

The effect of different planting methods on the yield and spad readings of sweet potato (*Ipomoea batatas* L.)

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Abstract: A small-plot field experiment was established to examine the sweet potato variety Ásothalmi 12 in the case of various planting methods on chernozem soil in 2017. The obtained experimental results showed that, by using appropriate agrotechnical solutions, sweet potato can be successfully grown with favourable yields also in Hungary (the marketable tuber yield was between 23.2-50.7 t ha⁻¹). As opposed to bibliographical references, higher yields were obtained in flat planting than in ridge planting at both row spacing values - 1.0 m (32.2 t ha⁻¹ and 23.2 t ha⁻¹, respectively) and 0.75 m (50.7 t ha⁻¹ and 39.4 t ha⁻¹). The 0.75 m row spacing was more favourable than 1.0 m. The proportion of non-marketable tubers was also more favourable (lower) in flat planting (9.97-10.9%) than in ridge planting (13.03-15.57%). During the growing season, the SPAD readings of the sweet potato leaves increased between July and August, reaching their peak in mid-August (39.61-50.31). SPAD readings decreased until harvesting (38.89-43.31 on 7th October). Positive correlation was observed between the marketable tuber yield and SPAD readings on 10th July (0.632xx) and 21st July (0,664xx).

Keywords: sweet potato, planting technology, yield, SPAD

Introduction

The structure of field crop production in Hungary greatly simplified during the past decades (Pepó 2011), resulting in the fact nearly 85% of cropland is occupied by the five main crops which are grown on the largest area (wheat, maize, sunflower, barley, rape). This simplified sowing structure significantly increased the ecological, agronomic and economic vulnerability of crop production. On the one hand, it would be necessary to increase the sown area of traditional crops (legumes, root and tuber crops, fodder crops) and to introduce new crops on the other. In the recent years, sweet potato (*Ipomoea batatas* L.) appeared as a new crop and its sown area has been (moderately) increasing (about 500 ha in 2017) as farmers from various regions started to grow it. Sweet potato is originally a perennial crop from South and Central America, produced as an annual crop in Hungary. Currently, sweet potato is the seventh most significant food crop in the world, while it is ranked the 4th in tropical countries (Julianti et al. 2017). Sweet potato is an essential food source in many countries of Africa and Asia (Bowell-Benjamin, 2007; Low, 2011). The tuberous root

of sweet potato contains valuable nutrients and carbohydrates and is rich in vitamins (C, B₁, B₂, B₆, E, Woolfe 1992). For this reason, sweet potato production is constantly becoming more widespread in developing countries (Hartemink 2003). Sweet potato is grown on very different (fertile and nutrient-deficient) soils around the world due to its favourable adaptation abilities (White and Zasoski, 1999; Yan et al., 2006; Zuo and Zong, 2011).

Due to the topical origin of sweet potato (a short-day plant in need of warm temperature and water), only early ripening varieties can be grown in a way that the vegetation period is lengthened with planting. Sweet potato prefers neutral – slightly acidic (pH: 6-7) soils with loose structure (Lebot 2009). It is mainly cultivated using the ridge planting method, but flat planting can also be carried out (Clark 2013). However, research focusing on the production technology of sweet potato is still relatively limited. Szarvas et al. (2017) concluded that the yield of sweet potato did not increase as a result of fertilisation on alluvial soil properly supplied with nutrients. The examinations of Kuepper (2014) showed the significance of the applied planting method.

According to the examinations performed by Szarvas et al. (2017), the yield of sweet potato was higher in flat planting than in ridge planting. The row spacing, plant density could effect the yield of batata because of different water utilization, canopy shading, radiant energy utilization (Funnah and Matsebullar, 1984; Ojikpong et al., 2007) and they could modify the photosynthesis, leaf production (Onunka et al. 2011). Nwokocha et al. (2000) and Ikerogu (2003) stated that neither row spacing nor density affected the batata yields.

The agronomical, morphologic and physicochemical attributes of sweet potato varieties were examined in detail mainly by researchers in subtropical and tropical countries (Solomon et al., 2015; Picha, 1985; Tairo et al, 2008; Loretan et al., 1989; Chen et al., 2006; Surayia et al., 2006). Sweet potato tuberous root is a commercially valuable organ that provides a high level of biomass and nutrients per hectare. Grafting experiments have suggested that the productivity of sweet potato is due to the sink strength of tuberous root i.e. its capacity to deposit and store the products of photosynthesis (Hozyo et al., 1971; Harn, 1977). Unfortunately there is little experimental data available in relation to how photosynthetic capacity (SPAD and LAI) affects the yield of sweet potato. Based on the analyses performed by Su et al. (2009), a strong positive correlation was observed between SPAD readings and the chlorophyll content of sweet potato leaves.

The aim of our experiments was to examine the effect of different planting methods on the yield of sweet potato and the proportion of marketable yield, as well as to seek correlation between SPAD readings obtained in the growing season and the tuber yield of sweet potato.

Material and methods

The small-plot field experiment was established in the Demonstration Garden for students of the Institute of Crop Sciences of the University of Debrecen with three replications in 2017. Size of plots was 4 m². The previous crop of

the experiment was winter wheat. Following the harvesting of the previous crop, the usual operations were performed on the soil (stubble cleaning and rolling, stubble maintenance, 30 cm deep autumn ploughing) and the properly loose structure and weed-free conditions of the soil were maintained with a cultivator in the spring. No nutrient replenishment was performed in the autumn. In the spring (22nd May 2017), complex artificial fertiliser (N:P₂O₅:K₂O = 13:19:19) and CAN (N = 27%) was applied on the plot. All fertilizers were applied in spring because of rainy weather in autumn. The following amounts of active ingredient were applied: N = 52+54 = 106 kg ha⁻¹, P₂O₅ = 76 kg ha⁻¹, K₂O = 76 kg ha⁻¹. No chemical weed control was applied on the experiment site. Sweet potato variety "Ásotthalmi 12" which adapted to Hungarian weather conditions was used in the experiment. The skin of its tuber is red and its pulp is orange, tasty and sweet. This variety grows long tendrils and provides good soil coverage. The cuttings were obtained from Bivalyos Tanya Kft. Planting was performed on 31st May 2017.

In the experiment, flat planting and ridge planting were performed in the case of both varieties. Row spacing values of 1.0 m and 0.75 m were examined in both production methods. The planting distance of plantlets was 0.3 m in the case of the different row spacing values.

4 mm irrigation water was applied (by sprinkling method) on the crop stand of the plots each day between 31st May-10th July 2017. Weather was very favourable from mid July up to the end of September so we did not used irrigation in this period of vegetation season.

Manual weed control was performed on four occasions in June 2017.

The experiment was harvested on 10th October 2017. During manual harvesting, the total tuber yield of the plots and the marketable and non-marketable tuber yield (tubers below 200g, damaged by insects and diseased tubers) were measured.

The main meteorological data before and during the growing season are shown in *Table 1*.

Table 1. The most important meteorological data of experimental site (Debrecen)

	Rainfall autumn and winter (mm) (09/2016- 02/2017)	Rainfall in spring (mm) (03-05/2017)	June	July	August	September	Sum (mm) Mean (°C) in vegetation period
<u>Rainfall</u>							
2017 year	210.2	86.3	61.0	66.5	55.1	74.0	256.6
30 years mean	186.7	134.7	79.5	65.7	60.7	38.0	242.9
<u>Temperature</u>							
2017 year	-	-	20.9	21.0	22.1	15.5	19.9
30 years mean	-	-	18.7	20.3	19.6	15.8	18.6

Table 2. The most important traits of experimental soil (Debrecen)

Humus (%)	Soil plasticity K_A	pH		$CaCO_3$ (%)	AL-soluble	
		H_2O	KCl		P_2O_5 (mg kg^{-1})	K_2O (mg kg^{-1})
2.57	42.0	7.0	6.5	-	100.0	165.0

The analytical results of the experiment soil (Table 2) showed that the calcareous chernozem soil is mid-heavy and belongs to the loam soil physical group. The humus content (2.57%), AL-soluble P_2O_5 content (100.0 mg kg^{-1}) and K_2O content (165 mg kg^{-1}) of the soil are average.

The relative chlorophyll content (SPAD readings) of sweet potato leaves was measured on four occasions (10th July, 21st July, 17th August and 7th October 2017) during the experiment. Konica Minolta 502 meter was used to obtain SPAD readings. During each measurement session, 30 readings were obtained per plot in all three replications.

Results and discussion

The moderate, continental climate of Hungary is only partially suitable for the ecological needs of sweet potato. Accordingly, the great heat demand of the plant needs to be satisfied with a special treatment for producing the cuttings and irrigation is necessary due to its great water need. Regarding the growing season of 2017,

precipitation of the previous periods also need to be considered (Table 1) due to the chernozem soil of the experiment site which has favourable water management. The amount of precipitation in the autumn-winter period (210.2 mm) was higher than the multiple-year-average (186.7 mm). In the spring months, the amount of rainfall was lower than the 30-year-average (86.3 mm and 134.7 mm, respectively). Both the amount and distribution of rainfall in the growing season (between June and September) were favourable in 2017. In addition to the proper amount of precipitation and irrigation, the development of sweet potato was also helped by the fact that the mean temperature in each month of the vegetation period was 1.3-2.5 °C higher than the multiple-year-average (Table 1), except in September. There was a significant amount of rainfall and average temperature in September, which had a favourable effect on tuber growth. As a result of the properly performed agrotechnical operations and the relatively good weather, favourable yield was obtained in the

Table 3. Effects of planting method and row spacing on the yields of sweet potato (Debrecen, 2017)

Planting method Row distance	Total (gross) yield (kg ha^{-1})	Marketable (net) yield (kg ha^{-1})	Ratio of non- marketable yield (%)
<u>Flat</u>			
1.0 m between rows	35 497	32 200	9.97
0.75 m between rows	56 816	50 689	10.90
<u>Ridge</u>			
1.0 m between rows	27 467	23 233	15.57
0.75 m between rows	45 352	39 356	13.03
LSD _{5%}	10 986	9 950	2.07

Table 4. Effects of planting method and row space on the SPAD readings of sweet potato (Debrecen, 2017)

Planting method Row distance	SPAD readings			
	10 July	21 July	17 August	07 October
<u>Flat</u>				
1.0 m between rows	44.05	50.31	49.72	38.89
0.75 m between rows	48.07	49.30	49.08	39.44
<u>Ridge</u>				
1.0 m between rows	40.14	39.61	48.93	42.47
0.75 m between rows	39.10	45.19	49.49	43.31
LSD _{5%}	2.16	3.07	1.07	1.15

small plot experiment established on chernozem soil in 2017 (Table 3). The marketable yield of Ásotthalmi 12 was between 23.2-50.7 t ha⁻¹, depending on the applied production method (flat planting or ridge planting) and row spacing (1.0 m and 0.75 m). As opposed to experimental and general practice, flat planting provided higher yield than ridge planting under the given ecological and agrotechnical circumstances. The reason should be a better start situation for cuttings in the flat plots comparing with the ridge plots. The marketable yield was 32.2 t ha⁻¹ in the case of flat planting and 1.0 m row spacing and 50.7 t ha⁻¹ in the case of 0.75 m row spacing. The respective yields obtained in ridge planting were 23.2 t ha⁻¹ and 39.4 t ha⁻¹. The obtained research results showed that the highest yields were produced at 0.75 m row spacing in the case of both production methods. The marketable yield in flat planting was 32.2 t ha⁻¹ and 50.7 t ha⁻¹ in the case of 1.0 m and 0.75 m row spacing, respectively (the difference in yield was 18.5

t ha⁻¹), while the respective values of ridge planting were 23.2 t ha⁻¹ and 39.4 t ha⁻¹ (i.e., the difference in yields was 16.2 t ha⁻¹). During harvesting, the weight of non-marketable tubers (tubers lighter than 200g, as well as damaged and diseased tubers) was also measured and the weight percentage of non-marketable tubers were calculated (Table 3). The obtained results showed that not only the amount of yield was higher in the case of flat planting, but the proportion of non-marketable tubers was also more favourable (lower) in the case of both row spacing values. In the case of flat planting, the proportion of non-marketable tubers was between 9.7-10.9%, while the respective range was 13.03-15.57% in ridge planting.

Within the same production method, row spacing did not have any significant effect on the proportion of non-marketable tubers. The obtained experimental results in relation to the different planting methods are in contrast

Table 5. Pearson correlation analysis among the planting method, row spacing, yield and SPAD of sweet potato (Debrecen, 2017)

	Marketable yield (kg ha ⁻¹)	SPAD readings			
		10 July	21 July	17 August	07 October
Planting method (ridge / flat)	-0.460 ^x	-0.789 ^{xx}	-0.847 ^{xx}	-0.062 ^{ns}	0.682 ^x
Row spacing (1,0 / 0,75)	0.784 ^{xx}	0.183 ^{ns}	0.261 ^{ns}	-0.015 ^{ns}	0.130 ^{ns}
Total yield (kg ha ⁻¹)	0.991 ^{xx}	0.609 ^x	0.613 ^x	-0.078 ^{ns}	-0.135 ^{ns}
Marketable yield (kg ha ⁻¹)		0.632 ^x	0.664 ^x	-0.089 ^{ns}	-0.131 ^{ns}
Proportion of non marketable yield (%)		-0.343 ^{ns}	-0.688 ^x	0.169 ^{ns}	0.061 ^{ns}

^x Correlation on LSD_{0.05} level

^{xx} Correlation on LSD_{0.01} level

^{ns} Non-significant

with most experimental results (Clark, 2013; Kuepper, 2014), i.e., higher yields were realised in flat planting, similarly to the findings of Szarvas et al. (2017). Relative chlorophyll content (SPAD readings) was measured on four occasions during the growing season (Table 4). The obtained measurement results showed that SPAD readings gradually increased during the growing season from early July to mid-August, followed by a reduction in October at the end of the vegetation period. SPAD readings were between 39.10-48.07 on 10th July, between 39.61-50.31 on 17th August and between 38.89-43.31 on 10th October.

According to the obtained results, SPAD readings in flat planting were higher than those in ridge planting in the case of both row spacing values. The canopy of plants in flat planting was better comparing with ridge planting.

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- Based on the results of Pearson's correlation analysis (Table 5), row spacing had a strong effect (0.784^{xx}) on sweet potato yield/ha, while there was only a moderate correlation (-0.460^x) in the case of the applied production method. Positive correlations were observed between tuber yield and SPAD readings on 10th July and 21st July (0.632^{xx} and 0.664^{xx}, respectively), while there was no correlation on 17th August and 7th October (-0.089 and -0.131, respectively). The research findings of 2017 gave some new results in comparison with previous occasions; but we intend to continue these experiments in the following year.

Acknowledgements

The work/publication is supported by the EFOP-3.6.3-VEKOP-16-2017-00008 project. The project is co-financed by the European Union and the European Social Fund.

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Impact of former long-term fertilization on springtail communities in a reforested experimental area

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Abstract: At the beginning of the 1970's, two fertilization experimental areas were established in Gödöllő. After different long-term fertilization treatments, sessile oak (*Quercus petraea*) and black locust (*Robinia pseudoacacia*) plantations were planted in the second half of the 1990's. In the present research, the effects of former fertilization were studied in these two different forests, with the help of soil Collembola communities as indicators. Soil cores and litter samples were collected from the chosen 30 plots to determine the most important soil parameters and to extract Collembola specimens. According to the results, soil organic carbon (SOC), AL-P₂O₅ and AL-K₂O contents were significantly higher in the two highest-dose treatments of the sessile oak plantation compared to the control. In the black locust plantation, only AL-P₂O₅ content differed significantly in the second highest-dose treatment when compared to the control. Among the two plantations significant differences can be observed in almost every parameter, except for AL-K₂O and nitrogen content. The effects of former fertilizer application are reflected in occasional differences in total Collembola abundance, species richness and diversity, mostly related to the treatments with the highest doses. Due to the relatively young age of the plantations, very few typical forest Collembola species were observed, while species characteristic for open habitats are still predominant. The higher SOC content in the black locust plots is well reflected in the higher Collembola abundance compared to the sessile oak plots. Nevertheless, species richness, diversity and equitability were higher in the plantations of the native sessile oak.

Keywords: black locust, sessile oak, afforestation, soil biota

Introduction

Fertilizers entering ecosystems have considerable impact on chemical and physical properties of the soil and the nutrient flow of the soil-plant system (e.g. Haynes and Naidu 1998; Kádár et al. 2007; Kovács and Füleky 1991). Therefore, reasonable use of soil nutrient stock is indispensable, as well as the investigations of nutrient flow and long-term effects of fertilization (Németh and Várallyay 1998). For this kind of researches, long-term fertilization experiments are the most appropriate due to the over-decade achievements providing important information on soil-plant nutrient turnover. With their help, long-term effects of treatments can also be investigated (Berzsenyi 2009) and useful information on changes in soil nutrient stock can be derived (Körshens 2006). Present researches often highlight also the environmental protection aspect of these types of experiments (e.g. Csathó et al. 2012; Kádár and Németh 2003; Szalókiné and Szalóki 2003; Szováti et al.

2006; Tolner et al. 2010). These considerations led to the reforestation of the area of former fertilization experiments established in 1970 in Gödöllő. These experimental areas have been used as arable land for centuries, but the natural vegetation corresponding to climatic and edaphic conditions was the forest. Over the course of the 48-year period experiment, research topics shifted towards the environmental effects of intensive fertilization (Kovács and Füleky 1991). In a unique way, on the plots of the former arable land, black locust (*Robinia pseudoacacia*) and sessile oak (*Quercus petraea*) plantations were established 20 years ago. Extending the area of forests including reforestation of low quality arable lands is an important objective worldwide (UN 2014; UNEP 2014), as well as in the European Union (CEU 2014). Reforestation can mitigate the effects of climate changing efficiently (Hooper et al. 2005), since forests can store a much larger amount of carbon compared to other land use types (Pan et al. 2011). Although plantations

lack the structural complexity of a mature forest, they mitigate the effects of radiation and wind (Cunningham et al. 2015), decrease soil erosion, increase biodiversity (Jackson et al. 2005), make connection among populations and thereby help gene flow (Gilbert-Norton et al. 2010) and participate in the biogeochemical cycle of carbon, oxygen and nutrients (Arneith et al. 2010). After forests were planted, only few results have been published from the experiment area of Gödöllő (Ockert 2006; Szováti et al. 2006; Tolner et al. 2010; Harta et al. 2016). Publications dealing with fertilization effects on biomass, growth, structure and nutrient flow of forests are scarce even worldwide (Burner 2005; Grunewald et al. 2007; Mäkipää 1994; Mirmanto et al. 1999; Plass 1972; Tanner et Kapos 1992; Turkington et al 1998). The presence of mesofauna is indispensable for the nutrient flow in soil (Giller et al. 1997). One of the most important groups is Collembola, which plays an important role in the mineralization of organic matter and also in the distribution of mycorrhiza. Through the responses reflected in their community characteristics, Collembola are considered as excellent test organisms for bioindication analyses. They can respond quickly to any type of soil degradations as well as the changes in land use (Paul et al. 2011; Sousa et al. 2006). The correlations between fertilization and soil biological characteristics are, however not fully clarified (Giller et al. 1997).

