Effects of set-aside management on certain elements of soil biota and early stage organic matter decomposition in a High Nature Value Area, Hungary

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Abstract

Agricultural intensification is one of the greatest threats to soil biota and function. In contrast, set-aside still remains a management practice in certain agri-environmental schemes. In Hungary, the establishment of sown set-aside fields is a requirement of agri-environmental schemes in High Nature Value Areas. We tested the effects of set-aside management on soil biota (bacteria, microarthropods, woodlice and millipedes), soil properties and organic matter decomposition after an initial establishment period of two years. Cereal – set-aside field pairs, semi-natural grasslands and cereal fields were sampled in the Heves Plain High Nature Value Area in Eastern Hungary, in May 2014. Topsoil samples were taken from each site for physical, chemical, microbial analyses and for extraction of soil microarthropods. Macrodecomposers were sampled by pitfall traps for two weeks. The biological quality of soil was estimated by the integrated QBS index ("Qualità Biologica del Suolo", meaning "Biological Quality of Soil") based on diversity of soil microarthropods. To follow early stage organic matter decomposition, we used tea bags filled with a site-independent, universal plant material (*Aspalathus linearis*, average mass 1.26 ± 0.03 g). Tea bags were retrieved after 1 month to estimate the rate of mass loss. We found significant differences between habitat types regarding several soil physical and chemical parameters (soil pH, K and Na content). The study showed positive effects of set-aside management on soil biodiversity, especially for

microarthropods and isopods. However, we did not experience similar trends in relation to soil bacteria and millipedes. There was higher intensity of organic matter decomposition in soils of set-aside fields and semi-natural grasslands (remaining mass on average: 74.17% and 76.6%, respectively) compared to cereal fields (average remaining mass: 81.3%). Out of the biotic components, only the biological quality of soil significantly influenced (even if marginally) plant tissue decomposition. Our results highlight the importance of set-aside fields as shelter habitats for soil biota, especially for arthropods. Set-aside fields that are out of a crop rotation for 2 years could be a valuable option for maintaining soil biodiversity, as these fields may simultaneously conserve elements of above- and below-ground diversity.

Introduction

The European Union (EU) is one of the most intensive agricultural regions per unit of surface area in the world (Monfreda et al. 2008). Agriculture is a dominant form of land management in Europe, with 40% of the total land area of the EU 28 used for crop production and for grasslands (European Commission 2013).

Agroecosystems and agricultural landscapes provide important soil related ecosystem services, i.e. the maintenance of soil fertility and structural properties, filtering and providing reservoir for water, nutrient cycling and climate regulation (Dominati et al. 2010). Production and maintenance of healthy soils in agricultural areas are therefore key elements in the development of sustainable agriculture. The importance of soil communities (microbiota, meso- and macrofauna) contributing to a very diverse range of biochemical and biophysical processes has long been recognised (Barrios 2007). However, the decomposer subsystem and soil related ecosystem services are still poorly understood (Bardgett and Wardle 2010). Several studies have demonstrated the importance of soil fauna in the maintenance of soil fertility (e.g. Brussaard et al. 2007). Soil microarthropods [springtails (Collembola), mites (Acari), proturans (Protura), diplurans (Diplura), pauropods (Pauropoda), symphylans (Symphyla) etc.] millipedes (Diplopoda) and terrestrial isopods (Isopoda: Oniscidea) have essential roles in litter decomposition, nutrient mineralisation and the improvement of soil properties (Culliney 2013). In addition to earthworms, these organisms are responsible for the first steps in the decomposition processes by fragmentation and inoculation of dead plant material (Lavelle and Spain 2001). They promote litter breakdown through their feeding and burrowing activities that support microbial decomposition (Lavelle and Spain 2001). Given their importance in nutrient cycling, the lack of knowledge on how agricultural practices affect these taxa and their functions is striking. The soil biological activity that can be measured e.g. by litter decomposition rate, depends largely on the diversity of soil organisms (Hättenschwiler et al. 2005) and is the result of complex interactions (Scheu 2002).

Numerous studies have shown that agricultural intensification represents a major threat to soil biodiversity and to the provision of ecosystem services (e.g. Altieri 1999).

Local land use, microclimate, pH, landscape diversity and habitat structure influence the species richness and abundance of soil detritivores (Hopkin and Read 1992, Hopkin 1997, Warburg 1987). Plant species richness and plant community structure greatly affect the above-ground microclimate which has indirect effects on soil biota and on decomposition dynamics of substrates through their chemistry, physiology, rhizodeposition and the quantity, quality and diversity of litter (Dudgeon et al. 1990, Smith and Bradford 2003, Hättenschwiler et al. 2005, Tripathi et al. 2013).

The establishment of semi-natural habitats (grassy strips, sown or naturally regenerated set-aside fields, hedgerows, treelines etc.) in agricultural landscapes is a common practice to enrich habitat diversity or to connect isolated habitats (e.g. Critchley et al. 2004, Smith et al. 2008, Kovács-Hostyánszki et al. 2011, Morris et al. 2011). These green patches in arable landscapes support high biodiversity and provide suitable environmental conditions for several plant and animal species (Altieri 1999, Kovács-Hostyánszki et al. 2011, Tóth et al. 2016). The presence of set-aside fields contributes to a productive and ecologically balanced soil environment through improving soil properties necessary for plant health (activation of soil biology, addition of organic matter, N fixation, microclimate modification etc.). Moreover, these habitats may have important impacts on the adjacent cropping systems through spillover (e.g. Blitzer et al. 2012).

European agricultural policy has long relied on agri-environmental schemes (AES) to alleviate the negative environmental impacts of agricultural intensification (Batáry et al. 2015). The first set-aside scheme was introduced by the EU in 1988 to reduce of production surplus. However, despite the positive environmental effects, set-aside management was abolished in most EU countries because of increasing production demands (Rowe et al. 2009). In countries where set-aside still remains a management practice, it serves as an essential component of agri-environment schemes (Batáry et al. 2015). However, the effectiveness of such measures on soil biodiversity and function is still questionable.

Insight in conservation management in Central and Eastern European countries could be particularly valuable, as their agrobiodiversity is still high compared to Western Europe (Báldi et al. 2013). In Hungary, rotational set-aside management has been present as part of the national agri-environment scheme since 2002 (Ángyán et al. 2003). The maximum period of setting aside a given arable field is three years. Set-aside fields are generally sown with a seed mixture of grass and leguminous species (Ángyán et al. 2003).

The present study aimed to test the following hypotheses:

(i)   set-aside management has profound effects on soil physical and chemical properties,
(ii) set-aside fields and semi-natural grasslands provide more favourable conditions for studied soil organisms compared to cereal fields,
(iii) plant tissue decomposition is higher in set-aside fields and semi-natural grasslands
(iv) decomposition rate is positively correlated with measures of soil biodiversity.

