Long-term changes in the herb layer of a southern Swedish broadleaf forest

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Abstract

The forest herb layer is reacting fast to the changes in the environment due to its sensitivity and short life cycle. The forest overstory has a strong influence over the availability of the environmental factors and also represents a powerful competitor for the available resources. The species of the herbaceous ground flora have different requirements and sensitivities in regard to the environment, therefore each of them has to be treated separately in order to detect the factors that influence and direct the changes in their abundance.

Measurements from 30 permanent sample plots extending during the period 1988-2015 in a hornbeam forest in Stenshuvud National park, southern Sweden, were used to quantify the inter-annual variation and long-term trends of the herb layer and its interactions with the environmental factors. Mean Ellenberg indicator values were used to assess the responses of the herb layer community to the variations in environmental factors.Results of paired t-tests show that the species richness is decreasing, while the evenness remained quite stable, probably partly due to the closing up of the canopy which is also reflected by the Ellenberg mean indicator values for light. Ellenberg mean indicator values also indicate that the temperatures have been increasing, while the climate became more oceanic. However, mean indicator values also suggest a decreasing soil moisture, soil reaction and available nitrogen. Overall, the total cover has increased, while some species like *Hedera helix* and *Hepatica nobilis* have manifested declining covers through time. Fifteen of the ground flora species present in 1988 were absent in 2015, while only two species colonised the area.

Roe deer has probably played an important role in shaping the abundance of several species, due to its rapid increase in population density until early 1990s. The regression analyses revealed that precipitation amounts during spring have quite strong relationships with both total cover and covers of some species.

Keywords: Ellenberg indicator values, herb layer, hornbeam forest, Stenshuvud National Park, vegetation change

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Introduction

The herbaceous layer of temperate forests is comprised of sensitive elements which live in an environment shaped, among others, by the overstory (Gilliam & Roberts, 2003) and are competing for the available resources not only with each other, but also with the trees forming the overstory.

Long-term studies can be used for detecting if any changes occurred on the abundance or diversity of the herb layer and to determine which and how environmental factors may have caused these changes (e.g. Bernhardt-Römermann et al., 2015; Brunet and Tyler, 2000; Corney et al., 2008). Assessing the variation of the vegetation composition is also an important step in the process of ecological restoration (Korb & Fulé, 2008) and may require the establishment of permanent plots and yearly measurements (Brunet & Tyler, 2000).

Long-term monitoring studies with yearly measurements are important for following the ecological development of communities (Korb and Fulé, 2008) and the effects of environmental factors that might not be immediate (Rogers, 1983). The effects of environmental factors can be cumulative, opposite or synergistic, therefore they need to be separated (Corney et al., 2008) in order to be able to determine the influence of each of them on the herb layer.

Also, the environmental factors influence each other, for example changes in light conditions can determine changes in temperature, moisture and water availability (Neufeld and Young, 2003). Further on, higher soil temperatures speed up the nitrogen mineralization processes which increases the soil nitrogen together with the atmospheric deposition (Diekmann et al., 1999). While increased availability of nutrients is beneficial for plant growth (Diekmann et al., 1999), it can in the same time increase the susceptibility to herbivory (Anderson, 2003).

Herbivory is affecting not only the cover of the herbaceous species in a certain year, but also their reproduction or biomass in the following years. The extent to which species are affected by herbivores is dependent not only on their palatability, but also on the proportion of preferred herb species for forage and their adaptations, with some species benefiting from being browsed (Anderson, 2003).

The rapid increase of roe deer densities in the past decades (Bergström & Danell, 2009) along with the absence of predators such as wolves (Naturvårdsverket, 2015) represent a potentially high influential factor in shaping the composition of the herb layer in many forests. The absence of predators may lead to overgrazing which can suppress the development of many plant species (herbs and trees), as it happened in Yellowstone National Park before the reintroduction of wolves (Ripple and Beschta, 2012).

In the previous decades, soil pH has decreased in northwestern Europe (Tyler et al., 2002), the species which are sensitive to very acid soils being expected to suffer on long term due to the acidification of the soil (Brunet et al., 1997; Diekmann et al., 1999) caused among others by pollution with sulphur dioxide (Ellenberg, 1988). According to Tyler et al. (2002), the most likely negative effects would be a decrease in cover of acid-sensitive species such as *Hepatica nobilis* and *Hedera helix*, while acid-tolerant species such as *Holcus mollis* would increase its cover, as a response to the soil reaction change.

In addition, current global warming has an array of cascading effects on the forest ecosystem, higher temperatures leading not only to changes in the flora composition, but also speeding up the processes taking place in the soil, affecting nutrient availability (Hedwall & Brunet, 2016).

Furthermore, long-term studies of the herb layer can be used also in conservation biology, for analysing trends in species diversity (Ricketts et al., 1999). Following the dynamics of the herbaceous layer and consequently determining the causal factors, represent useful tools for keeping track of the negative changes in habitat area (loss or fragmentation) and of the increase in habitat area of alien species with an invasiveness potential. In this manner, proactive measures can be taken in order to stop or slow down decreases in cover of native species or increases in cover of alien species.

Study aim

The aim of this study is to analyse the inter-annual variation and long-term change of the cover of herb layer species in a European temperate broadleaf forest and its causal relations with selected environmental factors (precipitation amount, soil chemistry, herbivore densities). The study is based on data collected between 1988 and 2015, including surveys of summer flora for 20 consecutive years (1988-2008). The variability of the cover of a number of selected field layer species and its causal factors, for the first decade, have been previously analysed (Brunet & Tyler, 2000), but this study includes a broader and more detailed analysis by including all the field layer species and data from the second decade, as well as an attempt to assess the influence of possible factors on the abundance, with the help of Ellenberg indicator values.

The following hypotheses have been formulated and tested throughout the study:

1) Inter-annual fluctuations are caused by short-term variation in precipitation and temperature.
2) Long-term changes are caused by long-term climatic trends.
3) Long-term changes are caused by changing soil conditions.
4) Long-term changes are caused by long-term changes in herbivore densities.

Materials and methods

Study site

The study has been performed in Stenshuvud National Park situated on the coast of the Baltic Sea in southern Sweden (55° 40' N, 14° 16' E). The permanent plots were set in a hornbeam (*Carpinus betulus* L.) stand of 60-90 years of age which developed from a formerly grazed hazel (*Corylus avellana* L.) stand. The soil is a dystric cambisol which developed from a non-calcareous silt-moraine (Tyler, 1994). In the last three decades there haven't been carried out any kind of silvicultural operations.

Data collection

In 1988 have been established 30 circular permanent sample plots (figure 1) as a 5x6 network, each having a 5 m^2 surface (Brunet & Tyler, 2000), in the down-slope the plots being placed at 4 m distance (between the centres of adjacent plots) and in the along-slope at 8 m (Tyler, 2001). The percentage cover of the vernal flora species was estimated in each plot, in April 1988 and 2015, the same measurements being performed for the summer flora in July of every year in the period 1988-2008 (except 2001) and in 2015 (Tyler & Brunet, unpublished data).

Figure 1. Examples of sample plots in the study area (Source: Ștefania-Elena Purcaru, 2016)

The total monthly precipitation measurements and monthly mean temperatures were obtained from the Swedish Meteorological and Hydrological Institute (SMHI) and are from the meteorological stations of Simrishamn, 12 km SE (1988-1995) and Brösarp, 12 km NW (1996-2015).

Chemical analyses of the soil were performed in 1988, 1998 and 2007. For that purpose, samples of soil were collected from the first 5 cm under the litter, at 4 opposite edges of all sample plots (n = 30). The sub-samples of soil corresponding to each sample plot were merged together and sifted through a 2 mm mesh. The next step was extracting (2 h) soil at field moisture (20g) with 100 ml 0.2 M KCl, which was left to rest for one hour. The pH was determined by the electrometric method, using ~10 ml from the supernatant suspension (Brunet & Tyler, 2000).

Data analysis

Assessing changes in diversity

Using spring and summer percentage covers of each species in each plot, from 1988 and 2015, the following diversity indices were calculated:

1) Species richness (S), the total number of species found in a sample plot;
2) Evenness (E), expresses the level of representation uniformity of the species (based on cover estimated), having values between 0 and 1, the value one signifying a completely equal distribution of all species;
3) Shannon diversity (H), measures diversity by taking into consideration both species richness and evenness (Bernhardt-Römermann et al., 2015).

The changes in species diversity were assessed by applying paired t-tests, using pairs of the same sample plots surveyed 1988 and 2015.

In addition, the lists of species present in the sample plots for 1988 and 2015 were compared with the purpose of identifying which species have disappeared or have colonised the study site.

Assessing changes in environmental factors

Ellenberg's indicator values are one of the methods used to describe a plant community from the ecological point of view (van der Maarel, 2009). For each vascular plant, there are indicator values for 6 environmental factors (3 climatic and 3 edaphic), expressed on a scale from 1 to 9, from low to very high value of the factor, and an additional x symbol representing indifference.

The climatic factors are:

- L – light (1- poor light conditions, 9- full light);
- T – temperature (1-arctic/alpine climate, 9- Mediterranean climate);
- K – continentality (1- Atlantic coast, 9- inland Eurasia).

The edaphic factors are:

- M – moisture (1- dry rocky slopes, 9- wet marshy ground, in addition 10-12 shallow to deep water);
- R – reaction (1- very acid, 9- very alkaline);
- N – nitrogen (1- low availability of mineral nitrogen, 9- excess of mineral nitrogen) (Ellenberg, 1988).

Using the indicator values of all the plant species in a community to calculate average factor values, results in a "rough interpretation of site conditions" (Ellenberg, 1988).

