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Effect of different agricultural management practices on soil biological parameters including glomalin fraction

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ABSTRACT

Gałązka A., Gawryjołek K., Grządziel J., Księżak J. (2017): Effect of different agricultural management practices on soil biological parameters including glomalin fraction. Plant Soil Environ., 63: 300–306.

The aim of the study was to determine the glycoproteins content (total glomalin (TG), easily extractable glomalin (EEG) and soil proteins related to glomalin (GRSP)) in soil under long-term monoculture of maize. Soil microbiological and biochemical properties, including microbial biomass and enzymatic activity were also assessed. The presence of total, easily-extractable glomalin and soil proteins related to glomalin was dependent on both the growth phase of the plant and tillage system. The highest content of glomalin was detected in the soils under maize in direct sowing and reduced tillage. The glomalin content was correlated with soil biological activity. The linear regression was observed between TG and GRSP content, but no linear relationship was found between GRSP and C_{org}. The principal component analysis showed the strong correlations between the parameters of soil quality and biodiversity indicators. Selected indicators of soil microbial parameters explained 52.27% biological variability in soils.

Keywords: *Zea mays*; fungi; soil glycoproteins; cultivation techniques; soil diversity

Glomalins are a thermostable, water-insoluble soil glycoproteins produced by arbuscular mycorrhizal fungi (AMF), measured in soils as glomalin-related soil proteins (GRSP) (Wright et al. 1996). AMF are ubiquitous root-symbiotic fungi from the phylum *Glomeromycota* (Rillig 2004) which form symbiotic relationships with roots of 85–90% land plants in natural and agricultural ecosystems. They are also known to be beneficial to plant nutrition, growth and survival (Bedini et al. 2007, Gałązka and Gawryjołek 2015). There is a hypothesis that mycorrhizal fungi produce large quantities of glomalins to improve the environment for growth of plants.

The GRSP proteins perform a fundamental role in making soil structure (Wright et al. 1996). They live relatively long in the soil and play a structural role in soil carbon dynamics. The content of glomalin in soil particles is significantly correlated with aggregate stability (Driver et al. 2005, Koide and Peoples 2013).

Plant cultivation in monoculture can lead to unfavourable changes in soil environment, connected with microbial diversity reduction and crop yield decrease (Bedini et al. 2007). Long-term plant cultivation in monoculture induces and accelerates soil degradation processes that can lead to a decrease in soil microorganism abundance and in the content of soil organic matter (Mijangos et al. 2006). Economic and environmental considerations encourage the use of reduced tillage or even direct

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sowing in maize cultivation (Wang et al. 2017). The highest savings can be obtained using direct sowing. No cultivation in the field is performed between the fore cropping and the sowing of the posterous crop. Maize is one of the most commonly cultivated crops in no tillage system with leaving plants residues on the surface of the field. In reduced tillage, the stubble cultivators are the most often used for surface cultivation instead of a plow. When using simplification in tillage systems, maize is sown in organic biomass that was decomposed during winter, after shallow surface tillage or direct sowing is employed.

The aim of the study was to demonstrate the presence of total and easily extractable glomalins in long-term monoculture of maize as well as to investigate the correlation of this glycoproteins contents with dynamics of the number of microorganisms and enzymatic activities in the rhizosphere of maize. More specifically, the objective was to assess the long-term trends in tillage-induced changes in soil biological quality, and to establish possible cause-effect relationships between management practices and soil biological activities due to monoculture maize cultivation.

MATERIAL AND METHODS

Field experiment. The study was conducted in the multi-year stationary field experiment established in 2004 which involved maize cropped continuously and rotated with other crops. The field experiment was carried out since 2014 to 2016 at the Agricultural Experimental Station (AES) of the Institute of Soil Science and Plant Cultivation in Grabow, the Mazowieckie voivodeship (51°23'N; 21°38'E), Poland. The experimental scheme involved four treatments: maize monoculture – direct sowing; maize monoculture – full tillage; cultivation in crop rotation (spring barley-winter wheat-maize) – full ploughing tillage and crop rotation and reduced tillage (grubbers). The research at AES in Grabow was conducted on a grey brown podsolic soil formed from light loam. The ploughing layer of soil was characterized by a low content of magnesium, a medium content of potassium and a high content of phosphorus (Gałązka et al. 2017). The experiment was established with the method of long strips with the mirror image of treatments.

