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LONG TERM EFFECTS OF AGRICULTURAL SYSTEMS ON SOIL PHOSPHATASE ACTIVITIES

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ABSTRACT

Long-term field trials can provide important information about the effects of soil management practices on soil properties but there are relatively few such trials available. The Agricultural Research and Development Station in Oradea (Bihor county) provided opportunity to study the effects of 18 years of cultivation on preluvosoil. The objective of the reported work was to determine at this site the effects of soil management practices on phosphatase activities as an index of soil biology. Phosphatase (phosphomonoesterase) activities were determined for two years, from 2008 to 2009, in the 0-20, 20-40 and 40-60 cm layers of a preluvosoil, from a long term trials with various tillage practices (no-till and conventional tillage), crop rotation (2 and 6 crop rotations) and fertilization [mineral (NP) fertilization and farmyard-manuring] experiment. The determined activities decreased with increasing sampling depth. No-till – in comparison with conventional tillage – resulted in significantly higher soil phosphatase activities in the 0-20 and in significantly lower activities in the deeper layers. The soil under maize or wheat was more enzyme-active in the 6 than in the 2 crop rotation. In the 2 crop rotation, higher phosphatase activities were recorded under wheat than under maize. Farmyard-manuring of maize - in comparison with mineral (NP) fertilization – led to a significant increase in enzyme activities. Maintenance of enzyme activities over tens of years in agricultural soils is partly attributed to traditional management practices including rotations with legumes, additions of animal manures, and minimum tillage.

Key words: crop rotation, fertilization, phosphatase activities, soil management, tillage.

INTRODUCTION

Phosphorus (P) represents a major nutrient element after nitrogen in higher plants (Turner et al., 2002). As an essential macro-nutrient for plant growth and development, P is found largely as phosphate esters, which possess a huge reserve of free hydrolyzing energy. P is implicated in processes of photo-synthesis and respiration, biosynthesis of proteins and complex carbohydrates, and is a component part of nucleic acids (McDowell and Sharpley, 2001). The supply of plants with the necessary P amount for their normal growth and development depends first of all, on the availability and solubility of P com-pounds in the soil (Ciobanu et al., 2009; Sttadon et. al., 1998).

In soil, P exists in both organic and inorganic forms. Up to 90% of the total soil P is found in the non-liable pool as results of its immobilization by soil organic and inorganic components (Pavlovschi and Ionescu, 1940; Borza, 2009; Rao et al., 1996). Besides this, and on a world wide scale, P represents the most often deficient nutrient in soil. A big part (20% to 90%) of total soil P exists as organic soil P. Therefore the organic P compounds have to be converted into inorganic P forms through the reactions that are mediated mainly by phosphomonoesterase (Olander and Vitousch, 2000).

Two types of phosphatases are known, the acid phosphatase (AcPA) and alkaline phosphatase (AlkPA), which occur in depen-dence of pH value of soils. It has been shown that phosphatases are concentrated in the sur-face layer and rhizosphere where most of the fresh and less rotted organic matter is found (Ştefanic and Dumitru, 1968; Criquet et al., 2004). Since soil rhizosphere represents a complex of living communities, it is consi-dered that soil AcPA activity and AlkPA that are responsible for organic P transformation in

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soil might be originating from extracellular and intracellular enzyme activities (Wright and Reddy, 2009). AcPA activity in soil originates from many sources, including plant roots (Baligar et al., 1988), fungi, micorrhizal fungi and bacteria (Todano et al., 1993). AlkPA is produced by soil microorganisms and soil fauna, whereas higher plants are devoid of alkaline phosphatase (Hysek and Sarapatka, 1998).

The activity of soil AcPA and AlkPA that are responsible for hydrolysis of both esters and anhydrous H_3PO_4 of soil organic matter depends on various factors as soil type and its fertility, type of fertilization and nutrient management, soil microbiological activity, organic matter, soil pH, soil moisture and varieties of higher plant species (Domuța, 2009).

Plants manifest different adaptive reactions to the impact of environment factors, thereby regulating their supply with necessary nutrients. Numerous data demonstrated that the soil activity of extracellular AcPA and AlkPA increase plant growth under P deficient conditions (Kang and Freeman, 1999; Senwo et al., 2007).