Un-weighted mean Ellenberg indicator values have been calculated for 1988 and 2015, by using the Ellenberg indicator values of each species for 6 environmental factors (light, temperature, continentality, moisture, acidity, nitrogen). The resulting values have been compared using the paired t-test for plot pairs 1988 and 2015.

Since the canopy is leafless in the spring and therefore the spring flora experiences different light conditions, the analysis of the Ellenberg's indicator values for light has been done only for the summer flora.

Assessing the inter-annual variations

For a more detailed view of the changes happening from year to year (1988-2008), the total summer covers in each plot have been calculated by summing up the percentage covers of all plant species in the respective plots. Furthermore, the trends for the cover of the six most frequent plant species (*Oxalis acetosella* L., *Stellaria holostea* L., *Lamium galeobdolon* (L.) Crantz, *Hedera helix* L., *Holcus mollis* L. and *Hepatica nobilis* Schreb.) have also been analysed. Linear regression analyses have been applied in the case of the six frequent species to highlight the nature of the variations.

Assessing the relationship between cover and precipitation

The influence of the amount of precipitation on the cover of the ground flora has been analysed through regression, separately for the two decades (1988-1998, 1998-2008). For this purpose were used, together and separately, the precipitation sums from July to September of the previous year and from April to June of the year taken into consideration. As for cover, it was used the mean cover including all species and also the mean cover of the six main plant species.

Results

Changes in species diversity

Comparing the species richness in 1988 (M=14.07, SD=2.02) and 2015 (M=9.27, SD=2.33) was found a statistically significant decrease of 4.8 (95% CI, 3.80 to 5.80), $t(29) = 9.80$, $p = 1.047 \cdot 10^{-10}$. As shown in figure 2, only in two sample plots was observed an increased species richness (plots 5 and 49) and two had an equal species diversity in both years (plot 15 and 37).

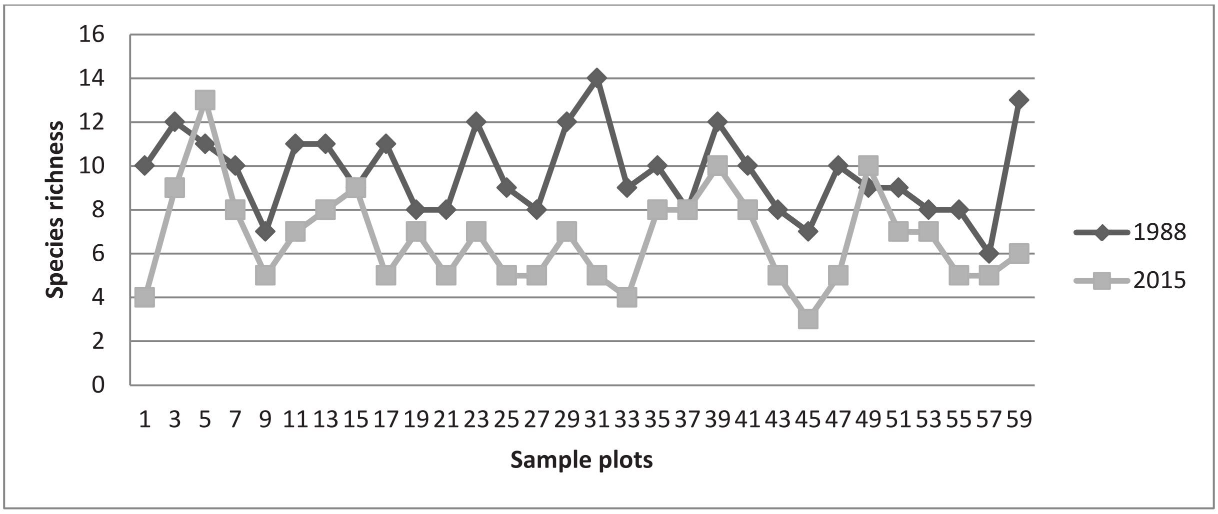

Figure 2. Changes in species richness in each sample plot (1988 and 2015)

A statistical significant decrease of 0.35 (95% CI, 0.23 to 0.48), $t(29) = 5.83$, $p = 2.571 \cdot 10^{-6}$ has been observed between the Shannon diversity index from 1988 (M=1.79, SD=0.27) and the Shannon diversity index from 2015 (M=1.43, SD=0.24). Three of the sample plots (5, 7 and 53) have had an increase, all the rest decreasing (figure 3).

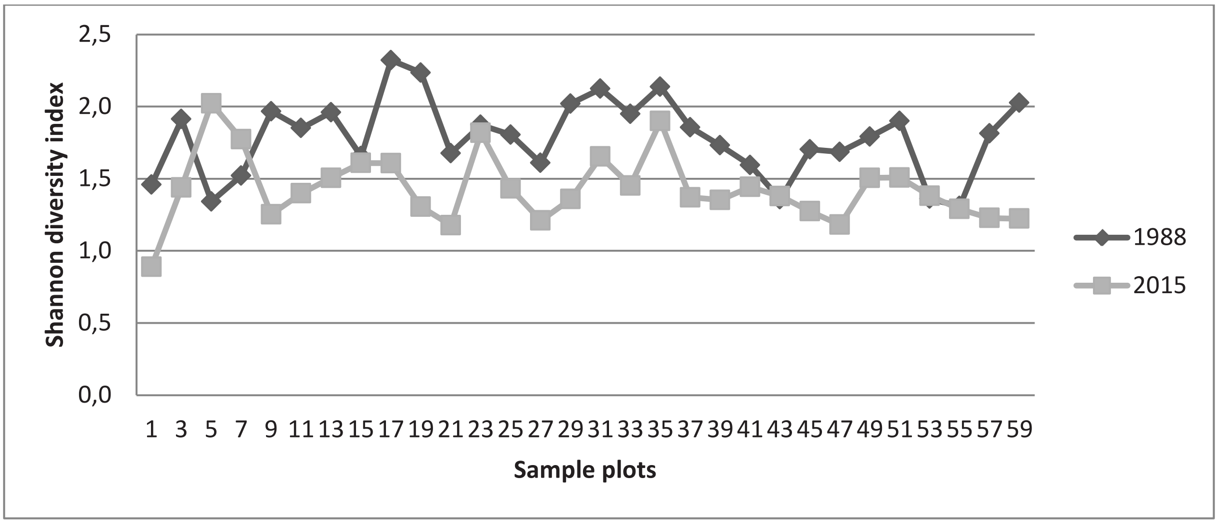

Figure 3. Changes in Shannon diversity in each sample plot (1988 and 2015)

However, the decrease of 0.02 (95% CI, - 0.01 to 0.06), t(29) = 1.32, p = 0.199 between the evenness in 1988 (M=0.68, SD=0.08) and in 2015 (M=0.65, SD=0.08) was not statistically significant.

Out of the species present in 1988, 15 species disappeared until 2015, of which 14 summer and one spring species. The summer species that are not present anymore in 2015 are: *Carex sylvatica* Huds., *Carpinus betulus* L., *Corylus avellana* L., *Fragaria vesca* L., *Geum urbanum* L., *Lathyrus linifolius* (Reichard) Bässler, *Lonicera xylosteum* L., *Pulmonaria obscura* Dumort., *Quercus robur* L., *Ribes alpinum* L., *Ulmus glabra* Huds., *Urtica dioica* L., *Viburnum opulus* L., *Vicia sepium* L. and the spring species *Corydalis intermedia* (L.) Mérat. Only two summer species have colonised the site, namely *Galium aparine* L. and *Melica uniflora* Retz.

Hornbeam was present in the ground layer throughout the years in most of the sample plots (27 out of 30), except for 1998-2000 and 2015 when it was absent. Pedunculate oak was present in 3 sample plots only in 1988. Hazel was found in 11 sample plots through time, but only until 1997. *Urtica dioica* existed in only one sample plot until 2007.

Changes in environmental factors

Ellenberg's indicator values for light

The paired t-test performed between the mean Ellenberg light indicator values for the summer species from 1988 and 2015 has pointed out a statistically significant decrease of 0.45 (table 1). As it can be observed in figure 4, most of the sample plots experienced a decrease in the mean Ellenberg light indicator values for summer flora.

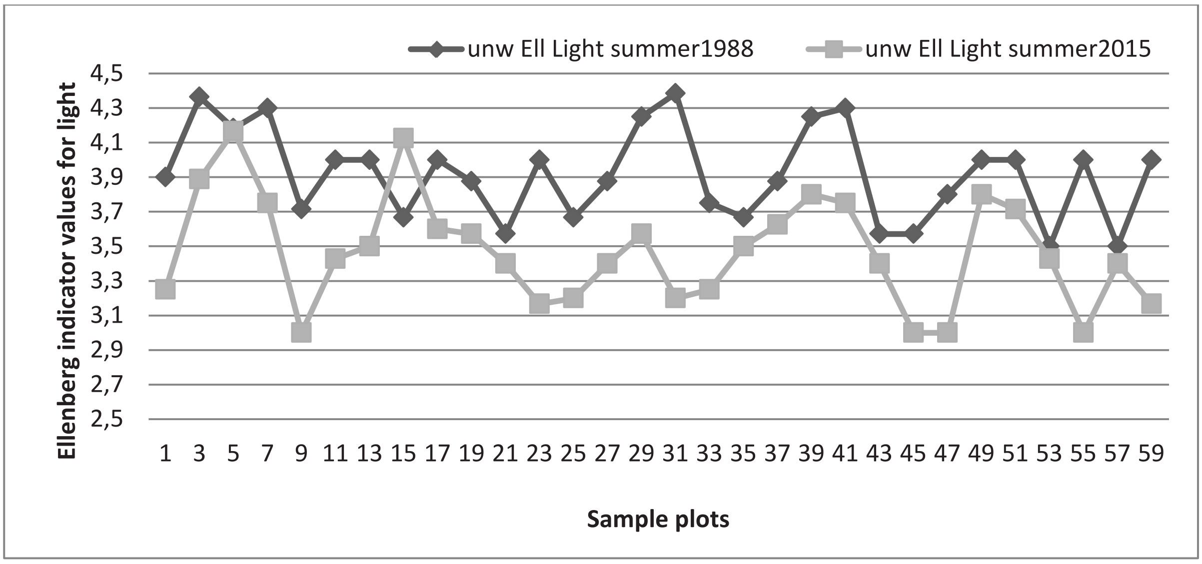

Figure 4. The variation of the mean Ellenberg light indicator values for summer flora (1988 and 2015)

Ellenberg's indicator values for temperature

The mean Ellenberg temperature indicator values in 1988 are statistically significant lower with 0.06 than in 2015 (table 1), the increasing trend being noticeable in figure 5.

