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Microbial and enzymatic characteristics of soils under pasture mixtures

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Abstract. In the years 2005–2007, twice a year, soil cores (0–20 cm deep x 2.5 cm diameter) were collected from three replicated plots of each of the selected treatments of a field experiment located at Grabów Experimental Station (51°20'58" N, 21°39'5" E). In this experiment pasture mixtures (white clover-grasses) were established on three fields (sites), about 500–600 m apart, and differing with respect to cropping history (preceding crops). Total numbers (cfu) of bacteria and fungi in soils under pasture mixtures were not significantly influenced by the studied factors (preceding crops and seeding rates). Numbers of bacteria from the genus *Azotobacter* were higher in the soil under pasture mixture grown after ploughed meadow (field III), than after spring barley (field I) and after potato (field II). The soil in field III was richest with respect to SOM and $N\text{-}NO_3$ contents and it had pH most favorable for bacteria. Activities of dehydrogenase and phosphatases (acid and alkaline) were highest in the soil under pasture mixtures grown in field III, lower in the soil of field II and the lowest in the soil of field I.

key words: bacteria, enzymatic activity, fungi, numbers, soil, pasture mixtures

INTRODUCTION

Beneficial environmental effects of growing grass or grass-legume mixtures (for forage or grazing) result mainly from large amounts of organic matter that are accumulated in soils under these mixtures (Ghani et al., 2003; Grzegorczyk, Grabowski, 2005). Ghani et al. (2003) compared chemical, biochemical and biological characteristics of some New Zealand soils of the same type but differing with respect to land uses and found that soils under sheep or dairy pastures contained 40–60% more the total organic C than adjacent cultivated soils. The pastoral soils were also richer in microbial biomass-C, labile fractions of soil organic matter (cold and hot-water extractable C) and mineralisable N contents. Moreover grass roots are known to harbor high populations of specific groups of beneficial microorganisms, e.g. arbuscular micorrhizal fungi or root ectotrophic fungus *Phialophora graminicola,* which play an important role in improving plant nutrition (particularly P uptake), soil structure formation or in controlling some plant pathogens (Deacon, 1976; Martyniuk, 1987, Martyniuk et al., 1991; Błaszkowski, 1991; Wright, Upadhyaya, 1998).

In this work we compared selected microbial and biochemical characteristics of soils under temporal pasture mixtures as influenced by preceding crops and seeding rates.

MATERIAL AND METHODS

For the purpose of this study soil samples were collected from selected treatments of a field experiment located at Grabów Experimental Station (51°20'58" N, 21°39'5" E) belonging to the Institute of Soil Science and Plant Cultivation in Puławy. In this experiment pasture mixtures (white clover-grasses) were established on three fields (sites), about 500–600 m apart, and differing with respect to cropping history (preceding crops). On field I pasture mixtures were sown after spring barley (grown after fodder grass), on field II after potatoes fertilized with farmyard manure and on field III after ploughed meadow. Fields I and II were located on a grey-brown podsolic soil and field III on a degraded black soil. Basic characteristics of these soils are given in Table 1. In the spring of 2004 mixtures of white clover seeds and grass seeds were sown at the following seeding rates: 10 mln, 20 mln and 30 mln seeds ha^{-1}, each containing either 20% or 40% of white clover seeds. Detailed information on this experiment are given by Harasim (2006, 2008). In the years 2005–2007, twice a year (in the spring – April or May, and in the fallow – October), soil cores (0–20 cm deep x 2.5 cm diameter) were

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Table 1. Basic chemical characteristics of soils.

Soils	Soil organic matter [%]	pH (H_2O)	Content [mg/100 g soil DM]			
			$N\text{-}NO_3$	P	K	Mg
I[A]	1.39	6.35	2.21	7.5	14.2	2.8
II	1,27	6.60	1.76	7.6	6.6	3.0
III	2.43	7.0	7.17	4.4	3.5	6.7

A – Preceding crops: field I – spring barley, field II – potato, field III – meadow

Table 2. Numbers of colony forming units [cfu g^{-1} soil DM] of *Azotobacter* spp., total bacteria and fungi in soils under white clover-grass pasture mixtures as influenced by preceding crops and some agro-technical factors.