Methods

Study site

The study was conducted in the region of North-eastern Hungary (Heves County) in 2014 (see map in Suppl. material 5). About 72% of the land was under agricultural management (ca. 60% arable and 12% grasslands) (Bükk National Park 2018). The study area can be characterised by a continental climate with extreme high temperature and low precipitation in summer. The study sites belong to the Heves Plain High Nature Value Area (HHNVA), which was established in the framework of the zonal action schemes of the National Agri-Environmental Programme in 2002 and covers around 40 000 hectares (Ángyán et al. 2003). The grasslands were extensively mown or grazed, mainly by cattle and sheep and no chemicals were applied. The most dominant species were Kentucky bluegrass (*Poa pratensis*), Pseudovina (*Festuca pseudovina*) and meadow foxtail (*Alopecurus pratensis*). Establishment of set-aside fields was part of the arable farming action plan. The main crops were cereals, sunflower and oilseed rape. Farmers' fields had to be managed by regular crop rotation during the 5-year long contract period: cereal 20–25%, alfalfa 20–30%, oilseed rape and other crops (pea, sunflower, corn etc.) 25–30%, set-aside 20–25%. Fields could be taken out of production for 1–3 years. The set-aside fields were sown with a three component seed mixture comprised of two parts grass (e.g. *Festuca pratensis*, *Festuca arundinacea*, *Poa pratensis*, *Dactilys glomerata*) and one part leguminous species (usually *Medicago sativa*) after the last harvest, in the autumn. Vegetation was mown once a year, after the 15th of June, leaving the cut vegetation on site.

Study design

Within the study area, two-year-old set-aside fields (Sa) were chosen, each with an adjacent cereal field (CSa) with seven replicates (Figure 1). Six semi-natural grasslands (G) and six cereal fields without set-asides (C) were also assigned as controls for comparisons. All cereal fields involved in the study were managed similarly, fertilised with about 90 kg nitrogen/ha/year and sown with winter wheat (*Triticum aestivum*) and, in one case, barley (*Hordeum vulgare*). Grasslands were managed extensively, without fertiliser application and grazed or mown once per year. The mean area (± SE) of the study sites was 30.21 ± 3.93 ha. The paired set-aside and cereal fields were of similar size and relief (difference in the field area within pairs: mean ± SE 8.14 ± 1.85 ha).

Soil sampling and analyses

Soil was sampled randomly by taking five soil cores from 0–15 cm depth in May 2014. Before soil analyses, soil cores corresponding to each site were pooled to obtain a composite sample. Physical and chemical analyses of soils were carried out on air-dried
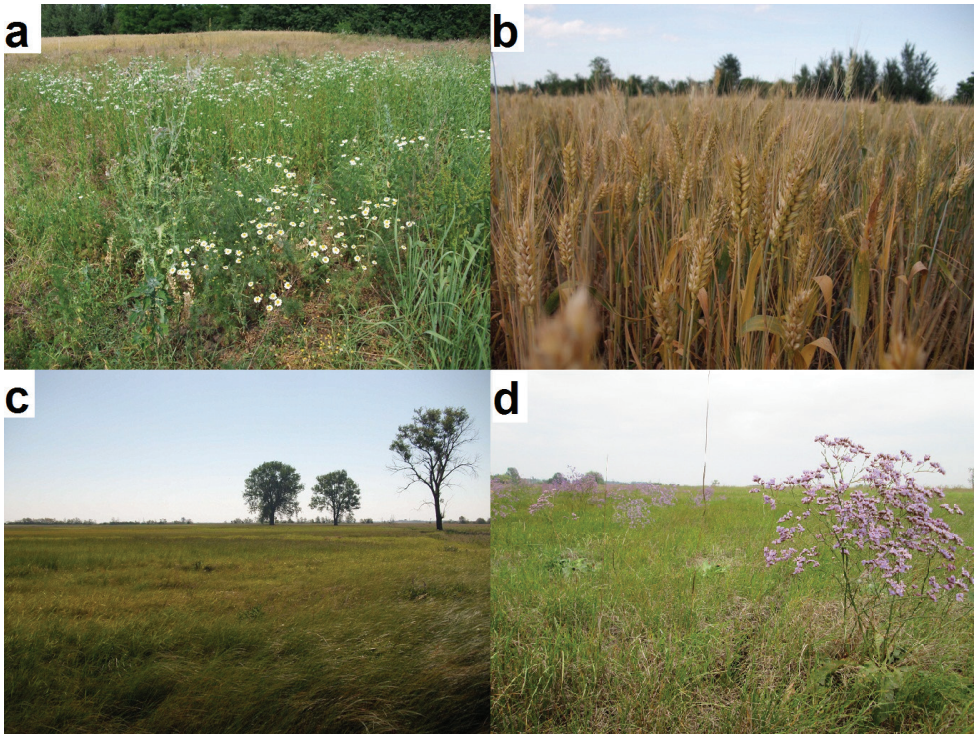
Figure 1. The main habitat types investigated in the Heves Plain High Nature Value Area, Hungary (**a** 2-year-old set-aside field **b** cereal field and **c–d** semi-natural grassland with a typical plant species, *Limonium gmelinii*).

samples from which crop residues, root fragments and rocks larger than 2 mm had been removed (MSZ 21470-2 1981). Soil plasticity, determined by the heaviness index according to Arany (K_A), concluded the soil physical condition and texture (MSZ 08-0205 1978). Soil pH was measured in 1 M KCl suspensions for 12 h after mixing (MSZ 08-0206-2 1978). Soil organic matter (SOM%) was determined using MSZ (Hungarian Standard) 08-0452 (1980). Total nitrite-nitrate nitrogen (NO_2-NO_3 N) was measured using a modified Kjeldahl method (MSZ 20135 1999). Plant-available phosphorus (P), potassium (K), sodium (Na) and magnesium (Mg) concentrations were extracted using AL (ammonium-lactate) (MSZ 20135 1999) and measured using inductively coupled plasma-atomic emission spectrometry (ICP-Thermo Jarrell Ash ICAP 61E). The total water soluble salt content was determined by MSZ (Hungarian Standard) 08-0206-2 (1978).

Microbial analyses: soil bacteria

To determine the effects of set-aside management on the bacterial community structure, composite soil samples were taken from each field in May 2014. Soil sampling

locations (five subsamples per field) were randomly chosen from the upper surface (0–5 cm). DNA was isolated from samples with NucleoSpin Soil kit (Macherey-Nagel, Düren, Germany). The quality of nucleic acids was assessed with 1% agarose gel electrophoresis stained with ethidium bromide. Nucleic acid quantification was undertaken with a Qubit 2.0 Fluorometer (Thermo Fischer Scientific, Waltham, MA, USA) throughout the study.