MATERIAL AND METHODS

The ploughed layer of the studied soil is of mellow loam texture, it has a pH value of 5.5, medium humus (2.32%) and P (22 ppm) contents, but it is rich in K (83 ppm).

The experiment started in 1992. The experimental field occupying 3.84 ha was divided into plots and subplots for comparative study of no-till and conventional tillage, rotations of 2 and 6 crops, and mineral (NP) fertilization and farmyard-manuring.

The crops of the two rotations are specified in table 1.

Table 1. Crops of the two rotations

Year	2 crop rotation		6 crop rotation					
	Plots		Plots					
	1	2	1	2	3	4	5	6
2008	Wheat	Maize	Maize (FYM)*	Clover	Wheat	Soybean	Maize	Oats – clover
2009	Maize	Wheat	Oats – clover	Maize (FYM)*	Clover	Maize	Wheat	Soybean

*FYM – farmyard-manured

Each plot consisted of two subplots representing the no-till and conventional tillage variants. The plots were annually NP-fertilized at rates of 120 kg N/ha and 90 kg P/ha, excepting in each year, a maize plot (in the 6 crop rotation) which received farmyard manure (50 t/ha) instead of mineral fertilizers. The plots (and subplots) were installed in three repetitions.

In October 2008 and 2009, soil was sampled from all subplots. Sampling depths were 0-20, 20-40 and 40-60 cm. The soil samples were allowed to air-dry, then ground and passed through a 2 mm sieve and, finally used for determination of phosphatase activities. Disodium phenylphosphate serve as enzyme substrate. Two activities were measured: acid phosphatase activity in reaction mixtures to which acetate buffer (pH 5.0) was added and alkaline phosphatase activity in reaction mixtures treated with borax buffer (pH 9.4). The buffer solutions were prepared as recommended by Őhlinger (1996).

The reaction mixtures consisted of 2.5 g soil, 2 ml toluene (antiseptic), buffer solution and 10 ml 0.5% substrate solution. Reaction mixtures without soil or without substrate solution were the controls. All reaction mixtures were incubated at 37°C for 2 hours.

After incubation, the phenol released from the substrate under the action of phosphatases was determined spectrophotometrically (at 614 nm) based on the colour reaction between phenol and 2.6-dibromoquinone-4-chloroimide. Phosphatase activities were expressed in mg phenol/g soil/2 hours. The activity values were submitted to statistical evaluation by the two-way-t-test (Sachs, 2002).

RESULTS AND DISCUSSIONS

Results of the determination of phosphatase activities are presented in Tables 2 and 3, and those of the statistical evaluation are summarized in table 4.

Variation of soil phosphatase activities in dependence of sampling depth

It is evident from Tables 2 and 3 that each phosphatase activity decreased with sampling depth in both subplots under all crops of both rotations. In addition, table 4 shows that the mean values of each of the two activities in both non-tilled and conventionally tilled subplots also decreased with increasing soil depth.

The effect of tillage practices on the phosphatase activities in soil

Each of the two determined phosphatase activities was significantly higher (at $p < 0.01$) in the upper (0-20 cm) layer of the non-tilled subplots than in the same layer of the conventionally tilled subplots. The reverse was true (at $p < 0.02$) in the deeper (20-40 and 40-60 cm) layers. These findings were also valid for subplots under each crop of both rotations (Table 4).

Table 2. The effects of soil management practices on phosphatase activities in 2008

Soil phos-phatase activity*	Soil depth (cm)	Rotation of 2 crops**				Rotation of 6 crops**											
		Wheat		Maize		Maize (FYM)		Clover		Wheat		Soybean		Maize		Oats-clover	
		N.t.	C.t.	N.t.	C.t.	N.t.	C.t.	N.t.	C.t.	N.t.	C.t.	N.t.	C.t.	N.t.	C.t.	N.t.	C.t.
Acid	0-20	0.263	0.206	0.221	0.200	0.304	0.296	0.280	0.246	0.336	0.316	0.352	0.328	0.290	0.278	0.323	0.308
	20-40	0.166	0.239	0.192	0.196	0.178	0.207	0.150	0.163	0.209	0.221	0.184	0.222	0.182	0.190	0.161	0.181
	40-60	0.122	0.165	0.115	0.139	0.161	0.162	0.122	0.146	0.122	0.158	0.114	0.111	0.143	0.153	0.128	0.148
Alkaline	0-20	0.202	0.194	0.258	0.173	0.314	0.250	0.240	0.195	0.268	0.241	0.258	0.213	0.263	0.243	0.244	0.232
	20-40	0.136	0.165	0.118	0.157	0.201	0.205	0.146	0.163	0.178	0.208	0.156	0.179	0.155	0.168	0.149	0.181
	40-60	0.050	0.081	0.044	0.079	0.052	0.040	0.064	0.092	0.082	0.095	0.080	0.085	0.055	0.064	0.053	0.077

