ENZYME ACTIVITIES IN THE PRELUVOSOIL OF ORADEA

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ABSTRACT. 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), crop rotation (2– and 6–crop rotations) and fertilisation [mineral (NP) fertilisation and farmyard-manuring] 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. In the 6–crop rotation, the enzymatic indicators of soil quality decreased, depending on the nature of crops and kind of fertilisers (mineral NP or farmyard manure), in the following order: minerally fertilised (m.f.) wheat > m.f. oats-clover > farmyard-manured maize > m.f. soybean > m.f. clover> m.f. maize. This order means that by determination of enzymatic activities valuable information can be obtained regarding fertility status of soils. It should be emphasised that farmyard-manuring of maize - in comparison with its mineral (NP) fertilisation – led to a significant increase in each of the five enzymatic activities determined. There were positive correlations between acid phosphatase activity and mobile phosphorus content under maize and wheat crops of the both rotations.

Keywords: catalase, crop rotation, dehydrogenase, phosphatase, tillage

INTRODUCTION

The degradation of plant and animal matter, the release and binding of nutrients and trace elements is one of the most important functions of soil organisms. The microorganisms are important for the enzymatic degradation of the complex organic substances to nutrients and for the release of nutrients and trace elements from the mineral soil fraction.

The number and activity of soil microorganisms are dependent on plant growth, soil type, soil treatment, soil cultivation as well as on the macro- and microclimate (Canarutto et al., 1995).

The effects of cultivation on soil biology (Farrell et al., 1994) can be modified by the type of tillage management that is used. Conservation tillage practices that have various degrees of soil disturbance and that leave significant amounts of plant residue on the soil surface can affect biological properties of soils. No-tillage systems result in an increase in the concentration of nutrients, organic matter and pesticides at the soil surface (Fenn et al., 1992).

Studies have shown that crop rotations have significantly higher levels of microbial biomass (Dick et al., 1994) and soil enzyme activities (Dick et al., 1988) than cropping sequences that are either continuously monocultured or have more limited crop rotations. Continuous monoculturing of a single crop species typically results in reduction of crop yields in comparison to the same species in rotation (Angers et al., 1993) and these reductions usually are not associated with fertility or pest interactions. Although it has been suggested that alleopathic toxins derived from decomposing plant residues may inhibit yields, this has yet to be clearly established (Breakwell and Turco, 1990).

In general, management practices that increase inputs of organic residue, plant or animal manures, increase biological activity. Addition of farmyard manure, usually increases microbial biomass and soil enzyme activities (Samuel and Kiss, 1999) over soils that have not received any organic or inorganic amendments. However, when comparisons have been made between soils amended with farmyard manure or organic fertilisers, there have been mixed results which vary with cropping system and biological index. Thus management practices that increase incorporation of organic residue typically increase biological activity. Use of inorganic fertiliser can increase the plant biomass production which in turn increases the amount of residue returned to the soil and stimulates biological activity (Dick, 1992).

The effects of tillage, crop rotation and fertilization on soil enzymatic activities were studied in many countries, including Romania (Balota et al., 2004; Bandick and Dick, 1999; Samuel et al., 2000; Domuta, 2005).

Soil enzymes are important for catalyzing innumerable reactions necessary for life processes of microorganisms in soils, decomposition of organic residues, cycling of nutrients and formation of organic matter and soil structure (Dick, 1997). Although enzymes are primarily of microbial origin it can also be

originate from plants and animals. These enzymes are constantly being synthesized, could be accumulated, inactivated and / or decomposed in the soil, assuming like this, great importance for the agriculture for their role in the recycling of the nutrients (Clarholm and Rosengren-Brinck, 1995).

Soil enzymes activities have successfully discriminated between a wide range of soil management practices (Deng and Tabatabai, 1997). Although there are a lot of information that show the relation between soil management and soil enzymes activities, very little is known about these effects under brown luvic soil. The first enzymological data on this soil were published by Ştefanic (1991). 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 brown luvic soil submitted to a complex tillage, crop rotation and fertilisation experiment at the Agricultural Research and Development Station in Oradea (Bihor county).

It is well known (Gupta and Germida, 1998) that the dehydrogenase and catalase 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 (Griffith et al., 1998).

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 ppm).

The experimental 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) fertilisation and farmyard-manuring.

