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Effect of cereal-legume intercrops on the soil enzymatic activity

Attila RÁCZ[1,2] – Melinda TAR[1] – Marianna VÁLYI NAGY[1] – Apolka UJJ[2] – István KRISTÓ[1]

1: Hungarian University of Agriculture and Life Sciences, Plant Production Scientific Institute, Crop Production Research Center, Crop Production and Agrotechnical Research Station, Alsó Kikötő sor 9. 6726, Szeged, Hungary, e-mail: Racz.Attila@uni-mate.hu
2: Hungarian University of Agriculture and Life Sciences, Institute for Sustainable Development and Management, Páter Károly u. 1. 2100, Gödöllő, Hungary

Abstract: Sowing and harvesting cereals with legumes is an old crop production practice. The main goal of cultivation is to make the best use of the area and to increase the quality and yield of the crops. The intercropping of cereals and legumes can stimulate the biological activity of the soil, thus increasing the recycling of soil organic matter. Competition between two or more plants has a positive effect on the nitrogen fixation of legumes. In organic form the soil enzyme activity increasing is more effective which is provided by the winter pea (*Pisum sativum* L.) in this crop production system. We set up our experiment at the experimental sites of the Hungarian University of Agriculture and Life Sciences, Plant Production and Agrotechnical Research Station, Szeged-Öthalom and Fülöpszállás in 2020/2021. The experiments were performed on 10 m^2 plots, in four replicates, with four cereals and one winter pea species, in different phenological phases. We were used fluorescein diacetate to determine the total microbial activity of the soil. As the phenophases progress, the enzymatic activity of the soil decreases, and activity is affected by soil type. The soil enzyme activity was lower on meadow chernozem soil and higher on calcareous meadow soil. Cereal-legume intercrop systems are better able to adapt to drought. The values are higher in cereal-legume intercropping system than in cereals sowed alone. Based on our results, it can be concluded that the enzymatic activity of the soil can be increased by using soil inoculation with bacteria and mycorrhiza fungi preparations.

Keywords: soil enzyme activity, cereal-pea intercrops, plant associations, winter pea

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Introduction

If we are looking at Hungary's arable land, it can be seen that five main crops (wheat, barley, maize, sunflower, rapeseed) dominate, of which almost three quarters of the crop area is dominated by cereals (KSH, n.d.). With the development of crop production, the yield of our cereals has tripled. For this reason, the area under cereals has started to increase, which has contributed to the increase in the number of animals consuming forage. Due to the feeding of the animals, there was a high demand for protein. Which was not satisfied by the dissonant sowing structure and the low yield of legumes (Bocz, 1996). Since the turn of the millennium, the demand for protein in the country has become even more serious. We do not currently have enough sources of animal (insect protein, fish meal, bone meal, meat meal) or vegetable protein. Hungary is only capable 15-20% self-supply, thus our country needs a large amounts of import (http1, 2019). Our demand are 600,000 tons of soybeans a year, which we often covered by low-quality genetically modified organism imports (http2, 2017). In the National Protein Program, the objectives include that increasing the sowed area of soybeans. However, the experience of

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recent years has made it clear that regardless of the vintage and the site of production, we do not produce enough protein despite the subsidies. The program also recommends the cultivation of our forgotten protein crops (winter pea, horse beans, grass pea, lupine etc.) that would perform better yield than soybean in some production sites (http3, 2018). The transformation of our sowing structure would be important from an economic and agronomic point of view. We need to increase the sowed area of protein crops economically (Kristó et al., 2021).

The choice of protein crops is large therefore we can think of winter pea as an alternative source of protein which has the second highest yield of our cultivated legumes in the world (Antal, 2005). Legumes are excellent preceding crops that stimulate the microbiological life of the soil by nitrogen fixation (Stefanovits et al., 1999). The role microorganisms in the nitrogen cycle are the most important of the nutrient cycling. Elemental nitrogen which makes up 78% of the air, enters the biological cycle through its uptake by bacteria (Stefanovits et al., 1999). The organic and chemical fertilizer use to improve the nutrient supply capacity of soils have a strong effect on the nitrogen content and microbial community (Bandick & Dick, 1999). Several groups of microorganisms, such as *Rhizobium* sp., *Bradyrhizobium* sp. are capable of biological nitrogen fixation in soil (Szabó, 2008). Peas are able to bind large amounts of nitrogen by the nitrogen fixing bacteria (*Rhizobium leguminosarum*) (Láng, 1976; Radics, 2002) The quantity of nitrogen fixation by legumes is important for maintaining the fertility of our soils (Füleky, 1999).