For bacterial community fingerprinting with the terminal restriction fragment length polymorphism (T-RFLP) method, 16S rDNA fragments were amplified with a 5' VIC-labelled 27F primer (VIC-5'-AGAGTTTGATCMTGGCTCAG-3') and a 518R primer (5'-ATTACCGCGGCTGCTGG-3'). Polymerase chain reactions (PCRs) were undertaken with 5 μl of DreamTaq PCR buffer, 0.3 μM of each primer, 0.2 mM of each dNTP, 30 ng DNA sample, 1 U DreamTaq DNA Polymerase (Thermo Fischer Scientific) and nuclease-free water up to the final reaction volume of 50 μl. Amplification conditions were as follows: 95 °C for 3 minutes, then 32 cycles of 94 °C for 30 s, 52 °C for 30 s and 72 °C for 30 s, then a final extension at 72 °C for 7 minutes. To obtain molecular fingerprints after the amplification, 16S rDNA amplicons were digested with the restriction enzyme, *Alu*I (AG↓CT) (Thermo Fischer Scientific) as described by Révész et al. (2006). Fragments were separated on a Model 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA), whereas primary evaluation of electropherograms was carried out with GeneMapper 4.0 software (Applied Biosystems). T-RF peaks with a peak height below 100 relative fluorescence units or with a peak abundance contribution below 1% were excluded from further analysis. For consensus T-RFLP profiles, duplicate electropherograms from each sample were aligned with each other by the T-Align programme (Smith et al. 2005) with a 0.5 bp confidence interval. Subsequently, the consensus profiles were aligned with T-Align programme for all samples; this step helps to eliminate background noise and to separate T-RFs properly that differ in one to two bases only in terms of their length. In the resulting data matrix, relative peak area ratio was calculated by dividing each individual T-RF peak area by the total peak area of each profile. Subsequently, this matrix was used for statistical analysis of the T-RFLP data.

Microarthropods sampling and QBS index

Soil microarthropods were collected by taking undisturbed soil cores (8 cm in diameter, 400 cm³, six per plot) to a depth of 15 cm at 0, 10 and 20 m from the field edge along a transect. In semi-natural grasslands, soil samples were taken at a distance of 10 m from each other and 2–300 m from the field edge.

Soil fauna was extracted using the Berlese-Tullgren funnel method. During a 12-day extraction period, microarthropods were collected and stored in vials containing 70% ethanol. All animals were counted under a dissecting microscope. Then they were classified into taxonomic groups and the QBS index ("Qualità Biologica del Suolo",

meaning "Biological Quality of Soil") was calculated according to Parisi et al. (2005). This is an integrated soil biological quality index based on eco-morphological types of edaphic microarthropods. The QBS index is the sum of the EMI (eco-morphological index) scores that increases with the degree of microarthropods' adaptation to soil environment (Parisi et al. 2005). Its concept is that high soil quality is associated with the number of microarthropod groups well-adapted to soil habitat. The strength of this indicator is its sensitivity to land use change and to short term variations in management practices. However, it is less sensitive to large variations in some soil parameters, such as SOM (Parisi et al. 2005).

Macrodetritivore sampling: Diplopoda and Isopoda

Macrodecomposers were sampled by pitfall traps for two weeks in May 2014. Traps were set along a 20 m transect 0, 5, 10 and 20 m from the field edge. In semi-natural grasslands, traps were placed at a distance of 10 m from each other and 2–300 m from the field edge. We applied funnel traps filled with ethylene glycol. They were sunk directly into the soil and covered with plastic roofs to shield from rain. Pitfall traps were returned to the laboratory and, after sorting for subsequent species identification, the samples were preserved in 70% ethanol. Millipedes (Diplopoda) and isopods (Isopoda: Oniscidea) were identified to species level. For identification of millipedes, the keys of Schubart (1934) and Korsós (2015) and for isopods, the key of Gruner (1966) were used.

Plant tissue decomposition: tea bag method

To follow microbial degradation of organic matter, the novel litter quality independent tea bag method was used (Keuskamp et al. 2013). In each plot, four pyramid-shaped, synthetic tea bags (mesh size: 280–300 μm) filled with rooibos (*Aspalathus linearis*; 1.26 ± 0.03 g) were placed at 3–5 cm depth under the soil surface in May 2014. A total of 104 tea bags were buried (four bags per plot × 26 plots). For further information about the tea and chemical descriptions, see Keuskamp et al. (2013). Before field application, tea bags were prepared by the protocol developed in the GLU-SEEN project (http://www.gluseen.org/protocols/preparing-teabags/) to eliminate water soluble materials (e.g. simple sugars and phenols). This is important in order to exclude abiotic mass loss from precipitation-induced leaching. Tea bags were retrieved after 1 month. After gently removing adhered soil from the outside of tea bags in the laboratory, they were soaked under tap water to eliminate soil particles that had passed through the mesh. All samples were air dried at room temperature and then in a climate cabin at 36 °C until they reached a constant weight. Dried samples were used to measure changes in the mass of organic matter through time and to estimate the rate of decomposition.

Statistical analyses

All statistical analyses were performed in R 3.3.1 (R Development Core Team 2016), using the R packages 'lme4' (Bates et al. 2015), 'mvabund' (Wang et al. 2012) and 'vegan' (Oksanen et al. 2017). Non-metric multidimensional scaling (NMDS) was carried out with the software PAST 3.10 (Hammer et al. 2001).

Outliers were identified and removed prior to data analysis. After fitting the full models for each dependent variable, we used Akaike Information Criterion (AIC) to select the most parsimonious model. The lack of spatial independence of the paired set-aside and cereal fields was treated by application of a random factor ('location'). Since there was significant intercorrelation between soil characteristics and habitat type, their effects were tested in separate models. Assumptions of normality and homoscedasticity of the residuals were verified visually using diagnostic plots. Statistical significance was determined at the level: $\alpha = 0.05$.

Soil physical and chemical properties

The effects of land use on soil physical and chemical properties were tested by linear mixed-effects model (LMM), with 'habitat type' as explanatory variable and 'location' as random factor.

Soil bacteria

Alpha diversity metrics (Shannon diversity [H'] and Evenness [J'] indices based on T-RFLP abundance data) were calculated to estimate the diversity of bacterial communities. We used LMMs to determine the effects of habitat type, soil physical and chemical properties on bacterial alpha diversity. A PERMANOVA (Bray-Curtis index, permutation = 999) was conducted to assess differences in the bacterial communities by habitat type.