Soil samples were taken for microbiological analyses at five dates: before sowing, 6 leaves stage, 12 leaves stage, flowering and after harvest the maize in years 2014–2016. The samples were collected in three replicates from the 0–15 cm layer and sieved through a 2 mm sieve and stored in a refrigerator (4°C) until analysis. The chemical soil properties [contents of: C_{org} (Tiurin method), P, K and Mg (Egner-Rhiem method), and pH_{H_2O}] were measured by the certified chemical laboratory of the Institute of Soil Science and Plant Cultivation in Puławy, Poland.

Microbial and biochemical properties. Microbiological counts were expressed as a number of colony forming units (CFUs) per g of dry soil:
- total number of bacteria (method on agarized soil extract);
- total number of fungi on the Martin's medium (Martin 2003);
- the ammonifying bacteria (AM), *Azotobacter* spp. (Azo) and phosphate solubilizing bacteria (PSB) according of Rodina (1968).

The enzymatic activities were determined spectrophotometrically: soil dehydrogenase activity using the triphenyl tetrazolium chloride (TTC) method (Casida et al. 1964), while phosphatase activity by the *p*-NPP method (Tabatabai 1982). Microbial biomass C (MBC) was determined by the chloroform-fumigation-extraction method (Ghani et al. 2003).

The glomalin content was determined according to Wright et al. (1996) methods. The easily extractable glomalin (EEG), total glomalin (TG) and soil proteins related to glomalin (GRSP) were extracted from soil subsamples. The EEG protein 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. The 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. After each autoclaving cycle supernatant was removed by centrifugation at 4000 rpm for 15 min and stored. Extracts from each cycle were pooled, centrifuged at 9000 rpm for 5 min to remove soil particles, and then analysed. The extraction of a sample continued until the supernatant showed none of the red-brown colour typical for glomalin. Extracts from each replicate were pooled, analysed, and the protein in the supernatant were determined by the Bradford dye-binding assay (Bradford 1976) with bovine serum albumin as the standard on a 96-plate reader (Victor, Perkin Elmer, USA).

Statistical analysis. The analysis of variance (ANOVA) was used in a proper model for the ex-

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perimental design. Tukey's test was used to evaluate the significance of differences of the means at a significance level of $P \leq 0.05$. Cluster analysis and principal component analysis (PCA) were applied to the standardized data to investigated similarities between different properties. Statistical analyses were performed using the packet Statistica.PL (10) (Stat. Soft. Inc., Tulsa, USA).

RESULTS AND DISCUSSION

The presence of glomalins (TG, EEG, GRSP) were found in soils (Table 1). The content of glomalin varied significantly depending on the cultivation technique (Table 1) and the sampling date (Table 2).

Table 1. The content of total glomalin (TG), easily extractable glomalin (EEG) and soil proteins related to glomalin (GRSP) (average for 2014–2016, $n = 9$)