The aim of the current research was to study the effect of former fertilization on two types of plantations, as well as to evaluate the effect of forests recultivation with the help of selected indicator organisms (Collembola) of mesofauna.

Materials and methods

Research area

The experimental area is situated in the “Szárítópuszta” research field of Szent István University in Gödöllő (Pest County, Hungary), located in the Gödöllő hills at 247 m a.s.l., where climatic condition is moderately dry continental (Dövényi 2010). The soil type is rust-brown forest soil (Luvic Calcic Phaeozem) according to

the Hungarian classification system (Stefanovits 1972), formed on bedrock typed sand mixed loess. Soil thickness is 60–90 cm. The texture in the 0–20 cm layer is loamy sand, where the total porosity is 51.2 % and soil density is 1.58 g·cm⁻³ on average (Ockert 2006). The ratio of the gravity pores is large (20.7%), indicating appropriate habitat for soil mesofauna. Nevertheless, the water holding capacity is weak. The nutrient management of this type of soil is basically low (Kovács and Füleky 1991).

Two plantations with different tree species, black locust (*Robinia pseudoacacia*) and sessile oak (*Quercus petraea*), were studied. The total area of the studied tree plantations is 11 900 m², whereof the assigned treatment plots cover 4622 m². In both plantations, 5 treatments were selected where different long term fertilization had been applied from the establishment of research fields until the plantation of trees. The extent of each treatment affected by a certain type of fertilization was at least 420 m². Treatments are indicated as SO1 (sessile oak – 1st treatment), SO2, SO3, SO4 and SO5, respectively; as well as BL1 (black locust – 1st treatment), BL2, BL3, BL4 and BL5, respectively. In both plantations, the 1st treatment always means the control, without any former fertilization.

The sessile oak plantation was established in a former crop rotation research field. The fertilizer doses changed depending on the cultivated crop. *Table 1* contains the fertilizer doses applied during the 25-year period of experiment before the sessile oak plantation was established (Kovács and Füleky 1991). The actual black locust plantation was a former maize monoculture where definite fertilizer doses were *Table 1*. Applied fertilizer doses in the plant rotation research field during the 25-year period before the establishment of the sessile oak (SO) plantation

Treatment	N	P ₂ O ₅	K ₂ O	Sum
kg * ha ⁻¹ * 25 y ⁻¹				
SO1	0	0	0	0
SO2	1660	1020	1288	3968
SO3	3270	1980	2418	7668
SO4	5400	3245	3923	12568
SO5	7530	4510	5428	17468

applied yearly in the assigned plots, depending on treatment category. *Table 2* contains the different fertilizer doses applied in this experiment during the 20-year period prior to the establishment of the black locust plantation.

Table 2. Applied fertilizer doses in the maize monoculture research field during the 20-year period, before the establishment of the black locust (BL) plantation

Treatment	N	P ₂ O ₅	K ₂ O	Sum
kg * ha ⁻¹ * 20 y ⁻¹				
BL1	0	0	0	0
BL2	1800	1200	1000	4000
BL3	3600	2400	2000	8000
BL4	5400	3600	3000	12000
BL5	7200	4800	4000	16000

Soil sampling and laboratory methods

From each selected plots, 5 soil cores (ca. 50 g) were collected randomly from the surface layer (0–20 cm). Composite samples were thoroughly mixed from every 5 soil samples collected from the same plots, thus, 30 samples were obtained in total. Soil samples were air-dried, crashed and sieved with a 2.0 mm grid size. During laboratory work, the following chemical parameters were determined from every soil samples; pH_{H₂O} in distilled water and pH_{KCl} in potassium-chloride suspension (Buzás 1988), soil organic carbon content (SOC) defined by wet oxidation with K₂Cr₂O₇ and H₂SO₄ according to the Tyurin method (Buzás 1988), accessible nitrogen (NO₃⁻ + NH₄⁺) by using potassium-chloride (Bacsó et al. 1972), ammonium-lactate soluble phosphorus (AL-P₂O₅) and potassium content (AL-K₂O) (Egnér et al. 1960), calcium-carbonate (CaCO₃) content (Buzás 1988) and soil texture type according to water binding capacity (K_A) (Buzás 1993).

Sampling and extraction of Collembola

From each selected plots, 5 soil cores of 100 cm³ volume (3.6 cm in diameter and 10 cm in depth) were sampled randomly. Collembola specimens were extracted from the soil samples within 14 days using Berlese–Tullgren funnels. Specimens

were identified to species level following main taxonomical keys by Deharveng (1982), Fjellberg (1980, 1998), Babenko et al. (1994), Zimdars & Dunger (1994), Weiner (1996), Jordana et al. (1997), Pomorski (1998), Bretfeld (1999), Potapov (2001), Thibaud et al. (2004) and Jordana (2012). Taxonomic classification is primarily based on the checklist of the Hungarian fauna (Dányi and Traser 2008).

Data analyses

To determine the differences among treatments, one-way ANOVA was performed for all soil parameters separately, while for the comparison of the two types of tree plantation two-way ANOVA was applied. Randomized experimental design ensured the independence of cases. Prior to ANOVA, the data were tested for normality and homoscedasticity (Chi square test, Levene's test, respectively). LSD_{5%} (Least Significant Difference) was counted to determine significant differences between the treatments, with a maximum allowed error rate of 5%.

For the evaluation of Collembola communities, three measures of species α diversity were calculated: the actual species richness, the Shannon–Weaver's index (Shannon and Weaver 1949) and Pielou's evenness index (Pielou 1966). The Shannon indices were compared according to the modified t-test proposed by Hutcheson (1970). Collembola abundance was expressed as the number of individuals per m². Dominance distribution was expressed by McNaughton's community dominance index (CDI) calculated as the sum of percentage abundance of the first and second most abundant species, respectively (McNaughton 1967). To compare abundance and species richness, Friedman's two-way analysis of variance (ANOVA) was used taking into account tree species and fertilization level simultaneously. Analyses were performed using the software SPSS vs20 (IBM Corp. Released 2011) and Past ver. 2.17b (Hammer et al. 2001).

Results

Soil parameters

Table 3 presents the defined soil parameters in

Table 3. Results of the measured soil parameters in the sessile oak (SO) plantations in the formerly fertilized plots (significant differences compared to the control are signed with bold numbers)

Treatment	pH _{KCl}	pH _{H₂O}	SOC %	AL-P ₂ O ₅	AL-K ₂ O	NO ₃ ⁻ + NH ₄ ⁺	K _A
				mg/kg			
SO1	5.45	6.28	1.51	80.03	165.00	10.23	26.33
SO2	5.00	6.01	1.57	95.83	183.33	12.47	27.00
SO3	4.61	5.70	1.66	148.67	184.33	9.97	27.00
SO4	4.72	5.70	1.87	211.33	221.00	12.63	26.67
SO5	4.56	5.57	2.11	280.00	270.33	12.83	27.00
LSD _{5%}	1.70	1.26	0.31	58.68	51.30	6.11	1.14

Table 4. Results of the measured soil parameters in the black locust (BL) plantations in the formerly fertilized plots (significant differences compared to the control are signed with bold numbers)

Treatment	pH _{KCl}	pH _{H₂O}	SOC %	AL-P ₂ O ₅	AL-K ₂ O	NO ₃ ⁻ + NH ₄ ⁺	K _A
				mg/kg			
BL1	6.35	7.00	2.21	10.57	217.00	15.13	29.00
BL2	6.37	6.98	2.30	24.74	209.67	8.97	28.67
BL3	6.30	6.95	2.19	27.17	212.67	15.60	29.67
BL4	5.96	6.68	2.11	63.47	191.00	9.37	29.00
BL5	6.26	6.85	2.22	45.60	188.33	9.00	28.33
LSD _{5%}	0.68	0.46	0.62	40.11	70.28	14.63	2.62

Table 4. Results of the measured soil parameters in the sessile oak (SO) and black locust (BL) plantations in the formerly fertilized plots (significant differences are signed with bold numbers)

	pH _{KCl}	pH _{H₂O}	SOC %	AL-P ₂ O ₅	AL-K ₂ O	NO ₃ ⁻ + NH ₄ ⁺	K _A
				mg/kg			
SO_mean	4.87	5.85	1.74	108.18	204.80	11.63	26.80
BL_mean	6.25	6.98	2.21	34.31	203.73	11.61	28.93
LSD _{5%}	0.52	0.38	0.23	22.00	27.50	4.45	1.13

the sessile oak plantation, divided by the former fertilizer types. According to the results, there is no significant difference among the two types of pH, however they show decreasing tendency towards to the higher fertilization doses. On the contrary, the amount of soil organic carbon (SOC) is higher in the soil where more intensive fertilization treatments occurred before the plantation; where treatments SO4 and SO5 are significantly different from the control (SO1). Both AL-P₂O₅ and AL-K₂O are increasing with the higher dose of fertilizers. Treatments SO3 (in the case of AL-P₂O₅ only), SO4 and SO5 (for both AL-P₂O₅ and AL-K₂O) differed significantly from the control. Among the nitrogen content and the K_A values no significant differences were observed.

Table 4 contains the defined soil parameters in the black locust plantation, divided by the former fertilizer types. Significant difference was found only in the AL-P₂O₅ content, between the treatment BL4 and the control (BL1).

The comparison results of the defined soil parameters of the two plantations (black locust and sessile oak) are presented in *Table 5*. Among the two plantations, significant differences can be observed in almost every parameter, except for AL-K₂O and nitrogen contents.

Both types of pH, the K_A value and also the SOC are higher in the black locust plantation, only the phosphorus content shows higher values in the soil of the sessile oak plantation.

Table 6. Mean abundance (ind. m⁻²) of Collembola species in the former fertilized plots of sessile oak and black locust plantations.

	sessile oak					black locust				
	SO1	SO2	SO3	SO4	SO5	BL1	BL2	BL3	BL4	BL5
Hypogastruridae										
<i>Ceratophysella luteospina</i> (Stach, 1920)	0	0	0	13	20	0	0	0	0	0
<i>Willemia virae</i> Kaprus, 1997	300	127	160	220	280	313	420	213	187	193
Neanuridae										
<i>Neanura muscorum</i> (Templeton, 1835)	0	13	0	7	27	0	0	0	0	0
<i>Pseudachorutes parvulus</i> Börner, 1901	27	0	27	47	33	27	0	0	0	7
<i>Pseudachorutes pratensis</i> Rusek, 1973	13	0	0	0	0	0	7	0	13	0
Odontellidae										
<i>Superodontella lamellifera</i> (Axelson, 1903)	0	0	0	7	13	0	0	0	0	0
Onychiuridae										
<i>Protaphorura armata</i> (Tullberg, 1869)	433	287	407	500	747	527	440	453	440	347
<i>Protaphorura campata</i> (Gisin, 1952)	0	13	0	0	20	20	7	13	0	47
Tullbergidae										
<i>Mesaphorura critica</i> Ellis, 1976	80	60	113	220	93	400	520	540	413	373
<i>Mesaphorura italica</i> (Rusek, 1971)	73	53	13	47	0	107	27	20	47	53
<i>Mesaphorura krausbaueri</i> Börner, 1901	360	100	387	133	100	347	513	467	487	453
<i>Mesaphorura macrochaeta</i> Rusek, 1976	33	33	13	40	27	320	173	267	253	180
<i>Metaphorura denisi</i> Simon, 1985	0	7	0	0	0	0	0	0	0	0
Entomobryidae										
<i>Entomobrya dorsalis</i> Uzel, 1891	0	7	20	13	13	0	7	0	0	0
<i>Entomobrya multifasciata</i> (Tullberg, 1871)	320	260	240	567	220	513	473	487	460	433
<i>Entomobrya muscorum</i> (Nicolet, 1842)	0	13	0	0	0	0	0	0	0	0
<i>Lepidocyrtus cyaneus</i> Tullberg, 1871	13	0	0	0	0	0	27	0	13	0
<i>Lepidocyrtus lanuginosus</i> (Gmelin, 1788)	173	207	320	267	427	147	180	133	140	133
<i>Pseudosinella alba</i> (Packard, 1873)	173	113	167	180	133	60	87	47	60	67
<i>Pseudosinella petterseni</i> Börner, 1901	0	13	20	20	33	0	20	33	13	20
<i>Heteromurus major</i> (Moniez, 1889)	13	0	0	0	0	0	0	0	0	0
<i>Heteromurus nitidus</i> (Templeton, 1835)	0	13	0	33	0	0	0	7	0	0
<i>Orchesella cincta</i> (Linnaeus, 1758)	187	193	160	200	147	167	233	227	120	153
<i>Orchesella flavescens</i> (Bourlet, 1839)	0	0	0	0	7	0	0	0	0	0
<i>Orchesella multifasciata</i> (Stscherbakow, 1898)	307	300	433	507	447	240	267	287	120	267
Isotomidae										
<i>Cryptopygus bipunctatus</i> (Axelson, 1903)	0	0	20	27	13	0	0	13	0	0
<i>Folsomia manolachei</i> Bagnall, 1939	613	700	573	533	567	1180	720	1267	1047	833
<i>Folsomia quadrioculata</i> (Tullberg, 1871)	360	427	413	420	373	920	1093	787	1027	973
<i>Isotomiella minor</i> (Schäffer, 1896)	327	340	387	440	347	120	120	160	200	133
<i>Parisotoma notabilis</i> (Schäffer, 1896)	633	700	600	507	627	1133	1707	1433	953	1727
<i>Proisotoma minuta</i> (Tullberg, 1871)	7	0	0	0	20	0	0	0	0	0
Neelidae										
<i>Megalothorax minimus</i> Willem, 1900	13	20	13	213	167	7	27	0	0	20
Katiannidae										
<i>Sminthurinus elegans</i> (Fitch, 1863)	40	13	80	7	53	20	47	27	33	7
Sminthurididae										
<i>Sphaeridia pumilis</i> (Krausbauer, 1898)	20	167	73	107	93	73	100	60	40	20
Σ	4520	4180	4640	5273	5047	6640	7213	6940	6067	6440

Collembola species richness, diversity and abundance

A total of 8,545 Collembola individuals,

representing 10 families, 21 genera and 34 species were extracted from the collected soil samples. Mean abundance of the species occurred are presented in Table 6, whereas

Table 7 shows the most important structural characteristics of Collembola communities found in the different plots of the studied sessile oak and black locust plantations.

Mean Collembola abundance was significantly higher ($p < 0.05$) in the black locust plantation, while according to the former treatments, abundance is significantly higher ($p < 0.05$) in the SO4 and SO5 treatments of the sessile oak plantation compared to the control (SO1).

of the black locust plantation, only the BL2 treatment was significantly higher ($p < 0.05$) compared to the control (BL1).

There were marked differences (Hutcheson t test, $p < 0.05$) in community diversity between the two plantation types (sessile oak and black locust), while only occasional differences have been observed between the different former treatments within the same plantation type (Table 8).

Table 7. Structural indices of Collembola communities in the sampled plots

	SO1	SO2	SO3	SO4	SO5	BL1	BL2	BL3	BL4	BL5
S_{total}	23	25	22	26	27	20	23	21	20	21
S_{mean}	17	18	18	19	20	17	19	17	17	18
H'	2.644	2.604	2.653	2.767	2.712	2.488	2.476	2.436	2.445	2.372
J	0.843	0.809	0.858	0.849	0.823	0.830	0.790	0.800	0.816	0.779
CDI	27.58	33.49	25.29	20.86	27.21	34.84	38.82	38.90	34.17	41.93

S – number of species; H' – Shannon–Weaver’s diversity index; J – Pielou’s evenness index; CDI – community dominance index (%)

With regard to the black locust plantation, no significant differences were revealed between the different former treatments.

Between the mean species richness there is no significant difference ($p < 0.05$) according to the type of plantation, while the total number of species identified was higher in the sessile oak plantation (34 species) compared to the black locust plantation (26 species). According to the former treatments, species richness was significantly higher ($p < 0.05$) in the SO4 and SO5 treatments of the sessile oak plantation compared to the control (SO1), while in case

Discussion

Soil properties

In most cases, canopy closure and thus the emergence of the forest structure mostly occurs in the first 20 years after reforestation (Oliver and Larson 1996). Suitable plant litter layer also develop within 20 years after planting (Cunningham et al. 2012), which corroborates the time-appropriateness of this research. According to the results obtained, some determined soil parameters in the sessile oak forest do not differ significantly among treatments, with the exception of SOC (%), AL- P_2O_5 (mg/kg)

Table 8. Results of Shannon diversity comparison (Hutcheson t test) in the sessile oak (SO) and black locust (BL) plantations. Significant differences are marked with* ($P < 0.05$) and ** ($P < 0.01$)

	SO1	SO2	SO3	SO4	SO5	BL1	BL2	BL3	BL4
SO2	0.956								
SO3	-0.247	-1.197							
SO4	-3.182**	-3.801**	-3.022**						
SO5	-1.583	-2.349*	-1.389	1.376					
BL1	3.907**	2.478*	4.286**	7.422**	5.355**				
BL2	4.105**	2.700**	4.473**	7.505**	5.506**	0.329			
BL3	5.164**	3.619**	5.572**	8.677**	6.535**	1.372	1.004		
BL4	4.831**	3.348**	5.220**	8.267**	6.201**	1.084	0.731	-0.262	
BL5	6.339**	4.803**	6.737**	9.613**	7.587**	2.865**	2.485*	1.575	1.796

and AL-K₂O (mg/kg) in the former larger doses of fertilizers (treatment 4th and 5th), which can be interpreted as a non-efficient nutrient utilization of the sessile oak trees. Most often, nutrient uptake is very efficient in forests due to mikorrhiza, but fertilization has negative impacts on this symbiotic relation and thus the nutrient supply of trees (Berki 1999). Moreover, according to a research in boreal forests, N-fertilization led to 54% decline of fungi and bacteria biomass in the soil (Wallenstein et al. 2006). Another study carried out in a boreal forest showed that N-fertilization raised soil organic matter content (Mäkipää 1994).

With respect to the soil parameters in the black locust plantation, no significant differences were found among former treatments, with the only exception of the AL-P₂O₅ (mg/kg) content in the 4th treatment (BL4) when compared to the control. Phosphorus can bound much longer in the soil than other macronutrients (Kovács and Füleky 1991), which is undoubtedly the consequence of the higher fertilization dose applied to the treatment in question before the tree plantation.

When comparing the two different tree plantations, significant differences were detected in almost all soil parameters except for AL-K₂O (mg/kg) and NO₃⁻ + NH₄⁺ (mg/kg). The organic carbon content of the soil is higher in the black locust plantation, while the phosphorus content is markedly higher in the soil of the sessile oak forest. The mineralization of the black locust leaves is much faster due to their more optimal C/N ratio (Tanteno et al. 2007), which generally result in higher SOC content. As a fast-growing species, the black locust can sequester atmospheric carbon more efficiently than the native sessile oak (Lindenmayer et al. 2003). Moreover, the closure of canopy and the development of structure are faster in the black locust stands (Hagggar et al. 1997). The remarkably lower phosphorus content of the black locust plantation can be explained by the much higher phosphorus demand of black locust as a legume (Kanzler et al. 2015; Loch 1999; Plass 1972). Rhizobium bacteria help N-fixation

for legumes (Mantovani et al. 2015) and their growth increases when K-fertilization occurs (Berki 1999). The loss of phosphorus is also confirmed as an accelerated growing of black locust in case of P-fertilization (Burner 2005; Grunewald et al. 2007). When NPK-fertilization applied, the amount of biomass and leaf litter can increase significantly (Tanner and Kapos 1992; Turkington et al. 1998).

