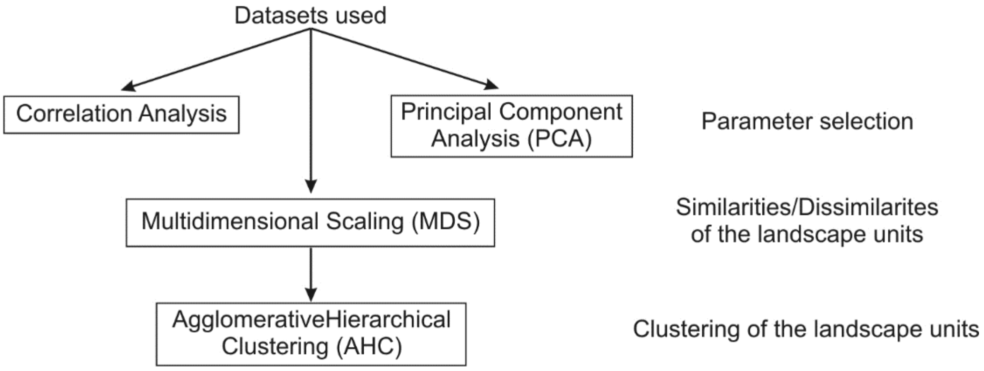

Figure 3. *Flowchart of the methods applied for the similarity assessment of the landscapes*

The correlation analysis was conducted to determine whether there were strong relationships among parameters. If such variables exist, one of the variables has to be removed from the MDS analysis because the axes of the dimensions representing these parameters are very similar, which influences the results. Based on the resulting correlation matrix, the key factors among the selected parameters in the landscape can be determined.

Principal component analysis (PCA) was used for the determination of the relationship between the applied factors based on Pearson's correlation matrix. The PCA method is mostly used for data reduction and data structure assessments. PCA uses an orthogonal transformation to convert a set of possibly correlated variables into a set of uncorrelated variables (F_1… F_n). The number of the resulting principal components is less than or equal to the number of original variables. The first principal component has the largest possible variance. Because the first few principal components describe a significant amount of the variance, dimension reduction is possible. If the calculated eigenvector of a principal component is less than 1, it is not considered in the evaluation. The cumulative percentage is the accumulated share of variation explained up to the last component (Joliffe, 2002).

Multidimensional scaling (MDS) refers to a class of techniques that uses proximities among any type of objects as input (Kruskal and Wish, 1978). According to Carroll et al. (2005), MDS is a family of models and methods for representing stimuli or other objects as points in multidimensional space based on proximity (e.g., similarity or dissimilarity) data. This method relies on the principle that distances (usually, but not necessarily, Euclidean) in that space are related via a simple (usually linear or monotonic) function of the proximities (Marcussen, 2014)

For multidimensional scaling, a data matrix was established considering the factors for each of the 230 landscape units. Based on this data set, a dissimilarity matrix ($\Delta\delta_{ij}$) was computed, where the dissimilarity coefficient (δ_{ij}) refers to the dissimilarity between i and j landscape units. Dissimilarity (distance) was calculated using Euclidean distance measures:

$$\text{Euclidean distance} = \sqrt{\sum_{t=1}^{p}(x_{it} - x_{jt})^2}$$

where x_{it} denotes the ith row and x_{jt} the jth column of the x_t data matrix (Hout et al., 2013; Rushton and Golledge, 1972).

Using MDS, multidimensional datasets can be reduced to 2 or 3 dimensions. During the analysis, the x, y and z co-ordinates of each landscape unit were computed; thus, the units could be visualised in a three-dimensional space. The location of the points and the distances between them describe the dissimilarity/similarity of the landscape units. Two approaches were considered during the evaluation. In version A, only natural factors were involved in the calculations, whereas in version B, both natural and anthropogenic factors were considered. Similarity was evaluated based on the taxonomic distances from the cluster centroids.

Kruskal's stress test (Kruskal, 1964) was used to measure the goodness of fit (S) before and after the dimension reduction (the difference between the original and the computed Euclidean distances).

The results of the test were described as excellent ($S<0.05$), good ($0.05<S<0.1$), medium ($0.1<S<0.15$), or poor ($0.15<S<0.2$). If $S>0.2$, the given number of dimensions is not acceptable due to the high variance (Kruskal, 1964; Kruskal and Wish, 1978).

Agglomerative Hierarchical Clustering (AHC) identifies clusters based on the dissimilarity matrix data of the landscape units. The AHC, as an iterative classification method, results in a hierarchical structure of the input datasets based on a bottom-up approach. In the AHC algorithm, the aggregation criterion is based on variance (Rolet and Seguin, 1986) using the Ward formulation (Ward, 1963), which ensures that at each step of the clustering, the overall heterogeneity is minimised, and the separation between classes is maximised. Using the AHC method, landscape units were clustered into 4 groups.

Results

According to the correlation matrix of the investigated parameters (*Table 2*), a strong positive relationship between the relief and forest cover (0.86) and a strong negative correlation between forest cover and hemeroby (-0.78) were identified. Avoiding duplications, the forest cover and hemeroby parameters were excluded from further investigations because they are partially involved in the vegetation, built-up areas and fragmentation parameters. The reason for this exclusion was confirmed by the results of the principal component analysis.

The principal component analysis based on all (9) parameters resulted in two main dominant variables (F1-F2). F1, the most dominant factor involved the relief, soil, forest cover, vegetation and hemeroby parameters, with relief as the most important factor. F2 involved built-up areas, referring to the anthropogenic effects. F3, F4 and F5 included fragmentation, vineyards and climate factors, respectively. These variables corresponded to the factor for which the squared cosine is the largest. The eigenvalues of F4-F9 were less than 1 (*Fig. 3*); thus, they were not considered.

Table 2. *Correlation matrix of the investigated parameters (Pearson correlation, n=230)*

Parameters	1	2	3	4	5	6	7	8	9
1 relief	1	-0.523	-0.431	0.677	0.860	-0.177	-0.008	-0.228	-0.711
2 soil		1	0.259	-0.203	-0.527	0.026	-0.073	0.014	0.691
3 climate			1	-0.492	-0.411	0.173	0.014	0.078	0.305
4 vegetation				1	0.769	-0.342	-0.051	-0.281	-0.458
5 ratio of forest cover					1	-0.290	-0.009	-0.140	-0.785
6 ratio of built-up areas						1	0.058	-0.237	-0.105
7 ratio of vineyards							1	-0.125	0.122
8 fragmentation								1	-0.015
9 hemeroby									1

Based on the results of the correlation and principal component analyses, 7 parameters were applied in the investigation of landscape similarity. In version A, natural factors (relief, soil, climate and vegetation) were included in the calculations, and the resulting 0.002 value of Kruskal's stress test indicates an excellent fit. In version B, both natural (relief, soil, climate and vegetation) and anthropogenic factors (ratio of built-up areas, ratio of vineyards and fragmentation) were included in the calculations, and the resulting 0.01 value of Kruskal's stress test also indicates an excellent fit. In both versions, four clusters were separated using the AHC method.

The clusters and their centroids (centroid landscape units) in version A were visualised in 3D in *Figure 4*, demonstrating the dense point cloud and the clusters. The calculated distances were shown in *Table 3*. Based on the results, 101, 33, 93 and 3 elements were clustered into Clusters 1, 2, 3 and 4, respectively.

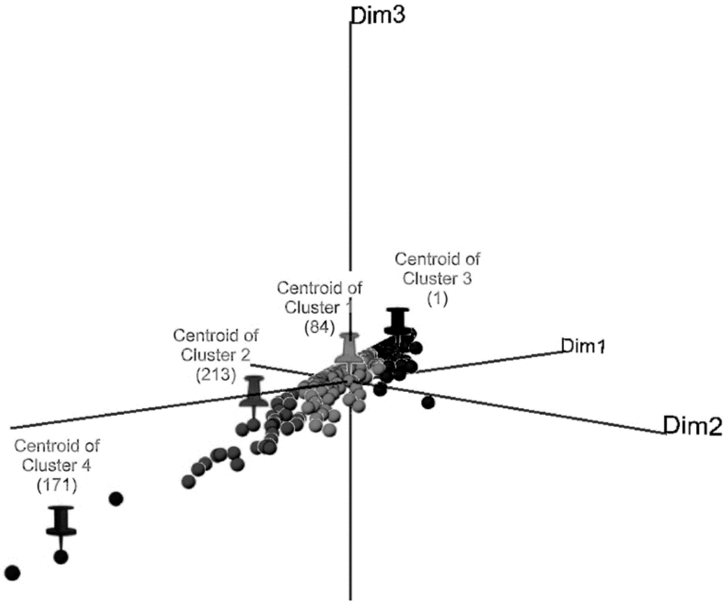

Figure 4. *Clusters in version A and their centroids in 3D space*

Table 3. *Distances between cluster centroids in 3D space in version A*

	Cluster 1 (84)	Cluster 2 (213)	Cluster 3 (1)	Cluster 4 (171)
Cluster 1 (84)	0	146.75	82.77	443.24
Cluster 2 (213)		0	229.33	298.33
Cluster 3 (1)			0	525.93
Cluster 4 (171)				0

The taxonomic distances from the cluster centroids were the basis of the determination of similarity; however, the definition of the boundaries for outliers in the clusters was not straightforward. The statistical analysis of the distances from the cluster centroids (excluding points of Cluster 4) indicated a frequency limit at 70% of the distances; thus, this value was applied as a criterion for similarity. Using this criterion, 25 landscape units were selected for which the revision of the grouping might be necessary (*Fig. 5*).

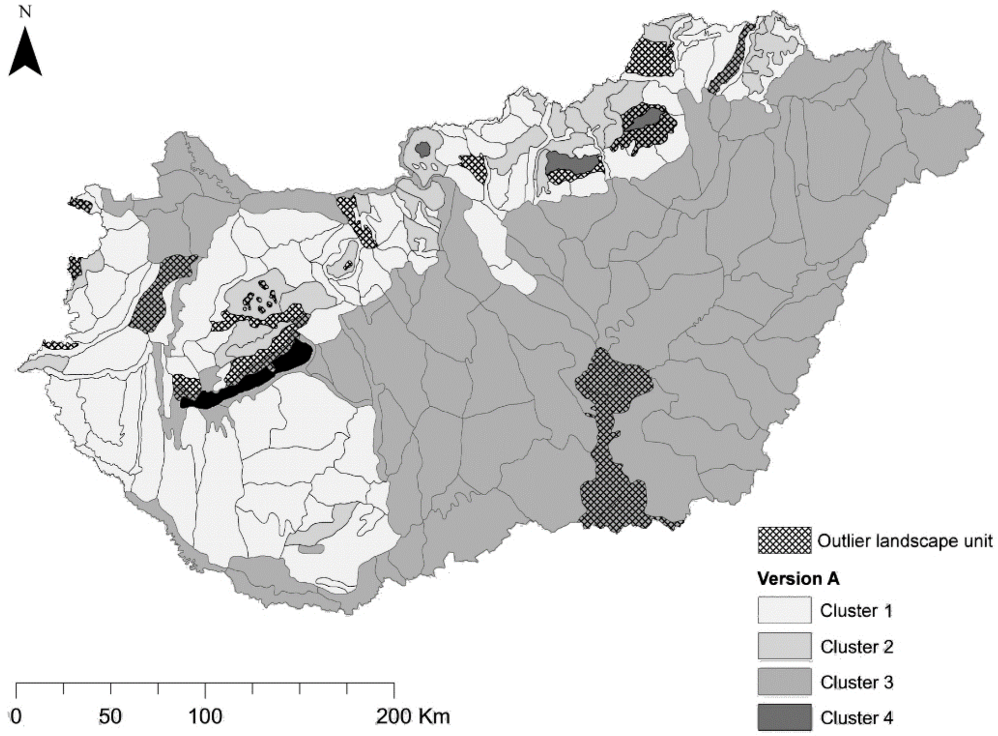

Figure 5. *Clusters in version A and outlier landscape units*

In version A, Cluster 1 was defined by medium relief and significant topsoil-thickness; Cluster 2 was characterised by high relief and high EVI averages indicating high forest cover; the lowest relief was observed in Cluster 3; and the landscape units of Cluster 4 were defined by the smallest topsoil thickness, highest relief and high EVI averages, also indicating high forest cover.

The version B analysis had similar results. The clusters and their centroids were visualised in 3D in *Figure 6*, and the calculated distances between them were given in

Table 4. According to the results, 101, 36, 88 and 5 elements were clustered into Clusters 1, 2, 3 and 4, respectively. In this version, 25 landscape units were selected for which the revision of the grouping might be necessary (*Fig. 7*).

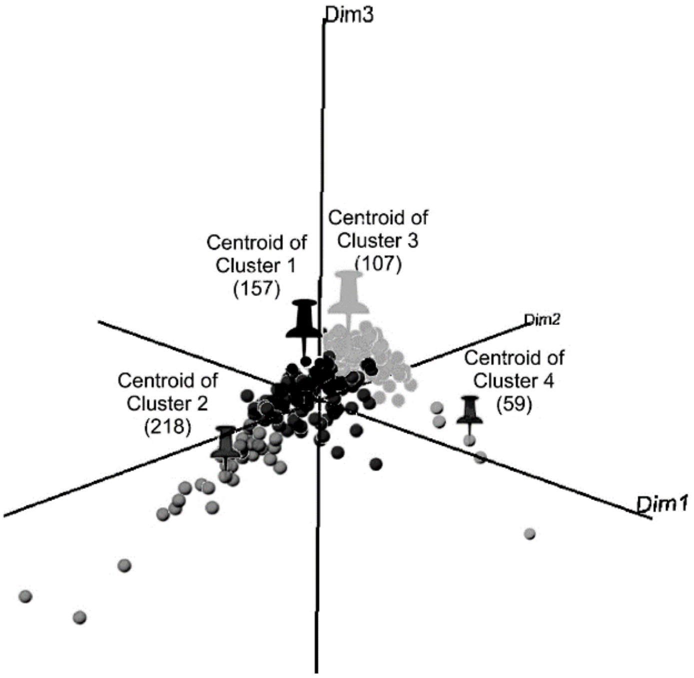

Figure 6. *Clusters in version B and their centroids in 3D space*

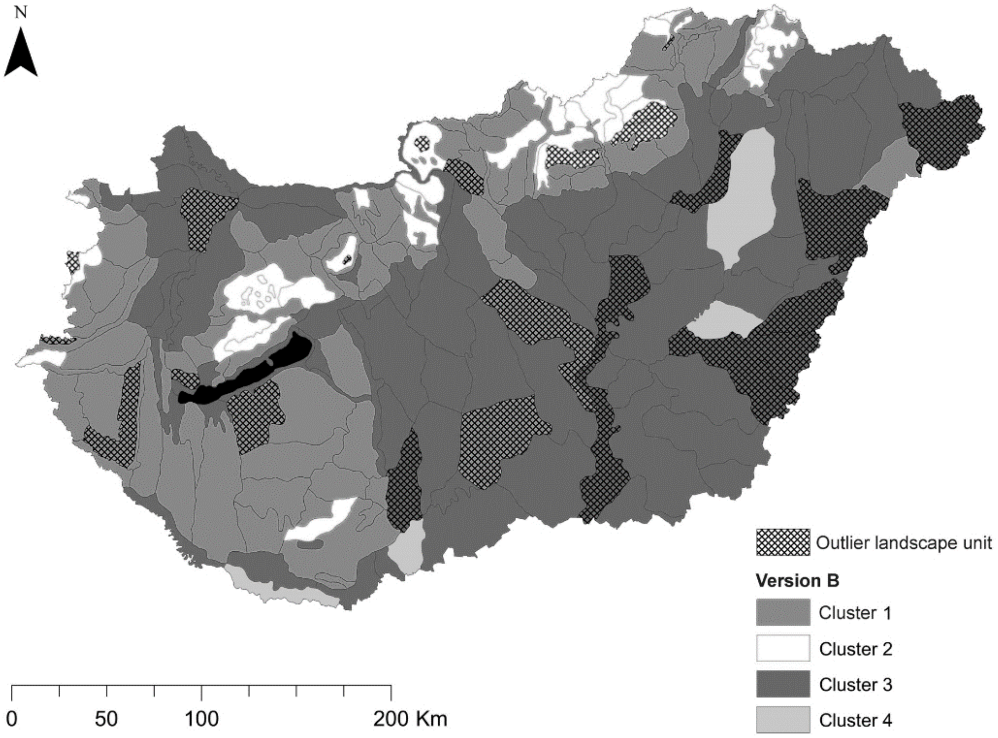

Figure 7. *Clusters in version B and the outlier landscape units*

Table 4. *Distances between cluster centroids in 3D space in version B*

	Cluster 1 (157)	Cluster 2 (218)	Cluster 3 (107)	Cluster 4 (59)
Cluster 1 (157)	0	172.10	81.45	294.45
Cluster 2 (218)		0	250.45	413.97
Cluster 3 (107)			0	283.77
Cluster 4 (59)				0

In version B, Cluster 1 was defined by medium relief and relatively high averages of built-up areas and vineyard cover; the highest relief was characteristic of the landscape units of Cluster 2; Clusters 3 and 4 were similarly characterised by low relief, high topsoil thickness, and a higher ratio of built-up areas, but cluster 4 was separated due to low fragmentation (higher effective mesh size). These areas were located along rivers without substantial linear infrastructure.

The A and B assessments defined outlier landscape units (marked with stripes in *Figures 5 and 7*); furthermore, certain landscape units were assigned to different clusters in the two different approaches. In these cases, further assessment of the linkages with the neighbouring landscape units should be considered.

Table 5 presents examples where taxonomic distances may reveal the dissimilarity of neighbouring landscapes or confirm that they should be merged based on their similarity. In several cases, clear answers cannot be obtained. For example, among the lowland landscapes, units 7 and 8 (Mohács Island and Mohács Terrace Plain) are relatively close, based on their taxonomic distance; thus, they might be merged according to version A.

Table 5. *Taxonomic distances (based on MDS) between selected landscape units*

Version A			Version B		
unit IDs	clusters	taxonomic distance	unit IDs	clusters	taxonomic distance
7-8	3-3	**4**	7-8	**4-3**	**139**
226-227	1-1	**18**	226-227	1-1	23
226-223	1-1	80	226-223	1-1	96
227-223	1-1	61	227-223	1-1	74
194-195	2-2	**16**	194-195	2-2	**16**
194-193	2-4	**274**	194-193	2-2	**245**
195-193	2-4	**262**	195-193	2-2	**256**

The consideration of more parameters in version B resulted in higher taxonomic distances between them and their placement into different clusters, confirming the opposite result (they cannot be combined). Among the hilly landscapes, similar results occur. In the case of the neighbouring landscape units 223, 226, and 227 (Sajó Valley, Rakaca Valley, and Eastern Cserehát), 226 and 227 can be combined based on the results of both versions of the analysis. However, the two units are both at greater distances to the neighbouring landscape unit, 223, in the same cluster, indicating their dissimilarity.

To analyse the mountainous landscapes, the landscape units of the Bükk Mountains are used as an example. Landscape units 194 and 195 (Northern and Southern Bükk) are characterised by low taxonomic distances and belong to the same clusters; thus, they can be merged. However, both units differ from the neighbouring unit, 193 (Bükk Plateau); thus, their separation is reasonable (they belong to different clusters in version A).

The results confirm the revised landscape unit classification in the new Hungarian National Atlas (publication is expected in 2016), where simplification of the previous classification and the mitigation of political influence were the basis of the reconsideration of the landscape units.

Discussion and conclusion

European landscapes and those of the Carpathian Basin are characterised by high diversity. The preservation of the mosaic character has become important and is increasingly a target of many countries in Europe (e.g., European Landscape Convention). In this study, the similarity of the landscape units was assessed. The focus of the assessment was not to highlight the similarity of landscapes but to develop a quantitative basis for landscape assessment.

In geoscientific studies, at least three different landscape concepts exist (focusing on the formation, the functions or the land use of the landscape), but these concepts are not clearly distinct approaches. Within these concepts, different factors describe the system; thus, the assessments integrate different parameters. Whether the land use or visual character of the landscape can be properly characterised based on traditional genetic or functional parameters is still under debate. The higher the naturalness of a landscape unit, the more obvious the relationship between the genetic type and the landscape character (Brunce et al., 2007; Li and Wu, 2007; Csorba, 2008; Breuste et al., 2009)

In the case of most European landscapes, the knowledge of the genetic processes (e.g., a hilly landscape formed by aeolian processes) or the functional parameters does not provide useful information for practical assessment. Practical application requires data on land use (e.g., arable lands) or visual characteristics for planning and development purposes, which can be obtained by applying assessments of landscape character and landscape indicators (LCA, 2014).

In the optimal case, the land use and the landscape character fit the potential natural attributes of the landscape (Pietrzak, 2000; Tress et al., 2003; Li and Wu, 2007). This match between character and land use would support increased economic sustainability, because adapting to the inherent potential causes the least impact on the system and also requires the least effort to maintain the land use. Land use was adapted to the natural attributes of the landscape for centuries, but many attributes have been irreversibly altered, and social needs have extended far beyond the natural potential.

The methods applied in this study provided a more objective statistical comparison of landscapes. A wide range of possible indicators exists (in addition to those used in this assessment) but with special care of the correlations of the parameters, furthermore several different approaches (e.g., function or use) can also be considered in landscape assessment. The grouping of the applied indicators may also allow more focused similarity assessments.

Statistically correct clustering does not guarantee that the resulting groups are clearly interpretable in the landscape classification. The clustering of similar landscapes into different meso-level landscape units indicates failure. This problem refers back to the landscape concept. If landscapes are considered the integration of natural and social factors, their describing parameters depend on the concept and the type of the landscape. Therefore, the selection of parameters describing the system cannot be 'standardised' because the most relevant parameters have to be involved in the assessment (Naveh and Lieberman, 1984). The integration of social factors that are not easily measurable is important, as demonstrated by the Hortobágy 'Pusta' (UNESCO world heritage) in Hungary. This landscape, typifying the ancient image of the Carpathian Basin, cannot be correctly described only by natural parameters because the social influence and grazing have contributed significantly to its present character.

The calculations in this study were based on integrated units and their hierarchical system. Other approaches exist for the joint evaluation of different factors and parameters; for example, the LANMAP project, which overlays four basic maps (climate, relief, lithology and land use), with the resulting map consisting of homogenous polygons that can be integrated into a system and grouped regionally or hierarchically (Wascher, 2005; Mücher et al., 2010). These homogenous units are not realistic for Hungarian territories, and several landscape character types are not represented. Moreover, the results differ significantly from the traditional classification, possibly because of the parameters applied and the European scale data. However, the results facilitated further research using other types and greater numbers of parameters. Based on the findings of the ELCAI (European Landscape Character Assessment Initiative), several landscape assessments applying new approaches were initiated in Europe (Pedroli et al., 2007; Krzywinski et al., 2009; Küster, 2010).

The problem with the landscape approach, which applies integrated units, is that the integration of ecological units into landscape unit classification is not well defined in every case. Therefore, the present landscape classification methods are still based primarily on modern geomorphology or social and economic factors (Haase, 1989; Wrbka et al., 2004), but they do not reflect the integrated character of the landscapes.

Multidimensional scaling has many applications, from geophysics to mental mapping. The assessment of functional vegetation clusters and multi-parameter assessments in ecology and zoology has confirmed its application in landscape ecological assessments (Clarke, 1993; García-Abad et al., 2010; Muenchow et al., 2013).

Quantifiable similarity can likely be effectively used for other theoretical landscape research and for practical application in landscape planning and protection. Landscape similarity results can contribute to the estimation of the regional scale effects of global changes. For example, landscape units can be classified based on landscape similarity to examine the regional scale effects of global climate change, trends in mass tourism or land use homogenisation (e.g. afforestation).

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REFERENCES

[1] AGROTOPO (1991). Agrotopográfiai Adatbázis. [Agrotopographic Database]. (MTA Talajtani és Agrokémiai Kutatóintézet http://maps.rissac.hu/agrotopo/, Accessed: 27.05.2015

[2] Bastian, O., Schreiber, K.F. (1999): Analayse und ökologische Bewertung der Landschaft. – Spektrum. (2nd ed.), Heibelberg

[3] Bastian, O., Kronert, R., Lipsky, Z. (2006): Landscape diagnosis on different space and time scales – a challenge for landscape planning. – Landscape Ecology 21: 359–374.

[4] Bata, T., Mezősi, G., Meyer, B. C. (2014): Landscape units for Hungary using multiresolution segmentation of geo-data and fuzzy analysis. – Carpathian Journal of Earth and Environmental Sciences 9(2): 45–56.

[5] Blaschke, T., Strobl, J. (2003): Defining landscape units through integrated morphometric characteristics. – In: Buhmann, E., Ervin, S. (Eds.) Landscape Modelling: Digital Techniques for Landscape Architecture. Berlin: Wichmann-Verlag, pp. 104–113

[6] Bölöni, J., Botta-Dukát, Z., Illyés, E., Molnár, Z. (2011): Hungarian landscape types: classification of landscapes based on the relative cover of (semi-)natural habitats. – Appl Veg Sci 14: 537–546.

[7] Breuste, J., Kozova, M., Finka, M. (2009): European landscapes in Transformation. Challenges for landscape Ecology and Management. European IALE Conference in Salzburg and Bratislava, Printed and Publ. House of the Slovak Univ. of technology in Bratislava 608 p

[8] Brunce, R. G. H., Jongman, R. H. G., Hojas, L., Weel, S. (Eds) (2007): 25 Years of Landscape Ecology: Scientic Principes in Practice. – In: Proceedings of the 7th. IALE World Congress I-I. 1184 p

[9] Carroll, J.D., Arabie, P., Hubert, L. J. (2005): Multidimensional scaling (MDS) – In: Kempf-Leonard, K. (Ed.) Encyclopedia of social measurement. New York: Elsevier, pp. 779–784

[10] Clarke, K. R. (1993): Non-parametric multivariate analyses of change in community structure. – Australian Journal of Ecology 18: 117–143

[11] Csorba, P. (2008): Indicators of landscape functioning, which mark the material and energy budgets in landscape. – In: Andreychouk, V. (Ed) Methodology of landscape research. Commission of Cultural Landscape of Polish Geographical Society, Sosnowiec, 128–140

[12] Csorba, P., Szabó, Sz. (2009): Degree of human transformation of landscapes: a case study from Hungary. – Hungarian Geographical Bulletin 58: 91–99

[13] CORINE (1990). CORINE Land Cover database, European Environment Agency http://www.eea.europa.eu/data-and-maps/data/corine-land-cover-1990-raster-3, Accessed: 27. 05. 2015

[14] Divíšek, J., Chytrý, M., Grulich, V., Poláková, L. (2014): Landscape classification of the Czech Republic based on the distribution of natural habitats. – Preslia 86: 209–231.

[15] Dövényi, Z. (ed) (2010): Magyarország kistájainak katasztere. [Inventory of Microregions in Hungary] Budapest, MTA FKI, 876 p. In Hungarian

[16] García-Abad, J.J., Malpica, J.A., Alonso, M.C. (2010): Detecting plant spatial patterns, using multidimensional scaling and cluster analysis, in rural landscapes in Central Iberian Peninsula. – Landscape and Urban Planning 95: 138–150.

[17] Jaeger, J. A. G. (2000): Landscape division, splitting index, and effective mesh size: New measures of landscape fragmentation. – Landscape Ecology 15 (2): 115–130.

[18] Joliffe, I. T. (2002): Principal Component Analysis. (2nd ed.) – New York: Springer.

[19] Haase, G. (1989): Medium scale landscape classification in the German Democratic Republic. – Landscape Ecology 3(1): 29–41.

[20] Hastie, T., Tibshirani, N., Reuter, H. I. (2001): The elements of statistical learning: data mining, interference and prediction. Spreinger Series in Statistics. New York: Springer-Verlag.

[21] Hout, M.C., Papesh, M.H., Goldinger, S.D. (2013): Multidimensional scaling. – Wiley Interdiscip Rev Cogn Sci 4(1): 93–103.

[22] Jongman, R. H. G. (Ed) (2003): The New Dimensions of the European Landscapes. – Netherlands: Springer

[23] Kruskal, J. B. (1964): Multidimensional scaling by optimizing goodness of fit to a nonmetric hypothesis. – Psychometrika: 29(1): 1–27.

[24] Kruskal, J. B., Wish, M. (1978): Multidimensional scaling. – Beverly Hills, CA: Sage Publications.

[25] Krzywinski, K., O'Commell, M., Küster, H. (2009): Europäische Kulturlandschaften. – Bremen: Aschenbeck Media, 217

[26] Küster, H. (2010): Geschichte der Landschaft in Mitteleuropa. – München: C.H. Beck

[27] LCA (2014). Landscape and seascape character assessments. Planning and development – guidance. – In. Landscape, Planning and development, Marine environment and Biodiversity and ecosystems. Natural England and Department for Environment, Food, Rural Affairs, 2 October 2014. Available at: (https://www.gov.uk/landscape-and-seascape-character-assessments):

[28] Li, H., Wu, J. (2007): Landscape pattern analysis: key issues and challenges. – In: Wu, J., Hobbs, R. (Eds) Key Topics in Landscape Ecology. Cambridge Studies in Landscape Ecology, Cambridge University Press, pp. 39–61

[29] Marcussen, C. (2014): Multidimensional scaling in tourism literature. – Tourism Management Perspectives 12: 31–40.

[30] Marosi, S., Somogyi, S. (Eds) (1990): Magyarország kistájainak katasztere I-II. [Landscape unit inventory of Hungary I-II.]. Budapest: MTA FKI,

[31] Mezősi, G., Meyer, B. C., Loibl, W., Aubrecht, Ch., Csorba, P., Bata, T. (2013): Assessment of regional climate change impacts on Hungarian landscapes. – Reg Environ Change 13: 797–811.

[32] Miklos, L. (2012): The concept of the landscape and its acceptance in the practice. – AGD Landscape & Environment 6 (2): 93–104.

[33] Minasny, B., McBratney, A. B. (2007): Incorporating taxonometric distance into spatial prediction and digital mapping of soil classes. – Geoderma 142: 285–293.

[34] Mosimann, T. (1984): Methodische Grundprinzipien für die Untersuchung von Geoökosystemen in der topologischen Dimension (Methodological principles for the investigation of the small scale geoecosystem) – Geomethodica 9: 31–65.

[35] Muenchow, J., von Wehrden, H., Rodrigez, E. E., Rodrigez, R.A., Bayer, F., Richter, M. (2013): Woody vegetation of a Peruvian tropucal dry forest along a climatic gradient depends more on soil than annual precipitation. – Erdkunde 67(3): 241–248.

[36] Mücher, C. A., Klijn, J. A., Wascher, D. M., Schaminée, J. H. J. (2010): A new European Landscape Classification (LANMAP): A transparent, flexible and user-oriented methodology to distinguish landscapes. – Ecological indicators 10: 87–103.

[37] Naveh, Z., Liebermann, A. (1984): Landscape Ecology—Theory and Application. – Berlin and New York: Springer-Verlag,

[38] OTAB (1990). Országos Térinformatikai Alapadatbázis [National Geoinformatics Database] http://epa.oszk.hu/02100/02154/00003/pdf/EPA02154_RSGIS_2012_01_14-38.pdf, Accessed: 27.05.2015)

[39] Pálfai, I. (2004): Belvízek és Aszályok Magyarországon [Inland excess water and drought in Hungary]. Budapest: VITUKI (in Hungarian):

[40] Pálfai, I., Herceg, Á. (2011): Droughtness of Hungary and Balkan Peninsula. – Riscuri si Catastrofe, An X 9(2): 145–154

[41] Pedroli, B., Doorn, A., Blust, G., Paracchini, L., Wascher, D., Bunce, F. (Eds) (2007): Europe's living landscapes. Essays exploring our identify in the countryside. Zeist: KNNV Publishing,

[42] Pietrzak, M. (Ed) (2000): Granice Krajobrazowe - Problemy Teoretyczne I Znaczenie Praktyczne/Landscape Boundaries - Theory And Applications. Poznan: Polskiej Asocjacji Ekologii Krajobrazu

[43] Rolet, P., Seguin, J. J. (1986): Traitement de données multivariables (application aux sciences de la Terre): Rapport BRGM no. 86 DT005ISA

[44] Rushton, R. G., Golledge, G. (1972): Multidimensional Scaling: Review and Geographical Applications. – Assn. Of American Geographers

[45] SRTM (2000). Shuttle Radar Topography Mission. http://www2.jpl.nasa.gov/srtm/, Accessed: 27.05.2015

[46] Tress, B., Tress, G., van der Valk, A., Fry, G. (2003): Interdisciplinary and Transdisciplinary Landscape Studies: Potential and Limitations. – Delta Series 2, Wageningen

[47] Ward, J. H. (1963): Hierarchical grouping to optimize an objective function. – J Am Stat Assoc 58: 236–244.

[48] Wascher, D.M. (Ed) (2005): European Landscape Character Areas – Typologies, Cartography and Indicators for the Assessment of Sustainable Landscapes. Final Project Report as deliverable from the EU's Accompanying Measure project European Landscape Character Assessment Initiative (ELCAI), funded under the 5th Framework Programme on Energy, Environment and Sustainable Development (4.2.2), 150 pp.

[49] WRB (2006). World soil resources reports. IUSS Working Group WRB. Vol.103. FAO, Rome

[50] Wrbka, T., Erb, K. H., Schulz, N. B. , Peterseil, J., Hahn, C., Haberl, H. (2004): Linking pattern and process in cultural landscapes. An empirical study based on spatially explicit indicators. – Land Use Policy 21: 289–306.

HAZARD ASSESSMENT OF MICROCYSTINS FROM THE HOUSEHOLD'S DRINKING WATER

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Abstract. Cyanobacteria produce toxins which are hazardous to the health of people who are using water that is contaminated. The hazard quotient suggested by United States Environmental Protection Agency (USEPA) was used to assess the human health hazard of using contaminated water containers. There was no statistically significant difference ($P = 0.5511$) in the level of microcystin between blooming and decaying seasons. Findings show that all treated water samples in the containers were below the 1.0 µg/L level, which underscore this important issue: drinking water from treated water in containers has no adverse health effect when compared to drinking water from non-treated water containers, whether used by adults or children. In conclusion, collecting and storing of either pre-treated or non-treated water using containers needs post-treatment before drinking.

Keywords: *hazard quotient, microcystins modelling, cyanobacteria, water containers*

Introduction

It is believed that recreational and occupational contact with contaminated water are the most common forms of exposure to cyanobacterial toxins in dams, rivers and marine water (Azevedo et al., 2008). In the reports of Hoffman (1976) and Duy et al. (2000) it was discovered that long-term exposure to low levels of cyanotoxins may also occur in areas which receive treated drinking water, as most of the conventional water treatment processes are ineffective in removing cyanotoxins (DWAF, 1996). Oberholster and Ashton (2008) reported that extra cellular toxin concentrations remained constant after flocculation and filtration in a conventional water treatment plant. Daly et al. (2007) evaluated the effect of chlorine on the cell integrity of toxic *Microcystis aeruginosa*. Flow cytometry determined live (viable) and dead (nonviable) Microcystis cells, and it was found to have a higher concentration of chlorine than what was suggested by Nicholson et al. (1994). The difference was that Daly et al. (2007) lysed the cell, whilst Nicholson et al. (1994) degraded the toxins directly. Tsuji et al. (1997) reported that chlorination and ozonation are effective means for the removal of microcystins. Nicholson et al. (1994) reported that a chlorine dose of 3 mg/L is effective in eliminating the presence of microcystins in drinking water if a residual of 0.5 mg/L is sustained for 30 minutes. This is because chlorine reacts with microcystin organic compounds to form trihalomethanes, which maybe also toxic and carcinogenic to

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humans (Muyodi, 2009). Chlorine bleach is inexpensive and readily available, in stores, for the public to purchase. All studies demonstrated that chlorine was effective in degrading microcystins in water (Tsuji et al., 1997 and Nicholson et al., 1994). The use of the correct concentration of bleach and the right pH has been proven to remove microcystins by up to 99% in drinking water (DWAF, 1996; Backer et al., 2008).

Toxic cyanobacteria are recognised as hazardous to human and animal health and assessments are carried out to determine environmental health problems (Ahmed et al., 2008). However, thorough investigation of human illnesses and deaths, following exposure to microcystins, are required. Some toxins produced by cyanobacteria are carcinogens. Non-carcinogenic microcystins have been the most widely investigated toxin group. The health risks posed by exposure to microcystins are difficult to quantify, since the actual exposure and the resulting effects have not been conclusively determined, especially in relation to humans (Chen et al., 2009). Microcystin exposure can also occur through contact with or ingestion of algal scums. Three potential sources of exposure to microcystins can be distinguished: direct contact of exposed parts of the body including ears, eyes, mouth and throat and the areas covered by a bathing suit (Pilotto et al., 1997), accidental swallowing (Turner et al., 1990) and inhalation of water spray (Chorus and Bartram, 1999). Fawell et al. (1994) studied the dose-exposure using pigs to determine the no-observed-adverse-effect-level (NOAEL), whilst Humpage and Falconer (2003) used mice to determine the LOAEL of microcystins. The recognised route of exposure of humans to microcystins is through water.

The most reported occurrence of human intoxication by microcystins was the outbreak of illness that occurred at a haemodialysis clinic in Caruaru, Pernambuca State, Brazil. Patients developed visual disturbances, nausea, vomiting and muscle weakness; some patients developed acute liver failure (Azevedo et al., 2002). The study outcome was confirmed by comparing the human symptoms with those of animals that were studied and led researchers to conclude that humans were exposed specifically to microcystins LR, YR and AR. A study conducted by Ding et al. (2006) demonstrated that microcystins cause stomach and intestinal inflammation, liver cancer and disease of the spleen in humans who drink contaminated water. Microcystins have been linked to promoting tumour development and have clearly demonstrated having an effect on the liver and the colon (Falconer, 2005). Unfortunately, there is no confirmation of microcystins exhibiting carcinogenic tendencies, as they would need very high intraperitoneal doses of toxin (Falconer, 2005; Ito et al., 1997). The USEPA (2005) reported a need to generate sufficient epidemiological data to be able to estimate the risks. Animals are generally used in toxicity studies and the results extrapolated to humans.

Masango et al. (2010) reported an increase of microcystins from an average of 49.41 mg L^{-1} in February during summer blooming of the cyanobacteria, to 103.16 mg L^{-1} in June in the dam water found in the Kruger National Park. This increase in microcystin concentration was the result of the dying-off of cells in winter. Most research studies have been conducted on animals (Oberholster et al., 2005; Masongo et al., 2010), but there are reported cases of human health effects (Jochimsen et al., 1998) caused by microcystin. Nobre et al. (1999) studied the physiopathologic effects of a rat kidney perfused with a microcystin-LR solution and Masango et al. (2010) reported the death of mice within one hour after injecting them with water samples from a dam following the blooming of cyanobacteria, during a follow-up study resulting from the death of wildlife in the Kruger National Park. It was shown that the death of wildlife, in the study area, was due to toxic *Microcystis*. Willen et al. (2011) studied the deaths of wild and domestic animals around the Rift Valley in Kenya and reported traces of microcystins produced by *Microcystis aeruginosa* as the dominant species. The toxins were found to exceed 1 µg L^{-1} levels. Moreno et al. (2004) compared the effects of microcystins between humans and animals. In this study, they assessed the degradation of microcystins in human gastrointestinal tract before absorption and found alterations in gastric conditions by all the toxin assays studied. Microcystin-RR was the most prevalent toxin, the degradation of which ranged between 49 and 64%, whilst for others, such as Microcystin-YR and –LR, degradation percentages were around 30%.

Human health risk characterisation is defined as an integration of the findings from the exposure to microcystins and the consequent effects. The carcinogenesis guideline to drinking water is not yet conclusive, however, the risk assessment procedure was adopted from the non-carcinogenic chemicals and there was a derivation of the drinking water Guideline Value for microcystins. Falconer (2005) reported that a preferential procedure uses epidemiological data from human exposure and poisoning from microcystins in drinking water. In their study, two doses were used to determine the higher and lower levels which would not have any effects on animals or humans if administered. The higher dose with no effects on animals is called the 'No Observed Adverse Effect Level' (NOAEL) and the lowest dose that causes minimum effect is called the 'Lowest Observed Adverse Effect Level' (LOAEL). The World Health Organization uses this procedure of toxin doses to calculate the acceptable guideline of microcystins in drinking water. The guideline is based on the body mass of the animals, when compared to reported different doses from other animal studies (TDI), and the total daily dose intake which is administered to the animals for up to 14 weeks. The total daily intake (TDI) equation was used to estimate doses for drinking water quality assessment (Codd, 2005). In order to derive to TDI, the LOAEL or NOAEL was used and divided by the appropriate safety or uncertainty factors (WHO, 2006).

In 1998, the World Health Organization (WHO) provided many countries with the provisional guideline of 1 µg L^{-1} in drinking water. It is documented as a provisional guideline because it only covers the Microcystin-LR and not all cyanotoxins, as there is very little data available about other toxins. Some of these countries, including Brazil, New Zealand, Japan and Spain, recently directly adopted the WHO provisional guideline for microcystin-LR for drinking water. Burch and Thomas (1998) compared the international guidelines for microcystins-LR. South Africa uses the range of 0-0.8 µg L^{-1} (DWAF, 1996), SANA 241 uses the WHO guideline value of 1 µg L^{-1}, while other countries, such as Australia and Canada, have developed guidelines of 1.3 µg L^{-1} and 1.5 µg L^{-1} respectively, using a range above the recommended level of the WHO.

Microcystin in one of the toxins produced by the cyanobacteria and mostly found in drinking water. Toxins are grouped into two, carcinogenic and non-carcinogenic toxins, and are reported to cause either acute or chronic health effects if ingested. Microcystin is still grouped under the non-carcinogenic group as there is no study, as of yet, to prove if it causes cancer only suggestions it may advance the development of cancer. Therefore, in order to determine whether microcystin can cause the adverse health effect of humans, if found in drinking water, the hazard quotient model should be applied. Human health risk assessments of microcystins were simulated using recreational water and studies conducted from animals, as there no human health epidemiological studies using microcystins as indicators in drinking water containers. This is because there is limited dose data which can be used to calculate the hazard quotient. The use of water containers as a water storage facility is a tertiary water point in most rural areas in developing countries such as South Africa. Therefore the adverse health effects of stored water containers at household levels is not yet understood.

The present study assesses the human health hazard modelling of microcystins in drinking water containers. This was determined by assessing the quality of water used for drinking purposes, which were grouped together by their respective water sources and water containers stored in households. Microcystin concentration was determined in both treated and non-treated water samples and this data were used to calculate the associated health hazard.

Materials and methods

Study area

The study was conducted in the communities situated around Hartbeespoort Dam, in the North West Province, South Africa. Four different water sources (communal tap, tank supply, groundwater and Rand-water), used by community members, were assessed at household level. The Rand Water Board supply water from the Vaal Dam, directly to people in the study area of Hartbeespoort Dam, through house connections. Water from Hartbeespoort Dam water, was treated by Schoemansville Water Treatment and supplied by trucks to the area through communal taps and tanks supplied to communities. Ground water was provided via private boreholes, which individuals drilled themselves in their yards.