Global climate change is a major problem that is also showing signs in agriculture (Birkás et al., 2008). Unfavorable climatic effects can be reduced by suitable variety selection, timely and professional agrotechnics (Jolankai & Birkás, 2007). By growing a mixture of species with nearly the same growing season but different environmental needs, we are able to adapt better to changing climatic and economic conditions. In Hungarian practice, it was a habit to sow pea with support crops (wheat, barley, oats etc.) (Kurnik, 1970). Winter wheat and winter pea are compatible for sowing and harvesting time. Suitable technology can be developed to increase the sowed area of winter pea by using cereal legumes plant associations (Kristó et al., 2021; Murray & Swensen, 1985). Higher plant diversity is needed in agricultural production for sustainable development (Baulcombe et al., 2009).

Growing legumes and cereals together is an ancient crop production practice. The main goal of cultivation is the best use of resources (area, light, nutrients) (Li et al., 2003) and to increase the quality and quantity (Dahmardeh et al., 2009; Mpairwe et al., 2002). By combining legumes and cereals we can effectively reduce the negative effects of water stress (drought or high rainfall). The plants which used in the intercropping system are morphologically diverse, thus they can more efficiently use of climatic conditions than as a sole crop (Singh et al., 2008). Winter wheat and winter pea plant association is a special form of cereal-legume intercropping system which based on complementarity, offers greater financial stability and allows lower inputs which reduce environment impacts of agriculture (Vályi-Nagy et al., 2021). The plant association increases biodiversity (Tscharntke et al., 2005) the plants used in the association provide suitable habitat for insects and soil organisms which are not present in monoculture system (Cai et al., 2010). The association of cereals and legumes could help increase soil biological activity, resulting in higher cycling of organic matter in the soil (Dick et al., 1988)ting effect on nitrogen fixation in legumes (Hardarson & Atkins, 2003). Determining the amount of fluorescein diac-

Table 1: Soil sampling data

		Soil depht (0-20 cm)	
Parameters	Units of measure	Szeged-Öthalom	Fülöpszállás
KA	KA unit	41	50
pH (KCl)	pH unit	7.5	7.9
All salt	m/m%	0.03	0.02
Humus	m/m%	2.5	2.8
$CaCO_3$	m/m%	1.7	18
P_2O_5	m/m%	235.1	266.4
K_2O	mg/kg	237.1	624.8
NO_3–N+NO_2–N	mg/kg	35.2	27.5
SO_4^{2-}-S	mg/kg	10.9	6
Na	mg/kg	60.4	249.3
Mg	mg/kg	195.1	429.4
Cu	mg/kg	1.4	2.3
Zn	mg/kg	5.8	1.9
Mn	mg/kg	11.9	23.5

Figure 1: Location of the experimental plots in four repetitions, Szeged-Öthalom (2021).

etate (FDA) is a widely accepted, simple, and sensitive method for measuring total microbial activity in soil. Colorless FDA is hydrolyzed by both free and membrane-bound enzymes, releasing a colored final product that can be measured with a spectrophotometer (Adam & Duncan, 2001). The results of the microbial enzyme activity method indicate the functionality of the total microbial mass which is to determine the amount of degrading organizations involved in processes (Biró et al., 2012).