Symbol (cultivation technique)	TG	EEG	GRSP
	(mg/g DM of soil)		
A1	2.87^{bcd}	1.12^{ab}	3.99^{abcd}
A2	2.00^{ab}	1.25^{abc}	3.25^{a}
A3	1.89^{a}	1.35^{abc}	3.24^{a}
A4	3.01^{cde}	1.11^{a}	4.12^{abcd}
B1	3.53^{de}	1.59^{c}	5.12^{f}
B2	3.54^{de}	1.39^{abc}	4.93^{def}
B3	2.78^{abcd}	1.22^{abc}	4.00^{abcd}
B4	2.85^{abcd}	1.18^{abc}	4.03^{abcd}
C1	3.03^{cde}	1.57^{bc}	4.59^{cdef}
C2	3.24^{cde}	1.15^{abc}	4.39^{bcde}
C3	2.75^{abcd}	1.12^{ab}	3.87^{abc}
C4	2.74^{abcd}	1.23^{abc}	3.97^{abcd}
D1	3.34^{cde}	1.27^{abc}	4.61^{cdef}
D2	3.19^{cde}	1.24^{abc}	4.43^{bcde}
D3	2.82^{abcd}	1.30^{abc}	4.11^{abcd}
D4	2.91^{bcde}	1.24^{abc}	4.15^{abcd}
E1	3.51^{de}	1.47^{abc}	4.98^{def}
E2	3.85^{e}	1.17^{abc}	5.02^{ef}
E3	2.98^{cde}	1.14^{abc}	4.12^{abcd}
E4	2.38^{abc}	1.08^{a}	3.46^{ab}

Treatment means, separated by different letters, are significantly different (Tukey's mean separation test, $P \leq 0.05$). A – before sowing; B – 6 leaves; C – 12 leaves; D – flowering; E – after harvest; 1 – direct sowing; 2 – reduced tillage; 3 – full tillage; 4 – crop rotation; DM – dry matter

Table 2. One-way analysis of variance ANOVA (average for 2014–2016)

Seasons/phases of plant grow	TG	EEG	GRSP
	(mg/g DM of soil)		
Before sowing	2.07^{b}	1.10^{a}	3.17^{a}
6 leaves	2.80^{a}	1.32^{ab}	4.12^{b}
12 leaves	2.86^{a}	1.39^{b}	4.26^{b}
Flowering	2.91^{a}	1.41^{b}	4.32^{b}
After harvest	2.79^{a}	1.28^{ab}	4.07^{b}

Treatment means, separated by different letters, are significantly different (Tukey's mean separation test, $P \leq 0.05$). TG – total glomalin; EEG – easily extractable glomalin; GRSP – soil proteins related to glomalin; DM – dry matter

The highest content of glomalin was found in the soil with direct sowing and reduced tillage. These techniques of cultivation are meant to interfere with the soil structure as little as possible, not to cause the tearing up the trunks of mycorrhizal fungi. An actively growing mycelium can freely penetrate soil, that is the presence of the glomalin can be observed throughout the whole soil profile (Gillespie et al. 2011). The content of glomalin in soil was correlated with its biological activity and physical properties (Table 3). Also a linear regression was observed between TG and GRSP content (Figure 1a), but no linear relationship was found between GRSP and C_{org} (Figure 1b).

Tillage systems can influence soil microflora. The highest dehydrogenases activities (Figure 2a), microbial biomass C (Figure 2b), acid phosphatases (Figure 2d) were observed in direct sowing and reduced tillage compared to full tillage and crop rotation systems. In addition, the lowest alkaline activity was observed in direct sowing and reduced tillage (Figure 2c).

It is known that biological activity is dependent on climate and temperature (Ngosong et al. 2010). The utilization of reduced tillage and direct sowing caused the increase in soil microorganism number and enzyme activity in soil, especially in the 0–20 cm layer. Other authors observed a decrease in enzymatic activity in the soil taken from the monoculture in comparison to plant shifting (Schindler et al. 2007).

Cultivation in monoculture usually causes higher qualitative and quantitative changes of soil microorganisms, promoting growth of certain micro-

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Table 3. The Pearson's correlation coefficient of selected microbiological, chemical and biochemical parameters and glomalin content