Soil arthropods

To characterise soil arthropod communities, QBS index, species richness (number of species in the sample) and abundance (number of individuals in the sample) were used. The LMMs were used to examine the effects of abiotic soil properties and habitat type on faunal richness and abundance. The influence of abiotic soil properties and habitat types on the species composition of isopod and millipede assemblages was tested by generalised linear mixed models (GLMMs) with the multivariate approach. As our data showed a negative binomial distribution, we thus used the 'manyglm' method (family = negative binomial). Then we conducted NMDS ordinations using the Bray-

Curtis dissimilarity index to visualise patterns of species composition of macrodetritivore assemblages. In the latter case, species with low relative abundance (*Trachelipus nodulosus*: 0.43%, *Porcellionides pruinosus*: 0.58%) were excluded from the analysis.

Plant tissue decomposition

Rates of decomposition were estimated with a single exponential decay model (Olson 1963):

$$M_t / M_0 = e^{-kt}, \text{ (1)}$$

where M_0 is the initial dry mass, M_t is the residual dry mass at time t and k is the decay constant.

The effects of habitat type and abiotic soil properties were tested by a LMM.

Soil biodiversity – plant tissue decomposition linkage

A soil biodiversity index was calculated from the average of all standardised soil community characteristics (bacterial diversity, QBS index, macrofauna species richness and abundance) and used as a general indicator (Wagg et al. 2014). To reveal the relationship between soil biodiversity (and its biotic components) and the decomposition rate of plant residue, LMMs were also used.

Results

Soil physical and chemical properties

We experienced significant differences in soil pH, K_2O and sodium (Na) content amongst habitat types. Soil pH ranged from 4.42 to 6.86 in the different habitat types. It had the lowest value in semi-natural grasslands (G) followed by set-aside (Sa) and cereal fields (C and CSa) (Table 1). Soil K_2O was significantly higher in cereal fields (C and CSa) compared to set-aside fields and semi-natural grasslands. Moreover, there were significant differences in soil sodium content between grasslands and other habitat types (Table 1).

Soil biota

Soil bacteria
The Shannon and Evennes indices showed relatively high variability amongst habitat types, with values ranging from 1.37 to 3.03 and from 0.28 to 0.71, respectively. Only

Table 1. Basic properties of soil samples taken from the 0–15 cm depth (mean ± SE). Letters indicate significant differences amongst the means at p < 0.05. Abbreviations – SOM: soil organic matter, C: cereal fields, CSa: cereal fields adjacent to set-asides, Sa: set-aside fields, G: semi-natural grasslands.

	Habitat types			
	C	**CSa**	**Sa**	**G**
pH	6.09±0.22 **(a)**	5.54±0.22 **(ab)**	5.29±0.20 **(bc)**	4.92±0.13 **(c)**
K_A	44.83±1.23 **(a)**	47.14±2.77 **(a)**	43.57±0.94 **(a)**	47.17±1.44 **(a)**
Salt (m/m %)	0.04±0.01 **(a)**	0.03±0.00 **(a)**	0.04±0.01 **(a)**	0.05±0.01 **(a)**
$CaCO_3$ (m/m %)	0.32±0.29 **(a)**	0.19±0.17 **(a)**	0 **(a)**	0 **(a)**
SOM (m/m %)	3.57±0.43 **(a)**	3.29±0.21 **(a)**	3.52±0.28 **(a)**	4.02±0.29 **(a)**
NO_2-NO_3 N (mg kg^{-1})	13.04±6.67 **(a)**	7.88±1.04 **(a)**	23.27±5.39 **(a)**	35.67±16.49 **(a)**
P_2O_5 (mg kg^{-1})	300±57.79 **(a)**	165.71±23.61 **(a)**	136.86±43.21 **(a)**	233.75±119.06 **(a)**
K_2O (mg kg^{-1})	682±99.12 **(a)**	677.14±71.93 **(a)**	461±65.2 **(b)**	378±32.76 **(b)**
Na (mg kg^{-1})	182.92±66.35 **(b)**	97.37±13.77 **(b)**	72.14±13.35 **(b)**	500.5±96.61 **(a)**
Mg (mg kg^{-1})	565.33±81.85 **(a)**	542.14±70.63 **(a)**	605.57±68.48 **(a)**	775.17±118.34 **(a)**
SO_4-S (mg kg^{-1})	41.5±6.64 **(a)**	38.79±4.36 **(a)**	42.39±4.40 **(a)**	58.2±7.84 **(a)**

Table 2. Effects of habitat type and soil physicochemical properties on microarthropods and macrodetritivores. $CaCO_3$ and Mg variables are not included in the table, since they had significant effects on none of the dependent variables. Abbreviations – QBS: soil biological quality index, SR: species richness, SOM%: soil organic matter %, K_A: soil plasticity index according to Arany; +: positive effect, –: negative effect, NS: not significant, ***: p ≤ 0.001, **: p ≤ 0.01, *: p ≤ 0.05, ˙: p ≤ 0.1

	Microarthropods			Macrodetritivores				
				Isopoda		Diplopoda		
	diversity (QBS)	abundance	composition	diversity (SR)	abundance	diversity (SR)	abundance	composition
habitat	**	*	NS	*	**	NS	*	**
pH	– ***	+ ***	NS	– *	NS	NS	NS	NS
SOM %	+ *	NS	˙	NS	NS	NS	NS	NS
K_A	+ **	NS	NS	NS	NS	NS	NS	*
salt	NS	– ***	NS	NS	NS	NS	NS	˙
K_2O	NS	– ***	NS	NS	– ˙	NS	NS	NS
NO_2-NO_3 N	NS	+ ***	NS	NS	– *	NS	NS	NS
SO_4-S	+ **	NS	NS	NS	NS	NS	NS	NS
Na	– *	– **	NS	NS	NS	NS	NS	NS

Evennes index was significantly influenced by the studied environmental variables: it decreased with the SOM% (t = -2.47, p = 0.05), whereas increased with soil Na content (t = 3.28, p = 0.022) (Table 2). In this study, habitat type did not significantly affect bacterial alpha diversity (see Table 2) and community composition ($F_{PERMANOVA}$ = 1.2951, p = 0.122).

Soil microarthropods

In total, 14385 specimens belonging to 19 taxa of microarthropods were sampled (Suppl. materials 1–2). The QBS index varied from 29 to 128 and showed significant differences amongst habitat types (Table 2, Suppl. materials 1–2). The highest values were found in semi-natural grasslands, while cereal fields without set-asides were characterised by the lowest QBS (Figure 2). Abundance of microarthropods was significantly affected by habitat type: it was the highest in set-aside fields compared to the other habitats (Figure 2). Nevertheless, all samples were dominated by mites, particularly oribatids (70.38% of the total microarthropods collected). Soil pH and Na content had negative, while soil plasticity and SO_4-S content had positive effects on the QBS index. There was a positive relationship between soil pH, NO_2-NO_3 N content and abundance of microarthropods. We found that the number of microarthropods decreased with total soluble salt concentration and with the amount of soil K_2O and Na (Table 2).