Water samples collection

Water samples were collected from the water containers of all the participating households, as well as from their respective water sources. Water samples were collected at different seasons, namely: blooming season (spring and summer) and decaying season (autumn and winter) between 2012 and 2013. The samples were collected using sterile 500ml Whirl Paks. These samples were immediately stored in cooler boxes at less than 4°C (Chorus and Bartam, 1999) and transported to the laboratory for analysis within 24 hours of sampling. Sodium hypochlorite was used from 40% of the selected households as the point of water treatment usage. During the collection of water samples, care was taken to ask the household member if water was treated with bleach (NaOCl) and at what time it was treated. This was done to assess whether water treatment by bleach at household level was done 30 minutes prior to usage, as this is as an acceptable time lapse for consumption of the water. Immediately, in the laboratory, two drops of Lugol's solution were added to the water samples and they were kept in a black plastic bag to prevent exposure to sunlight (Funari and Testai, 2008). Immediately on arrival in the laboratory, 2ml of the water sample was decanted into an Eppendorf tube and frozen at 80°C until further analysis of the toxins was necessary. The method of analysing microcystins is described fully below.

Microcystins analysis

Analyses of microcystins were performed using the Abraxis Microcystins-ADDA ELISA kit (Microtiter plate) from ToxSolutions kit in South Africa, following the Abraxis procedure (PN.520011) that has six standard solutions and one control. After the mixing, washing and incubation of the microcystins solution, the plate was placed into a micro-reader to read the results. Data was captured on Microsoft Excel Office and statistical analysis was done using Stat V-10.

Microcystin human health hazard assessment model

The standard human health risk assessment approach incorporates two steps: risk assessment and risk characterisation. Risk assessment methodology incorporates the following steps: hazard identifying, exposure assessment, toxicity assessment and quantifying risk and hazard.

Hazard identifying

In carrying out the hazard identification and dose-response assessment of microcystins in drinking water, studies identified the level of toxins in drinking water. Many studies have been conducted in order to relate human and animal health problems to the presence of microcystins in water; microcystins were analysed as indicators of contamination in drinking water. The study assesses the water quality used for drinking purposes for two seasons: the blooming and decaying seasons. Cases of microcystins health problems have being reported worldwide. From the studies conducted using animals, the adverse effects of microcystins were determined using both NOAEL and LOAEL. Hazard identification, microcystin hazard analysis, was started with the estimation of the total daily intake (TDI).

Exposure assessment

It was expected for household members to be exposed to microcystins via the oral route, through the ingestion of contaminated container water during their life time. The safe doses of microcystins in the drinking water were to be determined and used to estimate the daily dose intake. The toxicological data was used to calculate a Tolerable Daily Intake.

For non-carcinogenic substances, the dose can be calculated using the intake equation below:

$$TDI = \frac{MC * IR * EF * ED}{BW * AT} \qquad \text{(Eq. 1)}$$

TDI = tolerable daily intake, MC = microcystin concentration, IR = contact rate (L/day), EF = exposure frequency (in days), ED = exposure duration (in years), BW = Body Weight (in kg) and AT = average time (in days).

For the exposure of the microcystins contamination in drinking water, the HQ is based on the oral exposure to microcystins, considering the body weight of 70kg for adults and 15kg for children, at daily water ingestion volumes of approximately 2L/day see *Table 1*. For the microcystins concentration, the oral LD_{50} or NOEAL of the either pig or mice were considered.

Table 1. *Defines lists the symbols used in equation 1 and the study concentration used during TDI calculation.*

Parameter	Definition	Resident
IR	Contact rate (in L/day)	2L/day drinking water
EF	Exposure frequency (in days per year)	350 days/year
ED	Exposure duration (in years)	Actual event duration or 30 years if chronic
BW	Body weight (in kg)	70 kg (adult), 15 kg (child)
AT	Period over which exposure is averaged (in days)	Actual event duration if not carcinogenic, or 365 day/years * 70 years if carcinogenic.
MC	Exposure point concentration	Average concentration of contaminant (Microcystins) on exposure (in mg/L if in water) µg/L.

Toxicity assessment

It is also appropriate to consider the establishment of a reference dose (RfD). The RfD is an estimate of the amount of substance in drinking water, normally expressed in a body weight, which can be ingested in a period of 24 hours. Drinking water samples were assessed for the concentration of microcystins during blooming and decaying seasons. The concentrations of the microcystins were relative to the exposure of people to the water during their lifetime.

$$RfD = \frac{NOEALorLOEAL}{UF} \qquad \text{(Eq. 2)}$$

RfD = reference dose, NOEAL = no-observed-adverse-effect level, LOEAL = lowest-observed-adverse-effect level and UF = uncertain factor

Quantifying risk and hazard

The determination of the microcystins non-carcinogenic risk assessment in drinking water was carried out according to the exposure pathways of contaminants recommended by the United States Environmental Protection Agency (USEPA).

$$HQ = \frac{TDI}{RfD} \qquad \text{(Eq. 3)}$$

HQ = hazard quotient, TDI = tolerable daily intake and RfD = reference dose

Interpreting the results of non-carcinogenic risk assessment:

When HQ is greater than 1 there are further steps to be employed for adequate risk assessment. These steps (options) include: sources control, deed restrictions, institutional controls and remediation.

Ethics

The proposed project and tools to be used were submitted to the Tshwane University of Technology Ethics Committee and permission was obtained. The study involved interviewing household members and sample collection at their households, however before data collection could begin adult members were asked to sign a consent form should they wish to participate. The household members' involvement was entirely voluntary in nature. The water samples were analysed for the presence of microcystins and other water quality parameters.

Results and discussion

Communities around the Hartbeespoort Dam use different water sources which are likely to be contaminated by the toxins (Microcystins) produced by the cyanobacteria. Microcystins are produced by *Microcystis Aeruginosa* during blooming and decaying seasons (Pawlik-Skowronska et al., 2008; Chaturvedi et al, 2015). These microcystins can survive for, at least, 21 days under good conditions such as warm temperature, good nutrients and calm wind (Backer et al., 2008).

Hazard identification in water sources

The dam was the main source supplying water to different water points used for drinking purposes. Microcystins were found in almost all water sources used in the area, but especially in the Hartbeespoort Dam water, which was also reported by Oberholster and Ashton (2008). Data of water samples from different water sources are shown in *Table 2*. It was assumed there was a direct link between the surface water (Hartbeespoort dam) to the communal tap and communal tank water after exposure to the treatment plant and direct contamination of the ground water that was drilled a few kilometres away from the dam (Hiscock and Grischek, 2002). It was further assumed that water supplied by Rand Water would have no association with the surface water in the study area as this water was supplied from Lesotho through the Vaal Dam Water Treatment. The World Health Organization (WHO) suggests a provisional microcystins guideline, derived from the study of Microcystin-LR. Therefore, for the purpose of this study, the same microcystin guideline (1 $\mu g\ L^{-1}$) in drinking water was applied in the results discussion section.

Microcystins data showed that dam water was significantly ($P=0.9888$) more contaminated with microcystins when compared to the other four water sources used (*Table 2*). The four water sources (Groundwater, Rand-water, Tank water and Tap water), showed less contamination of microcystins than that of the dam water. Although the microcystins contamination median was below the acceptable limits (1 $\mu g\ L^{-1}$), there were still some water samples that had more than the acceptable value of microcystins concentration given the order of contamination shown, i.e. ground water > Rand Water > tank water > tap water. Following the treatment of water by the treatment plant, there was a decrease, by half, in the microcystins (to 2.3 $\mu g\ L^{-1}$ of tap water) during the blooming seasons. The removal of microcystins was not complete in the water treatment plant as toxins were also found in communal taps connected to the plant. Hence, there was difference in microcystins content between the decaying and the blooming seasons. In the decaying seasons, there was less than 1 $\mu g\ L^{-1}$ in the tap water sample when compared to 5.0 $\mu g\ L^{-1}$ in the dam water sample. The high level of microcystins in tap and tank waters during blooming season's treatments could be due to the fact that the treatment was unable to remove all the toxins produced (DWAF, 1996). In summer, the cyanobacteria cells from scums or mats resulted from the blooming of the cells in the surface water. The blooming of cyanobacteria is as a result of sufficient nutrients, calm wind, heavy rain and warm temperatures. Toxins are produced by the cells due to stress and overcrowding (Neilan et al., 2013). Therefore, water becomes highly turbid and it becomes a challenge for water treatment to remove, completely, all unwanted particles, including cells and toxins.

Table 2. *Mean concentration (µg L^{-1}) of microcystins in different water points.*

	Blooming - Microcystins non treated	**Decaying - Microcystins non treated**	**Microcystin guideline in Drinking water (WHO)**
Dam Water			1.0 µg L^{-1}
Min-Max	(0.00010 – 8.6)	(3.5 – 6.2)	
Mean±SD	4.3 ± 4.9	5.0 ± 1.4	
Groundwater			
Min-Max	ND	(0.4 – 0.4)	
Mean±SD	ND	0	
Rand-Water			
Min-Max	ND	ND	
Mean±SD	ND	ND	
Tank Water			
Min-Max	ND	(0.00010 – 4.2)	
Mean±SD	ND	1.4 ± 2.0	
Tap Water			
Min-Max	(0.00010 – 5.2)	(0.00010 – 1.3)	
Mean±SD	2.3 ± 1.9	0.6 ± 0.5	

ND = no data to be presented in that group

During the decaying seasons, the microcystin level was significantly ($P < 0.001$) reduced between dam water and tap water, from 5.0 to 0.6 µg L^{-1}. This could be due to the effect of the reduction in the turbidity levels from 9.5 NTU in the blooming seasons to 7.4 NTU in the decaying seasons, thus enabling the water treatment plant to treat the cyanobacterial cells and toxins at the lower level turbidity. A decrease in the levels of microcystin was observed in the communal tap water samples between blooming (2.3 µg L^{-1}) and decaying seasons (0.6 µg L^{-1}). The dam and tap waters' data confirmed that most water treatment plants are unable to treat microcystins completely, as reported (Daly et al., 2007), especially during the blooming season. Groundwater and Rand Water samples did not have microcystins that were detected in both seasons. There was a significant difference ($P = 0.4174$) in the water sources' quality between the blooming and decaying seasons.

Hazard identification in water containers

Water samples from the above mentioned sources were collected and stored using plastic containers (Sobsey, 2002) by the community members, who use it for drinking and domestic purposes. Water storage containers sizes differed depending on the needs of the household members and settlement types; in a private area such as Meerhof, most people used 10L light penetrating plastic containers to store drinking water, with only a few using 2000L tanks. In the informal settlements, such as Kosmos and Zandfontein, more than 95% households used 20L or 25L light penetrating and opaque plastic containers. People in the RDP area of Refense also use 20L or 25L light penetrating containers, but mostly 200L opaque plastic containers, because they get water from the tanker supplied by truck. Data of the water samples from these containers are shown in *Table 3*, which was grouped according to the water sources (where the water was collected). The data were further grouped according to the seasons. As there was no group of people using the dam water directly for domestic purposes, there was no result to be discussed.

Point-of-use water treatment was practiced in 29 (39%) of the households that participated in the study and these were strategically selected. In the water containers group, no significant difference (P = 0.9999) was found between the levels of microcystins in either treated or non-treated water (P = 0.8040), either in the blooming or the decaying seasons (P = 0.6141). Microcystins ranged from 95% confidence interval (0.034 to 0.187) and (0.0536 to 0.4058) for treated water samples of the blooming seasons and decaying seasons respectively (P = 0.2758), while 95% confidence interval (0.4595 to 1.6662) and (0.6402 to 1.6755) for non-treated water in the blooming and decaying seasons (P = 0.8215). There was a significant increase (P = 0.0028) in the microcystins concentration from treated (with bleach) to non-treated water samples within the confidence intervals (-0.02842 to 0.1588) and (0.4807 to 1.7237) in the blooming season. The same patterns were also observed in the decaying season, where there was a significant increase (P = 0.0012) in the microcystins concentration from treated with bleach to non-treated water samples within confidence intervals (-0.05364 to 0.4058) and (0.7847 to 1.9712) in the decaying season.

Blooming season water container quality

Drinking water container qualities used at household levels were compared using microcystins as an indicator of water contamination and the effect of bleach in its treatment. The mean level of microcystins of non-treated Rand Water containers was 0.21 $\mu g\ L^{-1}$ less than the acceptable limit. Water from the Rand Water source did not contain microcystins, however, the containers used to store the water did have microcystins in them. This could be as a result of using the same containers to collect water from other water sources at a time when water was not available at the settlement.

Table 3. *Mean concentration ($\mu g\ L^{-1}$) of microcystin in water containers at household level.*

	Blooming season - (Microcystin) Household water treatment	**Blooming season– (Microcystin) Household non water treatment**	**Decaying season – (Microcystin) Household water treatment**	**Decaying season– (Microcystin) Household non water treatment**
Groundwater				
Min-Max	ND	ND	ND	(0.00010 – 1.91)
Mean±SD	ND	ND	ND	0.5 ± 0.81
Rand-Water				
Min-Max	ND	(0.00010 – 1.26)	ND	(0.00010 – 4.42)
Mean±SD	ND	0.21 ± 0.51	ND	1.5 ± 2.09
Tank Water				
Min-Max	(0.00010 – 1.18)	(0.00010 – 4.33)	(0.00010 – 1.80)	(0.00010 – 3.26)
Mean±SD	0.08±0.28	1.16 ± 1.56	0.14 ± 0.47	1.26 ± 1.28
Tap Water				
Min-Max	(0.00010 – 0.22)	(0.00001 – 4.37)	(0.00010 – 1.89)	(0.00010 –3.36)
Mean±SD	0.032 ± 0.083	1.75 ± 1.98	0.27 ± 0.71	1.23 ± 1.50

ND = there were no data to be presented in that group

Residents also reported they stored water for long periods of time, as they were not sure when the water would be turned-off and when their taps would be turned on again.

Other households reported that they live in a lower water pressure area, therefore they often receive water only in the evening when demands are not too high, forcing them to store water in their containers. Microcystins in treated water containers from tanks were observed to be as high as 1.18 µg L^{-1}, which is greater than the acceptable limit, but a mean concentration of 0.08 µg L^{-1}, which is below the acceptable level, was observed. However, microcystins levels in non-treated water containers collected from the tank water were above the acceptable limits of 4.33 µg L^{-1} and 1.16 µg L^{-1} for maximum and mean concentration levels respectively. The microcystins concentration of non-treated water was four times the concentration of the treated water using bleach. This shows that the NaOCl was effective in treating water containers to an acceptable level. Microcystins in the treated water containers from taps were observed to be at a maximum concentration level of (0.22 µg L^{-1}), which is within the acceptable limit although a mean concentration of 0.032 µg L^{-1}, which is below the acceptable level, was observed. However, microcystins levels in non-treated water containers collected from the same tank were above the acceptable limit of 4.37 µg $L^{-1}$and 1.75 µg L^{-1} for maximum and mean concentration levels respectively. The same pattern of microcystin treatment was observed in the water containers from the tap as that from the tank. The point-of-use water treatment practices had a significant decrease ($P = 0.0028$) of microcystins in water containers when compared to those of untreated water.

Decaying season water container quality

The decrease of microcystins in water containers was also observed during the decaying season. The findings show that microcystins (0.14 µg L^{-1}) were below the acceptable limit of 1 µg L^{-1}, when treating water with bleach, at a maximum concentration of 1.80 µg L^{-1} in the tank water. However, from the non-treated tank water, the minimum microcystin concentrations were 1.26 µg L^{-1} and the maximum was 3.26 µg L^{-1}. The level of microcystins in treated water containers decreased more than three times compared to the non-treated ones. The microcystin concentrations in the treated water containers were observed to average at 0.27 µg L^{-1} and pealed at 1.89 µg L^{-1}. Those who did not treat their water kept in household containers were exposed to microcystins, with a mean concentration of 1.23 µg L^{-1} and a maximum concentration of 3.36 µg L^{-1}. Groundwater was used in the study area as an alternate water source since most of the wells available were privately owned. There were a few people who stored groundwater in containers for domestic use. Data were collected during decaying season and not all participants treated their groundwater with bleach. Data presented in *Table 3* shows that the mean microcystin concentration of groundwater was 0.5 µg L^{-1} and a maximum concentration of 1.91 µg L^{-1}. Microcystins concentration was also observed in Rand-Water water containers. The findings show that microcystins were detected from water containers at mean a concentration of 0.5 µg L^{-1}, which was less than the acceptable level, however the results also revealed a maximum concentration of 1.91 µg L^{-1}. This level of contamination by microcystins could have been due to the use of the same containers to collect water from other sources. Improper hygiene practices could also be one of the reasons for major water deterioration.

Discussion of water container quality in seasons

It was observed that water containers from all sources grouped together, shared no statistically significant difference ($P = 0.5511$) from blooming and decaying seasons.

Tap and tank water microcystins were further compared during these seasons; there was no significant difference (P = 0.5379) in water container contamination by microcystins in non-treated water from tap or tank. The microcystins exposure in water containers was similar (1.16 and 1.26; 1.75 and 1.23) for tap and tank respectively. This finding shows that water container contamination and the level of microcystins in storage containers were not determined by external factors such as favourable conditions, rain fall, low or high temperature. It can, therefore, be concluded that the types of containers used, such as light penetrating containers as reported by Fosso-Kankue et al. (2008), contribute to water quality deterioration. Jagals (2006) and Gundry (2004) also reported that further deterioration of water containers is due to the access to water by children and domestic animals, use of scooping vessels as well as time of storing water. However, microcystins were significantly reduced ($P < 0.0001$) in all water treated by bleach at the point of use when compared to non-treated water containers.

Microcystins human health hazard assessment models

There were 504 participants in the study, of which 55 (11%) were children less than 5 years. The chemical assessment of non-carcinogen health adverse health effects using hazard quotient (HQ) was applied to determine the microcystins human health hazard. If the HQ between the two is greater than 1, people or animals would be at high risk; this means that 50% of the population using the substance with the contamination of the toxins will experience sub-lethal effects (Sánchez-Bayo et al., 2011). The hazard quotient (HQ) for children and adults through exposure of drinking water containers (DWC) to microcystins is shown in *Figures 1 and 2* (*Figure 1* shows the HQs of adults and children during the blooming season and *Figure 2* shows the HQs during the decaying season). The figures further grouped the HQ results of DWC of water treated or non-treated, using bleach. The HQs through treated DWC to microcystins by children and adults in the blooming season were less than 1.0 for all DWC from the tap and tank sources. There was no data collected for water treated with bleach for the groundwater and Rand Water groups. HQs through non-treated DWC to microcystins by children and adults in the blooming season were greater than 1.0 for all DWC from the tap and tank sources. The groundwater and Rand Water's HQs through non-treated were less than 1.0 for both children and adults (*Figure 1*). HQs through non-treated DWC by children in the study area, had the maximum HQs of 5.7 and 3.8 at the tap water and tank water, respectively. Also, non-treated DWC for the adults' HQs were 8.20 and 5.46 for tap water and tank water, respectively.

Generally, the HQs through treated DWC to microcystins of the children and adults in the study area were less than 1.0 for all water sources (tap and tank) that were treated at point of use, while the HQs through non-treated DWC to microcystins by children and adults were above 1.0; this shows that non-treated water posed adverse health effects. It is reported by the USEPA risk assessment guidelines, with a hazard quotient greater than 1.0, that the probability for adverse health effects associated with exposure to such toxins is high. Only non-treated water containers produced hazard quotients of greater than 1.0 for adults and children. This shows the adverse health effect to people drinking water from containers can be experienced from collected and stored non-treated water. The toxin hazard can however be reduced to acceptable levels if water is treated using bleach at point of use.

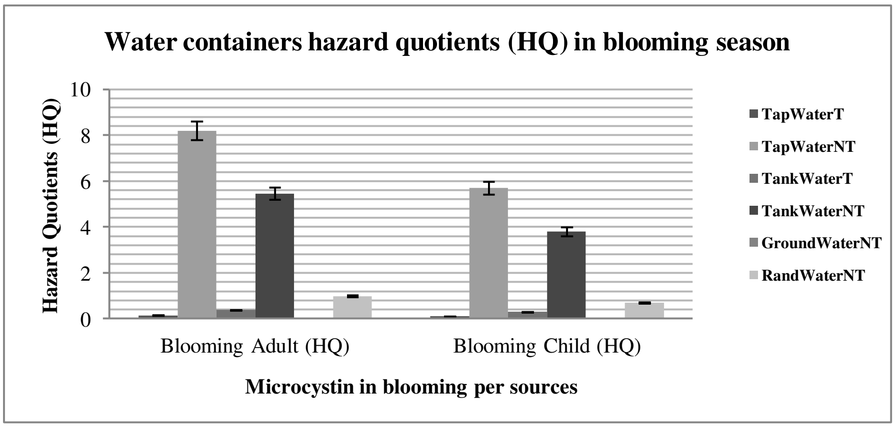

***Figure 1.** Hazard quotient (HQ) from exposure to microcystins by resident children and adults via water containers*

Figure 2 shows the HQs of decaying seasons grouped by the water sources used in the household for adults and children. All water container data was further grouped into treated or non-treated using bleach.

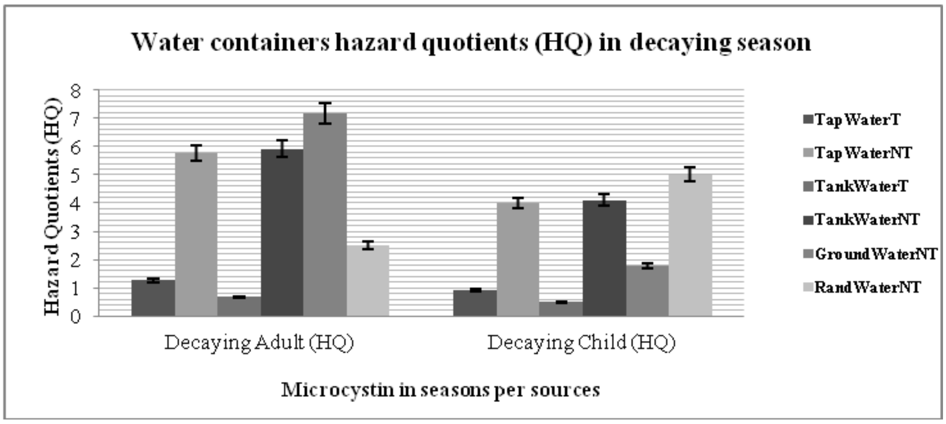

***Figure 2.** Hazard quotient (HQ) from exposure to microcystin by resident children and adults via water containers*

The HQs through treated DWC to microcystins for adults in decaying season were less than 1.0 for the tank sources and greater than 1.0 for tap sources, which was 1.27. However, the HQs through treated DWC to microcystins for children in decaying season were all less than 1.0 for both tap and tank water sources (*Figure 2*). A close look at the non-treated HQs through DWC to microcystins for children and adults in decaying season shows HQ greater than 1.0 for all water sources (tap, tank, groundwater and Rand Water). In adults, HQs were 5.76, 5.91, 7.18 and 2.51 for tap, tank, groundwater and Rand Water respectively. Similarly the HQs through DWC for the children were 4, 4.1, 1.8 and 5 for tap, tank, groundwater and Rand Water respectively.

Hypothetically speaking, the decaying season should have no toxins produced as it was believed that all cyanobacteria cells were removed during water treatment and, as there was lower turbidity, chlorination contact time with microcystins should be

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sufficient to degrade it (Zong et al., 2015), however, stored water containers recontamination and poor hygiene practices resulted in the growth of biofilm inside the containers (Fosso-Kankue et al., 2008). During dying and decaying, cells release toxins (Gélinas et al., 2014). This could be supported by the data (*Figure 2*) on the level of HQs non-treated water containers that were greater than 1.0 in all sources used. Furthermore, water container treatment using bleach was shown to be effective in treating microcystin. During the blooming and decaying seasons (*Figures 1 and 2*) of treated DWC, HQs data were all below 1.0, the fact that water was not free of toxins could be because some of cyanobacteria were resistant to chlorination or to the poor conditions, such as pH concentration and enough contact time (Zong et al., 2015). This could indicate that the hazard level of microcystins that people are exposed to may have no adverse effect on their health, while all HQs data of non-treated DWC above 1.0 could have possible adverse health effects.

The continuous ingestion of non-treated DWCs that are collected and stored by adults and children in the study area makes them susceptible to health hazards associated with exposure to microcystins. Such health symptoms manifest in two ways: exposure to low levels of microcystins for a long time could result in tumour promotion and kidney and liver problems, while exposure to a high concentration of microcystins for a short period of time could result in gastroenteritis disorder, respiratory tract infection, diarrhoea, vomiting, nausea, etc.

Conclusion

The presence of microcystins was shown in different water sources used by residents in the area, where such water was collected for drinking purposes. It had a direct link with the Hartbeespoort Dam water irrespective of the treatment process used, this included the tap and tank waters that were pre-treated before being supplied through communal taps or tankers and the groundwater that had an aquifer as the treatment process of most pathogen microorganisms. The presence of microcystins in water containers was proven to be above the acceptable limits. This was shown to be hazardous and, if exposed to the users as was done in most water containers, could have short or long term effects if there was continued use of the water in this state. The exposure to high concentration of MC results in gastroenteritis health problems (diarrhoea, vomiting, nausea and stomach pain) (Brookes, et al., 2008) and low concentration reportedly promoted tumour development and kidney problems (de Figueiredo et al., 2004). Water treatment at the point of use should always be practiced, as it was observed that the adverse health effect level was reached from all the non-treated water groups in both seasons. Furthermore, proper water treatment using bleach and educational campaigns by DWA and the Department of Health is a requirement in all areas that are exposed to cyanobacteria contaminated water. Further studies are needed on trihalomethanes and dissolved organic compounds, since chlorine reacts with microcystins to form trihalomethanes.

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REFERENCES

[1] Ahmed, M.S., Hiller, S., Luckes, B. (2008): Microcystis aeruginosa Bloom and the Occurrence of Microcystins (Heptapeptides Hepototoxins) From an Aquaculture Pond in Gazipur. - Turkish Journal of Fisheries and Aquatic Sciences 8: 37-41.

[2] Azevedo, S.M.F.O., Carmichael, W., Jochimsen, E.M., Rinehart, K.L., Lau, S., Shaw, G.R. Eaglesham, G.K. (2002): Human intoxication by microcystins during renal dialysis treatment in Caruaru-Brazil. - Journal of Toxicology 181-182: 441-446.

[3] Azevedo, S.M.F.O., Chernoff, N., Falconer, I.R., Gage, M., Hilborn, E.D., Hooth, M.J., Jensen, K., Macphail, R., Rogers, E., Shaw, G.R. Stewart, I. (2008): Cyanobacteria Harmful Algal blooms: Chapter 26: Human health effects working group report. - United States Environmental Protection Agency.

[4] Backer, L.C., Carmichael, W., Kirkpatrick, B., Williams, C., Irvin, M., Johnson, T.B., Nierenberg, K., Hill, V.R., Keiszak, S.M. Cheng, Y. (2008): Recreational Exposure to Low Concentrations of Microcystins during an algal bloom in a small lake. - Marine Drug 6: 389-406.

[5] Brookes, J.D., Daly, R., Regel, R.H., Burch, M., HO, L., Newcombe, G., et al. (2008): Reservoir management strategies for control and degradation of algal toxins. Denver, Colorado, USA. - American Water Works Association Research Foundation.

[6] Burch, J.D., Thomas, K.E. (1998): Water disinfection for developing countries and potential for solar thermal pasteurization. - Solar Energy 64: 87-97.

[7] Chaturvedi, P., Kumar-Agrawal, M. Nath-Bagchi, S. (2015): Microcystin-producing and non-producing cyanobacterial blooms collected from the Central India habour potentially pathogenic Vibrio cholera. - Ecotoxicology Environmental Safety 115: 67-74.

[8] Chen, J., Xie, P., Li, L., Xu, J. (2009): First Identification of the Hepatoxic Microcystins in the Serum of a Chronically Exposed Human Population Together with Indication of Hepatocellular Damage. - Toxicological Sciences 108(1): 81-89.

[9] Chorus, I., Bartram, J. (Eds.)(1999): Toxic Cyanobacteria in Water: A Guide to Their Public Health Consequences, Monitoring and Management. - St Edmundsbury Press, Bury St Edmunds, Suffolk. First pblished by E & FN Spon.

[10] Codd, G.A., Morrison, L.F., Metcalf, J.S. (2005): Cyanobacterial toxins: risk management and health protection. - Toxicology & Applied. Pharmacology 203: 264-272.

[11] Daly, R.I., Ho, L. Brookes, J.D. (2007): Effect of Chlorination on Microcystis aeruginosa Cell integrity and subsequent Microcystin release and degradation. - Environmental Science and Technology 41: 4447-4453.

[12] De Figueiredo, D., Azeiteiro, U., Esteves, S., Gonçalves, F., Pereira, M. (2004): Microcystin-producing blooms--a serious global public health issue* 1. - Ecotoxicology and Environmental Safety 59(2): 151-163.

[13] Department of Water Affair and Forestry (DWAF). (1996): South African Water Quality Guidelines. 2nd Edition. Volume 5., Agricultural water use: Livestock watering. - The Government Printer, Pretoria

[14] Ding, X.S., Li, X.Y., Duan, H.Y., Chung, I.K. Lee, J.A. (2006): Toxic effects of Microcystis cell extracts on the reproductive system of male mice. - Toxicon 48: 973-979.

[15] Duy, T.N., Lam, P.K.S., Shaw, G.R., Connell D.W. (2000): Toxicology and risk assessment of freshwater cyanobacterial (blue–green algal) toxins in water. - Reviews of Environmental Contamination & Toxicology 163: 113–185.

[16] Falconer, I.R. (2005): Cyanobacterial toxins of drinking water supplies: cylindrospermopsins and microcystins. - Boca Raton: CRC Press p. 279.

[17] Fawell, J.K., James, H.A., James. H.A. (1994): Toxins from blue-green algae: Toxicological assessment of Microcystin-LR and a method for its determination in water. - Water Research Centre, Medenham, England Report number FR 0359/2DoE 3358/2.

[18] Fosso-Kankeu, E., Jagals, P., Du Preez, H. (2008): Exposure of rural households to toxic cyanobacteria in container stored water. - Water SA 34: 631-636.

[19] Funari, E., Testai, E. (2008): Human health risk assessment related to cyanotoxins exposure. - Critical Reviews in Toxicology 38: 97-125.

[20] Gélinas, M., Fortier, M., Lajeunesse, A., Fournier, M., Gagnon, C., Barnabé, S., Gagné, F. (2014): Responses of freshwater mussel (Elliptio complanata) hemocytes exposed in vitro to crude extracts of Microcystis aeruginosa and Lyngbya wollei. - Ecotoxicology 23: 260-266.

[21] Gundry, S., Wright, J., Conroy, R. (2004): A systematic review of the health outcomes related to household water quality in developing countries. - Journal of Water and health 2 (1): 1-13.

[22] Hiscock, K.M., Grischek, T. (2002): Attenuation of groundwater pollution by bank filtration. - Journal of Hydrology 266: 139-144.

[23] Hoffman, J.R.H. (1976): Removal of Microcystins toxins in water purification processes. - Water SA 2: 58-60.

[24] Humpage, A.R., Falconer, I.R. (2003): Oral toxicity of the cyanobacterial toxin cylindrospermopsin in male Swiss albino mice: Determination of no observed adverse effect level for deriving a drinking water guideline value. - Environmental Toxicology 18: 94-103.

[25] Ito, E., Kondo, F., Terao, K. Harada, K.L. (1997): Neoplastic nodular formation in mouse liver induced by repeated intraperitoneal injections of microcystin-LR. - Toxicon 55: 1453-1457

[26] Jagals, P. (2006): Does improved access to water supply by rural households enhance the concept of safe-water at the point of use? A case study from deep rural South Africa. - Water Science and Technology 54: 9–16.

[27] Jochimsen, E.M., Carmichael, W.W., An, J., Cardo, D.M., Cookson, S.T., Holmes, C.E.M., et al., (1998): Liver failure and death after exposure to Microcystins at a Hemodialysis Center in Brazil. - New England Journal of Medicine 338(13): 873-878.

[28] Masango, M.G., Myburgh, J.G., Labuschagne, L., Govender, D., Bengis, R.G., Naicker, D. (2010): Assessment of Microcystins bloom toxicity associated with wildlife mortality in the Kruger National Park, South Africa. - Journal of Wildlife Diseases 46: 95-102.

[29] Moreno, I.M., Maraver, J., Aguete, E.C., Leao, M., Gago-Martinez, A. Camean, M. (2004): Decomposition of Microcystin LR, Microcystin-RR and Microcystin-YR in water submitted to in Vitro Dissolution Tests. - Journal of Agriculture, Food and Chemistry 52: 5933-5938.

[30] Muyodi, F.J., Hecky, R.E., Kitamirike, J.M. Odong, R. (2009): Trends in health risks from water-related diseases and cyanotoxins in Ugandan portion of Lake Victoria basin. - Lakes & Reservoirs: Research & Management 14: 247-257.

[31] Neilan, B.A., Pearson, L.A., Muenchhoff, J., Moffitt, M.C., Dittmann, E. (2013): Environmental conditions that influence toxin biosynthesis in cyanobacteria. - Environmental microbiology 15: 1239-1253.

[32] Nicholson, B.C., Rositano, J., Burch, M.D. (1994): Destruction of cyanobacterial peptide hepatotoxins by chlorine and chloramine. - Water Research 28: 1297-1303.

[33] Nobre, A.C.L., Jorge, M.C.M., Menezes, D.B., Fonteles, M.C. Monteiro, H.A.S. (1999): Effect of microcystin-LR in isolated perfused rat kidney. - Brazilian Journal of Medical and Biological Research 32: 985-988.

[34] Oberholster, P., Ashton, P.J. (2008): State of the nation report: An overview of the current status of water quality and eutrophication in South African Rivers and Reservoirs.

[35] Oberholster, P.J., Botha, A., Cloete, T.E. (2005): An overview of toxic freshwater cyanobacteria in South Africa with special reference to risk, impact and detection by molecular marker tools. - Biochemistry Nigerian Society for Experimental Biology 17(2): 57-71.

[36] Pawlik-Skowronska, B., Pirszel, J., Kornijow, R. (2008): Spatial and temporal variation in microcystin concentrations during perennial bloom of Planktothrox agardhii in a hypertrophic lake. Annales de Limnologie - Intentional Journal of Limnology 44(2): 145-150.

[37] Pilotto, L., Douglas, R., Burch, M., Cameraon, S., Beers, M., Rouch, G., Robinson, P., Kirk, M., Cowie, C., Hardiman, S., Moore, C. Attewell, R. (1997): Health effects of exposure to cyanobacteria (blue-green algae) during recreational water-related activities. - Australia and New Zealand Journal of Public Health 21: 562-566.

[38] Sánchez-Bayo, F., Van Den Brink, P.J., Mann R.M. (2011): Ecological Impacts of Toxic Chemicals. - Bentham Science Publishers Ltd.

[39] South African National Standard 241-1. (2011): Drinking water standard. By SABS Standard Division, Pretoria, ISBN 978-0-626-26115-3 p 1-46.

[40] Tsuji, K., Watanuki, T., Kondo, F., Watanabe, M.F., Nakazawa, H., Suzuki, M., et al. (1997): Stability of Microcystins from cyanobacteria–iv. Effect of chlorination on decomposition. - Toxicon 35(7) 7: 1033-1041.

[41] Turner, P.C., Gammie, A.J., Hollinrake, K., Codd, G.A. (1990): Pneumonia associated with contact with cyanobacteria. - British Medical Journal 300: 1440−1441.

[42] U.S. Environmental Protection Agency. (2005): Guidelines for Carcinogen Risk Assessment. Risk Assessement Forum U.S. - Environmental Protection Agency, Washington, DC. March 2005, EPA/630/P-03/001B.

[43] Willen, E., Ahlgren, G., Tilahun, Spoof, L., Neffling, M.R., Meriluoto, J. (2011): Cyanotoxin production in seven Ethiopian Rift Valley Lakes, G, Riina M. - Inland Water 1: 8-91.

[44] World Health Organization. (1998): Cyanobacterial toxins: Microcystin-LR in Drinking-water, Guidelines for drinking-water quality, 2nd ed. Addendum to Vol. 2. Health criteria and other supporting information. - World Health Organization, Geneva, 1998.

[45] World Health Organization. (2006): Guidelines for Drinking-water Quality: First addendum to third edition. - World Health Organization, Geneva.

[46] Zong, W., Sun, F., Pei, H., Hu, W., Pei, R. (2015): Microcystin-associated disinfection by-products: The real and non-negligible risk to drinking water subject to chlorination. - Chemical Engineering Journal 279: 498-506.

FOREST PATCH CONSERVATION INDICATORS

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Abstract. This work presents indicators to assess the conservation status of forest patches in fragmented landscapes. However, how can we evaluate the conservation status of forest patches in order to compare it to a hypothetical situation of well-preserved vegetation without human disturbance? It is possible to measure some ecological processes, like nutrient cycling, canopy, edaphic, topographic and hydrological parameters, as well as landscape attributes, and associate them to conservation status. When the attributes of original well-preserved forest are unknown because they are seldom found, it is possible to compare patches by assessing the variability of their attributes. To this end, parameters related to canopy, soil, topography and landscape were analyzed to establish indicators and their integrated analysis was developed to assess the conservation status of forest patches and identify possible causes of shortcomings or obstacles to reach the conservation status. The study area is located in a Semidecidual Forest region, in Brazil. The methodology was developed considering plots distributed in sites within forest patches. The results allowed the identification of patches in different conditions of conservation and provided information of the factors that contribute to this status, in order to help guide and select the most appropriate measures to mitigate degradation and identify forest conservation strategies.
Keywords: *vegetation assessement, decision support, forest sites, ecosystem parameters*

Introduction

Many strata of society have sought valuation, restoration and maintenance of forests and have set them as goals in political agendas. Protection strategies converge as lines of preservation, conservation, ecosystem restoration and, most recently, ecosystem services, such as nutrient flows into neighboring areas, capture and sequestration of carbon by biomass and soil, water storage and purification, organic waste decomposition and detoxification, flood and drought mitigation, soil fertility renewal, food production, biological pest control and conservation of pollinators and seed dispersers (Zhang and Swintow, 2009; Altieri, 1999).

The maintenance of forest ecosystems depends on physical factors which influence the colonization, permanence and dynamics of potential plant species, and also

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influence the biological rhythms of the plants in the biomes (Machado and Lopes, 2002; Araujo and Ferraz, 2003). Rainfall, as well as geographical location, topography configuration and soil conditions (fertility and organic matter, soil depth, among others) have a link with the distribution of species and biomass production (Sampaio, 2003). The factors that affect forests are human activities, which reduce biomass and floristic composition, changing vegetation structure and dividing forest ecosystems into mosaic forms (Oliveira, et al., 2000).

How can a forest patch be evaluated comparing it to its hypothetical full potential, in which it would have attributes with maximum values, without human interference or environmental constraints? At a forest ecosystem, assigning reference values for the productivity of sites is not possible because the combination of environmental constraints and ecological requirements of species result in the variability of the productivity in each site, even considering the cases they are almost undisturbed. Thus, it is not feasible to select a supposed preserved or a less changed forest patch and use it to know the succession stage of other patches. But the comparison among relative conditions of the sites seems a good way to understand the conditions of conservation of patches.

Then, the alternative to compare forest patches would be to measure some ecological processes, such as nutrient cycling, and landscape and canopy parameters (dendrometry, biophysics, diversity), as well as edaphic, topographical and hydrological processes. The variability of these parameters can be used to produce relative valuation of the patches of a certain type in the same biome on a local scale, considering a constant climate. This information can be used to assess the intensity of patch degradation.

These parameters can be considered indicators, with the ability to summarize, to focus and to condense the complexity of the dynamic environment to a manageable amount of meaningful information (Godfrey and Todd, 2001; apud Singh et al., 2009).

Many organizations are turning to a criteria and indicators (C&I) approach to assess and plan for forest sustainability. Under this approach, criteria define broad categories of sustainability, and indicators are specific measurements (quantitative or qualitative) within each category. A framework of criteria and indicators is a valuable tool when used for assessments, planning, issue management, inventory and monitoring, and communicating with others (USDA, 2003).

A set of indicators has to be wisely selected (Niemeijer and De Groot, 2008; Van Oudenhoven et al., 2012; apud Kandziora et al., 2013), based on specific management purposes, with an integrating, synoptical value, which (in the best case scenario) shows the difference between existing states and aspired target situations. Indicators are also comprehended as depictions of qualities, quantities, states or interactions that are not directly accessible (Kandziora et al., 2013).

For especific forest case a approach of indicators in large scale was proposed by Noss (1999). But when we think on evaluate the forest structure, biodiversity, associated with site quality, it is necessary increase detail to measure variables in the forest, that is, big scale.

The classification of vegetation in phytogeographic systems is a way to associate the knowledge of flora and vegetation types to climate and geomorphological conditions on a large-scale, in order to group similar ecosystems together (IBGE, 2012; Oliveira et al., 2010). Otherwise, on a local scale it is possible to indirectly assess the conservation status of these typologies by analyzing environmental and anthropogenic parameters (Costa, et al., 2009a; Costa et al., 2009b).

In forest inventories, the main dendrometric parameters used as aboveground biomass and forest development indicators are: basal area (B), density of individuals (D) and average height (h). The botanical identification of species makes it possible to obtain floristic diversity indexes such as the Shannon-Weaver (H') and the Simpson's Dominance (C). Among the biophysical indicators, there is the leaf area index (LAI), related to plants' physiological processes, such as photosynthesis, respiration and transpiration to produce biomass.

Nutrient cycling is one of the most studied ecological processes in forest ecosystems controlled by litter (Costa et al., 2014; Valenti et al., 2008; Moreira and Siqueira, 2002; Paula and Lemos Filho, 2001; Barichello et al., 2000; Poggiani and Schumacher, 2000; Brun et al., 1999; Feger and Raspe, 1998; Cunha, 1997). One of the purposes of measuring litter deposition is to estimate the efficiency of natural ecosystems to provide soil with the nutrients needed for its maintenance (Silva et al., 2009). Two parameters used to evaluate nutrient cycling are litterfall, decomposition rate and leaf nutrients.

Regarding the edaphic component, we can mention fertility and grain size parameters and decomposition activators, which are organisms of the meso-fauna and micro-fauna. A part of the decomposition process can be measured by enzyme activity, such as urease, arginase, acid phosphatase, alkaline phosphatase, hydrolases in general and basal soil respiration (Silveira and Freitas, 2007; Marriel, 2008; Aquino and Assis, 2005). In theory there is a relationship between the quantity of the fallen leaves of the canopy, the decomposition rate and the microbial activity, due to the lack of litter accumulation on forest soils.

Topographical conditions, such as altitude, slope, aspect and slope curvature determine microclimates, storage, runoff and the amount of incident solar radiation. Regarding the hydrological component, parameters such as distance and elevation of the sites in relation to water bodies are related to water availability.

