LONG TERM FERTILIZATION EFFECTS ON ENZYMATIC ACTIVITIES IN A PRELUVOSOIL

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Abstract

Agricultural practices that improve agricultural sustainability are needed particularly for brown luvic soil. Soil enzyme activities can provide information on how soil management is affecting the processes in soil such as decomposition and nutrient cycling. Soil enzyme activities (actual and potential dehydrogenase, catalase, acid and alkaline phosphatase) were determined in the 0–10, 10–20, and 20–30 cm layers of a brown luvic soil submitted to a complex fertilization experiment with different types of green manure. It was found that each activity decreased with increasing sampling depth. It should be emphasized that green-manuring of maize led to a significant increase in each of the five enzymatic activities determined. The enzymatic indicators of soil quality calculated from the values of enzymatic activities showed the order: lupinus + rape + oat > lupinus > vetch + oat + ryegrass > lupinus + oat + vetch > unfertilized plot. This order means that by determination of enzymatic activities valuable information can be obtained regarding fertility status of soils. There were significant correlations of soil enzyme activities with physical properties.

Key words: catalase, dehydrogenase, green manure, phosphatase, physical properties

INTRODUCTION

Soil microorganisms, the living component of the soil, usually occupy less than 1% of the soil volume, while their number and efficiency are very high. They colonize mainly the organic matter at the microsites (Balota et al., 2003). Clay minerals also serve as carrier of organisms, enzymes and metabolic products. The number and activity of soil microorganisms are dependent on plant growth (species composition, soil cover, root penetration of the soil), soil type, soil treatment, soil cultivation as well as on the macro- and microclimate at each location (Dick et al., 1994). The metabolic activity of soil microorganism is essential for organic matter turnover. The mobilization and immobilization of inorganic nutrients and trace elements are also mainly a result of microbial activities (Kandeler and Murer, 1993).

Special enzymes catalyze the organic matter turnover (Canarutto et al., 1986; Clarholm and Rosengren-Brinck, 1995). These enzymes are produced by the organisms and act intra- or extracellularly. Soil enzymes catalyze reactions in soils that are important in cycling of nutrients such as C, N, P, and S. Accumulated enzymes are primarily of microbial origin but may also originate from plant and animal residue. Soil enzymes form a part of the soil matrix as exoenzymes and as endoenzymes in viable cells. Soil enzyme activities commonly correlate with microbial parameters and have been shown to be a sensitive index of long-term management effects such as crop rotations, animal and green manures and tillage (Bandick and Dick, 1999; Campbell et al., 1986; Deng and Tabatabai, 1997).

The measurement of soil enzymes can be used as indicative of the biologycal activity or biochemical process (Dick et al., 1988). Soil enzyme activities have potential to provide an unique integrative biological assessment of soils because of their relationship to

soil biology, easy of measurement and rapid response to changes in soil management (Kirchner et al., 1993).

The effects of green manure on soil enzymatic activities were studied in many countries. In order to obtain new data on the soil enzymological effects of soil management practices we have determined some enzymatic activities in a brown luvic soil submitted to a complex fertilization experiment at the Agricultural and Research and Development Station in Oradea, Bihor county, Romania.

MATERIALS AND METHODS

The ploughed layer of the studied soil is of mellow loam texture, it has a pH value of 5.5 and medium humus content(23.2%). The experimental field was divided into plots for comparative study of green manure fertilization at rates of 47.8 t / ha lupinus (*Lupinus angustifolius* L.), 29.9 t / ha vetch (*Vicea dumetorum* L.) + oat (*Avena sativa* L.) + ryegrass (*Lolium perenne* L.), 39.7 t / ha lupinus + oat, 23.9 t / ha lupinus + rape (*Brassica rapa* L.) + oat, 20 t / ha rape, and 19.1 t / ha rape + lupinus. The green manure was maintained on the soil surface 7 days and after that the land was ploughed. The plots were installed in three repetitions.

In July 2007, soil was sampled from the 0–10, 10–20 and 20–30 cm depths of the plots under maize (*Zea mays* L.) crop. The soil samples were allowed to air dry, then ground and passed through a 2 mm sieve and, finally, used for enzymological analyses.

