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Microbial abundance and activity in chernozem under different cropping systems

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Summary: The study monitored microbial abundance and dehydrogenase activity in chernozem soil under different cropping systems. Soil samples were taken from the long-term trials Crop Rotations and IOSDV, at the Rimski Šančevi experimental field of the Institute of Field and Vegetable Crops. The soil samples were collected in two years from ten cropping systems at three sampling depths. Number of the examined microbial groups was assessed by the indirect dilution method, while dehydrogenase enzyme activity was measured spectrophotometrically. Number of the tested microbial groups and dehydrogenase activity varied significantly depending on the cropping system and year of study, while sampling depth significantly affected enzyme activity. The highest number of microorganisms was obtained in non-agricultural soil and unfertilized soil under 2-year and 3-year rotation, while the highest dehydrogenase activity was recorded in non-agricultural soil and soil under wheat grown in monoculture.
Keywords: chernozems, crop rotation, cropping systems, fertilization, microorganisms

Introduction

Soil quality is determined by its chemical, physical and biological components as well as their interaction (Kennedy and Smith, 1995). The biological component of the soil is mainly represented by microorganisms, which play an important role in soil formation and soil fertility (van der Heijden et al., 2008). There may be hundreds of millions to billions of microbes in a gram of soil. The most numerous microbes in soil are bacteria, followed by actinomycetes, fungi, algae and protozoa, in decreasing numerical order (Schloss and Handelsman, 2006). Soil microorganisms participate in decomposition of soil organic matter and cycling of nutrients (Gougoulias et al., 2014). Microorganisms also affect the physical properties of the soil via production of extracellular polysaccharides and other cellular debris, and thereby help maintaining soil structure (Bastida et al., 2008).

Soil microbial community is very dynamic and promptly affected by different soil uses regarding management, frequency and amounts of applied fertilizers, or any other disturbance (Cardoso et al., 2013). Besides microbial activity and biomass, biochemical parameters such as soil enzymes can also be useful indicators of soil fertility and soil health, because most of the soil enzymes have microbial origin (Yang et al., 2012). The preservation of soil microbial diversity is crucial for a balanced agro-ecosystem, especially under increasing agricultural intensification. In the past, agricultural practices have failed to promote populations of microorganisms, limit production yields and threaten sustainability. Agricultural practices such as organic farming, reduced soil tillage, crop rotation, intercropping, and land use extensification may help soil microorganisms to become more abundant and active (García-Orenes et al., 2013). Influence of management practices on the size, composition, and function of the soil microbial communities varies greatly depending on their interaction with other abiotic and biotic factors, such as soil type, plant species, and other environmental variables (Stamenov et al., 2016).

Long-term experiments are a valuable method for determining yield trends, changes in nutrient dynamics and balances, assessment of soil quality and system sustainability (Wang et al., 2010; Bi et al., 2014). Since soil microorganisms are vital for soil quality, improved understanding of the long-term responses of key microbiological and biochemical soil properties to different cropping systems will provide valuable information about the nutrient supplying capacity of the agro-ecosystem and crop demands (Enwall et al., 2007). Therefore, the objective of this study was to assess the abundance of microorganisms and dehydrogenase activity in chernozem under different cropping systems.

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Material and Methods

Soil samples for microbiological analyses were taken from a long-term trial with crop rotations (CR - Crop Rotations), and an international trial (IOSDV), during a 2-year period (in July of 2008-2009), ten cropping systems and three sampling depths (0-20 cm, 20-40 cm, 40-60 cm). The trials were established at the Rimski Šančevi experimental field of the Institute of Field and Vegetable Crops Novi Sad. The experimental site location is on the southern border of the chernozem zone of the Southern Pannonian Basin. Cropping technologies of the investigated trials are presented in Table 1.

The present study consisted in monitoring the abundance of microorganisms and dehydrogenase activity (DHA). Number of examined microbial groups was assessed by the indirect dilution method followed by plating of soil suspension on different selective media. The total number of microorganisms (TNM) was determined on soil agar, the number of nitrogen-fixing bacteria (NFB) on a nitrogen-free medium, ammonifiers (AMN) on meat peptone agar, actinomycetes (ACT) on a synthetic medium, and fungi (FNG) on Czapek-Dox agar. Incubation temperature was 28°C, while incubation time depended on the tested group of microorganisms. All microbiological analyses were performed in three replications and the average number of microorganisms was calculated at 1.0 g of absolutely dry soil (Briones and Reichardt, 1999). Dehydrogenase activity (DHA) (EC 1.1.1.) was determined spectrophotometrically (Cary 3E UV-VIS, Varian) by measuring the extinction of coloured triphenyl-formazan (TPF) formed by reducing a colourless triphenyl-tetrazolium chloride (TTC) (Casida et al., 1964). TPF concentration was measured at 485 nm and the average DHA for all samplings were calculated per 1.0 g of soil (μg TPF g^{-1} soil).