Patch size, shape and isolation are important to characterize landscape structure (Turner, 1989; Sih and Luikart, 2000; Nascimento and Laurence, 2006; Mcgarigal, 2011). All parameters described at site or landscape scale are related to the conservation and the succession stage.

All these parameters are affected by ecosystem heterogeneity which refers to inconsistencies of the spatial and temporal distribution of abiotic and other habitat parameters, indicated, for example, by landscape metrics related to soil conditions, geomorphologic items, microclimate or hydrological characteristics (Burkhard et al., 2011 and Eea, 2007 apud Kandziora et al., 2013).

Thus, considering the hypothesis that forest patches of the same biome, in better conservation conditions, show the best results for the parameters related to canopy, soil and landscape characteristics, the analysis of indicators associated to these characteristics can demonstrate the conservation status of forest patches, as well as the source of restrictions to its development. Also, considering the influence of local topographic variations on the type of vegetation, the inclusion of an indicator comprising topographic parameters completes this analysis.

Based on this premise, this study aims to analyze the behavior of various parameters related to canopy, soil, topography and landscape to establish indicators and their integrated analysis to assess the conservation status of forest patches and identify possible causes of shortcomings or obstacles to its full development.

Material and Methods

The study area is the experimental farm of Embrapa Milho e Sorgo, located in the city of Sete Lagoas, Minas Gerais, Brazil in a region with the following vegetation types: Savannah Park, Grassy-Woody Savannah, Savannah Tree, Savannah Woodland, Lowland and Semideciduous Forest and Evergreen Seasonal Forest (IBGE, 1988).

The Köppen climate classification is Cwa (Peel et al., 2007), indicating Savannah climate with dry winters and rainy summers. The average annual temperature is 21.1°C ± 6.0°C. The average annual rainfall is 1,384 mm and the average annual potential evapotranspiration is approximately 1,444 mm (Gomide et al., 2006).

The region is on the geological contact between the granites of the crystalline basement (Belo Horizonte Supergroup), and sedimentary rocks of the Bambuí Group; the latter is the dominant lithology of the study area. The regional topography consists of gently rolling hills.

Eight patches of forest physiognomy were selected for this study, characterized by the absence of grass in the understory layer, the presence of straight tree trunks, high canopy, non-coriaceous leaves, not in the early stages of the ecological succession, and at least 50 years old of recovering from pasture.

Twelve sites were selected in these patches (*Fig. 1*) and 27 plots, 20 x 20 meters, were marked in 100 meters from the edge of the patches. The number of sites and plots is proportional to the size of the patches. There are: Patch 1: three sites (11, 12 and 13) with two plots each; Patch 2: one site (21) with three plots; Patch 3: one site (31) with one plot; Patch 4: one site (41) with three plots; Patch 5: two sites (51 and 52) with two plots each; Patch 6: one site (61) with three plots; Patch 7: one site (71) with three plots; and Patch 8: two sites (81 and 82) with two plots each.

The phytosociological inventory was carried out measuring trees with diameters over 5 cm at breast height (DBH), and the botanical collection of material was sent to the PAMG-EPAMIG Herbarium of Minas Gerais, identified by the APGIII System (APG, 2009). The variables obtained by site were: density (D) ind.m^{-2}; basal area (B) m^{2}.ha^{-1}; average canopy height (h) m; Shannon diversity index (H') and Simpson dominance index (C). The sites were then classified by forest type using the species ecology information (IBGE, 2012).

In order to measure the leaf area index (LAI) we used the LAI 2200 (LI-COR, 2011). The methodological procedure is described by Costa et al. (2014). The LAI was measured per plot, then the average annual value per site was calculated, LAImd_anual (m^{2} of leaves.m^{-2} of land).

The soils at each site were classified with profiles until 1.6 meters deep. At the bottom of the profiles, boreholes were made to verify uniformity of the material. The thicknesses of each Horizon was measured, and fertility and grain size parameters were obtained accordingly to the brazilian official soil methods (DONAGEMA et al., 2011). As the first contribution of the literfall to soil is on A thickness, we used the depth of Horizon A, its fertility and its grain size parameters for the comparisons. And we used the agricultural and planted forest references for the interpretation of the soil fertility indicators due to the lack of detailed knowledge concerning the real nutritional needs of these native species.

Figure 1. *Location of the sites in each patch (F1/1 to F8/2) on the experimental farm of Embrapa, Sete Lagoas, Minas Gerais, Brazil. Source of image: Google Earth*

In order to characterize nutrient cycling, we evaluated the deposition of leaf litter (leaves), the dry weight of leaves $g.m^{-2}.year^{-1}$ (psfhanual) (described by Costa et al., 2014), the leaf decomposition rate (k) and microbial activity indicators: arginase (ARG, in μg $NH4.g^{-1}.h^{-1}$), fluorescein diacetate hydrolysis (FDA in $\mu g.g^{-1}.h^{-1}$), acid phosphatase and alkaline phosphatase (FOSFacid and FOSFbas, respectively, both in $\mu g.g^{-1}.h^{-1}$), urease (UREAS in $\mu g.g^{-1}.h^{-1}$), basal respiration (CO_2, in $ml.kg^{-1}.h^{-1}$).

To obtain the litter decomposition rate, samples of 10 g were introduced into litterbags which provide 75% shade, with a mesh of 1 mm x 5 mm, 25 x 25 cm total size. The litterbags were distributed at 11 sites on Aug. 11, 2011, 12 bags at each site (site 13, code for Path 1 and plot 3, was not sampled, because it is similar to site 11, code for path 1 and plot 1). Samples were collected on Feb. 15, 2012, May 15, Sep.14, and Dec.18 of the same year. Each collection had three repetitions and the collected material underwent a screening process to remove soil residues, dead animal remains and other impurities. After the cleaning, the material was placed in a kiln at 65°C for 48 hours. After weight was stabilized, the material was weighed. To estimate the litter decomposition rate we used the simplified model $x = b * e^{-kt}$ (Manzoni et al., 2012), adjusted for each site, in which x is the dry weight of remaining litter at time t; and -k is the rate of litter decomposition. The parameters evaluated were mean values and confidence intervals of k and b.

Microbiological analyses were performed by collecting four soil samples at each site at 0-20 cm depth. The samples were stored and kept in the refrigerator at temperatures between 7 and 10°C. These samples were obtained in the rainy season, in which there is higher biological activity. The methods applied to quantify the parameters of microbial activity are described by the authors mentioned: urease (Gerber and Kandeler, 1988), arginase (Alef and Keiner, 1986), acid and alkaline phosphatase (Tabatai and Bremmer, 1969), FDA (Adam and Duncan, 2001), and basal respiration (Silva et al., 2007).

The topography parameters were obtained from a digital elevation model with five-meter resolution, generated by the map of the farm with two-meter contour lines. The parameters of altitude (Alt), slope, slope orientation (aspect), curvature profile (crprof) and curvature plane (crplan) were generated after corrections to avoid spurious depressions and other inconsistencies in the model. The values per site and patch were then obtained using zonal statistic tools.

Considering that the regional climate has six dry months, the environments with favorable topography for water accumulation are usually colonized by plant communities of forest phytophysiognomies. The concavity and slope parameters were used as topography indicators of nutrient and water accumulation, which could be observed on concave surfaces.

Information on the hydrological influence obtained through water balance per site and hydrogeological studies have very high costs. In this study, the groundwater supply parameters used were the distance (distwat) and the altitude difference (altwat) between the site and the nearest water bodies - perennial or intermittent streams.

The landscape parameters were obtained using land use/land cover data on the experimental farm and its surroundings, delimited via visual interpretation of images available on Google Earth from August 22, 2011 to April 16, 2013. The classes delimited within the farm were: lake, lowland, railway, highway, urban (built area), bare soil, experimental field, pasture, forest plantation, Savannah Park, Grassy-Woody Savannah, Savannah Tree, Savannah Woodland, Semideciduous Forest and Evergreen Seasonal Forest. Outside the farm borders, only the natural vegetation was mapped and classified as Semideciduous Forest or Savannah.

The parameters that represent the extent and the shapes of patches of the sites as well as their position in relation to other patches in the landscape were obtained using the Fragstats program (McGarigal, 2011). To do so, the land use/land cover map was rasterized at the same resolution as the digital elevation model, and then the parameters, whose description can be found in McGarigal (2011), were calculated: Area (Area), Circumscribed Circle (CIRCLE), Contiguity Index (CONTIG), Euclidean Distance of Nearest Neighbor (ENN) and Proximity Index (PROX).

Whereas the use in patch surroundings affects the conditions of such patches (Werneck., 2001; Tabarelli et al, 2008), we calculated the percentage of the patch perimeters bordering natural vegetation (limAreNat), i.e., the land use/land cover that does not represent a negative impact on patch conservation.

We did not joined these data in one integrated multivariate analysis, for example Multiple Factor Analysis, due to the difficulty to extract relevant informations after the number of transformations and because of the possible loss of variation of the original data. And we noticed that some variables did not explain the expected relationships among parameters because of the complex interation of the factors that drive forest quality. Then, to compare the obtained parameters in the different patches and sites, we used Principal Component Analysis (PCA) applied by group of variable to assess the magnitude of the correlation and the direction of each variable.To analyze the sites, we worked with parameter groups:

- canopy - D, B, h, H', C and LAI;
- soil - pH in water (pH_{H2O}) with pHmeter (soil/water relation 1:2.5), phosphorus (P) extracted by the Mehlich 1 solution, potassium (K) by HCl 0,05 M solution, calcium (Ca) and magnesium (Mg) by the KCl 1 M extrator method and ICP-OES determination, aluminum (Al) by the KCl 1 M extrator and titration method, potential acidity (H+Al), sum of bases (SB), potential cation exchange capacity (CTC(T)) by the Ca acetate at pH 7 extrator method, effective cation exchange capacity (CTC(t)) by the KCl 1 M extrator method, organic matter (OM) by dry combustion method with an elemental analyzer, percentage of base saturation (V), percentage of aluminum saturation (m) , zinc (Zn), iron (Fe), manganese (Mn), copper (Cu), the last four extracted by the Mehlich 1 solution, gravel, fine soil, coarse sand, fine sand, silt, clay (by sieving and the pipete method) and horizon depth (Prof);
- nutrient cycling and microbial activity - leaves, k, ARG, FDA, FOSFacid, FOSFbas, UREAS and CO_2; and
- topograph and water proximity - crprof, crplan, slope, aspect, distwat and altwat.

Regarding the patches, we worked with the groups of parameters related to the landscape: Area, CIRCLE, CONTIG, ENN, PROX and LimAreNat.

After this exploratory analysis, knowing the a priori relevance relationship of each parameter and the conditions of the forest patches, we obtained the evaluation indicators that contained parameters of different types, units and scales and used simple support decision methodology (Eastman et al., 1995). As we did not judge which parameter is more important than others to conservation, we did not use weights, we considered the simple average of the parameters.

Due to this diversity, it was necessary to standardize the response of the parameters and establish relevant relationships to the goal (Eastman et al., 1995; Mello Filho et al., 2007). The standardization was performed on a 0-1 scale, and the relevance relationships adopted were linear and fuzzy trapezoidal with thresholds obtained from the literature or from the data series. To promote conservation (S goal), the conservation indicator was the average of the standardized parameters, $S = \text{mean}(x_i)$ considering that they have the same importance.

Results and Discussion

Canopy

Regarding canopy, the magnitude and direction of the parameters in Component 1 of the PCA (*Fig. 2*) show a cluster of sites 31, 81, 82, 41, 51 and 52 in relation to aerial biomass, parameters B, D, h, LAI; and in relation to diversity, parameters H' and C. The undesirable variable to conservation, C, is on the left, along with sites 11, 12, 13 and 71. Sites 21 and 61 showed heterogeneity in plots 213 and 613, because these plots have a more developed understore layer and higher density of individuals when compared to the other plots of the respective sites. Plot 411 is not close to the other plots of Site 41 due to unfavorable topographical conditions, which reflected negatively on canopy parameters.

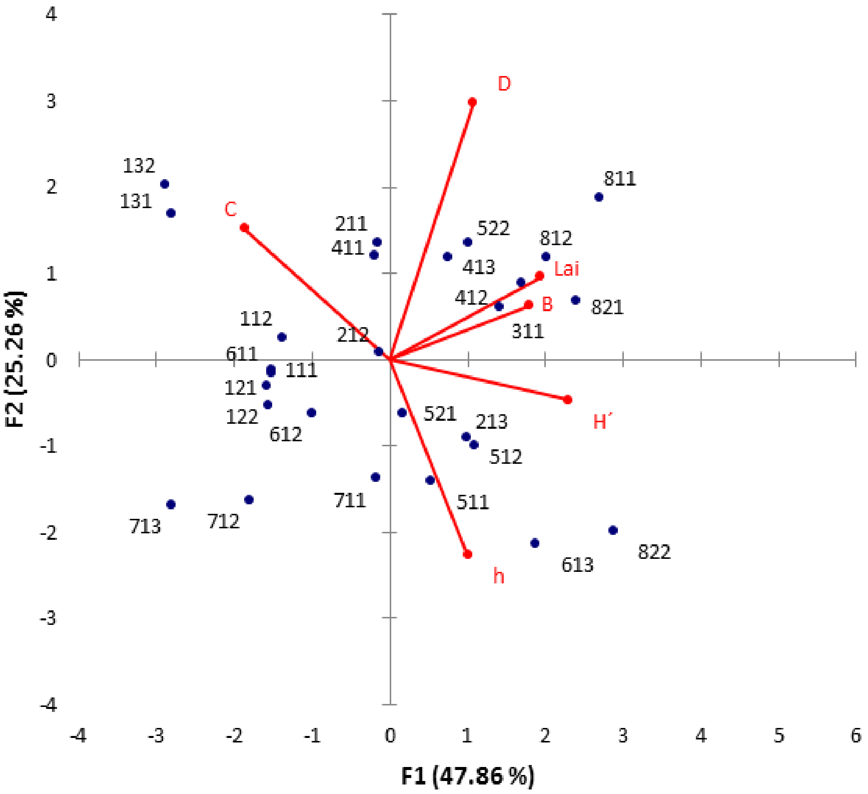

Figure 2. Joint dispersion of canopy parameters and plots in the sites for Components 1 and 2 (F1 and F2) of the PCA

Soils

In the evaluation of fertility, depth and grain size (*Fig. 3*), we found that the retention of cations (CTC(t), CTC(T)) is more related to organic matter content (MO) than to clay activity, because these soils, highly weathered, have low clay activity. *Figure 3* shows the vector of the proportion of clay in the direction approximately orthogonal to the main fertility parameters, including the vector of organic matter. This is evidence that the fertility of Horizon A is mainly supplied by nutrient cycling processes, not by the source material which forms the soils of the study area.

This indicates that the biotic ecosystem components affect the spatial and temporal patterns of the abiotic habitat components of ecosystems, which can be indicated by measuring ecosystem heterogeneity (Müller, 2005 apud Kandziora et al., 2013).

Comparing canopy parameters and those of fertility (*Fig. 4*), we found that the aerial biomass and diversity do not correlate to the fertility levels of Horizon A at the sites, indicating the existence of other factors which affect structure and floristic diversity. Sites 11, 13 and 71 have higher fertility levels in Horizon A, although their canopy parameters indicate an unfavorable condition for conservation. Sites 11 and 13, may have received fertilization for a long time, as there are records that this area has been used for pasture. Due to its favorable topographic conditions, it may also have been previously cultivated.

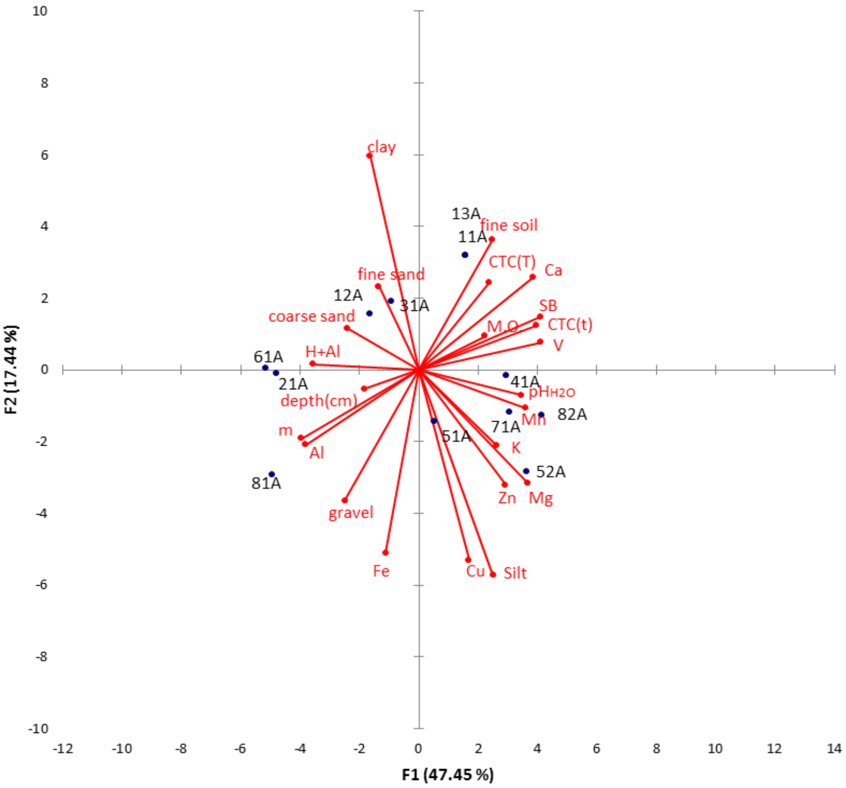

Figure 3. *Joint dispersion of particle size and fertility parameters in Horizon A and sites for Components 1 and 2 (F1 and F2) of the PCA*

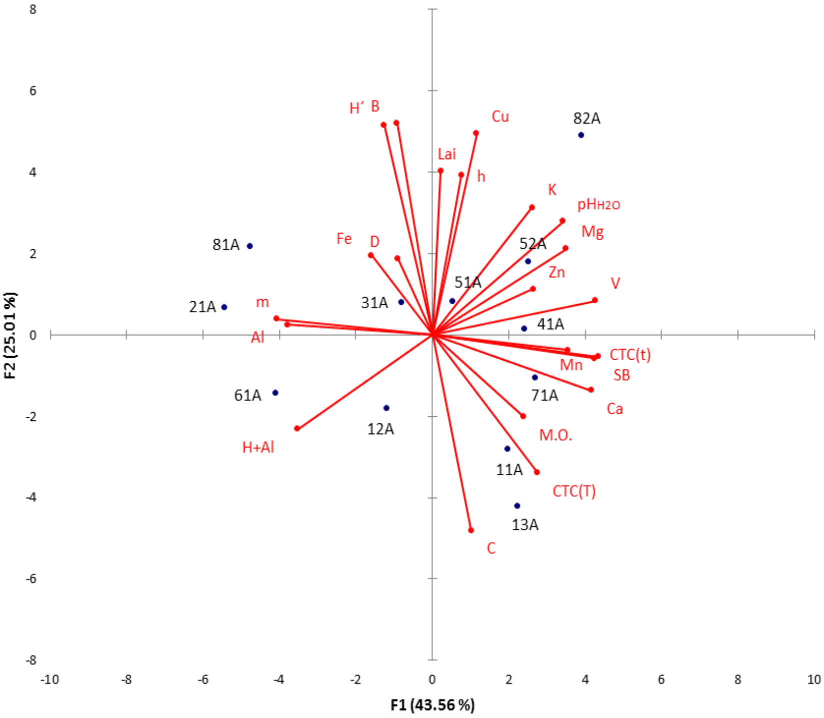

Figure 4. *Joint dispersion of canopy (average of plots) and fertility parameters in Horizon A and sites for Components 1 and 2 (F1 and F2) of the PCA*

The sites 21 (F 2/1), 61 (F 6/1) and 81 (F 8/1) have less favorable fertility characteristics. The site 21 (F 2/1) is on a Typic Haplustox (USDA, 1999) (weathered soil with low cation exchange capacity), site 61 (F 6/1) is on Humic Haplustox and site 81 (F 8/1) is on Humic Haplustox (soils with low base saturation). The vegetation in Site 21 is a transition from Semideciduous Forest to Savannah - common in weathered soils with surface acidity; Site 61 is Semideciduous Forest and Site 81 is Evergreen Seasonal Forest. One factor that differentiates Sites 61 and 81 is the presence of groundwater in most favorable conditions in Site 81. It is likely that fertility indicators and groundwater are compensations, as observed at Sites 31 (F 3/1) and 81, which present low fertility, but good water supply, which may have led to a greater structure and diversity of the vegetation in those sites.

Nutrient cycling and microbial activity

We did not find logical correlations in the vector positions when nutrient cycling parameters were evaluated (*Fig. 5*). We expected that the amount of annual deposition of leaf litter (leaves) would correlate positively with the leaf decomposition rate (k) and microbial activity parameters (ARG, UREAS, FDA FOSFacid, FOSFbas, CO_2), because microbial activity is a component in mineralization of organic matter. We expected all these vectors in the PCA to be oriented to sites with higher biomass, diversity and fertility.

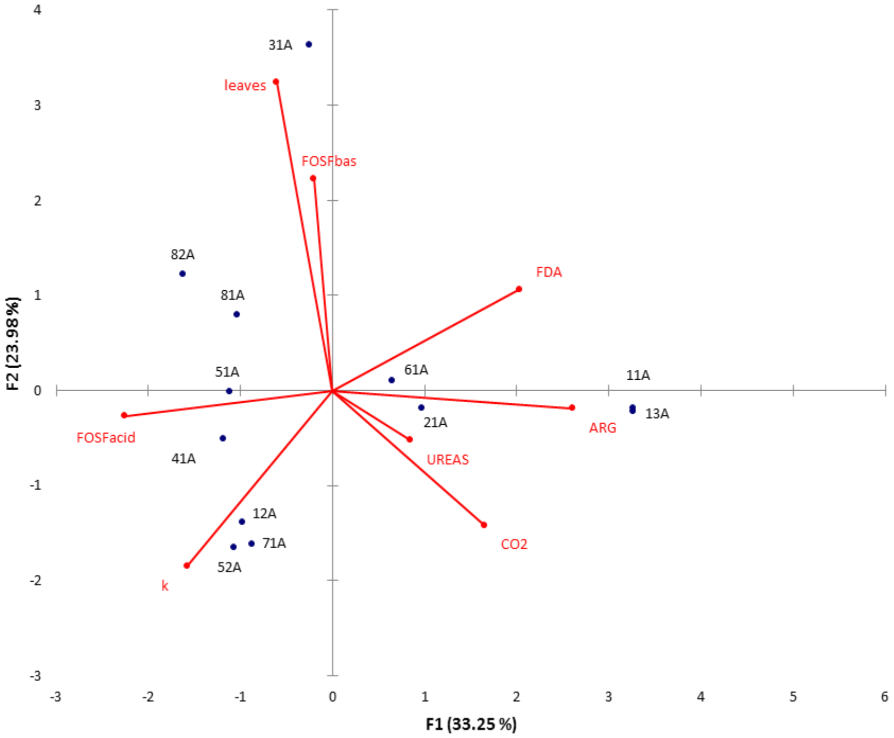

Figure 5. *Joint dispersion of cycling parameters and sites for Components 1 and 2 (F1 and F2) of the PCA*

The mismatch may be due to low sensitivity of the method to detect differences in the speed of leaf mineralization. *Figure 6* shows the leaf decomposition rate (k) for each site. We observed that, although rates vary from -0.002 to -0.005, their dispersion is high and the statistical difference only appears between Sites 81 and 52. The low sensitivity to detect differences in forest ecosystems is due primarily to the similarity between environments, although Sites 11, 12, 13 and 21 are secondary forests at an earlier stage. By using this method, Hayashi (2006) could detect higher annual litter production and higher decomposition rates in primary forests compared to regenerated forests. The accuracy of the procedure also may have affected the results. The material in the litterbags taken in each analysis did not return. Other samples were taken for analysis in the following period. Differences in the decomposition intensity occur because there are variations of fungi and bacteria colonies in the soil. And, finally, the longer the material remains on the ground, the harder it is to identify what leaves are, adding more uncertainty to the outcome. Therefore, the decomposition rate did not show good sensitivity to detect differences between forest ecosystems.

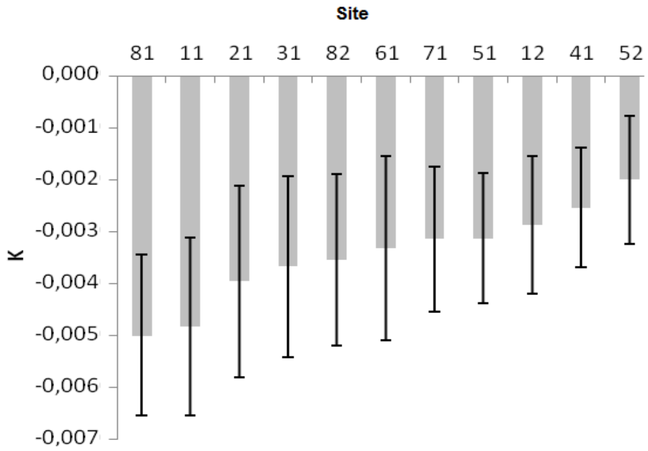

Figure 6. *Leaf decomposition rate (k) and its lower and upper limits at 95% confidence interval*

Regarding microbial activity, *Figures 7, 8 and 9* show the mean values and standard deviation for the ARG, UREAS, FDA, FOSFacid, FOSFbas and CO_2 parameters. Analyses of variance, considering treatments (sites) with four replicates per parameter, did not detect significant differences in the levels of UREAS, FDA, FOSFacid and CO_2. For ARG, the Scott – Knott means test detected differences between Sites 11/13 and others.

Microbial activity when comparing the different sites showed similarities. Regarding ARG and FOSbas parameters, there were no reasons for ARG to be an outlier at Sites 11/13, nor for the statistically equal results of FOSFbas at Sites 82, 61 and 51, which are different from the other sites.

We could not confirm the hypothesis, with use of this method that higher leaf deposition, nutrient content and microbial activity would lead to higher decomposition rate and soil fertility in Horizon A.

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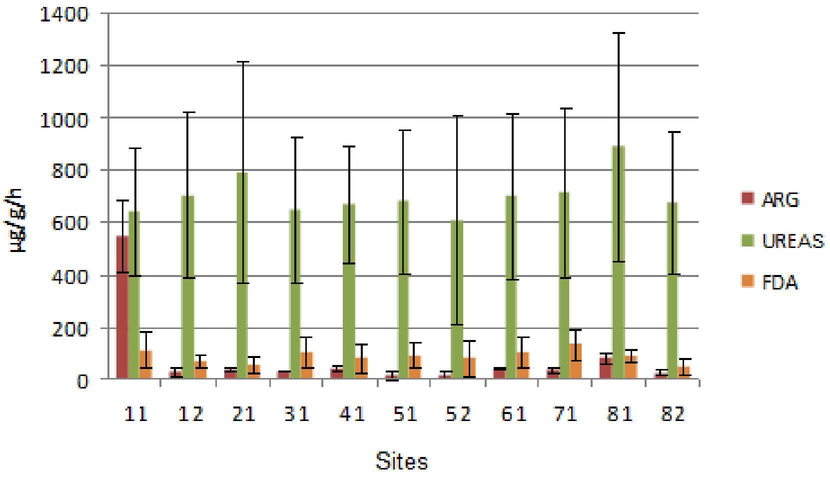

Figure 7. *Arginase activity, ARG (µg NH4/g/h); urease, UREAS (µg/g/h) and hydrolysis of fluorescein diacetate; FDA (µg/g/h) in soil samples at eleven sites (Site 13 is represented by Site 11)*

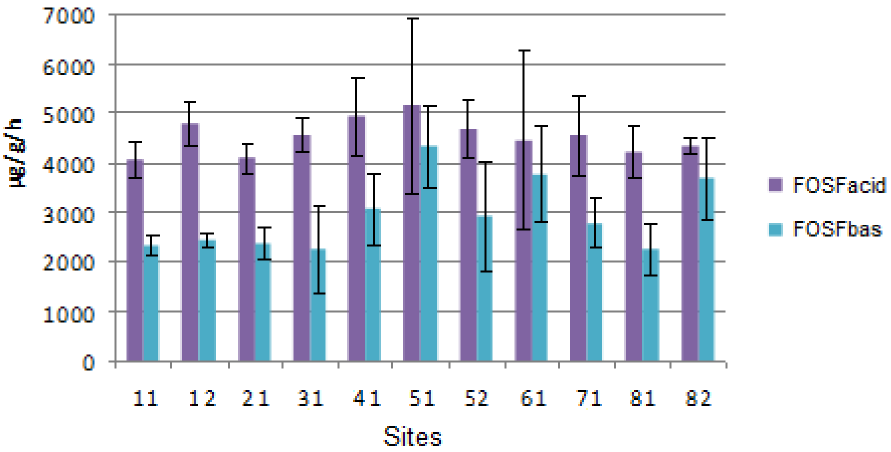

Figure 8. *Acid phosphatase activity (FOSFacid) and basic phosphatase (FOSFbas) (µg/g/h) in soil samples at eleven sites*

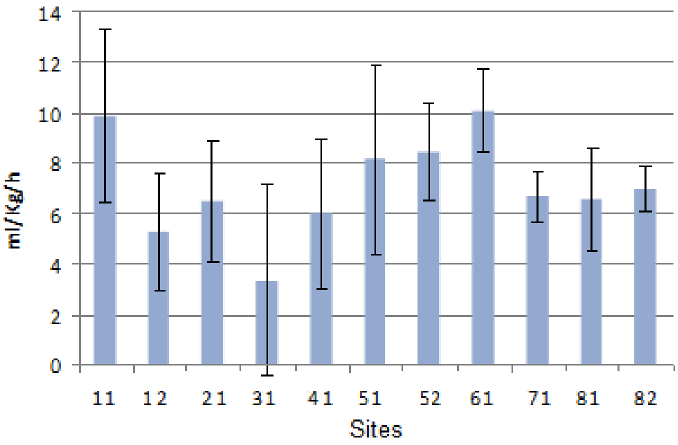

Figure 9. *Basal respiration, CO_2 (ml/kg/h) in soil samples at eleven sites*

Topography and water proximity

The main water bodies on the Embrapa farm are the Matadouro, Jequitibá, Marinheiro and Papuda streams. The Matadouro stream is at least 600 meters from the nearest sites (in Patches 4, 5, 6 and 7) with an altitude difference between 30 and 50 meters. The Jequitibá stream flows in Patch 8, 150 to 160 meters from the sites, with altitude differences between 7 and 10 meters. The sites of Patch 1 are close to the Papuda stream, between 100 and 200 meters, with altitude differences of 10 meters (Sites 12 and 13) and 30 meters (Site 11), and it is dammed 100 meters upstream from Site 13. In Patch 2, the distance to the Marinheiro stream is 400 meters with an altitude difference of 40 meters. In Patch 3, there is ground water two meters deep and a stream spring downstream. Except for Patches 2, 3 and 6, the others present intermittent drainage.

The parameters related to topography and water proximity (*Fig. 10*) show that there is a clear difference between Sites 13, 31, 81, 82, which are closer to water and have favorable topographic conditions, and Sites 41, 51, 52, 61 and 71, which are on sloping areas and far from water bodies. An inverse relation is also observed between curvature plane and profile. The sediment accumulation condition is not present simultaneously on both axes for most sites. Very often, one curvature is convex and the other is concave.

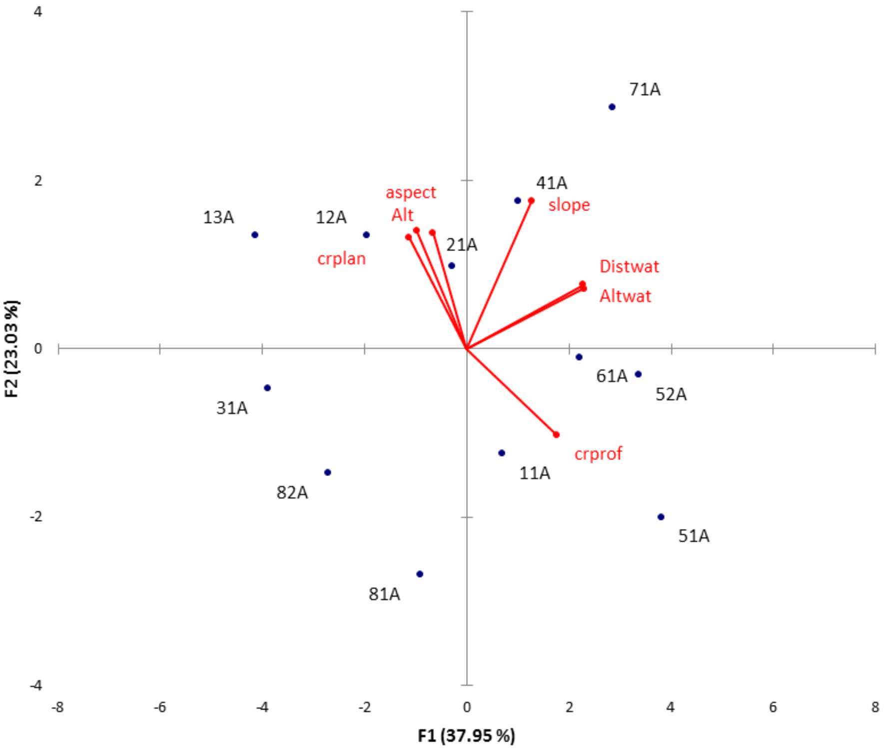

Figure 10. *Joint dispersion of topography and water availability parameters and sites for Components 1 and 2 (F1 and F2) of the PCA*

Analysis of the patches in the landscape

The metrics and topography for the forest patches and correlations between variables were strongly represented in the first two components, which concentrate 81.55% of the total variation (*Fig. 11*).

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The distribution of the parameters shows that the patches have different factors which are favorable to conservation. Regarding size (Area), the largest patches are 1 and 8, and Patches 4, 5 and 7 have a higher percentage of perimeter bordering natural vegetation (LimAreNat). The last three patches are also more aslope, which is a common feature in the Atlantic Forest landscape, where the remaining natural vegetation is located in areas hard to reach and restricted to human use due to their harsh topographic conditions. The metrics related to shape, CIRCLE and CONTIG did not stand out in any patch. Regarding unfavorable conservation features, we highlight Patch 3 which is the lowest and most isolated.

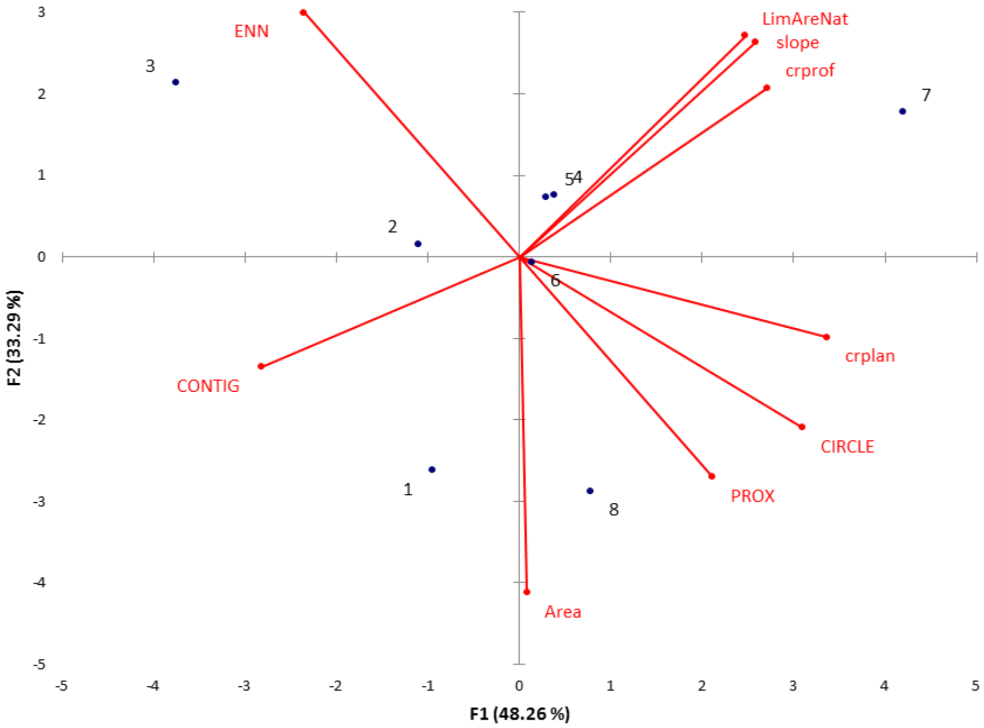

Figure 11. *Joint dispersion of topography parameters, landscape metrics and patches for Components 1 and 2 (F1 and F2) of the PCA*

Favorable indicators for conservation

After the PCA analysis, we found that the parameters considered were not enough to explain the current state of conservation of forest patches. Other factors such as water dynamics in soil and subsoil, human interventions, especially fires, wood removal and land use before regeneration may have contributed to the definition of current forest structures and floristic composition. Some of these factors are not available due to the lack of historical record.

However, that does not prevent the use of the selected parameters and their conversion into conservation indicators, as they constitute measurements of the ecosystem. The indicators show the relative degree of conservation of each patch as well as the most favorable or unfavorable factors for conservation. The thresholds for each parameter and their groupings are described in *Table 1.*

Table 1. *Selected parameters for conservation and ecological stability assessment of Semideciduous Forest patches, with minimum and maximum limits, and its relevance according to goals*

Group	Original Variable	Lim. Min.	Lim. Max.	Relation to conservation
Canopy	LAI ($m^2.m^{-2}$)	Data series		Linear increase
	h (m)			Linear increase
	D (Ind.m^{-2})			Linear increase
	B ($m^2.ha^{-1}$)			Linear increase
	H'			Linear increase
	C			Linear decrease
Soil fertility	pH_{H2O}	(1)a=3 (1)m=6	(1)n=6,5 (1)b=9	Trapezoidal fuzzy
	P ($mg.dm^{-3}$) Argila>60	0	15(2)	Sites 11,12,13,2,3,6 Linear increase
	P ($mg.dm^{-3}$) 41<Argila<60	0	20(2)	Sites 4,51,52,7,82 Linear increase
	P ($mg.dm^{-3}$) 21<Argila<40	0	30(2)	Linear increase Site 81
	K ($mg.dm^{-3}$) CTC_T>15	a=0	b=250(2)	Triangular fuzzy Sites 11,13,4,7
	K ($mg.dm^{-3}$) 5<CTC_T<15	0	200(2)	Triangular fuzzy Sites 12,2,3,51,52,6,81,82
	Ca ($cmol_c$ dm^{-3})	0	10(2)	Linear increase
	Mg ($cmol_c$ dm^{-3})	0	2(2)	Linear increase
	H+Al ($cmol_c$ dm^{-3})	0	9(2)	Linear increase
	V% (V)	0	100	Linear increase
	m% (m)	0	100	Linear decrease
	M.O. ($dag.kg^{-1}$)	0	15(2)	Linear increase
	Cu ($mg.dm^{-3}$)	0	2(2)	Linear increase
	Profundity H. A (prof)(cm)	Data series		Linear increase
Nutrient cycling	Leaves ($g.m^{2-}year^{-1}$)	100	900	Linear increase
	Decomposition rate (K)	0	-0.007	Linear decrease
Topography	Curvature plane (crplan)	-0.6 (3)	0.6 (4)	Linear decrease
	Curvature profile (crprof)	-0.6 (3)	0.6 (4)	Linear decrease
	Slope (^{o})	0	25	Linear decrease
Water proximity	Distance of water resources (distwat) (m)	Data series		Linear decrease
	Altitude difference in relation to water resources (altwat) (m)	Data series		Linear decrease
Patches in the landscape	Area (ha)	Data series		Linear increase
	CIRCLE	0	1	Linear decrease
	CONTIG	0	1	Linear increase

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ENN	0	100	Linear decrease
PROX	Data series		Linear increase
Proportion of patch perimeter bordering natural vegetation (LimAreNat)	0	100	Linear increase

Obs.

(1) a, b, m, n are inflection points of the curves.

(2) Minimum and maximum limits proposed by Ribeiro et al. (1999) and SBCS (2004).

(3) associated with concave forms, favoring water and sediment convergence.

(4) associated with convex forms, favoring water and sediment divergence.

After selecting the parameters, indicators were calculated for each group. For the canopy group, the canopy conservation indicator (canopy ind.) is presented in *Table 2*. The proximity of values of some sites groups them in a similar way to the distribution observed in *Figure 2*. For example, 81 and 82 with best canopy quality and Sites 11, 12, 13, 61, 71 with lower canopy quality.

Table 2. *Standardized canopy parameters (0-1) and canopy conservation indicator (canopy ind.) per site. Lower values close to zero indicate unfavorable for conservation and values close to 1, favorable for conservation*

Site	LAI	h	D	B	H′	C	canopy ind.
11	0.12	0.05	0.32	0.21	0.42	0.57	0.28
12	0.26	0.3	0.2	0	0.34	0.55	0.28
13	0.17	0	0.7	0.07	0	0.00	0.16
21	0.12	0.39	0.63	0.66	0.71	0.75	0.54
31	1	0.34	0.51	0.7	0.69	0.75	0.67
41	0.7	0.3	0.76	0.53	0.66	0.64	0.60
51	0.4	0.73	0.22	0.68	0.73	0.91	0.61
52	0.69	0.54	0.52	0.72	0.51	0.63	0.60
61	0.35	0.65	0.21	0.37	0.57	0.71	0.48
71	0	0.62	0	0.22	0.3	0.67	0.30
81	0.75	0.22	1	1	0.95	0.90	0.80
82	0.8	1	0.56	0.88	1	1.00	0.87

In order to calculate the soil fertility indicator (Soil ind.), we disregarded parameters Fe, Zn and Mn due to their high levels in the soil, and because there are no references to toxic levels for trees. Texture parameters were not considered because only Horizon A was studied and the parameters were unexpressive. The fertility indicator (*Table 3*) did not follow the same trend as the canopy indicator. Site 31 was in Typic Haplustox (soil with low base saturation and low fertility), but that soil sustained vegetation with good canopy and diversity, helped by the existence of groundwater 2 meters below it. On the contrary, Sites 41 and 51, with good fertility indicators, were among the most affected by drought, the distance from water bodies and their topographical position, with intermediate values of canopy indicators.

It is important that even the lower soil fertility indicators still had reasonable fertility levels in Horizon A supplied by nutrient cycling.