Two enzymatic activities (actual and potential dehydrogenase) were determined according to the methods described in (Kiss et al., 1990) .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 37ºC 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 has been determined using the permanganometric method (Kiss et al., 1990). The reaction mixtures consisted of 3.0 soil, 2.0 ml H_2O_2 3% and 10 ml phosphate buffer. It suffered incubation at 37ºC for 1 hour. Catalase activity is expressed as mg of H_2O_2 decomposed by 1g of soil in 1 hour.

For determination of phosphatase activities, 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 sol, 2 ml toluene (antiseptic), 10 ml buffer solution and 10 ml 0.5% substrate solution. All reaction mixtures were incubated at 37ºC 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.

Physical indicators were determined according to the methods described in (Egner et al., 1980).

The activity values were submitted to statistical evaluation by the two *t*-test (Sachs, 2002) and the correlations between the enzymatic activities and physical indicators were determined according to the methods described in (Kiss et al., 1990).

RESULTS AND DISCUSSIONS

Results of the enzymological analyses are presented in Table 1.

Variation of the enzymatic activities in dependence of sampling depth

It is evident from Table 1 that each enzymatic activity decreased with sampling depth in all plots under maize crop.

Enzymatic indicators of soil quality

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 type of activity and the nature of green manure. Based on these differences the following decreasing orders of the enzymatic activities could be established in the soil of the seven plots:

actual dehydrogenase activity: lupinus + rape + oat > rape + lupinus > lupinus > lupinus + oat > vetch + oat + ryegrass > rape > unfertilized plot;

potential dehydrogenase activity: lupinus + rape + oat > lupinus > rape + lupinus > lupinus + oat > vetch + oat + ryegrass > rape > unfertilized plot;

catalase activity: lupinus + rape + oat > vetch + oat + ryegrass > lupinus + oat > lupinus > rape > rape + lupinus > unfertilized plot;

acid phosphatase activity: lupinus + rape + oat > vetch + oat + ryegrass > lupinus > lupinus + oat > rape + lupinus > rape > unfertilized plot;

alkaline phosphatase activity: vetch + oat + ryegrass > lupinus + rape + oat > lupinus + oat > lupinus > rape > rape + lupinus > unfertilized plot

Table 1

The effect of different types of green manure on enzymatic activities in a brown luvic soil

Soil enzymatic activity*	Soil depth (cm)	Type of green manure**						
		V_1	V_2	V_3	V_4	V_5	V_6	V_7
ADA	0-10	9.01	6.95	7.31	11.82	6.10	11.56	5.52
	10-20	7.31	4.59	5.61	10.20	4.70	8.50	4.52
	20-30	5.10	2.72	3.91	5.76	3.40	5.10	2.72
PDA	0-10	22.78	16.66	14.28	24.28	11.22	16.32	10.60
	10-20	15.30	10.20	11.22	16.66	9.50	12.24	9.41
	20-30	8.33	8.16	10.37	15.30	8.67	9.86	7.88
CA	0-10	1.98	2.07	1.96	2.44	1.79	1.09	0.89
	10-20	1.79	1.95	1.85	2.23	1.33	1.07	0.83
	20-30	1.60	1.95	1.67	2.03	0.95	0.92	0.71
AcPA	0-10	2.85	2.94	2.81	2.96	2.81	2.79	2.69
	10-20	2.81	2.87	2.75	2.89	2.69	2.75	2.38
	20-30	2.74	2.81	2.69	2.85	2.20	2.32	2.30
AlkPA	0-10	1.72	1.97	1.90	1.94	1.85	1.71	1.67
	10-20	1.53	1.93	1.67	1.84	1.38	1.35	1.31
	20-30	1.40	1.83	1.51	1.76	1.34	1.31	1.29

* ADA – Actual dehydrogenase activity.
PDA – Potential dehydrogenase activity.
CA – Catalase activity.
AcPA – Acid phosphatase activity.
AlkPA – Alkaline phosphatase activity.

