Microbial community diversity and the interaction of soil under maize growth in different cultivation techniques

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ABSTRACT

Gałązka A., Gawryjołek K., Grządziel J., Frąc M., Księżak J. (2017): Microbial community diversity and the interaction of soil under maize growth in different cultivation techniques. Plant Soil Environ., 63: 264–270.

Soil microbial functional diversity under maize grown in different agricultural management practices was determined using the Biolog EcoPlates and other microbial and biochemical methods. Comparisons of substrate utilization and the diversity indices showed differences in community composition of microorganisms related to different cultivation techniques and seasons. The soil samples collected in spring were characterized by statistically significant lower indices of biological activity in comparison to the soil collected from the flowering stage of maize. The soils collected in spring from the plots with full tillage had a similarly high biological activity as the soils obtained from maize flowering season. The principal component of PC analysis, showed the strong correlation between the parameters of soil quality and biodiversity indicators. Selected indicators of soil microbial diversity explained 71.51% of biological variability in soils. Based on the PC analysis, two major groups of soils have been indicated. Management practices and seasons were two important factors affecting soil microbial communities.

Keywords: *Zea mays*; monoculture; community level physiological profiles; bioindicators; cultivation practices

Microorganisms regulate and influence important ecosystem processes and properties, such as nutrient transformation and decomposition, plant growth and promotion, and soil structure (Bowles et al. 2014). However, most of abiotic and biotic factors can change microbial community structure and also their ecosystem function. Both the total numbers and functions of microorganisms in soil are needed for better explanation and understanding of specific changes in microbial structure and diversity (Ghimire et al. 2014).

The microbial community structure may be also changed under different soil managements and tillage techniques (Järvan et al. 2014). These changes were measured using several parameters, such as microbial biomass, community level physiological profiles and enzymatic activities. Enzymes activities are essential in transformation of organic carbon

Table 1. Properties of the 0–30 cm layer of grey-brown podsolic soil (n = 3)

Cultivation techniques	pH_{KCl}	g C_{org} per 1 kg of soil	P_{Egner}	K_{Egner}	Mg
			(mg per 1 kg of soil)		
Direct sowing	5.9	7.0	71.16	87.95	28.2
Reduce tillage	6.5	6.7	211.96	117.25	21.0
Full tillage	6.7	6.9	103.47	68.62	24.6
Crop rotation	5.7	7.4	90.81	81.07	21.18

(C) and plant nutrients (Rillig 2004, Bowles et al. 2014). The Biolog EcoPlates method is widely accepted as a sensitive tool to indicate viable microorganisms and provide a fingerprinting for the microbial community composition. The numbers of different groups of microorganisms are also an indicator of changes taking place in the soil environment under different plant cultivation (Spedding et al. 2004, Frąc et al. 2012). On the other hand, tillage and cultivation practices and residue management have an important impact on soil degradation, biological activities and soil microbial community (Ghimire et al. 2014). In general, microbial community and their activity in the surface layer are more favourable under no tillage system with crop residue mulch rather than under the plough-till system without residue mulch. In crop rotation, plant production depends primarily on nutrient cycling in soils that are controlled by soil microorganisms and soil enzymes (Ngosong et al. 2010). Long-term monoculture may change soil parameters especially soil microorganism structure and their activity. Maize is one of the plants grown increasingly in no-tillage system with leaving plant residues on the surface of the field.

The aim of this study was to investigate changes in microbial communities in soil under maize crops in different cultivation techniques and growing seasons.

MATERIAL AND METHODS

Field experiment. The field experiment was carried out in 2013–2015 at the Agricultural Experimental Station (AES) of the Institute of Soil Science and Plant Cultivation in Grabow, (51°23'N, 21°38'E), Poland. The experimental scheme involved four treatments of maize monoculture: (1) direct sowing (DS); (2) reduced tillage (RT); (3) full ploughing tillage with cultivating measures (FT); (4) cultivation in crop rotation (spring barley-winter wheat-maize) (CR).

Research at AES in Grabow was conducted on a grey-brown podsolic soil formed from light loam (Table 1). The meteorological conditions during the growing season were presented in Table 2. The soil experiment was described in detail by Gałązka et al. (2017).