Table 3. *Soil fertility parameters of Horizon A selected and standardized (0-1) and soil fertility indicator (Soil ind.) per site. Result of Site 11 also represents Site 13*

Site	P	K	Ca	Mg	H+Al	M.O.	Cu	Prof	pH_{H2O}	V	m	Soil ind.
11	0.35	0.42	0.91	0.41	0.50	0.62	0.13	0.47	0.86	0.62	1.00	0.57
12	0.40	0.49	0.41	0.41	0.42	0.41	0.19	0.77	0.86	0.39	0.86	0.52
13	0.35	0.42	0.91	0.41	0.50	0.62	0.13	0.47	0.86	0.62	1.00	0.57
21	0.22	0.31	0.09	0.09	0.28	0.30	0.45	0.67	0.74	0.12	0.46	0.39
31	0.58	0.22	0.59	0.20	0.50	0.43	0.35	0.00	0.85	0.52	1.00	0.48
41	0.67	1.00	0.66	0.86	0.42	0.82	0.40	0.47	0.98	0.56	1.00	0.70
51	0.26	0.41	0.64	0.72	0.43	0.59	0.49	0.85	0.88	0.50	0.84	0.58
52	0.20	0.67	0.63	0.89	0.55	0.64	0.86	0.17	1.00	0.61	1.00	0.60
61	0.31	0.43	0.29	0.17	0.05	0.59	0.12	1.00	0.69	0.19	0.41	0.43
71	0.53	0.81	0.82	0.63	0.54	0.50	0.31	0.09	0.89	0.64	0.99	0.59
81	0.18	0.56	0.14	0.34	0.29	0.37	0.42	0.61	0.63	0.20	0.53	0.41
82	0.36	1.00	0.82	0.81	0.90	0.41	0.74	0.82	0.74	0.89	1.00	0.74

The nutrient cycling indicator (Cycl ind.) (*Table 4*) showed that the response to cycling was similar at most sites, having its lowest limit observed at Site 12, and its highest limit at Site 31.

Table 4. *Parameters of cycling selected and standardized (0-1) and the nutrients cycling indicator (Cycl ind.) per site*

Site	leaves	k	Cycl ind.
11	0.30	0.72	0.51
12	0.19	0.44	0.32
13	0.30	0.72	0.51
21	0.33	0.55	0.44
31	0.77	0.69	0.73
41	0.37	0.42	0.39
51	0.45	0.54	0.49
52	0.30	0.38	0.34
61	0.35	0.44	0.39
71	0.18	0.57	0.37
81	0.46	0.67	0.57
82	0.54	0.60	0.57

The topography indicator (Topo ind.) (*Table 5*) displayed no major differences between the sites. An exception occurred at Site 71, where unfavorable canopy parameters also were found. In that case, the low topography indicator may have contributed to the poor forest development at the site.

The water proximity indicator (Wat ind.) (*Table 5*) shows that Sites 21, 41, 51, 61 are less likely to have underground water supply, even though their ICds showed intermediate values. Sites of Patches 1 and 8 presented close Wat ind. values, although their responses, in terms of ICd, were very different, which shows that the proximity to an isolated water resource cannot respond to the level of forest conservation.

Table 5. *Standardized parameters of topography and water proximity (0-1) and topography (Topo ind.) and water proximity (ICwat) indicators per site*

Site	crprof	crplan	slope	Topo ind.	distwat	altwat	Wat ind.
11	0.36	0.52	0.70	0.53	0.75	0.26	0.51
12	0.64	0.51	0.35	0.50	0.75	0.79	0.77
13	0.76	0.41	0.74	0.64	0.75	0.79	0.77
21	0.69	0.37	0.71	0.59	0.33	0.00	0.17
31	0.71	0.25	0.73	0.56	1.00	1.00	1.00
41	0.64	0.41	0.54	0.53	0.00	0.00	0.00
51	0.36	0.74	0.68	0.59	0.00	0.00	0.00
52	0.55	0.92	0.42	0.63	0.00	0.00	0.00
61	0.55	0.52	0.61	0.56	0.00	0.00	0.00
71	0.42	0.07	0.11	0.20	0.00	0.00	0.00
81	0.52	0.53	0.76	0.60	0.75	0.84	0.80
82	0.53	0.48	0.90	0.64	0.75	0.84	0.80

The landscape indicator (Landsc ind.) was obtained from the selected parameters related to patches (*Table 6*). The highest value was that of Patch 8, due to its size and proximity to other patches, although its border with natural areas is not relatively high: 49%. The low value for this metric also shows the risk of harmful interference in forest conservation. In the case of the study area, livestock from a surrounding pasture area often escapes causing damage to patch regeneration. Patch 7, a smaller area, has a large border with natural areas, 90%, indicating that the change from Semideciduous Forest to Savannah Woodland, Grassy-Woody Savannah, may be a result of soil and topographical conditions -- in other words, natural constraints.

Table 6. *Standardized parameters and landscape indicator (Landsc ind.) per patch*

Frag.	Area	CIRCLE	CONTIG	ENN	PROX	LimAreNat	Landsc ind.
1	1.00	0.26	0.98	1.00	0.44	0.24	0.65
2	0.12	0.35	0.98	0.63	0.41	0.58	0.51
3	0.00	0.61	0.98	0.00	0.00	0.44	0.34
4	0.28	0.21	0.98	0.67	0.08	0.84	0.51
5	0.21	0.36	0.98	0.85	0.18	0.70	0.55
6	0.09	0.28	0.98	0.85	0.50	0.58	0.55
7	0.08	0.14	0.97	0.82	0.68	0.90	0.60
8	0.92	0.14	0.98	0.98	1.00	0.49	0.75

When considering the joint analysis of indicators (*Fig. 12*), we can assess their contribution, identifying weaknesses and strengths. Knowledge on the factors limiting conservation allows selecting the most appropriate measures to mitigate degradation and promote conservation of forest patches.

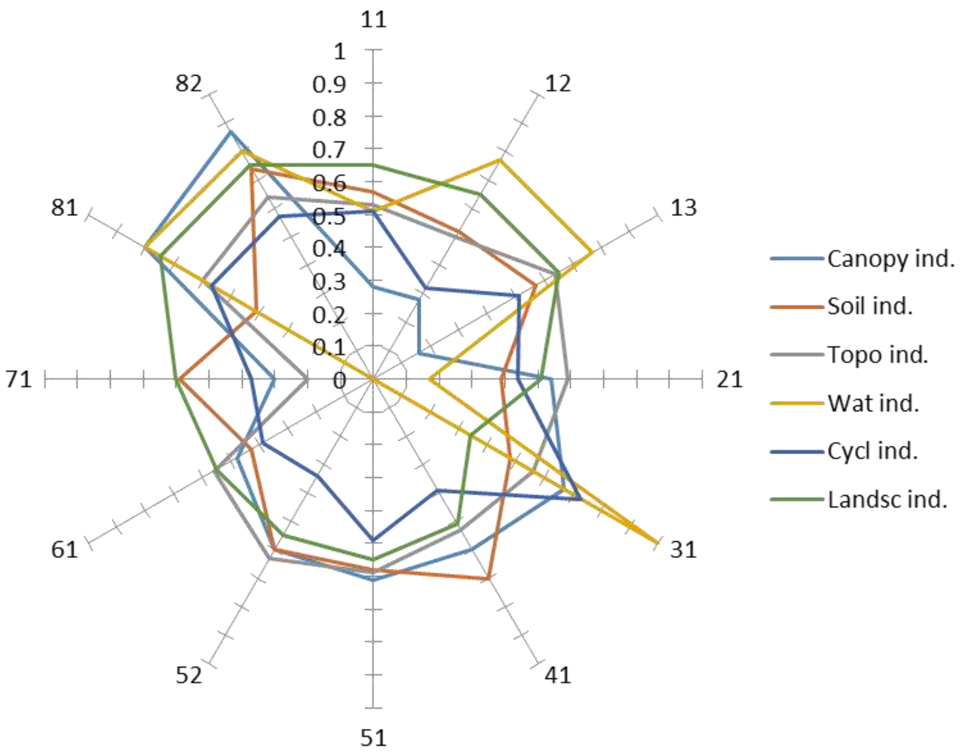

***Figure 12.** Favorable indicators for conservation at each site*

Concerning the canopy indicator, the lowest values were observed on Sites 11, 12, 13 and 71. With the exception of Site 71, which had great topographic limitations, the others had been pasture areas regenerating for the last 50 years. Sites 21 and 61 combined low values for the canopy, fertility and access to groundwater indicators. At Site 41, there is evidence that favorable fertility combined with unfavorable water conditions accounted for the moderate canopy indicator.

Site 82 coupled the best canopy indicator with good results for the other indicators, showing the positive effect of the set of indicators for conservation. Sites 51 and 52 had intermediate values for most indicators, including canopy.

The nutrient cycling indicator presented a tendency towards lower values in sites with higher slope parameters. Its best performance was at Site 31, which also performed well for the water proximity and canopy indicators.

We observe that the lowest value for landscape indicator was obtained at Patch 3, contributing to its small size and isolation in the landscape. Its high conservation indicator associated with canopy was probably due to the moisture caused by the 2-meter-deep groundwater and a likely recent isolation. This result shows that although this patch presented good conditions, it is at risk due to the edge effect, reduction of some fauna groups and barriers to gene flow.

Patch 1 had low conservation values for the proximity and percentage of border with natural areas parameters. However, the integrated result of the landscape indicator was satisfactory due to the positive influence of the area parameter.

Patch 8 had the best attributes on the site and landscape scales, showing favorable characteristics for conservation. In this case, the maintenance of these characteristics requires actions to prevent degradation by hunting, cattle invasion and fires.

The edaphic, water, topography and landscape qualities are not the only qualities affecting the structure and diversity of the patch, but also the age and history of human interventions such as logging and, especially, fire.

The age of the patches is unknown. Using aerial photographs from 1964 (photography n. 11591 / VM AST - 10 1370PMW R-82 of 09.02.1964) we observed that the remaining patches at that time were 2, 3, 4, 5, 7 and 8. Patch 6 was partially covered and Patch 1 was fully covered by pasture or other agricultural activity.

The farm has belonged to Embrapa Milho e Sorgo since 1976 and was formed by cotton test fields, corn fields for breeding and pastures at different times, as well as the acquisition of neighboring lands (Embrapa Milho e Sorgo, 2000). Therefore, we estimated that parts of Patches 1 and 6 are between 40 and 50 years old.

There are no historical records of anthropogenic interference in the natural environment. The reports are inaccurate, making it impossible to measure their influence on vegetation. All patches had traces of fire, some had small fragments of charcoal, bark or pieces of charred trunks in their first layers of soil, showing that fire has occurred at different times, but its intensity and scope could not be measured. The last fire was in September 2012 in Patches 1 and 2, causing burning of litter, the base of some trees, lianas and dead trees, reaching the treetops. That fire induced strong leaf fall because of stress. At the end of the rainy season, in March 2013, few traces of fire were visible.

Although the effects of human impacts and environmental conditions could not be isolated to know their influence on the current conservation status, the analysis showed some factors which contributed to the conservation of the patches. For example, favorable canopy parameters with unfavorable edaphic quality at Site 31 indicated the importance of considering a third factor, in this case, water proximity as a parameter related to groundwater supply.

The purpose of this study is not to clarify all the complex interactions between environmental components, which would require a long-term multidisciplinary survey and a control of all ecosystem dynamics phenomena. Our proposal is the relative evaluation of patch conservation by using groups of parameters, which indicate strengths and weaknesses for decision making regarding their conservation.

Conclusion

The methodology for the assessment of forest conservation in fragmented landscapes allowed gathering information on factors that express their weaknesses and strengths.

The results obtained from the application of the method did not express exactly the current conditions of the patches and sites studied in some cases. The limitations are inherent to natural conditions, lack of record of human activities in the area and restriction of sampling considering the diversity of the environmental conditions.

In the overall assessment, the applied method and the achieved results reached the proposed goal as its application allowed the identification of patches in better and worse condition, and provided information on the factors that contribute to this status. This knowledge can help guide and select the most appropriate measures to mitigate degradation and identify forest conservation strategies.

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REFERENCES

[1] A.P.G.[=Angiosperm Phylogeny Group] III (2009): “An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III”, Botanical Journal of the Linnean Society, vol. 161, pp. 105-121.

[2] Adam, G., Duncan, H. (2001): Development a sensitive and rapid method for the measurement of total microbial for the measurement of total microbial activity using fluorescein diacetate (FDA) in a range of soils. - Soil Biology and Biochemistry 33: 943-951.

[3] Alef, K., Keiner, D. (1986): Arginine ammonification,a simple method to estimate microbial activity potentials in soils. - Soil Biol. Biochem., 18(2):233-235.

[4] Altieri, M.A. (1999): The ecological role of biodiversity in agroecosystems. - Agriculture, Ecosystems and Environment 74: 19–31.

[5] Aquino, A. M., Assis, L. R. (2005): Processos biológicos no sistema solo-planta: ferramentas para uma agricultura sustentável. Embrapa Agrobiologia. - Brasília, DF: Embrapa Informação Tecnológica.

[6] Araujo, E.L., Ferraz, E.M.N. (2003): Processos ecológicos mantenedores da diversidade vegetal na caatinga: estado atual do conhecimento. – In: Claudino-Sales, V. (ed.). Ecossistemas brasileiros: manejo e conservação. Fortaleza, Expressão Gráfica e Editora, p. 115-128.

[7] Barichello, L. R.; Schumacher, M. V.; Vogel, H. L. M.; Dallago, J. S. (2000): Quantificação dos nutrientes no solo e serapilheira de diferentes estágios sucessionais em um sistema de agricultura migratória. - In: Reunião Sul Brasileira de Ciência do Solo, 3., 2000, Pelotas. Resumos expandidos... Pelotas, 1 CD-ROM.

[8] Brun, E. J., Schumacher, M. V., Vaccaro, S. (1999): Produção de serapilheira e devolução de nutrientes em três fases sucessionais de uma Floresta Estacional Decidual no município de Santa Tereza (RS). - In: Simpósio de fertilização e nutrição florestal, 1., 1999, Piracicaba. Anais... Piracicaba: ESALQ, p.348-364.

[9] Costa, T.C.C., Accioly, L.J.O., Oliveira, L.M.T., Oliveira, M.A.J., Guimarães, D.P. (2009b): Interação de fatores biofísicos e antrópicos com a diversidade florística na indicação de áreas para conservação do bioma Caatinga. - Sociedade & Natureza, Uberlândia 21 (1): 19-37.

[10] Costa, T.C.C., Oliveira, M.A.J., Accioly, L.J.O., Silva, F. H. B. B. da (2009a): Análise da degradação da caatinga no núcleo de desertificação do Seridó (RN/PB). - Rev. Bras. Eng. Agríc. Ambient. 13 (Suplemento):961–974.

[11] Costa, T.C.C., Viana, J.H.M., Ribeiro, J.L. (2014): Semideciduous Seasonal Forest Production of Leaves and Deciduousness in Function of the Water Balance, LAI, and NDVI. - International Journal of Ecology. V. 2014, 15 pages http://dx.doi.org/10.1155/2014/923027

[12] Cunha, G. C. (1997): Aspectos da ciclagem de nutrientes em diferentes fases sucessionais de uma floresta estacional do Rio Grande do Sul. (86 pp.) Dissertação (Mestrado em Engenharia Florestal). - Escola Superior de Agricultura “Luiz de Queiroz”, Piracicaba.

[13] Donagema, G. K. (2011): Manual de métodos de análise de solos / organizadores, Guilherme Kangussú Donagema. [et al.]. — Dados eletrônicos. — Rio de Janeiro: Embrapa Solos, 230 p. - (Documentos / Embrapa Solos, ISSN 1517-2627; 132)

[14] Eastman, J. R., Jin, W., Kyem, P. A. K., Toledano, J. (1995): Raster procedures for multi-criteria, multi-objetive decisions. - Photogrammetric Engineering and Remote Sensing, Bethesda 61(5): 539-547.

[15] Embrapa Milho e Sorgo (2000): Novas trilhas no sertão – história da pesquisa agropecuária em Sete Lagoas: das origens à Embrapa – Sete Lagoas,. 184 p. ISBN 858580-203-0.

[16] Feger, K. H., Raspe, S. (1998). Ökosystemforschung im chwarzwald: Auswirkungen von atmogenen einträgen und Restabilisierungsmassnahmen auf den Wasser- und Stoffhaushalt von Fichtenwäldern. - In: Raspe, Feger und Zotil (Hrsg). Verbundprojekt Arinus. Landsberg: Umweltforschung in Baden-Württenberg, p.1-18.

[17] Gomide, R. L., Albuquerque, P.E.P., Andrade, C.L.T., Durães, F.O.M., Viana, J.H.M. (2006): Caracterização climática e determinação da necessidade hídrica de culturas do sítio – específico de precisão de Sete Lagoas para fenotipagem de genótipos de cereais tolerantes a seca. Sete Lagoas, MG, 7p. Acesso em: 30 de out. 2014. Online. Disponível em: http://www.alice.cnptia.embrapa.br/handle/doc/490096.

[18] Hayashi, S.N. (2006): Dinâmica de serapilheira em uma cronossequência de florestas no Município de Capitão Poço – PA. Belém. Mestrado em Botânica Tropical. UFRAM/Museu Emílio Goeldi. 61p.

[19] IBGE. 2012. Manual técnico da vegetação brasileira: sistema fitogeográfico, inventário das formações florestais e campestres, técnicas e manejo de coleções botânicas, procedimentos para mapeamentos. 2ª Ed. IBGE-Instituto Brasileiro de Geografia e Estatística, Rio de Janeiro, 2012, 275p.

[20] IBGE. (1988): Mapa de vegetação do Brasil, SEPLAN/PR.

[21] Kandeler, E.; Gerber, H. (1988): Short term assay of soil urease activity using colorimetric determination ammonium. - Biol.Fertil. Soils 6:68-72.

[22] Kandziora, M.; Burkhard, B.; Muller, F. (2013): Interactions of ecosystem properties, ecosystem integrity and ecosystem service indicators: a theoretical matrix exercise. - Ecological Indicators 28: 54-78.

[23] Li-Cor. Lai-2200 (2011): Plant Canopy Analyzer. Instruction Manual. Li-Cor Biosciences, 2009. 3rd printing: May 2011.

[24] Machado, I.C.; Lopes, A.V. (2002): A polinização em ecossistemas de Pernambuco: uma revisão do estado atual do conhecimento. - In: M. Tabarelli; J.M.C. Silva (orgs.). Diagnóstico da Biodiversidade de Pernambuco. Recife: Secretaria de Ciência, Tecnologia e Meio-Ambiente, Fundação Joaquim Nabuco e Editora Massangana. p. 583-596.

[25] Manzoni, S.; Piñeiro, G.; Jackson, R.B.; Jobbágy, E.G.; Kim, J.H.; Porporato, A. (2012): Analytical models of soil and litter decomposition: Solutions for mass loss and time-dependent decay rates. - Soil Biology & Biochemistry 50: 66-76.

[26] Marriel, I.E.; Adelario, M.S.; Borges, A.L.; Oliveira, A.A.N.; Silva, U.C.; Guimarães, L.J.M. (2008): Variação da Atividade de Arginase e Urease na Rizosfera de Genótipos de Milho Contrastantes no Uso de Nitrogênio. - In: CONGRESSO NACIONAL DE MILHO E SORGO, 27.; SIMPOSIO BRASILEIRO SOBRE A LAGARTA-DO-CARTUCHO, SPODOPTERA FRUGIPERDA, 3.; WORKSHOP SOBRE MANEJO E ETIOLOGIA DA MANCHA BRANCA DO MILHO, Londrina. Agroenergia, produção de alimentos e mudanças climáticas: desafios para milho e sorgo: trabalhos e palestras. [Londrina]: IAPAR; [Sete Lagoas]: Embrapa Milho e Sorgo, No. 20814.

[27] Mcgarigal, K. Fragstats: user guideline, version 3. Disponível em: <http://www.umass.edu/landeco/research/fragstats/documents/User%20guidelines/User%20guidelines%20content.htm>. Accessed on: 09 Sept. 2011.

[28] Melo Filho, J. F.; Souza, A. L. V.; Souza, L. S. (2007): Determinação do índice de qualidade subsuperficial em um latossolo amarelo coeso dos tabuleiros costeiros, sob floresta natural. - R. Bras. Ci. Solo 31: 1599-1608.
[29] Moreira, F.M.S.; Siqueira, J.O. (2002). Microbiologia e bioquímica do solo. Lavras: UFLA, 625pp.
[30] Nascimento, H. E. M., Laurance, W. F. (2006): Efeitos de área e de borda sobre a estrutura florestal em fragmentos de floresta de terra-firme após 13-17 anos de isolamento. - Acta Amazônica 36(2): 183 - 192.
[31] Noss, R. F. (1999). Assessing and monitoring forest biodiversity: a suggested framework and indicators. - Forest Ecology and Management 15: 135-146.
[32] Oliveira, L. M. T., França, G. B., Nicácio, R. M., Antunes, M. A. H., Costa, T. C. C., Torres, A. R., França, J. R. A. (2010): A study of the El Niño-Southern Oscillation influence on vegetation indices in Brazil using time series analysis from 1995 to 1999. - International Journal of Remote Sensing 31 (2), DOI:10.1080/01431160902893477
[33] Paula, S. A.; Lemos Filho, J.P. (2001): Dinâmica do dossel em mata semidecídua no perímetro urbano de Belo Horizonte, MG. - Revista Brasileira de Botânica, São Paulo 24 (4) (suplemento): 545-551.
[34] Poggiani, F.; Schumacher, M.V. (2000): Ciclagem de nutrientes em florestas nativas. - In: Gonçalves, J.L.M.; Benedetti, V. (eds.) Nutrição e fertilização florestal. Piracicaba: Instituto de Pesquisas Florestais. p.287-308.
[35] Ribeiro, A.C.; Guimaraes, P.T.G.; Alvarez, V.H. (eds.) (1999): Recomendação para o uso de corretivos e fertilizantes em Minas Gerais: 5a. aproximação. - Comissão de Fertilidade do Solo do Estado de Minas Gerais, Viçosa:, 359p. (Titulo da capa: Recomendações para o uso...Biblioteca(s): Embrapa Amazônia Ocidental; Embrapa Arroz e Feijão; Embrapa Cerrados; Embrapa Hortaliças; Embrapa Milho e Sorgo; Embrapa Semiárido.)
[36] Oliveira, N.B.; McDougall, C.; Ritchie, B.; Hartanto, H.; Haggith, M.; Setyawati, T.(2000): Developing criteria and indicators of community managed forests as assessment and learning tools: objectives, methodologies and results. - CIFOR, 303p. https://books.google.com.br/books?id=rsyZM1To59YC.
[37] Sampaio, E.V.S.B. (2003): Caracterização da caatinga e fatores ambientais que afetam a ecologia das plantas lenhosas. p. 129-142. - In: V.C. Sales (ed.). Ecossistemas brasileiros: manejo e conservação. Fortaleza, Expressão Gráfica e Editora.
[38] SBCS (2004): Manual de adubação e de calagem para os Estados do Rio Grande do Sul e de Santa Catarina. Comissão de Química e Fertilidade do Solo. 10 ed. Porto Alegre, 400p.il.
[39] Sih, A., Jonsson, B. G., Luikart, G. (2000): Habitat loss: ecological, evolutionary and genetic consequences. - Trends in Ecology and Evolution 15:132-134.
[40] Silva, C.J., Lobo, F.A., Bleich, M.E., Sanches, L. (2009): Contribuição de folhas na formação da serrapilheira e no retorno de nutrientes em floresta de transição no norte de Mato Grosso. - Acta Amazonica 39 (3): 591-600.
[41] Silva, E.E., Azevedo, P. H. S., De-Polli, H. (2007): Determinação da respiração basal (RBS) e quociente metabólico do solo (qCO_2). - Comunicado Técnico 99, Seropédica/RJ.
[42] Silveira, A. P. D., Freitas, S. S. (2007): Microbiota do solo e qualidade ambiental. - Campinas: Instituto Agronômico, 312 p.
[43] Singh, R.K., Murty, H.R., Gupta, S.K., Dikshit, A.K. (2009): An overview of sustainability assessment methodologies. - Ecological Indicators 9: 189-212.
[44] Tabarelli, M., Lopes, A.V., Peres, C.A. (2008): Edge-effects drive Tropical Forest fragments towards an early-successional system. - Biotropica 40: 657–661.
[45] Tabatai, M.A., Bremmer, J.M. (1969): Use of pNitrophenyl phosphate for assay of soil phosphatase activity. - Soil Biology and Biochemistry 1: 301-307.
[46] Turner, M. G. (1989): Landscape ecology: The effect of pattern on process. - Annu. Rev. Ecol. Syst. 20:171 - 197.

[47] USDA (1999). Soil Survey Staff. Soil Taxononmy – a basic system of soil classification for making and interpreting soil survey. 2 ed., Washington, 871 p.

[48] USDA (2003). Base Indicators of Forest Sustainability: Metrics and Data Sources for State and Regional Monitoring. Forest Service Northeastern Area State and Private Forestry and Northeastern Forest Resource Planners Association In cooperation with: Northeastern Area Association of State Foresters, Washington, 70p.

[49] Valenti, M.W., Cianciaruso, M.V., Batalha, M.A. (2008): Seasonality of litterfall and leaf decomposition in a Cerrado site. - Brazilian Journal Biology 68 (3): 459-465.

[50] Werneck, M. S.; Pedralli, G.; Gieseke, L. F. (2001): Produção de serrapilheira em três trechos de uma floresta semidecidual com diferentes graus de perturbação na Estação Ecológica de Tripuí, Ouro Preto, MG. - Revista Brasileira de Botânica. 24:195-198.

[51] Zhang, W., Swinton, S.M. (2009): Incorporating natural enemies in an economic threshold for dynamically optimal pest management. - Ecological Modelling 220: 1315-1324.

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COMPARISON OF UNIVARIATE AND BIVARIATE APPROACHES TO MAP PRECIPITATION USING GEOSTATISTICS AND THE KALMAN FILTER

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Abstract. Measured rainfall data are very important in agriculture and environmental science. However, in many cases, the information gathered by existing rain gauges is insufficient for characterizing climatic variation within a study area. Thus, the use of interpolation techniques is necessary to predict values to unsampled sites. In this work, the performances of geostatistical algorithms, such as ordinary kriging and ordinary cokriging, and a proposed Kalman filter method were compared for mapping rainfall. The analysis was performed using both univariate and bivariate approaches. Natural terrain elevation was taken as the auxiliary variable for the bivariate case. The analysis was conducted for specific months of the dry and wet seasons in the Santiago River basin in Mexico. After comparison of the statistical errors, it was established that the geostatistical methods provided excellent results (especially cokriging) for the wet season months, with good correlation of 0.7 or above between rainfall and elevation, but not for the dry season months. Nevertheless, good results were achieved for the dry season months using the proposed Kalman filter methodology, due to the high normality and spatial dependence of the sample in this period.

Keywords: *climatic variation, correlation, auxiliary variable, cokriging, Kalman filter*

Introduction

Knowledge of spatiotemporal rainfall variation is essential in hydrology. In most basins in Mexico, the low density of weather stations that constitute the monitoring network is a hindrance to the realization of robust scientific investigation. Additionally, inconsistent field data and missing or short records are other common problems. The consequence of these shortcomings is the lack of sufficient data for the characterization of actual climatic variations, which limits the validity of the conclusions of climatological and hydrological studies (Costa and Soares, 2009).

Some studies require high-resolution data of the spatial variability of rainfall for accurate analysis of extreme hydrological events such as floods and droughts. To achieve this objective, a highly dense network of rain gauges would be required, which is the reason researchers adopt interpolation methods to obtain values at ungauged sites.

Geostatistics represents a set of widely used techniques based on the theory of regionalized variables. It capitalizes on the spatial correlation between neighboring observations to predict values at ungauged locations. Its application has become an increasingly important tool in climatic research, as demonstrated by several authors who have shown that such prediction methods provide better estimates than some conventional techniques (Awadallah, 2012; Béal et al., 2002; Soenario et al., 2010; Moral 2009; Shaghaghian and Abedini, 2012).

To obtain reasonable rainfall estimates, it is necessary to consider all the factors involved in conjunction with techniques that explicitly take into account the error associated with each measurement. One of these techniques is estimation theory, which represents a method for combining measurements and predicted values. The Kalman filter method is a special case of estimation theory that allows the calculation of the uncertainty of the independent variables involved.

The Kalman filter has been applied in many surface hydrology studies. For example, Morales et al. (2014) evaluated the applicability of the discrete Kalman filter algorithm for the prediction of short-term flows in reservoirs with very acceptable results. McMillan et al. (2013) used an ensemble recursive Kalman filter for information assimilation in an operational iterative runoff forecast system. This system improved the generated estimates considerably because of the enhancement of the initial conditions attributable to the Kalman filter. Komma et al. (2008) also used an ensemble Kalman filter for the prediction of real-time flows to update soil moisture data in a rainfall–runoff model, in addition to forecasting floods. Thus, the updating of states helped reduce the estimation errors substantially. Similar studies have been undertaken by Moradkhani et al. (2005), Srikanthan et al. (2015), and Weerts et al. (2006).

This study evaluated univariate and bivariate estimates of rainfall using the Kalman filter. These results were compared with estimates obtained using the geostatistical methods of ordinary kriging (OK) and ordinary cokriging (OCK). For the bivariate case, natural terrain elevation was adopted as the auxiliary variable.

Rain estimation

Different methods have been proposed for the interpolation of rainfall. The simplest method assigns an actual measurement to the nearest ungauged sites. Other methods commonly used include Thiessen polygons and the “inverse distance” method, which was developed by the US National Weather Service in 1972. In this method, ungauged rainfall is calculated based on the weighted average of the surrounding values. Another method, which uses isohyets, has the advantage of allowing the use of values at each measurement position, in conjunction with those factors that affect rainfall measurements, to generate lines of equal precipitation; nevertheless, a dense network of rain gauges is still required in order to obtain reasonable accuracy.

Geostatistics originated as a branch of statistics. Initially, it was used only in the analysis of spatial variability and modeling in earth sciences; however, its scope has been extended to include the analysis and prediction of any phenomena that vary in space and time. Specifically, geostatistics has become an important tool for the interpolation of weather data to unsampled sites (Waylen et al., 1995; Holawe and Dutter, 1999).

Geostatistical prediction methods such as kriging can provide better estimates than conventional techniques, although results have not been favorable for measurements with high dispersion. Dirks et al. (1998) showed that for stations covering areas >35 km^2, the simple kriging method showed no significant improvement over less complex techniques such as the “inverse distance” method. To address this problem, authors such as Hevesi et al. (1991), Borga and Vizzacaro (1997), Bostan and Akyürek (2009), Pardo (1998), Goovaerts (2000), Majani (2007), Murthy and Abbaiah (2007), Huang and Hu (2008), Moral (2009); Volkmann et al. (2010), and Diaz et al. (2002) have all used multivariate methods (e.g., cokriging and/or kriging) with external drift to fuse rainfall data with elevation data and/or radar data for estimations. Such auxiliary information is

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readily available and inexpensive and has dense distribution. In addition, these authors tested and compared these methods against univariate methods, and they agreed that multivariate geostatistical estimation methods provide reasonable results as long as the correlation between rainfall and the auxiliary variables is >0.70.

Static Kalman filter

The Kalman filter is a set of mathematical equations from which linear unbiased estimates can be obtained recursively, and the estimations are achieved with minimum variance for the state of a system using noisy data (Briseño et al., 2011). In particular, the static Kalman filter has been used to estimate pollutant concentrations and quality parameters in groundwater (Herrera, 1998; and Júnez, 2005) as well as to estimate the hydraulic head of an aquifer (Briseño et al., 2011; Júnez and Herrera, 2013). In such applications, the Kalman filter uses an initial covariance matrix derived from transport model flows (Herrera, 1998) or by means of geostatistical analysis of the data (Júnez, 2005). The main objective of such work was the optimum design of monitoring networks, based on the reduction of estimation error variance.

Some authors have used the Kalman filter for the spatial estimation of rainfall. For example, Diaz et al. (2002) obtained the best rainfall estimate with cokriging using radar data as the auxiliary variable. They recommended the consideration of other secondary variables such as topography, and they also proposed to use the Kalman filter in conjunction with a geostatistical approach in their future work. Valdés et al. (2015) compared the results of an application of the Kalman filter technique against kriging in the estimation of rainfall, finding that the Kalman filter provided reasonable estimates in real time and that kriging was suitable for the spatial distribution of rain. Urquiola et al. (2015) also proposed the use of a dynamic time–space model with restricted parameterized covariance matrices to predict rainfall amounts. This was achieved using algorithms such as the Kalman filter and smooth Kalman filter to estimate the unknown states of the system.

Materials and methods

Santiago River watershed case study

The Santiago River basin is located in the central northwestern region of Mexico. It covers an area of 76,274 km^2 with a perimeter of 1923.5 km. The basin partially occupies seven Mexican states: northern parts of Jalisco, southern Zacatecas, Aguascalientes, eastern Nayarit, and small parts of Durango, San Luis Potosi, and Guanajuato (*Fig. 1*). The basin type is exoreic because it discharges into the Pacific Ocean near the town of San Blas, Nayarit. Its highest topographic point is 3130 mamsl. The Santiago River basin belongs to Hydrologic Region number VIII "Lerma-Santiago." This occupies a wide variety of climates and biomes and it is subdivided into the hydrological subregions of Rio Alto Santiago and Rio Bajo Santiago.

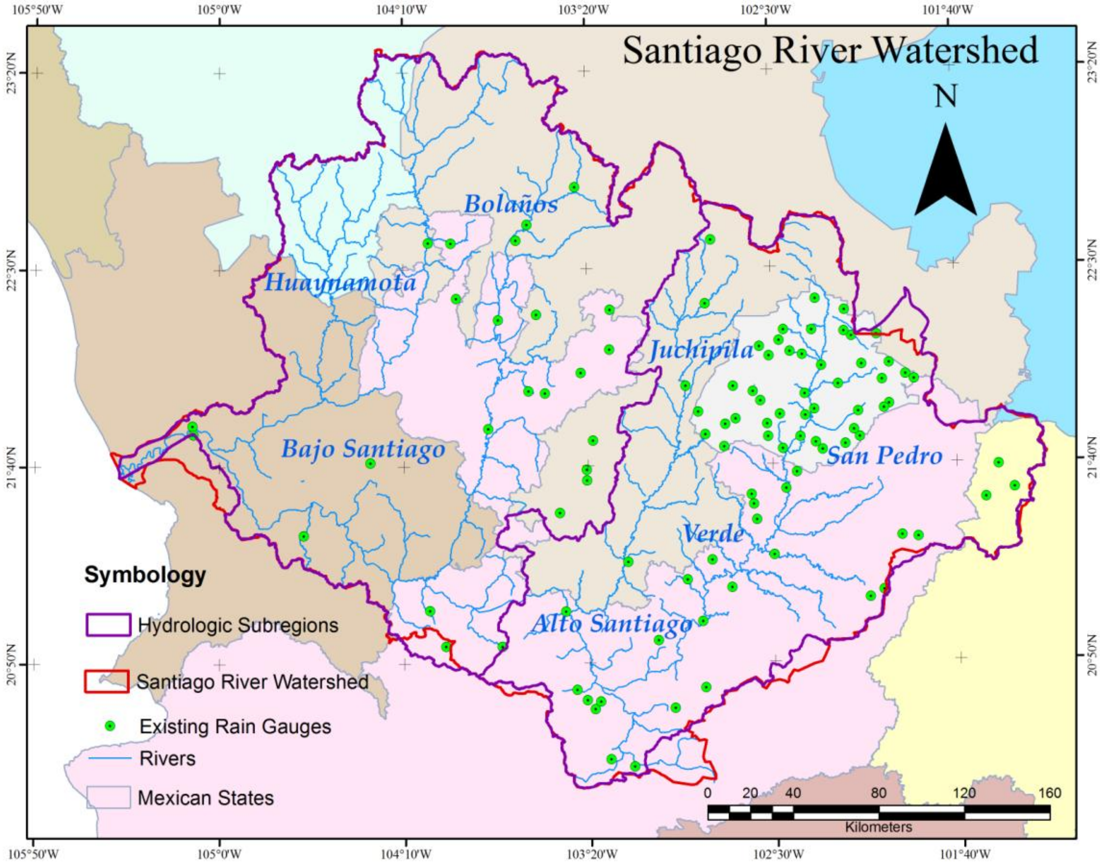

***Figure 1**. Santiago River basin and existing rain gauge network*

The Santiago River basin main collector starts from the controlled spills of the Poncitlán dam at the northeastern end of Lake Chapala. It transverses the states of Jalisco, Zacatecas, and Nayarit, to finally discharge into the Pacific Ocean after traveling 524 km. Its most important tributaries are the Verde, Juchipila, Bolaños, and Huaynamota rivers. There are several reservoirs within the basin, which are used primarily for irrigation and energy generation (Gomez et al., 2012).

According to the Mexican National Water Commission (CONAGUA, 2015a), the climate of the region is dry in northern parts of the basin, temperate humid in central regions, and warm and humid in the coastal region. The average annual temperature is 19 °C. The average rainfall is 822 mm/year, 80% of which is concentrated in the months of June–September. The average annual evaporation reaches 1831 mm/year.

Methodology

In the present work, the static Kalman filter was used for univariate and bivariate spatial estimations of rainfall for the Santiago River watershed. The required initial covariance matrix used by the filter was derived by geostatistical analysis. The resulting estimates are compared with univariate and bivariate estimates obtained using the OCK and OK geostatistical techniques, respectively. The methodology for the geostatistical analysis of each parameter followed three steps: exploratory data analysis, structural analysis, and predictions with respective validations.

Exploratory Data Analysis

The principal goal of the exploratory analysis is to characterize the sample of available data, while trying to retain as much information as possible. This analysis is based on conventional statistical techniques to obtain unknown data from a priori information of the sample. This is important in any statistical analysis but particularly in geostatistical analysis. It should also be guaranteed that the sample is not affected by outliers, both distributional and spatial. In other words, the sample should follow a normal function and be trend-free with a homogeneous spatial distribution.

Structural Analysis

The aim of structural analysis is to characterize the spatial structure of a regionalized variable. It is the process of estimating and modeling the function describing the spatial correlation of the involved variables, commonly called the semivariogram. Geostatistics reliability depends on the semivariogram (Subyani, 2015). It describes the spatial and/or temporal correlation of the studied variable through its components, which are the sill, range, and nugget.

Univariate geostatistics estimates a property using known values obtained from neighboring or nearby positions. The function describing the spatial continuity of the variable is the experimental variogram (Eq. 1), to which a theoretical variogram model is adjusted. There are various theoretical variogram models, the most common of which are the spherical, exponential, and Gaussian (Diaz, 2002).

$$\gamma(\mathbf{h}) = \frac{\mathbf{1}}{\mathbf{2N(h)}} \sum_{i=1}^{\mathbf{N(h)}} \{Z(x_i) - Z(x_i + h)\}^2 \qquad \text{(Eq.1)}$$

where $Z(x_i + h)$ and $Z(x_i)$ are the variable values at points $x_i + h$ and x_i, respectively, and N(h) is the number of pairs of data separated by distance h.

If two regionalized variables Z_{v1} (x) and Z_{v2} (x) are considered, the moment estimator function of cross semivariance is given by the cross variogram equation (Giraldo, 2001).

$$\gamma_{\upsilon 1 \upsilon 2}(\mathbf{h}) = \frac{\mathbf{1}}{\mathbf{2N(h)}} \sum^{\mathbf{N(h)}} \{\mathbf{Z}_{\upsilon 1}(\mathbf{x}+\mathbf{h}) - \mathbf{Z}_{\upsilon 1}(\mathbf{x})\}\{\mathbf{Z}_{\upsilon 2}(\mathbf{x}+\mathbf{h}) - \mathbf{Z}_{\upsilon 2}(\mathbf{x})\} \qquad \text{(Eq.2)}$$

The linear model of coregionalization assumes that all simple semivariograms (Eqs. 3 and 4) and cross variograms (Eq. 5) can be expressed as a linear combination of these theoretical models (Isaaks and Srivastava, 1989). For a case considering only two variables, the equations are:

$$\gamma_{v1}(\mathrm{h}) = \alpha_0 \gamma_0(\mathrm{h}) + \cdots + \alpha_m \gamma_m(\mathrm{h}) \qquad \text{(Eq.3)}$$

$$\gamma_{v2}(\mathrm{h}) = \beta_0 \gamma_0(\mathrm{h}) + \cdots + \beta_m \gamma_m(\mathrm{h}) \qquad \text{(Eq.4)}$$

$$\gamma_{v1v2}(\mathrm{h}) = \delta_0 \gamma_0(\mathrm{h}) + \cdots + \delta_m \gamma_m(\mathrm{h}) \qquad \text{(Eq.5)}$$

where γ_{v1} (h) and γ_{v2} (h) are the simple semivariograms, γ_{v1v2} (h) is the cross semivariogram, γ_0(h), γ_1(h), … , γ_m(h) are theoretical models of semivariance, and α_i, $\beta\, i$, and $\delta\, i$ with i = 1, 2, … , m, are constant values. This is expressed in matrix notation as follows:

$$\Gamma(h) = \begin{pmatrix} \gamma_{\upsilon 1}(h) & \gamma_{\upsilon 1 \upsilon 2}(h) \\ \gamma_{\upsilon 1 \upsilon 2}(h) & \gamma_{\upsilon 2}(h) \end{pmatrix} = \sum_{s=0}^{m} B_s \gamma_s(h) \quad \text{(Eq.6)}$$

where

$$B_s = \begin{pmatrix} \alpha_s & \delta_s \\ \delta_s & \beta_s \end{pmatrix} \quad \text{(Eq.7)}$$

$$\gamma_s(h) = \begin{pmatrix} \gamma_s(h) & 0 \\ 0 & \gamma_s(h) \end{pmatrix} \quad \text{(Eq.8)}$$

where $\Gamma(h)$ in Eq. (6) is called the coregionalization matrix.

As in the case of univariate geostatistics, the key is to have a tool that measures the spatial correlation of the variables involved. The spatial correlation of each of the involved variables is obtained through the covariance function or variogram. The joint spatial correlation, or the relationship, is obtained through the cross-covariance function generated from the measurements and from the marginal and cross variogram parameters of the linear model of coregionalization. The cross-covariance matrix of rainfall and topographic elevation data has the following form:

$$P_0 = \begin{bmatrix} \text{Rain} & \text{Rain} - \text{Elevation} \\ \text{Elevation} - \text{Rain} & \text{Elevation} \end{bmatrix} \quad \text{(Eq.9)}$$

Predictions

After the structural analysis has been performed, the prediction of values at the unsampled sites can be undertaken using the techniques for univariate and bivariate cases, respectively. In this work, the parameters of the variograms resulting from the geostatistical analysis were used to generate the a priori covariance matrices for the univariate and bivariate estimates using the Kalman filter.

Kalman filter estimations

The Kalman filter is a set of mathematical equations with which linear unbiased estimates can be obtained recursively. Minimum variance estimates are obtained for the state of a system using noisy data (Briseño et al., 2011). Furthermore, it can be used to update an estimate each time new data of the variable become available, without recourse to previous data. It is a recursive filter because the solution is recalculated when a new observation or measurement is incorporated into the system.

