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ENZYME ACTIVITIES IN A PRELUVOSOIL AS AFFECTED BY CROP ROTATION AND FERTILIZATION SYSTEMS

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ABSTRACT

A major agricultural research priority has been recently focused on sustaining soil health and quality, and identifying key parameters or processes for monitoring changes in soil properties induced by agricultural management practices. Enzymatic activities are sensors of soil stress to management practice that may sensitively warn us about soil degradation. In the present study, three key soil enzymes involved in intracellular metabolism of microorganisms and two soil enzymes involved in phosphorus metabolism were selected.

Actual and potential dehydrogenase, catalase, acid and alkaline phosphatase activities were determined in the 0–20 cm layer of a preluvosoil submitted to a complex crop rotation and fertilization experiment at the Agricultural Research and Development Station in Oradea (Bihor County). The soil under all crops was more enzyme-active in the 4-than in the other rotations or in the monoculture. In the 2-crop rotation, higher enzymatic activities were registered under maize than under wheat, only in the case of dehydrogenase activity. In the plots of the 3- and 4-crops rotations the enzymatic indicators of soil quality varied, depending on the nature of crops and kind of fertilizers. Additions of fertilizers increased the soil enzymatic activities because of an increased plant biomass production, which upon incorporation stimulates soil biological activity. It should be emphasized that farmyard-manuring of crops, in comparison with the mineral fertilization led to a significant increase in each of the five enzymatic activities determined.

Key words: soil enzymes, agricultural management practices, crop rotation, soil quality.

INTRODUCTION

Soil enzymes are biological catalysts of specific reactions depending upon a variety of factors such as pH, temperature and the presence or absence of inhibitors (Dick et al., 2000). Other factors including climate, type of amendment, cultivation techniques, crop type and edaphic properties also affect enzyme catalysed reactions (Caldwell, 2005). Soil enzymes are mainly of bacterial and fungal origin. Only a small fraction is excreted by plants and/or animals (Dick, 1992). Measurements of several enzymatic activities have been used to establish the indices of soil biological fertility (Tabatabai and Dick, 2002).

Soil enzymes are frequently linked with fertility dynamics because of their utmost sensitivity to management practices, although they undergo distinct changes long before any detectable changes in soil quality indicators (Yang et al., 2012).

Studies of enzyme activities provide information on the biochemical processes occurring in soil (Utobo and Tewari, 2014). There is growing evidence that soil biological parameters are undoubtedly potential and sensitive indicators of soil ecological stress or restoration. Soil enzymes regulate ecosystem functioning and in particular, play a key role in identifying nutrients (Yang et al., 2016). All soils contains a group of enzymes that determine soil metabolic processes which in turn, depend on its physical, chemical, microbiological and biochemical properties (Balota et al., 2004a, 2004b; Udawatta et al., 2009).

Soil management influences soil microorganisms and soil microbial processes through changes in the quantity and quality of plant residues entering the soil, their seasonal distribution, changes in nutrient inputs (Bowles et al., 2004) and the ration between above and below ground (Bielinka and Mocek-Ploniniak, 2012). Because crop

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residues are primary sources of organic matter, cropping systems and fertilizer regime may exert a significant influence on soil quality (Ziomek and Lemanowicz, 2016). Soils under monoculture systems, in general contain significantly lower concentration and qualities of soil organic matter, less soil structural stability, and reduced amounts of microbial biomass and activities compared with systems involving crop rotation (Silvestro et al., 2017). Crop rotations lead to significant effects on soil physical, chemical and biological properties by providing higher inputs and diversity of plant residues returned to soils (Samuel et al., 2000). Systems with high organic matter inputs and easily available soil organic matter compounds tend to have higher microbial biomass and enzyme activities (Lemanowicz, 2011), because they are preferred sources for microorganisms.

