ACID AND ALKALINE PHOSPHATASE ACTIVITIES OF AGRICULTURAL LAND

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Abstract

Agricultural soil contains phosphatases in variable amounts depending on microbial count, amount of organic materials, other macroscopic living organisms and their activities. The amount of phosphate released into the soil can then be directly co-related to soil fertility. 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 work reported 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 in the 0–20-, 20–40- and 40–60- cm layers of a preluvosoil submitted to a complex tillage (no-till and conventional tillage), crop rotation (2- and 6- crop rotations) and fertilization [mineral (NP) fertilization and farmyard-manuring] experiment. Each activity 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 each activity.

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. The phosphatases present in a soil sample are heterogeneous and might be utilized as a major parameter to assess soil fertility in an agricultural land.

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

INTRODUCTION

In nature, phosphorus cycle plays an important role in the survival of living organisms (Pankhurst C.E., 1997; Wang J.B. et al, 2011). Phosphatase is an enzyme that release inorganic phosphate from organic moiety and complex inorganic materials. It is known to play an essential role in phosphorus cycle (Samuel A.D. et al, 2011), even though, roles of other various physical and chemical factors cannot be ignored (Richardson A.E., and Simpson R.J., 2011). Soil receives various phosphatases from living organisms that play important roles in the solubilization of inorganic phosphates (Nannipieri P. et al, 2002). Enzymatic activities of a soil sample are critical index of soil fertility because enzymes play an important role in nutrient cycles (Olander L.P., and Vitousek P.M., 2000). Various minerals

such as Al, Fe, Mg and Ca are complexes with phosphorous and remain in soils in various forms (Dick W.A., and Tabatabai M.A., 1983). Rock phosphates of various kinds undergo solubilization due to microbial activities in the soil (Herrick J.E., 2000). In particular, phosphatases play a key role in phosphorous cycle by solubilizing organic and inorganic phosphates into available forms that support growth of crop plants (Dick W.A. et al, 2000; Freeman C. et al, 2008). Released inorganic form of phosphate is readily soluble in soil and plant system can easily uptake it as nutrient source. However, phosphorus also comes from death and decay of all living organisms that reside in soils (Hu C., and Cao Z., 2007; Huang W. et al, 2011).

Soil phosphatase activity depends heavily on moisture content and environmental temperature of the soil (Hysek K., and Sarapatka B., 1977; Lovell R.D. et al, 2005). Phosphatases are usually classified based on pH optim: these are neutral (EC 3.1.3.-), acid (EC 3.1.3.2.) and alkaline (EC 3.1.3.1). However, there are several subclasses, this classification is based on the type of substrate where it acts such as tyrosine specific phosphatase, serine-threonine specific phosphatase, dual specificity phosphatases, histidine phosphatase and lipid phosphatases (Acosta-Martinez V., and Tabatabai M.A., 2000; Balota E. et al, 2003). Soil phosphatases are heterogeneous in nature and the enzymes have tribal names, according to their substrates (Bandick A.K., and Dick R.P., 1999).

MATERIAL AND METHOD

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.

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 2012, 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 (Drăgan-Bularda M., 1983). 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 R., 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 37^0C 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 are expressed in mg phenol/g soil/ 2 hours.

The activity values were submitted to statistical evaluation by the two *t*-test (Sachs L., 2000).

RESULTS AND DISCUSSION

Results of the determination of phosphatase activities are presented in Table 1, and those of the statistical evaluation are summarized in Table 2.

Variation of soil phosphatase activities in dependence of sampling depth. It is evident from Table 1 that each phosphatase activity decreased with sampling depth in both subplots under all crops of both rotations. In addition, Table 2 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.

Table 1

The effects of soil management practices on phosphatase activities in 2012

Soil phos-phatase activity*	Soil depth (cm)	Rotation of 2 crops**				Rotation of 6 crops**					
		Wheat		Maize		Maize (FYM)		Wheat		Maize	
		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	0.336	0.316	0.290	0.278
	20-40	0.166	0.239	0.192	0.196	0.178	0.207	0.209	0.221	0.182	0.190
	40-60	0.122	0.165	0.115	0.139	0.161	0.162	0.122	0.158	0.143	0.153
Alkaline	0-20	0.202	0.194	0.258	0.173	0.314	0.250	0.268	0.241	0.263	0.243
	20-40	0.136	0.165	0.118	0.157	0.201	0.205	0.178	0.208	0.155	0.168
	40-60	0.050	0.081	0.044	0.079	0.052	0.040	0.082	0.095	0.055	0.064

* Expressed in mg phenol/g soil/2 hours.

** N.t. – no-till; C.t. – conventional tillage.

(FYM) – (farmyard-manured).

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.

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 W.A. et al, 2000) so that the source of this enzyme in soil seems to be exclusively from soil microorganisms. 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 B. et al, 2014) and soil enzyme activities (Turner B.L. et al, 2002) 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.

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. In the soil under wheat, only acid phosphatase activity 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. Acid 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 which was the same under both crops. 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 (Huang W. et al, 2004) and soil enzyme activities than cropping sequences that are either continuously monocultured or have more limitedcrop rotations.

Table 2

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

Management practices	Soil phospha-tase activity*	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	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
	AlkPA	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
The same crop in the two rotations						
Maize in 2 crop rotation (a) versus maize in 6 crop rotation (b)	AcPA	0-60	0.177	0.185	-0.008	0.01>p>0.002
	AlkPA	0-60	0.138	0.150	-0.012	0.0001>p
Wheat in 2-crop rotation (a) versus maize in 6 crop rotation (b)	AcPA	0-60	0.194	0.227	-0.033	0.10>p>0.05
	AlkPA	0-60	0.138	0.179	-0.041	0.002>p>0.001
Different crops in the same rotation 2 crop rotation						
Wheat (a) versus maize (b)	AcPA	0-60	0.194	0.177	0.017	0.01>p>0.002
	AlkPA	0-60	0.138	0.138	0.000	-
6 crop rotation						
Maize (FYM)** (a) versus maize (b)	AcPA	0-60	0.218	0.206	0.012	0.01>p>0.002
	AlkPA	0-60	0.181	0.158	0.028	0.05>p>0.02

* Expressed in mg phenol/g soil/2 hours. AcPA – Acid phosphatase activity. AlkPA – Alkaline phosphatase activity.

** (FYM) – (farmyard-manured).

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. It has been generally accepted that addition of farmyard manure usually increases soil enzyme activities (McDovell R.W., and Sharpley A.H., 2011). 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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