Collembola species richness, diversity and abundance

The application of fertilizers into the soil may affect the abundance of Collembola, the species richness, diversity and the dominance structure (Hopkin 1997). In a forest soil fertilization experiment, Geissen et al. (1997) found no relation between the treatment and collembolan abundance, while dominance of certain species varied between the P/K-fertilized and unfertilized control plots. As a consequence of a forest soil-liming and fertilization experiment, Geissen and Kampichler (2004) found no change in springtail community composition and no relationship between total and single species abundances and soil chemical parameters. In a pine plantation, effect of N fertilizer application was found to be weak on Collembola population, while in the same experiment Vilkamaa and Huhta (1986) emphasized the role of soil pH in relation of collembolan community composition and dominance structure. In our study, the effects of former fertilizer application are reflected in occasional differences in total Collembola abundance, species richness and diversity, mostly related to the treatments with the highest doses.

As a consequence of the geographical nature and climate of the area we recorded a number of typical xerothermophilic species (e.g. *Mesaphorura critica*, *Metaphorura denisi*, *Entomobrya multifasciata*, *Orchesella cincta*). Due to the relatively young age of the plantations, very few typical forest species were observed (*Ceratophysella luteospina*, *Neanura muscorum*, *Entomobrya muscorum*) while species characteristic for open habitats

(e.g. *Pseudachorutes pratensis*, *Lepidocyrtus cyaneus*) are still predominant. This kind of species composition can be explained by the absence of surrounding forest habitats and, at the same time, the slow dispersal ability and thus the lack of colonization of certain euedaphic species (Salmon and Ponge 1998, Huhta and Ojala 2006).

Paradoxically, both deforestation and afforestation can have strong negative impact on soil collembolan communities (Jordana et al. 1987; Deharveng 1996; Ponge et al. 2006). It is particularly true for our study area, where there was a shift from a long-term fertilized agricultural land to woodland habitat through reforestation. We have no former data on soil mesofauna, but literature usually report low diversity from arable fields (e.g. Kováč et al. 2001; Winkler and Traser 2017). Although the collembolan abundance detected in the sessile oak and black locust plantations is relatively low, diversity values (2.4–2.7) shows the signs of revitalization and diversification mostly owing to the vegetation cover and leaf-litter accumulation.

Choice of tree species for reforestation is crucial and can have major impact on soil and soil biota in the long term. Among the invasive tree species, black locust has high importance, since almost 25% of the Hungarian forest stands are covered

by this tree species (Bartha et al. 2008). Black locust has complex effects on soil characteristics, most notably, increasing total nitrogen and nitrate content and soil organic matter content (Rice et al. 2004; Tateno et al. 2007). Latter characteristic is well reflected in the higher Collembola abundance in the studied black locust plots compared to the sessile oak plots. Nevertheless, species richness, diversity and equitability were higher in the plantations of the native sessile oak. This phenomenon might be explained by the allelopathic effect of black locust, which can produce and release secondary metabolites (e.g. toxalbumins, robin and phasin) that can reveal inhibition effects on protein synthesis that certain species cannot tolerate (Hui et al. 2004; Rahmonov 2009; Lazzaro et al. 2018). Community structure as emphasized by the CDI index is more evenly distributed among species in the native sessile oak plots, while the few eudominants with higher abundance and the majority of species at relatively low numbers in the black locust plantation might also reflect unfavourable conditions for certain groups of Collembola.

Acknowledgements

This research was supported by the ÚNKP-17-3 new national excellence program of the ministry of human capacities.

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Soil metabolic activity profiles of the organic and conventional land use at Martonvásár

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Abstract: These days, increasing attention has been paid to understand the relationship between different farming systems and soil microbiological processes supporting sustainable land use. Soil microbiota have been considered as a priority component in organic land managements and sustaining soil health in long term. This statement should be supported by appropriately selected indicators - physiological properties or more precisely the metabolic activity profile of the soil microbes. A recently developed method, MicroResp™ gives a promising characterization of the catabolic activity pattern of the soil microbial communities. The goal of this study was to compare the catabolic profile of soil microbial communities of organic and conventional land management in a long-term experiment at Martonvásár (Hungary) from two consecutive years by two samplings (autumn and spring). MicroResp™ with 22 different substrates were used to characterize the catabolic activity patterns of these soils. Multivariate statistical analysis revealed a significant difference between the catabolic activity profiles of the soil microbial communities of the two management systems. Considering the soil chemical parameters, the $AL-P_2O_5-K_2O$, NO_3^-N were responsible for the divergence of the two farming systems according to the principal component analysis (PCA). The biotic (crop) and abiotic (EC, humus content, pH) parameters could affect not only the rate of soil respiration but the catabolic activity profiles as well. Organic farming increased the catabolic activity of soil microbes.

Keywords: organic, conventional, farming system, MicroResp

Introduction

Nowadays organic farming has a great significance because fertile soil supply the crops with essential nutrients thereby contribute to an active and diverse soil microorganisms community. Soil microbes have crucial role in ecosystem functions and in the sustainability of soil resources (Allison and Martiny 2008). Evaluation of physicochemical soil properties and determination of the soluble nutrient content available for plants is complicated because these factors has a very complex interaction with each other. It is largely depends on the efficient utilization of agricultural sources (Garbisu et al. 2011; Mäder et al. 2002).

The type of agricultural land management can have a remarkable impact on the activity of soil microbial communities. The organic land managements with green manure application and crop rotation can have an important influence on the soil microbial communities (Ge et al. 2013).

The MicroResp™ method is one of the CLPP (community level physiological profiles)

techniques (Campbell et al. 2003). This is based on the microbial utilization of different carbon sources. This method is widely used to indicate the activity in soil microbial communities (Bárány et al. 2014). It is a suitable method which can illustrate the quality of the agricultural farming systems and the degree of the fertility of soil (Romaniuk et al. 2011). The advantage is the rapid microbial identification of the total activity of the soil microorganisms (Chapman et al. 2007). Moreover MicroResp™ is an appropriate tool to detect the differences between the management practices because the metabolic fingerprints could discriminate between the different land management methods (Campbell et al. 2003; Ge et al. 2013). By adding 23 different substrates (simple sugars, amino acids and carboxylic acids) to the soil samples it is possible to get a catabolic fingerprint of the soil microbial community because the individual species of soil bacteria could have distinct capabilities to respire to the substrates (Mucsi et al. 2017). In this paper MicroResp™ method was tested on soil samples from two

farming systems under different vegetation types where soils were treated with fertilizer at conventional land managements and green manure at organic plots.

The activity of the soil microbial community is mainly influenced by the composition of plant's root exudates in the rhizosphere. In general the soil microorganisms utilize of the photosynthetic assimilates from the host plant including sugars (Bais et al. 2006).

Gunina and Kuzyakov (2015) showed that the application of glucose resulted in higher raise of the soil microbial activity in organic and conventional farming system. Sugars account about 52% of the organic substances of the root exudates of pea. The main sugar of root exudates is glucose with 50%, while fructose and saccharose have a lower contribution of 23% for both. Exudation of sugars could increase microbial activity and biomass in soil which later raises the available nitrogen for crop. The carboxylic acids could decrease the pH in the rhizosphere, the amino acids could mobilize micronutrients (Strickland et al. 2015).

Our goal was to compare the soil catabolic activity in different seasons (autumn and spring) under both organic and conventional land management systems. Further, our aim was to find relationship between the soil physicochemical parameters and catabolic activity results. We presumed that the organic land management will increase the activity of the soil microorganisms and will alter the community structure, which might result in a difference of the catabolic profiles of the samples from the two management systems.

Material and methods

Study area and soil sampling

The area is characterized by temperate climate, the annual precipitation was 308.6 mm and 405.3 mm, the mean annual air temperature was 11 °C and 12 °C in 2011 and 2012, respectively (<http://www.metnet.hu>).

The soil samples were collected in November of 2011 and May of 2012 from the upper 20 cm of the organic and conventional plots of a 15-year-long experiment, in the Centre for Agricultural Research, Martonvásár (Hungary) with a calcic chernozem soil (FAO 1998) (Table 1) with a loam soil texture. The GPS coordinates were the following: 47° 18' 38" N, 18° 46' 45" E. The soil samples were air-dried, ground and sieved through a 2 mm mesh for physical and chemical analysis, the other part of the samples were stored at + 4 °C for microbiological analyses.

The main soil physical and chemical properties

The soil texture was characterised by pipetting method, determining three fractions, sand, silt and clay. The humus content (%) was calculated from soil organic C measured by wet digestion and back titration. The total salt content was calculated from the electric conductivity (EC 2.5) of soil:water (1:2.5) suspensions. The pH_{H_2O} and pH_{CaCl_2} (0.01N $CaCl_2$) values, the NH_4-N ($mg\ kg^{-1}$), NO_3-N ($mg\ kg^{-1}$), the total N (%), C% values were measured. The ammonium-lactate (AL)-soluble nutrient content (Ca (m/m %), K_2O ($mg\ kg^{-1}$), Na ($mg\ kg^{-1}$), P_2O_5 ($mg\ kg^{-1}$) were determined. The above values were measured according to the Hungarian soil standards (Buzás 1988; Buzás 1993).

Table 1. Crops and fertilization in organic (OF) and conventional (CF) farming systems at Martonvásár

Years	OF		CF	
	crop	fertilization	crop	fertilization
2011	peas	green manure (peas)	spring wheat	250 $kg\ ha^{-1}$ NPK (0:10:24%)
2012	cereal	no	corn	300 $kg\ ha^{-1}$ NPK (15:15:15 %) 270 $kg\ ha^{-1}$ N (39%)

Legend: Complex NPK fertilizer was used with 15-15-15% active agent. 15% N:10 % ammonium nitrogen + 5 % urea N; 15 % P_2O_5 :P content 6.2 %; 15 % K_2O :K content 12.5 %, N fertilizer (calcium ammonium nitrate fertilizer (CAN)) was used which contain 27 % N, 5 % Ca (7 % calcium oxide) and 3 % Mg (5 % magnesium oxide).

Catabolic activity pattern of soil microbial communities by MicroRespTM, statistical analyses

MicroRespTM technique was used to evaluate the catabolic activity pattern of the microbial community of soils. It is based on the colorimetric detection of CO₂ evolved from the soil after addition of substrate solutions (Campbell et al. 2003). 23 different substrates (simple sugars, amino acids and carboxylic acids) and ultrapure distilled water (control) in four replications were distributed to each plate. Four plates were used for OF and four for CF samples, within the plates each substrates were tested in 4-4 replicates. The following substrates were used: D-galactose (Gal), trehalose (Tre), L-arabinose (Ara), D-glucose (Glc) and D-fructose (Fru) in 80 mg ml⁻¹, citric acid (Cit), DL-malic acid (Mal), Na-succinate (Suc), L-alanine (Ala) and L-lysine (Lys) in 40 mg ml⁻¹, L-glutamin (Gln) in 20 mg ml⁻¹, L-arginine (Arg), 3,4-dihydroxybenzoic acid (Dhb) and L-glutamic acid (Glu) in 12 mg ml⁻¹. Myo-inositol (Ino), D-xylose (Xyl), D-mannitol (Mat), D-mannose (Man), D-sorbitol (Sor), L-rhamnose (Rha) 80 mg ml⁻¹, L-asparagin-monohydrate (Asn) 20 mg ml⁻¹, D-gluconic-acid-potassium (Gla), L-ascorbic acid (Asa) 40 mg ml⁻¹. The pH of the substrate solutions was adjusted to 6.5 by 1N NaOH or HCl solutions. The plates were read before and after 6 h of incubation at 25 °C in dark with a plate reader (Anthos 2010, Biochrom, Cambridge, UK) at 570 nm for colorimetric detection. Then respiration rates were calculated from the normalized % CO₂ data after 6 h incubation period (Szili-Kovács et al. 2011).

Significant differences in the soil chemical, physical properties (n=12) between the OF and CF types were tested by two-sample tests. Similarity Percentage (SIMPER) test with Bray Curtis dissimilarities was used for statistical analysis to identify which physical-chemical parameters and substrates had the largest contribution to the average dissimilarity between the two different land use systems. MicroRespTM method was evaluated with n=4 soil samples per sites with four replicates. Ascorbic acid substrate resulted a remarkably high respiration rate at all samples, therefore it was excluded from the further statistical analyses. Principal component analysis (PCA) was used to compare the main soil chemical parameters and also the catabolic activity profile data between the two type of land managements. PCA was calculated by using the correlation matrix with disregard groups, and Bootstrap N parameters of 4. These statistics were made by Past3 software package (Hammer et al. 2001).

Results*Main soil physical and chemical properties*

The soil texture was classified as loam according to particle size distributions of soil samples (Table 2). The area was not salty based on the electric conductivity (EC) of the saturation extract (< 2 mS cm⁻¹) value and slightly alkaline (Table 3). The most important first three parameters for the autumn and spring soil samples were AL-P₂O₅, AL-K₂O and NO₃⁻-N on the basis of SIMPER test. According to the ammonium-lactate soluble

Table 2. The main physical properties of soil from organic (OF) and conventional (CF) fields of Martonvásár

Soil physical properties	Martonvásár farming system			
	Autumn		Spring	
	OF	CF	OF	CF
Sand (%) (0.05 - 2 mm)	33.74 ± 3.36	31.99 ± 4.05	32.7 ± 3.2	30.05 ± 4.55
Silt (%) (0.002 - 0.05 mm)	42.47 ± 2.45	42.74 ± 2.39	42.44 ± 2.56	43.00 ± 2.68
Clay (%) (< 0.002 mm)	23.79 ± 1.35	25.29 ± 2.22	24.86 ± 1.26	26.95 ± 2.77

Legend: Data are means ± standard deviation of the means; n = 12.

Table 3. The main chemical properties of soil from organic (OF) and conventional (CF) fields of Martonvásár

Soil chemical data	Martonvásár farming system							
	Autumn			Spring			SL among the OF's (A+S)	SL among the CF's (A+S)
	OF	CF	SL among OF and CF (A)	OF	CF	SL among OF and CF (S)		
AL-Ca (m/m %)	1.52 ± 0.62	1.23 ± 0.96	n.s.	1.62 ± 0.52	1.16 ± 0.95	n.s.	n.s.	n.s.
AL-P ₂ O ₅ (mg kg ⁻¹)	646.54 ± 130.94	569.2 ± 56.03	n.s.	737.83 ± 261.96	654.83 ± 46.10	n.s.	n.s.	***
AL-K ₂ O (mg kg ⁻¹)	539.67 ± 73.77	474.69 ± 53.05	*	605.33 ± 89.25	526.50 ± 58.21	**	**	*
AL-Na (mg kg ⁻¹)	16.38 ± 2.32	20.36 ± 3.00	**	11.19 ± 0.97	11.06 ± 3.20	n.s.	***	***
EC (mS cm ⁻¹)	0.28 ± 0.03	0.24 ± 0.03	**	0.21 ± 0.03	0.27 ± 0.05	**	***	n.s.
pH _{H2O}	7.79 ± 0.10	7.85 ± 0.12	n.s.	7.92 ± 0.14	7.75 ± 0.16	**	***	**
pH _{CaCl2}	7.91 ± 0.07	7.85 ± 0.07	n.s.	7.85 ± 0.10	7.71 ± 0.12	**	n.s.	***
NH ₄ ⁺ -N (mg kg ⁻¹)	4.95 ± 1.18	4.04 ± 0.93	n.s.	4.94 ± 0.67	8.84 ± 4.64	**	***	**
NO ₃ ⁻ -N (mg kg ⁻¹)	37.12 ± 10.56	23.14 ± 8.83	**	5.24 ± 0.98	40.24 ± 17.86	***	***	**
Total N (%)	0.20 ± 0.01	0.17 ± 0.01	***	0.19 ± 0.00	0.17 ± 0.01	***	***	n.s.
Humus content (%)	2.90 ± 0.20	2.51 ± 0.18	***	2.98 ± 0.26	2.66 ± 0.24	**	n.s.	*

Legends: Data are means ± standard deviation of the means; n = 12. SL= significance level. Significant differences among the different land managements with two-sample tests, p* = < 0.05; p** = < 0.01; p*** = < 0.001; n.s. = not significant between OF and CF). EC = electric conductivity, Total N = total nitrogen, AL= ammonium-lactate (AL)-soluble nutrient content, A = autumn, S = spring.

nutrient content, the AL-Ca content of soils were not differ significantly between OF and CF in both seasons. AL-P₂O₅ content of soils were not significantly different only between spring and autumn CF data with significantly higher value of the former. The AL-K₂O content of the soil from OF management was significantly higher in both seasons compared to the CF. The AL-Na content of the soils in the spring samples were not differ significantly between in organic and conventional managed plots, while in autumn samples the AL-Na content of soils of OF were significantly lower than that of the CF. The soil

NH₄⁺-N content between OF and CF were not differ significantly in autumn. However in spring the NH₄⁺-N level of soil was significantly higher in CF. Soil NO₃⁻-N was significantly higher at OF than at CF in the autumn samples, while it was significantly higher at CF in spring. The soil total N as well as humus content were significantly higher in OF in both seasons.

Catabolic activity pattern of soil microbial communities

The three most active substrates were Mal, Cit and Glc (Figure 1) for autumn and Glc, Fru,

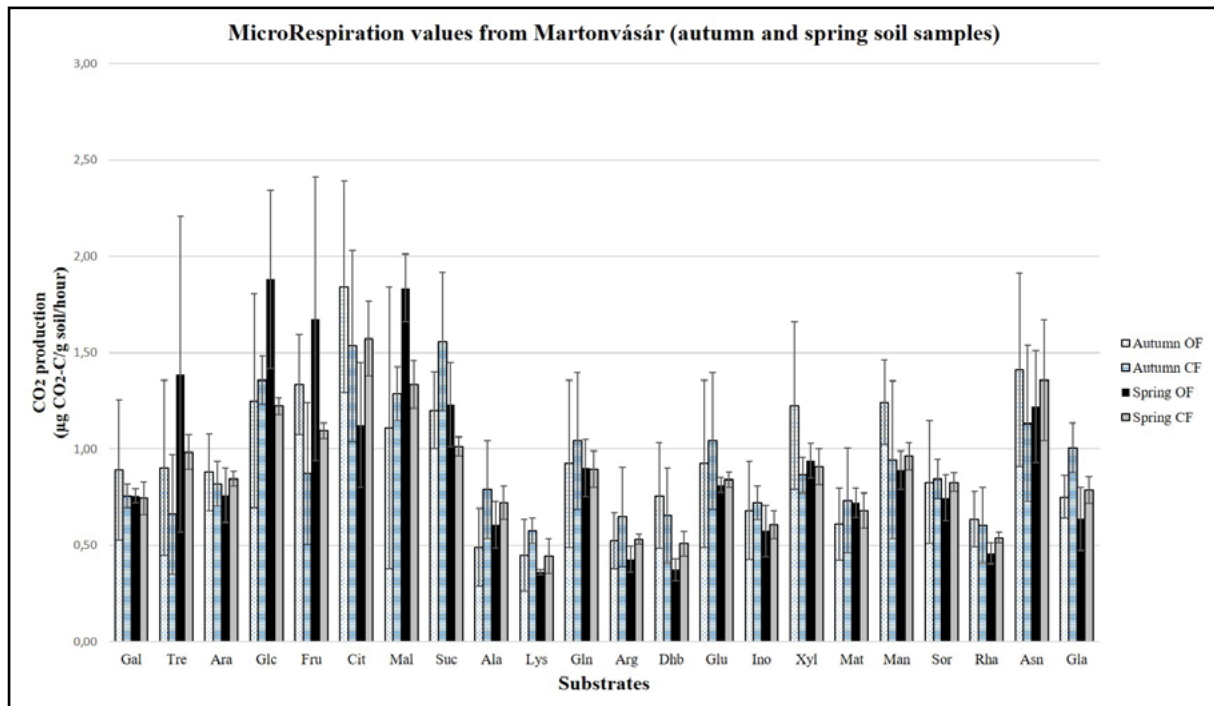


Figure 1. Mean catabolic response for 22 substrate sources in two land management systems, organic (OF) and conventional (CF) in autumn and spring by MicroResp™.