Any changes in management practices is reflected in the microbial biomass and soil enzyme (Mundaganur et al., 2016) in a short-period of time, long before measurable changes in soil chemical properties can occur (Nahas, 2015). Therefore, enzyme activities have been suggested as early indicators of changes in soil properties induced by agriculture practices. Thus, it is important to obtain new data about the effects of soil management practices on soil enzyme activities for better management of our preluvosoil.

MATERIAL 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 field was divided into plots for comparative study of monoculture and rotations of 2-, 3- and 4-crops and of different types of fertilization. The plots were not fertilized (N_0P_0), or NP-fertilized at rates of 120 kg of N/ha and 90 kg of P/ha, or received farmyard manure (10 t ha^{-1}) with mineral fertilizers. The plots were installed in three repetitions.

In October 2014, soil was sampled from all plots. Sampling depth was 0-20 cm. The soil samples were allowed to air dry, then ground and passed through a 2-mm sieve and, finally, used for enzymologic analyses.

Actual and potential dehydrogenase activities were determined according to the methods described in Drăgan-Bularda (1983). 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 37^{o}C for 24 hours. After incubation, the triphenylformazan produced was extracted with acetone and was measured spectrophotometrically at 485 nm. Dehydrogenase activities were expressed in mg of triphenylformazan (TPF) produced (from 2, 3, 5-triphenyltetrazolium chloride, TTC) by 10 g soil in 24 hours.

Catalase activity was determined using the permanganometric method (Drăgan-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 37^{o}C for 1 hour. Catalase activity was recorded as mg H_2O_2 decomposed by 1 g of soil in 1 hour.

Disodium phenylphosphate served as phosphate 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), 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 37°C for 2 hours. After incubation, the phenol released from the substrate under the action of phosphatases was determined spectro-photometrically (at 614 nm) based on the colour reaction between phenol and 2.6- dibromoquinone-4-chloroimide. Phosphatase activities were expressed in mg phenol/g soil/2 hours.

The activity values were submitted to statistical evaluation by the t-test (Sachs, 2002).

RESULTS AND DISCUSSION

Results of the determination of enzymatic activities are presented in Tables 1-5, and those of the statistical evaluation are summarised in Table 6.

Table 1. Actual dehydrogenase activity in a preluvosoil as affected by crop rotation and fertilization systems

Crop rotation		Fertilization		
		N_0P_0	$N_{120}P_{90}$	$N_{120}P_{90}$ + FYM*
Monoculture	Wheat	6.59	7.68	8.81
Rotation of 2-crops	Wheat	6.82	8.54	9.03
	Maize	7.44	8.60	9.19
Rotation of 3-crops	Peas	7.25	7.68	8.09
	Wheat	7.93	8.98	9.24
	Maize	8.25	8.98	9.46
Rotation of 4-crops	Peas	7.59	7.86	9.04
	Wheat	7.82	8.91	9.36
	Maize plot 3	8.14	8.99	9.50
	Maize plot 4	8.24	9.06	9.58

*FYM – farmyard-manured.

Table 2. Potential dehydrogenase activity in a preluvosoil as affected by crop rotation and fertilization systems

Crop rotation		Fertilization		
		N_0P_0	$N_{120}P_{90}$	$N_{120}P_{90}$ + FYM*
Monoculture	Wheat	19.41	21.80	22.93
Rotation of 2-crops	Wheat	20.14	22.30	23.02
	Maize	22.10	22.28	24.09
Rotation of 3-crops	Peas	22.24	23.80	24.31
	Wheat	22.01	22.95	25.13
	Maize	24.17	25.05	26.08
Rotation of 4-crops	Peas	22.43	23.98	24.89
	Wheat	22.13	23.00	25.01
	Maize plot 3	25.00	25.20	25.86
	Maize plot 4	25.63	25.81	26.07

*FYM – farmyard-manured.