Data were processed using a three-way analysis of variance (ANOVA). Means were compared using Fisher's least significant difference (LSD) test at the $p < 0.05$ level. All analyses were performed in STATISTICA 12 software.

Results and Discussion

The different cropping systems can have a positive or negative effect on microbiological number and activity, which directly reflect the fertility of the soil. Knowledge of the functions and activities of certain groups of microorganisms enables directing the microbiological processes either to synthesis or decomposition of organic and inorganic matter which gets into soil (Falkowski et al., 2008). Bacteria and other microorganisms participate in a variety of reactions that affect nutrient cycling, pH, as well as oxygen and CO_2 content (Singh et al., 2011). Bacterial groups selected for this study are suitable indicators of nitrogen (N) and carbon (C) cycling in soil, while dehydrogenase activity is an indicator of oxidation-reaction processes and a sensitive indicator of soil quality and soil fertility (Schmidt et al., 2011).

The analysis of variance of microbial number and activity based on the LSD test showed that the cropping system and the year of study significantly affected the number of the tested microbial groups and enzyme activity, while dehydrogenase activity was significantly affected by the sampling depth (Tab. 2).

Table 1. Cropping technologies of the investigated trials

System	Trial	Crop rotation	Fertilization
$4F_{mnr}$	IOSDV	4-year rotation	Manure (40 kg ha^{-1})
$4F_{N100+mnr}$	IOSDV	4-year rotation	Mineral fertilizer (100 kg ha^{-1} N) + Manure (40 kg ha^{-1})
$4F_{N200}$	IOSDV	4-year rotation	Mineral fertilizer (200 kg ha^{-1} N)
$4F_{N200+cr}$	IOSDV	4-year rotation	Mineral fertilizer (200 kg ha^{-1} N) + Crop residues
$1F_{N100}$	CR	Monoculture	Mineral fertilizer (100 kg ha^{-1} N)
$2UF_{cr}$	CR	2-year rotation	Crop residues
$3UF_{cr}$	CR	3-year rotation	Crop residues
$2F_{N100+cr}$	CR	2-year rotation	Mineral fertilizer (100 kg ha^{-1} N) + Crop residues
$3F_{N100+cr}$	CR	3-year rotation	Mineral fertilizer (100 kg ha^{-1} N) + Crop residues
NV	CR	Native vegetation	–

Monoculture – winter wheat; 4-year rotation – winter wheat/sugar beet/spring barley/maize; 3-year rotation – winter wheat/maize/soybean; 2-year rotation – winter wheat/maize.

Table 2. Analysis of variance of microbial abundance and activity of three factor experiment ANOVA (P values)

Source of variation	TNM	AMN	NFB	ACT	FNG	DHA
Cropping (C)	<.001	<.001	<.001	<.001	0.001	<.001
Depth (D)	0.105	0.853	0.899	0.166	0.056	<.001
Year (Y)	0.003	<.001	0.004	<.001	<.001	<.001
C × D	0.543	0.708	0.757	0.249	0.131	<.001
C × Y	<.001	<.001	0.006	0.104	0.242	<.001
D × Y	<.001	0.386	<.001	<.001	<.001	0.343
C × D × Y	0.466	0.731	0.554	0.355	0.310	0.228

TNM – total number of microorganisms; AMN – ammonifiers; NFB – nitrogen-fixing bacteria; FNG – fungi; ACT – actinomycetes; DHA – dehydrogenase.