Soil samples. Soil samples were collected according to the Polish Standard PN-ISO 1038-6 (1998),

Table 2. Meteorological conditions during the growing season

	Month					
	IV	V	VI	VII	VIII	IX
Sum of precipitation (mm)						
2013	29.9	112	116.3	20.8	11.6	63.9
2014	51.5	161.7	93.1	101.4	91.9	15.2
2015	34.8	107.0	30.3	51.7	6.2	93.9
Long-term average (1871–2000)	39	57	71	84	75	50
Average air temperature (°C)						
2013	8.3	15.3	18.6	19.7	19.2	11.8
2014	9.9	13.5	15.2	20.4	17.9	14.4
2015	8.1	12.7	16.9	19.7	22.1	15.0
Long-term average (1871–2000)	7.7	13.4	16.7	18.3	17.3	13.2

twice in the year: spring (before sowing of maize) and summer (flowering phase of maize growth). The soil samples in three replicates were taken from the 0–30 cm layer and sieved through a 2 mm sieve and stored in a refrigerator (4°C) until the analysis.

Bacterial community analysis. Microbiological counts were expressed as a number of colony forming units (CFUs) per g of dry soil. The total number of bacteria (Bacteria) was determined by the dilution method on agarized soil extract and *Azotobacter* number according to Fenglerowa (1965). The total number of fungi (Fungi) was determined on the Martin's medium (Martin 2003) and the ammonifying bacteria (AM) and phosphate solubilizing bacteria (PSB) according to Rodina (1968).

Microbial biomass C and N. Microbial biomass was determined by the chloroform-fumigation-extraction method. The results of microbial biomass C and nitrogen (N) were calculated according to the following formula:

$$C_{mic} = E_C/k_{EC}$$

Where: E_C – soluble C in fumigated samples – soluble C in control (un-fumigated) samples and $k_{EC} = 0.45$.

$$N_{mic} = E_N/k_{EN}$$

Where: E_N – soluble N in fumigated samples – soluble N in control (un-fumigated) samples and $k_{EN} = 0.54$ (Ghani et al. 2003).

Biolog EcoPlates analysis. Metabolic potential of soil communities was evaluated using Biolog EcoPlate (Biolog Inc., Hayward, USA) with 31 carbon sources. Each well of the plate was inoculated with 120 µL of soil inoculum and incubated at 28°C. Absorbance readings were taken every 24 h for 264 h at 590 nm with a plate reader Biolog MicroStation™. On the basis of data obtained at 120 h, Richness (S), Shannon-Weaver (H), Evenness (E) and average well colour development (AWCD) indices were calculated following Garland and Mills (1991).

Determination of glomalin content. The glomalin content was determined according to Wright and Upadhyaya methods (Wright et al. 1996). The easily-extractable glomalin (EEG), total glomalin (TG) and glomalin-related soil protein (GRSP) were extracted from soil subsamples. EEG was extracted from 1 g of ground dry-sieved soil with 8 mL of 20 mmol citrate, pH 7.0 at 121°C for 30 min. TG was obtained by repeated extraction from 1 g of ground dry sieved soil with 8 mL of 50 mmol citrate, pH 8.0 at 121°C for 60 min. The protein content in

the supernatant was determined by the Bradford assay with bovine serum albumin as the standard on 96-plate reader (Victor, Perkin Elmer, USA).

The enzymatic activities analysis. The enzymatic activities were determined spectrophotometrically: soil dehydrogenases activities using the TTC method (Polish Standard. PN-EN ISO 23753-1, 2011), phosphatases activity by p-NPP method (Tabatabai 1982).

Statistical analysis. Statistical analyses were performed using the packet Statistica.PL (version 10) (StatSoft Inc., Tulsa, USA). Collected data were assessed by a three-way (enzymes activities, microbial populations, biodiversity indices from EcoPlate) analysis of variance (ANOVA) for the comparison of means, and significant differences were calculated according to post-hoc Tukey's *HSD* (honest significant difference) test at $P < 0.05$ significant level. Cluster analysis, including grouping of treatments and features, was performed on standardized data from the average absorbance values at 120 h (Biolog EcoPlate). The dendrogram was prepared with scaled bond distances on the axis (Ward's method) and boundary marked according to Sneath's criteria.

