



**SZENT ISTVÁN UNIVERSITY**

**RELATIONSHIP BETWEEN BIOTIC, ABIOTIC  
FACTORS AND DECOMPOSING ACTIVITY OF  
DIFFERENT SOILS**

PHD THESIS

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# 1. INTRODUCTION AND OBJECTIVES

Ecosystem services provided by natural and human-modified ecosystems deserve more and more attention because of multiplying and unresolved global environmental problems. Therefore, their assessment, preservation and optimization are of paramount importance. Organic matter decomposition in soil is considered as an ecosystem services, being essential part of nutrient cycling. Our investigations focused on decay of plant residue which gives a significant fraction of the breakdown. A wide variety of soil organisms are involved in decomposition. The activities (comminution, burrowing and inoculation) of macrofauna [earthworms (Oligochaeta), land snails (Gastropoda), millipedes (Diplopoda), woodlice (Isopoda: Oniscidea)] support microbial decomposition of detritus. Soil mesofauna [especially mites (Acari) and springtails (Collembola)] also comminute plant debris into smaller pieces and inoculate ingested organic matter with microbial propagules. Nevertheless, fungi and bacteria are largely responsible for biodegradation of plant tissue. Without the necessary pre-digestion and surface-enhancing effect, however, their enzymes are less effective, leading to a delay in decomposition. Thus, organic matter decomposition necessary for the functioning of biogeochemical cycles constitutes an important successional network, which can be slowed down or even accelerated by the loss of certain functional groups. The accelerated release of CO<sub>2</sub> and other greenhouse gases (e.g. CH<sub>4</sub>) during organic matter decomposition might have a positive feedback effect on climate change. In addition to global warming, the effects of urbanization and intensive agriculture are not negligible because they greatly modify our ecosystems, including the state of our soils, thus affecting their biodiversity and functionality.

So, the main question of my PhD research is how the various anthropogenic impacts (urbanization, climate change, agricultural management) affect linkages between soil physicochemical properties, biodiversity and decomposition of plant detritus. I present my researches in four major chapters according to the following objectives and hypotheses:

**(i)** Investigation of the effects of urbanization on soil biodiversity and organic matter decomposition on the Buda side of the capital (GLUSEEN-Project: 'Global Urban Soil Ecology and Education Network'). Soil biota and organic matter decomposition were compared in four habitat types characterized by different level of anthropogenic disturbance and management. Our hypotheses were the following:

- (a) urbanization has significant effect on physicochemical soil properties;
- (b) the more natural (less disturbed) habitats (remnant and reference forests) are characterized by richer soil biota than degraded urban fields;

- (c) organic matter decomposition is more intensive in habitats with favourable conditions for soil life (remnant and reference forests);
- (d) there is significant correlation between soil biodiversity and plant tissue decomposition.

**(ii)** Investigation of the effects of extreme drought, on soil microbes and decomposition of plant tissue in a climate manipulation field experiment (ExDRain-Project: 'Extreme Drought and Rain Manipulation Experiment'-Fülöpháza). The main goal of this study was to determine changes in the rate of organic matter decomposition in response to an extreme drought event (5-month precipitation exclusion) and to assess its effects on structure of the microbial community 6 months after the dry period. Furthermore meso- and macrofauna were sampled to get some information about soil arthropods communities. Our hypotheses were the following:

- a) lack of water resulted from extreme drought decreases organic matter decomposition;
- b) extreme drought, even six months after precipitation exclusion, has significant effects on community structure of the soil microbiota; and
- c) bacterial diversity influences intensity of organic matter decomposition.

**(iii)** Investigation of the effects of set-aside management on macrodetritivore soil arthropods in the Heves Environmentally Sensitive Area (today called High Nature Value Area). We assessed the effects of habitat type (pairs of wheat and set-aside fields of different ages [1, 2, 3 years] and semi-natural grasslands), plant species richness and vegetation cover on species richness and abundance of millipedes and woodlice. Our hypotheses:

- a) the species richness and abundance of isopods and millipedes is higher in set-aside than in wheat fields;
- b) richness and/or abundance of isopods and millipedes increases in set-aside field during their 3-year duration;
- c) wheat fields, adjacent to older (2–3 years old) set-aside areas have higher diversity and/or abundance of millipedes and isopods than those adjacent to one-year-old fields;
- d) plant species richness, vegetation cover and habitat type have an effect on the abundance of individual isopod and millipede species and the composition of macrodecomposer communities.

**(iv)** Investigation of the effects of set-aside management on soil biodiversity and organic matter decomposition in the Heves Plain (LIBERATION-Project: 'Linking farmland Biodiversity to Ecosystem services for effective ecological intensification'), Semi-natural grasslands, cereal and 2-year-old

set-aside fields were compared regarding soil biota and organic matter decomposition. Our hypotheses:

- (a) farming practices influence soil physicochemical properties;
- (b) set-aside fields and semi-natural grasslands have richer soil biota than heavily managed cereal fields, and
- (c) organic matter decomposition is more intensive in habitats with favourable conditions for soil life (set-aside fields and semi-natural grasslands);
- (d) there is positive correlation between soil biodiversity and plant tissue decomposition.