In the first year of the study, the highest total number of microorganisms and the number of nitrogen -fixing bacteria were found in non-agricultural soil under native vegetation (NV) (Tab. 3). The highest number of ammonifiers and fungi was recorded in the soil under winter wheat grown in monoculture ($1F_{N100}$), while actinomycetes were the most abundant in unfertilized soil under 2- ($2UF_{cr}$) and 3-year rotation ($3UF_{cr}$) with incorporation of crop residues. Lower number of investigated microbial groups was obtained under cropping systems in the IOSDV trial, especially in soils under cropping systems with higher rates of applied mineral fertilizers ($4F_{N200}$ and $4F_{N200+cr}$). The highest dehydrogenase activity was recorded in non-agricultural soil (NV) and soil under winter wheat grown in monoculture ($1F_{N100}$), while the lowest values were measured in soils under manuring and mineral fertilization ($4F_{N100+mnr}$, $2F_{N100+cr}$ and $3F_{N100+cr}$).

In the second year of the study, the highest total number of microorganisms was found in the soil under unfertilized 2-year rotation ($2UF_{cr}$) (Tab. 4). Soils under unfertilized 3-year rotation ($3UF_{cr}$) were the most abundant in ammonifiers, while the highest number of nitrogen-fixing bacteria, fungi and dehydrogenase activity were found in non-agricultural soils under native vegetation (NV). Differences in the number of actinomycetes dependent on the cropping system were not observed. Microorganisms were the least numerous in soils with higher amounts of mineral fertilizers ($4F_{N200}$), while the lowest dehydrogenase activity was recorded in manured soil ($4F_{mnr}$), without the incorporation of crop residues.

Table 3. Microbial abundance and activity depending on cropping system at the depth of 0-20 cm in the first year of study (g^{-1} soil)

Cropping system	Total microbial number (CFU×10^6)	Ammoni-fiers (CFU×10^6)	N_2-fixers (CFU×10^6)	Fungi (CFU×10^4)	Actino-mycetes (CFU×10^4)	Dehydro-genase activity (μg TPF)
$4F_{mnr}$	108^{bc}	44^{ab}	249^{a}	12^{b}	69^{ab}	217^{bc}
$4F_{N100+mnr}$	141^{abc}	33^{ab}	181^{c}	13^{b}	66^{ab}	197^{c}
$4F_{N200}$	32^{c}	32^{ab}	141^{c}	10^{b}	66^{ab}	215^{bc}
$4F_{N200+cr}$	28^{c}	28^{b}	125^{c}	15^{ab}	69^{ab}	325^{bc}
$1F_{N100}$	167^{abc}	183^{a}	229^{b}	26^{a}	66^{ab}	402^{b}
$2UF_{cr}$	56^{c}	56^{ab}	361^{a}	24^{ab}	84^{a}	250^{bc}
$3UF_{cr}$	220^{ab}	37^{ab}	335^{a}	23^{ab}	81^{a}	385^{bc}
$2F_{N100+cr}$	162^{abc}	76^{ab}	266^{b}	23^{ab}	72^{ab}	186^{c}
$3F_{N100+cr}$	106^{bc}	169^{ab}	248^{bc}	20^{ab}	75^{ab}	197^{c}
NV	282^{a}	72^{ab}	401^{a}	15^{ab}	56^{b}	658^{a}
Average	130	73	254	18	70	303

*The different letters above the number indicate a significant difference at $P < 0.05$

$4F_{mnr}$ – fertilized 4-year rotation (manure); $4F_{N100+mnr}$ – fertilized 4-year rotation (mineral fertilizer, manure); $4F_{N200}$ – fertilized 4-year rotation (mineral fertilizer); $4F_{N200+cr}$ – fertilized 4-year rotation (mineral fertilizer, crop residues); $1F_{N100}$ – fertilized monoculture (mineral fertilizer); $2UF_{cr}$ – unfertilized 2-year rotation (crop residues); $3UF_{cr}$ – unfertilized 3-year rotation (crop residues); $2F_{N100+cr}$ – fertilized 2-year rotation (mineral fertilizer, crop residues); $3F_{N100+cr}$ – fertilized 3-year rotation (mineral fertilizer, crop residues); NV – native vegetation.

In general, the abundance of examined microbial groups across different cropping systems was high (10^4-10^6 per g^{-1} soil) (Tab. 3 and 4), indicating good quality of the tested soil. Favorable physical and chemical properties, stable structure and availability of nutrients of have contributed to a very high number microorganisms and high dehydrogenase activity in chernozem soil. Soils under wheat monoculture were more abundant in ammonifiers and fungi (first year), as well as actinomycetes (second year), compared to soils under wheat grown in crop rotation, contrary to the results obtained for other microbial groups. Comparison of cropping systems revealed the largest dehydrogenase activity in non-agricultural soils and soils under wheat grown in monoculture.