* Expressed in mg phenol/g soil/2 hours. ** N.t. – no-till; C.t. – conventional tillage. (FYM) – (farmyard-manured).

Table 3. The effects of soil management practices on phosphatase activities in 2009

Soil phos-phatase activity*	Soil depth (cm)	Rotation of 2 crops**				Rotation of 6 crops**											
		Maize		Wheat		Oats-clover		Maize (FYM)		Clover		Maize		Wheat		Soybean	
		N.t.	C.t.	N.t.	C.t.	N.t.	C.t.	N.t.	C.t.	N.t.	C.t.	N.t.	C.t.	N.t.	C.t.	N.t.	C.t.
Acid	0-20	0.294	0.204	0.318	0.250	0.352	0.245	0.429	0.362	0.310	0.246	0.387	0.312	0.344	0.298	0.303	0.247
	20-40	0.141	0.171	0.135	0.173	0.136	0.179	0.179	0.204	0.137	0.156	0.142	0.209	0.149	0.195	0.133	0.146
	40-60	0.097	0.108	0.069	0.137	0.117	0.123	0.115	0.133	0.090	0.136	0.104	0.107	0.112	0.156	0.118	0.126
Alkaline	0-20	0.094	0.082	0.102	0.086	0.116	0.082	0.136	0.102	0.100	0.084	0.124	0.096	0.098	0.086	0.104	0.086
	20-40	0.040	0.052	0.044	0.050	0.048	0.054	0.056	0.064	0.048	0.054	0.052	0.060	0.050	0.054	0.044	0.048
	40-60	0.022	0.032	0.022	0.038	0.034	0.042	0.038	0.044	0.036	0.046	0.030	0.040	0.028	0.040	0.036	0.044

* Expressed in mg phenol/g soil/2 hours. ** N.t. – no-till; C.t. – conventional tillage. (FYM) – (farmyard-manured).

Our observation is in agreement with other studies. The higher enzyme activity values in the surface profile increments of the no-till plots compared to the conventional tillage plots indicates that higher biological activity was established near the soil surface where long-term no-till had been practiced. Acid and alkaline phosphatase activities have not been observed in plant tissue (Dick et al., 2000) so that the source of this enzyme in soil seems to be exclusively from soil microorganisms. High microbial activity is

desirable in decomposing the plant residue deposited on the soil surface, so that the nutrients contained in the residue can be recycled. Parham et al. (2002) also suggested that increased enzyme activities may be responsible for higher residual nutrient concentrations and increased fertilizer use efficiency of crops, sometimes reported for no-till systems.

Table 4. Significance of the differences between phosphatase activities in a preluvosoil submitted to different management practices

