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SOIL MICROBIAL PROPERTIES UNDER DIFFERENT MANAGEMENT SYSTEMS IN SOYBEAN PRODUCTION

ABSTRACT: The aim of the study was to examine the effects of management practice on microbial properties of soil under soybean production. The study included 180 samples of soil under certified organic soybean production and 80 samples in conventional production system. An abundance of the examined microbial groups was assessed using the indirect dilution method, followed by plating of soil suspension on different selective media, while dehydrogenase and β -glucosidase activity was measured spectrophotometrically. Our data indicated that the management practice affected the structure and activity of microbial communities. A significant positive effect of organic farming on *Azotobacter* spp., free N-fixers and abundance of actinomycetes was identified. The influence of management system for the total number of bacteria, ammonifiers and fungi was not observed. Significantly higher dehydrogenase and β -glucosidase activity was recorded in the soils under organic farming compared to the conventional farming. The obtained results showed an increase in organic matter content, associated with organic soil management, and its positive correlation with soil microbial properties.

KEYWORDS: dehydrogenase, β -glucosidase, microbial abundance, organic and conventional management, soybean

INTRODUCTION

The presence and activity of microbial communities have a crucial role in numerous biochemical cycles important in the functioning of soil ecosystem. Soil microorganisms play the key role in organic matter decomposition, transformation, mineralization and release of carbon, nitrogen, phosphorus, sulfur and other nutrients important for plant nutrition. Soil microbes are also involved,

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either directly or indirectly, in many other processes which provide stability to the soil ecosystem, such as control of erosion through the formation of stable soil aggregates and soil structure, pesticide degradation, pest and disease regulation and bioremediation (Nannipieri et al., 2003).

Microbial abundance, diversity and activity are important indicators of soil quality. Relationships between the stability and functioning of the soil ecosystem and the changes in microbial community structure are very complex (Stagnari et al., 2014). Intensification of agriculture, use of cultivation techniques in conventional production and intensive anthropogenic activities cause serious soil degradation. Microbial community structure is mostly determined by soil chemical and physical characteristics, but soil management practices have the potential to modify the biomass, diversity and physiology of microbes in agricultural fields. Agricultural practice has a complex and diverse influence on soil microorganisms and composition of their communities (Wang et al., 2016). Conventional agriculture relies on the use of synthetic mineral fertilizers and pesticides, which cause adverse impacts on soil health and productivity. However, they are also regarded as the primary factors which induce negative changes in soil microbial population, while shaping their size and structure. Organic production minimizes the impact of agricultural practice on soil quality and the environment, and represents the best alternative to conventional production. Approximately 1% of the world's arable land is under organic soil management (Lori et al., 2017) with a 14% annual increase in surface area (Wang et al., 2016). Organic farming improves soil physical and chemical properties, while the application of different organic fertilizers enriches soil by providing it with organic matter, and thus enhances soil fertility. Organic farming system aims to close nutrition cycles and this concept relies heavily on microbial activity. Soils managed organically exhibit higher microbial diversity, biomass and activity. However, complex and diverse overall response of soil microbial populations to soil management type is still insufficiently understood (Stagnari et al., 2014).

The impact of agricultural management on soil microbial abundance, diversity and activity is very complex and diverse. For this reason, the aim of the study was to identify and compare microbial community abundance and enzymatic activity in soils under organic or conventional farming systems.

MATERIALS AND METHODS

Soil samples were collected from several fields under organic soybean production at the location Čurug–Gospodinci, AP Vojvodina. The largest areas under organic soybean production in AP Vojvodina were registered under Global Seed d.o.o. (Čurug), at the total area of 390 ha (soybean variety Rubin on 170 ha, NS Maximus on 110 ha, Fortuna on 40 ha, varieties Galina and Valjevka on 35 ha, each). Organic management practice had been applied for at least three consecutive years prior to sampling all the tested plots.

Samples of soil under conventional soybean production were collected at several fields at the location Rimski Šančevi – Čenej (AP Vojvodina). A total of 180 samples of soil under certified organic production and 80 samples of soil under conventional production were collected. Soil samples were collected randomly from soybean rhizosphere, at 0–20 cm, at the beginning of June, in the period of soybean full bloom (R3), while the number of samples depended on the size of the plot. Soil samples were stored for microbial analysis at 4 °C, and maintained until laboratory analysis.

Soil chemical analyses were conducted on air-dried samples, as previously described (Marinković et al., 2018).