The crops of the two rotations are specified in Table 1. 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 2007, 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 (Ohlinger, 1996b). 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 (Dragan-Bularda, 1983), 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 (Ohlinger, 1996a). 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 Ohlinger (1996a). The reaction mixtures consisted of 2.5 g soil, 2 ml toluen (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.

Chemical indicator was determined according to the method described in (Gupta and Germida, 1988).

The activity values were submitted to statistical evaluation by the two *t*-test and the correlations between the enzymatic activity and chemical indicator were determined according to the methods described in (Kiss et al., 1990).

RESULTS AND DISCUSSION

Results of the determination of enzymatic activities are presented in Table 1 and those of the statistical evaluation are summarised in Table2.

Table 1

THE EFFECTS OF SOIL MANAGEMENT PRACTICES ON ENZYMATIC ACTIVITIES IN PRELUVOSOIL

Soil enzymatic activity*	Soil depth (cm)	Rotation of 2 crops** Maize N.t.	Maize C.t.	Wheat N.t.	Wheat C.t.	Wheat N.t.	Wheat C.t.	Soybean N.t.	Soybean C.t.	Rotation of 6 crops Maize N.t.	Maize C.t.	Maize (FYM)*** N.t.	Maize (FYM)*** C.t.	Clover N.t.	Clover C.t.	Oats-clover N.t.	Oats-clover C.t.
ADA	0-20	4.68	4.50	7.36	6.02	7.76	6.80	7.56	6.72	5.76	4.88	5.82	5.12	6.16	5.32	8.68	6.16
	20-40	2.68	3.10	4.84	5.20	5.01	5.60	1.40	4.81	2.86	3.52	2.52	3.92	4.04	4.36	3.36	4.48
	40-60	1.12	1.80	1.36	1.84	2.80	3.01		2.52	1.02	1.84	1.40	2.40	2.24	2.80	2.40	3.90
PDA	0-20	23.52	22.36	22.96	21.20	25.20	23.20	26.60	20.90	24.12	22.96	27.16	25.48	24.92	20.72	29.68	23.24
	20-40	15.68	16.52	14.08	15.40	15.28	18.96	16.40	17.72	16.44	17.48	17.92	18.48	12.60	12.88	14.52	15.40
	40-60	2.52	3.36	5.32	5.72	5.60	6.76	7.00	7.56	5.64	7.00	6.01	6.72	4.88	5.60	5.20	6.36
CA	0-20	1.27	1.17	1.86	1.52	1.98	1.77	1.42	1.23	1.44	1.37	1.73	1.68	1.43	1.39	2.15	1.73
	20-40	0.62	1.06	1.17	1.45	1.37	1.49	0.22	0.90	1.02	1.16	1.05	1.10	0.93	1.27	0.74	1.59
	40-60	0.25	0.53	0.59	0.62	0.62	0.34		0.57	0.27	0.55	0.49	0.65	0.55	0.44	0.53	1.34
AcPA	0-20	0.221	0.200	0.263	0.206	0.336	0.216	0.35	0.328	0.280	0.246	0.304	0.296	0.290	0.278	0.323	0.308
	20-40	0.192	0.196	0.166	0.239	0.209	0.221	0.18	0.224	0.150	0.163	0.178	0.207	0.181	0.190	0.161	0.181
	40-60	0.115	0.139	0.122	0.165	0.122	0.158	0.11	0.111	0.122	0.146	0.161	0.162	0.143	0.143	0.128	0.148
AlkPA	0-20	0.258	0.173	0.202	0.194	0.268	0.241	0.25	0.213	0.240	0.195	0.314	0.250	0.263	0.243	0.244	0.232
	20-40	0.118	0.157	0.136	0.165	0.178	0.208	0.15	0.179	0.146	0.163	0.201	0.205	0.155	0.168	0.149	0.181
	40-60	0.044	0.079	0.050	0.081	0.082	0.095	0.08	0.085	0.064	0.092	0.052	0.064	0.055	0.064	0.053	0.077

** ADA – Actual dehydrogenase activity (expressed in mg TPF/10g soil/24 hours).*
PDA – Potential dehydrogenase activity (expressed in mg TPF/10g soil/24 hours).
CA – Catalase activity (expressed in mg H_2O_2 / g soil /1 hour).
****AcPA – Acid phosphatase activity (expressed in mg phenol / g soil / 2 hours).*
AlkPA – Alkaline phosphatase activity (expressed in mg phenol / g soil / 2 hours).