Legends: Error bars = standard deviation; Gal = D-galactose, Tre = trehalose, Ara = L-arabinose, Glc = D-glucose, Fru = D-fructose, Cit = citric acid, Mal = DL-malic acid, Suc = Na-succinate, Ala = L-alanine, Lys = L-lysine, Gln = L-glutamin, Arg = L-arginine, Dhb = 3,4 dihydroxybenzoic acid, Glu = L-glutamic acid, Ino = Myo-inositol, Xyl = D-xylose, Mat = D-mannitol, Man = D-mannose, Sor = D-sorbitol, Rha = L-rhamnose, Asn = L-asparagin-monohydrate, Gla = D-gluconic-acid-potassium.)

Mal for spring sampling originated from OF and CF. The malic acid and glucose were the most utilized substrates in both years and farming systems. The most differentiating substrates between OF and CF were glucose, fructose, malic acid, gluconic acid and citric acid ($p < 0.05$) according to the SIMPER test. The soil catabolic response to citrate substrate was significantly higher ($p < 0.05$) in CF than OF, while to malic acid, glucose and fructose it was significantly higher in OF than CF.

Principal component analysis (PCA) partially separated the soil samples to their own group according to OF and CF. Humus and total nitrogen correlate with each other (Figure 2). This PCA result was related to the Table 3, where the amounts of nitrate and ammonia were doubled in the conventional land management in spring while the total nitrogen and humus remained similar.

The PCA of the catabolic activity patterns resulted a clear separation of the OF samples

from CF in spring while they were not separated significantly in autumn (Figure 3).

Discussion

The influence of organic and conventional land managements on the main soil physical - chemical and microbial properties have been studied earlier (Monokrousos et al. 2006; Santoyo et al. 2017; Girvan et al. 2003; Clark et al. 1998). Nearly all of the soil chemical parameters studied varied significantly depending on the season and soil managements (Table 1). In our experiment, the soil AL-Ca content was the only one among the soil chemical parameters which resulted no significant difference between OF and CF. AL-soluble nutrient content and the available N of soils showed significant difference between OF and CF (Table 3). Marinari et al. (2006) also found significantly better soil nutritional conditions and enhanced soil microbial activity in OF managed soils in central Italy, after seven years of organic management. They used

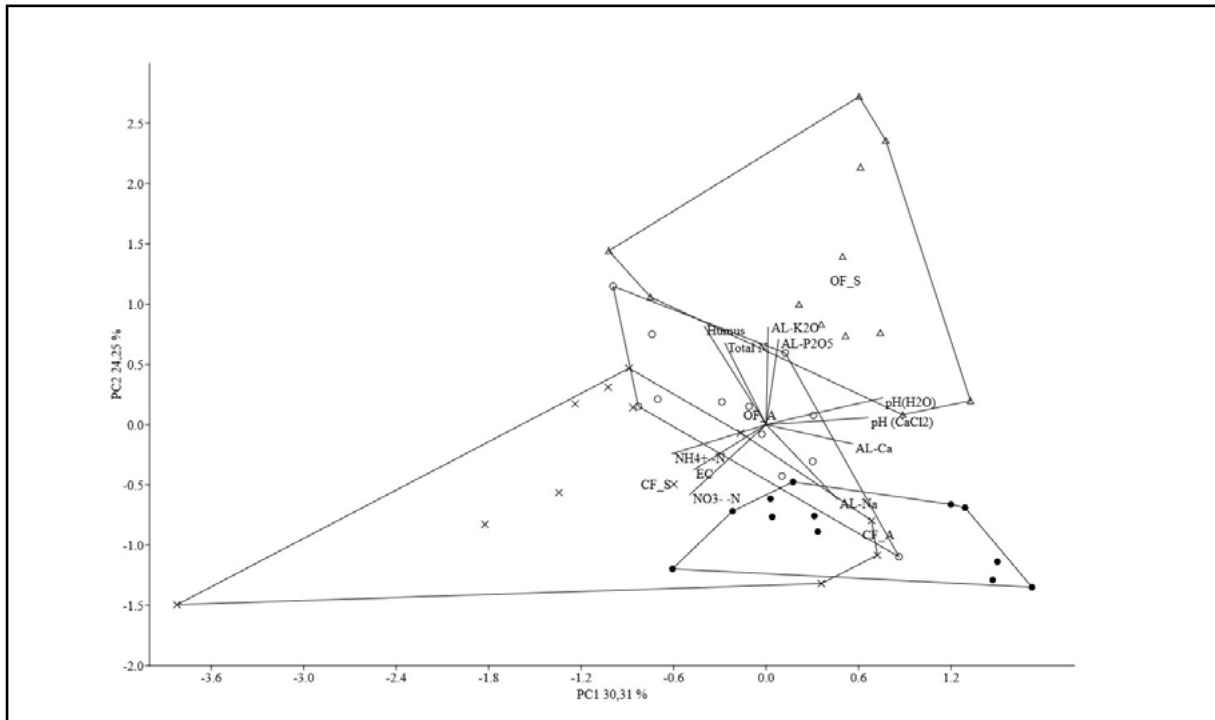


Figure 2. PCA analyses between the main soil chemical parameters according to OF and CF land managements from autumn and spring.
 Legends: OF = organic farming system, S = spring, A = autumn, AL = ammonium-lactate soluble nutrient content, EC = electric conductivity.

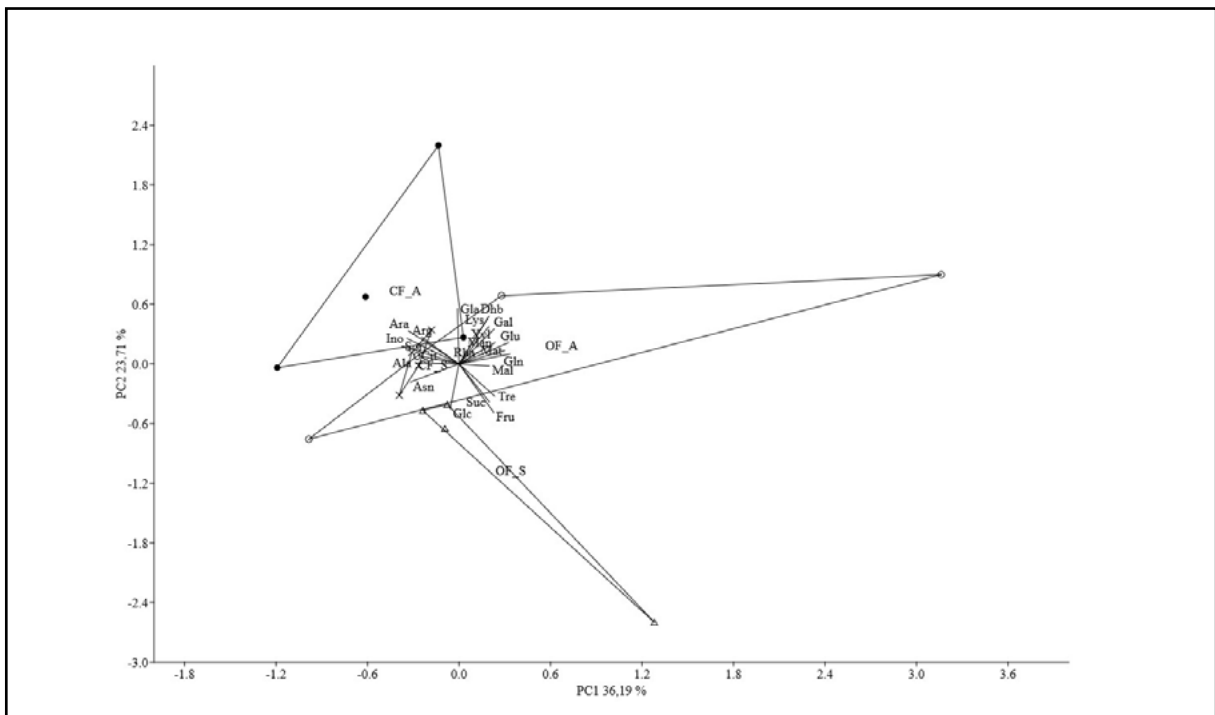


Figure 3. PCA analyses between the catabolic activity of OF and CF soils from both seasons.
 Legends: OF = organic farming system, S = spring, A = autumn, Gal = D-galactose, Tre = trehalose, Ara = L-arabinose, Glc = D-glucose, Fru = D-fructose, Cit = citric acid, Mal = DL-malic acid, Suc = Na-succinate, Ala = L-alanine, Lys = L-lysine, Gln = L-glutamin, Arg = L-arginine, Dhb = 3.4 dihydroxybenzoic acid, Glu = L-glutamic acid, Ino = Myo-inositol, Xyl = D-xylose, Mat = D-mannitol, Man = D-mannose, Sor = D-sorbitol, Rha = L-rhamnose, Asn = L-asparagin-monohydrate, Gla = D-gluconic-acid-potassium.)

composted poultry manure and green manure for OF and N, P fertilizer for CF. These values were mostly significantly higher in soils from the spring period. Indirectly, the season may affect the chemical parameters of the soil through fertilization, green manure and crop rotation (Meleora et al. 2006).

The EC, humus content, pH and the crops could be established affecting the rate of CO₂ evolution and the catabolic profiles. The metabolic respiration response is a useful method to classify the soil microbial communities. The results (Figure 1) showed that the catabolic activity of soil microbial communities was larger in OF land managements compared to CF. Gunapala and Scow (1998) got similar results in California, in loamy soil. They used mineral fertilizer for CF and green, -turkey manure for OF. Similarly to our results, Tautges et al. (2016) found that the substrate utilization was significantly higher in organic sites in wheat/pea crop rotations on OF and CF sites using EcoPlates™. Our results showed that OF and CF land managements also affect the soil substrate utilizing pattern. Glucose substrate resulted the most active catabolic activity in the spring at OF ($1.88 \pm 0.46 \mu\text{g CO}_2 - \text{C g soil}^{-1} \text{ hour}^{-1}$) however lysine was the least active substrate in the spring OF soil ($0.36 \pm 0.02 \mu\text{g CO}_2 - \text{C g soil}^{-1} \text{ hour}^{-1}$).

The MicroResp™ method is suitable for separating the soil microbes of the two different cultivated areas of Martonvásár according to their catabolic activity. PCA results (Figure 3) showed that the different farming systems were partially separated by season and cultivation. In autumn, the most responsive substrates were Mal, Cit, Glc, while in spring Glc, Fru, Mal acids and sugars. These substrates were the most responsive from the all examined substrates. Romaniuk et al. (2011) found also that, after a discriminant analysis of the catabolic response profile data, D-glucose was one of the most important substrates differentiating organic and conventional horticultural plots.

The results of our analyses proved that the organic cultivation in spring is more sustainable compared to the conventional land management. Higher soil microbial activity would be an appropriate marker of good soil quality which has got major effect on the nutrient cycling and the growth of vegetation as well (Ge et al. 2013; Creamer et al. 2016). The activity of substrate utilizing soil bacteria was influenced by the different farming systems. Our results showed that soil microbial communities have a large metabolic potential which can be easily activated by metabolisable substrates (Gunina and Kuzyakov 2015).

Conclusion

We have concluded that organic farming practice enhanced the catabolic activity of soil microorganisms. A divided pattern of catabolic activity profiles was observed by MicroResp™ according to the autumn and spring seasons and organic and conventional farming systems. The soil AL-P₂O₅, AL-K₂O and NO₃⁻-N were correlated to differences in catabolic activity profiles. Numerous environmental factors - EC, humus content, pH and the crops could also affect the generated amount of CO₂.

Acknowledgements

This research was financially supported by Research Institute of Organic Agriculture, the Hungarian Scientific Research Fund (OTKA) Grant K108572 and by János Bolyai Research Scholarship (BO/00948/15/4). Furthermore, the European Regional Development Fund and the Hungarian Government GINOP-2.3.2-15-2016-00028 and GINOP-2.3.2-15-2016-00056 have been provided financial support.

The data service of Péter Mikó and the technical assistance of Mariann Mózes were highly appreciated.

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Evolution of the absorption of heavy metals in function of nutrients

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Abstract: The condition of an eco-system greatly depends on the different biotic and abiotic factors. The area along the river “Tisza” is highly imperiled by random appearance of heavy metal pollutants originated from mining accidents or other sources. Heavy metals are dangerous because of the bioaccumulation and they could be toxic or poisonous even at low concentration. The aim of our research was to test the reaction of a simplified soil-plant pot experiment, consists of garden cress (*Lepidium sativum*) for metal pollution close to the sanitary limiting value.

Garden cress is considered one of the most important agricultural vegetables and its short reaction time for various treatments makes this plant ideal object of eco-toxicological tests. Based on the results it is realistic assumption to expect decrease the above-mentioned flexibility character. Garden cress (*Lepidium Sativum*) was chosen as a test plant to simulate the accessible pollutant uptake. Additionally, *Lepidium Sativum* is a possible carrier of heavy metals in food chain, since it is a many-sided green vegetable consumed by humans and animals as well. The present study is undertaken to examine the level of accumulation as it is modified by a plant, if the plant growing up under other conditions. It appears how for each factor as in mobilizing heavy metals, the plant laboratory water will affect the special nutrient solution or soil pollution. Responsive changes are relevant for all of the different conditions relevant for mobile heavy metals. The accumulation levels may undergo variations in function of them. In the present study, such change is characterized by the cress garden.

Keywords: heavy metals, Cd and Cu accumulation, garden cress environmental risk, heavy metals

Introduction

The heavy metal pollution is one of the leading issues of nowadays. Heavy metal pollution can cause enormous changes in the flora and fauna community decreasing of resilience capacity of the soil, although the estimation of its danger level alters according to loadability of the carrier medium.

Garden cress (*Lepidium sativum*) was chosen as a test plant to simulate the accessible pollutant uptake (Alvarenga 2008);(Herwijnen 2008). Additionally *L. sativum* is a possible carrier of heavy metals in food chain, since it is a many-sided green vegetable consumed by human beings and animals (Terbe 2000).

Nowadays alarming amount of pollution enters into the environment due to anthropogenic activities. Human health is directly endangered when these pollutants get into the food chain. Because of the environmental pollution luckily there is an increased attention on the potentially toxic elements and on the dangers related to heavy metals (Mileston 1994, Vermes

1994). Detailed and intensive examinations are imperative in order to avoid extended and extensive contaminations (Kádár 1995). Wide range of technologies are available to remediate the contaminated soils however, most of these methods are costly and non aesthetic solutions (Yoon et al. 2006).

The most polluting heavy metals include cadmium (Cd), copper (Cu), lead (Pb) and zinc (Zn), while iron (Fe) and manganese (Mn) can be contaminating in large amount (Fomes et al 2009, Filep 2002).

The metals and their compounds make a significant impact on most of the biological processes. The metals can be broken down biologically, accumulate in living organisms, toxic compounds in the subsequent biochemical reactions may occur over (Kádár and Morvai 2007; Smolinsk and Cedzyska 2007).

If large amounts of toxic materials get into the soil, the adsorption and precipitation reactions become dominant. Acidification of the soil in turn significantly increases the quantity of

mobile ions, the concentration of the metal ion in solution. Acidification is specially dangerous in already polluted areas, because the insoluble state of heavy metal compounds can cause serious environmental damage to the soil when they are mobilized (Yoon et al. 2006).

According to our knowledge the essential elements for the plants can be divided into two groups, those that are required in relatively large quantities (macro-elements H, C, N, O, Mg, P, S, K and Ca), and those which are needed only in a small amount (micronutrients: C, Cl, V, Mn, Fe, Cu, Zn and Mo). However, as the experimental techniques are getting more and more precise, the number of microelements that are important for the plants will continue to grow (Györi 1998, Luo and Rimmer 1995).

Materials and methods

Sampling

During earlier investigations it was found that those samples that had Cu and Zn concentrations exceeded the pollution limits or approached it. Into these soil samples were planted cress seeds and after 14 days the Cu and the Zn concentration were determined in the roots, stems and leaves with FAAS method. The physical parameters of the plants (e.g. root length, stem length, leaf surface) were determined in each case. In the experiment was placed 2 cm sample of the cress seeds were sowed into 50 ml beakers.

Table 1. The composition of ten times concentration of nitrogen-free Hoagland solution and content of Arnon-type solution

Composition of ten times concentration of nitrogen-free Hoagland solution			Content of Arnon-type solution	
Chemical	Concentration		Chemical	Concentration
	g dm ⁻³	mmol/dm ⁻³		
FeSO ₄ · 7 H ₂ O	0.05	0.18	H ₃ BO ₃	1.43
CaCl ₂	3.05	27.5	MnCl ₂	1.42
MgSO ₄ · 7 H ₂ O	2.46	10	CuSO ₄ · 5 H ₂ O	0.04
Na ₂ – EDTA	0.06	0.161	Na ₂ MoO ₄ · 2 H ₂ O	0.059
Arnon-type solution*			ZnSO ₄ · 7 H ₂ O	0.11
KH ₂ PO ₄ **				

* - Before filling the stock solution up to 1 cm³, 20 cm³ of Arnon-type solution was added to the flask.

** - From the potassium-dihydrogen phosphate we create a separated stock solution (111,62 dm⁻³), which was supplemented with trace elements, we only add it to the nitrogen-free Hoagland stock solution while diluting.

During the experiment, the soaked seeds were kept wet with distilled water.

The contamination happened with 0.56 mg Cu²⁺, and 1.965 mg Zn²⁺. The experiment happened in containers, that were specially designed for the rearing of plants occurred. Into the containers were sprayed 150-180 pieces of cress seeds by water. Previously these were soaked in distilled water (for 10-20 minutes) and then there were placed them on the web that layed in the containers. The cups had a 4 cm diameter and 2-3 cm thick. After the plants were contaminated, the liquid in the pot to the roots was always refilled until the net.