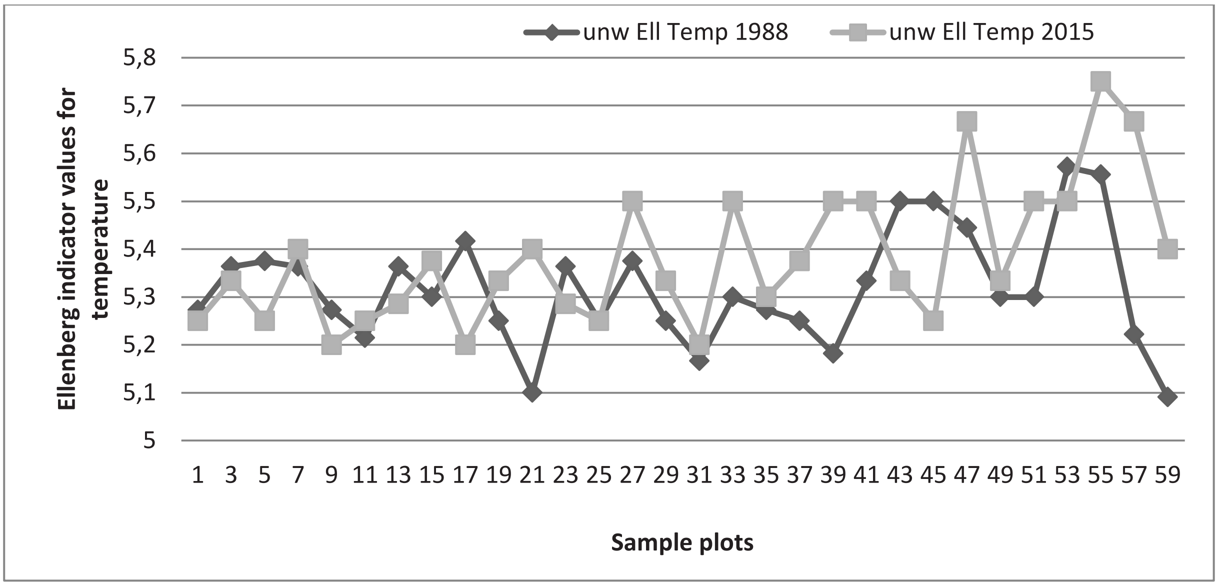

Figure 5. The variation of the mean Ellenberg temperature indicator values (1988 and 2015)

Ellenberg's indicator values for continentality

Comparing the mean Ellenberg continentality indicator values in 1988 and 2015, a statistically significant decrease of 0.16 was found (table 1), as shown in figure 6.

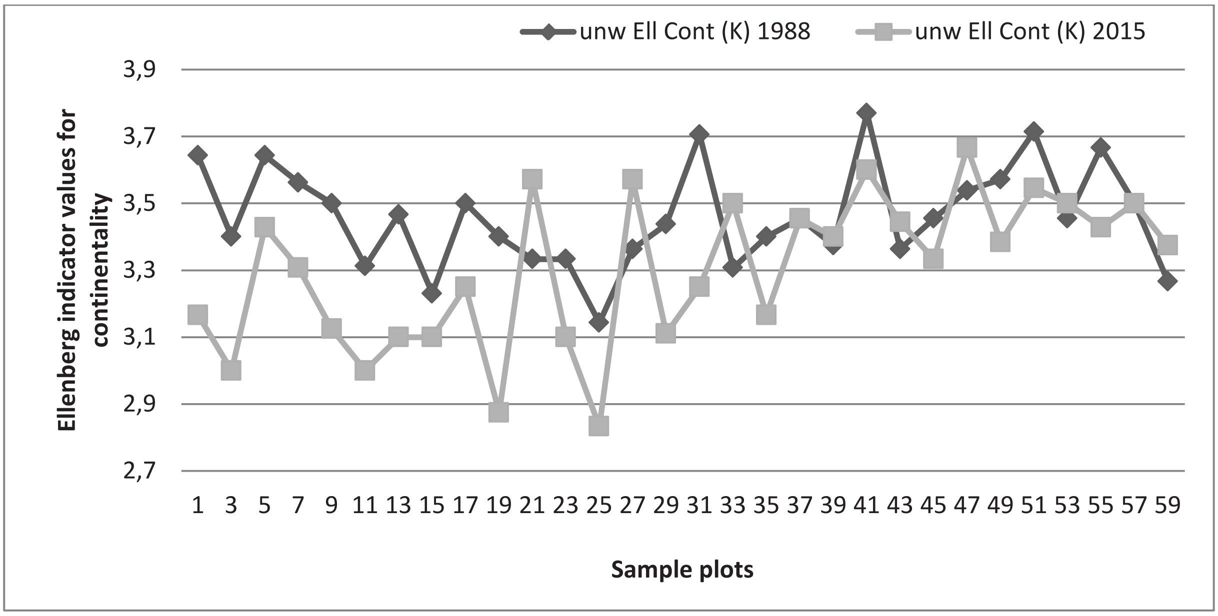

Figure 6. The variation of the mean Ellenberg continentality indicator values (1988 and 2015)

Ellenberg's indicator values for moisture

A statistical significant decrease of 0.09 (table 1) has been observed between the mean Ellenberg moisture indicator values from 1988 and the ones from 2015, as it can be observed in figure 7.

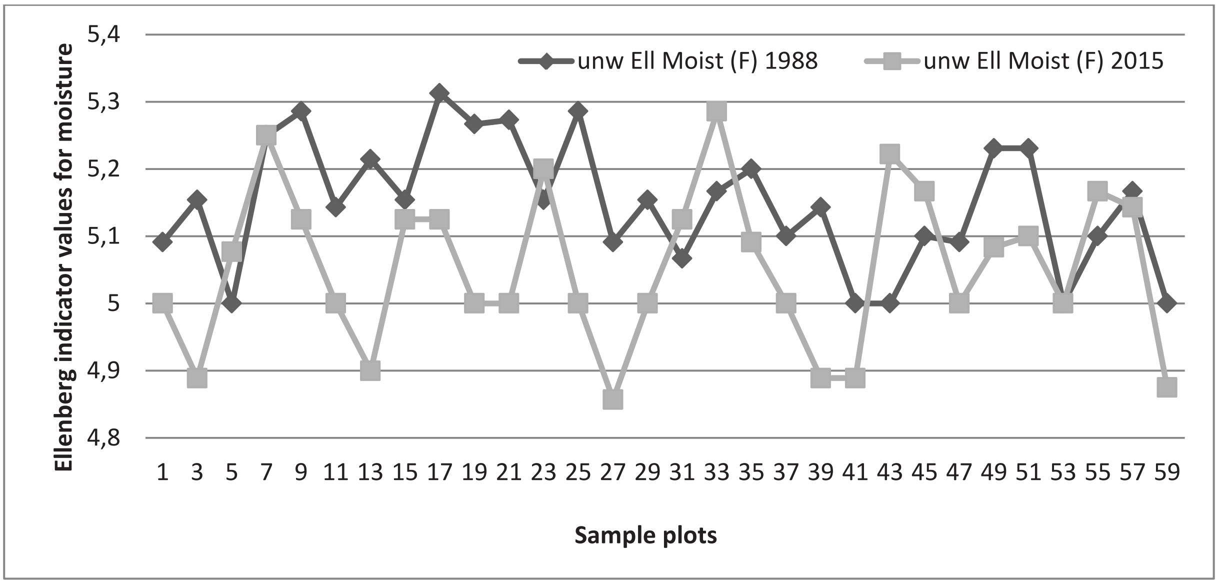

Figure 7. The variation of the mean Ellenberg moisture indicator values (1988 and 2015)

Ellenberg's indicator values for reaction

The paired t-test has revealed a statistically significant decrease of 0.38 between the mean Ellenberg reaction indicator values from 1988 and 2015, figure 8 illustrating the values for each plot.