Soil macrodetritivores

In total, 1391 individuals of 8 macrodecomposer species were identified from samples collected by the pitfall traps, including 783 individuals of four isopod species (*Armadillidium vulgare*, Latreille, 1804; *Porcellionides pruinosus*, Brandt, 1833; *Trachelipus rathkii*, Brandt, 1833; *Trachelipus nodulosus*, C. Koch, 1838) and 608 individuals of four millipede species (*Brachydesmus superus*, Latzel, 1884; *Brachyiulus bagnalli*, Brölemann, 1924; *Iulus terrestris*, Linnaeus, 1758; *Megaphyllum unilineatum*, C. Koch, 1838) (Suppl. materials 3–4). The most abundant species were *Armadillidium vulgare* (89.27%) and *Iulus terrestris* (59.38%) from isopod and millipede species respectively. The total abundance of the studied macrodecomposers was highest in semi-natural grasslands, with 645 individuals of isopods and 379 individuals of millipedes. The lowest abundance of detritivores was recorded within cereal fields for isopods (4 individuals of total) and cereal fields adjacent to set-asides for millipedes (30 individuals of total). In total, 98 isopod and 37 millipede specimens were collected in set-aside fields.

In the present study, species richness was significantly affected by the studied environmental variables only in the case of isopods. We experienced significant effects of habitat type and soil pH on isopod species number increasing with soil acidity (z = -2.236, p = 0.022) (Table 2). Semi-natural grasslands were characterised by the highest species richness, while cereal fields without set-asides proved to be the most species-poor habitats (Figure 2). There were significant differences in abundance of iso- and diplopods amongst habitat types. Isopod individual numbers were the highest in semi-natural grasslands and the lowest in cereal fields without set-asides, respectively. By contrast, millipedes were the most abundant in the latter habitats (Figure 2). Soil K_2O and N had negative effects on the abundance of woodlice (Table 2).

Species composition of iso- and diplopod assemblages was affected by habitat type, salt concentration and soil plasticity (Table 2, Figure 3). *Brachyiulus bagnalli* mostly occurred in cereal fields (Dev = 19.903, p = 0.009), while *A. vulgare* preferred semi-
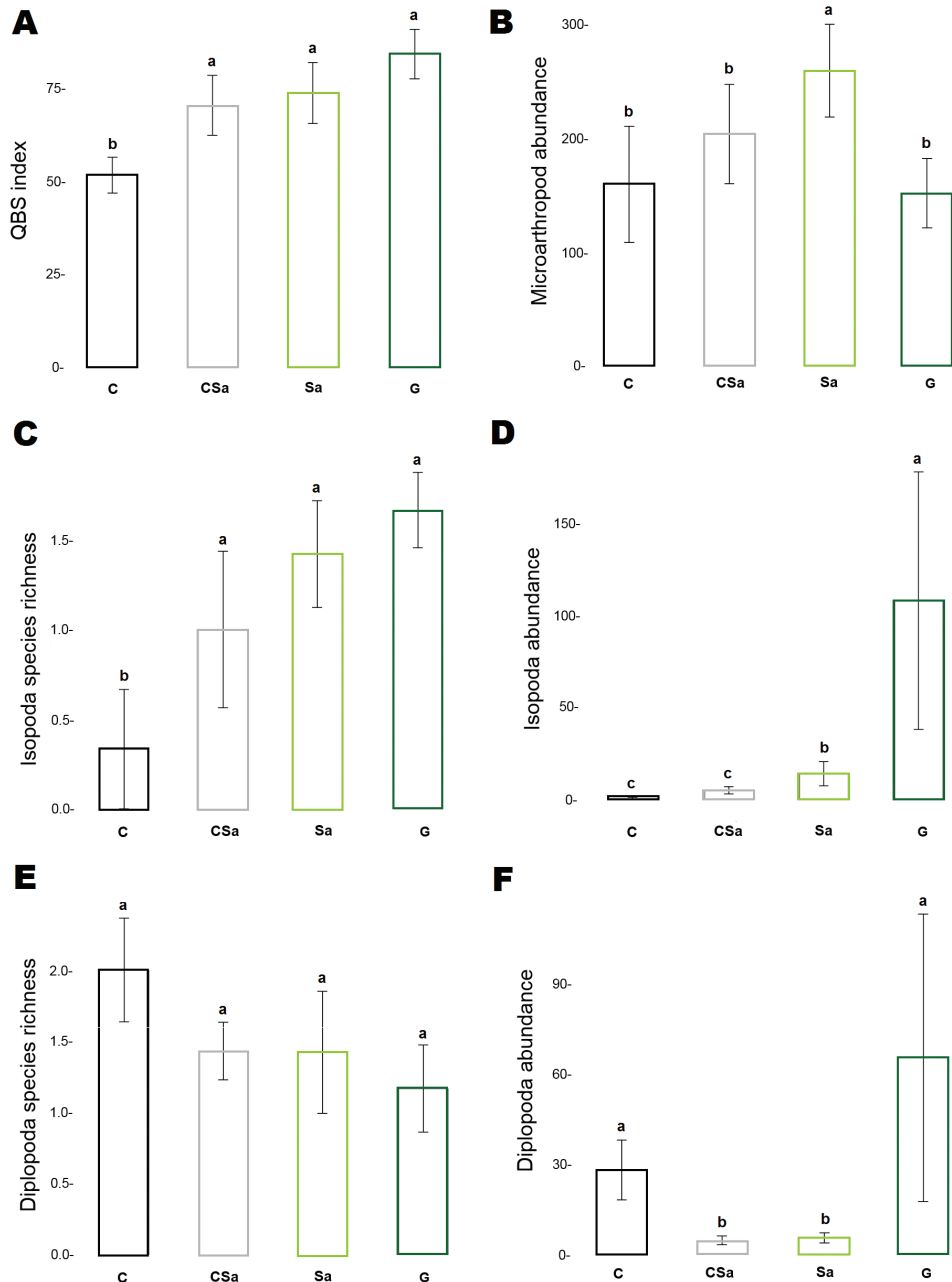
Figure 2. Diversity (expressed in species richness and QBS index) and abundance of microarthropods (**A–B**), isopods (**C–D**) and millipedes (**E–F**) in different habitat types. Error bars represent means and SE. Please note the different scaling for the Y axes. Letters indicate significant differences amongst the means. Abbreviations – C: cereal fields, CSa: cereal fields adjacent to set-asides, Sa: set-aside fields, G: semi-natural grasslands.
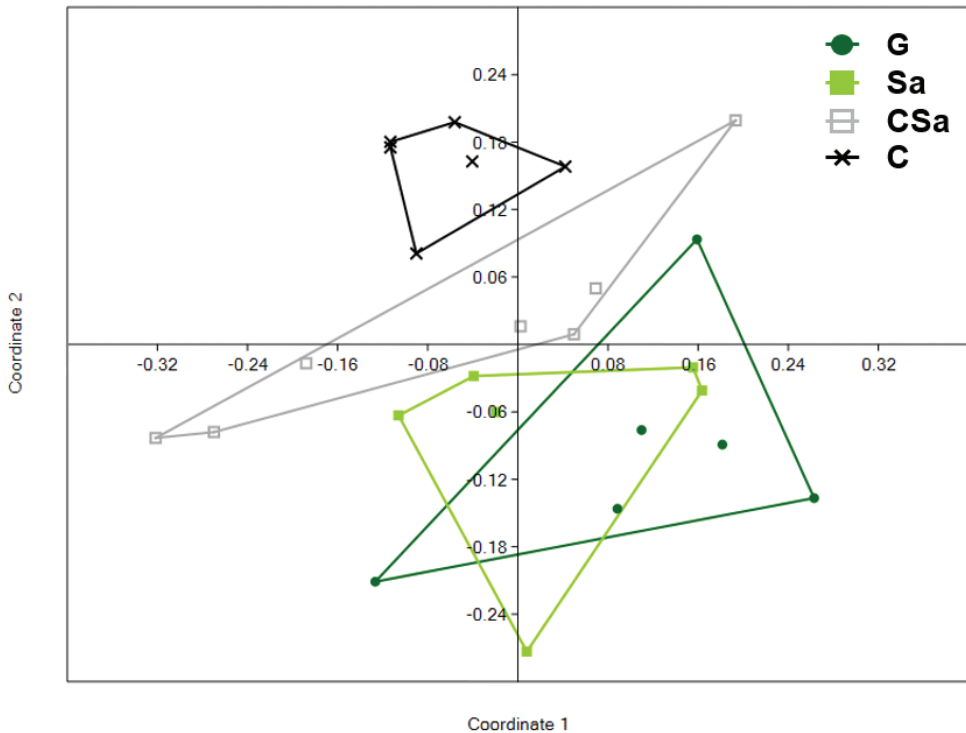
Figure 3. NDMS plot on species composition of macrodecomposer assemblages as related to habitat types. See Figure 2 for abbreviations.