Besides the above mentioned container cress was planted into petri dishes, too. The contamination occurred the same level, 0.56 mg and 1.965 mg Cu²⁺ Zn²⁺ solution. Within the petri dishes 6 grams of cress was groved. This is 15-20 times more than the plants mentioned at paragraph 2. The Petri dishes had 15 cm of diameter, lined with filter paper. The amount of seeds that was spread onto the surface was 6 g. The plants were kept wet by distilled water after the pollution. They were treated for three days.

Preparation of the Hoagland solution

The medium was prepared on the day of use: 100 cm³ Hoagland stock solution was diluted to 1 dm³, while KH₂PO₄ to 5 cm³ of the stock solution was added. The pH is could be set to approximately 6 with adding 1 mol dm⁻³ NaOH (approx. 0.6 cm³).

Monitoring of soil pollution

Sampling: 50 soil and 4 control samples were collected from 0-30 cm (A) and 70-100 cm (B) depth along Szabolcsveresmart settlement, which frequently called Rétközi reservoir (located in Szabolcs – Szatmár – Bereg county, GPS coordinates: N: 48.29340° E: 22.03357°). Samples were taken according to the sampling network based on standards no. MSz 21470-50 (Msz. 2007).

After transport to the laboratory, samples were left to dry by exposing to the air. In order to remove the bigger stones and remnants of roots, the samples were sifted through a 2 mm sieve.

Dry weight content and pH determination:

Determination of dry weight content and pH were made according to standards no. MSz-08-0205-1978; MSz-08-0206/2-1987 (Msz. 1978, Msz. 1987).

Phytoextraction experiment

Phytoextraction processes with *L. sativum* in soil were conducted under laboratory condition.

General treatment of the seeds:

4 containers were filled the soil derived from Rétközi reservoir (see on Figure 1.: 17-A; 20-A; T-A; T-B). Three replicated were used in all variants. The soil type is Eutric Cambisol (WRB reference). 4 soil samples (T-1 to T4) with outstanding value of concentration (compared

to the average value) were used. The bio cross seeds, produced by BIOrganic Ltd, were scattered over the soil. Small amount of soil was sprinkled lightly over them, just to cover. A spray bottle was used to keep the seeds evenly moist. They sprouted after 2-3 days. 0.2 g (138 pieces) seeds were used for one experiment. The seedling occurred at room temperature (between 18-23 °C). At the end of 14th day, the full-grown plants were removed and carefully washed off. The redundant water was done away and every plant was separated into roots, stems and leaves. The parts of the plants were digested as it is described below.

Microwave digestion

Sample preparation:

Soil: at first samples were sifted through a 0.2 mm sieve. 0.5 g dry matter soil was taken into each teflon bomb. Then 5 cm³ 65% HNO₃ and 2 cm³ 30 % H₂O₂ were added before starting the digestion program.

Plant: The preparation of plant samples happened the same way, but different quantity of acids were added: 6 cm³ 65% HNO₃, 1 cm³ 30 % H₂O₂.

Digestion:

Samples were digested by a MILESTONE 1200 Mega Microwave Digester (see the digestion program in Table 3. – (Terbe 2000)).

Determination of heavy metal content:

After the procedure described above the

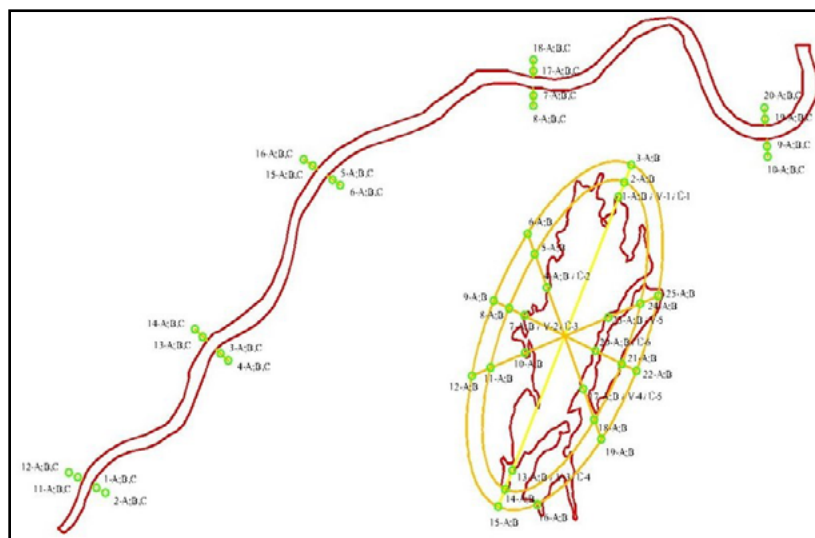


Figure 1. The “Rétközi” lake sampling points, which shows the location of the sampling grid patterns.

Table 2. Digestion program

Steps	Time [min]	Process [Watt]
	Soil / Plant	Soil / Plant
1.	5 / 2	Digesting, 250 / 250
2.	2 / 2	Aeration
3.	5 / 6	Digesting, 400 / 250
4.	5 / 5	Digesting, 250 / 400
5.	7 / 5	Digesting, 700 / 600
6.	5 / 5	Aeration

following elements were measured by ICP-OES and FAAS: Al, Ca, Cr, Cu, Fe, Ni, Mg, Mn, Pb, Zn in soil and Zn, Cu in garden cress.

Monitoring of the soil pollution

It became clear after completing the variance analysis (ANOVA one-way test) that the heavy metal content of the soil samples significantly depends on the place of sampling. Only one soil sample's Cu concentration (sample id.: 22-A, see on Figure 1.) $84,8 \text{ mg kg}^{-1}$ was higher than the limit value (75 mg kg^{-1}) based on the Governmental Regulation 6/2009. (IV. 17.) KVVMEÜM-FVM. In the case of other measured elements neither of them exceed the limit values after summarizing all the results. These results will be used as reference base of a long term monitoring system. The reason 22-A was not taken into account is that the seed sprouting was inhibited at that degree of pollution causing the insufficient quantity of plant dry matter for the analysis.

Other parameters

Soil tests should be taken into properties of soils, because the soil plays an important role in the biosphere of the transformation of materials, environmental protection (Györi 1998). Therefore, it is necessary to identify and take account of the different properties of the

soil. These parameters were performed (Table 2) on the basis of Györi (1998).

Phytoextraction experiment

It has to be taken into consideration, that soil parameters have notable effect on these experiments: metals, heavy metals, cations are not so mobile in carbonaceous soil (Kádár and Morvai 2007). The transfer coefficient (T %) was calculated, too (Formes et al. 2009). Although the organic matter content is determinant regarding the heavy metal concentration of the soil solution, straight correlation cannot be demonstrated by adsorptional experiments. The reason for this is pH affects the creation of metalorganic complexes and there is a strict correlation between pH and the degree of absorption. Another important factor beside this is that the structural diversity of the mould material, which is not mapped completely yet, cannot be regarded as a uniform material. Their absorption and cation-exchanging characteristics are different and depend on pH (Luo and Rimmer 1995).

It can be concluded that in almost every case the heavy metal concentration in the roots were significantly higher than in the other plant parts (see on Figures 2,3). It is favorable for the consumers because heavy metals accumulate only in a small proportion in leaves and stalks of the garden cress.

ATI UNICAM 939 AAS was used for determination of heavy metal concentration.

Results and Discussion

Evaluating the CU concentration

The soil samples for the enrichment of heavy

Table 3. Physical and physico-chemical characteristic and composition (dry weight basis) of studied soils

	The soil*					Concentrations (mg/kg in dry matter)			
	T-1	T-2	T-3	T-4		T-1	T-2	T-3	T-4
SP* (K_A)	30	28	55	44	Zn	165.6	32.2	189.9	42.3
CaCO ₃ (%)	1	1	1	1	Cd	23.0	10.8	41.4	14.7
Humus (%)	2.2	2.3	2.3	2.3	Cr	2.8	4.0	0.9	3.6
C/N ratio	16.7	12.4	15.0	14.4	Al	1630.7	3519.3	1031.5	2411.2
pH (H ₂ O)	5.70	6.20	7.12	5.97	Pb	3.4	2.0	1.4	1.6
pH (KCl)	5.69	6.19	7.13	5.89	Ni	1.9	2.2	0.6	1.9
DM* (%)	98	99	99	97					

* Values of the polluted sediment sample in the „Tisza” (T-1 to T-4);

SP = soil plasticity (K_A). DM = dry matter content

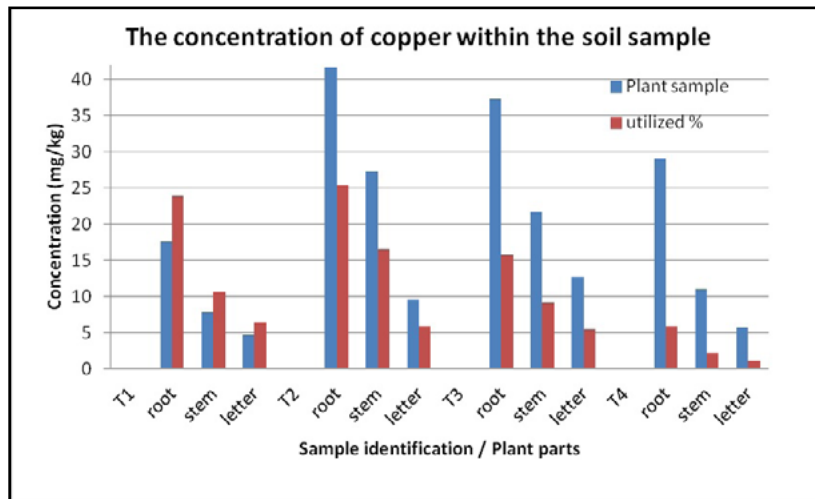


Figure 2. The concentration of copper in the soil pattern increased in plant.

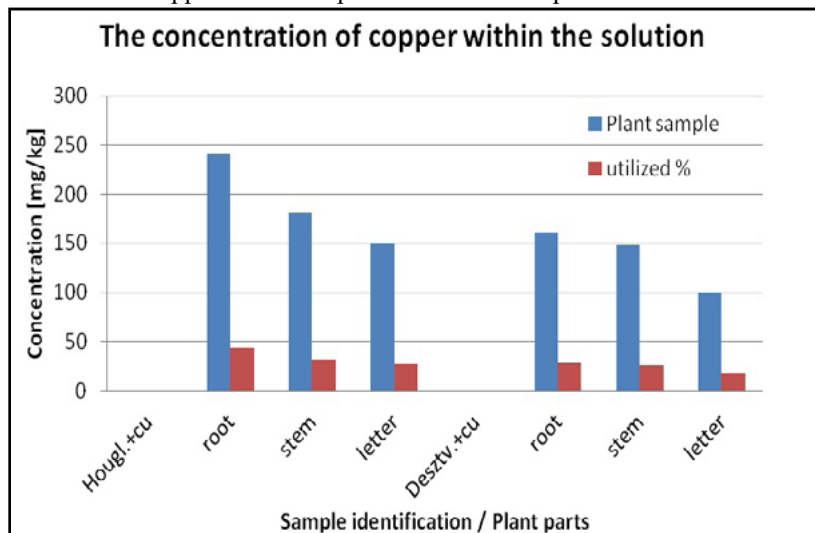


Figure 3. The concentration of copper in the solution pattern increased in plant.

metals in the accumulation of plants 20 mg / kg concentration limit varies. The higher the C / N ratio of soil, and the acid-base sensitivity of the soils neutral pH (5.6) produces the utilization value of around 25%. Declining C / N ratio and the effect of increased metal concentrations to around 5% decrease in utilization. The parts of the plant does not determine the extent of the accumulation. The root part of the largest accumulation, while the smallest part of the letter.

The liquid phase accumulation of heavy metals in plant samples grown Cu concentration ratio of deficit to alter the accumulation of plant parts. The parts of the plant does not determine the extent of the accumulation. The root portion of the maximum accumulation, but is similar to the degree of drying sections. The soil grown

plants stems the accumulation of Cu accumulates at higher rates.

The medium plant samples grown heavy metal accumulation does not alter the rate of accumulation of deficits Zn concentration in plant parts. The parts of the plant does not determine the extent of the accumulation. The same plants grown in soil accumulation rate takes place. The utilization rate minimal however, shows a utilization of around 1%. The pollution-free medium, this value of high C / N and the neutral pH plants grown in soil samples is identical.

Evaluating the Zn concentration

The soil samples for the enrichment of heavy metals in the accumulation of plants 10 mg / kg concentration limit varies. The higher the C/N

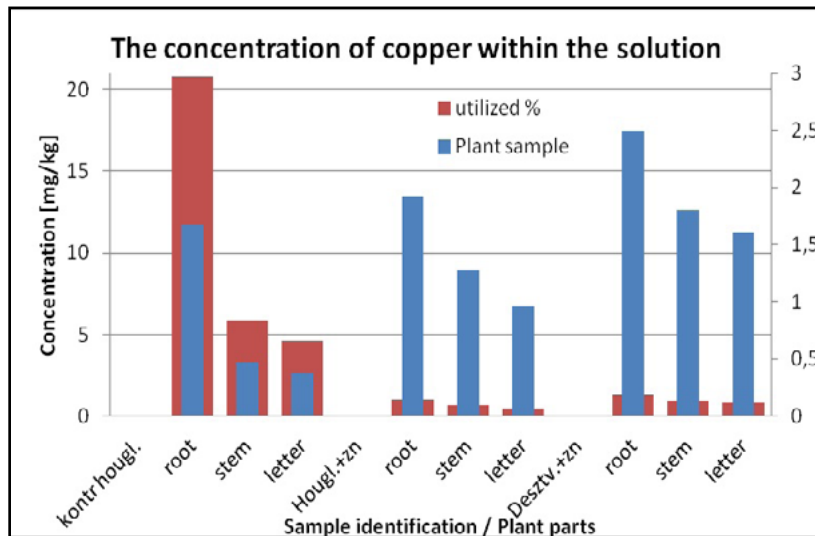


Figure 4. The utilize percentage of copper in the solution

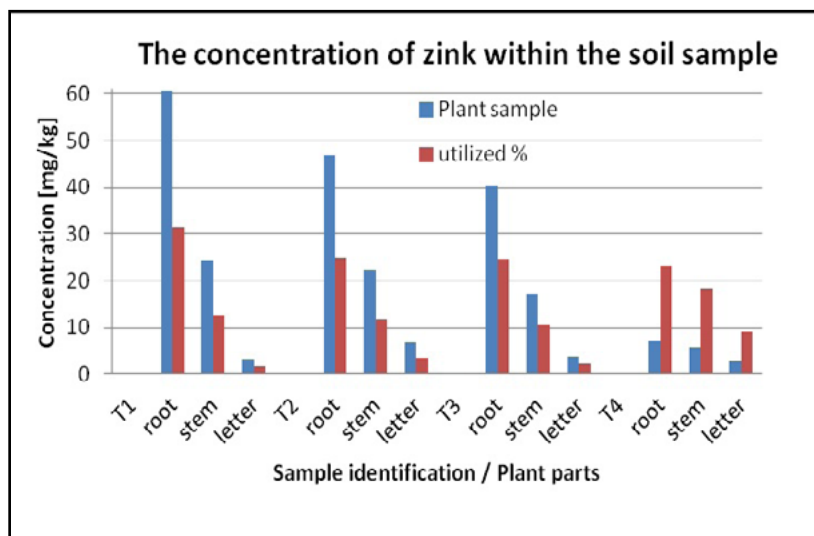


Figure 5. The concentration of zink within the soil sample

ratio of soil, and the acid-base sensitivity of the soils neutral pH (5.6) produces a value of around 32% utilization. Declining C / N ratio and the effect of increasing the metal concentration decreases utilization at around 21%. The parts of the plant does not determine the extent of the accumulation. The root part of the largest accumulation, while the smallest part of the letter.

The medium plant samples grown changing the ratio of Cu concentration of heavy metal accumulation in plant parts into deficit accumulation. The extent of the parts of the plant concentrates the accumulation of plant root and stem area. The utilization ratio of the control sample shows a high utilization of 20%. Contrary, the utilization is less than 5% of the

value of the distilled water and the contaminated medium.

The liquid phase plant samples grown heavy metal accumulation rate of accumulation of deficits Zn concentration in plant parts shifts. The extent of the parts of the plant accumulation shifts in the utilization rate of the same proportion. The root and stem part of the largest accumulation, but the minimum leaf sections. Accumulation in soil grown plants showed only a result of the control medium

Conclusions

The medium and the biological functions of plants grown in soil do not differ from each other.

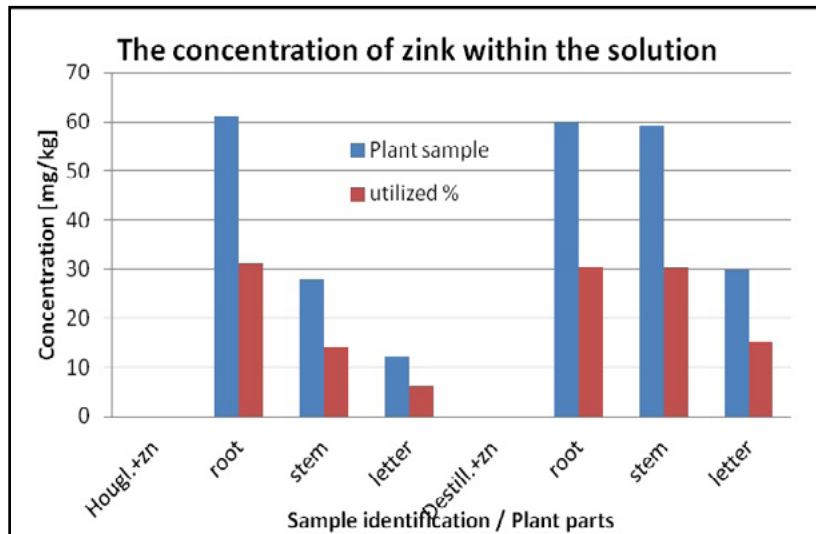


Figure 6. The concentration of zink within the solution

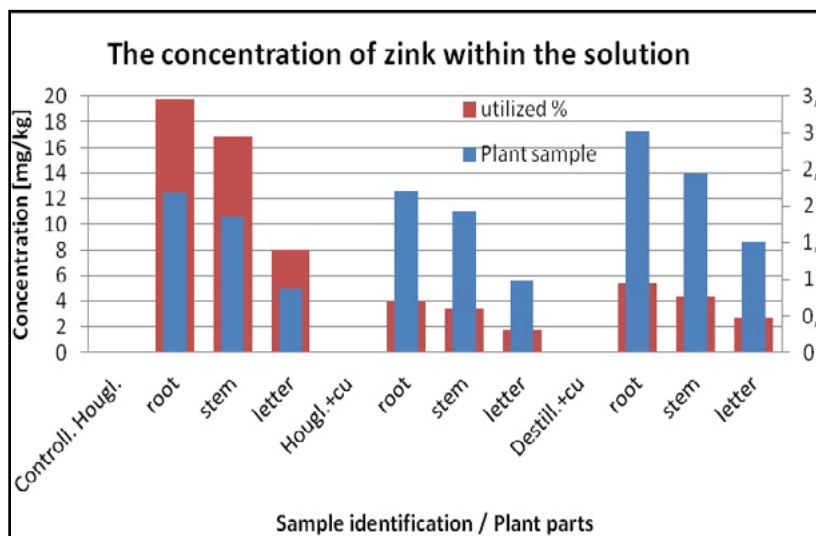


Figure 7. The utilize percentage of zink in the solution

The plants grown in different medium heavy metal contamination in soil more sensitive to the pest picture. The nutrient composition of the plants can positively influence the accumulation ability. The plant parts may be increased from the root and shank portions of the rate of accumulation and uptake rate of the contamination. In contrast to soil, the plant nutrient solution less sensitive to degree of contamination. Seedlings thus a higher ability to remove impurities of the contaminated medium. This investigation is in accordance with other authors's results of garden cress test, which show the highest accumulation

were happened in the root (Smolinks 2007). The test area can also specify the location of the akumulation for determining cress, but the concentration of pollutants. Case of both the heavy metal concentration results in a higher accumulation of the root.

