INFLUENCE OF DIFFERENT TYPES OF GREEN MANURE ON THE SOIL BIOLOGY

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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 chemical properties.

Keywords*: catalase, dehydrogenase, green manure, phosphatase, soil.*

Biologically mediated processes in soils are central to the ecological function of soils. Soil biotic activity is the driving force in the degradation and conservation of exogenous plant material and anthropogenic depositions, transformations of organic matter and evolution and maintenance of soil structure [2,3,4]. Energy obtained by the primary decomposers of organic matter supports the activity of a number of trophic levels in soils. In turn this activity plays a primary function in nutrient cycling and support of plant life [1,5].

Special enzymes catalyze the organic matter turnover. These enzymes are produced by the organisms and act intra- or extracellularly [6]. Soil enzymes catalyze reactions in soils that are important in cycling of nutrients such as C, N, P, and S [8]. 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 [9]. Soil enzyme activities commonly correlate with microbial parameters [11] and have been shown to be a sensitive index of long-term management effects such as crop rotations [6,8], animal and green manures [12], and tillage [4].

The measurement of soil enzymes can be used as indicative of the biologycal activity or biochemical process [8]. Soil enzyme activities have potential to provide a unique integrative biological assessment of soils because of their relationship to soil biology, easy of measurement and rapid response to changes in soil management [12].

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 2006, 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 [13]. 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[13]. 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[14]. Phosphatase activities are expressed in mg phenol/g soil/2 hours.

Chemical indicators were determined according to the methods described in [10].

The activity values were submitted to statistical evaluation by the two *t*-test [15] and the correlations between the enzymatic activities and chemical indicators were determined according to the methods described in [16].

RESULTS AND DISCUSSION

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.

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

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.

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 [7]. 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 that plots in which enzymatic activities were the highest occupy the first positions. 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 chemical properties in a brown luvic soil

Chemical properties	Soil depth (cm)	Type of green manure*						
		V_1	V_2	V_3	V_4	V_5	V_6	V_7
Available P (mg P_2O_5/100g soil)	0-10	37.4	38.7	38.8	38.9	37.5	37.6	34.1
Available K (mg K/ 100g soil)	0-10	210.1	213.1	213.9	214.0	211.4	212.0	209.2
$N\text{-}NO_3$ (mg N/kg soil)	0-10	1.03	0.80	1.05	1.08	0.70	0.71	0.68
$N\text{-}NH_4$ (mg N/kg soil)	0-10	4.25	4.41	4.61	4.80	3.12	3.30	2.50

*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 chemical properties in the 0-10 cm depth

Variables***	ADA	PDA	CA	AcPA	AlkPA	Available P	Available K	$N\text{-}NO_3$
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**	-	-	-	-
Available P	0.460**	0.522**	0.804**	0.843**	0.809*	-	-	-
Available K	0.404**	0.328**	0.660**	0.713**	0.863**	0.881**	-	-
$N\text{-}NO_3$	0.419**	0.750**	0.764**	0.580**	0.557**	0.621**	0.507**	-
$N\text{-}NH_4$	0.424**	0.230**	0.280**	0.850*	0.856**	0.397**	0.761**	0.876**

* Significantly at $P \leq 0.05$.
** Significantly at $P < 0.001$.
*** ADA – Actual dehydrogenase activity. PDA – Potential dehydrogenase activity.
CA – Catalase activity. AcPA – Acid phosphatase activity. AlkPA – Alkaline phosphatase activity.

CONCLUSIONS

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 chemical indicators.

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