Table 3. Catalase activity in a preluvosoil as affected by crop rotation and fertilization systems

Crop rotation		Fertilization		
		N_0P_0	$N_{120}P_{90}$	$N_{120}P_{90}$ + FYM*
Monoculture	Wheat	1.28	1.39	2.08
Rotation of 2-crops	Wheat	1.39	1.60	2.07
	Maize	1.28	1.57	2.09
Rotation of 3-crops	Peas	1.41	1.63	1.70
	Wheat	1.55	2.18	2.30
	Maize	1.57	2.19	2.46
Rotation of 4-crops	Peas	1.50	1.89	2.18
	Wheat	1.68	2.22	2.87
	Maize plot 3	1.71	2.19	2.94
	Maize plot 4	1.78	2.23	2.90

*FYM – farmyard-manured.

Table 4. Acid phosphatase activity in a preluvosoil as affected by crop rotation and fertilization systems

Crop rotation		Fertilization		
		N_0P_0	$N_{120}P_{90}$	$N_{120}P_{90}$ + FYM*
Monoculture	Wheat	5.26	9.78	10.07
Rotation of 2-crops	Wheat	7.30	7.47	9.16
	Maize	7.42	7.54	8.26
Rotation of 3-crops	Peas	7.58	8.44	9.56
	Wheat	7.35	8.81	9.89
	Maize	7.51	7.85	8.02
Rotation of 4-crops	Peas	7.62	7.95	8.08
	Wheat	7.40	9.24	9.16
	Maize plot 3	7.52	7.68	8.18
	Maize plot 4	7.55	7.80	8.20

*FYM – farmyard-manured.

Table 5. Alkaline phosphatase activity in a preluvosoil as affected by crop rotation and fertilization systems

Crop rotation		Fertilization		
		N_0P_0	$N_{120}P_{90}$	$N_{120}P_{90}$ + FYM*
Monoculture	Wheat	3.38	4.08	4.77
Rotation of 2-crops	Wheat	3.58	5.22	6.11
	Maize	3.58	5.20	6.11
Rotation of 3-crops	Peas	4.07	4.91	5.45
	Wheat	3.90	4.69	5.23
	Maize	3.92	4.58	5.08
Rotation of 4-crops	Peas	4.27	5.06	5.87
	Wheat	3.98	4.84	5.37
	Maize plot 3	3.99	4.26	5.40
	Maize plot 4	4.03	4.25	5.60

*FYM – farmyard-manured.

The effect of crop rotations on the enzymatic activities in soil

- Soil enzymatic activities as affected by the same crop in the three rotations. As wheat was a crop in the monoculture and in the 2-, 3-, and 4-crops rotations, it was possible to compare the effect of different rotations on soil enzyme activities. The difference between the monoculture and the 2-crops rotation was not significantly higher ($p>0.10$), in the case of each enzymatic activity, excepting acid phosphatase activity, which was significant higher ($0.05>p>0.02$) in the 2-crops rotation than in the monoculture. The difference between the monoculture and the 3-, and 4-crops rotations was significantly ($p<0.02$ and $p<0.01$, respectively) higher in the case of potential dehydrogenase and alkaline phosphatase activities in the 4-crops rotation than in the monoculture. The soil under wheat was more

enzyme active in the 4- than in the other rotations, excepting alkaline phosphatase activity, which was significantly higher (0.05>p>0.02) in the 2- than in the 4-crops rotation. In the soil under maize, only potential dehydrogenase and catalase activities were significantly higher (0.05>p>0.02 and 0.02>p>0.01, respectively) in the 4- than in the 2-crops rotations. In the soil under peas, the difference between the two rotations was insignificantly higher (p>0.10) in the 3- than in the 4-crop rotation in the case of acid phosphatase activity, whereas the other activities were not significantly higher (p>0.10) in the 4- than in the 3-crops rotation.

Many investigations have shown that crop rotations can provide higher input and diversity of organic materials to the soil and generally contain higher enzyme activities than under monoculture.