Parameter	C_{org}	P_{Egner}	K_{Egner}	Mg	TG	EEG	GRSP	DHA	AcP	AlP	Bacteria	Fungi	MBC
Cultivation practice	0.614	−0.101	−0.433	−0.663	−0.857*	−0.894*	−0.939*	−0.660*	0.745*	−0.898*	−0.856*	−0.896*	0.744*
pH	−0.809*	0.538	0.087	−0.093	0.353	−0.236	0.249	−0.060	0.423	−0.324	−0.043	−0.272	0.575
C_{org}	–	−0.674*	−0.527	−0.096	−0.835*	−0.222	−0.767*	−0.496	−0.032	−0.266	−0.296	−0.211	−0.008
P_{Egner}	–	–	0.823*	−0.635	0.599	−0.339	0.436	0.619	0.558	−0.033	−0.405	−0.310	0.184
K_{Egner}	–	–	–	−0.377	0.782*	0.104	0.694*	0.947*	0.088	0.480	−0.082	0.155	−0.393
Mg	–	–	–	–	0.201	0.866*	0.370	−0.081	−0.894*	0.578	0.954*	0.833*	−0.523
TG	–	–	–	–	–	0.545	0.981*	0.859*	−0.314	0.712*	0.477	0.563	−0.513
EEG	–	–	–	–	–	–	0.696*	0.413	−0.965*	0.908*	0.960*	0.998*	−0.853*
GRSP	–	–	–	–	–	–	–	0.831*	−0.489	0.818*	0.628	0.711*	−0.634
DHA	–	–	–	–	–	–	–	–	−0.238	0.737*	0.215	0.461	−0.658
AlP	–	–	–	–	–	–	–	–	–	−0.832	−0.923*	−0.961*	0.842*
AcP	–	–	–	–	–	–	–	–	–	–	0.768*	0.933*	−0.960*
Bacteria	–	–	–	–	–	–	–	–	–	–	–	0.939*	−0.673*
Fungi	–	–	–	–	–	–	–	–	–	–	–	–	−0.885*

*indicated statistically significant ($P \leq 0.05$), ($n = 9$, average of 2014–2016). C_{org} (g/kg of soil); P_{Egner}, K_{Egner}, Mg (mg/kg of soil); bacteria (10^8 CFU (colony forming units)/g DM (dry matter) of soil); fungi (10^4 CFU/g DM of soil); dehydrogenases (DHA) (ug formazan/g DM of soil/24 h); alkaline phosphatase (AlP) (ug p-nitrophenol/g DM of soil/h); acid phosphatase (AcP) (ug p-nitrophenol/g DM of soil/h); microbial biomass C (MBC) (ug/g DM of soil); total glomalin (TG) (mg/g DM of soil); easily extractable glomalin (EEG) (mg/g DM of soil); glomalin-related soil protein (GRSP) (mg/g DM of soil)

organisms and limiting growth of others (Bedini et al. 2007). However, these matters depend on plant species, which is probably connected to the composition of root secretion, quality and quantity of crop residues, and root system depth.

The principal component analysis PCA showed strong correlations between the basic microbial and biochemical parameters of soil quality and biodiversity indicators (Figures 2 and 3). Selected indicators of soil microbial parameters explained