The general form of the Kalman filter is based on two equations: a dynamic equation and a measurement equation. The discrete Kalman filter aims to solve the general problem of estimating the state of a discrete time process, which is represented by a linear stochastic equation as follows:

$$X_{k+1} = AX_k + w_k \quad \text{(Eq.10)}$$

The measurement of $z_k \in R^m$ is related linearly to the system state as follows:

$$Z_k = HX_k + v_k \quad \text{(Eq.11)}$$

where X_{k+1} is the system state at time *k* + *1*, X_k is the state at time *k*, *A* is an n × n dimensional matrix that relates the system at time *k* with the state at time *k* + *1*. The *H* matrix of dimensions n × m relates the state at time *k* with the measurement Z_k, while w_k and v_k represent the process error and measurement error, respectively. These errors should be independent of each other and should show a normal probability distribution.

This study used the static Kalman filter used by Herrera (1998), which considers only the measurement equation and incorporates time using space–time vectors (Briseño, 2012).

The linear measurement equation of the discrete Kalman filter relates the state vector *h* of the variable in the positions and times of the desired estimate with the sampled data *z*:

$$z_j = H_j h + \upsilon_j \tag{Eq.12}$$

where z_j (*j* = 1, 2,…) is a measurement sequence of the variable of interest, H_j is a matrix of 1 × N dimensions that is not only zero at the position that corresponds to the input of *h* where sample *j* is taken, and N is the vector of dimension *h*. H_j is the sampling matrix of *j*, $h = \{h_{ip}\}$ is the space–time vector with the estimated values of the variable in the positions and times of interest (where h_{ip} is the variable value at position x_i at time t_p), and $\{\upsilon_j, j = 1, 2,\ldots\}$ is a scalar value that represents the measurement error. This is a white Gaussian sequence with zero mean and variance r_j. The measurement error sequence (υ_j) and vector *h* are independent.

The error covariance matrix for each variable involved in the estimation is:

$$P^n = E\{(h - \hat{h}^2)(h - \hat{h}^2)^T\} \tag{Eq.13}$$

where $\hat{h}^n = E\{(h/z_1, z_2,\ldots,z_n)^T\}$ is the expected value of *h* given the measurements z_1, $z_2,\ldots,z_n$, and *T* stands for transpose. In this notation, the superscript identifies the number *n* of measurements used for the estimate (Briseño et al., 2011).

For the application of the Kalman filter, it is required to define an estimation mesh. This mesh has two types of nodes: those that correspond to the positions of the sampling points (S) and those points where the estimates of the variable (*E*) are required. This estimation method requires a priori estimates of the variable in space ($\hat{h}_0$) and the covariance matrix of the estimation error (P_0). Given these a priori estimates, the linear minimum variance estimate of *h* can be obtained sequentially through the following formulas:

$$\hat{h}^{n+1} = \hat{h}^n + K_{n+1}\left(z_{n+1} - H_{n+1}\hat{h}^n\right) \tag{Eq.14}$$

$$P^{n+1} = P^n - K_{n+1}H_{n+1}P^n \tag{Eq.15}$$

$$K_{n+1} = P^n H_{n+1}^T\left(H_{n+1}P^n H_{n+1}^T - r_{n+1}\right)^{-1} \tag{Eq.16}$$

where superscripts *n* and *n+1* represent the current time and forward time respectively (for this work, these formulas were applied only spatially), $\hat{h}$ is the estimated state, *K* is the Kalman gain, *P* is the variance state matrix, *H* is the measurement matrix, z_n stands for measurements, and r_n is the variance.

Validation

In order to compare the estimates obtained by different methods with the real values of sampling data, the errors can be computed. Error e_i is calculated as the difference of data values of measurement *h* and those of the estimated *h* points of the mesh with each of the different discussed methods. The calculated errors are the mean error (ME, mm), mean squared error (MSE, mm^2), and standard mean square error (SMSE, dimensionless). The ME is an indicator of the overestimation or underestimation of the values of *h*. The MSE is a measure of the magnitude of the estimation error. Theory has established that a good fit exists between the measurements of *h* and the estimates of $\hat{h}$ by the different methods the closer the values of ME and MSE are to zero. Conversely, values of SMSE closer to 1 indicate greater agreement between the values of *h* and $\hat{h}$ estimates. The corresponding equations are as below:

$$ME = \frac{1}{n}\sum_{i=1}^{n} e_i \qquad \text{(Eq.17)}$$

$$MSE = \frac{1}{n}\sum_{i=1}^{n} (e_i)^2 \qquad \text{(Eq.18)}$$

$$SMSE = \frac{1}{n}\sum_{i=1}^{n} \frac{(e_i)^2}{\sigma_i^2} \qquad \text{(Eq.19)}$$

where σ_i^2 is the error estimation variance at position *i*.

Results and discussions

Rainfall data were obtained from CONAGUA (2015b), specifically from the project entitled “Program of preventive measures and drought mitigation.” In order to obtain consistent and coherent records, data from these stations were checked and only those that did not show inconsistencies or missing data were selected. This resulted into a monitoring network of 89 weather stations distributed throughout the basin (*Fig. 1*). Then, the exploratory analysis of rainfall records (units: mm) was undertaken with the purpose of describing the sample. *Table 1* shows the obtained values of the principal statistics for monthly and annual periods. The correlation values between these rain gauges records and natural terrain elevation (units: mamsl) are also shown. These were obtained by fitting a simple linear model to the rainfall data and digital elevation model values.

Table 1. *Descriptive statistics of rainfall data for 89 selected rain gauges*

Period	Mean	Min.	Max.	Med.	Kurtosis	Skewness	Variance	σ*	Cor.**
January	19.91	11.00	37.56	19.28	5.04	1.12	28.85	5.37	0.0064
February	11.05	2.30	17.48	10.96	3.81	-0.18	7.36	2.71	0.0003
March	2.79	0.75	8.50	2.76	8.02	1.39	1.37	1.17	0.0739
April	5.48	0.96	17.38	5.34	5.41	0.96	7.47	2.76	0.2134
May	18.69	5.92	29.17	18.74	3.36	-0.42	22.07	4.72	0.2491
June	105.1	48.72	208.33	92.35	3.11	1.00	1471.49	38.5	0.2796
July	167.1	55.78	368.96	149.77	4.53	1.16	3704.12	61.1	0.6750
August	145.2	76.69	468.15	133.61	13.58	2.71	4260.49	65.4	0.8321

September	106.0	52.47	380.78	94.19	17.75	3.37	2496.69	50.0	0.7839
October	40.22	24.05	149.96	36.29	23.88	4.14	309.92	17.6	0.7065
November	9.74	4.71	25.19	9.06	8.74	1.75	9.59	3.10	0.3052
December	10.21	4.76	21.78	9.59	4.70	1.37	12.91	3.60	0.1151
Monthly	53.46	28.79	129.84	48.72	7.61	1.81	325.04	18.0	0.7568
Annual	641.5	345.4	1558.09	584.70	7.61	1.81	46806.19	216.9	0.7568

*σ = Standard deviation of the data sample
**Cor = Linear correlation expressed as the Pearson correlation coefficient (R^2) between rainfall and elevation.

Given the statistics shown in *Table 1*, it is easy to describe the histogram frequency of the monthly rainfall data recorded at the 89 analyzed rain gauges. It can be seen that for dry months favorable values in terms of normality were obtained; however, there is a marked difference between the mean and median values for the wet months. *Table 1* also shows the correlation between rainfall and terrain elevation for the different months. There is poor correlation during the dry season in comparison with the wet season. This occurs because the values of measurements of rain in these two periods are small, and thus so is the variation. Generally, a good linear correlation between precipitation and natural terrain elevation exhibits a value of the Pearson correlation coefficient (R^2) close to or greater than 0.7. The highest correlations were obtained for the wet season (July–October). These correlations were negative, indicating that large values of precipitation occur at low natural terrain elevations (*Fig. 2*). Similarly, we obtained negative correlation between terrain and elevation for average monthly and annual rainfall values.

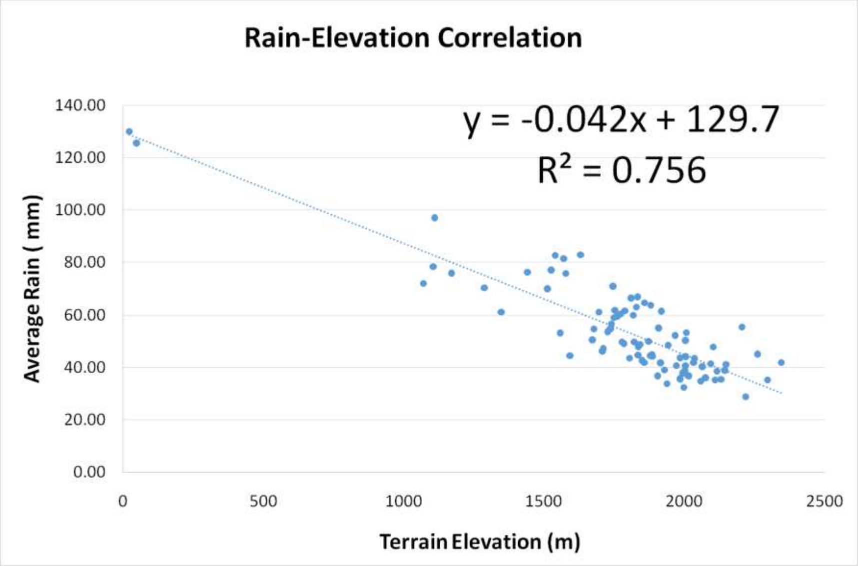

Figure 2. *Correlation of monthly precipitation and terrain elevation*

Geostatistical estimations

Two geostatistical interpolation methods were first evaluated for the monthly rainfall data: OCK and OK. These algorithms were used to obtain spatial estimates at unmonitored sites, and then the error statistics were evaluated. *Table 2* shows the adjusted marginal semivariograms and cross covariances for the OCK case, obtained in the structural analysis made for the different algorithms. This was performed using data from 45 of the 89 selected rain gauges. Using these data, rain estimates were obtained using the geostatistical algorithms, which were then evaluated and compared against the values of the remaining 44 rain gauges. The months of February and April were chosen for the dry season, and July and August selected for the wet season. For ease of comparison, maps of the estimates derived using OK and OCK are shown in *Figure 3a–d*, respectively.

Table 2. *Adjusted theoretical variograms for different months*

METHOD	MONTH	MODEL	NUGGET (m^2)	SILL (m^2)	RANGE (m)
Ordinary Kriging	February	Spherical	3.1495	9.065	201,470
	April	Spherical	0.63267	11.852	145450
	July	Spherical	140.63	14,020	448720
	August	Spherical	16.317	14,574	448720
Ordinary Cokriging	February	Spherical	3.0858	17.56	448720
		Spherical	31374	617220	448720
		CC: Spherical	-	-99.133	448720
	April	Spherical	1.5004	24.162	448720
		Spherical	31374	614220	448720
		CC: Spherical	-	372.28	448720
	July	Gaussian	140.62	14020.29	448718.013
		Gaussian	106889.18	-7682.76	448718.013
		CC: Gaussian	-	671729.35	448718.013
	August	Gaussian	16.317	14574.35	448718.013
		Gaussian	106889.18	-7864.511	448718.013
		CC: Gaussian	-	671729.35	448718.013

CC = Cross covariance

Different patterns were observed in the corresponding variograms for the months in each of the seasons (*Table 2*). Furthermore, the maps of monthly rain also illustrated some differences. In the dry season, which covers the months of February and April, small precipitation values were recorded in the mountainous areas in the northwestern part of the basin (*Fig. 3a* and *b*). However, during the months analyzed in the wet season, the maximum values were recorded in western coastal areas of the basin (*Fig. 3c* and *d*).

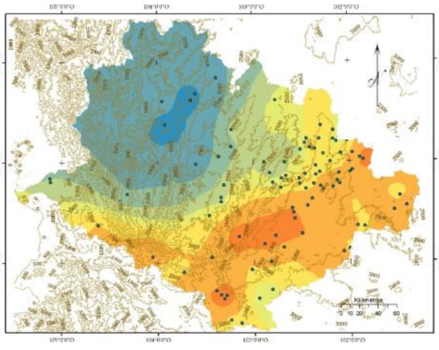

Figure 3a. *February rainfall estimate with OK*

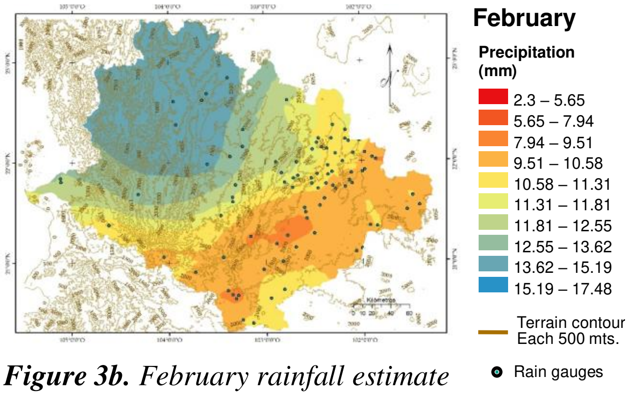

Figure 3b. *February rainfall estimate with OCK*

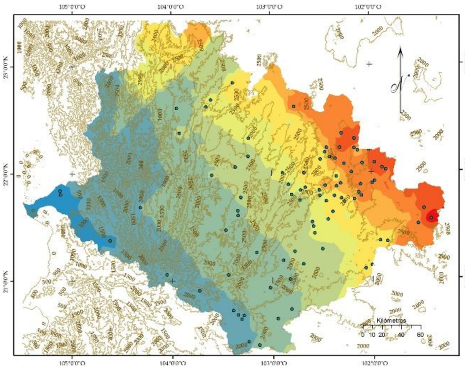

Figure 3c. *July rainfall estimate with OK*

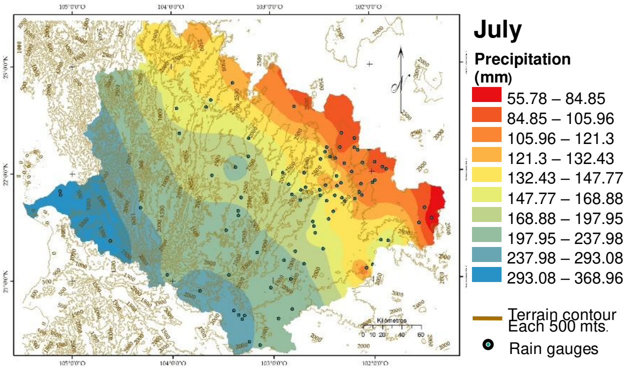

Figure 3d. *July rainfall estimate with OCK*

The results of the geostatistical methods are similar to those reported by other authors such as Goovaerts (2000), who showed that the contribution of secondary information in the OCK method depends not only on the correlation but also on its pattern of spatial continuity. When the nugget effect of primary information is high, the noise is higher, and therefore it contains less information while the secondary information gains more weight. The benefit of OCK over OK is increased when the spatial dependence between the observations of the primary variable is weak, as evidenced by the relatively high nugget effect in the variogram of rain that is most evident in the dry months (*Table 2*). Furthermore, the correlation is low with respect to the values of terrain elevation. The values of the obtained statistical errors (*Table 3*) show numerically better performance for OCK when compared with OK for humid months. This is because the rain–elevation correlation coefficient is favorable, and additionally, the spatial dependence of the primary variable is lower in the dry months.

Table 3. *Error statistics for OK and OCK*

Month	Statistic	Ordinary Kriging	Ordinary Cokriging
February	ME	0.385	0.443
	MSE	7.040	6.108
	RMSE	2.653	2.471
	SMSE	0.998	1.009
April	ME	-0.56	-0.526
	MSE	10.136	7.784
	RMSE	3.183	2.790
	SMSE	1.008	1.013
July	ME	0.757	0.027
	MSE	469.120	0.047
	RMSE	21.659	0.218
	SMSE	0.978	0.993
August	ME	1.927	0.022
	MSE	266.196	0.024
	RMSE	16.315	0.155
	SMSE	0.991	0.998

Static Kalman filter estimates

For the Kalman filter estimation method, a mesh consisting of 306 nodes spaced evenly over the entire basin was defined. This mesh, together with the 89 existing rain gauge positions, is shown in *Fig. 4*. Subsequently, the variograms for the univariate and bivariate cases were generated and the linear model of co-regionalization was computed for the bivariate case. Simple and cross-covariance matrices were generated using the variogram parameters, spatial coordinates of the sampling (S), and mesh of the estimation points (E). For the bivariate case, the data of the digital elevation model at the same positions of S and E were also used. This resulted in a square covariance matrix twice as large as in the univariate case.

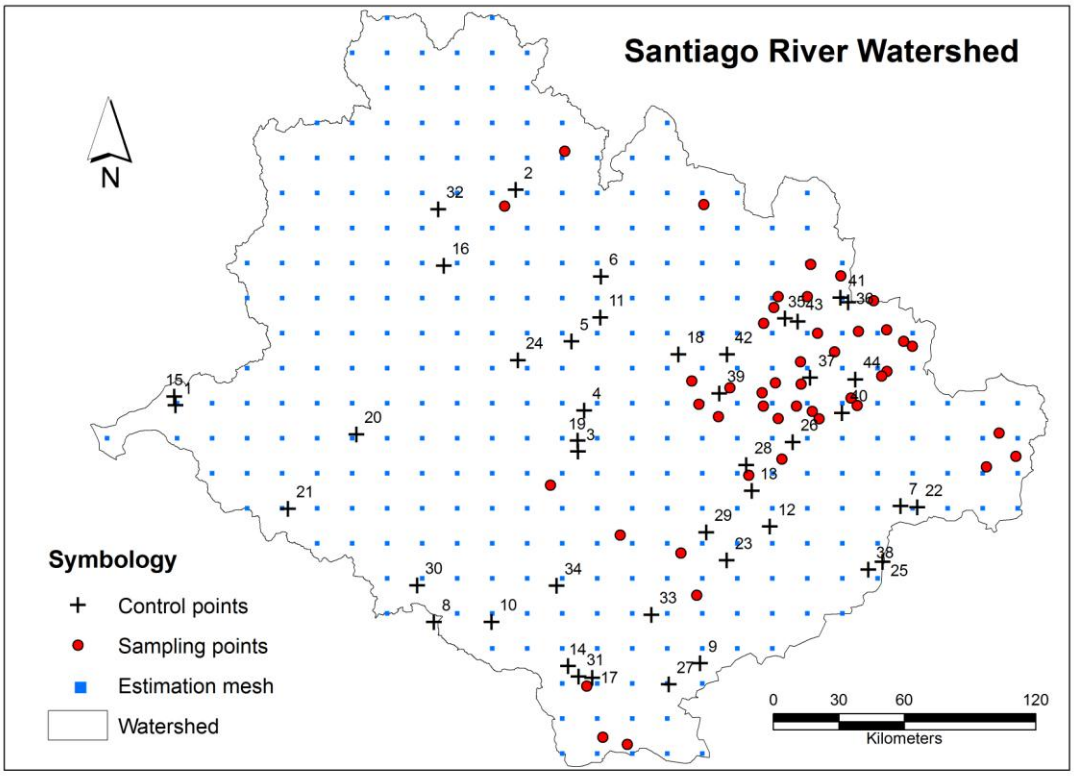

Figure 4. *Mesh of estimation points and locations of existing rain gauges*

The Kalman filter was applied as a programmed subroutine written in FORTRAN. This used as input data the covariance matrix (and for the bivariate case a cross-covariance matrix), average rain values at the sampled nodes, and UTM coordinates of the sampling and estimation points. As in the geostatistical case, to facilitate comparison, the months of February and April were chosen for the dry season and July and August were selected for the wet season. The results of the univariate and bivariate Kalman filter estimations were compared with the actual values recorded by 44 existing gauges which were left as control points and thus not considered in the estimation process (*Fig. 4*). The results are shown graphically in *Fig. 5a–d*.

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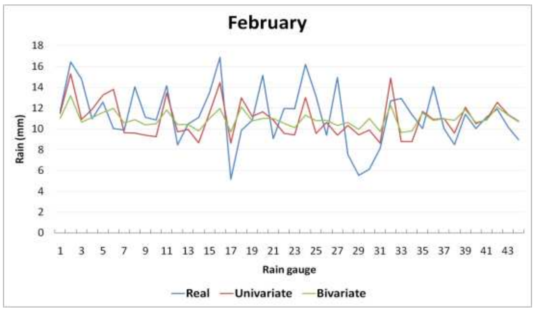

Figure 5(a). *February rainfall estimates*

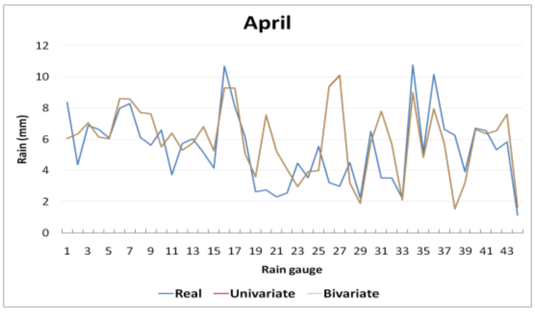

Figure 5(b). *April rainfall estimates*

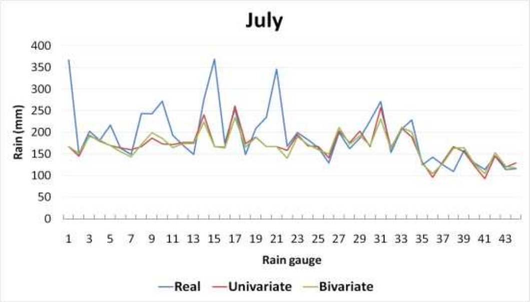

Figure 5(c). *July rainfall estimates*

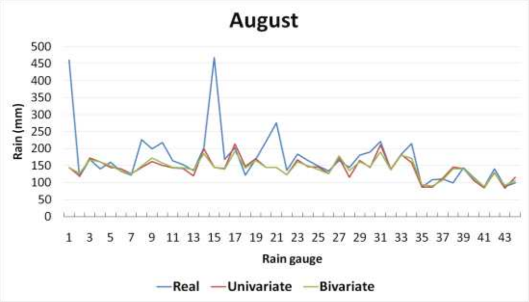

Figure 5(d). *August rainfall estimates*

Both the univariate and the bivariate methods show good performance for the dry months, especially February (*Fig. 5a*). April presents low rainfall values with greater variability, and in this case, the bivariate and univariate estimates appear the same (*Fig. 5b*). However, for the wet months of August and July, extreme values are observed, reflecting high spatial variability in these months (*Fig. 5c* and *d*). In this case, the Kalman filter was unable to model values for the raingauges 1, 8, 11, 15 and 21 which are located near the coast and in a low density sampling area (*Fig. 4*).

Table 4 shows the values of the error statistics for the estimates obtained using the Kalman filter. The estimates of the univariate and bivariate methods were compared with the actual values recorded by the 44 rain gauges not considered in the estimation process. The results show that the bivariate estimation method with the Kalman filter outperformed the univariate method in most cases. This is particularly noticeable when considering the MSE values.

Table 4. *Error statistics for univariate and bivariate Kalman estimates*

Month	Statistic	Univariate Kalman	Bivariate Kalman
February	ME	0.364	0.434
	MSE	7.063	5.634
	RMSE	2.657	2.373
	SMSE	0.995	1.011
April	ME	−0.539	−0.538
	MSE	5.079	5.035
	RMSE	2.254	2.244
	SMSE	1.036	1.037
July	ME	−6.078	2.056
	MSE	4338.875	3046.605
	RMSE	65.870	55.196
	SMSE	0.985	0.978

August	ME	2.180	4.792
	MSE	6165.38	4592.553
	RMSE	78.520	67.768
	SMSE	0.978	0.982

The comparison of the evaluated estimation methods revealed that estimates obtained using the Kalman filter were better for the dry season months of February and April. Conversely, the estimates obtained using the Kalman filter did not improve the performance of the geostatistical methods (OK and OCK) for the wet months (*Table 3*). This is because these months presented high variability.

Estimates made with the OCK method were significantly improved with respect to the OK method, as evidenced by the good correlation obtained between rainfall and terrain elevation in the humid months.

Generally, the Kalman filter estimation was affected by the chosen initial state vector. Thus, the estimates of the univariate and bivariate methods oscillated around the average value of the data, which was selected as the initial system state. Another factor affecting the accuracy of predictions especially for wet months (*Fig. 5c* and *d*), was the low density of sampling data for positions 1, 8, 11, 15 and 21 (*Fig. 4*). The highest errors produced for the lack of surrounding data points, affects the input covariance matrix values. This in turn increases the error statistics for the entire estimate (*Table 4*).

Conclusions

In this work, the performance of geostatistical and Kalman filter estimation methods for both univariate and bivariate cases were compared. The estimates were produced for rainfall data at ungauged sites.

The ME values for the Kalman methods indicate an accuracy advantage for the bivariate Kalman method over the univariate estimation method. This is less evident for the month of April, where a high nugget effect in the cross variogram indicated a low spatial dependence. The dry season presented values of lower spatial dependence than the wet season. In the wet season case, the values of ME and MSE were more favorable for the geostatistical estimation methods.

The error was provided in the first instance by the initial covariance matrix. This was obtained from a geostatistical analysis that should be undertaken carefully for both algorithms because the estimates made using the Kalman filter exhibited no difference with respect to the geostatistical methods in terms of sensitivity to the a priori covariance matrix.

In some cases, when the spatial dependence between data is low, as reflected by a higher relative nugget effect at the variogram of the primary variable, it could be possible to obtain better results with the Kalman filter, as long as there is normality in the sample and the semivariogram adjustment is optimum.

For the estimates made using the geostatistical methods, the correlation between rainfall and terrain elevation, as well as the spatial dependence of the primary variable, played a decisive role. This was particularly true for the OCK estimates, which significantly improved the estimations made using OK for the rainy season.

REFERENCES

[1] Awadallah, A. G. (2012): Selecting Optimum Locations of Rainfall Stations Using Kriging and Entropy. - International Journal of Civil & Environmental Engineering 12: 36-41.

[2] Béal, D., Guillot, G., Courault, D., Bruchou, C. (2002): Interpolation of rainfall at small scale in a Mediterranean region: relation between rainfall and altitude within a simple space-time model. - Geostatistics for environmental applications 13: 379-389, Kluwer Academic Publishers.

[3] Borga, M., Vizzacaro, A. (1997): On interpolation of hydrologic variables: formal equivalence of multiquadratic surface fitting and kriging. - Journal of Hydrology 195 (1-4):160-171.

[4] Bostan, P.A., Akyürek, Z. (2009): Spatio-temporal analysis of precipitation and temperature distribution over Turkey. - The International Archives of the Photogrammetry, Remote Sensing and Spatial Information Sciences 38: 92-97.

[5] Briseño, J.V. (2012): Método para la calibración de los modelos estocásticos de flujo y transporte en aguas subterráneas para el diseño de redes de monitoreo. - Tesis Doctoral. Recuperado de: http://www.ciencianueva.unam.mx/repositorio/handle/123456789/50

[6] Briseño, J. V., Herrera, G. S., Júnez H. E. (2011): Método para el diseño óptimo de redes de monitoreo de los niveles del agua subterránea. - Tecnología y Ciencias del Agua 2(4): 77-96.

[7] CONAGUA (2015a): Programa de medidas preventivas y de mitigación de la sequía. Consejo de Cuenca Río Santiago. 1a Versión. Recuperado de: pronacose.gob.mx/pronacose14/contenido/documentos/Río%20Santiago.pdf

[8] CONAGUA (2015b): Formulación del Programa Hídrico por Organismo de Cuenca, Visión 2030, de la Región Hidrológico-Administrativa VIII, Lerma-Santiago-Pacifico. INTEGRACIÓN DEL PROGRAMA HÍDRICO REGIONAL. Recuperado de: http://www.conagua.gob.mx/OCLSP07/NotaP/FINALaaResumenEjecutivo.pdf

[9] Costa, A. C., Soares, A. (2009): Homogenization of Climate Data: Review and New Perspectives Using Geostatistics. - International Association for Mathematical Geosciences 41: 291–305

[10] Dirks, K. N., Hay, J. E., Stow, C. D., Harris, D. (1998): High resolution studies of rainfall on Norfolk Island: Part II: Interpolation of rainfall data, - J. Hydrol. 208(3–4): 187–193

[11] Díaz, M. A. (2002): Geoestadística Aplicada, Instituto de Geofísica, UNAM, Instituto de Geofísica y Astronomía, CITMA, Cuba.

[12] Díaz. M., Herrera, G., Valdés, A. (2002): Estimación De La Precipitación En El Valle De México Usando Datos De Pluviómetros Y Radar Meteorológico. III Reunión Nacional de Ciencias de la Tierra, Puerto Vallarta México, Recuperado de: http://mmc2.geofisica.unam.mx/cursos/geoest/Presentaciones/Ejemplo Precipitacion.pdf

[13] Giraldo, H. R. (2001): Propuesta de un indicador como variable auxiliar en el analisis cokriging. - Revista Colombiana de Estadistica. V., 24(1): 1-12

[14] Gómez-Balandra, M. A., Díaz-Pardo, E. , Gutiérrez-Hernández, A. (2012): Composición de la comunidad íctica de la Cuenca del Río Santiago durante su desarrollohidráulico. - Hidrobiológica 22(1): 62-78.

[15] Goovaerts, P. (2000): Geostatistical approaches for incorporating elevation into the spatial interpolation of rainfall. - Journal of Hydrology 228: 113–129

[16] McMillan, H. K., Hreinsson, E. O., Clark, M. P., Singh, S. K., Zammit, C., Uddstrom, M. J. (2013): Operational hydrological data assimilation with the recursive ensemble Kalman filter. - Hydrol. Earth Syst. Sci. 17: 21–38

[17] Herrera, G. (1998): Cost Effective Groundwater Quality Sampling Network Design, Ph. D. Dissertation, University of Vermont, Burlington, Vermont. Recuperado de: http://mmc2.igeofcu.unam.mx/cursos/gest/Articulos/Geostatistics/Cost Effective Groundwater Quality Sampling Network Design.pdf

[18] Hevesi, J. A., Istok, J. D., Flint, A. L. (1991): Precipitation estimation in mountainous using multivariate geostatistics. Part II: Isohyetal Maps. - J. Appl. Meteor., 31: 677–688.

[19] Holawe, F., Dutter, R. (1999): Geostatistical study of precipitation series in Austria: time and space. - Journal of Hydrology 219: 70–82

[20] Huang, B., Hu, T. (2008): Spatial interpolation of rainfall based on DEM. Advances in Water Resources and Hydraulic Engineering, - Proceedings of 16th IAHR-APD Congress and 3rd Symposium of IAHR-ISHS, 1: 77-81.

[21] Isaaks, E.H., Srivastava, R.M. (1989): An Introduction to Applied Geostatistics. - Oxford University Press, New York, p. 561

[22] Júnez, H. E. (2005): Diseño De Una Red De Monitoreo De La Calidad Del Agua Para El Acuífero Irapuato-Valle, Guanajuato. - Tesis De Grado De Maestría. Universidad Nacional Autónoma De México. Facultad De Ingeniería. DEPFI (Campus Morelos):

[23] Júnez, H.E., Herrera, G.S. (2013): A geostatistical methodology for the optimal design of space–time hydraulic head monitoring networks and its application to the Valle de Querétaro aquifer. - Environmental Monitoring and Assessment 185(4): 3527-3549.

[24] Komma, J., Bloschl, G., Reszler, C. (2008): Soil moisture updating by Ensemble Kalman Filtering in real-time flood forecasting. - Journal of Hydrology 357: 228– 242

[25] Majani, B.S. (2007): Analysis of External Drift Kriging Algorithm with application to precipitation estimation in complex orography. - International Institute For Geo-Information Science And Earth Observation Enschede, The Netherlands. Thesis. Recuperado de: http://www.itc.nl

[26] Moradkhani, H., S., Gupta, H., Houser, P. (2005): Dual State-Parameter Estimation of Hydrological Models using Ensemble Kalman Filter. - Advances in Water Resources 28(2): 135-147.

[27] Moral, F. J. (2009): Comparison of different geostatistical approaches to map climate variables: application to precipitation. - International journal of climatology 30: 620–631.

[28] Morales, M.I., Aparicio, J., Valdés, J. B. (2014): Pronóstico de avenidas utilizando el filtro de Kalman discreto. - Tecnología y Ciencias del Agua 5(2): 85-110

[29] Murthy, K., Abbaiah, G. (2007): Geostatistical analysis for estimation of mean rainfalls in Andhra Pradesh, India. - International Journal of Geology 3: 35-55.

[30] Pardo, E. (1998): Comparison Of Geostatistical Methods For Estimating The Areal Average Climatological Rainfall Mean Using Data On Precipitation And Topography. - International Journal of Climatology 18: 1031–1047

[31] Shaghaghian, M.R., Abedini, M. J. (2012): Rain gauge network design using coupled geostatistical and multivariate techniques. - Transactions A: Civil Engineering, Scientia Iranica A. 20 (2): 259–269

[32] Soenario, I., Plieger, M., Sluiter, R. (2010): Optimization of Rainfall Interpolation - Intern rapport. De Bilt Netherlands: Koninklijk Nederlands Meteorologisch Instituut (KNMI)

[33] Srikanthan, R., Amirthananthan, G., Kuczera, G. (2015): Real-time Flood Forecasting Using Ensemble Kalman Filter. Recuperado de: http://www.mssanz.org.au

[34] Subyani, A. M. (2015): On the Space-Time Variogram Models-An Application to Seasonal Precipitation. http://www.kgs.ku.edu/

[35] Urquiola, A., Infante, S., Cedeño, F. (2015): Modelo Dinámico Espacio Temporal para Predecir Niveles de Precipitaciones en Venezuela. - Departamento de Matemáticas, FaCyT, Universidad de Carabobo, Valencia, Venezuela. Recuperado de: http://www.researchgate.net/publication/236214317_Modelo_Dinmico_Espacio_Temporal_para_Predecir_Niveles_de_Precipitaciones_en_Venezuela

[36] Valdés, A., Herrera, G., Díaz, M. (2015): Avances En El Monitoreo De La Precipitación En La Cuenca De México Usando Radar Meteorológico Y Pluviómetros. Recuperado de: http://www.inecc.gob.mx/cuencas-eventos/985-cuencas-con-nal2

[37] Volkmann, T. H. M., Lyon, S. W., Gupta, H. V., Troch, P. A. (2010): Multicriteria design of rain gauge networks for flash flood prediction in semiarid catchments with complex terrain. - Water Resources Research 46: 1-16.
[38] Waylen, P. R., Quesada, M. E., Caviedes, C. N. (1995): Temporal and spatial variability of annual precipitation in Costa Rica and the southern oscillation. - International Journal of climatology 16: 173-193.
[39] Weerts, A. H., El Serafy, G. Y. H. (2006): Particle filtering and ensemble Kalman filtering for state updating with hydrological conceptual rainfall-runoff models. - Water Resour. Res., 42, W09403, doi:10.1029/2005WR004093

THE EFFECT OF REAPING TIMES ON VOLATILE COMPONENTS OF NATURAL *PHLOMIS* L. (LAMIACEAE) TAXA IN THE LAKES DISTRICT OF TURKEY

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Abstract. This study performed SPME analysis in the Lakes District of Turkey between 2012-2015 and found that *Phlomis armeniaca includes* 54 volatile components, *P. bourgaei* 62, *P. grandiflora* var. *grandiflora* 60, *P. leucophracta* 70, *P. lycia* 62, *P. nissolii*' 54, *P. pungens* var. *pungens* 74 and *P. samia* 49. The main components were (E)-2-hexenal (12.12%), β-caryophyllene (16.63%) and germacrene-D (27.22%) for *P. armeniaca;* α-cubebene (16.04%), β-caryophyllene (21.98%) and germakren-D (15.12%) for *P. bourgaei*; α-pinene (26.40%), α-cedrene (28.15%) and α-curcumene (13.92%) for *P. grandiflora* var. *grandiflora*; (E)-2-hexenal (8.74%), limonene (14.56%) and β-caryophyllene (22.45%) for *P. leucophracta*, limonene (17.68%), β-caryophyllene (23.66%) and germacrene-D (21.88%) for *P. lycia*; limonene (23.75%), β-caryophyllene (12.50%) and germacrene-D (20.73%) for *Phlomis nissolii*; (E)-2-hexenal (17.60%), vinly amyl carbinol (20.44%) and germacrene-D (9.84%) for *P. pungens* and α.-copaene (10.59%), β-caryophyllene (15.20%) and germacrene-D (23.44%) for *P. samia*.

Keywords: *Phlomis, volatile component, SPME analyses, β-caryophyllene, germakren-D, Turkey*

Introduction

The flora in Turkey has approximately 11.466 plant taxa. As a comparison, the European continent has approximately 12.000 (Guner et al., 2012). Turkey also has species variety that is the gene center of several plants and there are many endemic species in different geographical regions (Tan, 1992). In particular for the endemics, aromatic and medical values of hundreds of plant types grown in Turkey are naturally higher (Baydar, 2009). This is especially the case for volatile oil content in medicinal and aromatic plant groups which has a separate importance. Volatile oils (perfumes, etheric oils) and aromatic extracts are commonly used for perfume production, to enhance smell and taste, as food additives, in cleaning products, in cosmetic and drugs, and as sources of aroma chemicals or identical natural and semi-synthetic aroma chemicals for the synthesis of starting materials. Currently, there is increasing demand for volatile oils in aromatherapy applications (Weiss, 1997).

The Lamiaceae family has generally sweet smelling one or multi perennial plants that are rarely briers and with some trees. This cosmopolite family is represented by 200 genera and approximately 3000 species. *Lamiaceae* (labiatate) family members that are represented by 45 genera and 546 species in Turkey are important for the pharmacology and perfumery industry due to their volatile and aromatic oils. Etheric oil is an example that is used as a spice is also grown as a decorative plant. The *Phlomis* taxa which has the most species of genera of the Lamiaceae family, has over 100 species all around the world. The taxa of this genus are

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distributed in Asia, South Europe and North Europe (Matthiesen et al., 2011). In Turkey, it is represented by 39 taxon and 13 hybrids, for a total of 52 taxon (Guner et al., 2012).

The discovery of new usage areas for medical and aromatic plants, increasing demand for natural products, increases the demand for these plants each passing day. The medical plant market is estimated to currently reach approximately 60 billion dollars (Kumar, 2009). Moreover, there is growing public interest towards such plants and for use of aromatic and medical plants.

Phlomis taxa have an important place in the natural distribution of medical plants and one of the species that has the most types of the Lamiaceae family. It has about 100 species all around the world. The length of species can vary between 30 cm and 2 meters. The sides of leaves are jagged and opposing in alignment but are not in a bulk condition. Feathers covering the surface of plant are stellate. Flowers are purple, pink, white or yellow colored (Huber-Morath, 1982). Leaves and flowers are used to make products more appetizing, as an anti-allergic, as a diuretic, for diarrhea prevention, against stomach aches, to relieve pain, as an anti-diabetic, herbal tea and tonic. The plant is also colloquially known to be used for respiratory tract diseases and hemorrhoid problems (Harput et al., 2006).

Although it is used in many areas, there is little research about the *Phlomis* taxa of Turkey. Research about the volatile oil of leaves is also limited. For these reasons, this study aimed to research the effect of different picking periods in terms of volatile oil compounds and to determine suitable picking periods for *Phlomis armeniaca* Willd., *P. bourgaei* Boiss., *P. grandiflora* H.S. Thompson var. *grandiflora, P. leucophracta* P.H.Davis & Hub.-Mor., *P. lycia* D. Don., *P. nissolii* L., *P. pungens* Willd. and *P. samia* L.

Materials and Methods

Phlomis samples were collected from the Lakes District of Turkey (C2, C3, C4 squares) between the years 2012 and 2015 (*Figure 1*). Thirty-three samples for *Phlomis* species were collected from the Lakes District in three different annual time periods: pre-bloom period, bloom period, and post-emergence period *(Table 1).*

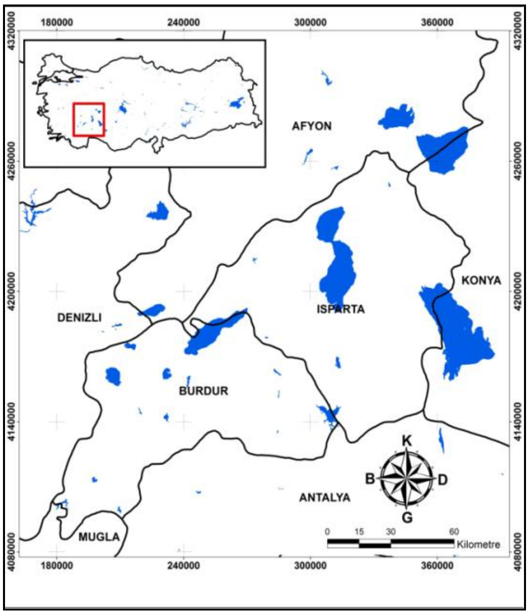

Figure 1. *Research area*

Table 1. *Ecological land informations of collecting samples*

Taxa	Province	Location	Altitude (m)
Phlomis armeniaca Willd.	Afyon-Çay county	37º 47' 08" N 30º 45' 54" E	830
	Konya-Beyşehir county, Kurucuova province	37º 50' 25" N 31º 06' 11" E	1155
	Isparta-Aksu county, Sorgun Yaylası province	37º 42' 11" N 31º 17' 38" E	1490
	Isparta- Davraz mountain, Ardıçdibi province	37º 48' 09" N 30º 45' 28" E	1604
	Isparta-Davraz mountain	37º 41' 55" N 31º 24' 18" E	1622
	Isparta-Yenişarbademli county, Melikler Yaylası province	38º 57' 02" N 31º 02' 19" E	1762
Phlomis bourgaei Boiss.	Isparta- Sığla (*Liquidambar orientalis*) Forest Nature Protected Area	37º 34' 15" N 30º 50' 06" E	379
	Isparta- Eğirdir county, Aşağıgökdere province	37º 37' 33" N 30º 43' 50" E	471
	Antalya-Isparta motoway, Dereboğazı province	37º 19' 48" N 30º 37' 22" E	590
	Isparta- Eğirdir county, Aşağıgökdere Akbelenli province	37º 33' 57" N 30º 51' 58" E	705
	Burdur- Karlık Kavaklık province	37º 44' 28" N 30º 33' 18" E	1228
	Isparta- Urban forest, Sidre province	37º 22' 31" N 30º 49' 18" E	1312
Phlomis grandiflora H. S. Thompson var. *grandiflora*	Antalya-Burdur motoway, Dağbeli province	37º 38' 44" N 30º 52' 09" E	855
	Antalya-Isparta motoway, Gökbel province	37º 38' 08" N 30º 42' 48" E	900
	Isparta- Kovada Lake National Park	36º 53' 03" N 29º 25' 38" E	920
	Burdur- Gölhisar county, Ballık province	37º 11' 11" N 30º 11' 02" E	1427
	Isparta-Sütçüler county, Tota mountain Böğülüyurt province	37º 35' 01" N 31º 04' 34" E	1580
Phlomis leucophracta P. H. Davis & Hub.-Mor.	Burdu- Bucak county, Kargı Taşdibi province	37º 34' 05" N 30º 50' 43" E	176

Taxa	Province	Location	Altitude (m)
	Isparta- Eğirdir county, Aşağıgökdere Akbelenli province	37º 38' 16" N 30º 43' 41" E	420
	Burdur- Ağlasun county, Çamlıdere village	37º 15' 30" N 30º 48' 39" E	690
Phlomis lycia D. Don	Burdur- Bucak county, Boğazköy province	37º 09' 15" N 30º 17' 35" E	783
	Burdur- Bucak county, Uğurlu province	37º 09' 22" N 30º 30' 05" E	823
	Burdur- Bucak county, Çubuk Beli province	37º 14' 23" N 30º 29' 50" E	908
Phlomis nissolii L.	Afyon- Çay county	37º 47' 08" N 30º 45' 54" E	830
	Isparta-Sav county, Yazısöğüt village	37º 46' 09" N 30º 37' 13" E	989
	Isparta-Şarkikaraağaç county, Belceğiz village	38º 00' 25" N 31º 17' 32" E	1203
	Isparta-Yalvaç county, İleği village	37º 41' 46" N 31º 22' 18" E	1224
	Isparta- Şarkikaraağaç county, Salur village	37º 58' 03" N 31º 17' 45" E	1236
	Isparta- Aksu county, Zindan cave province	38º 57' 02" N 31º 02' 19" E	1280
	Isparta- Urban forest, Sidre province	37º 44' 28" N 30º 33' 18" E	1316
Phlomis pungens Willd. var. *pungens* (Silvanok)	Isparta- Sorgun Yaylası province	37º 50' 12" N 31º 06' 15" E	1488
	Burdur- Gölhisar county	37º 44' 28" N 29º 24' 24" E	1649
Phlomis samia L.	Isparta- Kovada Lake National Park	37º 38' 53" N 30º 52' 08" E	942
	Isparta-Yenişarbademli county Pınargözü province	37º 42' 06" N 31º 18' 36" E	1529

Preparation of plant samples for GCMS analyses

Samples belonging to three different vegetation periods, pre-bloom, bloom, and post-emergence were taken from the determined areas. Collected leaves and flower samples were transported to the laboratory after placement in paper packages and without delay or exposure to sunlight. All materials were dried.