## 2. MATERIALS AND METHODS

### 2.1. Effects of urbanization on soil biodiversity and organic matter decomposition (GLUSEEN-Project, Budapest)

HABITAT SELECTION: During habitat selection, we took parent material, soil type, vegetation, size, accessibility and position of site into account. Twenty (4 habitat types × 5 replicates) urban green habitat patches were selected along a gradient of anthropogenic influence. The habitat types were the following: (1) highly disturbed, ruderal habitats [high disturbance/low management]; (2) turfs [high disturbance/medium management]; (3) remnant forest [low disturbance/low management]; and (4) reference forests.

SAMPLING METHODS: For soil physicochemical and microbial analyses, composite soil samples were taken to a depth of 10 and 5 cm in the fall of 2013. Soil pH, CaCO<sub>3</sub>, soil organic matter, N, P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O content were measured. Quantitative PCR and amplicon sequencing using Illumina MiSeq platform were conducted to determine soil microbial communities. Microarthropods were sampled by collecting soil (800 cm<sup>3</sup>/habitat) from the upper surface (0-10 cm) in May 2015. Soil animals were extracted using Berlese funnel. Extracted specimens were classified into higher taxa followed by calculation of QBS (“Qualità Biologica del Suolo”, meaning “Biological Quality of Soil”) index (Parisi *et al.* 2005). Macrofauna (earthworms, land snails, woodlice, millipedes) was also sampled in each habitat. For earthworm sampling, extraction method (using mustard powder and formalin) followed by hand sorting was applied in spring 2014 and 2015. The other taxa were sampled using time-restricted hand sorting in May 2015.

ORGANIC MATTER DECOMPOSITION: To determine decay of plant residue, a decomposition experiment was launched in November 2013, using pyramid-

shaped, synthetic tea bags filled with rooibos (*Aspalathus linearis*) tea (Keuskamp *et al.* 2013). Before application, tea bags were prepared to eliminate water soluble materials (e.g. simple sugars and phenolics). All samples were air dried at room temperature then in climate cabin at 35 °C. Per site, 20 tea bags were placed at a depth of 0-5 cm from the surface. Thus, altogether 400 tea bags were buried and retrieved five bags from each site after 4,6,8 and 12 months of field incubation. Changes in mass of organic matter through time were measured to estimate decomposition rate constant (k).

STATISTICAL ANALYSES: Urbanization intensity at study sites was quantified (calculation of urbanization index) as in Liker *et al.* (2008). To detect difference in soil physicochemical properties between habitat types, linear mixed effects models (LMM) were applied. Fixed factor was habitat type, while locality was added as a random factor. The effects of urbanization on soil parameters were tested LMMs in which urbanization index (UI) was response factor. Alpha diversity metrics (Shannon diversity (H') and Evenness (J') indices based on T-RFLP abundance data) and OTU (operational taxonomic unit) number were used to estimate the diversity of archaeal (Archaea) and bacterial (Bacteria) communities. Microbial abundance was expressed as gene copy numbers. Bacterial community comparisons were carried out by the SDR simplex approach (Podani and Schmera, 2011). To estimate the relation between the diversity/abundance of microbial communities and urbanization, habitat type and soil physicochemical properties, LMMs were applied. Habitat effect on bacterial and archaeal communities was analysed using Permutational Multivariate Analysis of Variance (PERMANOVA). This result was visualized by Principal Coordinates Analysis (PCoA). To determine relationship between edaphic factors and microbial community composition, Canonical Correspondence Analysis (CCA) was applied. OTUs with  $\geq 0,01$  % relative abundance were excluded from CCA and PERMANOVA. Because structurally very different habitats were sampled, we carried out individual-based rarefaction to reduce possible methodological bias ('iNext' R package, Hsieh *et al.* 2016). To characterize diversity of soil mesofauna, QBS index was calculated, while abundance was expressed as total number of individuals. For macrofauna diversity, species richness (number of species) was applied. To assess abundance of earthworms, biomass (g/m<sup>2</sup>) was measured. The above-mentioned variables were added as response variables to general and generalized linear mixed effects models (GLMM) to test the effects of urbanization and soil physicochemical properties on studied taxa. We applied GLMMs with multivariate approach ('manyglm' function) to measure the effects of urbanization, habitat type and soil physicochemical properties on the abundance of the individual species ('mvabund' R package, Wang *et al.* 2012). Rates of decomposition were estimated with a single exponential decay

model (Olson, 1963). Linkages between urbanization, habitat type, soil physicochemical parameters and organic matter decomposition were tested using LMM. A soil biodiversity index was calculated from the average of all standardized soil community characteristics and used as a general indicator of soil biodiversity (Wagg *et al.* 2014). This soil biodiversity index includes species richness of earthworms, gastro-, iso- and diplopods, QBS index, microbial diversity indices and abundance of microbes, earthworms, microarthropods. To estimate relationship between soil biodiversity and organic matter decomposition, LMMs was applied.

## **2.2. Effects of extreme drought on soil biodiversity and organic matter decomposition (ExDRain-Project, Fülöpháza)**

STUDY SITE AND DESIGN: This study is part of long-term experimental research on climate change (ExDRain, Extreme Drought and Rain Manipulation Experiment), which was carried out at the field site (Fülöpháza) of the Institute of Ecology and Botany, Hungarian Academy of Sciences. The experiment was designed to test the effects of an extreme drought event on organic matter decomposition. Six 3 m × 6 m experimental blocks were selected. Half (3 m × 3 m) of each block was covered with a transparent plastic roof to exclude rain (drought or ‘XC’ plots), whereas the other halves served as non-manipulated controls (control or ‘CC’ plots). Rain exclusion was applied from 24 April 2014 to 18 September 2014.