The case of minor concentrations of the same extent in all parts of the plant. Since it is proved the garden cress was able to absorb and translocate heavy metals, it creates an opportunity to apply it in fitoremediation processes. The heavy metal removal from the ecosystem via melioration in all case increases its resilience.

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Feeding preference of three Collembola species on two plant residues

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Abstract: This study formed the part of an extended climate change experiment that focuses on the ways climate change may affect the species composition and the ecological processes of vulnerable open sand grasslands. The project is conducted in a natural shrubland-grassland ecosystem in the Kiskunság National Park. This is an arid area, and the members of macrofauna are almost completely lacking from these soils. Collembola presumably plays an important role in the direct decomposition processes, beyond the indirect regulation of the microbiota. Previously, we found a higher decomposition rate for grass leaves as compared to grass roots and faster decomposition of one dominant grass species (*Festuca vaginata*) over the other (*Stipa borysthenica*) in this experimental system.

In the present study, we investigated in a laboratory experiment, whether higher decomposition rate may be related to food preference of potential decomposer species in the system, and posed the following questions: (i) is there a preference in the food choice of three springtails species (*Folsomia fimetaria*, *F. candida*, *Sinella magyari*) between the roots and leaves of the two dominant plant species (*Stipa borysthenica*, *Festuca vaginata*) and between the roots and leaves of the same species? (ii) can the C/N ratio and lignin content of plants as background variable explain such preference?

Significant differences were found in the food preference of all Collembola species between the two parts of the plants and in some case between the two plant species. The leaves and *Festuca vaginata* were more preferred food type as compared to roots and *Stipa borysthenica*. The chemical composition of the plant parts well explained the observed patterns, especially the N and lignin content of the leaves and roots.

Keywords: springtails, food preference, decomposition, fecal pellet count, *Folsomia candida*

Introduction

Dead organic matter (DOM) decomposition is of key importance in the nutrient cycle of grasslands. Changes in the DOM content of soils as a result of climate change is a priority research area as climate, particularly soil temperature and humidity, are important factors of the decomposition rate (Kirschbaum 1995; van Meeteren et al. 2008; Smith 2012). Besides, the quality, i.e. the chemical composition of the organic residuum entering into the soil is also a crucial parameter of the decomposition (Swift et al. 1979; Aerts 1997; Almagro and Martinez-Mena 2012). The third determining factor of the decomposition is the qualitative composition and the quantity of

organisms involved in the process (soil animals, microbes). Soil fauna plays a well-known role in the fragmentation of plant parts, making the organic matter accessible to microbes involved in chemical decomposition. Members of the macrofauna (annelids, woodlice and diplopods) take the main role in this process (Slade and Riutta 2012; Pant et al. 2017). In extremely dry habitats such as open sand grasslands, the majority of macrofauna is absent. According to literature data and field experience (Lawrence 2000; Filser 2002), it is assumed that springtails are not only indirectly involved in decomposing processes by controlling populations of microorganisms (bacteria, fungi) in this habitat but also directly, by consuming plant parts.

Most Collembola species have a wide food spectrum. They may consume, among others, different mosses, plant parts, dead organic matter, fungal spores or hyphae, bacteria, nematodes, alga cells and lichens (Anderson and Healey 1972; Bakonyi et al. 1994; Bakonyi 1998; Gilmore and Potter 1993). Moreover, they may play an important role in the dissemination of saprophytic and mycorrhizal fungi found in soil (Seres et al. 2007, 2009). Several studies have found that these microarthropods are able to choose between the foods provided on a fine scale and select their diet according to their needs, avoiding foods that are of insufficient quality (Bakonyi et al. 2006).

Study investigating the structure of springtail communities in the study area (Kiskunság National Park, central Hungary) found one species, *Entomobrya nigriventris*, with strong dominance in the community (Flórián et al. 2016). This species has very specific needs, so it is not easy to keep it in laboratory cultures. However, the same study detected the presence of *Folsomia candida*, a species easily kept under laboratory conditions, in the area.

Present study formed the part of an extended climate change experiment that focuses on the ways climate change may affect the species composition and the ecological processes of vulnerable open sand grassland. This experiment is conducted in a natural shrubland-grassland ecosystem in the Kiskunság National Park, central Hungary (N 46.871°, E 19.421°). Previously, we examined the decomposition of organic matter in this sand grassland through different methods (Seres et al. 2015). An important aspect of the study was that both plant species and plant parts had a significant effect on the rate of decomposition. A higher weight loss was detected in the case of *Festuca vaginata* than *Stipa borysthenica* and the leaves than the roots of the dominate grasses. This implies that the chemical quality of the plant strongly affects the rate of decomposition. The present study was designed in the light of the above results.

The questions were as follows: (i) is there a preference in the food choice of three springtail

species (*Folsomia fimetaria*, *F. candida*, *Sinella magyari*) between the roots and leaves of two dominant plant species (*Stipa borysthenica*, *Festuca vaginata*) and between the roots and leaves of the same species? (ii) can the C/N ratio and lignin content of plants as background variable explain such preference?

Methods

Folsomia fimetaria (Linnaeus, 1758), *Folsomia candida* Willem 1902 and *Sinella magyari* (Chen, 2002) individuals from the Collembola culture of the Department of Zoology and Animal Ecology of Szent István University (SZIE) were used. The animals are kept in the dark in a climate chamber at 20 ± 1 °C, on the surface of moistered plaster of Paris mixed with charcoal, and are fed on dried yeast. The animals were tested individually in Petri dishes of 3 centimetres in diameter. A wet filter paper was placed in each Petri dish. A previously printed figure (Figure 1) was placed on top of the filter paper and two types of food were placed at the centre of the circles. The animals were kept at a constant temperature of 20 ± 1 °C in the laboratory thermostat and the experiment was terminated after 10 days. The study was performed using the following combinations: 1. *Stipa borysthenica* leaf vs. *Stipa*

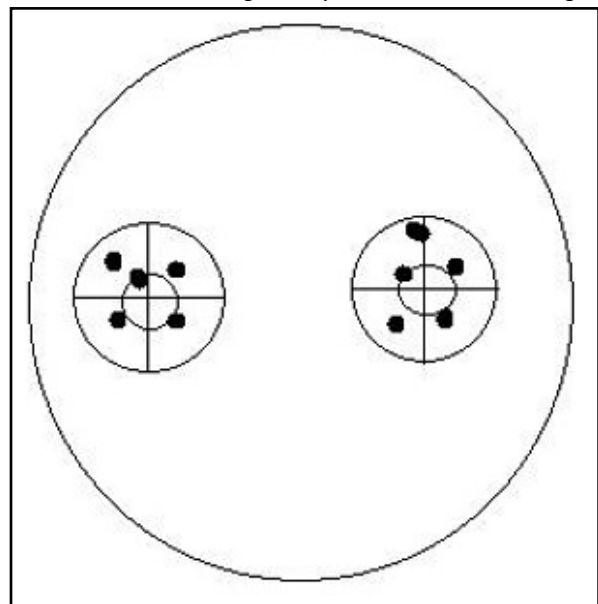


Figure 1. Experimental design. Outer circle: Petri dish of 3 cm. Food was placed at the centre of smaller circles and fecal pellets were counted within the larger ones. Black dots symbolises fecal pellets.

borythenica root, 2. *Festuca vaginata* leaf vs. *Festuca vaginata* root, 3. *Stipa borysthenica* leaf vs. *Festuca vaginata* leaf, 4. *Stipa borysthenica* root vs. *Festuca vaginata* root. The number of replicates was 20 to 30 with each Collembola species, using four treatments. The number of fecal pellets within the larger circle around the food was counted after 10 days of incubation. As the metabolism of springtails is high, they defecate where they feed; therefore, the number of fecal pellets in the direct vicinity of food sources may be a measure of the food (Bakonyi et al. 2006).

The C, N and lignin content of *Stipa borysthenica* and *Festuca vaginata* leaves and roots was determined. The analysis of C, N content was performed by the Department of Chemistry of SZIE, using a Carlo-Erba NA 1500 elemental analyser, while the analysis of lignin content took place at the Department of Animal Nutrition of SZIE, according to the method developed by van Soest (van Soest 1963).

Statistical analyses were performed using the R software package (R Core Team 2013); after checking assumptions for use of analysis, the C, N, and lignin content data of plants was analysed by one-way ANOVA followed by Tukey's post-hoc test. After confirming that the data distribution is normal, GLM was used with fix and random factors, with dependent variables being the number of fecal pellets. Fix factor was the plant type, while the number of Petri-dishes was used as random factor.

Results

Statistical analyses clearly found significant differences between plant parts in C/N ratio and lignin content (Figure 2). In terms of food preference, the two most important parameters seem to follow this pattern. The C/N ratio of plant parts increased in the following order: *Festuca* leaf, *Stipa* leaf, *Festuca* root, *Stipa* root (Figure 2A). The same order was observed for the increase of lignin content, which is a hardly digestible component in plant parts (Figure 2B). The C and N content were the following in the *Festuca* leaf, *Festuca* root, *Stipa* leaf and *Stipa* roots, respectively. The ANOVA (F-value: 39 and 328) found significant difference in these values. The results of post hoc comparison are in brackets. The C content: 45.19±0.71 (bc), 44.06±0.34 (b), 46.71±0.11 (c), 40.51±171 (a). The N content: 1.20±0.03 (c), 1.04±0.04 (b), 1.13±0.04 (c), 0.57±0.02 (a).

Significant differences were found in some cases in the food preference of Collembola between the two plant parts and the two species. Results show clearly that *F. fimetaria* had a preferred food in each combination (Figure 3A), *F. candida* (Figure 3B) and *S. magyari* (Figure 3C) showed preference in two and three out of four cases, respectively. *F. fimetaria* eat more on the leaves of both species than the roots, and preferred both the leaves and roots of *F. vaginata* rather than *S. borysthenica* (Figure 3A). *F. candida* did not select between the two grass species. The

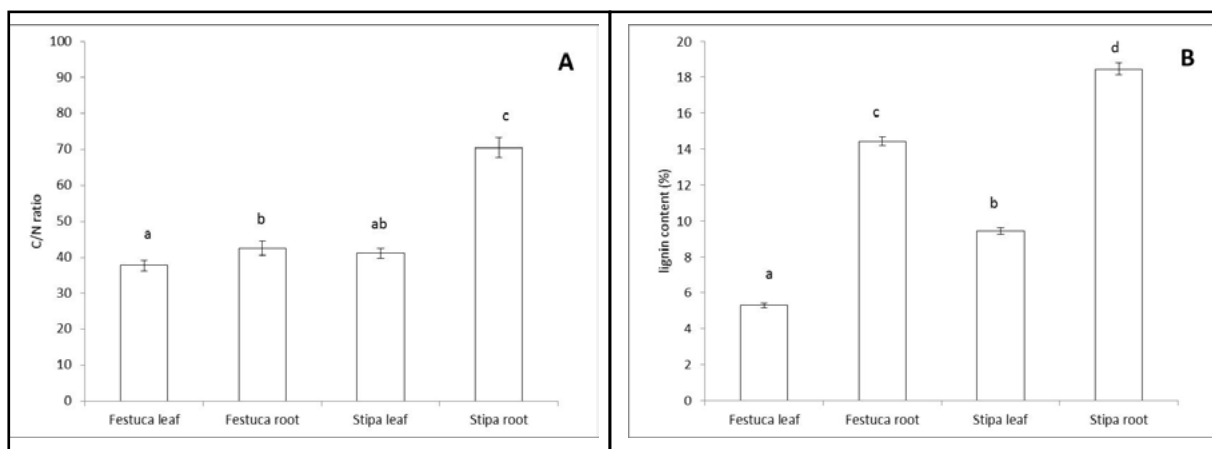


Figure 2. The C/N ratio (A) and lignin content (B) of plant parts provided during preference tests. Different letters show statistically confirmed differences for a given variable (Tukey's test, $p < 0.05$).

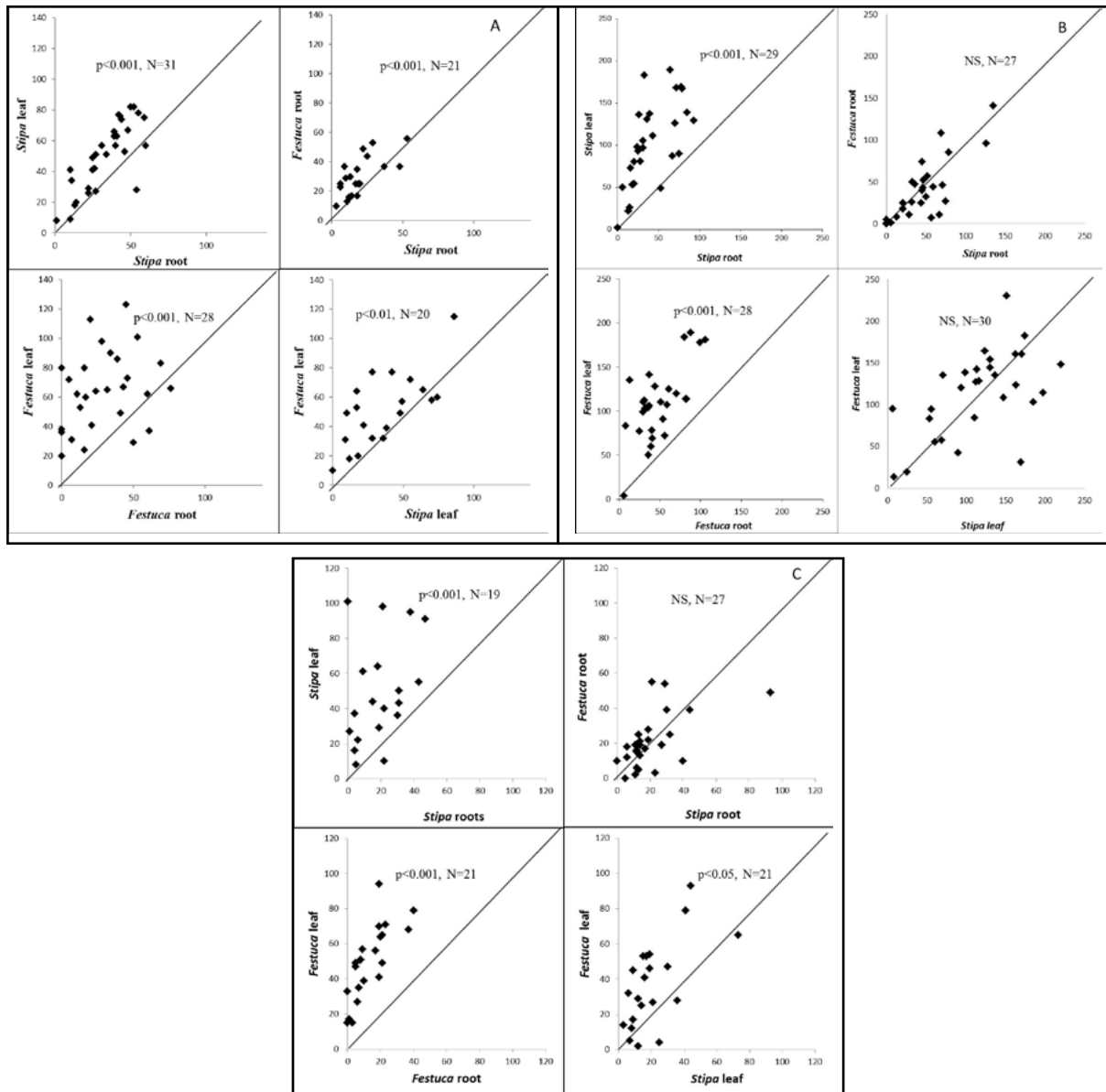


Figure 3. Number of fecal pellets near the two different food sources and results of the linear model ($p < 0.05$:*, $p < 0.01$:**, $p < 0.001$:***). One point means the data of one individually kept Collembola. A: *Folsomia fimetaria*, B: *Folsomia candida*, C: *Sinella magyari*.

preference of leaves was clear in the case of both species (Figure 3B). *S. magyari* had also great preference for the leaves of the grasses, rather than roots. They eat more on the leaves of *F. vaginata* than the leaves of *S. borysthenica*. But we did not find preference between the roots of the two grass species, in the case of *S. magyari*. The leaves (from the two plant parts) and *F. vaginata* (from the two plant species) were the more preferred food types, in that case where preference occurred.

Discussion

The results of the present food preference tests provide an adequate explanation to the findings of previous field studies (Seres et al. 2015) as a preference for grass species or plant parts with a higher rate of decomposition was clearly demonstrated. The food preference of springtails was driven in our study by two factors: the lignin content and the C/N ratio of food. According to Larsen et al. (2008), Collembola usually choose

foods with a higher N content and a lower C/N ratio. This pattern was evident in the present case. Collembola did not choose the food with the lowest N content (*Stipa* root) at all, while the food with the highest N content (*Festuca* leaf) was almost always preferred over the others. The other important aspect of food preference is the lowest possible amount of substances that are hard to digest (Rincon and Martinez 2006). As the content of lignin, a hardly digestible substance, increased in the same order in plant matter as N content decreased, the phenomenon described above stands for this component as well.

Springtails could be divided into different feeding guilds, but most of them feed on fungi in the soil (Berg et al. 2004). However, it has become well known that the composition of their food may be extremely diverse. An increasing number of studies have found in the past years that these animals consume dead plant matter and living plants as well (Malcika et al. 2017). This is confirmed also by the present study, although it should be noted that no alternative food was

available to animals in this experimental setting. According to N isotope studies by Chahartaghi et al. (2005), these animals cannot be classified into any specific feeding guild as they are able to switch to other food in the case of changes in food availability.

The Collembola species involved in this study avoided foods with high lignin (and low N) content under laboratory conditions, supporting field findings (Seres et al. 2015). Based on the above, it is assumed that the similar patterns seen under laboratory and field conditions are caused by the fact that springtails are directly involved in the decomposition of organic matter in this extremely dry habitat. The monitoring of changes in springtail populations is therefore particularly important in terms of the nutrient cycle of protected sand grasslands in Hungary.

Acknowledgment

This study was supported by the ÚNKP-17-4 New National Excellence Program of the Ministry of Human Capacities.