natural grasslands characterised by soils with high salt concentration (Dev = 10.426, p = 0.03). *Brachydesmus superus* was observed in sites with higher soil plasticity index (Dev = 15.422, p = 0.008).

Plant tissue decomposition and its relationship with soil biodiversity

On average, 22.41% of organic matter was decayed during a month. Mass loss was significantly different between habitat types (F = 10.8618, p < 0.001). We experienced the highest decomposition in set-asides (remaining mass: 74.17%, on average) while the lowest in cereal fields (remaining mass: 81.3%, on average). The decomposition rate was negatively influenced by SOM content (F= 12.3966, p= 0.002). However, soil pH had positive effects on the intensity of mass loss (F = 5.3119, p = 0.033). Organic matter decomposition did not change with soil biodiversity (t = 1.2589, p = 0.255). Nevertheless, we found marginally significant QBS index – decay rate relationship (t = 2.1076, p = 0.08) (Figure 4).
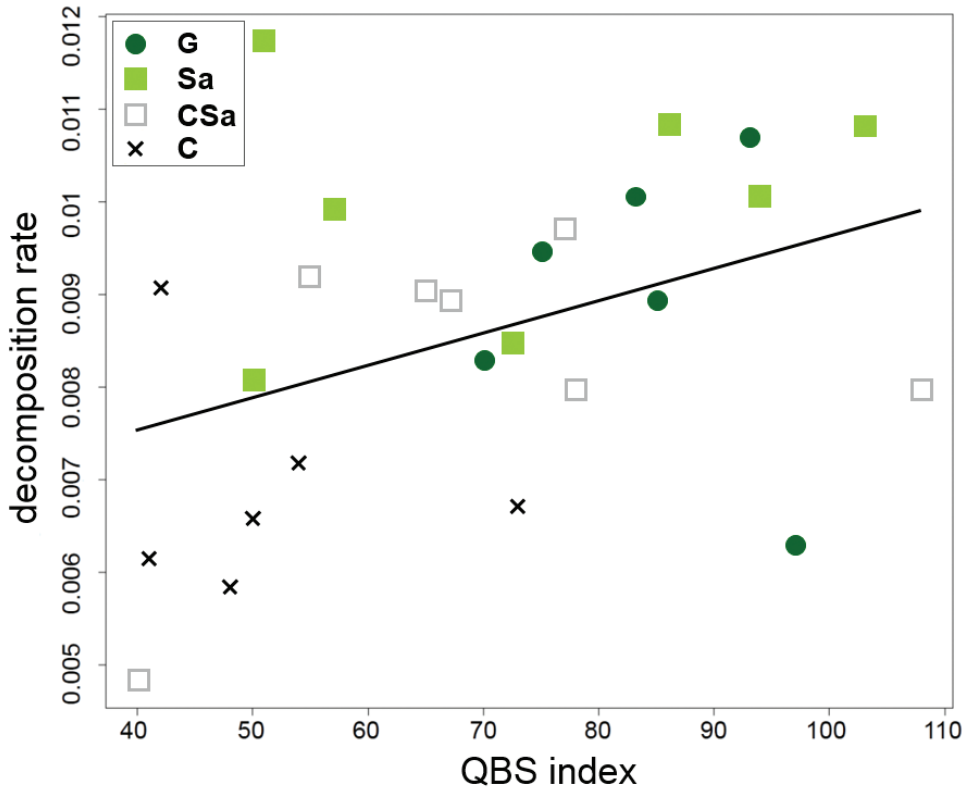
Figure 4. The relationship between decomposition rate of organic matter (g day^{-1}) and biological quality of soils (expressed in QBS index) based on the linear mixed-effects model. See Figure 2 for abbreviations.