SAMPLING METHODS: The soil temperature and moisture of each plot was measured throughout the year and data were stored on data loggers at 10-minute intervals. For microbial analyses, composite soil samples were taken to a depth of 5 cm from each plot in March 2015. Comparison of microbial communities (16S rDNA T-RFLP fingerprint and shotgun metagenome sequencing) was carried out in the laboratory of Regional University Center of Excellence in Environmental Industry, Szent István University. We collected soil arthropods by pitfall traps (Barber 1931) close to the experimental blocks (2 traps per block) for two weeks in April and October 2015.

ORGANIC MATTER DECOMPOSITION: The decomposition experiment started on 26 March 2014 (a month before drought treatment) with pyramid-shaped, synthetic tea bags filled with rooibos tea. The preparation of the tea bags was the same as previously described. In each plot, four tea bags were placed at 3–5-cm depth under the soil surface. A total of 48 tea bags were buried (four bags per plot × 12 plots) and retrieved after 2, 4, 6 and 12 months. Changes in mass of organic matter through time were measured to estimate decomposition rate constant (k).

STATISTICAL ANALYSES: Alpha diversity metrics ( $H'$  and  $J'$  indices based on T-RFLP abundance data) were calculated to estimate the diversity of bacterial communities. For beta diversity, bacterial community comparisons were carried out by the SDR simplex approach (Podani and Schmera, 2011). To identify the consequences of extreme drought treatment on soil temperature and moisture, LMMs were applied with the 'lme' function from the 'nlme' package (Pinheiro *et al.* 2015). The fixed factor was treatment with two levels (CC and XC), whereas the random factors were block and month. The variances of these microclimatic variables were analysed to consider intra-annual variability of soil moisture and temperature. The variances of soil temperature and moisture in CC and XC plots were compared by generalized least squares models (gls) in the package 'nlme' (Pinheiro *et al.* 2015). The effects of extreme drought, soil temperature and moisture on the rate of organic matter decomposition and on the diversity of bacterial communities were analysed by LMMs. Fixed factors were treatment, means and variances of soil microclimatic properties, whereas month and block variables were included as random factors. Structure of the error variance was modelled with month as a grouping criterion and 'varIdent' as a variance function. To consider time series, a temporal correlation structure (corAR1) was added to the LMMs. The T-RFLP data were subjected to a PERMANOVA to assess the effect of extreme drought treatment on the structure of the soil bacterial community ('vegan' package, Oksanen *et al.* 2015). This was followed by a nonmetric multidimensional scaling (NMS) and a hierarchical cluster analysis combined with a similarity profile permutation (SIMPROF) test ('clustsig' package) to show the significance of the branches in the cluster (Whitaker and Christman, 2014). CCA was used to examine the relation between microclimatic factors and the bacterial community compositions.

### **2.3. Effects of set-aside management on soil biodiversity and organic matter decomposition**

#### *2.3.1. I. Study: Effects of set-aside management on soil macrodecomposers (Heves Plain, 2008)*

The study was conducted as part of the doctoral research of Anikó Kovács-Hostyánszki (Kovács 2011). My work was limited to species identification of macrodecomposer taxa (woodlice, millipedes) and data evaluation.

HABITAT SELECTION: Soil macrodecomposer fauna of one, two and three years old set-asides, adjacent winter wheat fields and semi-natural grasslands were compared to each other. Within the study area, one-, two- and three-year-old



set-aside fields (Sa1, Sa2, Sa3) were chosen, each with an adjacent winter wheat field (W) and each pair with five (1- and 3-year-old) or six (2-year-old) replicates. Six semi-natural grasslands (G) were also assigned as a control for comparison.

SAMPLING METHODS: Ten  $2 \times 2$  m quadrates were recorded at each study site in the spring of 2008 to assess species richness and vegetation coverage (Kovács 2011). Macrodecomposers (Iso- and Diplopoda) were sampled by pitfall traps during their main activity period, totalling three periods of two weeks from the beginning of May until mid-June, in 2008. Five traps per site were placed at a distance of 20 m from each other and 50 m from the field edge.

STATISTICAL ANALYSES: Species richness and abundance of millipedes and isopods failed to meet normal distribution assumptions for parametric tests, thus non-parametric Wilcoxon-signed rank tests were used to compare those within field pairs. Because structurally very different habitats were sampled using pitfall traps, we carried out individual-based rarefaction to reduce possible methodological bias ('iNext' R package, Hsieh *et al.* 2016). Effectiveness of set-aside management was measured as the difference of the dependent variables (species richness and abundance of isopods and millipedes) between the wheat and the adjacent set-aside fields. To evaluate that and to detect the effect of set-aside age on paired wheat fields we used Kruskal-Wallis rank sum tests with Dunn's post-hoc tests at 5% Type-I error rate. Kruskal-Wallis rank sum test with Dunn's post hoc test was also applied to compare species richness and abundance of isopods and millipedes in different aged set-aside fields and grasslands. Since the number of collected species was relatively low, the Wilcoxon-signed rank tests and Kruskal-Wallis tests were applied at species level, too. We applied GLMMs with multivariate approach ('manyglm' function) to measure the effects of plant species richness, vegetation cover and habitat type (fixed factor with five levels: winter wheat field, semi-natural grassland, one-, two- and three-year-old set-aside field) on the abundance of the individual isopod and millipede species. The lack of spatial independence of the paired set-aside and wheat fields was treated by application of a random factor (location). The influence of plant species richness, vegetation cover and habitat type on the species composition of isopod and millipede assemblages was tested by redundancy analysis ('vegan' package, Oksanen *et al.* 2015).