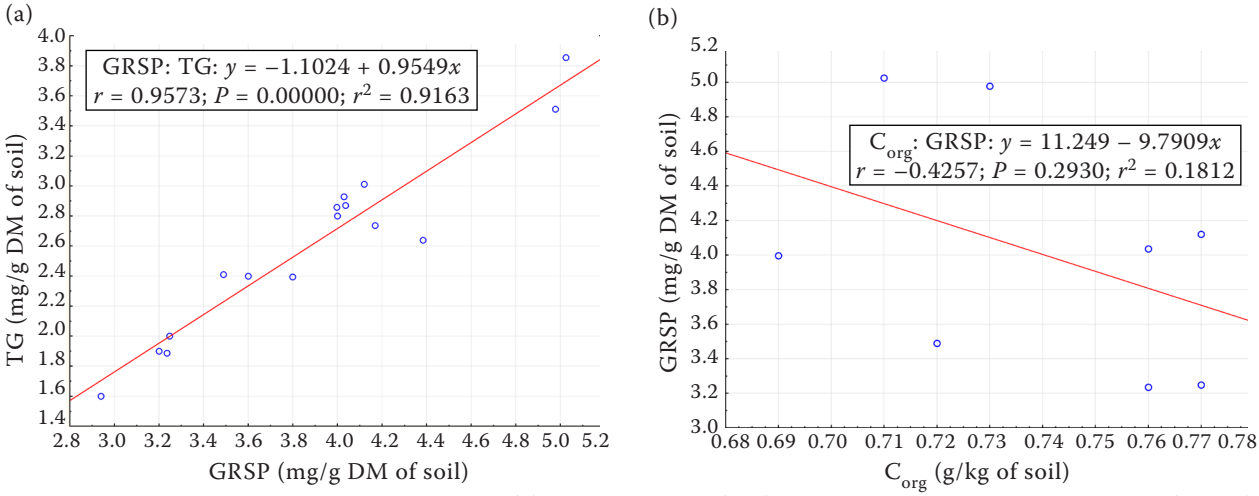

Figure 1. The simple regression analysis on (a) total glomalin (TG) and glomalin-related soil protein (GRSP) content, and on (b) GRSP and carbon content

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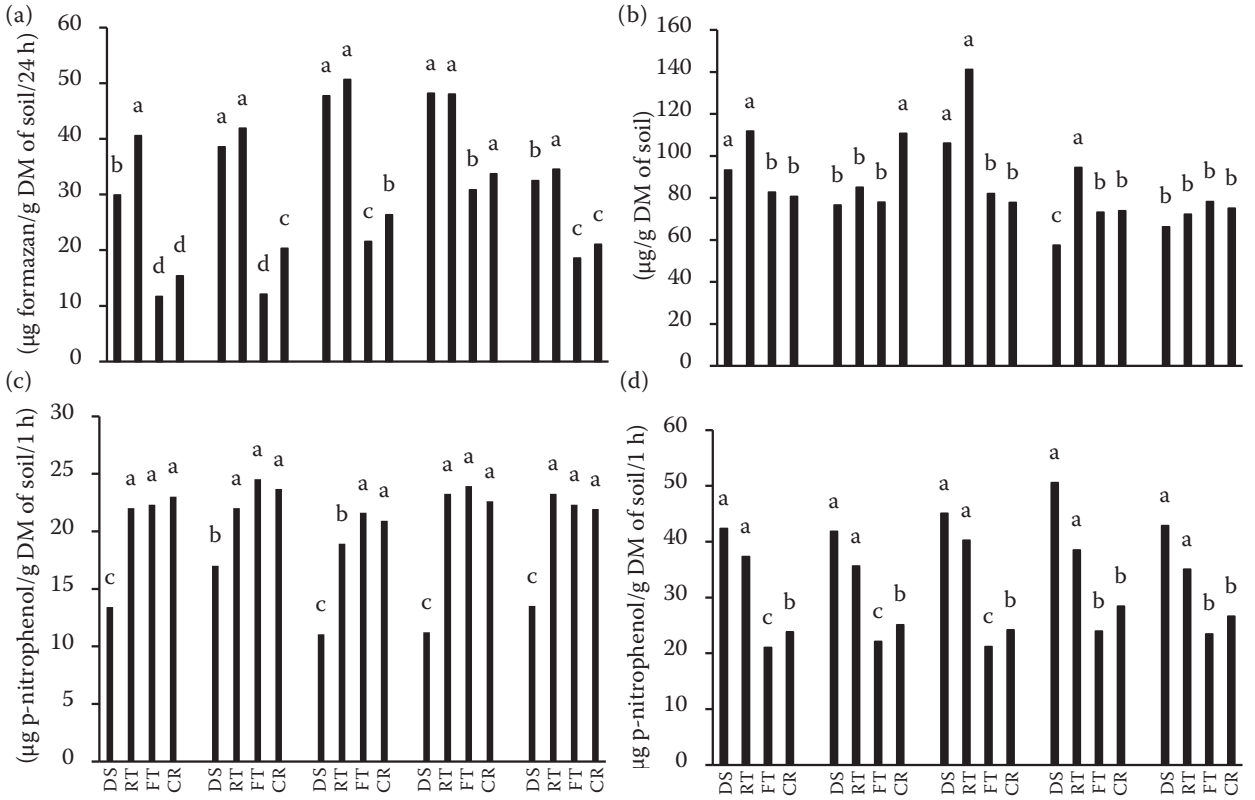