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Response of winter wheat to arbuscular mycorrhizal fungal inoculation under farm conditions

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Abstract: The effect of arbuscular mycorrhizal (AM) fungi inoculation was investigated on two winter wheat cultivars (*Triticum aestivum* var. Mv Nádor and var. Genius) grown under farm conditions in the neighbourhood of Nagyhörcsök in 2016. The soil was a chernozem with lime deposits (WBR classification: Calcic chernozem) with a mean humus content of 2.73%, AL-soluble P₂O₅ and K₂O concentrations of 181 mg kg⁻¹ and 149 mg kg⁻¹ and a pH_(KCl) value of 7.27. The AM inoculum contained reproductive units of *Rhizophagus irregularis* (previously *Glomus intraradices*) and *Glomus mosseae* (syn. *Funneliformis mosseae*). In addition to soil inoculation, some of the treatments were also given mineral fertiliser treatment (130 kg N ha⁻¹, 78 kg P₂O₅ ha⁻¹, 60 kg K₂O ha⁻¹).

Both AM inoculation and mineral fertiliser treatment were found to have a significant effect on the yield (at the p<0.05 level). The yield of plots with mycorrhizal inoculation averaged 8.17 t ha⁻¹, which was higher than that of non-inoculated plots (7.52 t ha⁻¹), while the yield of plots with fertiliser treatment averaged 8.31 t ha⁻¹, as compared with 7.38 t ha⁻¹ for non-fertilised plots. The yield-enhancing effect of AM inoculation was only manifested in plots given no mineral fertiliser. Plant protection measures were the same in all the treatments.

The results and the conclusions drawn from them were based on the data of a single year (2016). Data from experiments performed in several years with more cultivars and soils with diverse properties will be required to obtain better grounded, more reliable recommendations for farmers.

Keywords: AM fungi inoculation, *Rhizophagus irregularis*, *Glomus mosseae*, winter wheat, grain yield

Introduction

Arbuscular mycorrhizal (AM) fungi are beneficial microbes, ubiquitous in natural and agricultural ecosystems (Pellegrino et al. 2015). Under natural conditions the AM fungi living in symbiosis with plant roots make a substantial contribution to the nutrient (P, N, S, K, Ca, Fe, Cu, Zn) and water uptake of the plants (Smith and Read 2008). Phosphate ions are almost insoluble in soil because of interactions with soil cations, and are very poorly mobile. The traditional model of mycorrhizal function is based on the exchange of phosphate and carbon between plant and fungus (Fitter et al. 2011). In addition to this direct effect, AM fungi also have indirect effects, including the amelioration of the soil structure (Rillig and Mummey 2006; Miller and Jastrow 2010), interactions with other soil-borne microorganisms (Artursson et al. 2006; Veresoglou et al. 2016) and protection against plant pathogens (Hooker et al. 1994; Azcón-Aguilar and Barea 1996). Under intensive

agricultural production conditions AM fungi are of much less significance than in natural conditions. The reduction in the number of plant species found on a given area (e.g. wheat-maize rotation) (Sasvári 2017), the regular disturbance of the soil (Kabir, 2004), the use of mineral fertiliser (Kahiluoto et al. 2001) and the application of fungicides (Jin et al. 2013) all lead to a decline in the number and activity of AM fungi. On areas constantly used for agricultural production the number of AM fungal propagules in the topsoil (0-30 cm) is greatly reduced (Oehl et al. 2005; Posta 2013; Gottshall 2017).

With increasing soil depth, a decrease was found in the percentage of roots colonized by AM fungi, in the number of infective propagules and in the amount of extraradical AM fungi hyphae, but the reducing effect of agronomic practices could not be demonstrated. More remarkably, the AM fungi community composition changed towards deeper soil layers and a surprisingly high species richness was observed even in the deepest soil layers (50-70 cm) examined (Oehl et al. 2005).

Crop plants differ in the extent of their dependence on mycorrhizae for nutrient uptake (Smith and Read 2008). The mycorrhizal dependence of different cultivars of a given species may also be different. Azcon and Ocampo (1981) observed a wide range of dependence on mycorrhizas in experiments with 13 wheat cultivars. Hetrick et al. (1996) investigated the mycorrhiza dependence of ten wheat cultivars, six of which responded positively, while four responded negatively or were nonresponsive to mycorrhizal inoculation. The responses of the individual cultivars were consistent regardless of the inoculum source, suggesting that mycorrhizal responsiveness is an inherited trait rather than a response to individual fungi. Mycorrhizal responsiveness decreased with P fertilisation for cultivars that were dependent on the symbiosis, but it was unaffected by P fertilisation in cultivars that were negatively impacted by the mycorrhizae (Hetrick et al. 1996).

Hetrick et al. (1993) investigated modern wheat cultivars and their ancestors, and suggested that modern breeding practices had reduced dependence on mycorrhizal symbiosis. In contrast Lehman et al. (2012) found no evidence that new crop genotypes lost their ability to respond to mycorrhiza due to agricultural and breeding practices.

The advantages of AM fungi are obvious to crop producers, so the possibility of inoculating soils with these fungi has long been the subject of research. Inoculation with arbuscular mycorrhizal fungi is considered to be a sustainable crop production technology (Azcon and Ocampo 1981).

A number of reviews and meta-analyses have been published, most of which report on the yield increases demonstrated in field crops such as maize and winter wheat (Lekberg and Koide 2005; Hoeksema et al. 2010; Lehmann et al. 2012; Treseder 2013; Pellegrino et al. 2015). Based on these results, the effect of AM fungal inoculation depends on the available nutrient content of the soil, the type of cultivation, the fertilisers applied, the use of plant protection agents (particularly fungicides) and the weather.

The relationship between the available phosphorus (P) content of the soil and the level of mycorrhization has been examined in depth (Hetrick et al. 1996; Lekberg and Koide, 2005; Hoeksema et al. 2010; Treseder 2013; Suriyagoda et al. 2014). It is generally accepted that low available P content in the soil facilitates the development of mycorrhiza while high available P content inhibits it. A few researchers such as Hoeksema et al. (2010) stated that the N fertilisation was a more important predictor of plant responses to AM fungal inoculation than the P content in the soil. Hoeksema et al. (2010) also found that very few studies reported the available soil N and P concentrations or values for other important abiotic factors, such as ambient light or soil water availability. AM fungal communities are also influenced by climatic factors and the success of symbiosis depends on water availability and the temperature during early plant development (Augé 2001).

Most authors of reviews and meta-analyses (Hetrick et al. 1993; Lekberg and Koide 2005; Lehmann et al. 2012; Treseder 2013; Pellegrino et al. 2015) observed the yield-increasing effect of AM fungi. Pellegrino et al. (2015) conducted a well-documented meta-analysis of 38 field trials published between 1975 and 2013, involving a total 333 data, and reported that AM fungal inoculation led to a mean yield increase of 20%. However, the transferral of these results is complicated by the fact that the crop production was extensive (low-input), the grain yields were low (2-5 t ha⁻¹) in most experiments, and only three of the locations tested were in Europe (which means that the climates were different). This is true of all the meta-analyses published in this field.

The size of the experiment is also an important factor. Individual studies have shown that the effects of mycorrhizal fungi on plants are different in the field than in greenhouse or growth chamber experiments (Hoeksema et al. 2010). More specifically, Lekberg and Koide (2005) found that the beneficial effects of AM fungi on plants were smaller in field experiments than in greenhouse or growth

chamber experiments. Despite their scientific importance, field experiments with small plot size may lack agronomic relevance.

The aim of the present work was to determine whether AM fungal inoculation resulted in a grain yield increase in two cultivars with high grain yield potential of winter wheat (*Triticum aestivum*) (Mv Nádor and Genius) under field conditions on 1 ha plots with fertile soil (calcic chernozem) using the intensive crop production technology normally applied in Hungary.

Materials and Methods

The field experiment was set up on calcic chernozem soil in the neighbourhood of Nagyhorcsök, Hungary. The soil characteristics were as follows: upper level of plasticity according to Arany 43, mean humus content 2.73%, AL-soluble K_2O and P_2O_5 149 mg kg^{-1} and 181 mg kg^{-1} , respectively, $CaCO_3$ 5.05%, $pH_{(KCl)}$ 7.27. The soil analysis was performed in the Soil Protection Laboratory, Velence. The preceding crops on the experimental area were sunflower (2015), maize (2014) and pea (2013).

The climate is continental, with an annual mean temperature of 11 °C and annual mean precipitation of 590 mm. The weather conditions in 2016 were ideal for wheat production.

The AM fungi inoculant used in the experiment was the Aegis Sym Irriga microgranulate manufactured by Italtollina. This contains several AM fungi, principally the *Rhizophagus irregularis* (previously *Glomus intraradices*) and *Glomus mosseae* (syn *Funneliformis mosseae*) species. The inoculant has a concentration of 1,400 spores g^{-1} and the recommended dose is 1–2 kg ha^{-1} (internet 1).

Two winter wheat cultivars were used in the experiment, Mv Nádor and Genius. Mv Nádor (MTA ATK Martonvásár), an early–midseason cultivar, was state registered in 2012. It has a potential yield of 10–11 t ha^{-1} , low plant height (60–80 cm), excellent winter hardiness and good flour quality. Genius (Saaten-Union) has a high potential yield in case of extensive and intensive conditions alike. This cultivar has good

frost resistance, winter hardiness and drought tolerance. It has premium milling quality. No data are available on the mycorrhiza susceptibility of either cultivar.

Four treatments (mineral fertiliser + AM fungi inoculant, mineral fertiliser alone, AM fungi inoculant alone, no fertiliser or inoculant) were applied in three replications to both wheat cultivars. The mineral fertiliser dose was 130 kg ha^{-1} N, 78 kg ha^{-1} P_2O_5 and 60 kg ha^{-1} K_2O and the plot size 10,000 m^2 (40×250 m), arranged in a strip-split-plot design. All the plots were given the soil preparation and plant protection normal under farm conditions.

The sunflower crop in the previous year was harvested on 31 Aug. 2015, followed on 1 Sept. by disking and on 14 Sept. by the application of basic potassium fertiliser to the fertilised plots. The seedbed was prepared using a seedbed cultivator on 20 Oct. The AM fungi inoculant was applied on 21 Oct. as recommended by the manufacturer, using plant protection machinery, where the spraying device followed a short disk fitted with a bladed cylinder at a maximum distance of 20 cm. The sowing took place on 26 Oct. The seed was sown within 7 days of inoculation, according to the recommendations of the manufacturer. Ammonium nitrate (34%) was applied to the fertilised plots on 3 Nov. and Nitrosol (30%) on 22 Nov.

Two plant protection treatments were performed. The first, on 5 Apr., consisted of a 0.75 l ha^{-1} dose of the fungicide FalconPro (active ingredients: spiroxamine, tebuconazole and prothioconazole) and a 0.15 l ha^{-1} dose of the herbicide Sekator OD (active ingredients: amidosulfuron and iodosulfuron). On 9 May a 2 l ha^{-1} dose of the fungicide Cherokee (active ingredients: cyproconazole, propiconazole and chlortalonyl) was applied.

Samples were taken with a plot combine at harvest on 12 July 2016. The grain crop were dried to 14% water content.

Root samples were taken at a depth of 5–20 cm, washed, and stored in 0.05% lactoglycerol solution at 4°C. The root colonisation of the AM

fungi was checked under a light microscope after staining the roots with Trypan Blue. The ratio of mycorrhizal roots were measured by the grid line intersection method and were expressed in mycorrhization % (Brundrett 2008).

The statistical evaluation was performed using a paired t-test and multi-factor analysis of variance (ANOVA). Differences were considered to be significant at the $p < 0.05$ level. The differences between the samples means were compared with the value of the LSD with a 95% confidence interval.

Results

The effects of the treatments on the grain yield and mycorrhization of wheat roots were evaluated using analysis of variance for three factors (AM inoculation, mineral fertilisation, wheat cultivar). The grain yields averaged over the factors are given in *Table 1* together with the significance levels of factors. All three factors had a significant effect on the grain yield and (with one exception) interactions between the factors were also significant. The one exception was the interaction between cultivar and AM inoculation.

Mineral fertilisation increased the grain yield from 7.38 to 8.31 t ha⁻¹. AMF inoculation in the same way increased the yield from 7.52 to 8.17 t ha⁻¹. As the interactions between the factors were also significant, the yield averages were also plotted for each treatment (*Table 2*). The two cultivars had different reactions to AM inoculation. The AM inoculation in both cases increased the yield but in a different measure.

Yield of Mv Nádor increased from 6.96 to 8.55 t ha⁻¹, yield of Genius changed from 6.72 to 7.28 t ha⁻¹, the increasing was 123% and 108%, respectively. The reaction to mineral fertilization were also different in case of cultivars. Mineral fertilization increased the yield of Mv Nádor with 132%, while increased the yield of Genius with 108%.

The three factors and their interactions had also significant effects on the mycorrhization of winter wheat roots (*Table 3*). The fertilisation decreased, the AMF inoculation increased the percentages of mycorrhizal roots. The wheat cultivars had also significant effect, Mv Nádor had higher mycorrhization (14.6%) compared to Genius (4.3%).

Discussion

The results of the large (1 ha) plot experiment carried out under farm conditions showed that inoculation with arbuscular mycorrhizal fungi had a detectable yield-enhancing effect on two different cultivar (Mv Nádor, Genius) of winter wheat (*Triticum aestivum*).

Both the winter wheat cultivars tested (Mv Nádor, Genius) are modern, high-yielding cultivars (10–12 t ha⁻¹) recommended for cultivation under intensive conditions. The size of the yield increment, in case of cultivar Mv Nádor, was similar to the ~20% value given in the literature (Pellegrino et al. 2015). The higher yield achieved in response to inoculation with arbuscular mycorrhizal fungi confirms the results of Lehmann et al. (2012) and suggest that

Table 1. Yield averages of winter wheat for each factor (wheat cultivar, mineral fertilisation, AM inoculation). N= 12 (number of replications).

Factor	Yield average (kg ha ⁻¹)		Level of significance
	Mv Nádor	Genius	
Cultivar	8480	7200	0.000
Fertilisation	without 7380	with 8310	0.000
AMF inoculation	without 7520	with 8170	0.000
Cultivar * Fertilisation			0.000
Cultivar * AMF inoculation			0.089
Fertilisation * AMF inoculation			0.002
Cultivar * Fertilisation * AMF inoculation			0.014

Table 2. Yield averages and standard deviations of the wheat cultivars for each fertiliser and AM inoculation treatment. N=3 (number of replications). LSD value for all treatments is 342.

Treatment	Yield averages and standard deviations (kg ha ⁻¹)	
	Mv Nádor	Genius
No fertilisation, no AMF inoculation	6960 (207)	6720 (200)
No fertilisation, AMF inoculation	8550 (190)	7280 (410)
Fertilisation, no AMF inoculation	9160 (430)	7240 (160)
Fertilisation, AMF inoculation	9270 (290)	7570 (220)

even modern wheat cultivars have not lost their ability to form mycorrhizas. The yield averages of the two wheat cultivars differed significantly, the greater yield potential of cultivar Mv Nádor being confirmed in the experiment. Cultivar Genius responded to the AM inoculation in a smaller measure but this cultivar showed smaller yield increasing to the fertilization too. We don't have not enough data to decide that this cultivar has low AM susceptibility or it has limited P uptake potential.

The experimental area has been used for intensive crop production involving replenishment rates of mineral fertiliser (P and K) for several decades. Sasvári (2017) reported that wheat-maize rotation has pronounced detrimental effect on AM fungi. These factors were probably responsible for the fact that the number of arbuscular mycorrhiza-forming fungus propagules had dropped to such an extent that a single mycorrhizal inoculation was able to cause a detectable change in the yield. This was confirmed by the fact that small number

of structures characteristic of mycorrhization (arbuscules, hyphae) could be observed on the roots of plants that were not inoculated (Table 3). Direct spore counting from the soil was not happened.

Data in the literature suggest that the mycorrhization of plants is stimulated if the soil has a low content of available phosphorus, and inhibited by high P content. In the present experiment the available P content in the soil was measured with the method routinely used in Hungary as the quantity of AL-soluble phosphorus, which was found to be 181 mg P₂O₅ kg⁻¹ at the beginning of the experiment. According to the official recommendations (MÉM NAK, 1979) this represents a good phosphorus supply level. As the meta-analyses, quoted above in the introduction, generally either give no data on the available phosphorus content of the soil or measure it using a different method (other than AL-solubility), this complicates a comparison of the results. On the other hand

Table 3. Mycorrhization of winter wheat for each factor (wheat cultivar, mineral fertilisation, AM inoculation). N= 12 (number of replications).

Factor	Mycorrhization (%)		Level of significance
	Mv Nádor	Genius	
Cultivar	14.6	4.3	0.000
Fertilisation	without 18.9	with 0.03	0.000
AMF inoculation	without 0.85	with 18.1	0.000
Cultivar * Fertilisation			0.000
Cultivar * AMF inoculation			0.000
Fertilisation * AMF inoculation			0.000
Cultivar * Fertilisation * AMF inoculation			0.000

beside the soil P content it is necessary to take plant P demand into consideration.

The yield-enhancing effect of AM fungi inoculation could only be detected in treatments given no mineral fertiliser (*Table 2*). The mineral fertiliser treatments involved a total of 130 kg N ha⁻¹, 78 kg P₂O₅ ha⁻¹ and 60 kg K₂O ha⁻¹ active agents, based on the nutrient management calculations usually employed on the farm. As NPK mineral fertiliser was applied in the present work, therefore it is not possible to separate the effects of nitrogen and phosphorus fertiliser on mycorrhization. There is agreement in the literature that the application of phosphorus fertiliser to agricultural crops reduces the level of mycorrhization of the crops, as the crop itself is able to take up the necessary quantity of phosphorus and is not dependent on a fungal partner. Very few papers reached other conclusions.

One such is that of Cozzolino et al. (2013), who demonstrated the yield-enhancing effect of NP and NPK fertiliser in maize plants inoculated with mycorrhizal fungi. This could be due to the fact that the soil had very different characteristics to that used in the present experiment.

Our results support the idea usually accepted in the literature, that mineral fertilisation (mainly P) decreased the efficiency of AM inoculation.

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Conclusions and recommendations

The results and the conclusions drawn from them were based on the data of a single year (2016). Our research showed that AM fungi inoculation of winter wheat in field conditions can be an effective agronomic practice, although its economic profitability should still be questioned. Data from experiments performed in several years with more cultivars and soils with diverse properties will be required to obtain more reliable recommendations for farmers. The harmonisation of the production technology and the AM fungi inoculation is important for efficient crop production. Changes in the production technology could produce more favourable conditions for the fungi, making the AM fungi inoculation more successful. Many papers have reported on the favourable effects of reduced disturbance/ploughing, the use of vegetation cover and the incorporation of organic matter.

Acknowledgements

Thanks are due to the SZÉRA Kft. for performing the experiment, to the Hajnalpír Kft. for the background and to the staff of the following companies: Elitmag Kft., Hortiservice Kft., Italpöllina SPA, Bayer Hungária Kft., Eurofins Agroscience Services Kft. and Syngenta Kft.

We express our thanks for the support provided by EFOP-3.6.3-VEKOP-16 project.