Management practices	Soil phosphatase activity*	Year	Soil depth (cm)	Mean activity values in management practices			Significance of the differences
				a	b	a-b	
No-till (a) versus conventional tillage (b)	AcPA	2008	0-20	0.296	0.272	0.024	0.002>p>0.001
			20-40	0.178	0.202	-0.024	0.02>p>0.01
			40-60	0.128	0.148	-0.020	0.01>p>0.002
		2009	0-20	0.342	0.270	0.072	0.0001>p
			20-40	0.114	0.179	-0.035	0.001>p>0.0001
			40-60	0.102	0.128	-0.026	0.02>p>0.01
	AlkPA	2008	0-20	0.256	0.218	0.038	0.01>p>0.002
			20-40	0.155	0.178	-0.023	0.001>p>0.0001
			40-60	0.060	0.080	-0.020	0.001>p>0.0001
		2009	0-20	0.109	0.088	0.021	0.001>p>0.0001
			20-40	0.047	0.054	-0.007	0.001>p>0.0001
			40-60	0.030	0.040	-0.01	0.0001>p
The same crop in the two rotations							
Maize in 2 crop rotation (a) versus maize in 6 crop rotation (b)	AcPA	2008	0-60	0.177	0.185	-0.008	0.01>p>0.002
		2009	0-60	0.168	0.210	-0.042	0.10>p>0.05
	AlkPA	2008	0-60	0.138	0.150	-0.012	0.0001>p
		2009	0-60	0.053	0.067	-0.014	0.01>p>0.002
Wheat in 2 crop rotation (a) versus maize in 6 crop rotation (b)	AcPA	2008	0-60	0.194	0.227	-0.033	0.10>p>0.05
		2009	0-60	0.180	0.209	-0.029	0.01>p>0.002
	AlkPA	2008	0-60	0.138	0.179	-0.041	0.002>p>0.001
		2009	0-60	0.057	0.059	-0.02	0.02>p>0.01
Different crops in the same rotation 2 crop rotation							
Wheat (a) versus maize (b)	AcPA	2008	0-60	0.194	0.177	0.017	0.01>p>0.002
		2009	0-60	0.180	0.168	0.012	0.02 >p>0.01
	AlkPA	2008	0-60	0.138	0.138	0.000	-
		2009	0-60	0.057	0.053	0.004	0.02>p>0.01
6 crop rotation							
Maize (FYM)** (a) versus maize (b)	AcPA	2008	0-60	0.218	0.206	0.012	0.01>p>0.002
		2009	0-60	0.237	0.210	0.027	0.02>p>0.01
	AlkPA	2008	0-60	0.181	0.158	0.028	0.05>p>0.02
		2009	0-60	0.073	0.007	0.006	0.01>p>0.002

* Expressed in mg phenol/g soil/2 hours. AcPA – Acid phosphatase activity. AlkPA – Alkaline phosphatase activity.
** (FYM) – (farmyard-manured).

Soil management influences soil microorganisms and soil microbial processes through changes in the quantity and quality of plants residues entering the soil and its spatial distribution.

In conventional tillage systems, organic matter is more thoroughly distributed than in no-till systems, where crop residues are concentrated on the soil surface. The high concentration of residues and roots of previous

crops on the soil surface under no-till can effect the microbial activity. One of the beneficial effects due to no-till may be the „rhizosphere effect", which probably contribute significantly to higher enzyme activities when compared with conventional tillage systems (Tarafdar and Marschner, 1994).

The effect of crop rotations on the phosphatase activities in soil

For evaluation of this effect, the results obtained in the three soil layers analyzed in the two subplots of each plot were considered together.

Soil phosphatase activities as affected by the same crop in the two rotations

As maize and wheat were included in both rotations, it was possible to compare their effect on soil phosphatase activities. The soil under both crops was more phosphatase-active in the 6- than in the 2 crop rotation. In the soil under maize, the difference between the two rotations was significant (at $p < 0.01$) in the case of each phosphatase activity, excepting acid phosphatase activity in 2009, when this activity was not significantly higher ($p > 0.05$). In the soil under wheat, only acid phosphatase activity in 2008 was not significantly different in the 6- than in the 2 crop rotation ($p > 0.05$).

Soil phosphatase activities as affected by different crops in the same rotation

The 2 crop rotation. Each phosphatase activity measured in the wheat soil exceeded significantly (at $p < 0.01$), the corresponding activity recorded in the maize soil, excepting alkaline phosphatase activity in 2008 which was the same under both crops (Table 4).

The 6 crop rotation. Significant ($p < 0.05$ to $p < 0.001$) and not significant ($p > 0.05$ to $p > 0.10$) differences were observed in the soil phosphatase activities, depending on the type of activity and the nature of crop. Based on these differences the following decreasing orders of the enzymatic activities could be established in the soil of the six crops in the two consecutive years:

- in 2008:

  - acid phosphatase activity: wheat > soybean > maize (FYM) > oats-clover > maize > clover;

  - alkaline phosphatase activity: wheat > maize (FYM) > soybean > maize > oats-clover > clover.

- in 2009:

  - acid phosphatase activity: maize (FYM) > maize > wheat > oats-clover > clover > soybean;

  - alkaline phosphatase activity: maize (FYM) > maize > oats-clover > clover> soybean > wheat.