RESULTS AND DISCUSSION

The microbiological and biochemical indicators of quality were applied to assess the diversity of the soils. Biolog EcoPlates is a very sensitive and widely accepted method to characterize changes in
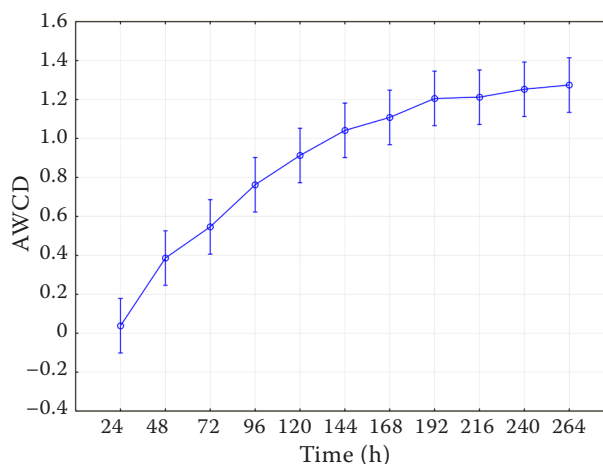

Figure 1. One-way analysis of variance (ANOVA) showing the changes of average well colour development (AWCD) index from time $P < 0.001$. Vertical bars represent 95% confidence intervals

Table 3. Effect of seasons and different tillage practices on microbial community catabolic diversity as evaluated by the Shannon's diversity index (H); substrate richness (S); substrate evenness (E) and average well-colour development (AWCD$_{590}$) in the Biolog EcoPlates incubated for 120 h

Season	Tillage practices	H	S	E	AWCD$_{590}$
Spring	DS	3.02 ± 0.12[ab]	23.33 ± 2.89[a]	0.960 ± 0.002[ab]	0.88 ± 0.12[ab]
	RT	2.98 ± 0.29[a]	23.33 ± 2.65[a]	0.958 ± 0.024[a]	0.75 ± 0.16[ab]
	FT	3.25 ± 0.04[ab]	27.33 ± 1.53[b]	0.981 ± 0.005[abc]	1.10 ± 0.14[ab]
	CR	2.98 ± 0.12[a]	22.33 ± 1.78[a]	0.965 ± 0.017[abc]	0.67 ± 0.18[b]
Summer	DS	3.34 ± 0.01[b]	29.00 ± 0.00[b]	0.992 ± 0.004[c]	1.22 ± 0.11[a]
	RT	3.34 ± 0.02[b]	29.33 ± 0.57[b]	0.989 ± 0.002[bc]	1.13 ± 0.07[ab]
	FT	3.29 ± 0.01[ab]	29.00 ± 1.00[b]	0.976 ± 0.006[abc]	1.18 ± 0.03[a]
	CR	3.32 ± 0.02[ab]	29.33 ± 0.57[b]	0.982 ± 0.001[abc]	1.21 ± 0.03[a]

The values are means ± standard error (*n* = 3). Treatment means separated by different letters are significantly different (Tukey's mean separation test, *P* < 0.05). DS – direct sowing; RT – reduced tillage; FT – full ploughing tillage with cultivating measures; CR – cultivation in crop rotation (spring barley-winter wheat-maize)