Table 6. Significance of the differences between enzymatic activities in a preluvosoil (0-20 cm) submitted to different management practices

Management practices	Soil enzymatic activity*	Mean activity values in management practices			Significance of the differences
		a	b	a-b	
The same crop in the three rotation					
Wheat in monoculture (a) versus wheat in 2-crops rotation (b)	ADA	7.69	8.13	-0.45	p>0.10
	PDA	21.38	23.36	-1.98	p>0.10
	CA	1.58	1.68	-0.10	p>0.10
	AcPA	8.37	7.97	0.40	0.05>p>0.02
	AlkPA	4.07	4.97	-0.90	p>0.10
Wheat in monoculture (a) versus wheat in 3-crops rotation (b)	ADA	7.69	8.71	-1.02	p>0.10
	PDA	21.38	26.36	-4.98	0.10>p>0.05
	CA	1.58	2.01	-0.48	p>0.10
	AcPA	8.37	8.68	-0.31	p>0.10
	AlkPA	4.07	4.60	-0.53	p>0.10
Wheat in monoculture (a) versus wheat in 4-crops rotation (b)	ADA	7.69	8.69	-1.00	p>0.10
	PDA	21.38	23.38	-2.00	0.05>p>0.02
	CA	1.58	2.25	-0.67	p>0.10
	AcPA	8.37	8.60	-0.23	0.10>p>0.05
	AlkPA	4.07	4.73	-0.66	0.01>p>0.001
Wheat in 2-crops rotation (a) versus wheat in 3-crops rotation (b)	ADA	8.13	8.71	-0.58	p>0.10
	PDA	21.82	23.36	-1.54	0.10>p>0.05
	CA	1.68	2.01	-0.33	p>0.10
	AcPA	7.97	8.68	-0.71	p>0.10
	AlkPA	4.97	4.60	0.37	p>0.10
Wheat in 2-crops rotation (a) versus wheat in 4-crops rotation (b	ADA	8.13	8.69	-0.56	p>0.10
	PDA	21.82	23.38	-1.56	0.10>p>0.05
	CA	1.68	2.25	-0.57	p>0.10
	AcPA	7.97	8.60	-0.63	p>0.10
	AlkPA	4.97	4.73	0.24	0.05>p>0.02
Maize in 2-crops rotation (a) versus maize in 3-crops rotation (b)	ADA	8.41	8.89	-0.48	p>0.10
	PDA	23.02	25.10	-2.08	0.10>p>0.05
	CA	1.64	2.07	-0.43	p>0.10
	AcPA	7.74	7.79	-0.05	0.10>p>0.05
	AlkPA	4.96	4.52	0.44	0.10>p>0.05
Maize in 2-crops rotation (a) versus maize in 4-crops rotation (b)	ADA	8.41	8.96	-0.55	0.10>p>0.05
	PDA	23.02	25.83	-2.08	0.05>p>0.02
	CA	1.64	2.30	-0.43	0.02>p>0.05
	AcPA	7.74	7.85	-0.05	0.10>p>0.05
	AlkPA	4.96	4.62	0.34	p>0.10