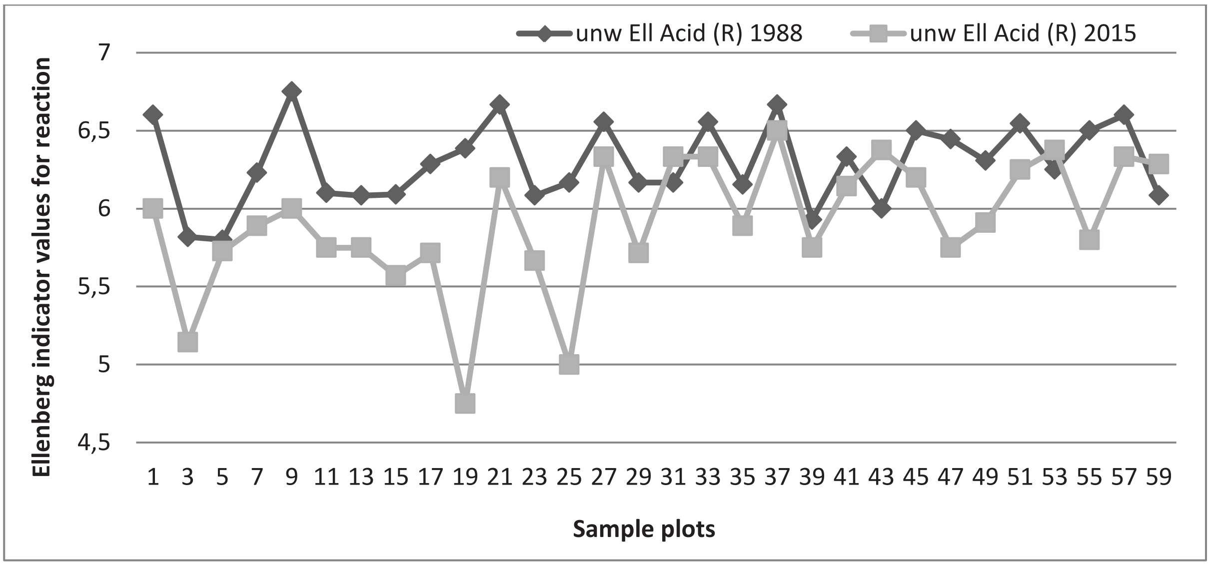

Figure 8. The variation of the mean Ellenberg reaction indicator values (1988 and 2015)

Ellenberg's indicator values for nitrogen

Comparing the mean Ellenberg nitrogen indicator values in 1988 and 2015, a statistically significant decrease of 0.36 was found (table 1), as shown in figure 9.

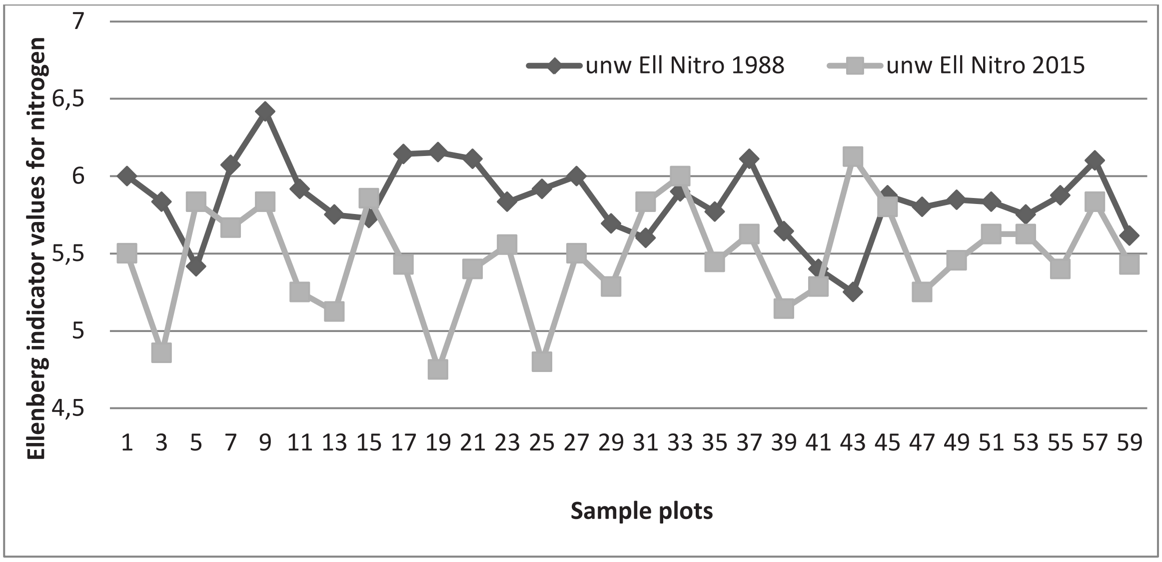

Figure 9. The variation of the mean Ellenberg nitrogen indicator values (1988 and 2015)

Table 1. Means, standard deviations, calculated probabilities (p-values) and confidence intervals of the Ellenberg indicator values for the environmental factors in 1988 and 2015

Ellenberg indicator values	Mean ± SD		p-value	95 % CI
	1988	2015		
Light (summer)	3.92 ± 0.27	3.47 ± 0.32	< 0.0001***	0.33 to 0.57
Temperature	5.32 ± 0.12	5.38 ± 0.14	0.044*	-0.13 to -0.002
Continentality	3.46 ± 0.15	3.30 ± 0.22	0.0004**	0.08 to 0.24
Moisture	5.15 ± 0.09	5.05 ± 0.12	0.0007**	0.04 to 0.15
Reaction	6.29 ± 0.26	5.91 ± 0.42	< 0.0001***	0.23 to 0.53
Nitrogen	5.85 ± 0.25	5.48 ± 0.34	0.0001**	0.19 to 0.53

*** $p < 0.0001$, ** $p < 0.001$, * $p < 0.05$

Chemical analysis of soil

The mean pH-KCl has fluctuated over the years. In 1988 the mean pH-KCl ± SD was 3.78 ± 0.13, the paired t-test applied uncovered a statistically significant decrease ($p < 0.0001$) in 1998, the value dropping to 3.67 ± 0.09 (figure 10). The pH-KCl has recovered until 2007 at the time of the third measurement, when the mean ± SD was 3.80 ± 0.13 (figure 11), the increase being statistically significant ($p < 0.0001$). The pH-KCl values of the years 1988 and 2007 are not statistically different ($p = 0.6$).

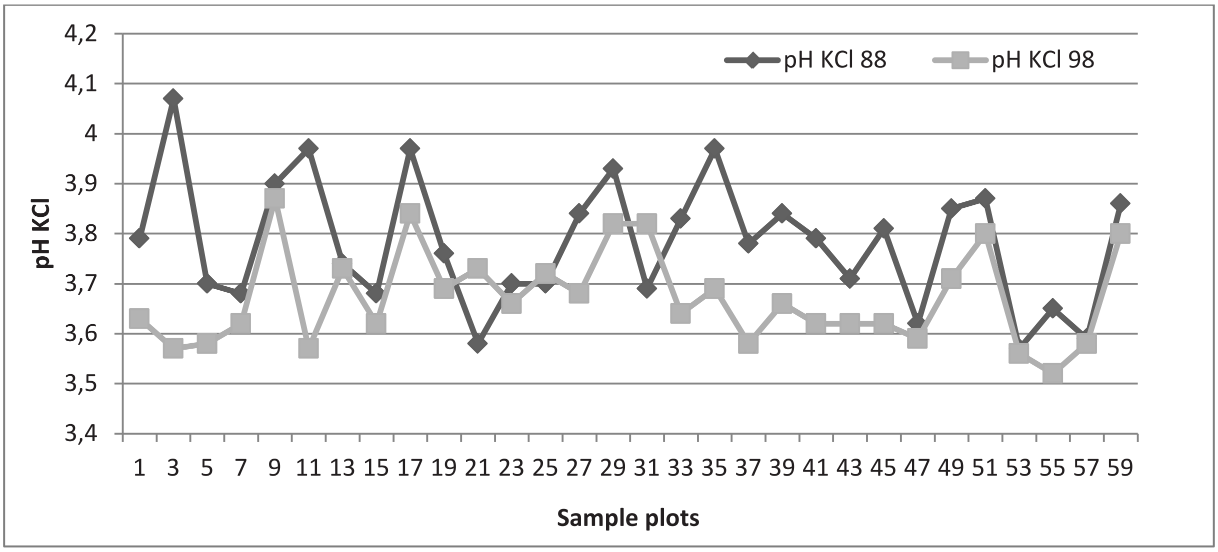

Figure 10. The variation of the pH KCl in 1988 and 1998

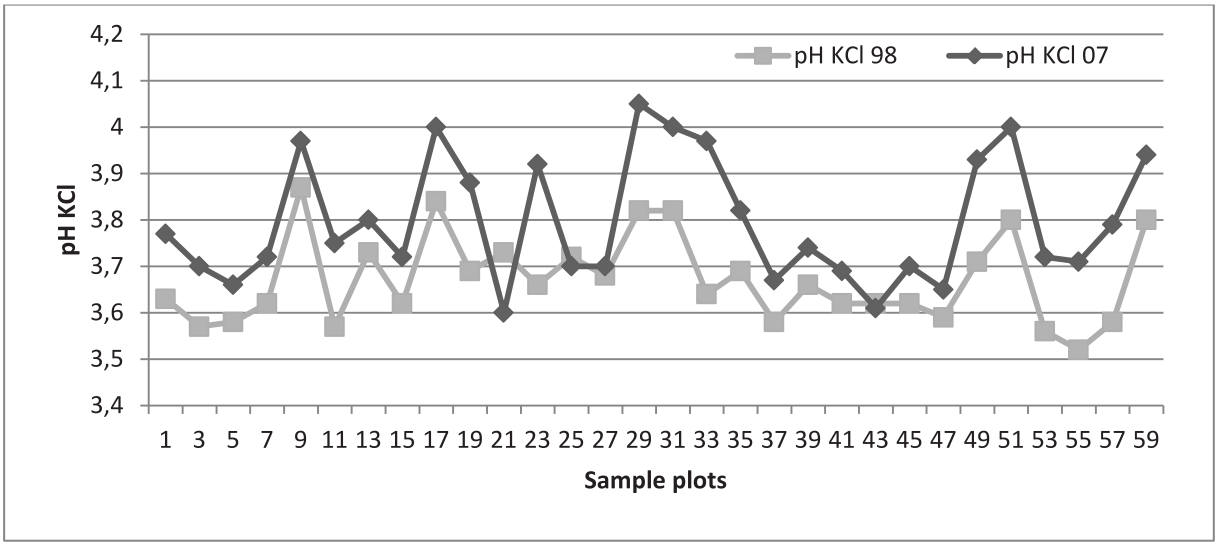

Figure 11. The variation of the pH KCl in 1998 and 2007

Inter-annual variations

During the first decade of the study, between 1988 and 1998, has been registered a statistically significant decrease in the cover percentage of 26%. In the next decade the mean cover has had a statistically significant increase of 36%.. This upward trend continued in the next period, 2008 to 2015, with a statistically significant increase of 15%. Considering the whole study period, there has been a statistically significant increase of 25%, as it can be observed also in figure 12 and table 2.