Figure 2. Microbiological properties of soil, statistically significant ($P \leq 0.05$), ($n = 6$, average of 2014–2016). (a) dehydrogenases activities (DHA); (b) microbial biomass C (MBC); (c) alkaline phosphatase (AlP), and (d) acid phosphatase (AcP); DS – direct sowing; RT – reduced tillage; FT – full tillage; CR – crop rotation

52.27% biological variability in soils (Figure 3a). Glomalin content in soil was strongly correlated with the date of sampling (Figure 3b). A strong positive correlation was shown between glomalin

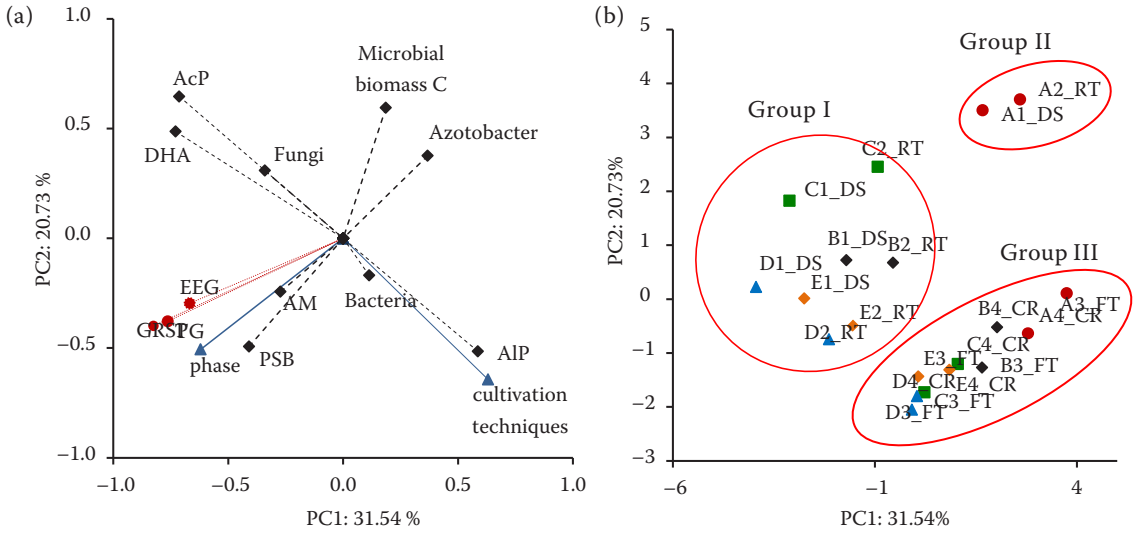

Figure 3. Principal component analysis (PC) (a) of microbial parameters, and (b) of cultivation techniques and phases. A – before sowing; B – 6 leaves; C – 12 leaves; D – flowering; E – after harvest; 1 – direct sowing (DS); 2 – reduced tillage (RT); 3 – full tillage (FT); 4 – crop rotation (CR); AcP – acid phosphatase; DHA – dehydrogenases activities; TG – total glomalin; EEG – easily extractable glomalin; GRSP – soil proteins related to glomalin; AM – ammonifying bacteria; PSB – phosphate solubilizing bacteria