Our research aimed to determine the cereal-legume intercrops how influenced the soil enzyme activity. In two different locations (Szeged, Fülöpszállás) examine the effects of pure cereals, pure pea, cereal-pea intercrops and different nitrogen treatments on

Table 2: Agrotechnical methods that were used in the experiment

Agrotechnical methods	Date	Ingredient	Preparation	Dose
Stubble cultivation	August, 2020	-	-	-
Fertilizer	October 2, 2020	NPK	NPK complex (15:15:15)	200 kg/ha
Grubber	October 10, 2020	-	-	-
Combinator	October 20, 2020	-	-	-
Soil inoculant + combinator	October 27, 2020	*Rhizobium leguminosarum*	Biofil	1 l/ha
Sowing	October 27, 2020	-	-	-
Weed control	October 28, 2020	pendimetalin	Stomp Aqua	3 l/ha
Fertilizer	March 10, 2021	ammonium-nitrate (34%)	ammonium-nitrate	30 kg/ha
Fungicide treatment	March 26, 2021	azoxistrobin	Blister	0,7 l/ha
Insecticide treatment	April 12, 2021	alfa-cipermetrin	Eribea	0,1 l/ha
Fertilizer	April 27, 2021	ammonium-nitrate (34%)	ammonium-nitrate	30 kg/ha
Insecticide treatment	May 12, 2021	gamma-cihalotrin	Rapid CS	0,08 l/ha
Harvesting	July 5, 2021	-	-	-

the soil life. Two microbial treatments, how influenced the total soil enzymatic activity on cereal-legume intercropping system in Szeged-Öthalom. In winter wheat – winter pea intercrops which is the best seed setting which can increases the soil's enzyme activity.

Materials and Methods

Our plant association investigations were set up at the experimental site of the Hungarian University of Agriculture and Life Sciences, Plant Production and Agrotechnical Research Station in Szeged-Öthalom and Fülöpszállás, in 2020/2021. The soil type of Szeged-Öthalom is meadow chernozem and Fülöpszállás has calcareous meadow soil (Table 1).

The cereal-legume intercropping systems were examined in four repetitions on random block plots of 10 m^2 (Figure 1). The agrotechnical methods were used in the experiment are summarized in Table 2.

The preceding crops of the plant association experiments was winter wheat (*Triticum aestivum* L.). At the end of October, the plots were sowed with parcel grain machine (Wintersteiger Plotman). Row width was 12.5 cm and sowing depth was approximately 4–5 cm. We sowed GK Csillag and Cellule winter wheat varieties in Szeged-Öthalom. In Fülöpszállás the winter wheat variety was GK Csillag as a basis for comparison. The winter barley (*Hordeum vulgare* L.) variety was GK Aréna, the winter triticale (*Triticosecale*) variety was GK Maros, and the winter pea (*Pisum sativum* L.) variety was Aviron at both production sites. They were also sowed in pure and mixed crops where cereals sowed in association with legumes (in-

Table 3: Overview table of treatments (pure cereals, pure pea, cereal-legume intercrop, nitrogen treatments) were used in Szeged-Öthalom and Fülöpszállás

Szeged-Öthalom	Fülöpszállás
Pure cereals	Pure cereals
• Winter wheat (GK Csillag)	• Winter wheat (GK Csillag)
• Winter barley (GK Aréna)	• Winter barley (GK Aréna)
• Winter triticale (GK Maros)	• Winter triticale (GK Maros)
Pure legume	Pure legume
• Winter pea (Aviron)	• Winter pea (Aviron)
Cereal-Legume	Cereal-Legume
• GK Csillag + Aviron	• GK Csillag + Aviron
• GK Aréna + Aviron	• GK Aréna + Aviron
• GK Maros + Aviron	• GK Maros + Aviron
Pure cereals + Nitrogen	Pure cereals + Nitrogen
• GK Csillag + 30 kg/ha nitrogen	• GK Csillag + 30 kg/ha nitrogen
• GK Csillag + 60 kg/ha nitrogen	• GK Csillag + 60 kg/ha nitrogen
• GK Aréna + 30 kg/ha nitrogen	• GK Aréna + 30 kg/ha nitrogen
• GK Aréna + 60 kg/ha nitrogen	• GK Aréna + 60 kg/ha nitrogen
• GK Maros + 30 kg/ha nitrogen	• GK Maros + 30 kg/ha nitrogen
• GK Maros + 60 kg/ha nitrogen	• GK Maros + 60 kg/ha nitrogen

tercropping system). In order to be able to monitoring the nitrogen effect and to be able to compare pure and plant associations, were set up plots where the cereal sowing alone was treated with half (30 kg / ha N) and full (60 kg / ha N) fertilizer (Table 3).