Determination of volatile compounds

Volatile components of *Phlomis* leaves and flowers were determined using a solid phase microextarction method (SPME) (Vichy et al., 2003). For this aim, after drying the plant materials at room temperature (25 °C), two grams from each sample were bottled and heated 15 minutes in 60 °C. A proper edge injector sank to the bottom and was absorbed for 30 minutes. Compounds held to the fiber edge were injected to a GC injection block and given five minutes for desorbing. The model for the used SPME Fiber was 75UMCAR/PDMSFUSED-SILICA, Supelco, USA, PA.

Gas chromatography-mass spectrometry equipment (GC-MS, Shimadzu QP 5050, Japan) was used in order to determine volatile compounds. HP-5 MS (30 m x 0.25 mm length and 0.25 um film was used in device and helium was used as the column and carrier gas (10 psi flow rate). The temperature of the injection block was 240 °C and detector temperature was 250 °C.

Method for statistical data

Non-parametrical tests were used, as the ratios determined for each volatile oil compound did not meet preconditions for parametric tests. The Kruskal-Wallis test, which is a non-parametrical test, was used in determining inter-species differences. The Friedman test, a non-parametrical test, was used for determining differences in different vegetation periods. The Bonferroni-Dunn method, which is a multi-compare method, was used for determining differences between the media.

Results

Leaf and flower volatile compounds of *Phlomis armeniaca*, *P. bourgaei*, *P. grandiflora* var. *grandiflora, P. leucophracta*, *Phlomis lycia*, *P. nissolii*, *P. pungens* var. *pungens*, *P. samia* were determined by SPME analysis (solid-based micro extraction method).

SPME analysis found 54 volatile components of *Phlomis armeniaca*, 62 of *Phlomis bourgaei*, 60 of *Phlomis grandiflora* var. *grandiflora*, 70 of *Phlomis leucophracta*, 62 of *Phlomis lycia*, 53 of *Phlomis nissolii*, 70 of *Phlomis pungens* var. *pungens* and 64 of *Phlomis samia*. These results were given in *Table 2*.

(E)-2-hexenal, β-caryophyllene and germakren-D were volatile components of *Phlomis armeniaca.* Volatile components *d*uring the pre-bloom period were: (E)-2-hexenal (11.64%), β-caryophyllene (15.73%) and germakren D (23.45%); during the bloom period (E)-2-hexenal (12.12%), β-caryophyllene (16.63%) and germakren-D (27.22%) and during the post-emergence period were: (E)-2-hexenal (10.07%), β-caryophyllene (11.55%) and germakren-D (25.03%).

α-cubebene, β-caryophyllene and germakren-D were volatile components of *P. bourgaei*. Specifically: α-cubebene (15.55%), β-caryophyllene (20.80%) and germakren-D (12.41%) ratios in the pre-bloom period, α-cubebene (16.04%), β-caryophyllene (21.98%) and germakren-D (15.12%) in the bloom period; and α-cubebene (13.92%), β-caryophyllene (14.73%) and germakren-D (11.21%) in the post emergence period.

Table 2. *Volatile components of leaf and flower according to different vegetation periods*

	Volatile components																							
	P. armeniaca			***P. bourgaei***			***P. grandiflora***			***P. leucophracta***			***P. lycia***			***P. nissolii***			***P. pungens* var. *pungens***			***P. samia***		
Components	**P.B..**	**B.P.**	**P.E.**	**P.B..**	**B.P.**	**P.E.**	**P.B..**	**B.P.**	**P.E.**	**P.B..**	**B.P.**	**P.E.**	**P.B..**	**B.P.**	**P.E.**	**P.B..**	**B.P.**	**P.E.**	**P.B..**	**B.P.**	**P.E.**	**P.B..**	**B.P.**	**P.E.**
Dimethyl sulfide	0.26	1.23	1.06	0.3	0.28	-	0.3	0.13	0.1	-	-	0.26	-	-	-	-	-	1.89	0.82	0.18	-	-	-	-
2-methyl-Propanal	-	0.86	0.66	-	-	-	-	0.26	-	-	-	0.14	-	-	-	-	-	0.26	0.95	-	-	-	-	-
Isobuttyl alcohol	0.3	-	-	0.59	0.43	-	0.6	-	-	-	-	-	-	-	-	-	-	-	-	0.17	-	-	-	-
2-Butenal	-	-	-	-	-	-	-	-	-	1.09	1	0.67	0.47	0.24	0.58	0.14	0.6	-	-	1.17	0.64	0.68	-	-
3-Methylbutanal	0.28	0.97	1.31	1.18	0.61	0.18	0.13	0.26	0.75	0.38	0.39	1.67	0.03	0.06	0.08	0.23	0.16	0.41	1.29	4.41	0.55	0.45	0.68	0.88
2-Methylbutanal	0.33	0.55	1.07	1.11	0.47	-	0.17	0.24	0.65	0.52	0.61	1.12	0.06	0.1	0.09	-	-	-	1.08	4.33	0.25	0.86	-	-
Methyl propyl ketone	-	1	0.55	-	-	-	-	-	-	-	-	-	-	-	-	1.06	-	-	-	-	-	-	-	-
n-Pentenal	-	-	-	-	-	-	-	-	-	0.54	0.47	0.56	0.45	0.15	0.33	-	-	-	-	1.16	0.32	0.7	-	-
Ethyl Vinly Carbinol	-	-	-	0.19	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.93	-	-	-	-
Ethyl Propyl Keton	-	-	-	-	-	0.13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Furan, 2-ethyl-	0.39	0.75	3.11	-	0.89	0.32	0.51	0.36	0.12	0.35	0.3	2.09	-	0.23	0.32	0.88	0.39	0.59	1.42	1.1	1.11	0.95	-	-
Ethyl vinly ketone	-	-	-	-	-	-	0.11	-	-	0.34	0.12	0.66	-	-	-	-	-	-	-	0.52	-	0.62	-	-
trans-3-Penten-2-one	-	-	-	-	-	-	-	-	-	0.16	0.18	0.1	-	0.04	0.17	-	-	-	-	-	-	-	-	-
3-Methyl-1-butanol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.28	1.57	-	-	-	-
2-Methyl-l-butanol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.55	-	-	-	-
(E)-2-Pentenal	-	0.24	0.25	-	-	-	-	-	-	0.18	0.2	0.75	0.1	0.12	0.21	0.45	0.32	0.37	0.31	0.57	0.59	0.56	-	0.34
(Z)-2-Pentenol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.44	0.32	0.14	-	-
3-Methyl-2-butanol	-	-	-	-	-	-	-	-	-	-	-	0.37	-	-	-	-	-	-	-	-	-	-	0.47	0.48
l-Pentanol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.77	-	-	-	-
n-Hexanal	0.74	1.36	1.95	0.79	0.85	0	0.39	0.11	0.2	4.89	3.54	3.27	2.95	1.56	3.88	0.58	1.87	1.27	2.68	4.86	3.73	2.69	2.5	4.1
(E)-2-Hexenal	11.64	12.12	10.07	5.52	3.82	0.5	4.34	0.51	1.02	7.5	8.74	6.1	3.78	3.92	6.85	8.57	10.57	9.05	16.87	17.6	12.68	9.04	2.98	3.66
Z-3-hexenol	-	0.93	-	-	-	-	-	-	-	0.22	0.44	1.91	0.11	0.18	0.35	0.76	0.18	-	-	0.33	2.52	1.24	-	-
cis-3-Hexene-1-ol	0.8	-	1.9	0.66	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2.65	3.88	-	-	-	-
cis-Hex-2-en-1-ol	0.27	0.2	1.83	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.34	1.3	-	-	-	-

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2-Hexenol, (E)-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.83	-	-	-	-	0.44	-	-	-
Hexanol <n->	0.34	0.5	3	-	-	-	-	-	-	0.16	0.25	0.51	0.05	0.17	0.09	0.81	0.05	1.17	1.3	2.17	2.2	0.3	-	0.42
Amyl methyl ketone	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.46	0.36
n-Heptanal	-	-	-	0.29	0.29	0.29	-	-	-	0.92	0.85	2.3	0.15	0.15	0.24	-	-	-	-	0.23	0.4	1.02	-	9.75
2,4-Hexadienal	-	-	-	-	-	-	-	-	-	0.42	0.56	0.2	0.26	0.1	0.43	-	-	-	-	0.54	-	0.19	-	-
Sorbaldehyde, (E,E)	-	-	-	-	-	-	-	-	-	-	-	2.15	-	-	-	-	-	-	0.68	-	-	-	-	2.61
α-Thujene	-	-	-	0.31	0.39	0.43	1.64	1.43	1.22	0.54	0.51	0.5	0.18	0.39	0.33	0.68	0.13	0.54	0.77	1.48	0.66	-	-	-
.α.-Pinene	0.74	0.93	1.44	0.11	2.13	2.8	25.97	26.4	18.95	2.85	2.72	1.92	1.26	2.24	2.03	6.86	6.93	6.43	4.91	4.07	3.85	0.26	2.2	1.1
(E)-2-Heptenal	-	-	-	-	-	-	-	-	-	1.33	1.26	1.25	0.67	0.35	0.79	-	-	-	-	-	0.23	0.39	-	-
Benzaldehyde	2.63	3.8	0.39	0.49	0.86	-	0.88	0.3	0.11	2.5	1.98	2.77	0.8	1.99	1.9	0.58	0.24	4.03	5.42	2.88	3.12	5.36	2.51	2.08
Methyl 2-hexenoate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.33	-	-	-	-	-	-
2-Hexenoic acid, methyl ester	-	1.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sabinene	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.4	0.23	-	-	-
.β.-Phellandrene	-	-	-	-	-	-	0.44	0.41	0.39	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
β-Pinene	-	-	-	-	-	-	0.79	0.13	0.66	0.15	0.22	0.1	-	-	-	-	-	-	-	0.16	0.33	-	-	-
Amyl vinyl ketone	-	-	-	-	-	-	-	-	-	0.24	0.23	0.39	0.15	0.05	0.17	-	-	-	-	-	-	-	-	-
Vinly amly carbinol	2.13	5.45	5.55	3.02	0.25	0.94	0.18	0.09	0.15	1.06	0.83	0.7	1.38	0.75	1.52	2.71	1.44	3.83	12.85	18.44	18.6	0.69	0.55	0.73
4-Pentene-l-yl acetate	-	-	0.38	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6-Methyl-5-hepten-2-one	-	-	-	0.21	0.45	0	0.38	0.31	0.17	0.42	0.44	1	0.2	0.24	0.35	-	-	-	-	-	-	0.42	0.44	0.52
Amyl ethyl ketone	0.3	0.78	1.75	-	-	0.66	-	-	-	-	-	-	-	-	-	1.96	-	0.82	1.79	2.37	-	-	-	-
β.-Myrcene	-	-	-	-	1.15	0.87	0.47	0.98	0.87	2.15	2.28	1.76	0.86	2.62	1.47	3.18	0.52	1.46	0.88	0.82	1.63	0.27	-	-
(R,S)-2-Propyl-5-Oxohexanal	-	-	-	-	-	-	-	-	-	-	-	0.57	-	-	-	-	-	-	-	-	-	-	-	1.26
Fenehone	0.32	-	-	0.16	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3-(2-methylpropyl) Ccyclohexene	-	0.6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4-Ethylcyclohexanol	-	-	1.54	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

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2-Octanone	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.85	-
n-Octanal	-	-	-	0.18	0.36	-	0.12	-	-	2.24	2.11	1.71	0.41	0.32	0.78	0.33	0.24	0.25	-	-	0.85	0.61	1.11	1.04
α-Phellandrene	-	-	-	-	-	0.21	0.14	-	-	0.44	0.64	0.77	0.06	0.58	0.18	0.66	0.23	-	-	-	0.18	-	-	-
2,4-Heptandienal	0.18	0.2	0.37	0.81	0.32	0	0	-	-	1.18	1.48	5.17	0.41	0.39	0.72	0.51	1.11	0.36	2.39	1.27	2.82	1.6	0	0.65
p-Dichlorobenzene	0.56	0.6	0.57	-	0.92	0.46	0.26	0.11	0.18	-	-	-	-	-	-	1.15	-	0.86	-	-	-	0.15	0.86	1.01
.α.-Terpinene	-	-	-	-	0.49	0.38	-	-	-	0.68	1	0.4	0.08	0.56	0.21	0.41	0.15	-	-	-	-	-	-	-
p-Cymene	-	-	-	-	0.31	0.3	0.42	0.34	0.4	1.03	1.29	1.05	0.33	0.55	0.6	1.88	0.32	1	0.3	0.53	1.51	-	-	-
Limonene	1.53	1.4	0.69	0.13	5.84	4.6	3.36	2.62	2.29	13.64	14.56	10.93	13.7	17.68	10.65	20.65	23.75	16.17	1.58	0.67	6.83	1.17	2.16	2.03
(E)-3-Octen-2-one	-	-	-	-	-	-	-	-	-	0.75	0.61	-	0.71	0.19	0.72	-	-	-	-	-	-	-	-	-
cis-Ocimene	-	-	-	-	-	-	1.47	0.82	2.03	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Benzeneacetaldehyde	0.58	0.69	-	0.41	0.6	-	0.26	-	-	-	-	-	-	-	-	-	-	0.32	0.78	0.46	-	-	-	-
Phenylacetaldehyde	-	-	-	-	-	-	-	-	-	0.63	0.71	-	0.21	0.1	0.63	-	-	-	-	-	0.51	0.76	-	-
β. Ocimene	-	-	-	-	-	0.1	0.18	0.15	0.14	0.24	0.39	-	0.05	0.39	0.11	0.42	0.04	-	-	-	-	-	-	-
2 Octenal	-	-	-	-	-	-	-	-	-	0.93	0.98	0.63	0.45	0.46	0.58	-	-	-	-	-	0.41	0.2	1.14	0.88
3,5-Octadien-2-one	-	0.21	-	-	-	-	-	-	-	0.36	0.34	-	0.26	0.1	0.48	-	-	-	-	-	0.49	0.55	-	-
γ-Terpinene	-	-	-	-	0.63	0.51	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Octanol	-	-	-	-	-	-	-	-	-	0.1	0.19	-	-	-	-	-	-	-	-	-	-	-	-	-
.α.-Terpinolene	-	-	-	-	-	-	-	-	-	1.55	1.29	0.5	0.14	0.89	0.36	1.1	0.42	0.32	-	-	-	-	-	-
Dimethylstyrene <α-para->	-	-	-	-	-	-	-	-	-	0.1	0.21	-	-	-	-	-	-	-	-	-	-	-	-	-
2-Nonanone	-	-	-	-	0.78	3.72	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.22	0.93	0.44
Methyl benzoate	-	-	-	-	-	-	-	-	-	0.26	0.27	-	0.16	0.07	0.28	-	-	-	-	-	-	-	-	-
Linalool	0.58	2.02	-	-	-	-	-	-	0.23	-	-	1.04	-	-	0.34	-	-	-	1.17	-	-	0.7	1.55	0.44
n-Nonanal	0.54	0.76	0.42	1.02	0.73	0.3	0.3	0.15	0.6	3.44	2.61	2.32	0.7	1.03	1.24	1.26	1.41	0.99	1	0.39	7.59	3.5	3.28	3.17
2-Hendecanone	-	-	-	0.19	3.27	29.87	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Phenethyl alcohol	0.54	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.52	0.32	-	0.25	-	-
.α.-Campholene aldehyde	-	-	-	-	-	-	-	0.12	0.09	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
trans-Alloocimene	-	-	-	-	-	-	-	0.08	0.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
D-Carvone	-	-	-	-	-	-	-	-	0.11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Trans-2-Nonenal	-	-	-	-	-	-	-	-	-	0.3	0.5	0.59	-	-	-	-	-	-	-	-	-	0.25	0.92	0.62

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Pinocarvone	-	-	-	-	-	-	-	0.09	0.09	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Methyl salicylate	1.17	2.15	0.34	-	-	-	-	-	-	-	-	0.6	-	-	-	-	-	1.29	1.36	-	-	-	-	-
(E)-2-Nonenal	-	-	-	-	-	-	-	-	-	-	-	0	0.23	0.17	0.23	-	-	-	-	-	-	-	-	-
n-Decanal	-	-	-	-	-	-	-	-	-	0.62	0.56	0.3	0.52	1.05	0.93	0.17	0.19	-	-	-	0.4	0.47	0.96	0.43
(E)-2-Decenal	-	-	-	-	-	-	-	-	-	0.24	0.46	0.15	0.09	-	0.11	-	-	-	-	-	-	-	-	-
Hendecanal	-	-	-	-	-	-	-	-	-	0.13	0.24	-	0.02	-	-	-	-	-	-	-	-	-	-	-
Nonyl methyl ketone	0.23	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2-Hendecanone	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.85	-
.α.-Cubebene	1.11	1.31	1.9	15.55	16.04	13.92	-	-	-	3.55	3.3	2.50-	1.86	0.29	2.75	0.27	0.45	0.27	0.38	0.41	0.3	0.42	1.29	0.92
Ylangene	0.18	-	-	-	0.22	0.23	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
.α.- Ylangene	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.59	-	-	-	-	0.75	-
.α.-Copaene	0.8	1.11	1.22	3.97	3.1	3.2	0.18	0.16	0.54	0.96	0.79	2.78	0.93	1.53	1.21	1.84	1.98	0.84	0.5	0.23	0.6	9.59	10.59	9.71
.β. Bourbonene	0.58	1.15	2.48	0.31	0.52	1.81	0.34	1.05	1.06	0.79	0.17	0.92	0.25	1.12	0.24	3.86	1.32	5.85	0.75	0.41	3.57	3.43	7.47	7.13
. β -Cubebene	-	0.22	-	6.55	4.59	-	-	0.11	-	0.89	0.2	-	1.07	0.6	1.26	0.52	0.99	0.88	-	-	0.54	0.7	-	-
(-)-.β.-Elemene	0.66	0.66	0.52	-	-	-	-	-	-	0.19	0.14	-	0.23	0.67	0.15	-	-	-	0.66	-	0.34	0.6	0.87	0.44
Sesquithujene <7-epi->	-	-	-	-	1.67	-	0.19	0.2	0.18	0	0	-	0.34	0	0.5	-	-	-	-	-	-	-	-	-
n-Tetradecane	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.36
α-Gurjunene	-	-	-	2.1	1.07	1.05	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.84	-	-
.α.-Cedrene	-	-	-	0.55	-	-	25.92	28.15	19.14	-	-	-	0.4	0.19	0.47	-	-	-	-	-	-	-	-	-
β-Caryophyllene	15.73	16.63	11.55	20.8	21.98	14.73	7.78	6.4	3.91	22.32	22.45	20.12	17.63	23.66	10.63	11.28	12.5	10.37	0.74	1.4	0.65	13.79	15.2	13.75
β-Cedrene	-	-	-	-	-	-	0.25	-	-	0.23	-	-	0.3	0.49	0.2	-	-	-	-	-	0.65	0.45	-	-
.γ.-Elemene	4.17	-	-	1.67	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4.37	2.36	2.2	-	-	-
.α.-Bergamotene	-	-	-	-	-	-	0.94	1.43	2.03	-	-	-	1.56	0.25	1.76	-	-	-	-	-	0.2	0.2	-	-
.α.-Cedrol	-	-	-	-	-	-	-	0.52	0.45	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
.δ.-Guaiene	-	-	-	0.61	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
.α.-Amorphene	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.47	-	-	-	-	0.85	-
Alloaromadendrene	-	-	-	4.63	-	-	0.17	-	-	1.17	0.56	0.77	-	0.43	0.41	1.64	0.29	-	-	-	0.29	-	-	-
(E)-β-Farnesene	14.87	0.21	6.87	-	-	-	1.8	-	1.28	3.57	2.96	2.4	4.76	3.71	10.23	3.86	4.05	5.86	11.32	0.43	1.94	-	-	-
Isoledene	-	-	-	-	0.22	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
.α.-Humulene	-	2.08	-	1.39	3.05	1.35	-	-	-	-	-	0.4	-	-	-	-	-	-	-	-	-	2.15	-	1.66

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Cyclosativene	-	-	-	5.23	-	-	0.64	-	0.68	-	-	-	-	-	-	2.66	-	-	-	-	-	-	-	-
.β.-Humulene	-	-	-	-	-	-	-	-	7.52	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Farnesol	-	-	-	-	-	-	-	1.19	1.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cadina-l(6),4-diene<10βH->	0.51	0.71	0.37	0.55	0.63	1.07	-	-	-	0.4	0.21	0.1	0.46	0.78	0.44	-	1.62	0.47	-	-	0.63	0.77	0.63	0.59
.β.-Selinene	-	-	-	-	-	0.15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Epi-Bicyclosesquiphellandrene	1.09	0.75	2.09	0.26	0.46	0.67	-	-	-	-	-	0.39	-	-	-	0.26	-	0.74	0.29	-	-	-	1.74	1.66
Germacrene-D	23.45	27.22	25.03	12.41	15.12	11.21	4	5.17	5.4	6.46	8.32	7.47	15.66	21.88	11.76	12.27	20.73	10.44	7.78	9.84	8.25	21.01	23.44	18.9
Ionone	-	-	-	-	-	-	0.16	-	1.28	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
α-Curcumene	-	-	-	-	-	-	11.96	13.92	13.24	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Bicyclogermacrene	0.63	1.34	2.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.14	1.22	-	0.6	-	-
Zingiberene	-	-	-	-	0.41	-	-	-	-	-	-	-	6.06	0.33	5.17	-	-	-	-	-	-	-	-	-
Valencene	-	-	-	1.59	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
α Bisabolol	-	-	-	-	-	0.81	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
α-Muurolene	0.53	0.61	0.8	0.3	0.37	0.37	-	-	-	-	-	-	-	-	-	0.19	0.91	0.27	-	-	-	0.59	1.5	0.58
β-Muurolene	-	-	-	-	-	-	-	-	-	-	-	-	0.25	0.54	0.2	-	-	-	-	-	-	-	-	-
Eremophilene	-	-	-	-	-	-	-	-	3.04	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cedr-8-e	-	-	-	-	-	-	0.26	2	3.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Caran-cis-3-ol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.61	-
β-Bisabolene	-	-	-	-	0.51	-	-	-	-	-	0.22	-	-	-	-	-	-	-	-	-	-	-	-	-
.α.-selinene	-	-	-	-	-	-	-	-	1.68	-	-	-	-	-	-	-	-	1.87	-	-	-	-	-	-
β- Bisabolene	-	-	-	-	-	-	-	-	-	-	-	-	4.96	-	4.33	-	-	-	-	-	-	-	0.66	-
.γ.-Cadinene	2.06	2	1.49	0.33	1.55	0.55	-	-	-	0.38	0.24	0.2	6.8	1.09	3.71	0.63	1.8	0.4	0.36	-	0.61	0.82	1.71	0.91
δ- Cadinene	1.39	1.44	2.46	1.31	1.07	1.27	-	0.08	0.1	0.71	0.82	1.18	0.83	1.35	0.62	0.64	1.26	0.61	0.82	-	1.02	6.6	5.29	3.55
β-Sesquiphellandrene	-	-	-	-	-	-	0.21	0.21	0.15	0.17	0.47	-	1.38	0.37	1.08	-	-	-	-	-	-	-	-	-
Germacrene B	4.48	1.24	-	2.26	-	-	-	-	-	-	-	-	-	-	-	0.55	0.22	4.69	4.11	-	1.28	-	-	-
(+)-spathulenol	0.17	-	-	-	-	-	-	-	0.37	-	-	-	-	-	-	-	-	0.39	-	-	-	-	-	-
Isolongifolene, 4,5,9,10-dehydro-	-	-	-	-	-	-	-	0.18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

(-)-Caryophyllene oxide	0.21	-	-	-	-	0.2	0.79	1.22	1.19	-	-	-	0.71	0.16	0.43	0.37	0.62	0.98	-	-	-	-	-	0.5
(2-Ethylhexyl)Ether	-	-	-	-	-	-	-	-	-	-	-	1.32	-	-	-	-	-	-	-	-	-	-	-	0.34
p-Menthane, 2,3-dibromo-8-phenyl-	-	-	-	-	-	-	0.29	1.21	0.7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
α-Elemol	-	-	-	-	-	-	0.21	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ar-tumerone	-	-	-	-	-	-	-	0.11	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Androstan-17-one. 3-ethyl-3-hydroxy-.(5.alpha.)-	-	-	-	-	-	-	-	0.29	0.22	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Nonadecane	-	-	-	-	-	-	-	-	-	0.44	0.59	-	1.12	0.38	1.12	-	-	-	-	-	0.71	0.18	-	-

Volatile components of *Phlomis grandiflora* var. *grandiflora* were: α-pinene (25.97%), α-cedrene (25.92%) and α-curcumene (11.96%) in the pre-bloom period; α-pinene (26.40%), α-cedrene (28.15%) and α-curcumene (13.92%) in the bloom period; and α-pinene (18.95%), α-cedrene (19.14%) and α-curcumene (13.24%) in the post-emergence period.

(E)-2-hexenal, limonene and β-caryophyllene were volatile components of *P. leucophracta.* Specifically in the pre-bloom period: (E)-2-hexenal (7.50%), limonene (13.64%) and β-caryophyllene (22.32%); in the bloom period (E)-2-hexenal (8.74%), limonene (14.56%) and β-caryophyllene (22.45%) and in the post emergence period (E)-2-hexenal (6.10%), limonene (10.93%) and β-caryophyllene (20.12%) were determined.

Limonene, β-caryophyllene and germacrene-D were volatile components of *Phlomis lycia*. Specifically limonene (13.70%), β-caryophyllene (17.63%) and germacrene-D (15.66%) in pre-bloom period; limonene (17.68%), β-caryophyllene (23.66%) and germacrene-D (21.88%) in the bloom period and also limonene (10.65%), β-caryophyllene (10.63%) and germacrene-D (11.76%) in the post-emergence period.

For *Phlomis nissolii* L., the volatile components were: limonene, β-caryophyllene and germacrene-D. Specifically, limonene (20.65%), β-caryophyllene (11.28%) and germacrene-D (12.27%) in the pre-bloom period; limonene (23.75%), β-caryophyllene (12.50%) and germacrene D (20.73%) in the bloom period; and limonene (16.17%), β-caryophyllene (10.37%) and germacrene D (10.44%) in the post-emergence period.

(E)-2-hexenal, vinly amyl carbinol and germacrene-D were volatile components of *Phlomis pungens* var. *pungens.* In the pre-bloom period, (E)-2-hexenal (16.87%), vinly amyl carbinol (12.85%) and germacrene-D (7.78%); in the bloom period (E)-2-hexenal (17.60%), vinly amyl carbinol (18.44%) and germacrene-D (9.84%) and in the post-emergence period (E)-2-hexenal (12.68%), vinly amyl carbinol (18.60%) and germacrene-D (8.25%).

The volatile components of *P. samia* were α.-copaene, β-caryophyllene and germacrene-D. Specifically α-copaene (9.59%), β-caryophyllene (13.79%) and germacrene-D (21.01%) in the pre bloom period; α-copaene (10.59%), β-caryophyllene (15.20%) and germacrene-D (23.44%) in the bloom period and α-copaene (9.71%), β-caryophyllene (13.75%) and germacrene-D (18.90%) in the post-emergence period.

The differences between the medians of *Phlomis* species were statistically important as found by the Kruskal-Wallis test in respect to volatile components per rates for (E)-2-hexenal, α-pinene, vinly amly carbinol, limonene, α-cubebene, α-copaene, α-cedrene, β-caryophyllene, germacrene-D and α-curcumene *(Table 3).*

While there was no statistically important difference between *P. pungens* and *P. armeniaca* in respect to the ordinal method for an (E)-2-hexenal compound per the multi-comparison Bonferroni-Dunn test that was applied to determine the differences between the medians, there was a statistically significant difference between *P. pungens* and *P. bourgaei*, *P. grandiflora*, *P. leucophracta*, *P. lycia*, *P. nissolii* and *P. samia.* There was no statistically significant difference between *P. bourgaei* and *P. grandiflora.* There was no statistically significant difference between these two species and *P. lycia* and *P. samia.* There was a statistically significant difference between *P. bourgaei* and *P. grandiflora* and *P. armeniaca*, *P. leucophracta*, *P. nissolii* and *P. pungens.*

There was a statistically significant difference between *P. grandiflora* and *P. armeniaca*, *P. bourgaei*, *P. leucophracta*, *P. lycia*, *P. pungens* and *P. samia,* however, there was no statistically significant difference for ratios of α.-pinene compounds according to the ordinal method between *P. grandiflora* and *P. nissolii.* While there was no statistically significant different between *P. armeniaca* and *P. samia* and *P. bourgaei* and *P. lycia*. There was a statistically significant difference between *P. armeniaca* and *P. samia* and *P. grandiflora*, *P. leucophracta*, *P. nissolii* and *P. pungens.*

Table 3. *Kruskal-Wallis analyses results acccording to Phlomis taxa*

Component	Taxa	N	N*	Mean	SEMean	StDev	Median	P	Rank
(E)-2-Hexenal	*P. armeniaca*	3	0	11.277	0.619	1.072	11.640	0.009	19.7ab
	P. bourgaei	3	0	3.28	1.47	2.55	3.82		6.0e
	P. grandiflora var. *grandiflora*	3	0	1.96	1.20	2.08	1.02		4.7e
	P. leucophracta	3	0	7.447	0.763	1.321	7.500		13.0cd
	P. lycia	3	0	4.85	1.00	1.73	3.92		8.7de
	P. nissolii	3	0	9.397	0.603	1.044	9.050		16.7bc
	P. pungens var. *pungens*	3	0	15.72	1.53	2.66	16.87		23.0a
	P. samia	3	0	5.23	1.92	3.32	3.66		8.3de
Component	**Taxa**	**N**	**N***	**Mean**	**SEMean**	**StDev**	**Median**	**P**	**Rank**
α.-Pinene	*P. armeniaca*	3	0	1.037	0.209	0.362	0.930	0.008	4.7e
	P. bourgaei	3	0	1.680	0.808	1.400	2.130		8.3de
	P. grandiflora var. *grandiflora*	3	0	23.77	2.41	4.18	25.97		23.0a
	P. leucophracta	3	0	2.497	0.291	0.504	2.720		12.0cd
	P. lycia	3	0	1.843	0.298	0.516	2.030		9.0de
	P. nissolii	3	0	6.740	0.156	0.271	6.860		20.0ab
	P. pungens var. *pungens*	3	0	4.277	0.323	0.559	4.070		17.0bc
	P. samia	3	0	1.187	0.562	0.973	1.100		6.0e
Component	**Taxa**	**N**	**N***	**Mean**	**SEMean**	**StDev**	**Median**	**P**	**Rank**
Vinly amly carbinol	*P. armeniaca*	3	0	4.38	1.12	1.95	5.45	0.006	19.0ab
	P. bourgaei	3	0	1.403	0.833	1.442	0.940		11.0de
	P. grandiflora var. *grandiflora*	3	0	0.1400	0.0265	0.0458	0.1500		2.0f
	P. leucophracta	3	0	0.863	0.105	0.182	0.830		9.7de
	P. lycia	3	0	1.217	0.237	0.410	1.380		12.3cd
	P. nissolii	3	0	2.660	0.690	1.196	2.710		16.7bc
	P. pungens var. *pungens*	3	0	16.63	1.89	3.27	18.44		23.0a
	P. samia	3	0	0.6567	0.0546	0.0945	0.6900		6.3ef
Component	**Taxa**	**N**	**N***	**Mean**	**SEMean**	**StDev**	**Median**	**P**	**Rank**
Limonene	*P. armeniaca*	3	0	1.207	0.261	0.452	1.400	0.011	4.7b
	P. bourgaei	3	0	3.52	1.73	3.00	4.60		9.3b
	P. grandiflora var. *grandiflora*	3	0	2.757	0.316	0.548	2.620		11.0b
	P. leucophracta	3	0	13.04	1.09	1.89	13.64		18.3a
	P. lycia	3	0	14.01	2.04	3.53	13.70		19.0a

	P. nissolii	3	0	20.19	2.20	3.81	20.65		22.7a
	P. pungens var. *pungens*	3	0	3.03	1.92	3.33	1.58		8.0b
	P. samia	3	0	1.787	0.311	0.538	2.030		7.0b
Component	**Taxa**	**N**	**N***	**Mean**	**SEMean**	**StDev**	**Median**	**P**	**Rank**
α.-Cubebene	*P. armeniaca*	3	0	1.440	0.237	0.411	1.310	0.008	15.0bc
	P. bourgaei	3	0	15.170	0.641	1.110	15.550		22.0a
	P. grandiflora var. *grandiflora*	3	0	0.000000	0.000000	0.000000	0.000000		2.0f
	P. leucophracta	3	0	3.425	0.125	0.177	3.425		19.5ab
	P. lycia	3	0	1.633	0.719	1.246	1.860		13.3cd
	P. nissolii	3	0	0.3300	0.0600	0.1039	0.2700		6.7ef
	P. pungens var. *pungens*	3	0	0.3633	0.0328	0.0569	0.3800		8.0de
	P. samia	3	0	0.877	0.252	0.437	0.920		12.0cde
Component	**Taxa**	**N**	**N***	**Mean**	**SEMean**	**StDev**	**Median**	**P**	**Rank**
α.-Copaene	*P. armeniaca*	3	0	1.043	0.126	0.218	1.110	0.006	11.3b
	P. bourgaei	3	0	3.423	0.275	0.476	3.200		20.0a
	P. grandiflora var. *grandiflora*	3	0	0.293	0.123	0.214	0.180		2.7c
	P. leucophracta	3	0	1.510	0.637	1.103	0.960		12.0b
	P. lycia	3	0	1.223	0.173	0.300	1.210		12.7b
	P. nissolii	3	0	1.553	0.359	0.622	1.840		14.0b
	P. pungens var. *pungens*	3	0	0.443	0.111	0.191	0.500		4.3c
	P. samia	3	0	9.963	0.315	0.546	9.710		23.0a
Component	**Taxa**	**N**	**N***	**Mean**	**SEMean**	**StDev**	**Median**	**P**	**Rank**
α.-Cedrene	*P. armeniaca*	3	0	0.000000	0.000000	0.000000	0.000000	0.006	9.0b
	P. bourgaei	3	0	0.183	0.183	0.318	0.000		13.0b
	P. grandiflora var. *grandiflora*	3	0	24.40	2.71	4.69	25.92		23.0a
	P. leucophracta	3	0	0.000000	0.000000	0.000000	0.000000		9.0b
	P. lycia	3	0	0.3533	0.0841	0.1457	0.4000		19.0a
	P. nissolii	3	0	0.000000	0.000000	0.000000	0.000000		9.0b
	P. pungens var. *pungens*	3	0	0.000000	0.000000	0.000000	0.000000		9.0b
	P. samia	3	0	0.000000	0.000000	0.000000	0.000000		9.0b
Component	**Taxa**	**N**	**N***	**Mean**	**SEMean**	**StDev**	**Median**	**P**	**Rank**
β-Caryophyllene	*P. armeniaca*	3	0	14.64	1.57	2.71	15.73	0.009	14.3bc
	P. bourgaei	3	0	19.17	2.25	3.89	20.80		18.3ab
	P. grandiflora var. *grandiflora*	3	0	6.03	1.13	1.96	6.40		5.0de
	P. leucophracta	3	0	21.630	0.756	1.309	22.320		21.3a
	P. lycia	3	0	17.31	3.76	6.52	17.63		16.7ab
	P. nissolii	3	0	11.383	0.617	1.069	11.280		9.0cd
	P. pungens var. *pungens*	3	0	0.930	0.236	0.410	0.740		2.0e

	P. samia	3	0	14.247	0.477	0.826	13.790		13.3bc
Component	**Taxa**	**N**	**N***	**Mean**	**SEMean**	**StDev**	**Median**	**P**	**Rank**
Germacrene-D	*P. armeniaca*	3	0	25.23	1.09	1.89	25.03	0.004	23.0a
	P. bourgaei	3	0	12.91	1.16	2.00	12.41		13.3c
	P. grandiflora var. *grandiflora*	3	0	4.857	0.433	0.751	5.170		2.0e
	P. leucophracta	3	0	7.417	0.538	0.931	7.470		5.7de
	P. lycia	3	0	16.43	2.95	5.10	15.66		16.0bc
	P. nissolii	3	0	14.48	3.17	5.49	12.27		13.7c
	P. pungens var. *pungens*	3	0	8.623	0.623	1.080	8.250		7.3d
	P. samia	3	0	21.12	1.31	2.27	21.01		19.0ab
Component	**Taxa**	**N**	**N***	**Mean**	**SEMean**	**StDev**	**Median**	**P**	**Rank**
α-Curcumene	*P. armeniaca*	3	0	0.000000	0.000000	0.000000	0.000000	0.002	11.0b
	P. bourgaei	3	0	0.000000	0.000000	0.000000	0.000000		11.0b
	P. grandiflora var. *grandiflora*	3	0	13.040	0.575	0.995	13.240		23.0a
	P. leucophracta	3	0	0.000000	0.000000	0.000000	0.000000		11.0b
	P. lycia	3	0	0.000000	0.000000	0.000000	0.000000		11.0b
	P. nissolii	3	0	0.000000	0.000000	0.000000	0.000000		11.0b
	P. pungens var. *pungens*	3	0	0.000000	0.000000	0.000000	0.000000		11.0b
	P. samia	3	0	0.000000	0.000000	0.000000	0.000000		11.0b

*a,b,c,d,e,f show differences between *Phlomis* taxa.

There was no statistically significant difference between *P. pungens* and *P.armeniaca* for vinly amly carnibol compound ratios, while there was a statistically significant difference between *P. pungens* and *P. bourgaei*, *P. grandiflora, P. leucophracta*, *P. nissolii* and *P. samia.* While there was no statistically significant different between *P. grandiflora* and *P. samia.* There was a statistically significant difference between *P. grandiflora* and *P.armeniaca*, *P. bourgaei*, *P. leucophracta*, *P. lycia*, *P. nissolii* and *P. pungens samia.*

There was no statistically significant difference between *P. leucophracta*, *P. nissolii* and *P. samia* for Limonene compound ratios according to the ordinal method, however there was a statistically significant difference between these three species and *P. armeniaca*, *P. bourgaei*, *P. grandiflora*, *P. lycia*, *P. nissolii* and *P. pungens.* Also there was no statistically significant difference between *P. armeniaca*, *P. bourgaei*, *P. grandiflora*, *P. lycia*, *P. nissolii* and *P. pungens.*

While there was no statistically significant difference between *P. bourgaei* and *P. leucophracta* for α-cubebene compound ratios according to the ordinal method, there was a statistically significant difference between these two species and *P. armeniaca*, *P. grandiflora*, *P. lycia*, *P. nissolii*, *P. pungens* and *P. samia.* While there was no statistically significant difference between *P. grandiflora* and *P. nissolii*, there was a statistically significant difference between these two species and *P. armeniaca*, *P. bourgaei*, *P. leucophracta*, *P. lycia*, *P. pungens* and *P. samia.*

There was a statistically significant difference between these two species and *P. armeniaca*, *P. grandiflora*, *P. leucophracta*, *P. lycia*, *P. nissolii* and *P. pungens,* however, there was no statistically significant difference between *P. bourgaei* and *P. samia* for α-cubebene compound ratios according to the ordinal method, while there was no statistically significant difference between *P. armeniaca*, *P. leucophracta*, *P. lycia* and *P. nissolii*, while there was a statistically significant difference between these species and *P. bourgaei*, *P. grandiflora*, *P. pungens* and *P. samia.* Also there was no statistically significant difference between *P. grandiflora* and *P. pungens.*

While there was no statistically significant difference between *P. grandiflora* and *P. lycia* for α-cedrene compound ratios according to the ordinal method, there was a statistically significant difference between these two species and *P. armeniaca*, *P.bourgaei*, *P. leucophracta*, *P. nissolii*, *P. pungens* and *P. samia.* There was no statistically significant difference between *P. armeniaca*, *P.bourgaei*, *P. leucophracta*, *P. nissolii*, *P. pungens* and *P. samia.*

There was no statistically significant difference between *P.bourgaei*, *P. leucophracta* and *P. lycia* for β-caryophyllene compound ratios according to the ordinal method, while there was a statistically significant difference between these species and *P. armeniaca*, *P. grandiflora*, *P. nissolii*, *P. pungens* and *P. samia.* While there was no statistically significant difference between *P. grandiflora* and *P. pungens,* there was a statistically significant difference between these two species and *P. armeniaca*, *P.bourgaei*, *P. leucophracta*, *P. lycia*, *P. nissolii* and *P. samia*

There was no statistically significant difference between, *P. armeniaca* and *P. samia* for germacrene-D compound ratios according to the ordinal method. There was a statistically significant difference between *P. armeniaca* and *P.bourgaei*, *P. grandiflora*, *P. leucophracta*, *P. lycia*, *P. nissolii*, *P. pungens* and *P. samia.* While there was no statistically significant difference between *P.bourgaei* and *P. nissolii* and *P. lycia,* statistically significant difference between *P.bourgaei* and *P. nissolii* and *P. armeniaca*, *P. grandiflora*, *P. leucophracta*, *P. pungens* and *P. samia* While there is not statistically significant difference between *P. grandiflora* and *P. leucophracta,* there was a statistically significant difference between *P. grandiflora* and *P. armeniaca*, *P.bourgaei*, *P. lycia*, *P. nissolii*, *P. pungens* and *P. samia*

There was a statistically significant difference between *P. grandiflora* and *P. armeniaca*, *P.bourgaei*, *P. leucophracta*, *P. lycia*, *P. nissolii*, *P. pungens* and *P. samia* for α-curcumene component ratios per the ordinal method. There was no statistically significant difference between *P. armeniaca*, *P.bourgaei*, *P. leucophracta*, *P. lycia*, *P. nissolii*, *P. pungens* and *P. samia.*

Differences between the essential compounds of β-caryophyllene and germacrene-D were statistically important per the Friedman test in respect to volatile components by rates. The multi comparison Bonferroni-Dunn test was applied to determine the difference between the medians and found that there was a statistically significant difference between the pre bloom period, bloom period and post-emergence period for β-caryophyllene compound ratios according to an ordinal method. There was no statistically significant difference between the pre bloom period, bloom period and post-emergence period for germacrene-D component ratios according to an ordinal method *(Table 4).*

***Table 4.** Friedman testi results according to vegetation periods*

Component	Period	N	N*	Mean	SEMean	StDev	Median	P	Rank
β-Caryophyllene	Before flowering	8	0	13.76	2.51	7.10	14.76	0.001	17.0b
	Flowering	8	0	15.03	2.83	7.99	15.91		23.0a
	After flowering	8	0	10.71	2.16	6.12	11.09		8.0c
Component	**Period**	**N**	**N***	**Mean**	**SEMean**	**StDev**	**Median**	**P**	**Rank**
Germacrene-D	Before flowering	8	0	12.88	2.44	6.89	12.34	0.010	12.0b
	Flowering	8	0	16.47	2.84	8.03	17.93		23.0a
	After flowering	8	0	12.31	2.31	6.52	10.82		13.0b

*a,b,c show differences between vegetation periods

Discussion and Conclusion

Yaşar et al. (2010) determined 12 volatile components for *P. armeniaca.* The main components were germakren-D (35.68%), β-caryophyllene (18.08%), caryophyllene oksit (13.35%), (E)-β-farnesene (7.24%) and hexahydrofarnesyl aseton (6.99%). They also determined that germakren-D was the dominant distinguishing compound of *P. armeniaca.* The results supported our thesis. Germakren-D was determined as dominant distinguishing compound.