soil community (Garland and Mills 1991, Ghimire et al. 2014). The values of average well colour development index in the Biolog EcoPlate incubated from 0 to 264 h were presented at Figure 1. The AWCD index was increased in proportion to 120–168 h, therefore the time of 120 h, as the
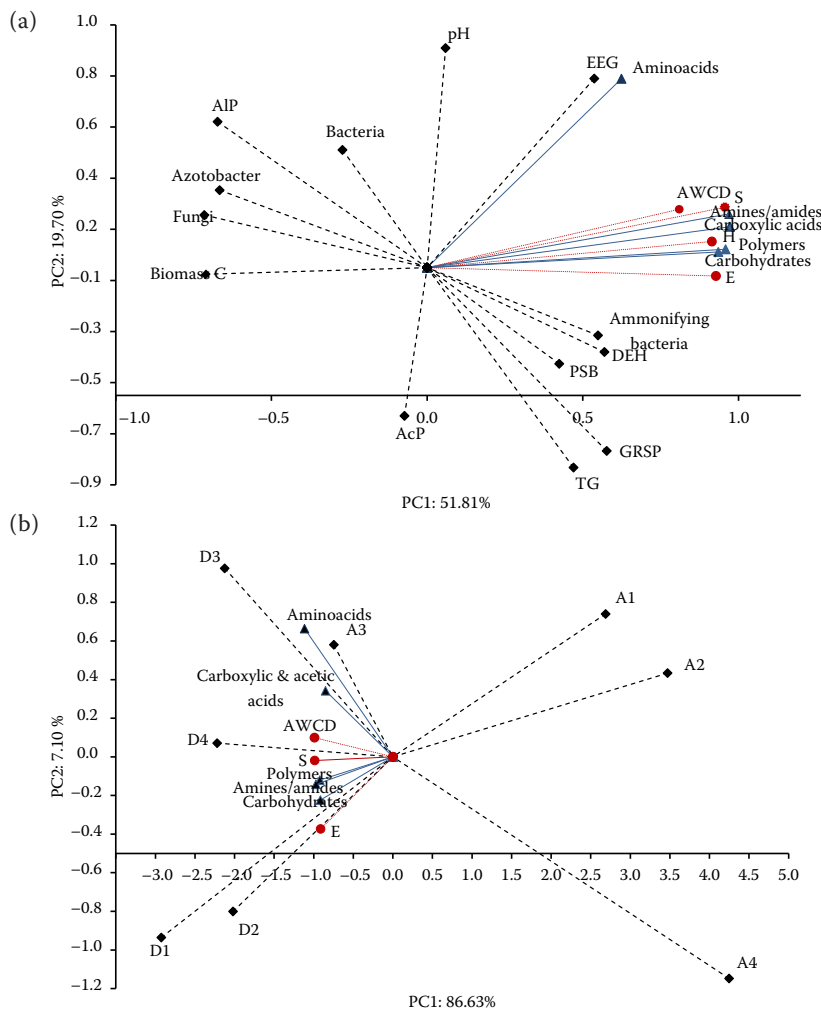

Figure 2. Principal component analysis of microbial parameters, biodiversity indexes and Biolog EcoPlates data incubated for 120 h from soil samples. Two times of soil taken under maize growth (A – before sowing; D – flowering); four cultivation techniques: 1 – direct sowing; 2 – reduce tillage; 3 – full tillage; 4 – crop rotation; AIP – alcaline phoshpatase; AWCD – average well colour development; S – Richness; H – Shannon-Weaver; E – Evenness; DEH – dehydrogenases activities; PSB – phosphate solubilizing bacteria; AcP – acid phosphatase; TG – total glomalin; EEG – easily-extractable glomalin; GRSP – glomalin-related soil protein

Table 4. Correlation of carbon source with the first (PC1) and second (PC2) components in soil

	PC1 (47.27%)	PC2 (22.99%)
Carbohydrates		
β-methyl-ᴅ-glucoside	–0.794	
Pyruvic acid methyl ester	–0.903	
i-Erythritol	–0.843	
ᴅ-Mannitol	–0.764	–0.635
ᴅ-Cellobiose	–0.916	
Glucose-1-phosphate	–0.767	
α-ᴅ-lactose	–0.732	
Amines and amides		
Phenylethylamine	–0.700	0.541
Amino acids		
ʟ-Arginine		–0.838
ʟ-Serine	–0.953	
Glycyl-ʟ-glutamic acid	–0.822	
Carboxylic and acetic acids		
ᴅ-Galactonic acid γ-lactone	–0.543	–0.824
2-Hydroxy benzoic acid	–0.950	
4-Hydroxy benzoic acid		–0.744
ᴅ-Glucosaminic acid	–0.964	
Itaconic acid	–0.821	
ᴅ-Malic acid	–0.859	
Polymers		
Tween 80	–0.738	
α-Cyclodextrin		0.803

most optimal time, was selected for the calculation of other biodiversity indices. The effect of different cultivation techniques and seasons on the catabolic diversity of microbial community as evaluated by substrate utilization in the Biolog EcoPlate incubated for 120 h were measured. The soils collected in spring from the plots with full tillage showed similarly high biological activity as soils obtained from maize flowering season (Table 3).