Plant associations were treated with microbiological preparations also examined in Szeged-Öthalom. Before sowing our experiment, we performed seed treatment with mycorrhiza fungi (*Glomus* sp.) and soil inoculant with *Rhizobium leguminosarum* bacterium. (Table 4).

Plant associations and pure sowings were sowed with 3–3 seed numbers. The sowing density of 5 million seed / ha was considered 100 % for cereals and 1 million seed /ha for winter pea. In our experiment the 75 seeds/m^2 quantity for cereals was 3.75 million seed/ha and 750 000 seed/ha for winter pea. In cereals, 50 seeds/m^2 was 2.5 million seed/ha and 500 000 seed/ha for winter pea. All possible combinations of 100 seeds/m^2, 75 seeds/m^2 and 50 seeds/m^2 sowing density were set up in our experiment (Table 5).

Soil samples were collected at three different times: before sowing (October), during pea flowering (May), before harvest (end of June). Soil samples were collected from each plot from the upper (0–20 cm) layer of the soil in four replicates. The name of the process is the determination of total microbial activity using fluorescein diacetate. First of all, 1 gram of soil was weighed from the samples into plastic test tubes. After potassium phosphate buffer solution was added to the soil. The samples were placed in a shaker which was heated to 30 °C for 30 minutes. After fluorescein diacetate solution was added to the samples and placed in shaker which was heated at 30 °C for 1 hour. As a result of time and heating the soil solution becomes discoloured. The process was stopped with acetone these coloured end-

Table 4: Extension of previous treatments with microbial preparations were applied in Szeged-Öthalom

Szeged-Öthalom
Pure cereal
• Winter wheat (Cellule)
• Winter barley (GK Aréna)
Pure legume
• Winter pea (Aviron)
Cereal-Legume
• Cellule + Aviron
• GK Aréna + Aviron
Pure cereals + Nitrogen
• Cellule + 30 kg/ha nitrogen
• Cellule + 60 kg/ha nitrogen
• GK Aréna + 30 kg/ha nitrogen
• GK Aréna + 60 kg/ha nitrogen
Cereal-Legume + Microbial preparations
• Cellule + Aviron + Soil inoculant
• Cellule + Aviron + Seed treatment
• Cellule + Aviron + Soil inoculant + Seed treatment
• GK Aréna + Aviron + Soil inoculant
• GK Aréna + Aviron + Seed treatment
• GK Aréna + Aviron + Soil inoculant + Seed treatment

Table 5: Agrotechnical methods that were used in the experiment

		Pea seed numbers (million/ha)			
		0	0.50	0.75	1
Cereal seed numbers (million/ha)	0	-	0:50	0:75	0:100
	2.5	50:0	50:50	50:75	50:100
	3.75	75:0	75:50	75:75	75:100
	5	100:0	100:50	100:75	100:100

products can be measured by spectrophotometry. The absorbance at 490 nanometer was measured in the samples and the results were recorded in Excel spreadsheet (Adam & Duncan, 2001).

Statistical processing of data obtained was performed using SPSS 27 statistical program. I used Microsoft Excel program to make the figures and tables. The change of the soil enzyme activity was determined by One-Way ANOVA analysis and T-test, refer to $p < 0.05$ significance level.

Results

The five figures in the results show the averages of soil enzyme activity in different

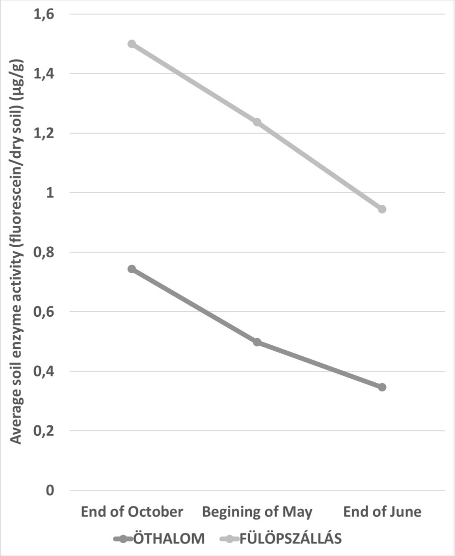

Figure 2: The change of the soil enzyme activity at winter wheat and winter pea intercrops in Szeged-Öthalom and Fülöpszállás.