### 2.3.2. II. Study: *Effects of set-aside management on soil biodiversity and organic matter decomposition (LIBERATION-Project, Heves Plain)*

**HABITAT SELECTION:** The study sites were located in the Heves Plain. Seven cereal – set-aside field pairs, six-six semi-natural grasslands and cereal fields without set-asides were chosen close to Poroszló and Besenyőtelek.

**SAMPLING METHODS:** For soil physicochemical analyses, composite soil samples were taken to a depth of 10 cm in May 2014. Soil pH,  $K_A$  (Arany-type texture coefficient), total salt,  $CaCO_3$ , soil organic matter,  $NO_2+NO_3-N$ ,  $P_2O_5$  and  $K_2O$ , Mg and  $SO_4-S$  content were measured. Composite soil samples were taken to a depth of 5 cm in May 2014 to compare microbial communities (using T-RFLP fingerprint method). Microarthropods were sampled (along a transect with samples at 0, 10 and 20 m from the field edge) by collecting six soil cores ( $400\text{ cm}^3$ ) per site from the upper surface (0-10 cm) in May 2014. In semi-natural grasslands, soil samples were taken randomly at a distance of 10 m from each other and 2-300 m from the field edge. Soil microarthropods were extracted, using Berlese-funnel. After classification into taxonomic groups we calculated QBS index according to Parisi *et al.* (2005). Macrodecomposers were sampled by pitfall traps for two weeks in May 2014. Four traps per site 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 10m from each other and 2-300 m from the field edge.

**ORGANIC MATTER DECOMPOSITION:** To follow microbial degradation of organic matter, the tea bag method was used as previously described. In each site, four pyramid shaped, synthetic tea bags were placed at 3–5-cm depth under the soil surface in May 2014. Tea bags were retrieved after 1 month before the harvest. Changes in mass of organic matter through time were measured to estimate decomposition rate constant ( $k$ ).

**STATISTICAL ANALYSES:** To detect difference in soil physicochemical properties between habitat types, linear mixed effects models (LMM) were applied. Fixed factor was habitat type, while locality was added as a random factor.  $H'$  and  $J'$  indices were calculated to characterize soil bacterial diversity. Bacterial community comparisons were carried out by the SDR simplex approach (Podani and Schmera 2011). To estimate the relation between bacterial diversity and habitat type, soil physicochemical properties, LMMs were applied. Habitat effect on bacterial communities was analysed using PERMANOVA that was visualized by PCoA. To characterize diversity of soil mesofauna, QBS index was calculated, while abundance was expressed as total number of individuals. For macrofauna diversity, species richness (number of species) was applied. The bacterial diversity indices, QBS index,

species richness/abundance of isopods and diplopods were added as response variables to LMMs and GLMMs to test the effects of habitat type and soil physicochemical properties on studied taxa. CCA was used to examine the relation between abiotic factors and species composition of isopod and millipede assemblages. Because structurally very different habitats were sampled, we carried out individual-based rarefaction to reduce possible methodological bias ('iNext' R package, Hsieh *et al.* 2016). Linkages between habitat type, soil physicochemical parameters and organic matter decomposition were tested using LMM. The soil biodiversity index includes species richness and abundance of iso- and diplopods, QBS index, abundance of microarthropods and bacterial diversity indices. To estimate relationship between soil biodiversity and organic matter decomposition, LMMs was applied.

### 3. RESULTS

#### 3.1. Effects of urbanization on soil biodiversity and organic matter decomposition (GLUSEEN-Project, Budapest)

Urbanization index ranged from -3,56 to 3,53. According to this index, study sites did not show distinct groups or patterns. Vegetation coverage was the highest in reference forests with low extent of built-up and anthropogenic impacts. The most urbanized sites belonged to the ruderal and turf habitat types.

We found significant differences in several soil properties between habitat types. Ruderal and turf habitats were generally characterized by more alkaline soils compared to remnant and reference forests. In contrast to soil plasticity (expressed as  $K_A$ ), SOM and N content, amount of  $CaCO_3$  showed similar trend. Soil pH,  $CaCO_3$  and K content increased, while soil plasticity and N level decreased with urbanisation intensity.

A total of 61 019 archaeal (1,91 %) and bacterial (98,09 %) OTUs were found in the soil samples. We obtained a total of 2 509 732 archaeal (5,22 %) and bacterial (94,78 %) sequences.  $\alpha$  diversity and abundance of Archaea was significantly affected by habitat type. Number of archaeal OTUs and sequences were the greatest in soils of turfs followed by ruderal habitats, remnant and reference forests. Turf habitats showed the highest H' index similar to remnant forests. However, ruderal and reference habitats were characterized by lower diversity. Evenness of archaeal communities was the highest in soils of remnant forests differed significantly from ruderal habitats. OTU number and abundance decreased with urbanization intensity. In contrast,  $CaCO_3$  and K content had positive effects on archaeal diversity and

abundance. Soil nitrogen level negatively affected H' and J' indices. In case of Shannon index, P content showed similar effect. Habitat type also had a marginally significant effect on species composition of Archaea ( $F_{\text{PERMANOVA}} = 1,698$ ;  $p = 0,07$ ). Ruderal and turf habitats were characterized by more similar archaeal communities compared to remnant and reference forests. SOM content also proved to be significant factor relating to community structure of Archaea ( $F = 1,7600$ ;  $p = 0,039$ ). Habitat type had marginally significant effect on bacterial OTU number. OTU number was the highest in ruderal habitats, while the lowest values were found in reference forests. Bacterial abundance decreased with soil pH and  $K_A$ , while increased with SOM content. Community structure of Bacteria was also influenced by habitat type ( $F_{\text{PERMANOVA}} = 1,523$ ;  $p = 0,06$ ).