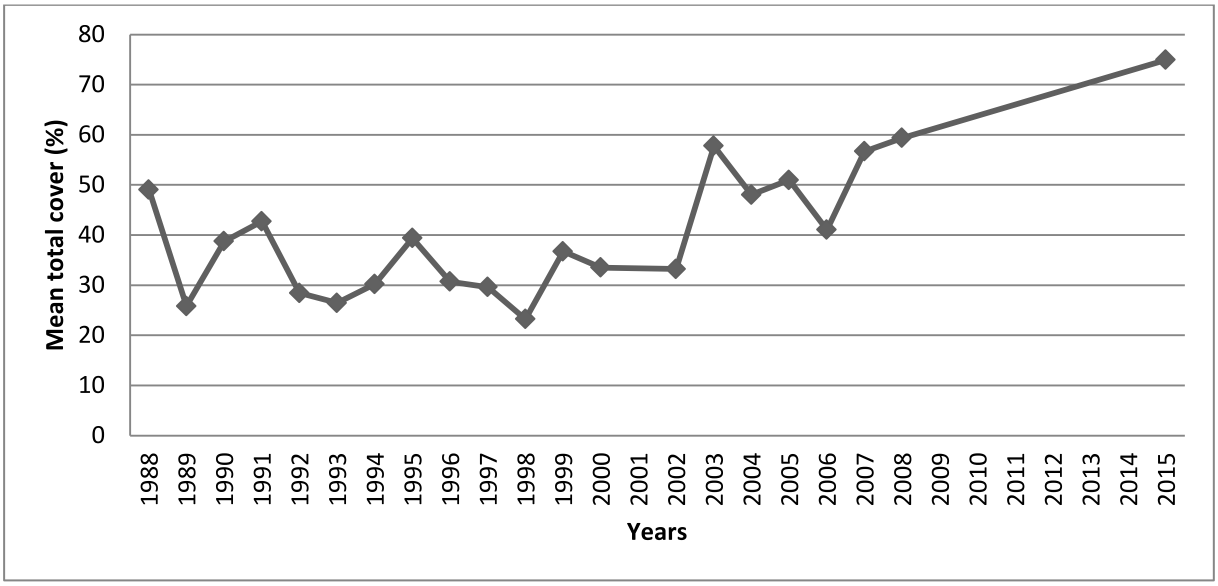

Figure 12. Inter-annual variation of the mean total cover for the summer species

Table 2. Means, standard deviations, calculated probabilities (p-values) and confidence intervals of the variations in mean total cover (%) of summer flora between decades

Year	Mean ± SD	p-value	95 % CI
1988	49.1 ± 13.2	< 0.0001***	19.8 to 31.9
1998	23.2 ± 14.9	< 0.0001***	- 42.6 to - 29.7
2008	59.4 ± 20.2	< 0.0001***	- 19.1 to - 11.6
2015	74.7 ± 17.1		

As for the 6 species considered, not all of them registered an increasing cover percentage in the period 1988-2015, as it can be observed in table 3 and table 4. *Holcus mollis* has had a statistically significant increase of 3.6%, the increase being steep in the first 15 years of the study period, later on the variation got mellower (figure 13). *Stellaria holostea* has manifested a statistically significant, long-term increase of 15.9%, the cover values fluctuating in the first decade followed by a fast growth until 2005 after which it started stagnating (figure 13). A statistically significant increase of 5.9% has occurred for *Oxalis acetosella*, which is however due to the fluctuating nature of the cover variations (low r^2) as it can be observed also in figure 14. *Lamium galeobdolon* has registered a fluctuating but statistically significant increase of 11.1% in the period 1988 to 2015, in the past 13 years displaying more abrupt changes (figure 14). *Hedera helix* has registered a statistically significant, long-term decrease of 5.8%, the evolution of the cover changes consisting of a fast initial decline in the first decade of the study followed by a very slow further decline (figure 15). *Hepatica nobilis* has had a similar decline pattern, characterised by a long-term statistically significant decrease of 1.4%, as it can be observed in figure 15.

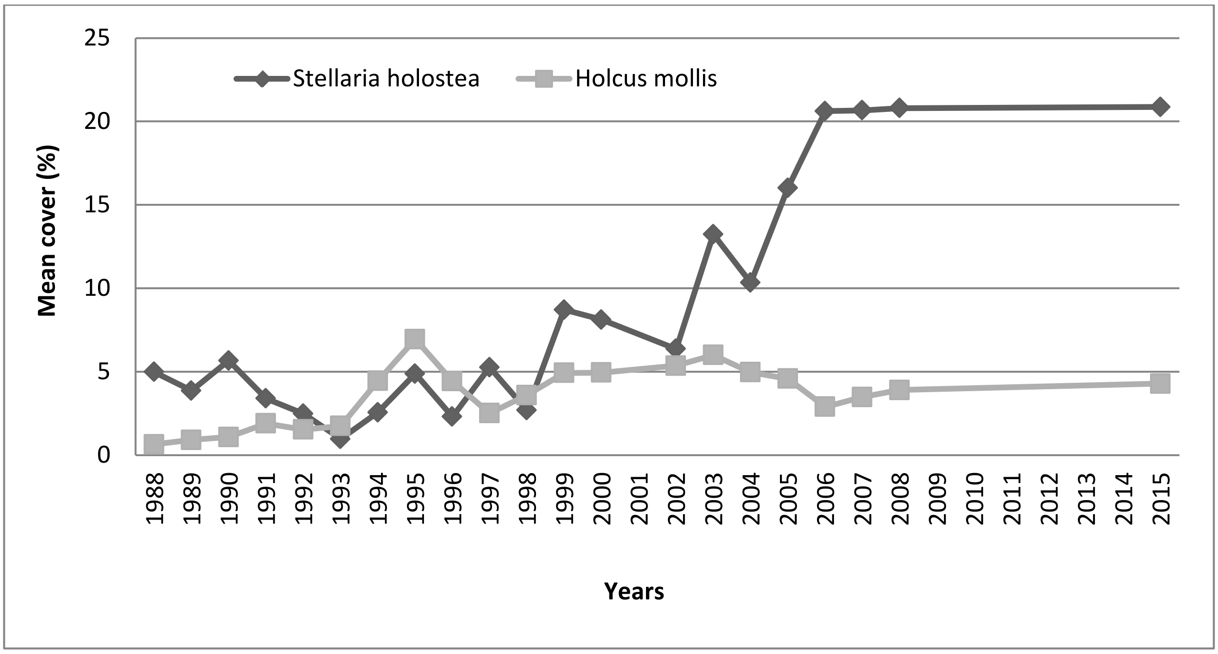

Figure 13. Inter-annual variation of the mean cover of *Stellaria holostea* and *Holcus mollis*

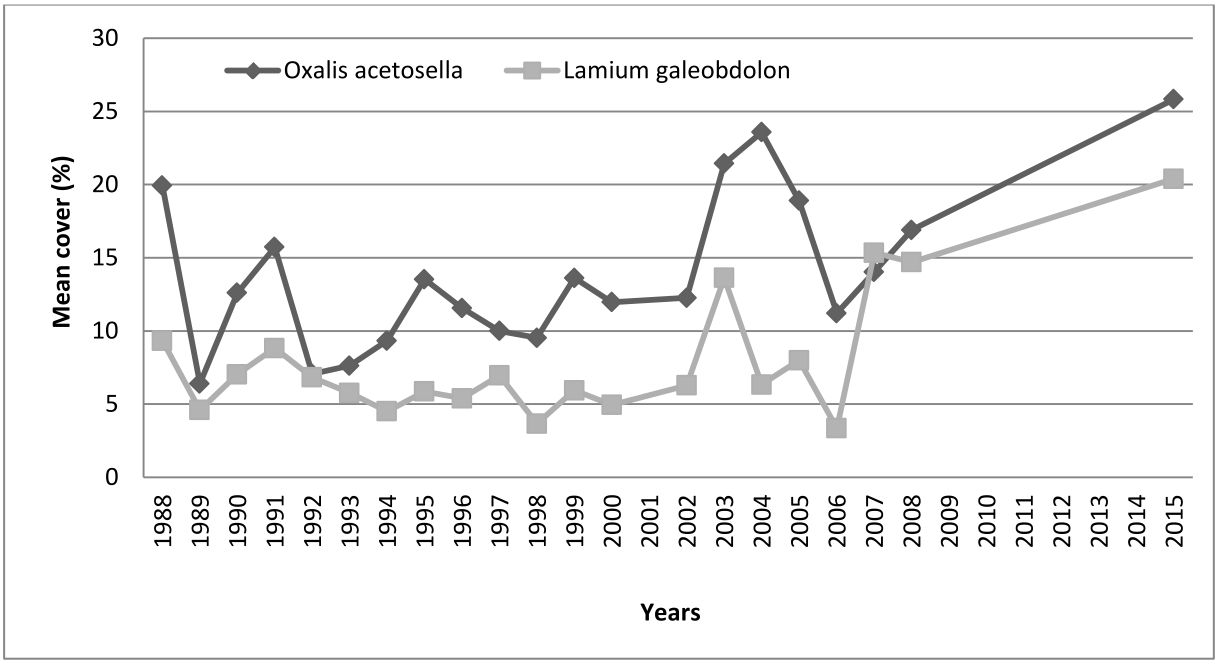

Figure 14. Inter-annual variation of the mean cover of *Oxalis acetosella* and *Lamium galeobdolon*

