

Effect of different soil usage on microbial properties in soils of Central Serbia

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Summary: Aim of the study was to examine the effects of soil use on soil microbial properties at five locations in Central Serbia. The study included 42 samples of soil under certified organic system, 8 samples under conventional system, and 30 samples of abandoned soils. The number of examined microbial groups was assessed using the indirect dilution method followed by plating of soil suspension on different selective media, while dehydrogenase activity was measured spectrophotometrically. The number and activity of microorganisms at the tested plots primarily depended on the soil pH. The acidic soil reaction caused more rapid fungal growth and lower presence of actinomycetes and *Azotobacter* sp. There were no significant alterations in the number of microorganisms, ammonifiers and fungi under the specific soil use system. Significantly higher dehydrogenase activity was recorded in the abandoned soils and soils under organic systems compared to the conventional farming.

Key words: ammonifiers, conventional farming, dehydrogenase, fungi, microbial number, microorganisms, organic farming, soil pH, soils

Introduction

Soil is a complex and dynamic ecosystem where substantial physical, chemical, and biological processes take place. Soil biological phase is predominantly formed by microorganisms, involved in the processes of soil formation, and in the processes essential for the functioning of this ecosystem. A gram of soil may contain more than ten billion microorganisms and thousands of different species (Schloss and Handelsman, 2006). The most important biological processes in soil (80-90%) occur due to microbial enzyme systems (Nannipieri et al., 2003). The most numerous among the soil microorganisms are bacteria (10^8 - 10^9 g⁻¹ soil), actinomycetes (10^7 - 10^8 g⁻¹ soil), fungi (10^6 - 10^7 g⁻¹ soil), and algae (10^4 - 10^5 g⁻¹ soil) (Chen et al., 2003). However, reliable description of the soil microbial community structure, diversity and their function in soil ecosystems remains still unclear (Stagnari et al., 2014).

The presence of various systematic and physiological groups of microorganisms, the abundance of specific genera and species, and the activity of

microbial enzymes, are indicators of overall soil microbiological activity. Soil microorganisms are found in specific relationships that are characteristic of each soil type and climate area. Balanced community structure is essential for ecological functioning of soil microorganisms (Stagnari et al., 2014), while changes in soil microbial diversity have a great impact on the soil ecosystem stability. Decreased diversity, abundance and activity of microorganisms indicate contaminated or degraded soil and its low fertility.

Soil chemical and physical characteristics are major determinants of soil microbial community structure (Rousk et al., 2010). The most favourable conditions for microbial development and biochemical activity were found in neutral soils, with a good soil structure, suitable water-air regime, and organic matter content above 2% (Riches et al., 2013). Bacteria were most frequently found in neutral soils, increased presence of fungi and acidophilic bacteria was found in acidic soils, while alkalophilic bacteria and actinomycetes were present in alkaline soils (Rousk et al., 2010; Castaneda et al., 2015).

The indigenous microbial communities of each soil are unique for the particular soil, as they have been shaped and evolved over time in accordance with the dynamics of the local habitat (Nazir et al., 2013). Soil microorganisms play a crucial role in biochemical cycling of nutrients, maintaining soil health and fertility (Wang et al., 2016). The essential nutrition elements in

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the soil are predominantly found in forms that are not directly available to plants. Through the activity of their enzymes, microorganisms provide plants with assimilates and directly participate in the formation of crop yield. Consequently, successful plant production should provide conditions for undisturbed development and biochemical activity of microorganisms in order to achieve high yields and preserve the ecological balance in the soil. Increase and overuse of inorganic fertilizers and pesticides, and intensive human activity significantly affects the environment. The accumulation of chemical inputs leads to soil degradation, loss of agricultural soil productivity in the long run, and reduce availability and quality of groundwater. Fertility and crop protection management in conventional agricultural systems exert significant negative impact on soil microbial communities with far-reaching consequences. Organic farming systems, as an alternative to conventional agriculture, suggest reduced impact of agricultural measures on environment and soil quality (Pelletier et al., 2008; Wang et al., 2016). Integrated results obtained from monitoring of appropriate biological, chemical and physical indicators provide more reliable information about the influence of the agricultural systems on microbial biodiversity and consequently on soil fertility (Stagnari et al., 2014).

The complex response of microbial diversity and activity to soil usage is still poorly understood, so the aim of this research was to evaluate and compare microbial properties in abandoned soils and in soils under conventional and organic agricultural management.

