INFLUENCE OF LONG -TERM FERTILIZATION ON SOIL ENZYME ACTIVITIES

Alina Dora SAMUEL*

*University of Oradea, Faculty of Science, Department of Biology, Oradea, Romania
Corresponding author: Alina-Doria Samuel, University of Oradea, Faculty of Science, Department of Biology, 1 Universitatii Str., 410087 Oradea, Romania, tel.: 0040259408161, fax: 0040259408461, e-mail: samuelalina@rdslink.ro

Abstract. 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

INTRODUCTION

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 biological activity or biochemical process [8]. 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 [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. 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 [13]. 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 [14] and the correlations between the enzymatic activities and chemical indicators were determined according to the methods described in [15].

RESULTS

Results of the enzymological analyses are presented in Table 1.

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**						
		V1	V2	V3	V4	V5	V6	V7
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.

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.

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 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 chemical properties in a brown luvic soil

Chemical properties	Soil depth (cm)	Type of green manure*						
		V1	V2	V3	V4	V5	V6	V7
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		$N\text{-}NO_3$
						P	K	
ADA	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
PDA	0.758*	N/A	N/A	N/A	N/A	N/A	N/A	N/A
CA	0.248**	0.646**	N/A	N/A	N/A	N/A	N/A	N/A
AcPA	0.645*	1.559**	0.909**	N/A	N/A	N/A	N/A	N/A
AlkPA	0.034**	0.457**	0.815*	0.824**	N/A	N/A	N/A	N/A
Available P	0.460**	0.522**	0.804**	0.843**	0.809*	N/A	N/A	N/A
Available K	0.404**	0.328**	0.660**	0.713**	0.863**	0.881**	N/A	N/A
N-NO3	0.419**	0.750**	0.764**	0.580**	0.557**	0.621**	0.507**	N/A
N-NH4	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.

DISCUSSION

Our observation is in agreement with other studies. For example [1] observed that soil management influences soil microorganisms and soil microbial processes through changes in the quantity and quality of plant residues entering the soil, and its spatial distribution. Mulching, generally, increases enzymes activities in soils. With the increasing of mulch there is an increased of the supply of the readily available substrate for microorganisms as well as soil enzymes. While N fertilization in the no-till treatments slightly increased microbial populations and activities [3, 8] green manuring with crimson clover had a much greater impact on soil microbial properties. Microbial activity as measured by soil enzymes was significantly greater in the clover plots than the non-clover plots. Differences in enzyme activities between the two treatments were significant for alkaline phosphatase. While green-manuring with crimson clover significantly affected soil biological properties in the 0 to 7.5 cm zone, few significant differences were found between treatments in the 7.5 to 30 cm zone. Soil enzyme activities can be more influenced by type of organic matter than the quantity of organic matter [2, 7], once had been observed that mineralization of plant residue added to soil is controlled by C:N ratio. The larger and more active microbial biomass in the surface soil from the green manure plots results from the green manure providing a source of C and N for microbial growth. Cropping systems that increase inputs of C through green manures have been shown to have more microbes and greater microbial activity than that found in systems that utilize only fertilizer inputs [7, 9]. Green manuring increases soil organic matter levels, leading to improved soil structure, infiltration, fertility and water-holding capacity [4]. [12] compared the effects of applications of green manure, animal manure and varying rates of N fertilizer on six soil enzymes. Organic amendments stimulated activity but increasing rates of inorganic N decreased activity of urease and amidase enzymes.

Our results on a preluvosoil are consistent with previous studies on other soils. Long-term management of plant nutrients and organic amendments does affect soil biological properties [8, 9, 11, 12]. In general management practices that increase imputs of organic residue, plant or animal manures, increase biological activity. Addition of green manure usually increases microbial biomass [4] and soil enzyme activities [6, 7, 8] over soils that have not received any organic or inorganic amendmets. Other indexes that increase with long-term green manure applications are N mineralization potential and soil respiration. Although [5] found no significant increases of microbial biomass C due to organic soil amendments, they did find higher rates of N mineralization potential green manure soils. They attributed these results to the soil type which was relatively high in organic matter.

The increase in soil enzyme activities may be the result of soil chemical changes, so there is a direct expression on microbial biomass and soil enzyme activities [9]. While the significant correlation between enzyme activities and chemical properties is likely due to higher nutrients levels supporting greater microbial biomass. Furthermore, higher organic matter provides a better environment for stabilizing and protecting enzymes.

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