Preceding crops A	Agro-technical factors B	*Azoto-bacter* spp.	Total bacteria [cfu x 10^7]	Total fungi [cfu x 10^5]
Field I	1	6.0	2.07	1.20
	2	0	1.93	0.79
	3	2.0	1.70	1.30
	4	2.0	1.97	1.40
	mean	2.50	1.92	1.17
Field II	1	13.0	1.97	1.73
	2	7.0	1.63	1.53
	3	1.0	2.10	2.07
	4	9.0	1.77	1.47
	mean	10.30	1.87	1.70
Field III	1	5.0	1.93	1.10
	2	3.0	1.54	1.27
	3	18.0	1.67	1.51
	4	71.0	1.68	1.15
	mean	24.30	1.70	1.26
LSD for:	A	20.5	ns	ns
	B	49.1	ns	ns
	Interaction AxB	ns	ns	ns

A – see Table 1
B – agro-technical factors:
1 – 10 mln seeds ha^{-1}, 20% of white clover seeds in seeding mixture,
2 – 30 mln seeds ha^{-1}, 20% of white clover seeds in seeding mixture,
3 – 10 mln seeds ha^{-1}, 40% of white clover seeds in seeding mixture,
4 – 30 mln seeds ha^{-1}, 40% of white clover seeds in seeding mixture
ns – non significant

collected from three replicated plots of each of the selected treatments (Table 2). The soil samples consisting of 9 cores taken from each treatment were sieved through a 2 mm sieve and stored in a refrigerator (4°C). The soils were analyzed for: total numbers (colony forming units) of bacteria counted on 1/10 PCA medium (Difco) and fungi on Martin's medium (Martyniuk et al., 2001), numbers of bacteria from the genus *Azotobacter* on nitrogen-free agar medium, soil dehydrogenase activity using TTC method (Casida et al., 1964), phosphatases activity by *p*-NPP method (Tabatabai, Brenner, 1969), pH (in H_2O) and water content (gravimetrically, after drying in 105°C). Data were statistically analyzed by two-way analysis of variance (ANOVA).

RESULTS AND DISCUSSION

The soils in fields I and II were similar with respect to almost all, with the exception of K content, chemical properties, as shown in Table 1. In comparison to these soils the soil in field III contained markedly higher amounts of soil organic matter (SOM), $N\text{-}NO_3$ and Mg but lower amounts of P and K. This soil had also the highest pH in water (about 7.0). Field III was located on a meadow, which was ploughed down in 2003 and after seedbed preparation it was re-seeded with the pasture mixture in the spring of 2004. Higher contents of SOM and $N\text{-}NO_3$ in the soil of this field resulted from grass sod transformation and mineralization processes that took place after meadow cultivation. Data on yields and herbage composition of the pasture mixtures grown in these fields have been analyzed by Harasim (2006, 2008). The average yields of herbage obtained in this experiment were in the order of field I > field II > field III. The share of white clover stalks in swards was also of similar order.

Numbers of soil bacteria and fungi shown in Table 2 represent mean results of six analyses performed twice a year during three growing seasons (2005–2007). Total numbers (cfu) of bacteria and fungi in soils under pasture mixtures were not significantly influenced by the studied factors (preceding crops and seeding rates). The mean number of bacteria from the genus *Azotobacter*, which are able to fix atmospheric nitrogen, was higher in the soil under pasture mixture grown in field III, than in the soils from fields I and II (Table 2). The soils in field III was richest with respect to SOM and $N\text{-}NO_3$ contents, which resulted from transformation and mineralization of the plowed-down plant residues of previous meadow. This soil had also the pH most favorable for bacteria from the genus *Azotobacter* (Barnes et al., 2007; Martyniuk, Martyniuk, 2003). Populations of *Azotobacter* spp. in the examined soils were generally low and ranged from 0–1 cell (cfu) g^{-1} in the soil samples taken from field I and II to 71 cfu in the soil from field III. These numbers are comparable with those found in our previous study on the occurrence of *Azotobacter* spp. in Polish soils. In this survey bacteria from the genus *Azotobacter* were not detected in about 50% of Polish soil and in other soils numbers of these bacteria were generally below 100 cfu g^{-1} (Martyniuk, Martyniuk, 2003).