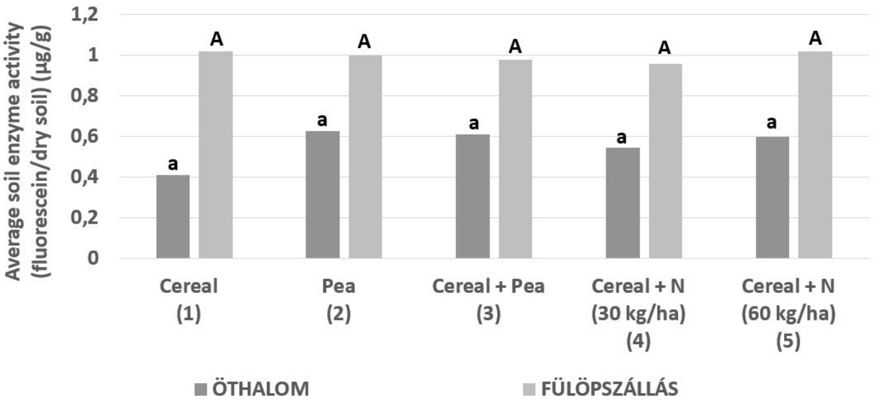

Figure 3: The effect of locations and different treatments on soil enzyme activity in Szeged-Öthalom and Fülöpszállás.
(1) Averages of GK Csillag, GK Aréna, GK Maros
(2) Average of Aviron
(3) Averages of GK Csillag + Aviron, GK Aréna + Aviron, GK Maros + Aviron
(4) Averages of GK Csillag + 30 kg/ha N, GK Aréna + 30 kg/ha N, GK Maros + 30 kg/ha N
(5) Averages of GK Csillag + 60 kg/ha N, GK Aréna + 60 kg/ha N, GK Maros + 60 kg/ha N

treatments. In the second figure, we compared winter wheat – winter pea intercrops datas at two locations which has different soil types. The second figure shows that there is a large difference between the production sites in measurement of the total microbial enzyme activity in the soil. Between sowing and the beginning of the harvest, the experiments set up in Szeged-Öthalom had the lowest values of soil enzyme activity. However, the soil enzyme activity in Fülöpszállás higher values could be measured during the vegetation period. The progressed time, increased the distance between the values of soil enzyme activity at two experiment sites. In Fülöpszállás we measured 2.0 times higher values at the end of October, 2.4 times higher values at the beginning of

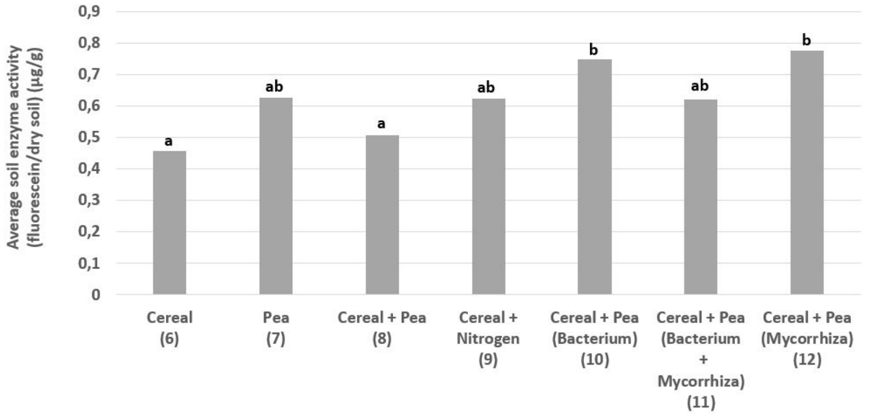

Figure 4: The effects of pure cereals, pure pea, cereal-pea intercrops, nitrogen treatment and microbial products on soil enzyme activity in Szeged-Öthalom.