Materials and Methods

Soil samples

Soil samples were collected at five locations on the territory of Central Serbia. Areas were determined based on the concentration of abandoned arable land with regard to the number of organic producers (data from the database Serbia Organica). Field survey and soil sampling, which was conducted in the period from August to October 2013, collected a total of 80 soil samples of annual plant species, namely 42 soil samples under certified organic production, 8 samples under conventional production, and 30 samples of abandoned soil. Soil samples were collected randomly in five replicates at depth 0-30 cm. Soil samples for microbiological analysis were immediately stored at 4°C, and maintained until laboratory analysis.

Soil chemical analysis

Soil chemical analyses were conducted on air-dried samples by the following methods: determination of the active acidity - pH in H₂O was determined in a suspension of soil with water (10 g; 25cm³), potentiometrically; determination of the potential acidity - pH in 1 M KCl - determined in a suspension of soil with potassium chloride (10g; 25cm³), potentiometrically; CaCO₃ content - with Scheibler calcimeter; humus content

- by the method of Tjurin (Potassium dichromate - K₂Cr₂O₇); total content of nitrogen - by CHNS analyzer; available phosphorus (extraction with ammonium lactate - AL method); phosphorus content by the blue method in a spectrophotometer; available potassium (extraction with ammonium lactate - AL method); potassium content determined by the flame photometer.

Soil microbiological analysis

The abundance of examined microbial groups was assessed by a dilution plate method on the appropriate nutritive media. Soil dilutions were prepared by adding 10 g of soil to 90 ml of sterile water. The total number of microorganisms was determined on a soil agar (5 days, at 28°C), the number of *Azotobacter* sp. and free N₂-fixers on nitrogen-free medium (Fyodorov's medium) (48 h and 5 days, respectively, at 28°C). The number of ammonifiers was determined on a meat peptone agar (3 days, at 28°C), actinomycetes on Krasilnikov's agar (7 days, at 28°C), and fungi on Czapek-Dox agar (5 days, at 28°C). All microbiological analyses were performed in three replications, and the average number of colony forming units (CFU) was calculated per 1.0 g of soil dry weight. Soil enzyme dehydrogenase activity - DHA (EC 1.1.1) was done according to the Casida et al. (1964) method using TTC (2,3,5-triphenyltetrazoliumchlorid), and following the formation of TPF (1,3,5 triphenylformazan) spectrophotometrically at 485 nm. The results were expressed as µg TPF g⁻¹ dry soil.

The variables were analysed using two-way analysis of variance (ANOVA), followed by mean separation according to Tukey's test at the P<0.05 level of probability.

Results and Discussion

Soil chemical properties

Chemical properties of soils are presented in Table 1. The average results showed that soil samples were acidic (pH 5.00) or slightly acidic (pH 5.83); slightly (0.27%) to moderate in CaCO₃ content (2.20 and 4.00); adequate (3.5-4.3%) and low (2.86%) in organic matter content; low (5.13 mg 100 g⁻¹) to moderate (12.21; 19.20 mg 100 g⁻¹) in phosphorus, adequate (20.57-29.08 mg 100 g⁻¹) in potassium content.

Substitutional acidity test results confirm the fact that acidic soils are the predominant soil type in central Serbia, which is primarily associated with geological substrates and other natural factors. Abandoned soils are mostly acid, and soils under organic and conventional production are slightly acid (Table 1). The highest numbers of samples on abandoned soils are in the class of highly acid and acid soils (23 soil samples). Neutral and slightly alkaline soils are less frequent, while the largest share of these soils was found at plots under organic and conventional production (18 soil samples).

Humus is a significant part of soil as it provides soil nutrients and plays a role in maintaining soil fertility.

Soil organic matter enhances soil physical and chemical properties, increases cation exchange capacity, ameliorates soil aggregate and structure, binds contaminants into unavailable forms (Al, Cu, Pb), provides energy for soil microbial community and has the key role in beneficial biological processes (Wolf & Wagner, 2005; Salazar et al., 2011). Humic and highly humic soils (60%) were mostly found in the tested abandoned plots and under organic production system. Lower humus amount (below 3%) was found at soils under conventional production (Table 1).

Soil microbial properties

In terms of diversity of the tested soils, the number and enzymatic activity of microorganisms depended on soil pH, but also on soil usage. Previous studies highlight a significant correlation between soil pH and organic matter content and composition of soil microbial communities (Nazir et al., 2010; Pereira et al., 2011).