The abundance of the total and specific bacterial and fungal communities was assessed by an indirect dilution spread-plating method on an appropriate nutritive media. The total number of bacteria was determined on a soil agar, ammonifiers on a nutrient agar (NA), *Azotobacter* spp. and free N-fixing bacteria on a nitrogen-free medium (Fyodorov's medium), fungi on a Czapek-Dox agar, and actinomycetes on a synthetic medium (Krasilnikov's agar). Plates were incubated at the temperature of 28 °C, while incubation time depended on the tested microbial group. The number of *Azotobacter* spp., ammonifiers and actinomycetes was detected after 2, 3 and 7 days respectively, while the total number of bacteria, free N-fixing bacteria and fungi was recorded after 5 days. After incubation, the average number of colony forming units (CFU) was calculated at 1.0 g of absolutely dry soil (Briones and Reichardt, 1999). Dehydrogenase activity (DHA) (EC 1.1.1.) was done by measuring the extinction of colored triphenylformazan (TPF) formed by reducing a colorless triphenyl-tetrazolium chloride (TTC) (Casida et al., 1964). TPF concentration was measured at 485 nm, and the results were expressed as $\mu\text{g TPF g}^{-1}$ dry soil. Activity of β -glucosidase (β -glc) (EC 3.2.1.21) was determined by measuring the extinction of colored p-nitrophenol, formed by reducing a colorless p-nitrophenyl β -d-glucoside (PNG) (Hayano, 1973). The intensity of the yellow colored p-nitrophenol was measured at 400 nm and the average β -glc was expressed as an enzyme unit. One unit of enzyme was defined as 1 μmol of p-nitrophenol released per min at 30 °C. All microbiological analyses were performed in three replications.

The variables were analyzed 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 DISCUSSIONS

Chemical characteristics of soils under organic and conventional agriculture production are presented in Table 1. The results showed that soil samples under both organic and conventional production were slightly alkaline (pH 7.26–7.42), and only one sample was acidic (pH 5.43). Soils under organic farming belong to the class of humic soils (> 3%), while soils samples in conventional fields were characterized by the lower humus content (< 3%) (Table 1).

Table 1. Chemical soil properties of the examined fields

Sample no.	Agricultural 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					
1	Organic	7.33	8.10	6.76	3.57	0.245	22.9	24.00
2	Organic	7.26	8.06	4.22	3.52	0.241	16.2	22.00
3	Organic	7.27	8.07	5.07	3.45	0.235	12.10	21.00
4	Organic	7.27	8.03	8.44	3.52	0.241	12.20	25.00
5	Organic	5.43	6.78	0.76	3.47	0.238	4.50	19.00
6	Conventional	7.24	8.29	7.16	2.19	0.163	27.76	29.23
7	Conventional	7.38	8.22	3.38	2.58	0.192	27.90	25.00
8	Conventional	7.42	8.26	4.64	2.62	0.195	26.90	23.91
9	Conventional	7.38	8.21	3.80	2.65	0.197	27.40	39.81
10	Conventional	7.37	8.16	4.64	2.38	0.177	25.50	32.37

Organically managed soils enhance microbial community structure, abundance, diversity and activity.

Table 2. Microbial abundance and activity depending on the management system

Sample no.	Agricultural management	Soybean variety	<i>Azoto-bacter</i> spp.	CFU g ⁻¹ soil							β-glc (mU g ⁻¹ soil)
				AMN ×10 ⁷	TNB ×10 ⁷	FNB ×10 ⁶	FNG ×10 ⁴	ACT ×10 ⁴	DHA (μg TPF g ⁻¹ soil)		
1	Organic	Rubin	1310 a	173 ab	162 a	230 a	19 a	17 a	318 a	74.6 a	
2	Organic	Maximus	1150 a	211 a	148 ab	231 a	20 a	20 a	217 ab	75.7 a	
3	Organic	Galina	690 b	206 a	160 a	194 ab	17 a	20 a	309 a	82.5 a	
4	Organic	Valjevka	740 b	192 a	193 a	243 a	14 a	19 a	314 a	79.0 a	
5	Organic	Fortuna	2 c	135 b	121 ab	77 c	28 a	3 b	35 c	71.4 a	
	Organic	Average	778 A	183 A	157 A	195 A	20 A	16 A	239 A	76.6 A	
6	Conventional	Rubin	0 c	204 a	157 a	190 ab	25 a	7 ab	60 bc	47.2 b	
7	Conventional	Maximus	2 c	147 b	143 ab	105 b	17 a	6 ab	73 bc	34.7 b	
8	Conventional	Galina	0 c	191 a	192 a	122 b	18 a	12 ab	110 b	35.6 b	
9	Conventional	Valjevka	1 c	213 a	163 a	138 b	28 a	8 ab	83 bc	39.8 b	
10	Conventional	Fortuna	1 c	197 a	136 ab	125 b	16 a	11 ab	63 bc	50.4 b	
	Conventional	Average	1 B	190 A	158 A	136 B	21 A	9 B	78 B	41.5 B	

AMN – ammonifiers; TNB – total number of bacteria; NFB – nitrogen-fixing bacteria; FNG – fungi; ACT – actinomycetes; DHA – dehydrogenase; β-glc – β-glucosidase.