Non-agricultural soils as no-tillage systems under native vegetation had a stimulatory effect on the investigated microbial parameters. Similarly, Liang and Balser (2011) reported that the no-tillage or reduced tillage systems, in addition to adequate crop rotation, has shown to improve microbial properties associated to soil health and fertility. Cropping systems that keep organic material on the soil surface maintain higher levels of soil microbial and biochemical activities, thereby pointing to greater environmental sustainability (McGuire and Treseder, 2010). Soils exposed to disturbance by tillage can be more susceptible to reductions in soil microorganisms due to desiccation, mechanical destruction, soil compaction, reduced pore volume, and disruption of access to nutrient resources (Huang et al., 2013). Ploughing and tillage operations facilitate aeration in soil and exposure of soil to light and thereby increase the biological activity of organisms, particularly of bacteria (Roger-Estrade et al., 2010). A reduced tillage system reduces soil erosion by leaving 15 to 30% of residual material to cover the soil (Kumar and Goh, 1999).

Present study also showed that microbial abundance and activity varied with the crop rotation. There are similar studies reporting a significant effect of seasonal inputs of crop roots, rhizosphere products, and crop residues on soil microbial biomass and mineralizable C and N of this soil, illustrating the dependence of N dynamics on short-term C inputs and association of soil C/N ratio with changes in microbial community composition across different treatments (Carney and Matson, 2006). According to Coleman (2011), roots and above-ground plant growth are the most important sources of organic matter, so it is necessary to choose varieties and crop rotations considering how much residue will be generated, and how many months each year plants will be growing and creating organic matter. Sowing of certain crops also exerts a direct influence on soil microorganisms because the plants through root excrements stimulate the development of certain microbial communities and increase microbial biomass, especially in the rhizosphere soil. Our results are in agreement with the study of Yu et al. (2014) who reported that crop rotation with legumes maintains the favourable microbial population balance and thereby improve soil fertility.

Table 4. Microbial abundance and activity depending on cropping system at the depth of 0-20 cm in the second year of study (g^{-1} soil)

Cropping system	Total microbial number (CFU×10^6)	Ammoni-fiers (CFU×10^6)	N_2-fixers (CFU×10^6)	Fungi (CFU×10^4)	Actino-mycetes (CFU×10^4)	Dehydro-genase activity (μg TPF)
$4F_{mnr}$	104^{d}	50^{d}	80^{c}	8^{c}	28^{a}	136^{d}
$4F_{N100+mnr}$	297^{c}	84^{d}	180^{bc}	14^{bc}	37^{a}	170^{cd}
$4F_{N200}$	13^{d}	13^{d}	57^{c}	6^{c}	30^{a}	171^{cd}
$4F_{N200+cr}$	615^{a}	615^{b}	112^{bc}	10^{bc}	29^{a}	301^{cd}
$1F_{N100}$	301^{c}	369^{c}	153^{bc}	17^{bc}	38^{a}	706^{b}
$2UF_{cr}$	622^{a}	622^{b}	91^{c}	14^{bc}	34^{a}	336^{c}
$3UF_{cr}$	122^{d}	916^{a}	107^{c}	9^{bc}	37^{a}	436^{bc}
$2F_{N100+cr}$	446^{b}	302^{c}	274^{b}	25^{a}	37^{a}	705^{b}
$3F_{N100+cr}$	349^{c}	325^{c}	262^{b}	21^{ab}	28^{a}	540^{b}
NV	513^{ab}	370^{c}	516^{a}	32^{a}	24^{a}	972^{a}
Average	338	367	183	16	32	447

*The different letters above the number indicate a significant difference at $P < 0.05$

$4F_{mnr}$ – fertilized 4-year rotation (manure); $4F_{N100+mnr}$ – fertilized 4-year rotation (mineral fertilizer, manure); $4F_{N200}$ – fertilized 4-year rotation (mineral fertilizer); $4F_{N200+cr}$ – fertilized 4-year rotation (mineral fertilizer, crop residues); $1F_{N100}$ – fertilized monoculture (mineral fertilizer); $2UF_{cr}$ – unfertilized 2-year rotation (crop residues); $3UF_{cr}$ – unfertilized 3-year rotation (crop residues); $2F_{N100+cr}$ – fertilized 2-year rotation (mineral fertilizer, crop residues); $3F_{N100+cr}$ – fertilized 3-year rotation (mineral fertilizer, crop residues); NV – native vegetation.