The abundance of all examined groups of soil microorganisms as well as dehydrogenase activity has been changed along the pH gradient. The highest number of all physiologically different groups of bacteria (total number, ammonifiers, and free N fixers) was found in soils with pH > 6.51 (Table 2).

Species within the genus *Azotobacter* are one of the most important aerobic, free-living, nitrogen fixing bacteria in agricultural soils in our climate region. The presence, distribution and abundance of *Azotobacter* sp. is mainly determined by environmental factors, soil characteristics (pH, nutrients and the organic matter content), the presence of heavy metals and pollutants, and vegetation composition (Bhatia et al., 2009;

Kizilkaya, 2009). The optimum pH for growth of *Azotobacter* strains is 7-7.5, therefore its population is the most numerous in neutral or alkaline soils (up to 10^4 g⁻¹ of soil). Since these bacteria are especially susceptible to soil acidification, *Azotobacter* strains are generally absent or present in very low number in soils with pH < 5.0 (Barnes et al., 2007). In the examined soils, *Azotobacter* sp. was not detected at pH < 4.5, a notable presence (340 CFU g⁻¹ soil) was recorded at pH 5.51-6.50, with significantly higher abundance (730 CFU g⁻¹ soil) at pH > 6.51 (Table 2). Considering that *Azotobacter* strains are sensitive to adverse environmental conditions, the presence and size of *Azotobacter* sp. population are indicators of soil quality, soil health and fertility, and have a substantial role in agricultural practices and management application (Kizilkaya, 2009)

Filamentous fungi are very important in the decomposition of complex organic compounds, mineralization of organic matter and humus synthesis. These are mostly acidophilic microbes which prefer soils with a lower pH, but they are also abundant in neutral environments (Rousk et al., 2008). Most of investigated soils are characterized by acidic pH, which led to intensive development of fungi. Soil pH affected the abundance of both fungal and bacterial communities, with stronger effects on the latter. The significant presence of filamentous fungi ($36-70 \times 10^3$ CFU g⁻¹ soil) was recorded in all soil samples (Table 2).

This result corroborates previous studies that showed strong influence of soil pH on bacterial community composition and the positive correlation between bacterial diversity and higher soil pH (Aciego Pietri & Brookes, 2009; Lauber et al., 2009; Rousk et al.,

Table 1. Chemical soil properties of examined fields according to soil use management

	pH		CaCO ₃ %	Humus %	Total N %	AL-P ₂ O ₅ mg 100 g ⁻¹	AL-K ₂ O mg 100 g ⁻¹
	in KCl	in H ₂ O					
Abandoned soils	5.00	6.17	0.27	3.50	0.242	5.13	20.57
Organic cropping system	5.83	6.95	4.00	4.30	0.290	19.20	29.08
Conventional cropping system	5.83	6.87	2.20	2.86	0.207	12.21	20.86

Table 2. Microbiological soil properties of examined fields according to soil pH

pH gradient	<i>Azoto-</i> <i>bacter</i> sp.	Total microbial number ×10 ⁵	Ammonifiers ×10 ⁵	Free N fixers ×10 ⁴	Fungi ×10 ³	Actino- mycetes ×10 ³	Dehydrogenase activity
< 4.5	0 b	276 ab	99 b	211 b	59 ab	2 b	343 b
4.51-5.50	20 b	310 ab	114 ab	220 b	70 a	6 b	806 ab
5.51-6.50	340 ab	305 ab	145 ab	246 ab	36 b	10 b	1059 a
> 6.51	730 a	397 a	166 a	328 a	50 ab	29 a	1117 a

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

2010). The highest bacterial growth rate was observed at the neutral pH and declined toward the lower pH values. The hypothesis which could explain bacterial community structure due to soil pH is that most bacterial taxa exhibiting a relatively narrow pH optimum. Deviations of 1.5 pH units from in situ pH reduce bacterial activity by 50% (Fernandez-Calvino & Baath, 2010). In contrast, fungal species which usually exhibit a wider pH for optimal growth, ranged from 5 to 9 pH units with no significant growth inhibition (Nevarez et al., 2009). The weaker pH influence on the fungal community is consistent with pure culture studies (Rousk et al., 2010).