The season caused profound changes in biotic and abiotic factors such as temperature, humidity, vegetation and nutrient concentrations, which are very important for microbial structure (Bowles et al. 2014). The microorganisms have a high adaptive capacity and physiological flexibility to survive in different environment (Spedding et al. 2004).

The PC analysis showed the strong correlations between the parameters of soil quality and biodiversity indicators. Selected indicators of soil community accounted for 71.51% biological variability in soils (Figure 2).

Positive correlations have been shown between the analysed indicators of soil biodiversity and the main groups of compounds in the Biolog EcoPlate analysis (except amino acids group) (Figure 2a). The amino acids are a group of nutrient compounds which are used by soil microorganisms much later than other groups (carboxylic acids or amines). The total content of glomalines demonstrated strongly negative correlations with other parameters (total bacteria count, pH value and phosphatase activity) (Figure 2a). Based on the PC analysis, two major groups of soils have been indicated. The season was the main differentiating factor (Figure 2b). Also, the soils collected in summer (crop rotation and reduced tillage) showed the highest biological activity and diversity. Strong positive correlations between dehydrogenase activity, PSB and AM bacteria numbers and content of GRSP were observed (Figure 2b). Since GRSPs contain a certain amount of organic carbon and nitrogen, there is the hypothesis that exogenous EEG also influenced soil enzyme activities (Ngosong et al. 2010). The activity of dehydrogenases and phosphatase directly relates to carbon, nitrogen, and phosphorus availability to plants.
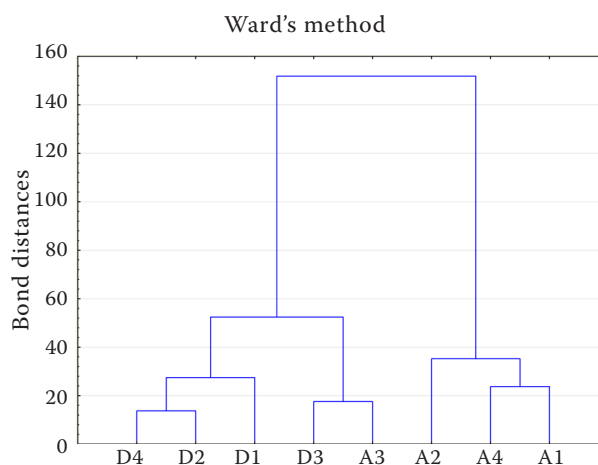

Figure 3. Dendrogram of the bond distances between the carbon utilization patterns of the substrates located on the Biolog EcoPlates and microbiological indicators. A – before sowing; D – flowering; four cultivation techniques: 1 – direct sowing; 2 – reduce tillage; 3 – full tillage; 4 – crop rotation