In total 6 833 specimens belonging to 18 taxa of microarthropods were extracted from soil samples. The highest biological quality of soils (expressed as QBS index) were found in remnant forests. There were significantly lower values in ruderal and reference forests. However, we experienced no significant difference between habitat types regarding abundance of microarthropods. In addition to habitat types, soil K content affected QBS index.

Earthworms sampling resulted in 172 specimens of seven species. We found significant difference in community composition between the two samplings. *Aporrectodea* sp. was the most frequent in 2014, while *Octolasion lacteum* was the most common species in 2015. Species richness was significantly influenced by SOM and  $\text{CaCO}_3$  content. There was also significant difference in biomass between the two samplings.

In total 18 species belonging to 12 families of Gastropoda were identified. The most common species were *Aegopinella pura*, *Granaria frumentum* and *Macrogastra ventricosa*. We found three protected species: *Cepaea hortensis*, *Helix pomatia*, *Orcula dolium*. There was significant difference in species richness between habitat types. Ruderal habitats were characterized by lower species richness compared to remnant forests ( $z = 2,257$ ;  $p = 0,024$ ). Additionally, soil pH,  $\text{CaCO}_3$ , P and N content had significant effects on diversity of gastropods. However, we found no significant variable regarding species composition.

In total three species of isopods and 13 species of diplopods were identified. The most common species were *Porcellium collicola*, *Ophiulus pilosus* and *Cylindroiulus boleti*. Both taxa were dominated by disturbance tolerant, generalist or/and synanthropic species. In case of millipedes, habitat type, urbanization index, soil plasticity and N content proved to be significant variables. However, in case of isopods none of them showed significant effects. Species richness of millipedes were highest in reference forests significantly differed from ruderal habitats. It decreased with urbanisation intensity and  $K_A$ , while increased with soil nitrogen content.

On average, 32.89 % of organic matter was decayed during a year. Decomposition intensity showed significant difference between habitat types: it was the highest in soils of turfs. We experienced positive correlation between soil biodiversity and organic matter decomposition: decay rate increased with soil biodiversity ( $t = 2,0565$ ;  $p = 0,064$ ). Of biotic components, species richness of earthworms, abundance of microarthropods, archaeal/bacterial diversity and abundance had significant effects on decomposition rate.

### **3.2. Effects of extreme drought on soil biodiversity and organic matter decomposition (ExDRain-Project, Fülöpháza)**

During the extreme drought treatment, 523 mm of precipitation (61,24 % of the total annual rainfall) was excluded by roof covers on the XC plots. The manipulation of humidity was successful because the LMMs showed significant differences in soil moisture ( $t = -6,357$ ;  $p < 0,001$ ) and soil temperature ( $t = 10,577$ ;  $p < 0,001$ ) between CC and XC plots as a result of the extreme drought treatment. In drought-treated plots, soil moisture was less by an average of 41,82%, whereas soil temperature was higher by an average of 1,56 °C than in the control plots after 1 year. The variance of soil moisture in the XC plots was diminished ( $p < 0,001$ ) and that of soil temperature was also significantly different ( $p = 0,043$ ) between the CC and XC plots on average.

Data from the T-RFLP fingerprinting revealed the mid-term effects of the extreme drought treatment on the composition of the entire soil bacterial communities according to PERMANOVA testing ( $F_{\text{PERMANOVA}} = 10,36$ ,  $p = 0,002$ ). The extreme drought treatment had a marginally significant mid-term effect on the estimated diversity indices. The bacterial communities in the control plots appeared to be more diverse than those of the drought plots. The points from the SDR simplex analysis are concentrated on the right side of the triangle. This suggests that bacterial communities are characterized by a strong similarity in species richness. The SDR scores obtained from pairwise comparison of the CC and XC soils moved towards species replacement (R corner), which resulted in a larger beta diversity than for pairs within similar CC or XC plots. The CCA analyses of the relation between bacterial communities and microclimatic soil properties showed that the structure of the bacterial community was significantly affected by the variance of soil temperature ( $p = 0,05$ ) and the mean of soil moisture ( $p = 0,03$ ).

In the case of both soil samples (1XC and 6CC) analysing by shotgun metagenomics sequencing Bacteria domain affiliated sequences were dominant (86.1% and 83.3% of the metagenomic sequences, respectively), while the amount of Archaea domain affiliated sequences was almost equal (2.26% and 2.33%, respectively). On the other hand, significant difference