(6) Averages of Cellule, GK Aréna
(7) Average of Aviron
(8) Averages of Cellule + Aviron, GK Aréna + Aviron
(9) Averages of Cellule + N, GK Aréna + N
(10) Averages of Cellule + Aviron + Soil inoculant, GK Aréna + Aviron + Soil inoculant
(11) Averages of Cellule + Aviron + Soil inoculant + Seed treatment, GK Aréna + Aviron + Soil inoculant + Seed treatment
(12) Averages of Cellule + Aviron + Seed treatment, GK Aréna + Aviron + Seed treatment

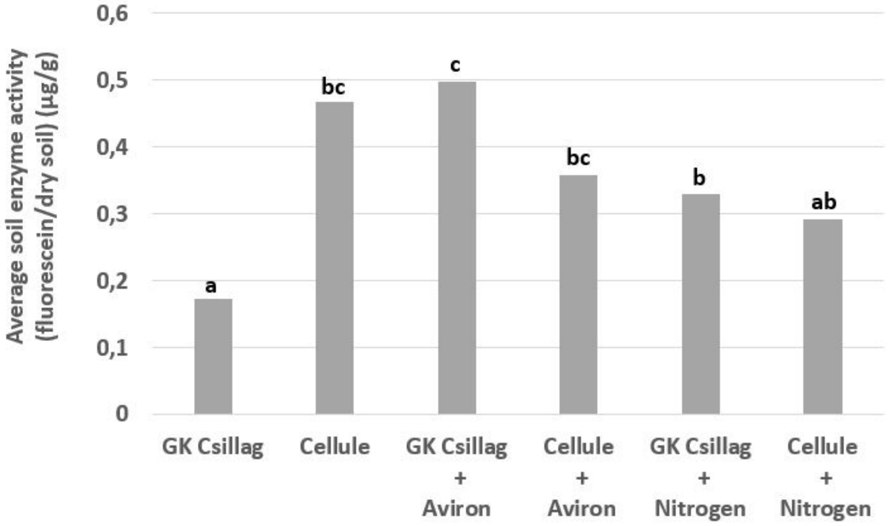

Figure 5: The effects of different winter wheat varieties (GK Csillag and Cellule), wheat-pea intercrops and nitrogen treatments on soil enzyme activity in Szeged-Öthalom.

May, and 2.7 times higher values at the end of June than in Szeged-Öthalom. The highest value of soil enzyme activity (1.49 μg/g) was measured in Fülöpszállás and the lowest value (0.34 μg/g) was measured in Szeged-Öthalom. Based on T-test, there are also significant differences ($p < 0.05$) between the locations in October, May and June enzyme activity values (2).

The soil enzyme activity values were higher in Fülöpszállás than Szeged-Öthalom. In Fülöpszállás we measured almost 2.5 times

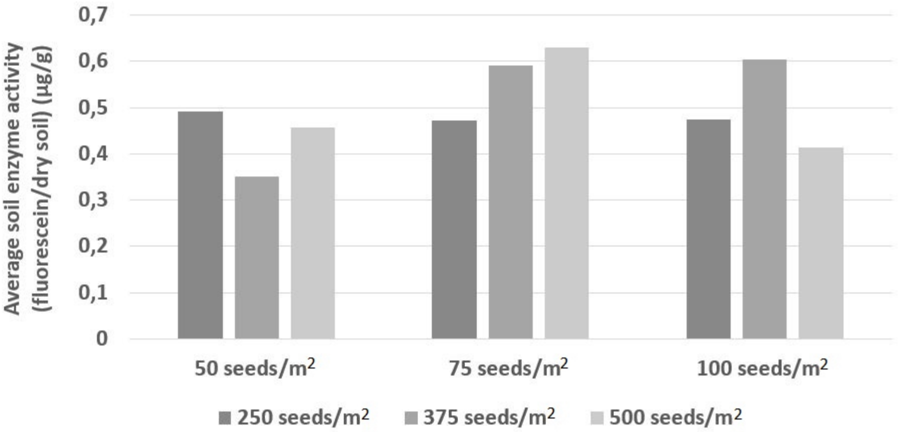

Figure 6: Different seed number effect in winter wheat – winter pea intercrops (GK Csillag + Aviron) on the soil enzyme activity.