Figure 4. HeatMaps for the carbon utilization patterns of the substrates located only on the Biolog Eco-Plates data incubated for 120 h from soil samples. A – before sowing; D – flowering); four cultivation techniques: 1 – direct sowing; 2 – reduce tillage; 3 – full tillage; 4 – crop rotation
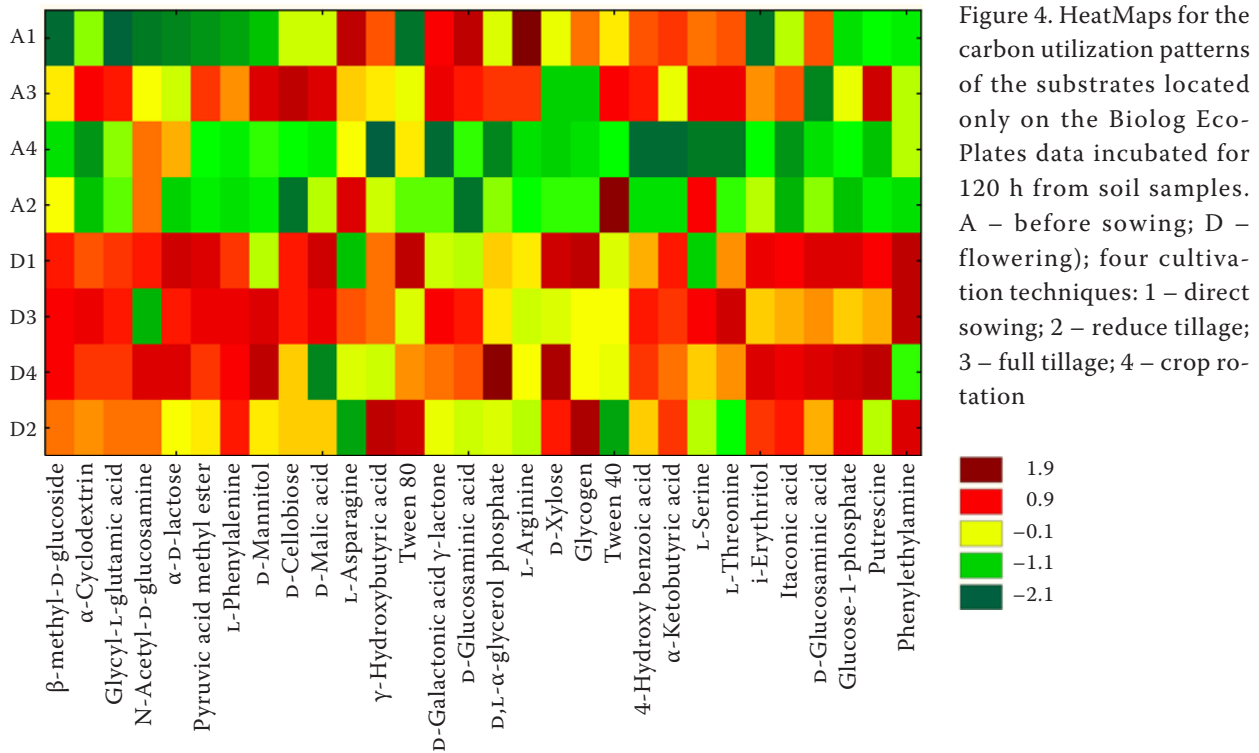
The correlations of carbon source with the first (PC1) and second (PC2) components are shown in Table 4. The carbon sources that give a statistically significant correlations could be a biochemical markers characteristic for soil cultivated with different techniques. The similar division was obtained in the analysis for the carbon utilization patterns of the substrates located on the Biolog EcoPlates (Figures 3 and 4).

On average, the lowest yields of maize were found in the treatments with direct sowing. The yield of maize grown in the full tillage system was by 22%

Table 5. Effects of different cultivation techniques on grain and straw yields of maize (t/ha)

Cultivation techniques	2013		2014		2015		Average of 2013–2015	
	grain	straw	grain	straw	grain	straw	grain	straw
Direct sowing	5.72[a]	4.75[a]	6.54[a]	3.95[c]	3.30[c]	4.9[b]	5.17[c]	4.53[c]
Reduce tillage	6.57[a]	5.22[a]	8.09[b]	5.65[b]	3.20[c]	5.6[a]	5.97[b]	5.48[b]
Full tillage	6.83[b]	4.82[a]	12.80[d]	6.92[a]	1.00[a]	4.3[b]	6.87[a]	5.35[b]
Crop rotation	5.59[a]	4.48[b]	11.13[c]	7.36[a]	2.30[b]	5.4[a]	6.30[a]	5.75[a]
Mean	6.15	4.81	9.63	4.13	2.45	5.05	6.07	5.27

Table 6. The Pearson's correlation coefficient on grain and straw yields of maize with selected parameters of the biological activity of soils (average of 2013–2015)

	Bacteria	Fungi	Biomass C	AlP	AcP	TG	EEG	GRSP
Grain	−0.672	0.886*	0.996*	0.820*	−0.975*	−0.579*	−0.852*	−0.691*
Straw	−0.996*	−0.964*	0.773*	0.948*	−0.811*	−0.488	−0.980*	−0.642*

*statistically significant ($P \leq 0.05$); AlP – alkaline phosphatase; AcP – acid phosphatase; TG – total glomalin; EEG – easily-extractable glomalin; GRSP – glomalin-related soil protein

higher than with the use of direct sowing. The yields of maize from full tillage system, rotation and reduce system were similar (Table 5).

In addition, soil collected from the cultivation of maize in reduce tillage was characterized by a high biological activity. Soil microorganisms play a fundamental role in promoting growth and development of plants (Scherer et al. 2011, Zhang et al. 2012). The yield of maize (grain, straw) correlated with some biological activity of soil (Table 6).