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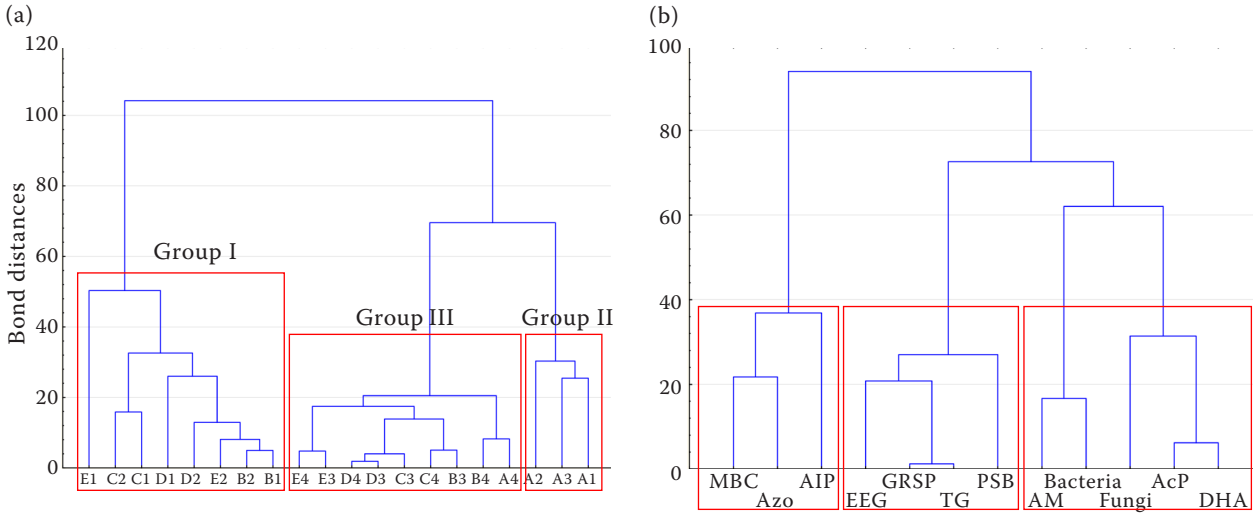

Figure 4. Dendrogram of the bond distances from the hierarchical cluster analysis of soil properties, different cultivation techniques, seasons and microbial activities (a). A – before sowing; B – 6 leaves; C – 12 leaves; D – flowering; E – after harvest; 1 – direct sowing; 2 – reduced tillage; 3 – full tillage; 4 – crop rotation; (b) Azotobacter (Azo) (10^1 CFU (colony forming units)/g DM (dry matter) of soil). MBC – microbial biomass carbon; AIP – alkaline phosphatase; EEG – easily extractable glomalin; GRSP – glomalin-related soil protein; TG – total glomalin; AM – ammonifying bacteria; PSB – phosphate solubilizing bacteria; AcP – acid phosphatase; DHA – dehydrogenases

content (TG, EEG, GRSP) and direct sowing and reduced tillage (Figure 3). PCA analysis showed three main groups: I – soils from direct sowing and reduced tillage, taken in three phases of maize growth and after harvest; II – soils from direct sowing and reduced tillage taken before sowing and III – soils taken from maize cultivated in full tillage and rotation system from all sampling dates. The same division was observed with Ward cluster analysis, too (Figure 4a). In hierarchical cluster analysis, the biological properties were grouped on the basis of their role in the transformation processes of soil organic matter. Generally, two main clusters and three subclusters were obtained from the cluster analysis that was performed on the biological properties being studied (Figure 4b). One included the total number of bacteria, fungi, ammonifying and PSB bacteria, AcP, DHA activities and glomalins content and second included microbial biomass C, *Azotobacter* and AlP activity.

An increased biological activity in soil resulting from direct sowing system and simplified cultivation is probably connected to the lower disturbance of its structure and the accumulation of crop residues on the surface that are the main carbon source for microorganisms (Wang et al. 2017). On the other hand, agrotechnical treatments in conventional tillage lead to the improvement of soil climate on the appropriate level of aeration and humidity of soil, which should increase its biological activity (Schindler et al. 2007). Biochemical environment under the reduced tillage is much less aerated than in the case of conventional crop system. Limitation of aeration with simultaneous increase of organic matter decomposition lead to the changes in the soil atmosphere, especially to the increase in CO_2 content. Further research is needed to determine the soil microbial community composition, to identity key organisms and their dynamics under maize growth in different agricultural management practices. It can be assumed that the content of glomalins may be such a factor identifying the quality of soil.

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