Actinomycetes are a specific group of bacteria which produce a number of enzymes that are involved in organic matter turnover, decompose of complex carbon sources (cellulose, lignin, pectin, chitin), and participate in humus formation (Fließbach et al., 2007; Li et al., 2012). Unlike fungi, actinomycetes are usually more abundant in neutral and slightly alkaline soils (Sreevidya et al., 2016). Predominantly acidic pH of the examined soils caused poor presence of actinomycetes, therefore on certain plots their notable presence was not recorded ($< \times 10^3$). The highest number of actinomycete colonies (29×10^3 CFU g^{-1} soil) was observed in soil samples where pH was higher than 6.51 (Table 2).

Dehydrogenases are among the most important enzymes in soil ecosystems because of their significant function in biological oxidation of organic matter (Zhang et al., 2010). Dehydrogenase activity is proportional to biomass of soil microorganisms and represents a reliable marker of soil biological properties. Dehydrogenases are constitutive enzymes of all living microorganisms, therefore its activity is considered as an indicator of overall soil microbial activity (Salazar et al., 2011; Yuan & Yue, 2012). Environmental factors, such as temperature, oxygen availability, soil moisture, pH, lack of organic matter, contamination with heavy metals, fertilizers and pesticides could impact dehydrogenase activity. The research indicated significant connection between dehydrogenase activity and soil pH. The lowest

DHA activity ($343 \mu g$ TPF g^{-1} soil) was recorded in very acid soils (pH < 4.5), and raised along with increased pH, reaching its maximum ($1117 \mu g$ TPF g^{-1} soil) between 6.51-8.2. The obtained results confirmed previous investigations which noted significantly positive correlations between dehydrogenase activity and soil pH (Fernandez-Calvino et al., 2010; Natywa & Selwet, 2011). The optimum soil pH for DHA ranged between 6.6–7.8 (Brzezinska et al., 2001; Ros et al., 2003), suggesting that acidic conditions suppress enzyme activity which results intense DHA inhibition (Levyk et al., 2007; Fernandez-Calvino et al., 2010). These findings emphasize the pH value as one of the best predictor of dehydrogenase activity in the soil (Moeskops et al., 2010).

The overall response of microbial communities to agricultural management is highly complex and diverse (Stagnari et al., 2014). Organic farming improve soil ecosystem quality (Cavigelli et al., 2013) through enhancement of soil nutrient status and soil enzyme activity, but the overall impact of agricultural management on composition, richness and diversity of soil microbial populations is still insufficiently understood (Stagnari et al., 2014).

The results in our study showed that soil usage significantly affected the abundance of *Azotobacter* sp., free-living N fixing bacteria and actinomycetes, as well as dehydrogenase activity. The greatest number of these bacterial groups was observed in organic agriculture fields, significantly higher than in abandoned soils (Table 3). Free-living N fixers and actinomycetes are considered to be sensitive to acid pH and lack of mineral nutrients (especially phosphorus). Soil pH was < 5.51 in most of the abandoned soils (77%), while P content was very low, which could explain the obtained results.

However, very subtle changes within abundance of fungi and all tested bacterial groups were observed due to agricultural management practices (Table 3). Some previous studies showed that soils under conventional management had a significantly higher diversity and number of free-living diazotrophic bacteria than those under organic production (Orr et al., 2011). Other

Table 3. Microbiological soil properties of examined fields according to soil usage

	<i>Azoto-</i> <i>bacter</i> sp.	Total microbial number $\times 10^5$	Ammoni- fiers $\times 10^5$	Free N fixers $\times 10^4$	Fungi $\times 10^3$	Actino- mycetes $\times 10^3$	Dehydro- genase activity μg TPF g^{-1} soil
	CFU g^{-1} soil						
Abandoned soils	140 b	282 a	136 a	189 b	60 a	6 ab	835 a
Organic cropping system	450 a	341 a	129 a	298 a	53 a	18 a	851 a
Conventional cropping system	340 ab	329 a	121 a	268 ab	48 a	11 ab	561 b

contrast observations denoted that abundance and diversity of some microbial groups increased under organic systems (Klaus et al., 2013; Wang et al., 2016), and pointed out fertility source (organic manure and mineral fertilizer) as the dominant factor in shaping bacterial communities (Esperschütz et al., 2007) and bacterial diversity (Jangid et al., 2008). Studies that are more similar with our results indicate that two management systems have little effect on the diversity and number of both the nitrogen fixing and the total bacterial community (Sugiyama et al., 2010; Li et al., 2012; Orr et al., 2012).