Sixty-two volatile components of *Phlomis bourgaei* were found thru SPME analysis and these components of *P. bourgaei* had the following ratios: α-cubebene (16.04%), β-caryophyllene (21.98%) and germakren-D (15.12%). Sarıkurkcu et al. (2013) determined the chemical compounds of volatile oils of *P. bourgaei* by hydro distillation and examine anti-toxicant potentials. The main components were: β-caryophyllene (37.37%), (Z)-β-farnesene (15.88%) and germakren-D (10.97%). Baser et al. (2008) determined the components of *P. bourgaei* as germakren-D (11.3%) and β-caryophyllene (112%). β-caryophyllene and germakren-D were the main compounds. These findings support our research results. The only difference was that we determined that the α-cubebene compound was the dominant compound.

In our research α-pinene (26.40%), α-cedrene (28.15%) and α-curcumene (13.92%) were volatile components of *Phlomis grandiflora* var. *grandiflora* among 60 components as determined by SPME analysis. Celik et al. (2005) determined components of *P. grandiflora* var. *grandiflora as* germakren-D (%45.4%), β-caryophyllene (22.8%) and bicycle germakren (4.9%). Demirci et al. (2008found that β-eudesmol (42%) and α-eudesmol (16%), which are oxygenic sesquiterpenes as the most important compounds of *P. grandiflora* var. *grandiflora.* Ozcan et al. (2009) determined 32 components that represent 92.1 % oil obtained from flowers. They determined the essential components as β-eudesmol (61.48%), β-curcumene (5.81%), E-β-farnesene (2.35%), α-zingiberene (2.18%) and α-cedrene (1.94%). They found 39 compounds that represent 87.7% of oil obtained from leaves. Volatile components were β-eudesmol (62.04%), β-curcumene (3.43%), α-curcumene (2.20%) and linalool (2.03%). It has been characterized that there are high percentages of β-eudesmol in both oils. The results of our study differ from the study of Celik et al. (2005). Meanwhile, Ozcan et al. (2009) found α-cedrene and α-curcumene as the main compounds in their studies. This result supports our study. In other research, α-pinene has been found as the dominant component.

Seventy volatile components of *Phlomis leucophracta* were determined by SPME analysis and the essential compounds were (E)-2-hexenal (8.74%), limonene (14.56%) and β-caryophyllene (22.45%). Celik et al. (2005) found β-caryophyllene (20.2%), α-pinen (19.2%) and limonene (11%) in *P. leucophracta.* β-caryophyllene and limonene were the volatile components. The result of this research supports our study. In other studies, (E)-2-hexenal was among the main component.

Sixty-two volatile components of *Phlomis lycia* were determined with SPME analysis and the main components of *P. lycia* were Limonene (17.68%), β-caryophyllene (23.66%) and germacrene-D (21.88%).

Fifty-three volatile components of *Phlomis nissolii* were determined with SPME analysis and the main components of *P. nissolii* were limonene (23.75%), β-caryophyllene (12.50%) and germacrene-D (20.73%) Kirimer et al. (2006) found 18 compounds thru GC/MS analysis. They found that the main components were germacrene-D (33.9%), bicycle germacrene (15.3%) and (Z)- β- farnesene (10.7%). Their results differ from our study. However, germacrene-D was found as a main component in both studies.

Seventy volatile compounds of *Phlomis pungens* var. *pungens* were determined and the main compounds were (E)-2-hexenal (17.60%), vinly amyl carbinol (18.44%) and germacrene-D (9.84%). Masoudi et al. determined 24 compounds in Iran for *P. pungens* var. *pungens* thru GC/MS analysis. The volatile components included bicycle germacrene (14.1%), α-pinen (13.5%) and (E)-β-farnese (13.5%). These results differ from our study.

A total of 64 volatile components of *Phlomis samia* were determined by the SPME analysis. The main components were α-copaene (10.59%), β-caryophyllene (15.20%) and germacrene-D (23.44%). Aligiannis et al. (2004) determined 72 components of upper sections of *P.samia* which grow in Greece and was represented at 67.4% by α-pinen, limonen, β- caryophyllene, linaol, (E)-β-farnesene, germacrene -D, (Z)-γ-bisabolene and cis-β-ocimene. Demirci et al. (2006) determined germacrene -D (33.8%) and β- caryophyllene (6.4%) as the main components of *P. samia* with GC/MS analysis. The above mentioned results support our results. Additionally, the α-copaene compound was found.

The result of Kruskal-Wallis test found; 2-butenal, n-pentenal, trans-3-penten-2-one, 3-methyl-1-butanol, n-hexanal, (E)-2-hexenal, cis-hex-2-en-1-ol, hexanol <n->, amyl methyl ketone, n-heptanal, 2,4-hexadienal, α-thujene, α-pinene, (E)-2-heptenal, sabinene, .β-phellandrene, β-pinene, amyl vinyl ketone, vinly amly carbinol, 6-methyl-5-hepten-2-one, β-myrcene, n-octanal, α-phellandrene, 2,4-heptandienal, p-dichlorobenzene, α-terpinene, p-cymene, limonene, (E)-3-octen-2-one, cis-ocimene, 2 octenal, γ-terpinene, γ-terpinene, octanol, α-terpinolene, dimethylstyrene <α-para->, 2-nonanone, methyl benzoate, n-nonanal, 2-hendecanone, α-campholene aldehyde, trans-alloocimene, trans-2-nonenal, pinocarvone, (E)-2-nonenal, (E)-2-nonenal, n-decanal, (E)-2-decenal, α-cubebene, α-copaene, (-)-.β-elemene, sesquithujene <7-epi->, α-gurjunene, α.-cedrene, β-caryophyllene, γ.-elemene, α-bergamotene, (E)-β-farnesene, farnesol, epi-bicyclosesquiphellandrene, germacrene-D, ionone, α-curcumene, bicyclogermacrene, zingiberene, α-muurolene, β-muurolene, cedr-8-e, .γ-cadinene, δ-cadinene, β-sesquiphellandrene, (-)-caryophyllene oxide, p-menthane, 2,3-dibromo-8-

phenyl, androstan-17-one ve 3-ethyl-3-hydroxy and nonadecane components, and the differences between medians of *Phlomis* species were statistically significant.

The differences between medians of vegetation periods were statistically significant for hexanol <n->, n-heptanal, β-caryophyllene and germacrene-D components by Friedman test.

As a result, it is significantly important to reap samples of *Phlomis* taxa in the bloom period in respect to volatile compound productivity. These results are important in providing guidance for local growers and traders to reduce untimely plant picking and to reduce economic losses.

The leaves and flowers of *Phlomis* are used for several purposes, from making products more appetizing, as an anti-allergic, as a diuretic, for diarrhea preventive, against stomach aches, to relieve pain, anti-diabetic, and for herbal teas and tonics. Additionally, taxa that have yellowish flowers are important for food coloring. As such, research and further studies should be pursued in order to investigate how various plants can be used as a raw material for medicine or as food and cosmetic products. Importantly, future research will promote further application.

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REFERENCES

[1] Aligiannis, N., Kalpoutzakis, E., Kyriakopoulou, I., Mitaku, S., Chinou, I. B. (2004): Essential oils of *Phlomis* species growing in Greece: chemical composition and antimicrobial activity. – Flavour and Fragrance Journal 19: 320–324.

[2] Baser, K.H.C., Demirci, B., Dadandi, M.Y. (2008): Comparative Essential Oil Composition of the Natural Hybrid *Phlomis x vuralii* Dadandı (Lamiaceae) and its Parents. – Journal of Essential Oil Research 20: 57–62.

[3] Baydar, H. (2009): Tıbbi ve Aromatik Bitkileri Bilimi ve Teknolojisi. – Süleyman Demirel Üniversitesi Ziraat Fakültesi.

[4] Baytop, T. (1997): Türkçe Bitki Adları Sözlüğü. – TDK. Yayınları.

[5] Celik, S., Gokturk, R.S., Flamini, G., Cioni, P.L., Morelli, I. (2005): Essential oils of*Phlomis leucophracta*, *Phlomis chimerae* and *Phlomis grandiflora* var. *grandiflora* from Turkey. – Biochem Syst Eco 33: 617-623.

[6] Demirci, B., Dadandi, M.Y., Paper, D.H., Franz, G., Baser, K.H.C. (2003): Chemical composition of the essential oil of *Phlomis linearis* Boiss. & Bal. and biological effects on the CAM-Assay: A safety evaluation. – Verlag der Zeitschriftfür Naturforschung 58: 826-829.

[7] Demirci, B., Baser, K.H.C., Dadandi, M.Y. (2006): Composition of The Essential Oils of *Phlomis rigida* Labill. and *P. samia* L. – Journal of Essential Oil Research 18: 328-331.

[8] Demirci, F., Guven, K., Demirci, B., Dadandi, M.Y., Baser, K.H.C. (2008): Antibacterial activity of two *Phlomis* essential oils against food pathogens. – Food Control 19: 1159-1164.

[9] Guner, A. (2012): Türkiye Bitkileri Listesi, Damarlı Bitkiler. – Nezahat Gökyiğit Botanik Bahçesi Yayınları.

[10] Harput, Ü.Ş., Calıs İ., Saracoglu, I., Donmez, A.A., Nagatsu, A. (2006): Secondary Metabolites from *Phlomis syriaca* and Their Antioxidant Activities. – Turkish Journal of Biology 30: 383-390

[11] Huber-Morath, A. (1982): *Phlomis* L. in Flora of Turkey and East Aegean Islands. – In:. Davis, P.H. (ed.) Flora of Turkey and the East Aegean Islands, Edinburg University Press., Edinburg.

[12] Kirimer, N., Baser, K.H.C., Kurkcuoglu, M. (2006): Composition of the Essential Oil of *Phlomis nissolii* L. – Journal of Essential Oil Research 18: 600-601.

[13] Kumar, S.A. (2009): Plants-based Medicines in India. http://pib.nic.in/feature/feyr2000/fmay2000 /f240520006.html. Accessed: 12.05. 2015

[14] Masoudi, S., Rustaiyan, A., Aberoomand, P. (2006): Composition of the Essential Oils of *Cyclotrichium straussii* (Bornm.) Rech. f. and *Phlomis pungens* Willd. from Iran. – Journal of Essential Oil Research 18: 16-18.

[15] Mathiesen, C., Scheen, A. C., Lindqvist, C. (2011): Phylogeny and biogeography of the lamioid genus *Phlomis* (Lamiaceae). – The Board of Trustees of the Royal Botanic Gardens, Kew 66: 83-89.

[16] Ozcan, M.M., Tzakou, O., Couladis, M. (2009): Essential oil composition of the turpentine tree (*Pistacia terebinthus* L.) fruits growing wild in Turkey. – Food Chemistry 114: 282-285.

[17] Sarikurkcu, C., Ozer, M.S., Cakir, A., Eskici, M., Mete, E. (2013): GC/MS Evaluation and In Vitro Antioxidant Activity of Essential Oil and Solvent Extracts of an Endemic Plant Used as Folk Remedy in Turkey: *Phlomis bourgaei* Boiss. –Hindawi Publishing Corporation Evidence-Based Complementary and Alternative Medicine.

[18] Tan, A. (1992): Türkiye'de Bitkisel Çeşitlilik ve Bitki Genetik Kaynakları. – Anadolu Journal of AARI 2: 50-64.

[19] Vichy, S., Castellote, A. I., Pizzale, L., Conte, L. S., Buxaderas, S., Lopez-Tamames, E. (2003): Analysis of virgin olive oil volatile compounds by headspace solid-phase microextraction coupled to gas chromatography with mass spectrometric and flame ionization dedection. – Journal of Chromatography 983:19-33.

[20] Weiss, E.A. (1997): Essential Oil Crops. – The Journal of Agricultural Science 129: 121-123

[21] Yasar, S., Fakir, H., Erbas, S. (2010): Gas chromatographic (GC-GC/MS) analysis of essential oil of *Phlomis armeniaca* Willd. from Mediterranean region in Turkey. –Asian Journal of Chemistry 22: 2887-2890.

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AN ANALYSIS OF LAND USE AND LAND COVER DYNAMICS AND CAUSATIVE DRIVERS IN A THICKLY POPULATED YAMUNA RIVER BASIN OF INDIA

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Abstract. Understanding the land use and land cover (LULC) change processes is of great importance for formulating the land use policy. In the present study, Remote Sensing, Geographic Information System (GIS) and statistical techniques are used to understand the LULC dynamics and possible driving forces in a thickly populated Yamuna river basin of India. The National Capital Territory of Delhi, one of the world's largest urban agglomerations, occurs in this river basin. The LULC maps on 1:250,000 scales prepared at decadal interval (1985, 1995 and 2005) using satellite images are used for studying the LULC dynamics. The major LULC changes observed during the study period are increase in cropland (65.7% to 67.9%) and built-up (0.9% to 1.9%) and decrease in forest (17.1% to 16.4%) and water bodies (2.3% to 1.9%). The dominant processes of the LULC changes include transitions from forest, grassland, shrub land, water bodies and wasteland to cropland; transitions from cropland, shrub land, wasteland, grassland and forest to built-up; conversion of forest to cropland, shrub land, plantation and built-up; and transitions from water bodies to cropland, shrub land and wasteland. Most of the transitions are reversible in nature. The statistical analysis (logistic regression) between decadal LULC maps and drivers indicates that LULC changes are driven by a multitude of factors in different combinations and with different importance. Socioeconomic, demographic and proximity drivers mainly influence the LULC change processes in the study area in decreasing order of importance. The insights gained are important for simulating future LULC scenarios as input for land management policy making.

Keywords: *land use and land cover (LULC); remote sensing; geographic information system; logistic regression; drivers of LULC change; Yamuna basin*

Introduction

Land is the most valuable natural resource that has been transforming since ages to meet the demands of human kind in pursuit of better life. Some of the major landscapes changes across the world have arisen due to human interference (Viles and Goudie, 1997; Lambin et al., 2001). A strong trend of urban development can be observed throughout the world (United Nations Centre for Human Settlements, 1996) and India is no exception, which has witnessed phenomenal development in different sectors since

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independence. It occupies 2.3% of the world's land area and supports more than 17.5% of the world's population. Indian agriculture has grown at the rate of about 2.6% per annum in the post-independence era which is double the rate of pre-independence era (Tripathi and Prasad, 2010). Significant land use and land cover (LULC) changes have occurred in the country during the last few decades wherein demography, rapid development, urbanisation and agriculture expansion has exerted an increasing pressure on land. Historically, it has been observed that most of the developed cities in India are located near the river banks. Considering this fact, the present study lays emphasis on understanding the interaction of causative driving forces and LULC change in Yamuna river basin of India.

The Yamuna is the longest tributary of river Ganga in the Lower Himalaya. After flowing for about 200 km in the Himalaya, it then emerges into the Indo-Gangetic plains (Rai et al., 2012) where it flows almost parallel to river Ganga and ultimately meets river Ganga at Allahabad in the state of Uttar Pradesh. While flowing in Indo-Gangetic plains, it creates a large (~69,000 km^2) fertile zone called Ganga-Yamuna Doab. The drainage basin is highly vegetated and populated area that needs attention towards LULC change analysis. The study area covers part of seven states of the country namely, Uttar Pradesh, Himachal Pradesh, Madhya Pradesh, Uttarakhand, Haryana, Rajasthan and National Capital Territory of Delhi. It is thickly populated with highest population density in Delhi. Yamuna waters feed a large population and are the source of irrigation. Studying LULC change processes and understanding their linkages with socioeconomic, demographic and physical factors are thus critical for formulating land use policy in this river basin.

The driving factors responsible for LULC change in an area may be socioeconomic, demographic, physical, proximate or a combination of all these. Also, these driving factors may operate independently at different levels in a simultaneous manner that may vary in time and space (Geist and Lambin, 2002). The changing patterns of LULC and their driving factors have been studied in different countries at various scales. In India, the changing patterns of LULC with decrease in forest cover, expansion of cropland and increase in built-up areas have been identified from 1880 to 2010 (Tian et al., 2014). Recently, in another study on LULC changes in India (Roy et al., 2015), significant increase in built-up areas and cropland and decrease in fallow land, forest and wasteland have been identified during 1985 to 2005 using satellite images. In a similar study in Northeast China (Shen et al., 2013), changes in built-up, cropland, forest, grassland and wetland were analysed from 1970 to 2004 that highlighted the effectiveness of forest and wetland protection and restoration projects. Decrease in ecosystem services value were found in Su-Xi-Chang region of Yangtz river delta, East China, due to the loss of cropland and water bodies by urban expansion (Yirsaw et al., 2016). In Bonsa catchment of Ankora river basin in Ghana, West Africa, land cover transitions from 1986 to 2011 showed that increase in population growth, agriculture expansion and increased surface mining activities were responsible for increased deforestation rate (Aduah, 2014). In Poland, the effect of LULC on ecological quality of rivers were analysed which showed decline in population of bryophytes due to the loss of forest (Zgola, 2014). Human induced LULC changes were revealed in Kagera Basin of Lake Victoria (Wasige et al., 2013). Role of socioeconomic and physical drivers on land degradation were identified in Vietnam (Vu et al., 2014).

It is difficult to link the drivers of LULC change in a heterogeneous landscape because of uncertainty associated with misclassification and availability of data at

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diverse scales (Martinez et al., 2011). LULC change is dynamic in nature and it is difficult to obtain the real time information of LULC change through conventional methods. Satellite remote sensing along with GIS that can bring different types of data at one platform for analysis has brought a new dimension to study LULC changes at varied scales (Wang et al., 2013).

In the present study, we investigate LULC change processes and the possible driving forces of such change in the Yamuna river basin of India using remote sensing, GIS and statistical techniques.

Materials and Methods

Study Area

Yamuna River originates from Yamunotri glacier at 30.98°and 78.45° in the Mussoorie range of the Lower Himalaya in the state of Uttarakhand, India. It covers an area of 3,66,223 km^2 which is about 42.5% of the total area of Ganga basin and 10.7% of the total country's land area. In the present study, the drainage area of Chambal river, which is a major tributary of river Yamuna, has been excluded. The study area of Yamuna basin (~1,98,890 km^2) delineated from 90 m digital elevation model (DEM) obtained from Shuttle Radar Topography Mission (SRTM) as shown in *Fig.1*. Tons, Chambal, Hindon, Sind, Betwa, Ken, Baghein and Mandakini are major tributaries and Rishi Ganga, Uma, Hanuman Ganga, Giri, Karan, Sagar and Rind are smaller tributaries of Yamuna. The basin landscape is characterized by hills, valleys, plains, lakes and network of river streams. Many protected areas like sanctuaries (Bhindawas bird sanctuary, Okhla wildlife sanctuary, Hastinapur wildlife sanctuary, National Chambal wildlife sanctuary, etc.) and National parks (Ranthambore national park, Kalesar national park, Keoladeo Ghana national park) are situated in the study area. The basin has a huge variety of flora and fauna that varies with respect to the location and environment. The elevation in the basin varies from 6320 m above mean sea level near Yamunotri Glacier to around 100 m near the confluence with the river Ganga at Allahabad with highly varied topography from steep, rugged mountains in the Himalaya to almost flat in Indo-Gangetic plains. About 42% of the area is covered by alluvial soil and over 50% of the area is cultivated. The irrigated land area in the basin is about 12.3 million hectares. There are four irrigation canals in the basin namely, Western Yamuna canal, Eastern Yamuna canal, Agra canal and Gurgaon canal that delivers the Yamuna water to the command areas (Rai et al., 2012).

Methodology

The methodology includes three parts viz, LULC change analysis and accuracy assessment; preparation and integration of socioeconomic, physical, demographic and proximity driver datasets; and statistical analysis to derive relationship between drivers and LULC changes (*Fig. 2*). Different datasets used in the present study and their sources are listed in *Table 1.*

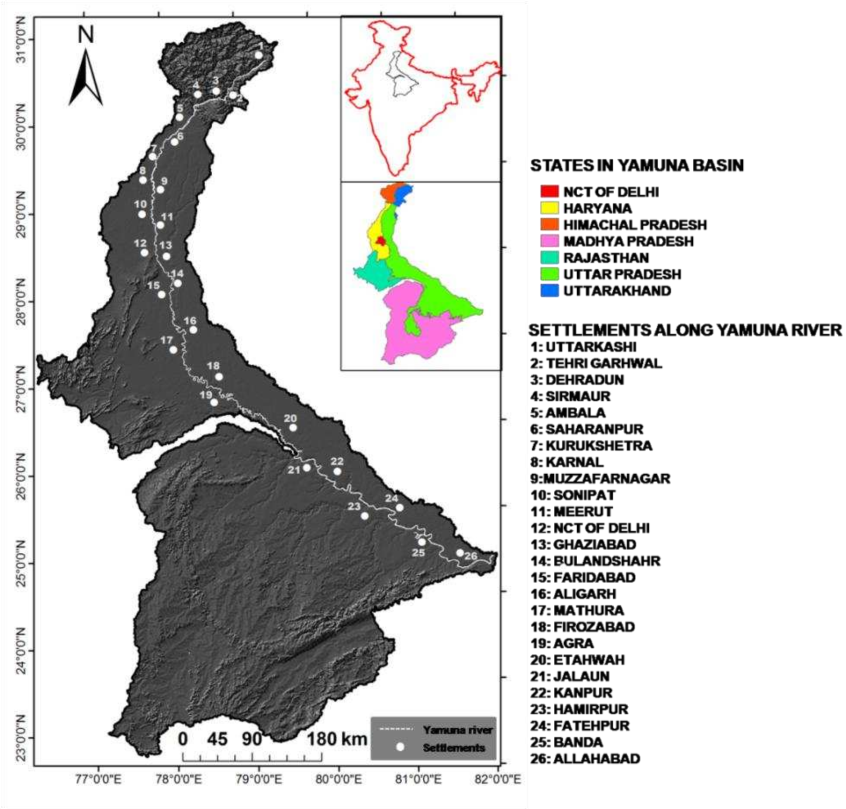


***Figure 1**. Study area taken for the present study*

***Table 1.** Datasets used in the study*

LULC maps			
Dataset	**Time period**		**Source**
LULC Maps	1985, 1995 and 2005		ISRO Geosphere Biosphere (GBP) Programme (Roy et al. 2015)
Satellite data			
Dataset	**Resolution**	**Time period**	**Source**
IRS LISS-III	23.5m	2005	ISRO-GBP
IRS LISS-I	30m	1995	ISRO-GBP
Landsat-MSS	56.25m	1985	Global Land Cover Facility (GLCF)
Physical data			
Dataset	**Resolution**	**Time period**	**Source**
Elevation	90 m	1995 and 2005	Shuttle Radar Topography Mission (SRTM) DEM
Slope	90 m	1995 and 2005	SRTM DEM
Mean annual temperature	1^0	1995 and 2005	India Meteorological Department (IMD)
Annual rainfall	0.5^0	1995 and 2005	IMD
Soil depth	1:1 million	1995 and 2005	Soil map of National Bureau of Soil Survey and Land use planning (NBSSLUP), Nagpur
Demographic and Socioeconomic data			
Dataset	**Scale**	**Time period**	**Source**
Total population	Taluka	1995 and 2005	Census of India, State Development Reports, Census handbooks, Indiastat.com, Ministry of Statistics and Programme Implementation
Total working population	District	1995 and 2005	
Number of agriculture workers	District	1995 and 2005	
Road length	District	1995 and 2005	
Proximity data			
Dataset	**Time period**		**Source**
Distance to built-up	1995 and 2005		LULC maps of 1995 and 2005
Distance to forests	1995 and 2005		
Distance to water bodies	1995 and 2005		

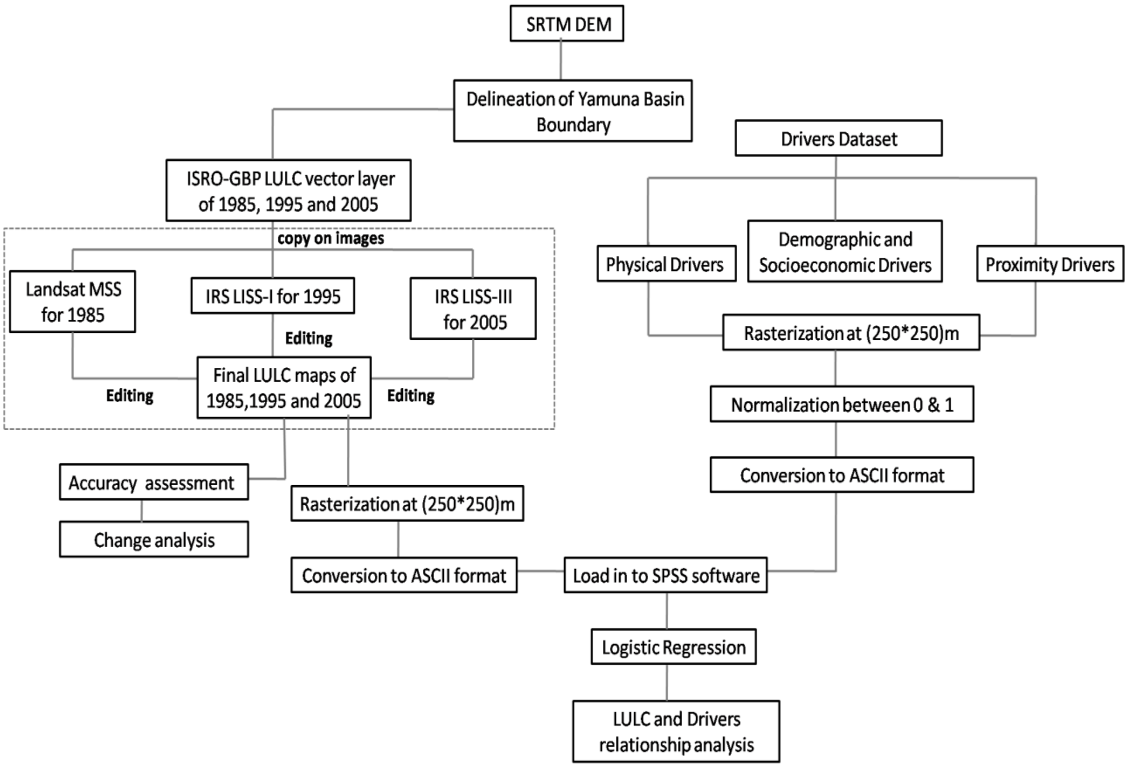


Figure 2. *Methodology followed in the present study*

Change analysis and accuracy assessment

Decadal LULC maps of 1985, 1995 and 2005 were prepared at 1:250,000 scales formed the basic inputs (Roy et al., 2015).The LULC maps follow IGBP (International Geosphere Biosphere Programme) LULC classification scheme (Loveland and Belward, 1997). In the present study, LULC maps were prepared by merging different types of forest classes to one forest class and cropland and fallow land to cropland class (*Table 2*). A total of ten LULC classes, i.e. built-up, cropland, plantation, forest, shrub land, grassland, baren land, wasteland, water bodies, permanent wetland were then refined wherever required using satellite images of Landsat-MSS, IRS LISS-I and IRS LISS-III satellite images respectively for 1985, 1995 and 2005 to produce modified LULC maps (*Fig. 3*). Snow and ice class was masked for the present study. Accuracy assessment is then carried out for LULC map of 2005 with aid of confusion matrix by using Google Earth as reference data. We have generated a total of 9003 random points with minimum of 3 points per polygon. From the confusion matrix, individual class accuracy, overall accuracy and kappa coefficient were computed (Congalton, 1991). The overall accuracy and kappa coefficient of 2005 LULC map is 89.82% and 0.88, respectively (*Table 3*). The accuracy assessment was performed for 2005 LULC map only and assumed to be of the same order for LULC maps of 1985 and 1995 considering the mapping procedures followed in preparing decadal LULC maps (Roy et al., 2015). After the refinement of LULC maps and accuracy assessment, the change area matrices are prepared for three periods, i.e. 1985-1995, 1995-2005 and 1985-2005. These matrices were used to analyse the transition of one LULC class to other LULC class.

Table 2. *LULC classification scheme adopted in the study*

IGBP LULC Classes (Roy et al. 2015)	**LULC Classes used in study**	**Code**
1.0 Built-up (both urban and rural)	1. Built-up	BU
2.0 Crop Land	2. Cropland	CL
2.1 Fallow land		
2.2 Plantation	3. Plantation	PL
3.0 Evergreen needle forest	4. Forest	F
3.1 Evergreen broad leaf forest		
3.2 Deciduous needle forest		
3.3 Deciduous broad leaf forest		
3.4 Mixed forest		
3.5 Shrub land	5. Shrub land	SL
3.7 Grassland	6. Grassland	GL
4.0 Barren land	7. Barren land	BL
4.1 Wasteland	8. Wasteland	WL
5.0 Water bodies	9. Water bodies	WB
5.1 Permanent Wetland	10.Permanent Wetland	PW

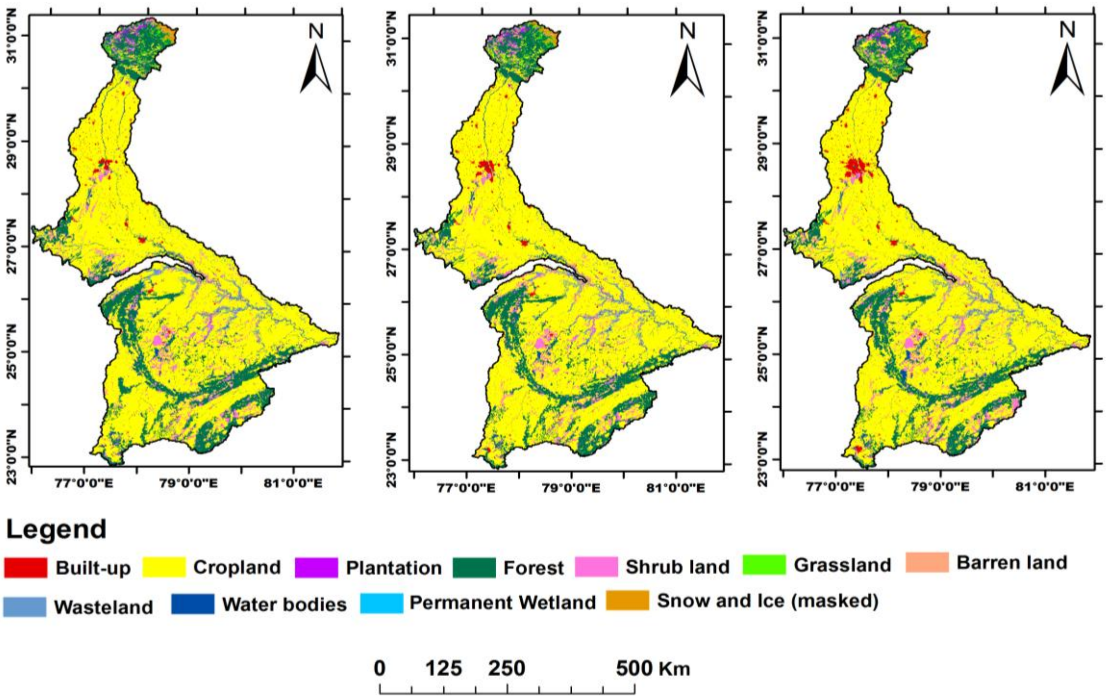


Figure 3. *Decadal LULC maps: (a) 1985, (b) 1995 and (c) 2005*

Preparation of driver datasets

The socioeconomic, demographic, physical and proximity driver datasets were prepared for 1995 and 2005 (*Table 1*). The demographic driver includes total population and socioeconomic drivers include total working population, number of agriculture workers and road length. The physical drivers include slope, mean annual temperature, annual rainfall and soil depth whereas proximity drivers include distance to built-up, distance to forests and distance to water bodies. These drivers were used as independent variables in the statistical analysis.

Table 3. *Accuracy assessment of LULC map of 2005*

	Reference data												**Users accuracy (%)**
	LULC Classes	**BL**	**BU**	**CL**	**F**	**GL**	**PL**	**PW**	**SL**	**WB**	**WL**	**Total**	
Classified data	**BL**	519	1	23	87				3			633	81.9
	BU	13	1634	72	2			1	7	2		1731	94.4
	CL		4	1147	24		1		25	5	3	1209	94.8
	F		5	109	1544		4		18		1	1681	91.9
	GL		96	17	2	315	6			1		437	72.1
	PL		3	12		2	415					432	96.1
	PW			8				116				124	93.5
	SL	12	2	75		46			946		4	1085	87.2
	WB			116	6	10			3	764		899	85.0
	WL	3	2	52	7	19			1	1	687	772	89.0
	Total	547	1747	1631	1672	392	426	117	1003	773	695	**9003**	
Producer accuracy (%)		94.9	93.5	70.3	92.3	80.4	97.4	99.1	94.3	98.8	98.8		
Overall accuracy: 89.82 %													
Khat or Kappa Coefficient: 0.88													

BL- Barren land, BU- Built-up, CL- Cropland, F- Forest, GL- Grassland, PL- Plantation, PW- Permanent Wetland, SL- Shrub land, WB- Water bodies, WL- Wasteland.

The socioeconomic data were collected at district level with the exception of population data which was collected at taluka (sub-district) level. The data set were collected from Census of India, Indiastat.com, Census handbooks, State development reports and Statistical diaries. The slope and elevation has been derived from the SRTM DEM. The annual rainfall and mean annual temperature were obtained from IMD (India Meteorological Department). The rainfall and temperature data were available at half and one degree grid, respectively. The soil depth was extracted from the soil map prepared at 1:1 million scale by National Bureau of Soil Survey and Land Use Planning, Nagpur. The soil depth was segregated from this map and reclassified and appended with the map as a new field. The proximity drivers viz., distance to built-up, distance to forests and distance to water bodies were derived from LULC maps of 1995 and 2005 using Euclidean distance in Arc GIS software. All the datasets were rasterized at (250m×250m) and normalized between 0 and 1, so as to balance the range of data values (*Fig. 4*).

Normalization of data values is important because of the sensitivity of data transformation in statistical analysis (logistic regression in the present case). Normalization is done by dividing the value of each grid cell with the highest value of cell data to achieve similar data range. Moreover, in the multivariate analysis such as logistic regression, the independent variables should have a similar scale to avoid discrepancy in outcome (Veldkamp and Fresco, 1997; Verburg et al., 2004; Nefeslioglu et al., 2008). After normalization, the raster data were converted to ASCII files and used for statistical analysis in SPSS software.

Statistical analysis between drivers and LULC change

To analyse the relationship between LULC classes (dependent variables) and possible driving factors (independent variables), logistic regression has been used. The analysis was performed between LULC classes and drivers of 1995 and 2005, respectively. The regression was run specifically for classes which are undergoing significant changes like built-up, cropland, forest, water bodies, barren land, wasteland

and shrub land. Since the driver datasets were prepared at coarse resolution, the regression was not performed for classes having smaller areas like permanent wetland, grassland and plantation to avoid meaningless results due to difference in scales. The total number of independent variables used to explain the spatial patterns of LULC are twelve. The regression was run separately for each class with individual drivers. Logistic regression is adopted for analysis because of dichotomous nature of LULC classes (Mac Nally, 2000; Millington et al., 2007; Martinez et al., 2011). The SPSS package is used to carry out regression analysis.

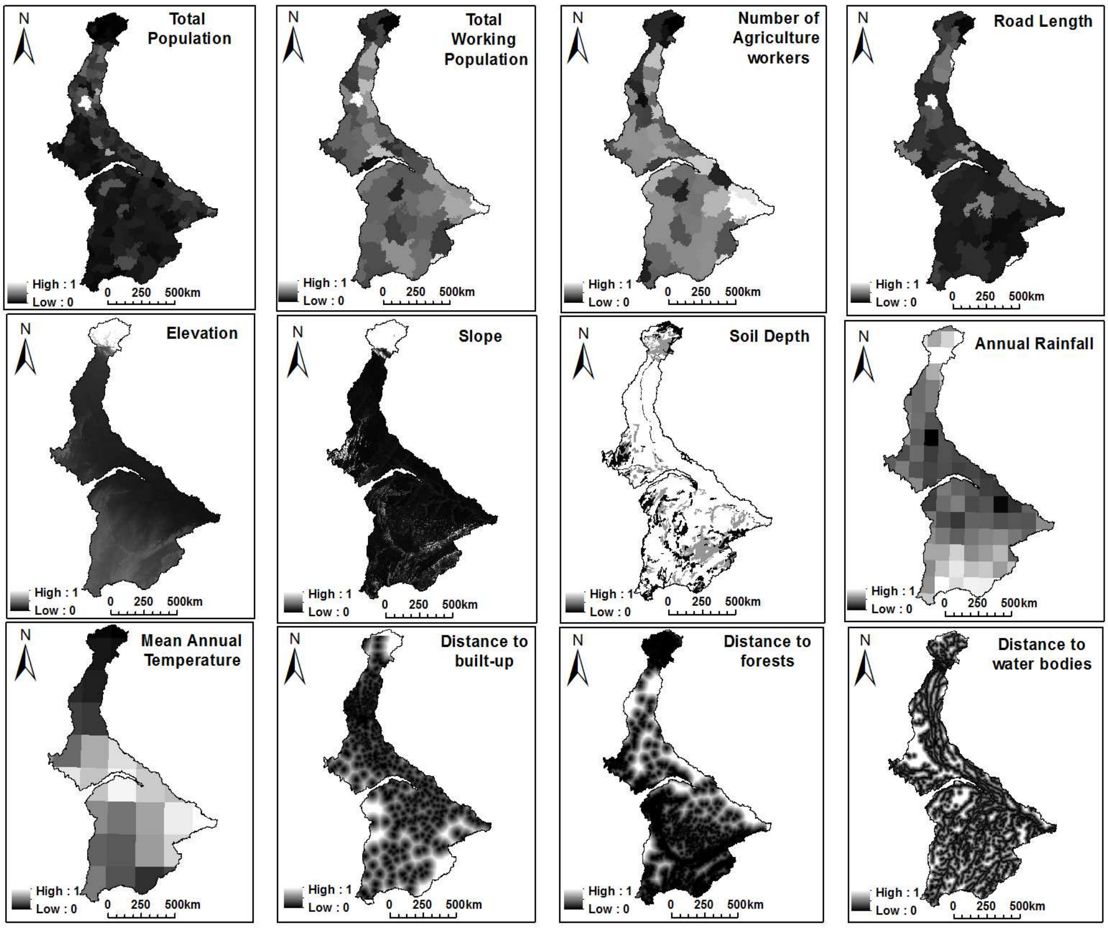


Figure 4. *Normalized drivers datasets used in the study*

The β values were used to express the possible relationship between LULC classes and driving forces. Multi-collinearity between driving factors has been checked before running the regression in order to reduce the errors in parameter estimates and biasness in the outcome (MacNally, 2000). For testing multi-collinearity between independent variables, tolerance values $(1 - R^2)$ and variance inflation factors (VIF) were used. If VIF values are smaller than 10 and the tolerance values are greater than 0.1, it indicates that there is no multi-collinearity issue between the drivers (Obrien, 2007).

The goodness of fit of the model is evaluated by relative operating characteristic (ROC) Curve. The ROC indicates whether the spatial pattern of LULC classes can be easily explained by the independent variables. The ROC value above 0.5 is good, above

0.6 is better and value closer to 1 is categorized as perfect for the logistic regression model performance (Hosmer and Lemeshow, 2000; Pontius and Schneider, 2001).

Results

Land use and land cover change analysis

The areal extent of LULC classes in the study area is shown in *Table 4.* The results of LULC changes from 1985 to 1995, 1995 to 2005 and 1985 to 2005 are summarized in *Tables 5a, 5b and 5c*, respectively. It is observed from *Table 4* that cropland is the dominant LULC type with 66.1% area in 1985, 67.2% area in 1995 and 67.9% area in 2005. Cropland and built-up classes show increasing trend from 1985 to 2005. There is a continuous increase in built-up areas around major cities like Delhi during study period as a result of development in various sectors like employment opportunities, better socioeconomic conditions for living and technology. On the other hand, forest shows decreasing trend from 1985 to 2005. This decrease in forest cover is mainly attributed to fragmentation, urbanisation, cropland expansion and construction of reservoirs and dams. Grassland, barren land, permanent wetland and water bodies also show decreasing trend from 1985 to 2005. In case of plantation class, its areal extent decreases marginally from 1985 to 1995 and increases from 1995 to 2005. An increasing trend is observed in case of shrub land and wasteland, while water bodies show decreasing trend.

