SOIL ENZYME ACTIVITIES UNDER LONG-TERM TILLAGE AND CROP ROTATION SYSTEMS

INFLUENȚA AFÂNĂRII ŞI ROTAȚIEI CULTURILOR ASUPRA ACTIVITĂȚII ENZIMATICE A SOLULUI

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Abstract: *Agricultural practices that reduce soil degradation and improve agricultural sustainability are needed particularly for preluvosoil. No-tillage planting causes minimal soil disturbance and combined with crop rotation may hold potential to meet these goals. Soil enzyme activities can provide information on how soil management affects the soil potential to perform processes, such as decomposition and nutrient cycling. Soil enzyme activities (actual and potential dehydrogenase, catalase, acid and alkaline phosphatase) 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) and crop rotation (2– and 6–crop rotations) experiment. Each activity in both non-tilled and conventionally tilled soil under all crops of both rotations decreased with increasing sampling depth. No-till – in comparison with conventional tillage – resulted in significantly higher soil enzymatic 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 enzymatic activities were recorded under wheat than under maize. The enzymatic indicators of soil quality were calculated from the values of enzymatic activities determined in the plots of the 6-crop rotation. The results obtained show that the different hierarchies of the six plots as registered in 2008 may be related to the different nature of crops and kind of fertilisers. This means that by determination of enzymatic activities, valuable information can be obtained regarding fertility status of soils.*

Rezumat: *Activitățile enzimatice studiate (dehidrogenazică, catalazică şi fosfatazică) au fost utilizate ca indicatori ai nivelului activității microbiene globale şi respective specifice a solului. S-au urmărit efectele lucrării de bază a solului (nearat şi arat profund) şi a rotației culturilor (2, şi, respectiv 6 culturi) asupra activității: dehidrogenazică actuală şi potențială, catalazică, fosfatazică acidă şi alcalină la trei adâncimi: 0 – 20, 20 – 40 şi 40 – 60 cm. Activitățile enzimatice studiate scad cu adâncimea, sub toate culturile atât în solul nearat cât şi în cel arat profund. Nearatul în comparație cu aratul profund determină niveluri mai ridicate ale activităților enzimatice în stratul superficial (0-20 cm) şi activități mai scăzute în straturile intermediar şi profound din ambele rotații. Activitățile enzimatice sunt mai ridicate sub culturile de porumb şi grâu din rotația de 6 culturi în toate straturile analizate. În rotația de 6 culturi, mediile activităților enzimatice pe adâncimea de 0-60 cm sunt mai ridicate sub cultura de grâu față de cultura de porumb. Cu ajutorul indicatorilor enzimatici ai calității solului care iau în considerare toate enzimele studiate, am stabilit o ierarhie a parcelelor cultivate din rotația de 6 culturi. In anul 2008 luând în considerare activitățile dehidrogenazice, catalazică şi fosfatazice din sol am obținut următoarea ierarhie a parcelelor : grâu fertilizat mineral (fm) > ovăz + trifoi (fm) > porumb + (gunoi de grajd) > soia (fm) > trifoi (fm) > porumb (fm).*

Key words: *catalase, crop rotation, dehydrogenase, phosphatase, preluvosoil, tillage*
Cuvinte cheie: *afânarea, catalaza, dehidrogenaza, fosfataza, rotația culturilor*

INTRODUCTION

Soil enzymes activities have successfully discriminated between a wide range of soil management practices (BALOTA et al.,2003; CANARUTTO et al., 1995; DICK, 1992). Although there is a lot of information that show the relation between soil management and soil enzymes activities, very little is known about these effects under preluvosoil. The first enzymological data on this soil were published by Ştefanic and his collaborators. They studied the soil enzymological effect of mineral (NP) fertilisation and liming and found that catalase activity was higher while dehydrogenase, invertase and phosphatase activities were lower in the NP-fertilised and liming soil samples than in the unfertilised limed ones.

In order to obtain new data on the enzymological effects of soil management practices, we have determined some enzymatic activities in a preluvosoil submitted to a complex tillage and crop rotation experiment at the Agricultural Research and Development Station in Oradea (Bihor county).

It is well known that the dehydrogenase (BANDICK and DICK, 1999; DICK et al.,1994) and catalase (DICK et al., 1988; KANDELER and MURER, 1993) activities are considered as indicators of the global and respiratory activity of soil, whereas phosphomonoesterase enzymes play an important role in P cycling in soil and, consequently, in P nutrition of plants (CLARHOLM and ROSENGREN-BRINCK, 1995; DENG and TABATABAI, 1997; KIRCHNER et al.,1993).