In this study, fertilization did not positively affect the total number of microorganisms. Among different types of applied fertilizers, the best effect on microbial number and DHA enzyme activity was obtained by ploughing down crop residues, followed by a combination of manure and smaller doses of mineral fertilizers, and the lowest effect of sole use of mineral fertilizer or manure. The decline in microbial number and activity with inadequate use of mineral and organic fertilizers was also observed in numerous studies. The intensive use of soil, as well as excessive use of mineral fertilizers and pesticides, lead to loss of soil fertility, soil erosion and ground water pollution and can drastically modify the function and structure of soil microbial communities, thereby altering the normal functioning of agro-ecosystems (Girvan et al., 2004). Earlier studies have shown that incorporation of organic amendments increases biomass, activity and diversity of soil microorganisms (Gelsomino et al., 2004). According to Hartmann et al. (2015), fertilization scheme, the application and quality of organic fertilizers in particular, is the major determinant of soil microbial diversity. The addition of animal or green manures on organic plots provides a significantly greater input of organic carbon which increases microbial population (Kong et al., 2011). However, repeated application of organic fertilizers may present environmental hazards, as they introduce faecal microbial flora into soil and have the potential to alter the endogenous microbial structure (García-Orenes et al., 2013).

On average, significantly higher total number of microorganisms, number of ammonifiers and dehydrogenase activity were determined in the second year of the study, while the opposite results were obtained for nitrogen-fixing bacteria and actinomycetes. The number of fungi did not show significant differences over the years. Precipitation was higher in first year of study (data not shown), possibly indicating a positive impact on the microbial number in the investigated groups which are more sensitive to the optimal soil moisture.

Soil microorganisms are in balanced relationships which are directly related to the type of soil. The decreased number of microorganisms in deeper soil layers is characteristic of all soil types. In this study, increase in soil profile depth resulted in reduced values of the tested parameters of microbial activity (data not shown). The highest number and activity of microorganisms is concentrated in the surface layer of the soil (0-40 cm), which has the highest organic matter content, sufficient moisture and oxygen. Aerobic microorganisms, whose activity is of major importance for plant production, are the most frequent in the surface layer (Marinković et al., 2012). Similarly, Wardle et al. (2004) suggested that the number of microorganisms per soil depth is primarily affected by the content of organic matter and soil aeration. Deep layers contain fewer nutrients, environmental conditions are more unfavourable, and the number of microorganisms decreases. This could be an explanation for the small number of the microorganisms in deeper soil layers in this study.

Conclusions

Microbial abundance and activity were promoted by reduced tillage and fertilization, while incorporation of crop residues demonstrated a higher effect in comparison with other cropping technologies. Increase in microbial number and activity most likely occurred due to increases in organic matter and changes in organic matter quality. Development of sustainable agricultural strategies, based not only on crop productivity but also on ecological principles, is crucial to maintaining soil microbial communities as well as soil quality and fertility.

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Brojnost i aktivnost mikroorganizama u černozemu pri različitim sistemima gajenja

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Sažetak: Cilj ovih istraživanja bio je utvrđivanje mikrobiološke brojnosti i dehidrogenazne aktivnosti u černozemu u zavisnosti od sistema gajenja. Uzorci zemljišta su prikupljeni na višegodišnjim ogledima Plodoredi i IOSDV, na eksperimentalnom polju Rimski šančevi Instituta za ratarstvo i povrtarstvo. Zemljište je uzorkovano tokom dve godine iz deset sistema gajenja i tri dubine. Brojnost mikroorganizama utvrđena je indirektnom metodom razređenja, dok je aktivnost dehidrogenaze određena spektrofotometrijski. Brojnost ispitivanih grupa mikroorganizama i dehidrogenazna aktivnost značajno su varirali u zavisnosti od sistema gajenja i godine ispitivanja, dok je dubina uzorkovanja značajno uticala na aktivnost enzima. Najveća brojnost mikroorganizma utvrđena je u nepoljoprivrednom zemljištu i zemljištu pod neđubrenim dvopoljem i tropoljem, dok je najviša dehidrogenazna aktivnost zabeležena u nepoljoprivrednom zemljištu i zemljištu pod monokulturom pšenice.

Ključne reči: černozem, đubrenje, mikroorganizmi, plodored, sistem gajenja

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