Discussion

Soil physical and chemical properties

The general consensus in literature is that agricultural practices, particularly soil cultivation and manuring, can lead to drastic changes in soil physical and chemical properties (e.g. Bronick and Lal 2005, De Paul Obade and Lal 2014). Consistent with our first hypothesis, we found significant differences amongst habitat types in case of soil pH, K_2O and Na content, confirming the close relationships between agricultural management and abiotic soil conditions. The significantly lower soil pH in cereal fields compared to semi-natural grasslands and set-asides is probably due to previous soil correction by liming practice, while the relatively high K_2O content of soil experienced in former habitats could be the result of fertilisation. The extremely high amount of Na in soils of semi-natural grasslands is probably due to the nature of the typical saline soil type in the region (Stefanovits et al. 1999).

Soil biota

Soil bacteria

Contrary to our expectations, we found no significant differences amongst habitat types regarding bacterial alpha diversity and community composition. The majority of studies showed that microbial species richness increases both with plant diversity and reduction of anthropogenic disturbances (e.g. Swift et al. 1996), which mainly characterises set-aside fields and semi-natural grasslands. In addition to vegetation characteristics, soil type and land use are of particular importance in influencing microbial diversity (e.g. Garbeva et al. 2004a,b). Most of the cases (84%), reviewed by Allison and Martiny (2008), reported the sensitivity of microbial communities to N, P, K fertilisation. O'Brien et al. (2016) experienced higher microbial α diversity in fertilised fields. However, there are also contrary experiences (in accordance with our results): several previous reports did not reveal a correlation between plant species richness and bacterial diversity, emphasising rather the importance of soil properties (e.g. Fierer and Jackson 2006, Zul et al. 2007). In addition, agricultural treatments such as fertilisation, have no consistent effects on the diversity of bacterial communities as evidenced by, inter alia, the results of Fierer and co-workers (Fierer et al. 2012).

Out of the α diversity indices, the evenness of the bacterial communities was significantly influenced by SOM and sodium contents of soils. This corresponds with findings in which significant effects of soil pH, organic matter content, moisture and nutrient availability on microbial community structure have been reported (e.g. Fierer et al. 2012, Kuramae et al. 2012). The evenness index increased with soil sodium content which mainly characterised semi-natural grasslands. This could be attributed to the better quality of these undisturbed habitats supporting more stable and balanced bacterial communities.

Soil microarthropods

The more diverse and complex vegetation creates more favourable microclimatic conditions for soil microarthropods (Adejuyigbe et al. 1999): the structure of vegetation largely determines the moisture and temperature conditions of the soil, which has a serious effect on the studied animal groups (e.g. Hopkin 1997, Tsiafouli et al. 2005). It provides a better quality food source for microarthropods, predominantly determined by the C / N ratio of dead plant material (Seastedt 1984). Furthermore, these habitats positively affect the presence of soil animals providing refuges for them due to the lower degree of human disturbance resulting from agricultural activities (Barbercheck et al. 2008).

The higher microarthropod diversity and abundance in cereal fields adjacent to set-asides compared to cereals without set-aside fields are probably attributable to the spillover effect of set-aside fields: several studies have proved the positive role of semi-natural habitat patches as propagule sources, affecting favourably the adjacent areas as well (e.g. Blitzer et al. 2012). In addition to the above-mentioned environmental

factors, the soil physical and chemical properties are of great importance for soil arthropods: soil pH, salt concentration, organic matter and nutrient content proved to be significant factors. Soil pH had a variable effect on the QBS index and the number of microarthropods: the former showed a positive relationship with pH, while the latter increased with soil acidity. Although most of the soil arthropods do not prefer acidic soils (Swift et al. 1979), the abundance of certain taxa (e.g. Oribatida) decreases with soil pH (e.g. Maraun and Scheu 2000). This might explain the higher microarthropod numbers found in soils with lower pH: most of the samples were dominated by mites (mainly oribatids). We experienced positive effects of soil organic matter and nitrogen content on microarthropods. The first is supported by the results of several studies (e.g. Edwards and Lofty 1969). This is likely to be closely related to the fact that soil organic matter serves as an energy and nutrient source for them (Swift et al. 1979). The observed positive relationship between soil nitrogen content and microarthropod diversity was probably due to their food preference. Although there were no significant differences between habitats, the nitrogen supply of soils of set-aside fields and grasslands was generally higher, which might be largely caused by the N-rich vegetation. The role of legumes, which characterised these habitats, is also essential in this respect as they make a significant contribution to atmospheric N fixation. Therefore, they increase soil fertility and return high quality litter to soil organisms (Mulder et al. 2002). The chemical composition of dead plant material is particularly important for the detritivore arthropods, remarkably affecting their diversity and abundance. It is well-known that most of them prefer N-rich detritus (e.g. Seastedt 1984), which might be found mainly in set-aside fields and semi-natural grasslands. Potassium also proved to be a significant soil nutrient: its increase resulted in unfavourable change in soil biological quality (expressed in the QBS index). Since there were significantly higher K_2O values in cereal fields compared to set-asides and grasslands, presumably fertilisation remains in the background. We can find examples of beneficial and adverse effects of fertilisation on soil microarthropods supporting the relevance of this issue (Bardgett and Cook 1998). The relatively high total soluble salt and Na concentrations refer unfavourable soil conditions not tolerated by the majority of soil organisms. Therefore, the negative effects of these soil parameters on soil arthropods are not surprising. The higher number of individuals found in clay soils (with higher K_A values) may be attributed to beneficial soil conditions (e.g. soil moisture, nutrient and organic matter). Nevertheless, it is important to emphasise that heavily-bound soils can lead to opposing changes, as limited pore space impedes movement of the soil microarthropods (O'Lear and Blair 1999).

Soil macrodetritivores

Species richness of isopods and millipedes reflected the regional species pool. All millipede and isopod species found in the sampling sites are rather common in the Hungarian Great Plain (Korsós and Hornung, unpublished results). In human modified habitats, such as agroecosystems, a wide range of millipede species generally occurs in relatively low species richness, but in high density (Golovatch and Kime 2009). Except for *Leptoiulus cibdellus* and *Porcellionides pruinosus*, the observed species were almost

the same as found by Tóth et al. (2016). The latter can be regarded as synanthropic: appears in all kinds of anthropogenic habitats in Hungary (Vilisics et al. 2007, Hornung et al. 2008). In the present study, it occurred only in cereal fields similarly to a millipede species, *Brachydesmus superus*. This is not surprising as both species have broad tolerance to anthropogenic disturbance (Schubart 1934, Schmalfuss 2003). The significant effects of abiotic soil properties on isopods shown by LMM are probably related to habitat type. The beneficial effect of grasslands and set-aside fields on isopods might be mainly due to the more favourable microclimatic conditions, the better quality food source and the lesser anthropogenic disturbance (Tóth et al. 2016). However, we did not find such a clear habitat preference in the case of millipedes. In the present study, the habitat type was almost irrelevant regarding species richness. Nevertheless, it had significant effect on their abundance: cereal fields without set-asides proved to be the most favourable habitats with almost the same values as semi-natural grasslands. Differing habitat preference of isopods and millipedes can be explained by physiological attributes: millipedes are less sensitive to microclimatic effects being more drought resistant (Morón-Ríos et al. 2010) than isopods. Soil temperature and moisture content are the main abiotic background factors influencing the presence and abundance of the animals in question, especially that of terrestrial isopods. Their exoskeleton is permeable to water and so the desiccation threat restricts their occurrence to habitats with higher humidity and suitable shelter sites (e.g. Warburg 1987, Hopkin and Read 1992).