was observed in the amount of Eukaryote domain affiliated sequences (1.7% in sample 1XC and 5.7% in sample 6CC). The shotgun metagenomic sequencing of DNA from soil samples 1XC and 6CC has shown the presence of similar compositions of bacterial communities. At the genus level, the pattern of composition of the bacterial community was still comparable, although there were characteristic differences in the abundance of certain notable, under-mentioned genera. In both samples, *Streptomyces*-affiliated sequences were the most plentiful; moreover, they were predominant in sample 6CC, which represented almost 4.5% of the total metagenomic sequence. In contrast, their abundance in sample 1XC was markedly less, and accounted for just 3.1% of metagenomic sequences. Similar phenomena appeared in the case of sequences affiliated to the actinobacterial genus, *Mycobacterium* (3.2% of the total metagenomic sequence in sample 6CC, whereas it was 2.1% in sample 1XC). Interestingly, the ratio of the actinobacterial genus, *Rubrobacter*-affiliated sequences, was almost the same in the two samples (~2.7% in both). By comparing the two metagenomes, a striking difference was observed in the abundance of *Acinetobacter*-affiliated sequences. In the control sample, *Acinetobacter*-related sequences showed a notable abundance by representing ~1.4% of metagenomic sequences, whereas in sample 1XC, limited amounts of such sequences were detected (0.1% of metagenomic sequences). In addition, the most appreciable difference between the structures of the bacterial community, the larger amount of *Candidatus Solibacter*, *Candidatus Koribacter*, *Gemmata* and *Methylobacterium*-affiliated sequences, could be seen clearly for sample 1XC. Regarding Archaea domain affiliated sequences the two samples showed considerably similar community composition. In both samples phylum Thaumarchaeota affiliated sequences were the most abundant with sequences representing genera *Nitrosopumilus*, *Cenarchaeum* and *Nitrososphaera*. By comparing the fungal communities of the two samples it is striking that class Eurotiomycetes affiliated sequences were highly dominant in the control sample 6CC mainly due to the predominance of genera *Aspergillus* and *Penicillium* affiliated sequences. Contrarily, in sample 1XC no predominant fungal genus was observable. Interestingly, class Dothideomycetes affiliated sequences (genera *Phaeosphaeria* and *Pyrenophora*) were much more abundant in the extreme drought-treated sample 1XC than in the control sample 6CC (18.7% and 9.2% of fungal sequences, respectively).

Totally 5861 specimens belonging to 13 species of the taxon Collembola were collected during 2-week period of pitfall trapping in April and October 2015. Regarding species composition and abundance, there was significant difference between the two seasons. In spring, *Entomobrya nigriventris* was the most common species (almost 90 % of the total individuals), while *Brachystomella curvula* was the most frequent (with 64 % relative abundance)

in autumn. Of the macrodecomposer arthropods, only one drought tolerant millipede species (*Megaphyllum unilineatum*) was found in autumn.

Rates of organic matter decomposition in CC plots were significantly larger by an average of 93,73% than for the XC plots. Following the extreme drought period, however, the rate of decomposition showed an increasing trend in the XC plots. The masses remaining after 12 months (mean $\pm$ SE) in CC and XC plots were 80,19  $\pm$  2,4 and 87,66  $\pm$  2,44%, respectively. The soil microclimate variables suggested significant effects on decomposition except for the mean of soil temperature. The rate of decomposition followed the same pattern as mass loss, with the same microclimatic factors showing a statistically significant effect. In this study, there was no significant relation between rates of organic matter decomposition and soil bacterial diversity.

### **3.3. Effects of set-aside management on soil biodiversity and organic matter decomposition**

#### *3.3.1. I. Study: Effects of set-aside management on soil macrodecomposers (Heves Plain, 2008)*

In total 2362 individuals of 8 macrodecomposer species were identified from samples collected by the pitfall traps. The most abundant species were *Leptoiulus cibdellus* (Diplopoda) and *Armadillidium vulgare* (Isopoda: Oniscidea). We found the lowest average number of isopods per field in 1-year-old set-aside (Sa1: 3,6/field) and winter wheat fields (W: 4/field). The average number of individuals increased with the age of set-aside areas: 3-year-old set-aside had the highest average number of isopods per field (Sa3: 43,6/field). Millipedes showed the highest mean values in 2-year-old set-aside fields (Sa2: 139,5/field). Grasslands proved to be the poorest habitats regarding average numbers of millipedes (G: 14/ field). Results of the Wilcoxon-signed rank tests showed that species richness ( $p = 0,045$ ) and abundance ( $p = 0,008$ ) of isopods was significantly higher in set-aside than in wheat fields. No significant effects were observed in the case of millipedes. At species level we found that almost all species occurred in higher number in set-aside fields within the field pairs. The species richness and abundance of both decomposer taxa showed a similar increasing trend with the age of set-aside fields. In 3-year-old set-aside fields isopods showed similar or higher values than in grasslands. In the case of millipedes there were significantly higher species richness and abundance in 2- and 3-year-old set-aside fields than in grasslands. At group level, the effectiveness of management increased significantly with the age of set-aside fields with regard to species richness and abundance of isopods. In the case of millipedes, we found a similar trend with significant difference. Abundance of millipedes was highest in the case of 2-year-old set-aside fields. We found significantly higher effectiveness of

set-aside management in the case of almost all species in 3-year-old set-aside fields than in younger ones. Species richness and abundance of isopods was significantly different between wheat fields adjacent to different aged set-aside fields ( $B1 > B3 > B2$ ). Wheat fields adjacent to 2- and 3-year-old set-aside fields had higher millipede abundance and species richness than those next to 1-year-old ones. Habitat type significantly affected all isopod and millipede species. Plant species richness had a significant effect on each species (except for *B. bagnalli* and *I. terrestris*). Wheat fields were the poorest in terms of plant species richness, while the 3-year-old set-aside fields were the richest. In the case of *B. bagnalli*, *I. terrestris* and *L. cibdellus* there was a significant effect of vegetation cover. The proportion of bare ground was lowest in wheat fields. Vegetation cover decreased with the age of set-aside fields. Plant species richness had a significant effect on the composition of macrodecomposer communities ( $F = 4,27$ ;  $p = 0,042$ ). Habitat type showed a similar trend ( $F = 2,43$ ;  $p = 0,071$ ) but vegetation cover had no effect on the species composition of macro-decomposers.