Table 3 shows activities of dehydrogenase and phosphatases (acid and alkaline) in soil samples collected in autumn seasons of the years 2005, 2006 and 2007. For the spring samples similar results were obtained (data not presented). The mean activities of the enzymes shown in Table 3 were the highest in the soil under pasture mixtures grown in field III, lower in the soil of field II and the lowest in the soil of field I. These differences were generally significant at $\alpha < 0.05$. Although the total numbers of bacteria and fungi in the soils under pasture mixtures were not significantly influenced, with the exception of *Azotobacter* spp. (Table 2), by chemical properties of the soils, it seems that

Table 3. Activities of dehydrogenase [µl H_2/100 g soil DM] and phosphatases [µg pNP/g soil DM] in soils under white clover-grass pasture mixtures as influenced by preceding crops and some agro-technical factors.

Preceding crops A	Agro-technical factors B	Dehydrogenase			Acid phosphatase			Alkaline phosphatase		
		2005	2006	2007	2005	2006	2007	2005	2006	2007
I	1	12.5	10.0	12.1	56.9	64.3	63.6	35.9	43.2	34.0
	2	10.6	8.5	7.5	50,3	72.8	56.4	45.3	44.1	34.5
	3	10.4	7.6	8.9	57.1	58.0	53.8	39.8	47.1	39.3
	4	9.2	9.8	10.9	56.0	68.5	57.8	38.7	44.5	40.1
	Mean	10.7	9.0	9.9	55.1	65.9	57.9	39.9	44.7	37.0
II	1	15.2	14.8	19.6	73.1	94.3	79.8	36.6	57.3	49.2
	2	13.3	11.5	17.5	73.1	98.5	76.7	36,1	40.7	39.7
	3	14.5	13.2	15.6	83.0	83.5	73.2	39.7	54.5	39.4
	4	9.2	18.2	14.9	75.9	83.9	76.4	35.7	43.0	43.4
	Mean	12.9	14.4	16.9	76.3	90.0	76.5	37.0	48.9	42.9
III	1	29.0	39.1	38.9	169.7	213.7	196.5	126.9	185.6	145.8
	2	34.9	45.1	40.3	158.8	210.3	184.1	122.9	185.2	136.2
	3	31.9	36.7	36.7	158.5	209.9	186.4	119.6	163.0	140.6
	4	34.6	42.3	40.0	152.7	198.1	181.0	140.2	183.0	152.2
	Mean	32.6	40.8	39.0	159.9	208.0	186.2	127.4	179.2	143.7
LSD for:	A	1.4	1.1	1.3	3.0	5.3	4.4	2.5	4.9	3.8
	B	1.8	1.4	1.6	3.8	6.8	5.7	3.2	6.3	4.9
	interaction AxB	3.1	2.4	2.8	6.6	11.8	ns	5.6	11.0	8.5

A, B – see Tables 1 and 2
ns – non significant

the activity of soil microorganisms might be affected by these properties. The soil under pasture mixture grown in field III containing the highest amounts of organic matter and NO_3-N displayed also the highest activity of soil microorganisms, as manifested by dehydrogenase and phosphatases activities (Table 3). There are numerous studies showing close relationships between various soil fertility indicators (SOM and nutrient contents) and soil microbial and biochemical properties (Barnes et al., 2007. Ghani et al., 2003; Kandele, Murer, 1993; Martyniuk et al., 2001). Activities of the enzymes in soils under pasture mixtures were also influenced by the agro-technical factors (Table 3) but the variability of these effects does not allow for a conclusive statement which of these factors had the most important influence on the biological activity of the soils.

Concluding, the results of this study have shown that microbial and biochemical characteristics of the soils under pasture mixtures were influenced mainly by their physicochemical properties such as pH, organic matter and nutrient contents, but not by the studied agro-technical factors (preceding crops and seeding rates).

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