The transition of one LULC class to other LULC class can be seen from *Tables 5a, 5b and 5c,* respectively. The observation indicates that four LULC classes i.e. built-up, cropland, forest and water bodies showed major conversions. There is a gain in built-up and cropland areas. The change in built-up includes transitions from cropland, shrub land, wasteland, grassland and forest. The gain in cropland includes transitions from forests, grassland, shrub land, water bodies and wasteland. The forest and water bodies also represent important transitions. The forest transition mainly includes transition to cropland, shrub land, plantation and built-up areas. With respect to water bodies, it is converted to cropland, shrub land and wasteland. The rest of LULC classes show minor conversions. The change in areal extent of LULC classes and a few locations of LULC change from 1985 to 2005 are shown in *Fig. 5 and Fig. 6.*

Table 4. *Areal extent of LULC classes in 1985, 1995 and 2005*

LULC Classes	**km^2** **1985**	**1995**	**2005**	**% of Total Area** **1985**	**1995**	**2005**
BL	7487	7396	6463	3.8	3.7	3.2
BU	1754	2481	3729	0.9	1.2	1.9
CL	131899	133795	135119	66.1	67.2	67.9
F	33951	32090	30570	17.1	16.1	15.4
GL	846	663	522	0.4	0.3	0.3
PL	683	678	692	0.3	0.3	0.3
PW	117	101	98	0.1	0.1	0.0
SL	12215	12385	12414	6.1	6.2	6.2
WB	4623	4020	3738	2.3	2.0	1.9
WL	4565	4531	4795	2.3	2.3	2.4

Table 5. *Change area matrix of LULC classes: (a) from 1985 to 1995, (b) 1995 to 2005 and (c) 1985 to 2005.*

(a) 1985 to 1995

	Area (km^2) 1995											
	Classes	**BL**	**BU**	**CL**	**F**	**GL**	**PL**	**PW**	**SL**	**WB**	**WL**	**Total**
Area (km^2) 1985	**BL**	7197	111	0	0	0	0	0	167	6	6	7487
	BU	0	1754	0	0	0	0	0	0	0	0	1754
	CL	0	491	130250	0	0	16	0	508	579	55	131899
	F	0	22	1184	32090	0	23	0	543	67	22	33951
	GL	0	23	150	0	661	0	0	12	0	0	846
	PL	0	0	47	0	0	633	0	3	0	0	683
	PW	0	6	11	0	0	0	100	0	0	0	117
	SL	112	62	951	0	0	0	0	10865	31	194	12215
	WB	0	0	1077	0	2	6	1	120	3321	96	4623
	WL	87	12	125	0	0	0	0	167	16	4158	4565
	Total	7396	2481	133795	32090	663	678	101	12385	4020	4531	

(b) 1995 to 2005

	Area (km^2) 2005											
	Classes	**BL**	**BU**	**CL**	**F**	**GL**	**PL**	**PW**	**SL**	**WB**	**WL**	**Total**
Area (km^2) 1995	**BL**	6226	477	0	0	0	0	0	346	70	277	7396
	BU	0	2481	0	0	0	0	0	0	0	0	2481
	CL	0	509	132438	0	0	77	0	282	440	49	133795
	F	0	10	610	30570	0	35	0	790	75	0	32090
	GL	0	2	117	0	517	0	0	27	0	0	663
	PL	0	0	89	0	0	545	0	44	0	0	678
	PW	0	0	6	0	0	0	95	0	0	0	101
	SL	192	163	960	0	0	31	0	10610	34	395	12385
	WB	0	0	726	0	5	4	3	121	3080	81	4020
	WL	45	87	173	0	0	0	0	194	39	3993	4531
	Total	6463	3729	135119	30570	522	692	98	12414	3738	4795	

(c) 1985 to 2005

	Area (km^2) 2005											
	Classes	**BL**	**BU**	**CL**	**F**	**GL**	**PL**	**PW**	**SL**	**WB**	**WL**	**Total**
Area (km^2) 1985	**BL**	6232	410	0	0	0	0	0	352	0	493	7487
	BU	0	1754	0	0	0	0	0	0	0	0	1754
	CL	0	1300	129746	0	0	21	0	157	593	82	131899
	F	0	112	1719	30570	0	61	0	1396	93	0	33951
	GL	0	27	299	0	463	0	0	57	0	0	846
	PL	0	0	115	0	0	521	0	47	0	0	683
	PW	0	0	15	0	0	0	98	0	0	4	117
	SL	186	93	1248	0	52	84	0	10161	27	364	12215
	WB	0	0	1144	0	7	5	0	169	2996	302	4623
	WL	45	33	833	0	0	0	0	75	29	3550	4565
	Total	6463	3729	135119	30570	522	692	98	12414	3738	4795	

BL- Barren land, BU- Built-up, CL- Cropland, F- Forest, GL- Grassland, PL- Plantation, PW- Permanent Wetland, SL- Shrub land, WB- Water bodies, WL- Wasteland.

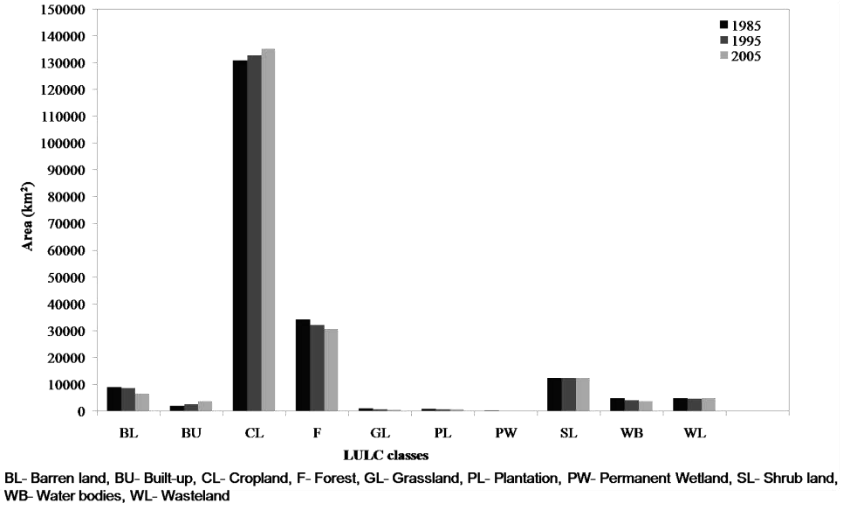
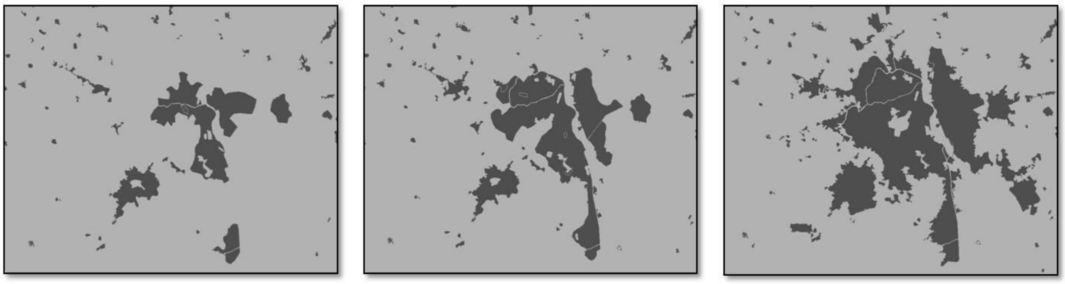
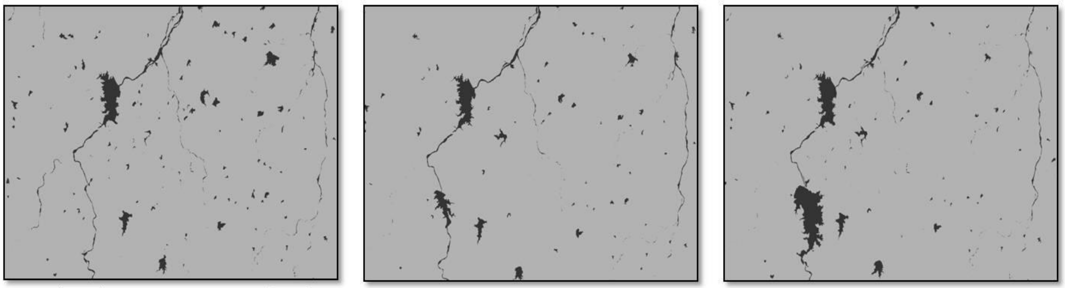


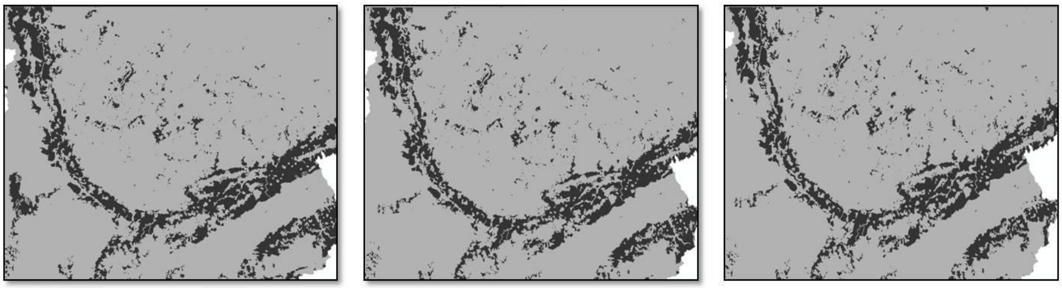
Figure 5. *Changes in the areal extent of LULC classes during 1985, 1995 and 2005*



(a) ■ **Built-up** ■ **Others**



(b) ■ **Water bodies** ■ **Others**



(c) ■ **Forest** ■ **Others**

Figure 6. *A few locations of LULC changes in the study area: (a) increase in the built-up areas at the expanse of shrub land, agricultural and forest around Delhi; (b) changes in part of water bodies of Madhya Pradesh; and (c) decrease in forest in part of Madhya Pradesh.*

Regression analysis

Before running logistic regression we have tested multi-collinearity between independent variables in SPSS software. No multi-collinearity issues between drivers are found as variance inflation factor (VIF) and tolerance values for all drivers are smaller than 10 and greater than 0.1, respectively. The VIF and tolerance values to check the multi-collinearity for drivers of 1995 are shown in *Table 6*. The performance of logistic regression was also evaluated on the basis of ROC curve and it has been observed that ROC values for all the classes lie between 0.5 and 1 indicating that the that performance of logistic regression is good enough to explain the patterns of LULC change by the drivers. The results of logistic regression with β and ROC values for 1995 and 2005 are summarized in *Table 7.*

Table 6. *VIF and tolerance values to check multi-collinearity between drivers of 1995*

DRIVERS		**TW**	**POP**	**AGR**	**RD**	**D_BU**	**D_FRS**	**D_WB**	**RAIN**	**TEMP**	**SLOPE**	**SOIL DEPTH**	**ELEV**
TW	**VIF**		1.9	1.7	3.5	1.1	1.2	1.0	1.9	3.4	4.4	1.0	4.5
	tolerance		0.5	0.5	0.2	0.8	0.7	0.9	0.5	0.2	0.2	0.9	0.2
POP	**VIF**	2.7		1.7	3.5	1.1	1.2	1.0	2.0	3.4	4.4	1.0	4.5
	tolerance	0.3		0.5	0.2	0.8	0.7	0.9	0.4	0.2	0.2	0.9	0.2
AGR	**VIF**	3.8	2.7		3.5	1.1	1.2	1.0	1.9	3.4	4.4	1.0	4.5
	tolerance	0.2	0.3		0.2	0.8	0.7	0.9	0.5	0.2	0.2	0.9	0.2
RD	**VIF**	6.0	4.3	2.8		1.1	1.2	1.0	1.8	3.4	4.4	1.0	4.5
	tolerance	0.2	0.2	0.3		0.8	0.7	0.9	0.5	0.2	0.2	0.9	0.2
D_BU	**VIF**	6.0	4.3	2.8	3.5		1.2	1.0	1.9	3.3	4.4	1.0	4.5
	tolerance	0.2	0.2	0.3	0.2		0.7	0.9	0.5	0.2	0.2	0.9	0.2
D_FRS	**VIF**	6.0	4.3	2.8	3.5	1.1		1.0	1.8	3.3	4.4	1.0	4.5
	tolerance	0.2	0.2	0.3	0.2	0.8		0.9	0.5	0.3	0.2	0.9	0.2
D_WB	**VIF**	6.0	4.3	2.8	3.5	1.1	1.2		2.0	3.4	4.4	1.0	4.5
	tolerance	0.2	0.2	0.3	0.2	0.8	0.7		0.4	0.2	0.2	0.9	0.2
RAIN	**VIF**	6.0	4.3	2.7	3.3	1.1	1.1	1.0		3.3	4.4	1.0	4.5
	tolerance	0.2	0.2	0.3	0.2	0.8	0.8	0.9		0.2	0.2	0.9	0.2
TEMP	**VIF**	6.0	4.3	2.8	3.5	1.1	1.2	1.0	1.9		3.9	1.0	4.0
	tolerance	0.2	0.2	0.3	0.2	0.8	0.8	0.9	0.5		0.2	0.9	0.2
SLOPE	**VIF**	6.0	4.3	2.8	3.5	1.1	1.2	1.0	2.0	3.0		1.0	3.1
	tolerance	0.2	0.2	0.3	0.2	0.8	0.7	0.9	0.4	0.3		0.9	0.3
SOIL DEPTH	**VIF**	6.0	4.3	2.8	3.5	1.1	1.2	1.0	2.0	3.4	4.4		4.5
	tolerance	0.2	0.2	0.3	0.2	0.8	0.7	0.9	0.4	0.2	0.2		0.2
ELEV	**VIF**	6.0	4.3	2.7	3.5	1.1	1.2	1.0	1.9	3.0	3.0	1.0	
	tolerance	0.2	0.2	0.3	0.2	0.8	0.8	0.9	0.5	0.3	0.3	0.9	

VIF- Variance inflation factor, TW- Total working population, POP- Total population, AGR- Number of agriculture workers, RD- Road length, D_BU- Distance to built-up, D_FRS- Distance to forests, D_WB- Distance to water bodies, RAIN- Annual rainfall, TEMP: Mean annual temperature, ELEV- Elevation.

The logistic regression results for built-up class show that total population (β: 4.23, 4.75), total working population (β: 4.59, 5.10) and road length (β: 3.58, 2.16) for 1995 and 2005 have strong positive influence for built-up area to change. The number of agriculture workers (β: -4.11, -5.43) show strong negative relation with built-up class. With respect to proximity drivers, distance to forests (β: -0.10, -0.57) have negative and distance to water bodies (β: 4.01, 2.58) have positive relation. Physical drivers, i.e. slope (β: -6.52, -6.16), elevation (β: -15.82, -13.21), annual rainfall (β: -5.15, -4.57) and mean annual temperature (β: -0.64, -0.68) have negative effect for the probability of finding built up areas.

The cropland class has positive relation with total population (β: 0.26, 0.15), total working population (β: 2.23, 0.52) and number of agriculture workers (β: 2.28, 1.45).

The negative β values for road length (β: -0.14, -2.91), distance to built-up (β: -1.42, -2.39) and distance to forests (β: -0.05, -0.61) show that as distance to built-up and forests increases, the probability of finding cropland class decreases. Distance to water bodies (β: 5.03, 2.09) is positively related to cropland class that increases the probability of finding cropland areas near the water bodies. With regard to physical drivers, slope (β: -17.29, -10.98), elevation (β: -16.91, -12.17) and mean annual temperature (β: -2.62, -2.60) have negative influence on the probability of finding cropland class; whereas, annual rainfall (β: 9.04, 8.13) and soil depth (β: 0.22, 0.21) show positive influence for the probability of finding cropland class.

Table 7. *Result of logistic regression between LULC classes and drivers of 1995 and 2005*

CLASSES	**TW**	**POP**	**AGR**	**RD**	**D_BU**	**D_FRS**	**D_WB**	**RAIN**	**TEMP**	**SLOPE**	**SOIL**	**ELEV**	**ROC**
	β values for 1995												
BU	4.59	4.23	-4.11	3.58	-8.11	-0.10	4.01	-5.15	-0.64	-6.52	0.16	-15.82	0.82
CL	2.23	0.26	2.28	-0.14	-1.42	-0.05	5.03	9.04	-2.62	-17.29	0.22	-16.91	0.80
F	-7.80	-9.19	-2.77	-3.85	-1.50	-8.29	0.50	4.12	-8.31	6.40	-0.31	6.89	0.84
SL	-0.88	-0.63	-0.60	-0.29	1.09	-3.73	0.20	0.67	0.43	1.32	-0.03	0.99	0.62
BL	1.95	-4.67	-3.94	-1.16	-8.96	3.32	-0.29	-5.86	2.65	2.13	-0.16	1.22	0.84
WL	-0.06	-4.28	1.96	1.60	-0.47	2.72	-1.85	-3.39	53.56	-4.06	0.13	-90.93	0.87
WB	-0.14	-1.11	-1.64	-0.27	0.88	0.33	-0.47	-0.11	0.34	-1.56	0.02	-7.26	0.63
CLASSES	**TW**	**POP**	**AGR**	**RD**	**D_BU**	**D_FRS**	**D_WB**	**RAIN**	**TEMP**	**SLOPE**	**SOIL**	**ELEV**	**ROC**
	β values for 2005												
BU	5.10	4.75	-5.43	2.16	-8.94	-0.57	2.58	-4.57	-0.68	-6.16	0.19	-13.21	0.82
CL	0.52	0.15	1.45	-2.91	-2.39	-0.61	2.09	8.13	-2.60	-10.98	0.21	-12.17	0.79
F	-4.10	-7.55	-1.41	-35.19	-1.67	-4.13	2.36	3.95	-8.18	6.16	-0.86	6.58	0.84
SL	-1.25	-0.34	-0.71	-4.67	1.12	-1.40	0.44	1.32	0.79	1.16	-0.06	0.98	0.62
BL	0.98	-15.67	-3.56	-0.30	-1.47	3.20	-2.36	-6.22	21.98	0.87	-0.08	1.51	0.89
WL	-0.09	-1.34	0.99	0.80	-1.88	0.69	-0.38	-3.36	51.54	-4.03	0.15	-88.30	0.85
WB	-0.22	-2.58	-0.73	-0.14	0.15	0.50	-0.75	-0.23	1.78	-1.94	0.01	-7.06	0.59

***Significant at 0.05 (95% confidence level)**

β is coefficient of independent variable; ROC (Area under Curve) is a measure of how well the independent variables are correctly predicts the value of dependent variable

Dependent Variables: **BL- Barren land, BU- Built-up, CL- Cropland, F- Forest, SL- Shrub land, WB- Water bodies, WL- Wasteland.** ***Independent Variables:*** **TW- Total working population, POP- Total population, AGR- number of agriculture workers, RD- Road length, D_BU- Distance to built-up, D_FRS- Distance to forests, D_WB- Distance to water bodies, RAIN- Annual rainfall, TEMP: Mean annual temperature, ELEV- Elevation.**

With regard to forest class, total population (β: -9.19, -7.55), total working population (β: -7.80, -4.10), number of agriculture workers (β: -2.77, -1.41) and road length (β: -3.85, -35.19) have negative effect for the presence of forest class. The physical drivers like annual rainfall (β: 4.12, 3.95), slope (β: 6.40, 6.16) and elevation (β: 6.89, 6.58) strongly influence presence of forest class in a positive way whereas temperature with high β values (β: -8.31, -8.18) show negative effect for the presence of forest class. Distance to water bodies (β: 0.50, 2.36) has positive influence while distance to built-up (β: -1.50, -1.67) has negative influence for the presence of forest class. The total population, total working population and road length with high negative β values and physical drivers (elevation, slope and annual rainfall) with high positive β values influence the probability of presence of forests.

In case of water bodies, total population (β: -1.11, -2.58), total working population (β: -0.14, -0.22), number of agriculture workers (β: -1.64, -0.73) and road length (β: -0.27, -0.14) have negative correlation which shows that as values of these drivers increases, there is a possibility of decrease in water bodies areas. Distance to built-up (β: 0.88, 0.15) and distance to forests (β: 0.33, 0.50) have positive effect for the presence of water bodies. Physical drivers like annual rainfall (β: -0.11, -0.23), slope (β: -1.56, -1.94) and elevation (β: -7.26, -7.06) have negative and mean annual temperature (β: 0.34, 1.78) has positive relation with water bodies. The total population and number of agriculture workers with negative β values indicate pressure on the water bodies.

In case of shrub land class, total population (β: -0.63, -0.34), total working population (β: -0.88, -1.25), number of agriculture workers (β: -0.60, -0.71) and road length (β: -0.29, -4.67) have negative effect for the presence of shrub land class. Proximity drivers like distance to built-up (β: 1.09, 1.12) and distance to water bodies (β: 0.20, 0.44) have positive relation while distance to forests (β: -3.73, -1.40) has negative relation with shrub land class. Physical drivers like annual rainfall (β: 0.67, 1.32), mean annual temperature (β: 0.43, 0.79), slope (β: 1.32, 1.16) and elevation (β: 0.99, 0.98) show positive relation while soil depth (β: -0.03, -0.06) show negative relation for the presence of shrub land.

In case of barren land, socioeconomic drivers like total population (β: -4.67, -15.67), number of agriculture workers (β: -3.94, -3.56) and road length (β: -1.16, -0.30) show negative relation while total working population (β: 1.95, 0.98) show positive relation. The increase in distance to built-up (β: -8.96, -1.47) and distance to water bodies (β: -0.29, -2.36) decreases the probability of finding barren land decreases. Distance to forests show positive (β: 3.32, 3.20) relation with barren land. Physical drivers like annual rainfall (β: -5.86, -6.22) and soil depth (β: -0.16, -0.08) have negative relation while mean annual temperature (β: 2.65, 21.98), slope (β: 2.13, 0.87) and elevation (β: 1.22, 1.51) have positive relation for the presence of barren land.

In case of wasteland class, total population (β: -4.28, -1.34) and total working population (β: -0.06, -0.09) have negative influence while number of agriculture workers (β: 1.96, 0.99) and road length (β: 1.60, 0.80) have positive influence. Distance to water bodies (β: -1.85, -0.38) and distance to built-up (β: -0.47, -1.88) show negative effect whereas distance to forests (β: 2.72, 0.69) shows positive effect for the presence of wasteland class. Very strong positive relation (β: 53.56, 51.54) with temperature and negative relation (β: -90.93, -88.30) with elevation are observed for the wasteland class. Thus, physical and proximity drivers strongly influence barren land and wasteland classes.

In summary, the above results indicate that LULC in the Yamuna river basin is influenced by a multitude of drivers (socioeconomic, demographic, physical and proximity drivers) in different combinations. Since physical drivers generally have very low temporal variability, socioeconomic, demographic and proximity drivers mainly control the LULC change processes in the study area in decreasing order of importance.

Discussion

The results indicate that significant LULC changes have occurred in the basin during the study period. The major LULC changes include the continuous expansion of built-up and cropland areas and shrinkage of forest areas. The LULC transitions in the study are of both reversible and irreversible nature. For example, transitions like barren land to shrub land, cropland to shrub land, cropland to water bodies, cropland to plantation, forest to plantation, grassland to cropland, plantation to cropland, shrub land to barren land, water bodies to cropland are some of reversible transitions; whereas, forest to built-up, cropland to built-up, grassland to built-up and shrub land to built-up can be considered as irreversible transitions.

It is likely that these changes will affect the biodiversity, soil erosion and soil and water quality of the area; however, the magnitude of impact will depend mainly upon the direction of land conversion. Conversion of forest to cropland can result in soil erosion, if good farming techniques are not adopted (Wasige et al., 2013). Decrease in forest may lead to loss of indigenous plant species and reduction of biodiversity (Aduah, 2014). It can be observed from change matrices that decrease in forest class are mainly due to conversion to cropland and built-up as a result of anthropogenic pressure. Forests with fertile soil, particularly in Indo-Gangetic plains, are generally more prone to deforestation for agriculture expansion (Geist and Lambin, 2004). While expansion of agricultural activity, as observed during the study period, is expected to have impact on freshwater ecosystem (Tilman, 1999), urban expansion and agriculture activities affect the water quality of the streams and reservoirs via increase in concentration of nutrients (Bonansea et al., 2016). Habitat loss and degradation are the major causes of biodiversity loss (Convention on biological diversity, 2010). The demand for additional land increases with increase in population that further causes the conversion of forests and cropland to built-up. Land cover changes like increase in built-up can reduce infiltration by virtue of impervious nature leading to increased soil erosion, sedimentation and pollution of river waters (Rogers, 1994).

The LULC changes in the basin are attributed to mainly socioeconomic, demographic and proximity drivers. The high β values of total population, number of agriculture workers, total working population and road length obtained from statistical analysis indicate the importance of these drivers on LULC changes. Among physical and proximity drivers, annual rainfall, slope, elevation and distance to water bodies are the main drivers contributing to LULC changes in the study area. The high negative β values for elevation and slope have a negative effect for the presence of water bodies in areas with high slope and elevation while the presence of forest is positively related with an increase in elevation and rising slope (Oprsal et al., 2016). Further, total population and number of agriculture workers with negative β values indicate pressure of population increase and agricultural expansion on water bodies. With agricultural expansion and increase in population, it is obvious that the demand for water supply has also increased in the basin over time. To meet this increased water demand, the Yamuna water is abstracted at different locations like Dakpathar, Hathinikund/ Tajewala, Wazirabad, Okhla and Gokul barrage for drinking water supply, irrigation, hydropower generation, etc. Various dams and reservoirs (e.g. Renuka, Lakhawar, Vyasi on Yamuna, Keshua on Tons, Rajghat on Betwa) have been constructed on river Yamuna and its tributaries. Such man-made interventions along the river courses may have an adverse impact on the overall quality of river water and aquatic ecosystem (Mishra, 2010).

On one hand, the basin has undergone phenomenal development with great advancement in different sectors like employment, health, education, transport, agriculture and technology, its population has also increased manifold simultaneously. So, there is a need to adopt sustainable development strategy to bring harmony between development, environment and society. The findings of the present study provide an understanding of LULC change processes which can be used as an input for sustainable land management not only in Yamuna river basin but also in other similar river basins.

One of the major limitations of the present study is that the drivers, particularly socioeconomic, demographic and meteorological (rainfall and temperature), were available at different scales (resolutions). It required a considerable effort in collating and converting the socioeconomic and demographic data available in tabular form to geo-referenced spatial format before carrying out statistical analysis to explore dependencies of drivers on LULC change. Despite limitations, the present study based on remote sensing, GIS and statistical analysis clearly brings out the linkage between LULC change and the drivers of such change at river basin scale. The statistical analysis at different scales (i.e. grid size) may further improve understanding the causes of LULC change. The results of this study provide first and foremost input towards simulating future LULC scenario.

Conclusions

In this study, we have investigated LULC change and driving forces causing such change in the Yamuna river basin of India. The major LULC changes observed during 1985-2005 at decadal interval are increase in cropland and built-up and decrease in forest and water bodies. The dominant processes of the LULC changes include transitions from forest, grassland, shrub land, water bodies and wasteland to cropland; transitions from cropland, shrub land, wasteland, grassland and forest to built-up; and conversion of forest to cropland, shrub land, plantation and built-up. The decrease in forest cover is mainly attributed to fragmentation, urbanisation, cropland expansion and construction of reservoirs and dams. Further, it is noticed that most of LULC transitions in the study are reversible in nature.

The statistical analysis suggests that LULC changes are driven by a combination of socioeconomic, demographic, physical and proximity drivers. However, in general it is found that the socioeconomic, demographic and proximity drivers mainly influence the LULC change processes in decreasing order of importance. The population increase with accompanied development exerted pressure on existing land in pursuit of better living standards leading to LULC change.

The present study is an example to understand the dynamics and causes of LULC change using multiple techniques, i.e. remote sensing, GIS and statistics. Despite limitations of having driver datasets at coarse and different scales, the study clearly brings out the linkage between LULC change and the drivers of such change in a large study area of river basin size. The statistical analysis at different scales (i.e. grid size) may further improve understanding the causes of LULC change. The insights gained through this study will be useful in simulating future LULC scenarios and thus in providing inputs to sustainable land management policy making.

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REFERENCES

[1] Aduah, M.S., Warburton, M.L., Jewitt, G. (2015): Analysis of land cover changes in the Bonsa catchment, Ankobra basin, Ghana. –Applied ecology and environmental research 13(4): 935-955.

[2] Bonansea, M., Ledesma, C., Rodriguez, M.C. (2016): Assessing the impact of land use and land cover on water quality in the watershed of a reservoir. –Applied ecology and environmental research 14(2): 447-456.

[3] Congalton, R.G. (1991): A review of assessing the accuracy of classifications of remotely sensed data. –Remote sensing of environment 37(1): 35-46.

[4] Hosmer, D.W., Lemeshow, S. (2000): Applied Logistic Regression. –John Wiley and Sons, New Jersey, USA.

[5] Lambin, E.F., Turner, B.L., Geist, H.J., Agbola, S.B., Angelsen, A., Bruce, J.W., Coomes, O.T., Dirzo, R., Fischer, G., Folke, C., George, P.S. (2001): The causes of land-use and land-cover change: moving beyond the myths. –Global environmental change 11(4): 261-269.

[6] Loveland, T.R., Belward, A.S. (1997): The IGBP-DIS global 1km land cover data set, DISCover: first results. –International Journal of Remote Sensing 18(15): 3289-3295.

[7] Mac Nally, R. (2000): Regression and model-building in conservation biology, biogeography and ecology: the distinction between–and reconciliation of–‘predictive’and ‘explanatory’models. –Biodiversity & Conservation 9(5): 655-671.

[8] Martinez, J.M.A., Suarez-Seoane, S., Calabuig, E.D.L. (2011): Modelling the risk of land cover change from environmental and socio-economic drivers in heterogeneous and changing landscapes: The role of uncertainty. –Landscape and Urban Planning 101(2): 108-119.

[9] Millington, J.D., Perry, G.L., Romero-Calcerrada, R. (2007): Regression techniques for examining land use/cover change: a case study of a Mediterranean landscape. –Ecosystems 10(4): 562-578.

[10] Mishra, A.K. (2010): A River about to Die: Yamuna. –J. Water Resource and Protection 2: 489-500.

[11] Nefeslioglu, H.A., Gokceoglu, C., Sonmez, H. (2008): An assessment on the use of logistic regression and artificial neural networks with different sampling strategies for the preparation of landslide susceptibility maps. –Engineering Geology 97(3): 171-191.

[12] Obrien, R.M. (2007): A caution regarding rules of thumb for variance inflation factors. –Quality & Quantity 41(5): 673-690.

[13] Oprsal, Z., Kladivo, P., Machar, I. (2016): The role of selected biophysical factors in long-term land-use change of cultural landscape. –Applied ecology and environmental research 14(2): 23-40.

[14] Outlook, G.B. 3 (2010): Secretariat of the convention on biological diversity. Montreal 94.

[15] Pontius, R.G., Schneider, L.C. (2001): Land-cover change model validation by an ROC method for the Ipswich watershed, Massachusetts, USA. –Agriculture, Ecosystems & Environment 85(1): 239-248.

[16] Rai, R.K., Upadhyay, A., Ojha, C.S.P., Singh, V.P. (2012): Salient Features of the Yamuna River Basin. –Springer, Netherlands.

[17] Rogers, P. (1994): Hydrology and Water Quality. – In: Meyer, W.B., Turner Ii, B.L. (eds.) Changes in Land Use and Land Cover: A Global Perspective, Cambridge University Press, Cambridge, UK.

[18] Roy, P.S., Roy, A., Joshi, P.K., Kale, M.P., Srivastava, V.K., Srivastava, S.K., Dwevidi, R.S., Joshi, C., Behera, M.D., Meiyappan, P., Sharma, Y. (2015): Development of decadal (1985-1995-2005) land use and land cover database for India. –Remote Sensing 7(3): 2401.

[19] Shen, Z., Lu, C., Yin, R., Qi, J. (2013): Land cover changes in Northeast China from the late 1970s to 2004. –Applied ecology and environmental research 11(1): 67-78.

[20] Tian, H., Banger, K., Bo, T., Dadhwal, V.K. (2014): History of land use in India during 1880–2010: Large-scale land transformations reconstructed from satellite data and historical archives. –Global and Planetary Change 121: 78-88.

[21] Tilman, D. (1999): Global environmental impacts of agricultural expansion: the need for sustainable and efficient practices. –Proceedings of the National Academy of Sciences 96(11): 5995-6000.

[22] Tripathi, A., Prasad, A.R. (2010): Agricultural development in India since independence: A study on progress, performance, and determinants. –Journal of emerging knowledge on emerging markets 1(1): 8.

[23] United Nations Centre for Human Settlements (1996): An urbanizing world: global report on human settlements. –Oxford University Press, USA.

[24] Veldkamp, A., Fresco, L.O. (1997): Reconstructing land use drivers and their spatial scale dependence for Costa Rica (1973 and 1984). –Agricultural systems 55(1): 19-43.

[25] Verburg, P.H., Van Eck, J.R.R., De Nijs, T.C., Dijst, M.J., Schot, P. (2004): Determinants of land-use change patterns in the Netherlands. –Environment and Planning B: Planning and Design 31(1): 125-150.

[26] Viles, H., Goudie, A. (1997): The Earth Transformed: An Introduction to the Human Impact on the Environment. –Blackwell, Oxford, UK.

[27] Vu, Q.M., Le, Q.B., Frossard, E., Vlek, P.L. (2014): Socio-economic and biophysical determinants of land degradation in Vietnam: An integrated causal analysis at the national level. –Land Use Policy 36: 605-617.

[28] Wang, D., Gong, J., Chen, L., Zhang, L., Song, Y., Yue, Y. (2013): Comparative analysis of land use/cover change trajectories and their driving forces in two small watersheds in the western Loess Plateau of China. –International Journal of Applied Earth Observation and Geoinformation 21: 241-252.

[29] Wasige, J.E., Groen, T.A., Smaling, E., Jetten, V. (2013): Monitoring basin-scale land cover changes in Kagera Basin of Lake Victoria using ancillary data and remote sensing. –International Journal of Applied Earth Observation and Geoinformation 21: 32-42.

[30] Yirsaw, E., Wu, W., Temesgen, H., Bekele, B. (2016): Effect of temporal land use/land cover changes on Ecosystem Services Value in coastal area of China: the case of Su-Xi-Chang region. –Applied ecology and environmental research 14(3): 409-422.

[31] Zgola, T. (2014): The response of aquatic plants to catchment land use for different types of lowland rivers. –Applied ecology and environmental research 12(1): 143-162.

APPENDICES

***Appendix 1.** VIF and tolerance values to check multi-collinearity between drivers of 2005*

DRIVERS		**TW**	**POP**	**AGR**	**RD**	**D_BU**	**D_FRS**	**D_WB**	**RAIN**	**TEMP**	**SLOPE**	**SOIL DEPTH**	**ELEV**
TW	**VIF**		2.2	3.9	1.2	1.4	1.3	1.1	1.9	3.7	4.3	1.0	5.4
	tolerance		0.4	0.3	0.9	0.7	0.8	0.9	0.5	0.3	0.2	1.0	0.2
POP	**VIF**	3.4		2.4	1.2	1.4	1.3	1.1	1.8	3.7	4.3	1.0	5.4
	tolerance	0.3		0.4	0.8	0.7	0.7	0.9	0.5	0.3	0.2	1.0	0.2
AGR	**VIF**	8.9	5.7		1.2	1.4	1.3	1.1	1.8	3.7	4.3	1.0	5.4
	tolerance	0.2	0.2		0.8	0.7	0.8	0.9	0.5	0.3	0.2	1.0	0.2
RD	**VIF**	9.1	6.0	5.6		1.4	1.3	1.1	1.9	3.7	4.3	1.0	5.4
	tolerance	0.2	0.2	0.2		0.7	0.8	0.9	0.5	0.3	0.2	1.0	0.2
D_BU	**VIF**	9.2	6.0	5.7	1.2		1.3	1.0	1.8	3.6	4.3	1.0	5.1
	tolerance	0.2	0.2	0.2	0.8		0.8	1.0	0.6	0.3	0.2	1.0	0.2
D_FRS	**VIF**	9.2	6.0	5.6	1.2	1.4		1.1	1.8	3.6	4.2	1.0	5.4
	tolerance	0.2	0.2	0.2	0.8	0.7		0.9	0.5	0.3	0.2	1.0	0.2
D_WB	**VIF**	9.1	6.0	5.6	1.2	1.4	1.3		1.8	3.7	4.3	1.0	5.4
	tolerance	0.2	0.2	0.2	0.8	0.7	0.7		0.5	0.3	0.2	1.0	0.2
RAIN	**VIF**	9.2	5.9	5.2	1.2	1.3	1.3	1.1		3.4	4.3	1.0	5.0
	tolerance	0.2	0.2	0.2	0.8	0.8	0.8	1.0		0.3	0.2	1.0	0.2
TEMP	**VIF**	9.1	6.0	5.6	1.2	1.4	1.3	1.1	1.7		3.7	1.0	4.4
	tolerance	0.2	0.2	0.2	0.8	0.7	0.8	0.9	0.6		0.3	1.0	0.2
SLOPE	**VIF**	9.2	6.0	5.6	1.2	1.4	1.3	1.1	1.9	3.3		1.0	4.0
	tolerance	0.2	0.2	0.2	0.8	0.7	0.8	0.9	0.5	0.3		1.0	0.2
SOIL DEPTH	**VIF**	9.2	6.0	5.7	1.2	1.4	1.3	1.1	1.9	3.7	4.3		5.4
	tolerance	0.2	0.2	0.2	0.8	0.7	0.7	0.9	0.5	0.3	0.2		0.2
ELEV	**VIF**	9.2	6.0	5.5	1.2	1.3	1.3	1.1	1.7	3.1	3.2	1.0	
	tolerance	0.2	0.2	0.2	0.8	0.7	0.7	0.9	0.6	0.3	0.3	1.0	

VIF- Variance inflation factor, TW- Total working population, POP- Total population, AGR- Number of agriculture workers, RD- Road length, D_BU- Distance to built-up, D_FRS- Distance to forests, D_WB- Distance to water bodies, RAIN- Annual rainfall, TEMP: Mean annual temperature, ELEV- Elevation

***Appendix 2.** Mathematical expressions, formulae used for accuracy assessment*

Generate an error matrix, which sometimes is also known as confusion matrix or contingency table. In this matrix, classified data are given as rows and reference data are given as columns for each sample point. An example is illustrated below:

		Reference data										
	LULC Classes	**BL**	**BU**	**CL**	**F**	**GL**	**PL**	**PW**	**SL**	**WB**	**WL**	**Row Total**
Classified data	**BL**	519	1	23	87				3			633
	BU	13	1634	72	2			1	7	2		1731
	CL		4	1147	24		1		25	5	3	1209
	F		5	109	1544		4		18		1	1681
	GL		96	17	2	315	6			1		437
	PL		3	12		2	415					432
	PW			8				116				124
	SL	12	2	75		46			946		4	1085
	WB			116	6	10			3	764		899
	WL	3	2	52	7	19			1	1	687	772
	Column Total	547	1747	1631	1672	392	426	117	1003	773	695	**9003**

BL- Barren land, BU- Built-up, CL- Cropland, F- Forest, GL- Grassland, PL- Plantation, PW- Permanent Wetland, SL- Shrub land, WB- Water bodies, WL- Wasteland.

Overall Accuracy

[Sum of the classes present diagonally/ Total no. of classes] × 100

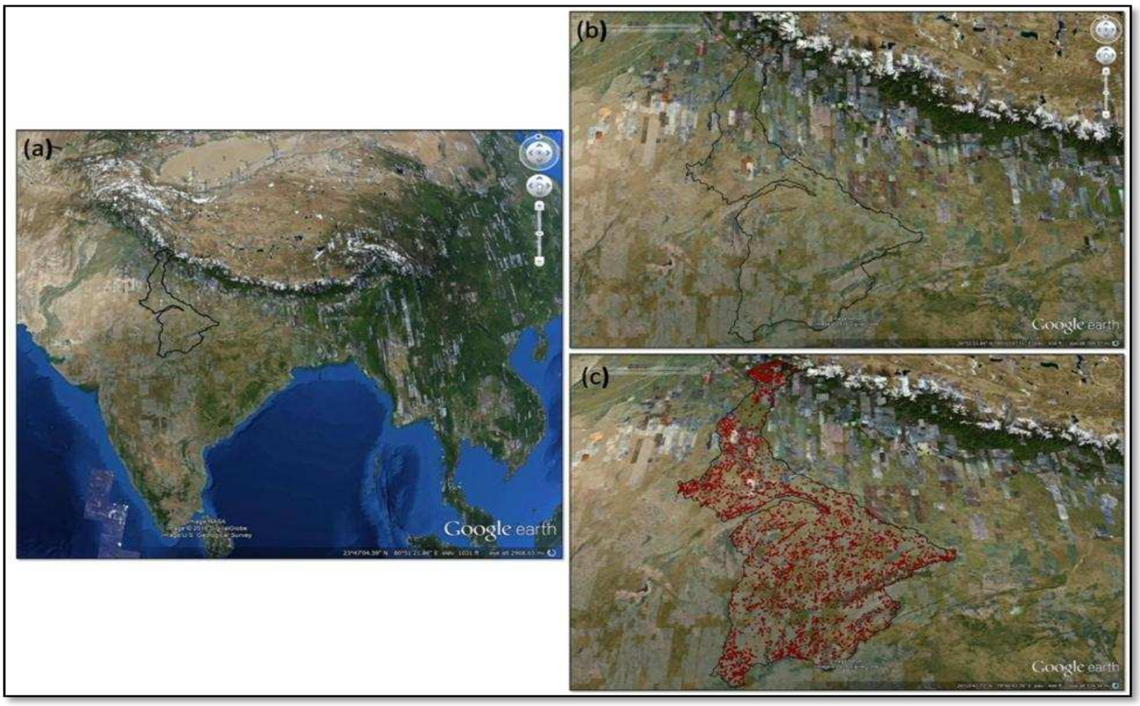
$$= [(519+1634+1147+1544+315+415+116+946+764+687)/9003] \times 100$$

$$= 8087/9003=89.82\%$$

Users accuracy
[Individual class present diagonally/ Row total of that class] × 100
For example user accuracy of Barren land class:
$= [519/633] \times 100 = 81.9\%$

Producer accuracy
[Individual class present diagonally/ Column total of that class] × 100
For example producer accuracy of Barren land class:
$= [519/547] \times 100 = 94.9\%$

Kappa coefficient
[Observed accuracy – Chance agreement]/ [1– Chance agreement]
Chance agreement = ∑[Product of row and column marginals for each class]



Appendix 3. *(a) Yamuna basin boundary overlaid over Google earth imagery of India, (b) Extended view of Yamuna basin boundary over Google earth imagery, (c) Random points (total no. 9003) used for accuracy assessment of LULC classes overlaid above the Google earth imagery.*

Appendix 4. *Important web resources*

http://glcf.umd.edu/data/landsat
http://data.gov.in/keywords/land-use-statistics
http://bhuvan.nrsc.gov.in
http://www.india-wris.nrsc.gov.in
http://www.indiastat.com
http://www.censusindia.gov.in

DOI: http://dx.doi.org/10.15666/aeer/1403_773792