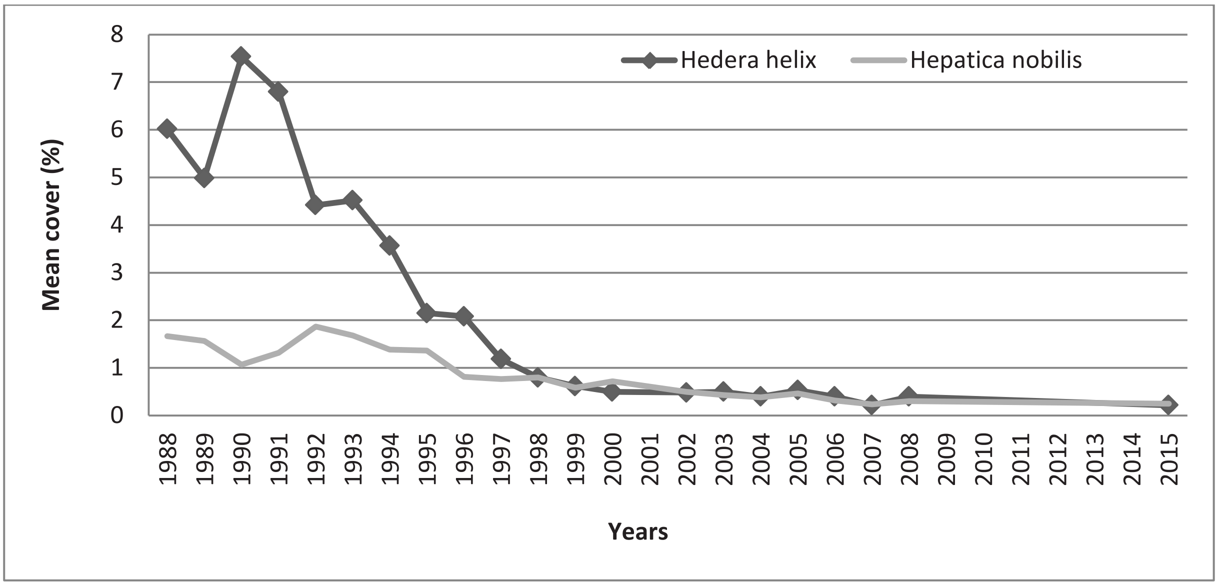

Figure 15. Inter-annual variation of the mean cover of *Hedera helix* and *Hepatica nobilis*

Table 3. Means, standard deviations, calculated probabilities (p-values) and confidence intervals of the variations in mean total cover (%) of the selected species between 1988 and 2015

Plant species	Mean ± SD		p-value	95 % CI
	1988	2015		
Holcus mollis	0.7 ± 1.3	4.3 ± 7.3	0.006*	- 6.2 to – 1.1
Stellaria holostea	5.0 ± 5.4	20.9 ± 9.4	< 0.0001***	- 18.9 to – 12.9
Oxalis acetosella	19.9 ± 7.2	25.8 ± 7.7	0.004*	- 9.8 to – 2.0
Lamium galeobdolon	9.3 ± 5.8	20.4 ± 5.3	< 0.0001***	- 13.8 to – 8.4
Hedera helix	6.0 ± 4.3	0.2 ± 0.3	< 0.0001***	4.2 to 7.5
Hepatica nobilis	1.7 ± 1.1	0.3 ± 0.3	< 0.0001***	1.1 to 1.8

*** p < 0.0001, ** p < 0.001, * p < 0.05, n.s. – not significant

Table 4. Correlation coefficients (r), calculated probabilities (p-values) and coefficients of determination (r^2) of the variations through time in mean total cover (%) of the selected species between 1988 and 2015

Plant species	r	p-value	r^2
Holcus mollis	0.5459	0.0105*	0.2980
Stellaria holostea	0.8661	< 0.0001***	0.7501
Oxalis acetosella	0.5600	0.0083*	0.3136
Lamium galeobdolon	0.5871	0.0051*	0.3447
Hedera helix	- 0.8408	< 0.0001***	0.7070
Hepatica nobilis	- 0.8868	< 0.0001***	0.7864

*** p < 0.0001, ** p < 0.001, * p < 0.05, n.s. – not significant

The relationship between total summer cover and precipitation

During the first decade, the relationship between cover and spring precipitation amounts (April - June) has been the most significantly described with the help of a second-degree polynomial regression equation with a coefficient of determination equal to 0.390 (figure 16).

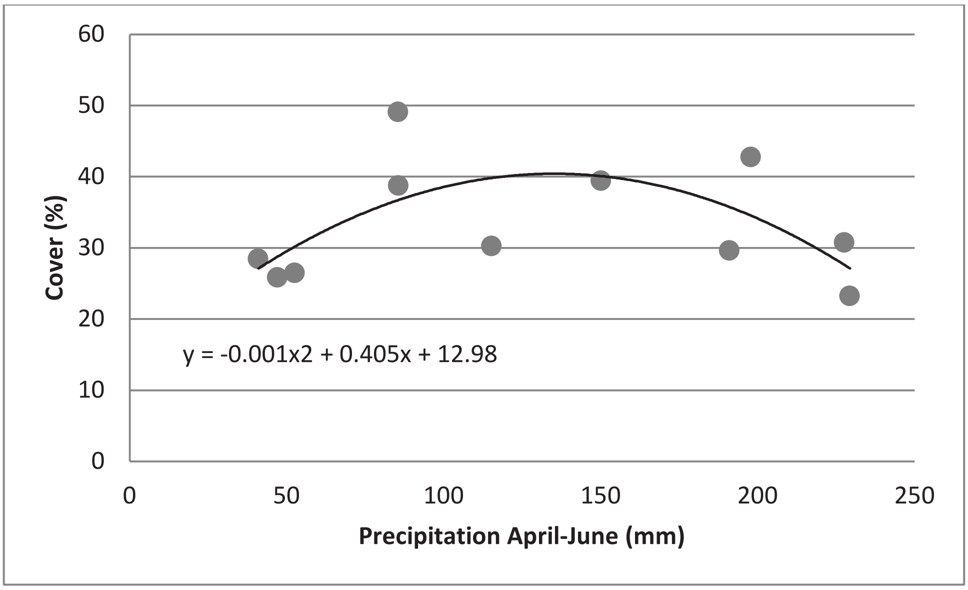

Figure 16. The relationship between summer cover and April- June precipitation (1988-1998)

Running the regression analysis between the total summer cover and spring precipitation in the second decade (1998-2008), a second-degree polynomial regression model was found to best describe the relationship between the two variables, for which the coefficient of determination is 0.470 (figure 17).

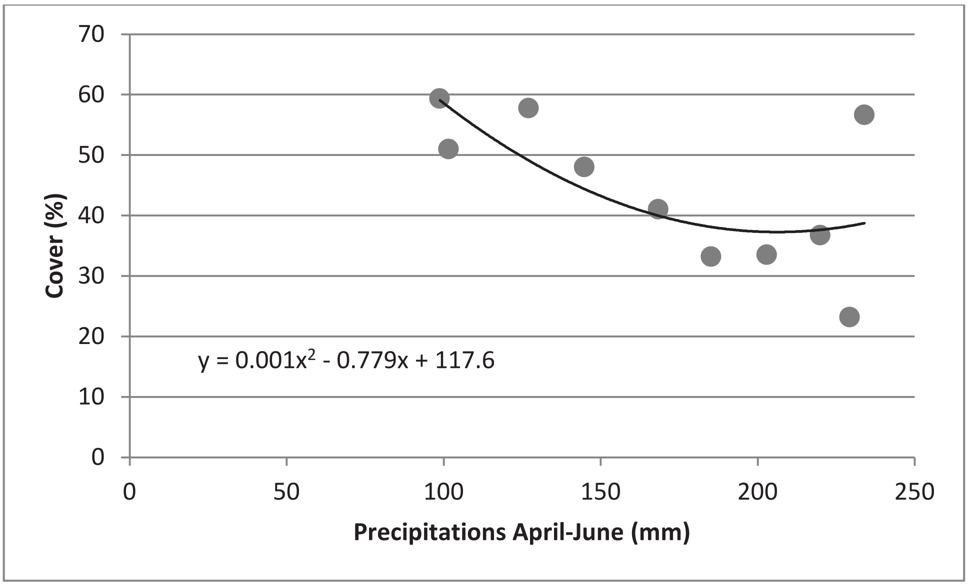

Figure 17. The relationship between summer cover and April- June precipitation (1998-2008)

During the first decade of the study, the cover of *Hepatica nobilis* has manifested a negative linear relationship with the precipitation amounts during April-June, the coefficient of determination being 0.697 (figure 18). The correlation analysis revealed a correlation coefficient of – 0.8353 and a p-value of 0.0014.