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

The results of the study showed significant increase in the abundance of *Azotobacter* spp., free N-fixing bacteria, actinomycetes and dehydrogenase and β-glucosidase activity in soils under organic management. Influence of

soil management system was not significant for the total number of bacteria, ammonifiers and fungi (Table 2). The two different soil management systems did not significantly affect the total bacterial population and fungal abundance in the previous studies (Scullion et al., 1998; Stagnari et al., 2014; Marinkovic et al., 2018), which is in accordance with our results. The total number of bacteria and fungi were similar in both systems due to their diversity and ability to adapt and grow under various environmental conditions (Anand et al., 2006).

Azotobacter spp. is among the most important free-living, heterotrophic, aerobic soil bacteria, capable of fixing an average of 20 kg N ha⁻¹ per year (Jnawali et al., 2015). *Azotobacter* strains are very sensitive to adverse environmental conditions, and their presence is therefore considered an indicator of soil quality, health and fertility (Kizilkaya, 2009). Favorable conditions in the soils under organic production – slightly alkaline pH and organic matter content above 3%, resulted in an abundance of *Azotobacter* spp. (690–1310 CFU g⁻¹ soil) and free N-fixers (194–243×10⁶ CFU g⁻¹ soil) (Table 2). A significant decrease in the presence of *Azotobacter* spp. (2 CFU g⁻¹ soil), free N-fixers (77×10⁶ CFU g⁻¹ soil) and low phosphorus content were noted in sample no. 5 with pH < 5.43. *Azotobacter* strains are sensitive to acidic pH and, for this reason, its population is the most abundant in neutral and slightly alkaline soils (Barnes et al., 2007). Samples of soil under conventional production also had slightly alkaline pH, so *Azotobacter* spp. strains were recorded in extremely low number or not detected at all (Table 2). The average abundance of free N-fixers (136×10⁶ CFU g⁻¹ soil) was also significantly lower compared with their presence in fields under organic production (Table 2). Previous studies showed that populations of different bacterial N-fixing genera were more abundant under organic soil management (Jangid et al., 2008; Orr et al., 2012; Stagnari et al., 2014). Extensive use of mineral fertilizers and pesticides may reduce the number of *Azotobacter* spp. and other nitrogen fixing bacteria in the soil (Khudhur and Askar, 2013; Jnawali et al., 2015; Shaid et al., 2019). Considering that soil chemical analysis conducted in our research did not reveal high concentrations of P and K in fields under conventional management, excessive pesticide application is assumed to have an extreme negative effect on *Azotobacter* spp. population. Nitrogen fixing bacteria are known for their sensitivity to pesticides (Moreno et al., 2009; Orr et al., 2012). Some pesticides may therefore cause deleterious impact to *Azotobacter* populations (Shahid et al., 2019) and nitrogen fixers (Walvekar et al., 2017).

Actinomycetes are involved in organic matter turnover, nutrient recycling, humus synthesis, but they are also known for their ability to produce antimicrobial compounds (Aislabie and Deslippe, 2013). Through the production of extracellular enzymes, these microbes are able to degrade complex organic compounds, including lignin, cellulose, chitin and pectin, into less recalcitrant molecules (Eilers et al., 2010; Li et al., 2012). Actinomycetes have a strong negative relationship with soil pH, thus being more abundant in neutral and slightly alkaline soils (Sreevidya et al., 2016). The significantly lowest number of actinomycetes (3×10⁴ CFU g⁻¹ soil) (Table 2) was recorded in the acidic soil sample. Soil management significantly affected the number of actinomycetes, while organic farming showed higher average values (16×10⁴ CFU g⁻¹ soil) than

the conventional (9×10^4 CFU g^{-1} soil) (Table 2). Increase in organic carbon sources, associated with organic fertility management, had a positive correlation with the population of actinomycetes (Chen et al., 2018). Recent studies confirmed a significant influence of soil management system and an enhanced population of actinomycetes in soils under organic farming (Stagnari et al., 2014; Chen et al., 2018).