** V_1 – Lupinus.
V_2 – Vetch + oat + ryegrass.
V_3 – Lupinus + oat.
V_4 – Lupinus + rape + oat.
V_5 – Rape. V_6 – Rape + lupinus. V_7 – Unfertilized plot.

It is clear from these orders that seven plots presented either a maximum or a minimum value of the six 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 (Kiss et al., 1990). 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 position of plot is in the hierarchy. Table 2 shows that the first positions are occupied by those plots in which enzymatic activities were the highest. The soil under unfertilized maize plot occupying the last position can be considered as the last enzyme-active soil.

Table 2

Enzymatic indicators of soil quality

Position	Plot	Enzymatic indicator of soil quality
1	Lupinus + rape + oat	496.32
2	Lupinus	417.43
3	Vetch + oat + ryegrass	401.66
4	Lupinus + oat	389.11
5	Rape + lupinus	370.60
6	Rape	331.57
7	Unfertilized plot	290.48

Results of the chemical analyses are presented in Table 3. Simple correlation between enzymatic activities and chemical properties in the 0-10 cm layer (Table 4) showed that soil enzyme activities were significantly correlated with chemical properties. This indicates that enzyme activities were associated with active microorganisms in soil which are the major source of soil enzymes. The activities of all five enzymes were significantly intercorrelated which suggest that green manure has similar effects on the activities of those enzymes involved in intracellular metabolism and in P cycling in soil

Table 3

The effect of different types of green manure on physical properties in a brown luvic soil

Physical properties	Soil depth (cm)	Type of green manure*						
		V_1	V_2	V_3	V_4	V_5	V_6	V_7
Soil density (g/cm^3)	0-10	1.41	1.40	1.39	1.38	1.43	1.42	1.44
Porosity (%)	0-10	12.7	13.2	14.0	14.5	11.4	12.0	10.9
Resistance to penetration (kg/cm^2)	0-10	20.6	20.1	20.5	19.5	21.7	21.5	25.6
Coefficient of filtration (mm/h)	0-10	15.97	16.19	16.91	16.57	14.32	14.36	13.85

*V_1 – Lupinus. V_2 – Vetch+oat+ryegrass. V_3 – Lupinus+oat. V_4 – Lupinus+rape+oat. V_5 – Rape. V_6 – Rape+lupinus. V_7 – Unfertilized plot.

Table 4

Simple correlations (r) between soil enzyme activities and physical properties in the 0-10 cm depth

Vari-ables**	ADA	PDA	CA	AcPA	AlkPA	SD	Po	RP
ADA	-	-	-	-	-	-	-	-
PDA	0.758*	-	-	-	-	-	-	-
CA	0.248*	0.646*	-	-	-	-	-	-
AcPA	0.645*	1.559*	0.909*	-	-	-	-	-
AlkPA	0.034*	0.457*	0.815*	0.824*	-	-	-	-
SD	0.509*	0.689*	0.833*	0.690*	0.644*	-	-	-
Po	0.623*	0.684*	0.848*	0.868*	0.820*	0.981*	-	-
RP	0.505*	0.754*	0.862*	0.938*	0.837*	0.836*	0.824*	-
CF	0.277*	0.793*	0.870*	0.832*	0.903*	0.927*	0.950*	0.797*

* Significantly at $P \leq 0.05$.

** ADA – Actual dehydrogenase activity. PDA – Potential dehydrogenase activity. CA – Catalase activity. AcPA – Acid phosphatase activity. AlkPA – Alkaline phosphatase activity. SD – Soil density. Po – Porosity. RP – Resistance to penetration. CF – Coefficient of filtration.

CONCLUSION

1. The soil enzymatic activities decreased with increasing sampling depth.

2. The enzymatic indicators of soil quality calculated from the values of enzymatic activities determined in the plots under maize crop showed the order: lupinus + rape + oat > lupinus > vetch + oat + ryegrass > lupinus + oat > rape + lupinus > rape > unfertilized plot.

3. Each of the five enzymatic activity was positively correlated with the physical indicators.

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