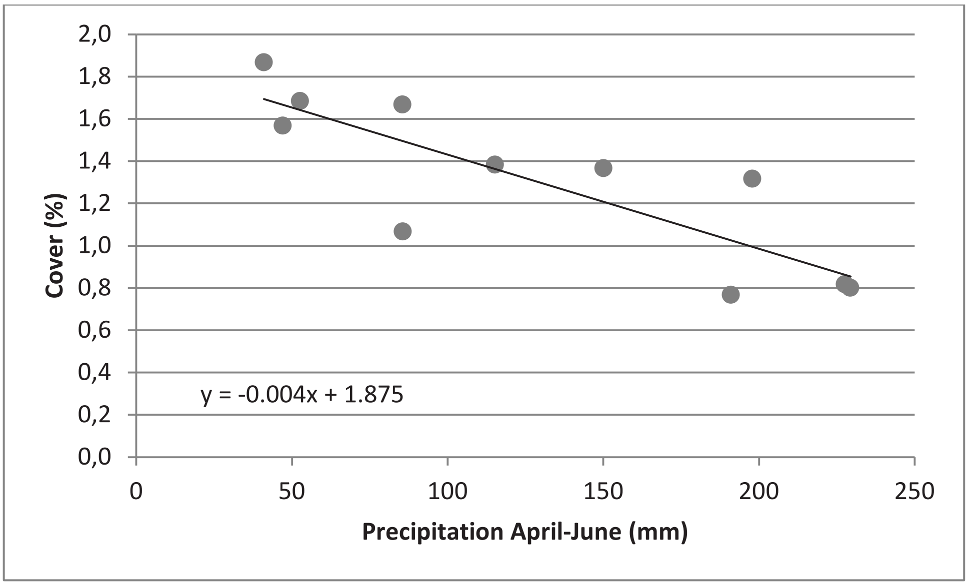

Figure 18. The relationship between the cover of *Hepatica nobilis* and April-June precipitation (1988-1998)

An exponential regression model (R^2=0.500) was found to best describe the relationship between the cover of *Oxalis acetosella* and precipitation during April-June, for the period of the second decade of the study (figure 19).

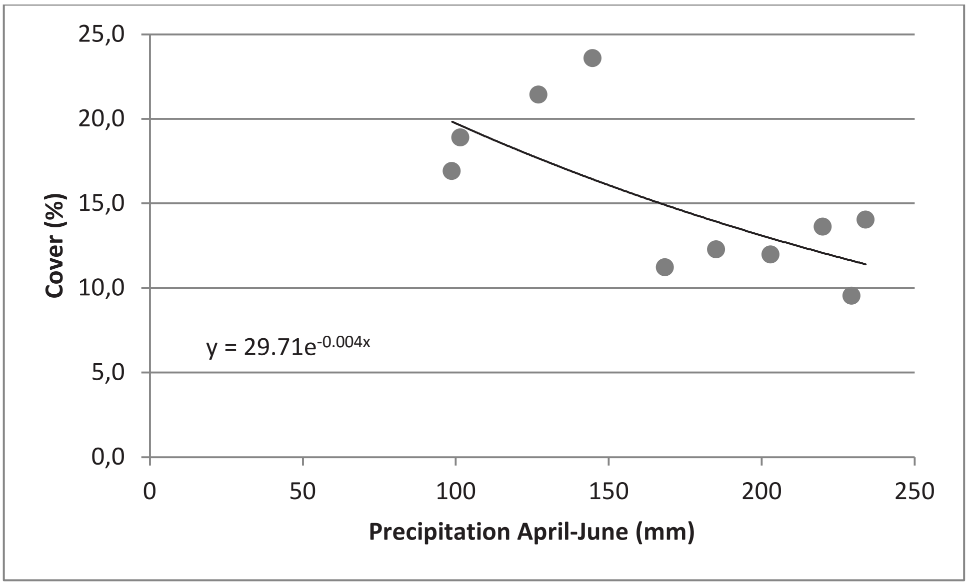

Figure 19. The relationship between the cover of *Oxalis acetosella* and April-June precipitation (1998-2008)

The relationship between the cover of *Hedera helix* and the precipitation amounts from April until June during 1988-1998 is best described by an exponential regression model with a coefficient of determination of 0.445 (figure 20).

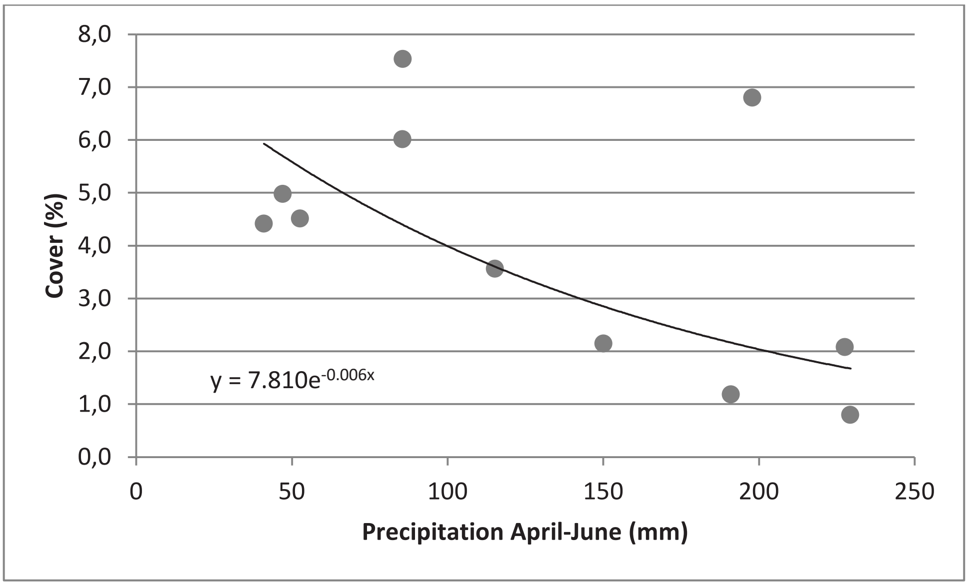

Figure 20. The relationship between the cover of *Hedera helix* and April-June precipitation (1988-1998)

In the case of *Holcus mollis*, the relationship during the first decade between its cover and the spring precipitation amounts (April-June) has been the most significantly described with the help of a power regression equation with a coefficient of determination equal to 0.389 (figure 21).

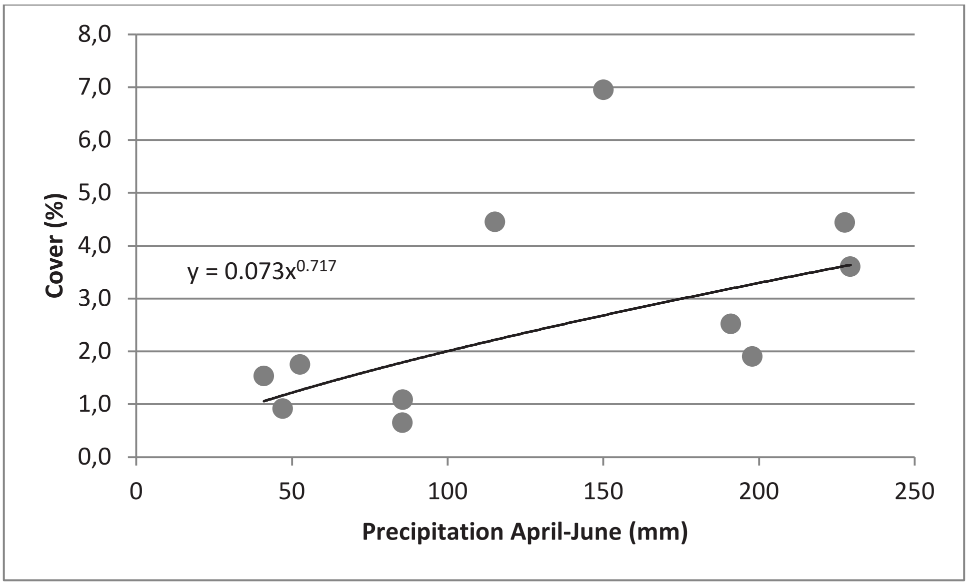

Figure 21. The relationship between the cover of *Holcus mollis* and April-June precipitation (1988-1998)

Discussion

The decrease in species richness combined with the relative stability of the evenness levels, which translate into decreasing number of species and constancy in uniformity of species distribution, indicate that the former is not due to an invasive plant species which would've had as effect a strong decrease in evenness also. A more likely cause would be a decrease in light availability due to the closing up of the canopy. The decrease in mean Ellenberg light indicator values for the summer species indicates a decrease in number of the light demanding species.

The increase of mean Ellenberg temperature indicator values indicates an increase in number and cover of species of with higher temperature requirements.

The decrease of the mean Ellenberg continentality indicator values points to a decrease in the number of more continental species, and an increase in the cover of coastal species.

In terms of moisture conditions, from the decrease in mean Ellenberg moisture indicator values it can be inferred that the number of species that prefer moist soils has decreased and that the species adapted to drier conditions have taken over the space left free after the disappearance or cover reduction of species preferring moist conditions.

As it concerns the reaction of the soil, a decrease in number of species which prefer soils with more alkaline reaction can be deducted from the decreasing mean Ellenberg reaction indicator values. The fluctuating values of the pH-KCl through the years may be explained by the fluctuations in emissions of SO_2. Sulphur dioxide causes acid rains, in years with increased precipitation amounts it determines a decrease in soil pH (Ellenberg, 1988). In the past 25 years the emission amounts of sulphur dioxide have drastically decreased (European Environment Agency, 2014), therefore the negative effects started to disappear and consequently the soil pH recovered to its initial level. However, the herb layer species which have a preference towards more alkaline reaction of the soil may need more time in order to re-colonize the site after the recovery of the soil pH levels.