Our present report contains the first data on the effects of complex management practices on the enzymatic activities in this preluvosoil.

MATERIALS AND METHODS

The ploughed layer of the studied preluvosoil 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 pp). The experimental field occupying 3.84 ha was divided into plots and subplots for comparative study of no-till and conventional tillage and rotations of 2 (maize, wheat) and 6 [wheat, soybean, maize, maize (FYM), clover, oats-clover] crops.

Each plot consisted of two subplots representing the no-till and conventional tillage variants. The plots were annually NP-fertilised at rates of 120 kg N / ha and 90 kg of P / ha, excepting, in each year, a maize plot (in the 6–crop rotation) which received farmyard manure (50 t/ha) instead of mineral fertilisers. The plots (and subplots) were installed in three repetitions. In October 2008, 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 enzymological analyses.

Actual and potential dehydrogenase activities were determined according to the methods describe in SAMUEL and KISS (1999). The reaction mixtures consisted of 3.0 g soil, 0.5 ml TTC (2,3,5- triphenyltetrazolium chloride) and 1.5 ml distilled water or 1.5 ml glucose solution, respectively, for potential dehydrogenase. All reaction mixtures were incubated at 37º C for 24 hours. After incubation, the triphenylformazan produced was extracted with acetone and was measured spectrophotometrically at 485 nm. Dehydrogenase activities are expressed in mg of triphenylformazan (TPF) produced (from 2,3,5- triphenyltetrazolium chloride, TTC) by 10 g of soil in 24 hours.

Catalase activity was determined using the permanganometric method (EGNER et al., 1980), the reaction mixtures consisted of 3.0 g soil, 2 ml H_2O_2 3% and 10 ml phosphate buffer. It suffered incubation at 37º C for 1 hour. Catalase activity is recorded as mg H_2O_2 decomposed by 1 g of soil in 1 hour.

For determination of phosphatase (phosphomonoesterase) activity, disodium phenylphosphate served 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), 10 ml distilled water or 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 37º 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 are expressed in mg phenol / g soil / 2 hours. The activity values were submitted to statistical evaluation by the two *t*-test (SACHS, 2002).

RESULTS AND DISCUSSION

Results of the statistical evaluation are summarised in Table1.

Variation of soil enzymatic activities in dependence of sampling depth

It is evident that each enzymatic activity decreased with sampling depth in both subplots under all crops of both rotations. In addition, Table 1 shows that the mean values of each of the five activities in both non-tilled and conventionally tilled subplots also decreased with increasing soil depth.

The effect of tillage practices on the enzymatic activities in soil

Each of the five enzymatic activities determined was significantly higher (at least at $p < 0.02$) 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 least at $p < 0.02$) in the deeper (20–40– and 40–60–cm) layers. These findings are also valid for subplots under each crop of both rotations.

The effect of crop rotations on the enzymatic activities in soil

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

The soil enzymological effect of the same crop in the two rotations

As maize and wheat were crops in both rotations, it was possible to compare the soil enzymological effect of the 2– and 6–crop rotations. The soil under both crops was more enzyme-active in the 6– than in the 2–crop rotation. In the soil under maize, the difference between the two rotations was significant (at least at $p < 0.05$) in the case of potential dehydrogenase, acid and alkaline phosphatase activities whereas in the soil under wheat, each activity was significantly higher (at least at $p < 0.02$) in the 6– than in the 2–crop rotation, excepting acid phosphatase activity.

The soil enzymological effect of different crops in the same rotation

The 2–crop rotation. Actual and potential dehydrogenase activities were significantly higher ($p < 0.05$ and $p < 0.01$, respectively), while catalase activity was unsignificantly higher ($p > 0.05$) in the wheat soil than in the soil under maize. Acid phosphatase activity measured in the wheat soil exceeded significantly ($p < 0.01$) the corresponding activity recorded in the maize soil. In the case of alkaline phosphatase activity weren't differences between the crops.

The 6–crop rotation. Significant ($p < 0.05$ to $p < 0.001$) and unsignificant ($p > 0.05$ to $p > 0.10$) differences were registered in the soil enzymatic activities depending on the kind of enzymatic 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 plots:

Actual dehydrogenase activity: wheat > oats-clover > soybean > clover > maize (FYM) > maize;

Potential dehydrogenase activity: maize (FYM) > soybean > wheat > oats-clover > maize > clover;

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

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

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

It is evident from these orders that each of the six plots presented either a maximum or a minimum value of the five soil enzymatic activities.