### 3.3.2. II. Study: Effects of set-aside management on soil biodiversity and organic matter decomposition (LIBERATION-Project, Heves Plain)

We experienced significant difference in soil pH, potassium (K) and sodium (Na) content among habitat types. Soil pH was the lowest in semi-natural grasslands (G) followed by set-aside (Sa) and cereal fields (C and CSa). Soil potassium was significantly higher in cereal fields (C and CSa) compared to set-aside fields and semi-natural grasslands. Moreover, there was significant difference in soil sodium content between grasslands and other habitat types.

Bacterial diversity showed relatively high variability among habitat types. However, only Evenness index was significantly influenced by the studied environmental variables: it decreased with SOM% (t-value=-2,47; p-value = 0,05), whereas increased with soil Na content (t-value = 3,28; p-value = 0,022).

In total 14 385 specimens belonging to 19 taxa of microarthropods were sampled. QBS index varied from 29 to 128 and showed significant difference among habitat types. The highest values were found in semi-natural grasslands, while cereal fields without set-asides were characterized by the lowest QBS. Abundance of microarthropods was significantly affected by habitat type: it was highest in set-aside fields compared to other habitats. However, there was no significant difference within the field pairs regarding QBS index and abundance of microarthropods. Soil pH and Na content had negative, while soil plasticity and  $SO_4$ -S content had positive effects on QBS index. There was positive relationship between soil pH,  $NO_2+NO_3$ -N content and abundance of microarthropods. We found that number of microarthropods decreased with amount of soil K and Na.



In total 1391 individuals of 8 macrodecomposer species were identified from samples collected by the pitfall traps, including 783 individuals of four isopod species and 608 individuals of four millipede species. The most abundant species were *Armadillidium vulgare* (89,27 %) and *Iulus terrestris* (59,38 %). In present study, species richness only in case of isopods was significantly affected by studied environmental variables. We experienced that habitat type and soil pH had significant effects on species number of isopods. It increased with soil acidity ( $z = -2,236$ ;  $p = 0,022$ ). Semi-natural grasslands were characterized by the highest species richness, while cereal fields without set-asides proved to be the poorest habitats. There was significant difference in abundance of iso- and diplopods between habitat types. Isopods were highest in semi-natural grasslands while were lowest in cereal fields without set-asides. By contrast, millipedes were the most abundant in the latter habitats. Soil K, N had negative effects on abundance of woodlice. We experienced no significant difference in species richness and abundance of both studied taxa within set-aside – cereal field pairs. Species composition of iso- and diplopod assemblages were also affected by habitat type (besides salt concentration, SOM and Mg content). *B. superus* and *B. bagnalli* mostly occurred in cereal fields, while *M. unilineatum*, *A. vulgare* and *T. rathkii* preferred semi-natural grasslands and set-aside fields with Mg- and SOM-rich soils. *I. terrestris* was observed more frequently in sites characterized by soils with high salt concentration.

On average, 22.41 % of organic matter was decayed during a month. Mass loss was significantly different between habitat types. We experienced the highest decomposition in set-asides while the lowest in cereal fields. Decomposition rate was negatively influenced by salt concentration, K and SOM content. However, soil pH and amount of  $P_2O_5$  had positive effects on intensity of mass loss. There was marginally significant relationship between biological quality of soil (expressed as QBS index) and decomposition rate ( $t = 2,1076$ ;  $p = 0,08$ ).

### **3.4. Summary of new scientific results**

1. My investigations provide comprehensive picture on structure and functioning of decomposer subsystems in different human-modified ecosystems.
2. To follow organic matter decomposition in soil, I applied and modified/improved the tea bag method which is innovative on international level, too.
3. Highly disturbed, urban fields of the capital city proved to be the most carbon-emitting habitats. On the other, they have essential role in preservation and maintenance of soil biodiversity.

4. As part of a climate change experiment, I demonstrated the effects of extreme drought on soil microbes (Bacteria, Archaea, Fungi). I found significant difference in microbial communities between soil samples six months after the five-month precipitation exclusion.

5. Based on my results, it can be concluded that set-aside fields established in the framework of the agri-environmental program are of great importance in maintenance of soil biodiversity. They provide favourable conditions for the majority of soil organisms. Furthermore, this positive effect was more profound with duration of set-aside management.

6. I experienced a positive relationship between soil biodiversity and organic matter decomposition which might contribute to clarify the scientific debates in this topic.

7. During the research in Fülöpháza, a new springtail species (*Orchesella taurica*) for domestic fauna could be detected.

## 4. CONCLUSIONS AND SUGGESTIONS

Soil is increasingly degrading as a result of human activities both in the EU and at global level, and this is further exacerbated by global environmental problems. Therefore, actions against degradation processes should be primary object to reduce pressure on soils resulting from unsustainable farming and forestry practices, environmental pollution, urbanization and climate change. According to FAO, towards 2050 per capita cropland is expected to drastically decrease (Conforti 2011). In the Communication of the European Commission, eight main threats to soil were identified: erosion, loss of organic matter, local and diffuse contamination, salinization, compaction, loss of biodiversity, sealing, floods and landslides (European Commission 2002). In general, soil degradation is not result of a factor, but combined effects of several processes.