From the decrease of the mean Ellenberg nitrogen indicator values, it can be deducted that both the number and cover of species that require higher levels of nitrogen have decreased. Therefore it can be inferred that the soil nitrogen levels have decreased due to the decrease in light availability and a decrease of nitrogen emissions (European Environment Agency, 2010).

With the help of Ellenberg's indicator values, a sketch of the changes produced in the environmental conditions can be drawn. Compared to 1988, the hornbeam stand offers now for the ground vegetation less light, the climate became more coastal and is characterised by higher temperatures which have led to a decrease in soil moisture. The soil has also become more acidic and poorer in nitrogen.

The regression analysis of the relationship between the cover and precipitation amounts during the period April to June has revealed a trend valid in both decades. The cover increases over 40% when the precipitation in April-June sums roughly between 100 and 175 mm, which could represent an optimum for the local flora community.

The species-wise relationship of cover with precipitation is simpler. During the first decade, the cover of *Hepatica nobilis* and *Hedera helix* has a negative relation with the spring precipitation, while *Holcus mollis* displays a positive relationship. The cover of *Oxalis acetosella* had a positive relation with the precipitation amounts during spring in the first decade (Brunet & Tyler, 2000), however in the second decade it developed a negative relationship with the precipitation. The inconsistency can be explained by the fact that *Oxalis acetosella* actually has a parabola shaped relationship with precipitation amounts, the cover minimums being observed in years with extreme low and high amounts of precipitation. This was observed after analysing the data from the both decades together.

As for the inter-annual variations, the overall increase in total cover during the study period, despite the decrease in number of species, could be explained by a change in competition, namely the species that disappeared may have been suppressing others that now got more space and resources.

Roe deer's (*Capreolus capreolus* L.) food consists mainly of herbaceous plants and grasses, during spring and early summer representing 80% of their food and 65% during autumn and winter in Białowieża Primeval Forest (Gębczyńska, 1980). Tixier et al. (1997) found in an oak-beech woodland from western France that *Hedera helix* was preferred for grazing by roe deer during winter and autumn. In Białowieża Primeval Forest, during all seasons with slight variable proportions, among the most frequently grazed tree species by roe deer were hornbeam and pedunculate oak, while in the shrub layer hazel was preferred. As for herbaceous species, during autumn *Urtica dioica* was among the favourite forage species, being chosen by 39% of the deer analysed, while in the winter hazel and ivy were preferred by 17% of the deer (Gębczyńska, 1980). These general browsing preferences in conjunction with the sharp increase in roe deer population of Sweden since mid 1950s until early 1990s and continued increase until today (375% increase in population size since 1955 until 2005) (Bergström & Danell, 2009) may be contributing factors in the dynamics of the ground flora cover. Thus, winter grazing by an increased roe deer population could contribute to the cover decrease of the evergreen *Hedera helix* (and *Hepatica nobilis*) and could be one of the factors that determined the disappearance of some species.

Quercus robur was present only in 1988 and its disappearance could be caused by roe deer browsing or by the decrease in light availability due to canopy closure, oak being known to need overhead light.

Urtica dioica, which was present until 2007 in just one of the sample plots, could be gone due to the roe deer feeding on it or due to the decrease in available soil nitrogen, having the highest requirements for it.

Once abundant and present in many of the sample plots, hazel has vanished after 1997, possible causes being the decrease in light availability, roe deer browsing or the natural course of succession, from open pasture with hazel to hornbeam forest.

As for hornbeam seedlings, which were present in almost all of the sample plots, except in 2015 when they were completely missing, they might have not survived the aggressive *Operophtera brumata* caterpillar outbreak.

The disappearance of the other species could be explained by the changes in one or more environmental factors as reflected by the mean Ellenberg indicator values, mainly the decrease of pH and available nitrogen for which those species have high requirements. To the changing environmental factors may be assigned also the colonisation of the two species, more exactly to the increase in temperature and decrease in continentality.

Oxalis acetosella's cover has fluctuated throughout the years, developing a slight increase which may be explained by the decrease in light availability, its lowest light requirements on the Ellenberg indicator values scale and ability to thrive under the thick canopy of hornbeam (Packham & Willis, 1977). Another factor that might have helped *Oxalis acetosella* to persist and have a slight increase in cover, despite the decrease in soil moisture and nitrogen (having quite high requirements for them) is the roe deer grazing, according to Landi et al. (2016) being one of the few species that flourishes in heavy grazing conditions due to its unpalatability.

The increase in abundance of *Stellaria holostea* could be caused by the temperature increase, since it has quite high Ellenberg indicator value for temperature. The same explanation may be valid for the slight increase in the cover of *Lamium galeobdolon*, together with the increase in shading (Ellenberg indicator value for light = 3).

In the case of *Holcus mollis*, the increase in cover was explained by Tyler et al. (2002) as to be due to the acidification of the soil, being a plant species that prefers acid soils. The combined effects of other environmental factors may also explain the increase, namely the increasing temperatures and spring precipitation together with the decreasing continentality and nitrogen.

Lastly, according to Tyler et al. (2002), the previous decrease in soil pH might have contributed to the decrease in abundance of *Hepatica nobilis*, preferring more alkaline soils. Also the negative linear relationship between its cover and the spring precipitation amounts could partly account for the variation.

Corney et al. (2008) identified similar changes in Witham Woods, such as lower nitrogen deposition after 1990 and influence of the roe deer grazing on the herb layer composition, the palatable species decreasing, however there was found a decrease in deep shade tolerant species and no statistical significant changes in species richness.

In a similar study performed in Dalby Söderskog, von Oheimb & Brunet (2007) found similar results, namely decreasing trends for the 1976-2002 period in the case of total species richness, cover of hazel and pedunculate oak and mean Ellenberg indicator values for moisture and soil reaction, but opposite trends compared to the results from this study in the case of mean Ellenberg indicator values for light and nitrogen and the cover of *Lamium galeobdolon* and *Urtica dioica*. This supports furthermore the hypotheses that low light availability promotes the increase in cover of *Lamium galeobdolon* and that nitrogen levels correlate positively with the abundance of *Urtica dioica*.

The findings of this study concerning the changes in environmental conditions in Stenshuvud National Park align with the changes found by Hedwall & Brunet (2016) for the temperate southern Sweden, with the exception of the trend for available soil nitrogen which is increasing according to the latter.

The importance of performing long-term studies is demonstrated by the differences in observed trends during the first decade of the study presented in Brunet & Tyler (2000) and the ones that include the following decade and a half also. *Stellaria holostea* had a fluctuating cover during the first decade, but later developed a long-term increase which would have been overlooked if the study wouldn't have continued. *Hedera helix* has had a fast decline during the first decade and later fluctuated slightly, so if the data collection would've started after 1998, its decline wouldn't have been registered.

According to von Oheimb & Brunet (2007) a decrease in species richness is to be expected during the succession process from wooded pasture to forest ecosystem due to the decrease in grazing and light. However, the intense grazing in the study site in combination with low light levels seems to have caused even a higher decrease in species richness. Thus, the following dilemma appears: should active management be applied in protected areas or not? If some kind of management is to be applied, in this case maintaining grazing in pastures, the ecosystem would hopefully maintain its initial state and biodiversity, but how long back was this the natural ecosystem? If no management is applied the ecosystem will follow the natural succession steps, in this particular case the pasture becoming a forest and loosing from the initial diversity. Also, if the ecosystem is unbalanced, having a missing component, as it is the case with the absence of predators which led to persistent high densities of roe deer, it can determine a much more different path of succession.

In my opinion, for this ecosystem to be balanced, there are two pathways that could be followed in order to regulate the herbivory, which I think has a high influence on the composition of the herb layer as long as the roe deer densities maintain high. One of the options would be to reduce the roe deer densities by hunting, but this would not solve the entire problem since the roe deer would still have a sedentary feeding habit. The second option is the reintroduction of wolves in the area which would re-establish the missing interactions and so would make the ecosystem more balanced.

Conclusions

Long-term studies offer a better overview over the changes in herb layer communities, helping to distinguish inter-annual variation from long-term trends and facilitate the understanding of the factors of influence.

Light has a higher influence than other environmental factors on the plant community due to its influence on some of the other factors, having both direct and indirect influence.

The rainfall during spring it proved to be more important in influencing the abundance than the rainfall during the summer of the previous year.

The interpretation of the mean Ellenberg indicator values to describe the variations of the environmental factors through time matches the actual changes in the field for the environmental factors that could and were measured. Therefore, Ellenberg indicator values could be used to determine the changes that occurred in environmental factors, but they should be used with caution due to the slowness in reaction of the plant community in the case of some environmental factors.

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Ștefania-Elena Purcaru

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Appendix

Photo 1. General outlook of the study site (Source: Ștefania-Elena Purcaru, 2016)

Photo 2. Close view on the herb layer (Source: Ștefania-Elena Purcaru, 2016)

Photo 3. Canopy closure (Source: Ștefania-Elena Purcaru, 2016)

Photo 4. Spring components of the herb layer (Source: Ștefania-Elena Purcaru, 2016)

Photo 5. Summer components of the herb layer (Source: Ștefania-Elena Purcaru, 2016)

Photo 6. *Dentaria bulbifera* (L.) Crantz (Source: Ștefania-Elena Purcaru, 2016)

Photo 7. *Oxalis acetosella* L. (Source: Ștefania-Elena Purcaru, 2016)

Photo 8. *Lamium galeobdolon* (L.) Crantz (Source: Ștefania-Elena Purcaru, 2016)

Photo 9. *Stelaria holostea* L. (Source: Ștefania-Elena Purcaru, 2016)

Photo 10. *Hepatica nobilis* Mill. (Source: Ștefania-Elena Purcaru, 2016)