higher values in the control plot (pure cereals) than in the soil of Szeged-Öthalom. The lowest value (0.41 μg/g) measured in the control (pure cereals) plot was found in Szeged-Öthalom. The highest (0.62 μg/g) value was in pure winter pea, which had almost the same value in cereal-pea intercrops (0.61 μg/g). The values of soil enzyme activity were lower in the plots treated with fertilizer, 0.54 μg/g in the plots treated with 30 kg/ha nitrogen and 0.59 μg/g in the plots treated with 60 kg/ha nitrogen. In Fülöpszállás calcareous meadow soil's values had approach 1 μg/g. As a result on the One-Way ANOVA analysis there is no significance differences between different treatments. Columns marked with different letters, are not significantly different at the $p < 0.05$ significance level (3).

Microbial preparations were applied in cereal-pea intercrops in Szeged-Öthalom, 4 showed the results. The control plot (pure cereals) had the lowest value (0.45 μg/g) of soil enzyme activity and the highest value (0.77 μg/g) was in the cereal-pea intercrops were treated with mycorrhiza fungi. Comparing pure cereals and cereal-pea intercrops, it can be seen that the soil enzyme activity is higher in plant associations (0.50 μg/g). Higher values (0.62 μg/g) were measured when plant associations were treated with nitrogen (0.62 μg/g). Furthermore, the values are even higher (0.74 μg/g) when we applied soil bacterial inoculants in cereal-pea intercrops. According to the result of the One-way ANOVA analysis, columns marked with different letters, are significantly different at the $p < 0.05$ significance level (4).

Of the two winter wheat varieties which were applied in plant association, the highest value (0.49 μg/g) of soil enzyme activity was measured in GK Csillag was associated with Aviron. The lowest value (0.17 μg/g) of soil enzyme activity was measured in GK Csillag (pure cereal). The microbial enzyme activity of the soil was lower in the plots were treated with fertilizer (0.32 μg/g and 0.29 μg/g) than in the cereal-legume intercropping systems. For plant associations with two different winter wheat varieties, GK Csillag + Aviron 0.49 μg/g and Cellue + Aviron produced 0.35 μg/g when measuring soil enzyme activity. According to the result of the One-way ANOVA analysis, columns marked with different letters, are significantly different at the $p < 0.05$ significance level (5).

6 shows the change in soil enzyme activity of plant association combinations were sowed with different seed counts in GK Csillag + Aviron. Increasing trend could be measured

in the plots with sowing densities of Aviron 75 seeds / m^2 and GK Csillag 250, 375 and 500 seeds / m^2. The highest activity was in the treatments of 75 seeds / m^2 of winter pea and 500 seeds / m^2 of winter wheat (0.62 μg/g). The lowest (0.35 μg/g) was in plots sowed with 50 seeds / m^2 of winter pea and 375 seeds / m^2 of winter wheat (6).

Discussion

In cereal-pea intercrop system, the microbial activity of the soil decreases as the phenological phases progress. Based on the research of Adam and Duncan (2001), it can be said that the change in soil enzyme activity is greatly influenced by soil type. We can also support this statement by our experiment, according to which the total microbial activity of the soil is lower in the meadow chernozem of Szeged-Öthalom and higher in the calcareous meadow soil of Fülöpszállás. Plant associations have higher measured values than pure cereals. Comparing the two winter wheat varieties (GK Csillag, Cellule), it can be stated that the measure of enzyme activity of the soil is higher in the plant associations when GK Csillag variety is sowed with Aviron winter pea variety. However, the activity was lower in the nitrogen treated plots than plant associations. For sowing with different seed counts, the optimal sowing density is 75 seeds/m^2 for winter pea and 500 seeds/m^2 for cereals. Bacterial inoculation and mycorrhiza fungi can stimulate the total microbial activity of the soil.

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