Enzymatic activity has been proposed as an integrative determinant of soil quality, because of the key role in numerous biochemical and nutrient-cycling processes in soils (Stott et al., 2010). Dehydrogenase is widely used as a reliable indicator of the total oxidative activity of soil microorganisms and overall soil metabolic activity (Nannipieri et al., 2003). Unfavorable environmental conditions, such as low/high temperature, low/high pH, lower content of organic matter, and presence of heavy metals, fertilizers and pesticides, could have an adverse impact on dehydrogenase activity. The lowest DHA ($35 \mu\text{g TPF g}^{-1}$ soil) (Table 2) was recorded in the acidic soil sample, confirming significant relation between dehydrogenase activity and soil pH (Fernandez-Calvino et al., 2010). Microbial communities in soils under organic production showed DHA values up to three times higher than those in soils under conventional production. Significantly higher average DHA was recorded in organic ($239 \mu\text{g TPF g}^{-1}$ soil) than in conventional systems ($78 \mu\text{g TPF g}^{-1}$ soil) (Table 2). Significant differences in DHA between production systems could be due to lower soil organic matter content in conventional fields. Previous research emphasized a positive correlation between soil DHA and organic matter content (Zhao et al., 2010; Yuan and Yue, 2012). The β -glucosidase enzyme catalyzes the final step of cellulose hydrolysis, and plays a major role in degradation of plant residues (Stott et al., 2010). The activity of β -glucosidase is sensitive to changes in crop residue management, but could also be an early indicator of changes in soil organic matter content (Stott et al., 2010). The average β -glucosidase activity in the fields under organic management was significantly higher (76.6 mU g^{-1} soil) than under conventional management (41.5 mU g^{-1} soil) (Table 2). The results confirm that β -glucosidase activity is sensitive to changes caused by differences in soil management systems. Application of organic materials in organic production results in increased β -glucosidase activity. Elevated levels of β -glucosidase activity are confirmed in the previous research (De la Hora et al., 2003; Acosta-Martínez et al., 2007; Stott et al., 2010).

Previous studies showed that soil pH is one of the most important factors affecting abundance and diversity of bacterial populations, with the optimum pH close to neutral (Lauber et al., 2009). Our study confirmed significantly the lowest number of azotobacter, free N-fixers, actinomycetes, and dehydrogenase activity in the acidic soil sample. Soil organic matter substrate to soil microorganisms and it is an essential plant nutrient source through mineralization (Haynes, 2005). Soils in AP Vojvodina naturally have a high content of organic matter, but inadequate cultivation practices applied during a longer period caused significant reduction in soil organic matter content (Vasin et al., 2013). Organic matter declines significantly after an extended time of agricultural production (Powlson et al., 2012), and it is lower in some agricultural soils

than in abandoned soils of the same type (Marinković et al., 2018). Organic management has a positive effect on soil organic matter content through incorporation of manure, compost, green manure, and plowing crop residues (Wang et al., 2016). Increased organic matter content in organic farming positively influences microbial growth, biomass and enzymatic activity (Lori et al., 2017). Significantly higher average number of azotobacter, free N-fixers and actinomycetes, as well as dehydrogenase and β -glucosidase activity found in our study, confirm the positive correlation with soil organic matter content.

CONCLUSIONS

Organic soil management practices improved soil nutrient status and impacted positively most of the investigated microbial indicators. Humus content was higher in all organic fields than on conventional plots. The abundance of *Azotobacter* spp., free N-fixing bacteria and actinomycetes was significantly enhanced in fields under organic farming. There were no significant changes in the total number of microorganisms, ammonifiers and fungi under specific agricultural management practices. The average dehydrogenase and β -glucosidase activity was significantly higher in soils under organic management than in conventional fields. These results allow better understanding of linkage between soil management and soil nutritional status, as well as structure and activity of soil microbial populations.

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МИКРОБИОЛОШКЕ КАРАКТЕРИСТИКЕ ЗЕМЉИШТА У РАЗЛИЧИТИМ СИСТЕМИМА ГАЈЕЊА СОЈЕ

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РЕЗИМЕ: Циљ рада био је да се испита утицај система гајења на микробиолошке карактеристике земљишта које је засејано сојом. Истраживање је обухватило 180 узорака земљишта под сертификованом органском производњом соје и 80 узорака у конвенционалној производњи. Бројност испитиваних група микроорганизама одређена је индиректним методом разређења, засејавањем земљишне суспензије на одговарајуће селективне подлоге, док је активност дехидрогеназе и β -глукозидазе утврђена спектрофотометријски. Добијени резултати указују да је систем гајења утицао на структуру и активност микробних заједница. Значајни позитивни ефекти органске производње одразили су се на бројност *Azotobacter* spp., слободних азотофиксатора и актиномицета. Утицај система гајења није запажен у промени укупног броја бактерија, амонификатора и гљива. Значајно виша активност дехидрогеназе и β -глукозидазе забележена је под органском производњом у поређењу са конвенционалном. Добијени резултати указују да је повећање у садржају органске материје, које је повезано са органским системом ђубрења, у позитивној корелацији са микробиолошким особинама земљишта.

КЉУЧНЕ РЕЧИ: бројност микроорганизама, дехидрогеназа, β -глукозидаза, органска и конвенционална производња, соја