In conclusion, soils collected in summer (crop rotation and reduced tillage) had higher biological activity and functional diversity than that under full tillage treatment. Soil microbial communities shifted with agricultural management practices and season. Management practices and seasons were two important factors affecting soil microbial communities. This study demonstrated that tillage systems under maize influence soil microbial functional diversity along with soil chemical and other microbiological properties. Further research is needed to determine the soil microbial community composition, to identify key organisms and their dynamics under maize growth in different agricultural management practices.

REFERENCES

Bowles T.M., Acosta-Martínez V., Calderón F., Jackson L.E. (2014): Soil enzyme activities, microbial communities, and carbon and nitrogen availability in organic agroecosystems across an intensively-managed agricultural landscape. Soil Biology and Biochemistry, 68: 252–262.

Fenglerowa W. (1965): Simple method for counting *Azotobacter* in soil samples. Acta Microbiological Polonum, 14: 203–206.

Frąc M., Oszust K., Lipiec J. (2012): Community level physiological profiles (CLPP), characterization and microbial activity of soil amended with dairy sewage sludge. Sensors, 12: 3253–3268.

Gałązka A., Gawryjołek K., Perzyński A., Gałązka R., Księżak J. (2017): Changes in enzymatic activities and microbial communities in soil under long-term maize monoculture and crop rotation. Polish Journal of Environmental Studies, 26: 39–46.

Garland J.L., Millis A.L. (1991): Classification and characterization of heterotrophic microbial communities on the basis of patterns of community-level sole-carbon-source utilization. Applied and Environmental Microbiology, 57: 2351–2359.

Ghani A., Dexter M., Perrott K.W. (2003): Hot-water extractable carbon in soils: A sensitive measurement for determining impacts of fertilization, grazing and cultivation. Soil Biology and Biochemistry, 35: 1231–1243.

Ghimire R., Norton J.B., Stahl P.D., Norton U. (2014): Soil microbial substrate properties and microbial community responses under irrigated organic and reduced-tillage crop and forage production systems. PLoS ONE 9: e103901.

Järvan M., Edesi L., Adamson A., Võsa T. (2014): Soil microbial communities and dehydrogenase activity depending on farming systems. Plant, Soil and Environment, 60: 459–463.

Martin J.P. (2003): Use of acid, rose Bengal, and streptomycin in the plate method for estimating soil fungi. Soil Science, 69: 215–232.

Ngosong C., Jarosch M., Raupp J., Neumann E., Ruess L. (2010): The impact of farming practice on soil microorganisms and arbuscular mycorrhizal fungi: Crop type versus long-term mineral and organic fertilization. Applied Soil Ecology, 46: 134–142.

Rillig M.C. (2004): Arbuscular mycorrhizae and terrestrial ecosystem processes. Ecology Letters, 7: 740–754.

Rodina A. (1968): Microbiological Methods for the Study of Water. Państwowe Wydawnictwo Rolnicze i Leśne, Warszawa. (In Polish)

Scherer H.W., Metker D.J., Welp G. (2011): Effect of long-term organic amendments on chemical and microbial properties of a luvisol. Plant, Soil and Environment, 57: 513–518.

Spedding T.A., Hamel C., Mehuys G.R., Madramootoo C.A. (2004): Soil microbial dynamics in maize-growing soil under different tillage and residue management systems. Soil Biology and Biochemistry, 36: 499–512.

Tabatabai M.A. (1982): Soil enzymes. In: Page A.L., Miller R.H., Keeney D.R. (eds.): Methods of Soil Analysis, Part 2. Madison, American Society of Agronomy and Soil Science Society of America.

Wright S.F., Franke-Snyder M., Morton J.B., Upadhyaya A. (1996): Time-course study and partial characterization of a protein on hyphae of arbuscular mycorrhizal fungi during active colonization of roots. Plant and Soil, 181: 193–203.

Zhang B., He H.B., Ding X.L., Zhang X.D., Zhang X.-P., Yang X.M., Filley T.R. (2012): Soil microbial community dynamics over a maize (*Zea mays* L.) growing season under conventional- and no-tillage practices in a rainfed agroecosystem. Soil and Tillage Research, 124: 153–160.