In my PhD dissertation, I investigated the relation between plant residue decomposition and soil biotic/abiotic properties in relation to anthropogenic impacts. Accordingly, the study shows the effects of urbanization, extreme drought (that is expected more frequent as a consequences of climate change) and set-aside management on decomposer subsystem in four main chapters.

### 4.1. Effects of urbanization on soil biodiversity and organic matter decomposition (GLUSEEN-Project, Budapest)

We found significant changes in many soil physicochemical properties (pH, CaCO<sub>3</sub>, KA, N, H) correlated with urbanization and anthropogenic

disturbance. Our study proved that highly disturbed, typical urban habitat patches (ruderal and turf habitats) have important role in maintaining soil biodiversity. However, unlike the expectations, soil biodiversity in total was the greatest in these habitats. The more disturbed habitat types (ruderal and turf habitats) were characterised by more diverse archaeal and bacterial communities than remnant and reference forests. However, several taxa (e.g. Gastropoda, Diplopoda) showed higher diversity in more natural habitat types. Therefore, soil biota was influenced in different ways by urbanization and soil abiotic parameters. There was the greatest decomposition rate of plant tissue in the more disturbed habitats that leads higher carbon emission. Moreover, we experienced that organic matter decomposition increased with soil biodiversity. Therefore, we need better understanding of urban ecosystems' decomposer subsystem to support the measures against climate change, too.

#### **4.2. Effects of extreme drought on soil biodiversity and organic matter decomposition (ExDRain-Project, Fülöpháza)**

Our planet is currently facing multiple global environmental challenges, including rising temperatures, changes in precipitation regimes and a depletion of biological diversity. The present study investigated the effect of a 5-month drought on organic matter decomposition within a 1-year period and its mid-term effect on microbial community structure. Here, we have provided evidence to show that lack of water affects soil microbial composition and decomposition, even after 6 months. Our results are in accord with previous hypotheses on the effect of climatic factors on SOM turnover. Although rates of decomposition of plant residue in our study did not show a positive relation with bacterial diversity, it was markedly affected by soil temperature, moisture and their variability. As a consequence of the rainfall manipulation (that is extreme drought), the rate of decay of organic matter was reduced by almost half compared with the control plots. More specific research on microbial community structure is required, however, to explain the mechanisms that underpin variation in the decomposition processes. Overall, we have emphasized the need to consider soil microbiota in climate change experiments to enable predictions of the consequences of extreme weather events for ecosystem services, such as the nutrient cycle.

#### **4.3. Effects of set-aside management on soil biodiversity and organic matter decomposition**

To investigate the effects of set-aside management (established as part of Hungarian agri-environmental scheme) on soil biodiversity, two researches were carried out.

#### 4.3.1. I. Study: *Effects of set-aside management on soil macrodecomposers (Heves Plain, 2008)*

Results showed that set-aside fields having more diverse and complex vegetation provided more favourable conditions (food quality, shelter sites etc.) for macrodecomposer soil invertebrates than cereal fields. The species richness and abundance of isopods and millipedes increased with the age of set-aside fields, in some cases exceeding the diversity of semi-natural grasslands. Wheat fields adjacent to 2- and 3-year-old set-aside fields had higher millipede abundance and species richness than those next to 1-year-old ones. However, isopods did not show similar patterns. Habitat type and plant species richness had significant effects on the composition of macrodecomposer communities.

#### 4.3.2. II. Study: *Effects of set-aside management on soil biodiversity and organic matter decomposition (LIBERATION-Project, Heves Plain)*

There was significant difference between habitat types (cereal fields without and neighbored set-asides, set-aside fields and semi-natural grasslands) regarding several soil physicochemical parameters. This study also 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 compared to cereal fields. This may be explained by richer soil fauna of the more natural habitats (set-aside fields and grasslands) since these habitats were characterised by the most diverse assemblages of microarthropods and isopods. In contrast to soil biodiversity, soil biological quality (expressed in QBS index) increased decomposition rate of organic matter.

Our studies highlight the importance of set-aside management system with regard to soil biota. The results presented support the effectiveness of set-aside management to ensure higher species richness. Set-aside fields function as semi-natural habitats providing favourable conditions for macrodecomposers particularly after an initial establishment period of two years, supporting the regeneration of soil biological resources. Set-aside fields that are not part of a crop rotation for more than 2 years could be a valuable option for establishing ecological focus areas under the CAP (Common Agricultural Policy) 2013 reform in the EU, as these fields may simultaneously conserve elements of above- and belowground diversity. However further research is required to look for the optimum management regimes for soil related organisms supporting the most abundant and diverse

arthropod populations, particularly in relation to the establishment methods of set-aside or other semi-natural habitat types in agricultural landscapes.

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## PUBLICATIONS RELATED TO THE THESIS

### *1. Peer-reviewed research articles*

1.1. With impact factor (according to WEB OF SCIENCE), in English

1.1.2. International publisher

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**TÓTH Zs**, HORNUNG E, BÁLDI A & KOVÁCS-HOSTYÁNSZKI A (2016): Effects of set-aside management on soil macrodecomposers in Hungary. *Applied Soil Ecology*, 99: 89-97. DOI: 10.1016/j.apsoil.2015.11.003 (IF = 2,67)

1.2. Without impact factor, in English

1.2.1. Hungarian publisher

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5.3. Conference abstracts in English and Hungarian

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