For establishing a hierarchy of the plots in the 6 crop rotation admitting equal importance for the two phosphatase activities, we have used the method, referred to in Samuel et al. (2008), to calculate the enzymatic indicators of soil quality (EISQ). Briefly, by taking the maximum mean value of each activity as 100%, we calculated the relative (percentage) activities. The sum of the relative activities was the enzymatic indicator which was considered as an index of the biological quality of the soil in a given plot. The higher the enzymatic indicator of soil quality, the higher the position of plots was in the hierarchy.

The obtained results show that the different hierarchies of the six plots, based on their phosphatase indicators of soil quality as observed in 2008 and 2009, may be related to the different nature of crops and kind of fertilizers (mineral NP or farmyard manure) (Figure 1).

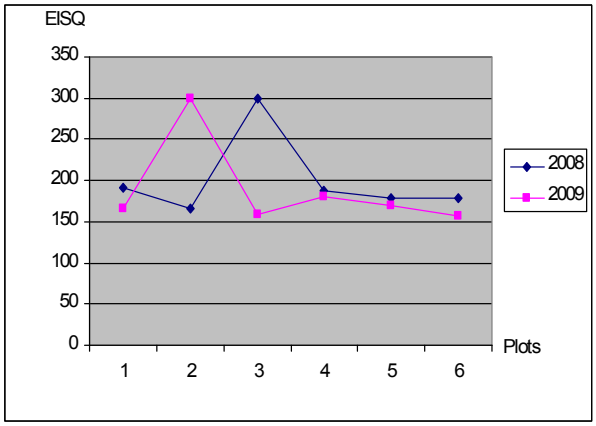

Figure 1. Enzymatic indicators of soil quality in plots of the 6 crop rotation

Our results on a preluvosoil are consistent with previous studies on other soils. Studies have shown that crop rotations have significantly higher levels of microbial biomass (Eichler et al., 2004) and soil enzyme activities (Tarafdar and Claassen, 1988) than cropping sequences that are either continuously monocultured or have more limited crop rotations.

Long-term management with plant nutrients and organic amendments does affect soil biological properties. In general, management practices that increase inputs of organic residue, plant or animal manures, increase biological activity.

Soil phosphatase activities as affected by fertilization

The two maize plots in the 6 crop rotation could serve for comparing the effect of mineral (NP) fertilization and farmyard-manuring on the soil phosphatase activities. Each activity was higher in the farmayard-manured maize plot than in the other minerally fertilized maize plot. The differences were significant (at $p < 0.01$) in the farmyard-manured plot than in the minerally fertilized plot. In concordance with these findings, figure 1 shows that the farmyard-manured maize plot occupies higher position, whereas the other maize plots are placed on lower positions in the hierarchy of plots in the 6 crop rotation.

It has been generally accepted that addition of farmyard manure usually increases soil enzyme activities (Parham et al., 2002). Also, management practices that increase incorporation of organic residue typically increase biological activity. Use of inorganic fertilizer can increase the plant biomass production, which in turn increases the amount of residue returned to the soil each year and stimulates biological activity.

CONCLUSIONS

Cultivation of soil, besides affecting soil chemistry and structure, also affects soil biology. Tillage reduces biological activity and there is evidence that this is due to the reduction of macro-aggregates with long-term cultivation practices. Macro-aggregates provide an important microhabitat for microbial activity. Conservation tillage practices that keep residue on the surface can maintain biological activity in the surface soil, but subsurface activity may be equal or lower in these systems compared with tilled soils.

Indirect evidence suggests that soil amendments such as animal manures and plant diversity (crop rotations) may be more important in maintaining soil microbial activity than conservation tillage in monocultural systems. There is increasing evidence that crop rotation affects crop productivity via suppressing deleterious microorganisms that flourish under monoculture. This also has implications for suppressing root disease organisms, where practices that promote soil biodiversity may inhibit certain disease organisms.

These studies have been useful in assessing the long-term effects of how agricultural practices change the soil biology. There is interest in developing a universal "soil quality index" that could be used to assess the „health" of a given soil. As shown by this paper, soil biological indices can be sensitive indicators to management practices. However, because soil biological parameters naturally vary widely among soil types it is necessary to have a reference point in time. Therefore at this time it is not possible to simple measure a series of soil biological parameters independent of a comparative control or treatment at a given site to determine the "soil health". This reaffirms the continuing need for the maintenance of existing long-term experimental sites.

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