Table 1

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

Management practices	Soil enzymatic 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)	ADA	0-20	6.72	5.69	1.03	0.01 > p > 0.002
		20-40	3.67	4.37	-0.70	0.002 > p > 0.001
		40-60	1.72	2.51	-0.79	0.001 > p > 0.0001
	PDA	0-20	25.52	22.51	3.01	0.02 > p > 0.01
		20-40	15.36	16.61	-1.25	0.02 > p > 0.01
		40-60	5.27	6.14	-0.87	0.001 > p > 0.0001
	CA	0-20	1.66	1.48	0.18	0.01 > p > 0.002
		20-40	1.00	1.25	-0.25	0.01 > p > 0.002
		40-60	0.44	0.63	-0.19	0.02 > p > 0.01
	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 (b) versus maize in 6– crop rotation (b)	ADA	0-60	2.98	3.31	-0.33	0.10 > p > 0.05
	PDA		13.99	15.61	-1.62	0.05 > p > 0.02
	CA		0.82	0.97	-0.15	0.10 > p > 0.05
	AcPA		0.177	0.185	-0.008	0.01 > p > 0.002
	AlkPA		0.138	0.150	-0.012	0.0001 > p
Wheat in 2– crop rotation (b) versus in wheat 6– crop rotation (b)	ADA	0-60	4.44	5.16	-0.72	0.02 > p > 0.01
	PDA		14.11	15.83	-1.72	0.02 > p > 0.01
	CA		1.20	1.26	-0.06	0.02 > p > 0.01
	AcPA		0.194	0.227	-0.033	0.10 > p > 0.05
	AlkPA		0.138	0.179	-0.041	0.002 > p > 0.001
Different crops in the same rotation						
2– crop rotation Maize (a) versus wheat (b)	ADA	0-60	2.98	4.44	-1.46	0.05 > p > 0.02
	PDA		13.98	14.11	-0.13	0.01 > p > 0.002
	CA		0.82	1.20	-0.38	0.10 > p > 0.05
	AcPA		0.177	0.194	-0.017	0.01 > p > 0.002
	AlkPA		0.138	0.138	0.000	-
6– crop rotation Maize (a) versus maize(FYM)** (b)	ADA	0-60	3.31	3.53	-0.22	0.01 > p >0.002
	PDA		15.61	16.96	-1.35	0.002 > p > 0.001
	CA		0.97	1.17	-0.20	0.02 > p > 0.001
	AcPA		0.185	0.218	-0.033	0.001 > p > 0.0001
	AlkPA		0.150	0.181	-0.031	0.01 > p > 0.002

* ADA – Actual dehydrogenase activity.
PDA – Potential dehydrogenase activity.
CA – Catalase activity.
AcPA – Acid phosphatase activity.
AlkPA – Alkaline phosphatase activity.
**(FYM) – (farmyard-manured).

Consequently, these orders do not make it possible to establish such an enzymatic

hierarchy of the plots which takes into account each activity for each plot. For establishing such a hierarchy, we have applied the method suggested in (SAMUEL and KISS, 1999). Briefly, by taking the maximum mean value of each activity as 100% we have calculated the relative (percentage) activities. The sum of the relative activities is the enzymatic indicator which is 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 is in the hierarchy. Table 2 shows that the first three positions are occupied by those plots in which dehydrogenase, catalase and phosphatase activities were the highest. Thus, position 1 was occupied by the mineral fertilised wheat plot, whereas the farmyard-manured maize plot and the mineral fertilised legumes (soybean and clover) were placed on the positions 3, 4 and 5, respectively. The mineral fertilised maize plot occupied the last position could be considered as the least enzyme-active soil.

Table 2

Enzymatic indicators of soil quality in plots of the 6–crop rotation

Position	Plot	Enzymatic indicator of soil quality
1	Minerally fertilised (M.f.) wheat	485.73
2	M.f. oats-clover mixture	464.17
3	Farmyard-manured maize	447.31
4	M.f. soybean	435.05
5	M.f. clover	413.39
6	M.f. maize	392.22

CONCLUSIONS

The soil enzymatic activities decreased with increasing sampling depth.

No-till – in comparison with conventional tillage - resulted in higher enzymatic activities in the 0–20– layer and in lower activities in the 20–40– and 40–60–cm soil layers under each crop of both rotations.

The 6–crop rotation – as compared to the 2–crop rotation – led, in general to higher enzymatic activities in the soil layers under maize or wheat.

In the 2–crop rotation, the soil layers under wheat were more enzyme active than those under maize.

The enzymatic indicators of soil quality calculated from the values of enzymatic activities determined in the plots of the 6–crop rotation showed the order: mineral fertilised (m.f.) wheat> m.f. oats-clover mixture > farmyard-manured maize > m.f. soybean > m.f. clover > m.f. maize.

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