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Agronomic benefits of long term trials

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Abstract: Long term trials have been established in favour of exploring and observing plant and soil interrelations on site. We may determine long term trials as live instruments providing *ceteris paribus* conditions in temporal sequences. This review is dealing with the introduction to major long term trials in the World and in Hungary. Giving a brief summary on plant nutritional research roots beginning with some data from Homer, and the fabulous initial willow tree experiment of van Helmont, as well as the basic inventions of physiological processes by von Liebig, Lawes and Boussingault. The most profound long term trials like Padova's Orto Botanico, the Linné Garden of Uppsala and the Broadbalk of Rothamsted are presented in the lecture.

Agronomic, educational and scientific benefits of the major Hungarian long term trials are also discussed from Westsik 1929 via Maronvásár and the National Plant Nutrition Trials (OMTK) founded in 1963. There is a list of experimental sites giving information on the most important recent long term trial locations and the activities.

Keywords: long term trials, plant nutrition, crop physiology, adaptability

Roots

Field trials in general and long term trials in particular have been established in favour of exploring and observing plant and soil interrelations on site. We may determine long term trials as live instruments providing *ceteris paribus* conditions in temporal sequences (Kellogg 1957). The utility of such trials is in their exact layout and the length of period they are operated. Simply, the older is the better regarding the mathematical plausibility of such scientific instrument (Jolánkai 2017).

The first written information on manure is almost dated back to three millennia. Homer (BC 11th Century) presents a story in the Odyssey about the homecoming of Odysseus to Ithaka, when he is recognized by his old dog ... “ ... *lying on a heap of dung with which the thralls were wont to manure the land*”. Homer's description is of an appropriate agronomic phenomenon giving the evidence, that people of his age knew about the fertilising value of animal excreta, the collection and storage of that material, and the means of dispensing that to the field.

The fabulous initial willow tree experiment of van Helmont was recorded in 1635. It was the

first scientific plant analysis approach detecting physiology of plant growth. A young willow tree of determined weight was placed into a pot containing measured amount of soil. The pot's surface was sealed to provide any alien material to access the growth substance. Then water was applied regularly to the system over two years. Terminating the trial all measurements were done precisely. van Helmont stated that there was a significant growth and development of the willow tree, and since there was no major difference between the soil data – he assumed that the few ounces loss could be due to measurement mistakes - and upon the results he concluded, that the only material source of life is water alone. However the experiment had no plausible results, it can be assumed as the dawn of a new period.

The development of chemistry and almost two Centuries were needed afterwards to the basic inventions of physiological processes by von Liebig, Lawes and Boussingault. Justus von Liebig has described the level of development with the help of a barrel, where staves are uneven in size. According to his thesis plant growth is determined by the minimum level of a certain plant nutrient. His theses were clear

and widespread, however they were immediately doubled by Sir John Bennet Lawes who tried the theses in his replicated exact field experiment and could not verify them. Who was right and who failed? Both of them were right, however the discrepancies were induced by the system of approach. Liebig as a chemist has followed a static model, and Lawes as a practicing landlord made a dynamic assessment – say, the proof of the pudding is in eating that. A third person and a new invention was needed to formulate the physiological process. Jean Baptiste Boussingault was the man who described first the Nitrogen cycle and so gave an explanation to diverse situation. Also, it was the time when urgent need appeared in establishing exact field trials to provide in vivo conditions for research in plant physiology.

Historical long term trials

The first long term trials were not established by agriculturists, but botanists. The basic task of these trials was to provide „*ceteris paribus*” (equal measures) conditions for scientific observations. It had long been known, that to study the behaviour, growth and development of any living creature, identical conditions and replicated methods are needed (Cserháti 1901). It is difficult to identify which trial in the World can be labelled as the first, because there are at least a dozen of candidates for that. Even we may have problems to define what is a trial, and what do we mean on long term. From a point of view regarding plant growth physiology the oldest long term trial should be Padova’s Orto Botanico, founded in 1545. Various plant species have been tested here since that time under rainfed and irrigated conditions in a controlled system.

Another long term trial, with some similarities to the previous one is the Linné Garden of Uppsala founded in 1728. Carolus Linné was a botanist, and he was keen to exclude environmental effects that may have altered plant morphology, so he set up a design for parallel observations. Linné Garden is not in operation anymore, it is a sort of a live museum of science maintained nicely by the local community.

The oldest long term trial dealing with plant nutrition as well as monocropping problems is the Broadbalk of Rothamsted. Sir John Bennet Lawes an English nobleman, entrepreneur and agricultural scientist established that in 1843 with an aim among others to clarify plant nutrition principles. He was the man who employed Joseph Henry Gilbert with whom developed the superphosphate fertiliser. He founded an experimental farm at his home at *Rothamsted* Manor that eventually became the *Rothamsted* Experimental Station, one of the most powerful scientific research institutions in agriculture.

National long term trials

Hungary has always been a land dedicated to agricultural activities and so to agronomic research. For historical reasons most of the 19th Century trials did not survive. What are the longterm trials in Hungary like? Our national longterm trials have been founded in favour of to explore various scientific hypotheses and to observe agronomic techniques (Várallyay 2006). The oldest site maintained since 1929 is the Westsik long term trials at Nyíregyháza. It was set up by Vilmos Westsik to study agronomic methods suitable for sandy soils. The trial has been introducing various crop rotation systems. The main problem of that area is twofold; water scarcity and poor soil properties.

The Martonvásár trials were initiated by Professor Béla Győrffy in 1958. The main purpose of the trial was to study plant nutrition and crop rotation versions as well manure and chemical fertiliser interactions in relation with the variety specific reactions of field crops. The trials have been designed in a polyfactorial structure with replications.

The National Plant Nutrition Trials (OMTK), founded in 1963. The OMTK plant nutrition trials were established to gain reliable data on mineral fertilization of field crops in all regions of Hungary. The experimental network was initiated by Professor Géza Láng with the active participation of Béla Debreczeni, József Antal and Ernő Bocz.

Crop production and soil tillage trials. During the past half of a Century several crop production and soil tillage trials were established in favour of better understanding of crop physiological processes within given agri-environments. Valuable results have been obtained in the field of crop yield quantity and quality, in abiotic and biotic stress tolerance, soil pollution and remediation, and climate change phenomena.

training and extension services may benefit from the research results obtained from long term trials. Some of today's long term trials were originally designed for educational purposes. Such is the above mentioned Westsik trial at Nyíregyháza demonstrating sand-soil tillage methods for farmers. Other long term trials were designed to solve certain specific scientific problems, but most of them can also be used

Table 1. Main data of the Hungarian National long term trials, 2017 (Source: Kismányoky and Jolánkai 2017)

Location and number of long term trials	Established
Nyíregyháza 3	1929
Fülöpszállás 1	1982
Keszthely 12	1963
Debrecen (Látókép, Hajdúböszörmény) 4	1967
Iregszemcse, Bicsérd 2	1967
Karcag 3	1984
Szarvas 1	1989
Kompolt 2	1967
Gödöllő (Józsefmajor, Nagygombos) 3	1972
Nagyhörcsök 6	1967
Órbottyán 2	1959
Nyírlugos 1	1962
Martonvásár 15	1958

Long term trials are not only scientific curios or honoured relics of a museum, but high value live ecological models that can never be replaced or restarted whenever ceased or suspended, nevertheless terminated. These trials provide valuable and dynamic databases for solving scientific problems.

Long term trials are therefore „major tools” in basic research on crop science, agro-chemistry, soil science and agro-ecology. Their role is similar to any of the man-made tools, instruments or implements, such as phytotrons, lysimeters, hydroponics or reactors.

The benefit of long term trials in education

Regardless to their scientific value and utility, long term trials play a major role in education. Various branches of education, including undergraduate and PhD courses, vocational

for educational purposes. All long term trials in Hungary whether owned and operated by research institutions or universities, are involved in one or more accredited educational programmes. They can also be visited, studied and examined within the framework of regular scientific and extension programmes.

Economic contribution

Long term trials may have a role in the implementation of certain economic processes, as well as in prevention or handling of hazards and disasters. Without the databases of long term trials most of crop production technologies and plant protection applications would be less efficient. Even in the case of adaptation of international research results, materials, instruments and technologies, long term trials may help us in a more accurate, successful and plausible application regarding the local

conditions. Also, long term trials often contribute to the success of avoiding and managing the consequences of natural hazards, catastrophes, climatic extremes, anomalies, epidemics, gradations (eg. flood, water logging, drought, eutrophication, cyanid pollution, red sludge flood etc), just to mention some of these from recent years. Environmental protection and nature conservation should be based on long term trials. Over 80 % of the territory of Hungary is covered by terrestrial ecosystems, the vast majority of which are agro-ecosystems, a situation that is unique in Europe.

Key to international cooperation

Hungarian long term trials represent a virtual network and most of them have a role in national programmes. They also provide the basis for regional cooperation as well. Within the Carpathian basin many of the transfrontier collaborations are based on research activities of long term trials. Research results contribute to solve problems in the field of natural and social sciences. Some examples to highlight the latter: Keszthely (Hungary) - Nitra (Slovakia), Látókép-Nyíregyháza (Hungary) - Livada (Romania), or

Nagyhörcsök (Hungary) - Eszék (Croatia) joint research programmes, or the utilization of the OMTK (National Fertilization Long-term Trials) results and data in EU climate change research projects are essential for scientific cooperation (Jolánkai 2008).

The Alps-Adria scientific cooperation provides a scientific forum for presenting and discussing the research results obtained in longterm trials in the region. At these conferences during the past decade more than a thousand scientific papers have been presented, around 200 of which were based on long term trials (Jolánkai 2009). International cooperation in this field is extremely valuable and will continue to contribute to plant and soil research in the future.

Acknowledgement

This paper presents research results gained from a long term trial supported by TÁMOP, NVKP and VKSZ funds of the Government of Hungary.

This paper is based on the presentation delivered at the International Conference on Long-term Field Experiments 27-28th September 2017, Nyíregyháza, Hungary

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Environmental and hereditary effects in plant nutrition: our collaboration with RISSAC Budapest

Dedicated to the memory of Prof. Dr. Imre Kádár: 1943 -2018

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Abstract: Imre Kádár (29th July 1943 - 1st March 2018), a world renowned retired scientist in the field of agrochemistry and plant nutrition from the Research Institute of Soil Science and Agricultural Chemistry (RISSAC) of the Hungarian Academy of Science (HAS) in Budapest, passed away suddenly in March 2018. Our collaboration has about a 30 year-long tradition. Intensive collaboration started in connection with the beginning of the Homeland war in Croatia (1991-1995). Imre's broad -minded soul was shown by his offer to make plant and soil analyses in RISSAC agrochemical laboratory for a price covering only the costs of chemicals and sample preparing. He made the offer after visiting Osijek and witnessing the total destruction of lecture rooms in the new building of Faculty and Institute, located on the southern outskirts of Osijek, and distanced only 0.5 km from the frontline. Until the end of 2012, we recorded about 6000 plant and soil samples for elemental analyses sent from the Faculty of Agriculture and Agricultural Institute Osijek to RISSAC Budapest. The outcome of this collaboration is numerous scientific and review articles. Until the end of 2016, Imre was included in 51 scientific, review articles and abstracts in total. Topics of our common articles can be divided into three parts: fertilization and liming effects on soil and plants, heavy metals and harmful elements in soil and plants, and hereditary impacts in maize nutrition. There is still unpublished data from the analyses made in RISSAC. We hope that, in the near future, some of the articles will be published including Imre in our team. Imre may have passed away, but his soul remains with us until our last breath. His rich legacy of development of environmental and genetic aspects of plant nutrition at the Faculty of Agriculture in Osijek and Agricultural Institute Osijek will be inspirational for many generations to come.

Key words: RISSAC, Kádár Imre, plant nutrition, Faculty of Agriculture in Osijek, Agricultural Institute Osijek

Introduction

Imre Kádár (29th July 1943 - 1st March 2018), a world-renowned retired scientist in the field of agrochemistry and plant nutrition (Picture 1.), from the Research Institute of Soil Science and Agricultural Chemistry (RISSAC) of the Hungarian Academy of Science (HAS) in Budapest, died suddenly in March 2018. The aim of this study is to review our collaboration with RISSAC and to list our common published articles. Imre Kádár and Vlado Kovacevic met each other for the first time at the 7th Congress on Chemistry in Agriculture, which was held 22-24. 06. 1987 in Nitra (the former Czechoslovakia). They exchanged experiences regarding plant nutritional problems, particularly potassium (K) deficiency as a result of K fixation. A similar field of scientific interest became a basis for the future collaboration. At the beginning of the Homeland war in Croatia (1991-1995) and during very difficult conditions at our Faculty and Institute,

without a possibility for activities in destroyed laboratories, the great Imre expressed his highly sentimental human heart. He decided to help us by analysing about 6000 plant and soil samples using ICP AES technique by Jobin-Yvon Ultrace 238 ICP-OES spectrometer in the Agrochemical laboratory of the RISSAC with a payment at a low price covering only the costs of chemicals and the preparation of samples. The costs of the chemical analyses were covered by Agricultural Institute Osijek. Domagoj Šimić and Ivan Brkić from the Department of Maize Breeding and Genetics at Agricultural Institute Osijek were included in the collaboration after 1995. From 2007, Zdenko Lončarić from the Department of Agroecology, Faculty of Agriculture was included in the collaboration for the realization of two bilateral projects. Very successful collaboration resulted by 51 common scientific and review articles and abstracts in total, along with two PhD theses. Posthumously the article was accepted for publishing (Kovacevic et al, 2018). Also, two

Hungarian - Croatian bilateral projects (the national principal investigators Imre Kádár and Zdenko Lončarić) were realized as follows: “Soil chemical properties impact on heavy metals availability and concentrations in field crops” (01.08.2007 – 31.07.2009: Picture 2) and “Heavy metals from farm to fork (protection of food chain)” (01.09.2009 – 31.08.2011). Also, Imre Kádár was an exterior co-worker in our national project “Overcoming limits of maize growing on acid soils by fertilization and genotype” (the 2006-2013 period – code 079-0730463-0447, the principal investigator Vlado Kovačević).

Material and methods

We reviewed our common articles with Imre Kádár published in the period from 1996 to 2016. The articles can be divided into three topics: fertilization and liming effects on soil and plant, heavy metals and harmful elements in soil and plants, and hereditary impacts in maize nutrition. The subject of some articles includes two topics. Also, some of the titles were first published as an abstract only, and then completed as an article with the same title. Also, two Hungarian - Croatian bilateral projects (principal investigators Imre Kádár and Zdenko Lončarić) supported by both State Ministries of Science (from 2007 to 2011) were shown.

Results and discussion

The topic of majority of our articles with Imre Kádár is the impact of fertilization and liming on soil and plant properties (Andrić et al., 2016; Kádár et al., 1997, 1998, 2010; Iljkić et al., 2013; Kovačević and Kádár, 1998, 1999; Kovačević et al., 1996a, 1998, 1999, 2000, 2002a, 2009a, 2009b, 2010a, 2010b, 2010c, 2010d, 2011a, 2011b, 2013a, 2013b, 2014, 2015a, 2015b; Rastija et al., 2009; Lončarić et al., 2011b; Regalyi et al., 2010; Karalić et al., 2010, 2011; Rekasi et al., 2010). Thanks to these and many other articles that originated from our collaboration, a great step was made in the knowledge of soil and plant nutritional problems on some less fertile soils in Croatia: potassium (Kádár et al., 1998; Kovačević et al., 2010a), phosphorus (Kovačević et al., 2010c)

and zinc (Kovačević et al., 1998, 2015a, Šimić et al., 2012) deficiencies.

Heavy metals and other harmful elements in soil and plants were elaborated mainly with the aspect of environmental pollution and some articles were focused on the role of hereditary factors (Kádár et al., 2010, 2011; Kovačević et al., 2002a, 2002b, 2002c, 2002d, 2004, 2010d, 2011a, 2011b, 2012; Lončarić et al., 2010, 2011a, 2011b, 2012a, 2012b, 2012c; Rastija et al., 2016; Rekasi et al., 2010; Šimić et al., 2004, 2012). Concentrations of heavy metals in ecosystems are continuously increasing due to industry and traffic, power plants and agriculture. In general, arable soils in the eastern Croatia are clean unpolluted soils well below the limits for conventional and organic agriculture, especially considering total concentrations of toxic heavy metals Pb and Cd. Excessive concentrations of heavy metals were found mainly in the soils of urban areas of Croatia. Preserving the fertility of soils, liming, adequate fertilization, and plant protection, the choice of plant species and genotypes, of feed and animal products are the basis for a lower input of toxic heavy metals into the food chain (Lončarić et al., 2012a, 2012b; Rastija et al., 2016). Genotype aspects of maize mineral nutrition alone or in combination with soil and fertilization, are the topics of some common articles (Andrić et al., 2016; Brkić et al., 2003, 2004; Kovačević et al., 1996a, 1996b, 1997, 1998, 2002b, 2002c, 2002d, 2004, 2011a, 2011b, 2012; Šimić et al., 2002, 2004, 2005, 2012). Two PhD theses, focused on the genetic aspect of maize nutrition, were developed from the results made in RISSAC and defended at the Faculty of Agriculture in Osijek as follows: Environmental and heredity effects on yield and concentrations on phosphorus, potassium, manganese and zinc in maize (Rastija Mirta – July 11, 2006); Impacts of genotype and soil on phosphorus and potassium status in maize (Vragolović Antun - October 27, 2010). Our findings regarding considerable differences in contents of micronutrients in grain of maize hybrids could be used to alleviate micronutrient malnutrition, particularly by widely extended



Picutre 1. Imre Kadar (1st June 2007: Joint International Conference on Long-term Experiments): the Nyírlugos Field Trials



Picutre 2. visit to RISSAC (April 8, 2008): from left to right: Vlado Kovacevic, Blazenka Bertic, Imre Kádár and Zdenko Loncaric

iron and zinc (Šimić et al., 2005). Namely, we found significant differences among 121 maize genotypes in grain, iron and zinc ranging from 11.0 to 60.7 mg Fe/kg and from 11.9 to 33.2 mg Zn/kg on dry matter basis (Brkić et al., 2004). Based on some results, hereditary aspects of maize nutrition were used for improvement of yields on soils of limited fertility. For example, choice of maize hybrids characterized by specific K and Mg uptake under soil conditions of K-fixation and excessive amounts of available Mg contributed to yield increases without a considerable cost of potassium fertilization (Kovacevic et al., 1996a). Importance of boron in seed-production of maize hybrids and fertility of their parents was elaborated by Andrić et al. (2016).

Conclusion

The collaboration between the RISSAC and the Faculty of Agriculture and Agricultural Institute

Osijek has over a 30 year-long tradition. As result of this collaboration, numerous scientific, review articles, and two doctor theses were published. Until the end of 2016, Imre was included in 51 scientific, review articles and abstracts in total.

These contributions cover fertilization effects on soil and plant nutritional status, environmental and hereditary effects on maize nutrition and heavy metals status in soil and plants as affected by environment and heredity.

On behalf of the Faculty of Agriculture in Osijek and Agricultural Institute Osijek, we would like to express our sincere gratitude to professor Kádár for his work and results, inspiration, his friendly personality, and at the same time we would like to say farewell.

Dear Imre, rest in peace!

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