We identified that habitat type, salt concentration and soil plasticity (expressed in K_A) were the main factors influencing the species composition of the macrodecomposer assemblages (Figure 2). *Brachyiulus bagnalli* mainly occurred in cereal fields that can be explained by its habitat preference: it favours disturbed, open habitats, tolerating a wide range of drought and human presence (Schubart 1934, Korsós 1992). In contrast, *A. vulgare* preferred grasslands and its occurence was connected to soils with higher salt content, indicating a relatively high salinity tolerance. *Brachydesmus superus* was significantly influenced by soil plasticity. There may be several explanations for this phenomenon: for example, it strongly affects the soil moisture regime, soil chemistry, overall substrate availability and the movement of millipedes.

Plant tissue decomposition and its relationship with biodiversity

Plant tissue decomposition was the highest in set-aside fields and semi-natural grasslands during the studied period. There was also a significant difference in mass loss of organic matter between cereal fields with and without set-asides. We experienced the lowest degree of decomposition in the latter habitats.

It has long been proven that characteristics of ecosystems (e.g. physical, chemical and biological properties) basically determine their functionalities (Wiens 1972). Concerning the carbon cycle, the carbon sink and source ecosystems can be distinguished. Almost all natural habitats, characterised by a high amount of plant bio-

mass, such as enhanced primary production and moderate carbon emissions, belong to the former category. By contrast, anthropogenic conversion of natural habitats results in degraded ecosystems becoming carbon sources (Bardgett and Wardle 2010). Consequently, more intensive decomposition processes and, thus, higher carbon emissions, could be expected in soils of cereal fields compared to grasslands and set-asides. However, we found higher organic matter decay in soils of the less disturbed habitats, which suggests greater rates of soil respiration. Nevertheless, it is important to emphasise that it is not possible to draw far-reaching conclusions from this one-month period of the examination. The dynamics of organic matter decomposition shows great spatial and temporal variation (Swift et al. 1979). At least a one-year period would have been necessary to seek a better picture, but agricultural activities in the sampling areas did not allow this.

In addition to the habitat type, two abiotic soil properties (soil pH and SOM%) also significantly affected the decomposition rates. The greater biodegradation observed in the more alkaline soils is probably attributed to the fact that the acidic soil pH is not favourable to the majority of soil organisms, which can lead to reduced organic matter decomposition. The negative relationship between SOM content and decay rates could infer less intensive mineralisation and immobilisation than humification, resulting in a higher SOM level due to the gradual accumulation of soil organic matter.

There was no significant correlation between soil biodiversity and organic matter decomposition in our research, despite the positive trend between the two variables. Reasons for the lack of significance may be related to one-off sampling, short period of the organic matter decomposition test, forced skip of key groups of decomposer organisms (fungi, earthworms) etc. In the case of microbes, we could estimate the diversity only of soil bacteria, although it is possible that habitats with acidic soils were dominated by fungi. This is also supported by the fact that soils with low pH and higher organic matter content – such as semi-natural grasslands and set-aside fields in our study – generally have a fungal-dominated food web (Bardgett and Wardle 2010). However, agricultural practices (fertilisation, grazing, ploughing etc.) generally lead to a shift from fungal-based soil food webs to more bacterial-based soil food webs (Bardgett and Wardle 2010). Ideally, therefore, both microbial groups should be taken into account as their role in breakdown of organic matter may differ depending on habitat type.

Out of the biotic components, only soil biological quality (expressed in the QBS index) significantly influenced (even if marginally) plant tissue decomposition. The positive effects of microarthropods on decay of organic matter have already been demonstrated in a number of studies (e.g. Crossley and Hoglund 1962). According to Seastedt's (1984) estimate, soil microarthropods consume 20–30% of the total annual litter, thus directly and indirectly promoting the breakdown of detritus. This is in line with the results of De Graaff and co-workers (2015) who found a decline in decomposition rates with the decrease of soil faunal diversity, while the reduction of microbial diversity has not affected the decay intensity.

Conclusions

Our results indicate that set-aside management under agri-environment schemes has profound effects not only on certain soil physical and chemical properties, but on soil biodiversity and function as well. The present study highlights the importance of set-aside fields particularly for the conservation to surface dwelling invertebrates. Set-aside fields function as semi-natural habitats providing favourable conditions especially for micro- and macroarthropods, supporting the regeneration of soil biological resources. Set-aside fields that are not part of a crop rotation for at least 2 years could be a valuable option for establishing ecological focus areas under the Common Agricultural Policy (CAP) in the EU, as these fields may help to conserve soil biodiversity.

However further research is required to look for the optimum management regimes for all soil-related organisms supporting the most abundant and diverse soil biota, particularly in relation to the establishment methods of set-aside or other semi-natural habitat types in agricultural landscapes.

Finally, we emphasise that evaluation of agri-environmental schemes, regarding soil biodiversity and function, is of high practical and theoretical importance. For example, data on soil biota, plant tissue decomposition and/or their relationship are essential to better understand mechanisms influencing biogeochemical cycles. Therefore, the biological and functional aspects of soil need to be better taken into account in a national or/and European soil monitoring scheme.

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Supplementary material 1

Table S1
Authors: Zsolt Tóth, Elisabeth Hornung, András Báldi
Data type: occurence

Link: https://doi.org/10.3897/natureconservation.29.24856.suppl1

Supplementary material 2

Table S2
Authors: Zsolt Tóth, Elisabeth Hornung, András Báldi
Data type: occurence
Link: https://doi.org/10.3897/natureconservation.29.24856.suppl2

Supplementary material 3

Table S3
Authors: Zsolt Tóth, Elisabeth Hornung, András Báldi
Data type: occurence
Link: https://doi.org/10.3897/natureconservation.29.24856.suppl3

Supplementary material 4

Table S4
Authors: Zsolt Tóth, Elisabeth Hornung, András Báldi
Data type: occurence
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Link: https://doi.org/10.3897/natureconservation.29.24856.suppl4

Supplementary material 5

Map file
Authors: Zsolt Tóth, Elisabeth Hornung, András Báldi
Data type: KML file
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