***N.t. – No-till.*
C.t. – Conventional tillage.
**** (FMY) – (farmyard-manured).*

Table 2

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 a	b	a-b	Significance of the differences
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	ADA	0-60	2.98	3.31	-0.33	0.10 > p > 0.05
rotation (b) versus	PDA		13.99	15.61	-1.62	0.05 > p > 0.02
maize in 6– crop	CA		0.82	0.97	-0.15	0.10 > p > 0.05
rotation (b)	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	ADA	0-60	4.44	5.16	-0.72	0.02 > p > 0.01
rotation (b) versus in	PDA		14.11	15.83	-1.72	0.02 > p > 0.01
wheat 6– crop rotation	CA		1.20	1.26	-0.06	0.02 > p > 0.01
(b)	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	ADA	0-60	2.98	4.44	-1.46	0.05 > p > 0.02
Maize (a) versus	PDA		13.98	14.11	-0.13	0.01 > p > 0.002
wheat (b)	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	ADA	0-60	3.31	3.53	-0.22	0.01 > p >0.002
Maize (a) versus	PDA		15.61	16.96	-1.35	0.002 > p > 0.001
maize(FYM)** (b)	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).*

Variation of soil enzymatic activities in dependence of sampling depth

It is evident from Table 2 that each enzymatic activity decreased with sampling depth in both subplots under all crops of both rotations. In addition, Table 3 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.

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 3 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 minerally fertilised wheat plot, whereas the farmyard-manured maize plot and the minerally fertilised legumes (soybean and clover) were placed on the positions 3, 4 and 5, respectively. The minerally fertilised maize plot occupied the last position could be considered as the least enzyme-active soil.

The effect of fertilisation on the enzymatic activities in soil

The two maize plots in the 6–crop rotation could serve for comparing the soil enzymological effect of mineral (NP) fertilisation and farmyard-manuring. One can see from Table 1 that the enzymatic activities were always higher in the 20–40– and 40–60–cm layers of the farmyard-manured subplots in comparison with the subplots that had received mineral (NP) fertilisers. When the three soil layers were considered together (Table 2), each of the enzymatic activities was found to be significantly higher (at least at $p < 0.02$) in the farmyard-manured plot than in the minerally fertilised plot. In concordance with these findings, Table 3 shows that the farmyard-manured maize plot occupies position 3, whereas the other maize plot is placed on the last position in the hierarchy of plots in the 6–crop rotation.

Results of the determination of mobile phosphorus content are presented in Table 4.. It was found that acid phosphatase activity was positively correlated ($r = 0.821$ to $r = 0.935$) with the mobile phosphorus content.

Our results are in good agreement with the literature data and constitute novelties for the enzymological characterization of preluvosoil submitted to complex management practices.

Table 3

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

Table 4

THE EFFECTS OF SOIL MANAGEMENT PRACTICES ON CHEMICAL INDICATOR IN A PRELUVOSOIL

Chemical indicator	Soil depth (cm)	Rotation of 2 crops*				Rotation of 6 crops			
		Maize		Wheat		Maize		Wheat	
		N.t.	C.t.	N.t.	C.t.	N.t.	C.t.	N.t.	C.t.
P_2O_5	0-20	12.0	11.6	11.8	11.6	14.2	13.6	13.8	13.8
(mg P_2O_5 / kg soil)	20-40	10.5	9.7	8.8	11.7	11.0	10.0	9.6	11.1
	40-60	8.7	10.1	7.5	8.6	8.8	10.7	9.8	9.5

** N.t. – No-till.*
C.t. – Conventional tillage.

CONCLUSIONS

The soil enzymatic activities and the chemical indicator decreased with increasing sampling depth.

No-till – in comparison with conventional tillage - resulted in higher enzymatic activities in the 0–10– and 10–20–cm layers and in lower activities in the 20–30– and 30–40–cm soil layers under each crop of both rotations. These findings are valid for chemical indicator.

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

Farmyard-manuring in comparison with mineral fertilisation proved to be more efficient in increasing enzymatic activities in soil layers under maize in the 6-crop rotation.

Acid phosphatase activity was positively correlated with the mobile phosphorus content.

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