In a contrast, dehydrogenase activity was lowest in conventional fields (561 $\mu\text{g TPF g}^{-1}$ soil), significantly lower than activity of both, organic fields (851 $\mu\text{g TPF g}^{-1}$ soil) and abandoned soils (835 $\mu\text{g TPF g}^{-1}$ soil). Reduced humus content is most often caused if organic manure is not applied and harvest residue are burnt during a longer period, which ultimately results in reduced humus mineralization. Fresh organic matter is introduced into the soil through application of organic fertilizers (manure, compost, green manure). Higher organic matter content increased microbial activity, resulting in intense dehydrogenase activity in soils in organic cropping system (Table 3) and high humus synthesis (humus content 4.3%, Table 1). Significant dehydrogenase activity, observed in abandoned soils (Table 3) could be explained by intense activity of autochthonous populations of microorganisms in undisturbed, natural environment. Higher humus content (3.5 %, Table 1) is characteristic of the soils which are not under continuous cultivation, more intensive humification process, and overall organic matter production remains at the same place. Increased soil microbial respiration, under organic compared to conventional management was already reported (Araujo et al., 2009). These results suggest high biological activity and rapid decomposition of organic residues that make nutrients available for plants. Soil organic matter content has significant effects on microbial growth, biomass and enzymatic activities. Previous studies emphasized a positive correlation between soil DHA and organic matter content (Zhao et al., 2010; Yuan & Yue, 2012).

High humus content and high dehydrogenase activity in soils under organic agriculture management indicate a positive influence of organic fertilizer application and reduced use of chemicals on soil characteristics. On the other hand, lower content of organic matter and significantly lower dehydrogenase activity in soils under conventional production system indicate a negative impact of intensive human activities on soil ecosystem.

In Serbia, organic production occupies an area of more than 11,000 ha (0.25% of total arable land). The recorded 400,000 hectares of arable land (10% of the cultivated land) is under the category of uncultivated land (Vasin et al., 2013). Since this land had spent a long

period of time at rest without application of agrochemicals, it therefore has good potential for use in organic production.

Conclusions

Soil pH had the highest effect on the diversity, number and activity of microorganisms on the tested plots. The highest abundance of the tested bacterial groups, actinomycetes and dehydrogenase activity was found in soils with pH > 6.51. Predominantly acid soil pH caused more rapid fungal growth. The highest number of *Azotobacter* sp., free-living N fixing bacteria and actinomycetes was recorded in fields under organic production system, while the total number of microbes, ammonifiers and fungi was not changed due to soil use. Dehydrogenase activity was significantly lower in soils under conventional management system than in organic fields and abandoned soils.

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Efekat različitih načina korišćenja zemljišta na mikrobiološka svojstva zemljišta centralne Srbije

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Sažetak: Cilj ovih istraživanja bio je da se ispita uticaj načina korišćenja zemljišta na mikrobiološka svojstva. Istraživanja su sprovedena u pet oblasti na teritoriji centralne Srbije. Analizirana su 42 uzorka zemljišta pod sertifikovanom organskom proizvodnjom, 8 uzoraka zemljišta u konvencionalnoj poljoprivrednoj proizvodnji i 30 uzoraka napuštenog zemljišta. Brojnost mikroorganizama određena je indirektnom metodom, zasejavanjem suspenzije zemljišta odgovarajućeg razređenja na selektivne hranljive podloge, dok je aktivnost enzima dehidrogenaze određena spektrofotometrijski. Na raznovrsnost, brojnost i aktivnost mikroorganizama na ispitivanim parcelama prvenstveno je uticala pH reakcija zemljišta. Kisela pH reakcija na većini ispitivanih lokaliteta uslovlila je intenzivniji razvoj gljiva i manje prisustvo aktinomiceta i *Azotobacter* sp. Ukupan broj mikroorganizama, brojnost amonifikatora i gljiva nije se značajno menjao u zavisnosti od načina korišćenja parcele. Značajno viša prosečna aktivnost dehidrogenaze zabeležena je u napuštenim zemljištima i zemljištima u organskom sistemu gajenja u poređenju sa zemljištima u konvencionalnoj proizvodnji.

Ključne reči: amonifikatori; brojnost mikroorganizama; dehidrogenaza; gljive; konvencionalna proizvodnja; mikroorganizmi; organska proizvodnja; pH zemljišta; zemljište

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