Peas in 3-crops rotation (a) versus peas in 4-crops rotation (b)	ADA	7.67	8.16	-0.49	p>0.10
	PDA	23.45	23.36	-0.31	p>0.10
	CA	1.58	2.01	-0.27	p>0.10
	AcPA	8.37	8.68	0.64	p>0.10
	AlkPA	4.07	4.60	-0.25	p>0.10
Different crops in the same rotation 2 -crops rotation Wheat (a) versus maize (b)	ADA	8.13	8.41	-0.28	0.10>p>0.05
	PDA	21.82	23.02	-1.20	0.10>p>0.05
	CA	1.68	1.64	0.04	0.10>p>0.05
	AcPA	7.97	7.74	0.23	0.10>p>0.05
	AlkPA	4.97	4.96	0.01	0.10>p>0.05
3-crops rotation Peas (a) versus wheat (b)	ADA	7.67	8.71	-1.04	0.05>p>0.02
	PDA	23.45	23.36	0.09	p>0.10
	CA	1.58	2.01	-0.43	0.10>p>0.05
	AcPA	8.52	8.68	-0.16	p>0.10
	AlkPA	4.81	4.60	0.21	p>0.10
Peas (a) versus maize (b)	ADA	7.67	8.89	-1.22	0.01>p>0.001
	PDA	23.45	25.10	-1.65	0.02>p>0.01
	CA	1.58	2.07	-0.49	0.10>p>0.05
	AcPA	8.52	7.79	0.73	p>0.10
	AlkPA	4.81	4.52	0.29	0.05>p>0.02
Wheat (a) versus maize (b)	ADA	8.71	8.89	-0.18	p>0.10
	PDA	23.36	25.10	-1.74	0.10>p>0.05
	CA	2.01	2.07	-0.06	p>0.10
	AcPA	8.68	7.79	0.89	p>0.10
	AlkPA	4.60	4.52	0.08	p>0.10
4-crops rotation Peas (a) versus wheat (b)	ADA	8.16	8.69	-0.53	p>0.10
	PDA	23.76	23.38	0.38	p>0.10
	CA	1.85	2.25	-0.40	p>0.10
	AcPA	7.88	8.60	-0.72	0.10>p>0.05
	AlkPA	5.06	4.73	0.33	0.10>p>0.05
Peas (a) versus maize (plot 3) (b)	ADA	8.16	8.87	-0.71	0.10>p>0.05
	PDA	23.76	25.35	-1.59	0.10>p>0.05
	CA	1.85	2.28	-0.43	p>0.10
	AcPA	7.88	7.79	0.09	p>0.10
	AlkPA	5.06	4.55	0.51	0.10>p>0.05
Peas (a) versus maize (plot 4) (b)	ADA	8.16	8.96	-0.80	0.10>p>0.05
	PDA	23.76	25.83	-2.07	0.10>p>0.05
	CA	1.85	2.30	-0.45	0.10>p>0.05
	AcPA	7.88	7.85	0.03	p>0.10
	AlkPA	5.06	4.62	0.44	p>0.10
Wheat (a) versus maize (plot 3) (b)	ADA	8.69	8.87	-0.18	p>0.10
	PDA	23.38	25.35	-1.97	0.10>p>0.05
	CA	2.25	2.28	-0.03	0.10>p>0.05
	AcPA	8.60	7.79	0.81	p>0.10
	AlkPA	4.73	4.55	0.18	p>0.10
Wheat (a) versus maize (plot 4) (b)	ADA	8.69	8.96	-0.27	0.10>p>0.05
	PDA	23.38	25.83	-2.45	0.10>p>0.05
	CA	2.25	2.30	-0.05	p>0.10
	AcPA	8.60	7.85	0.75	p>0.10
	AlkPA	4.73	4.62	0.11	p>0.10

Maize (plot 3) (a) versus maize (plot 4) (b)	ADA	8.87	8.96	-0.09	p>0.10
	PDA	25.35	25.83	-0.48	0.10>p>0.05
	CA	2.28	2.30	-0.02	p>0.10
	AcPA	7.79	7.85	-0.06	p>0.10
	AlkPA	4.55	4.62	-0.07	p>0.10
Fertilization system N_0P_0 (a) versus $N_{120}P_{90}$ (b)	ADA	7.72	8.64	-0.92	0.001>p
	PDA	22.87	23.81	-0.94	0.01>p>0.001
	CA	1.54	1.96	-0.42	0.001>p
	AcPA	7.47	8.08	-0.61	0.10>p>0.05
	AlkPA	3.92	4.77	-0.85	0.001>p
N_0P_0 (a) versus $N_{120}P_{90}$ + FYM** (b)	ADA	7.72	9.16	-1.44	0.001>p
	PDA	22.87	24.94	-2.07	0.001>p
	CA	1.54	2.39	-0.85	0.001>p
	AcPA	7.47	8.72	-1.25	0.01>p>0.001
	AlkPA	3.92	5.57	-1.65	0.001>p
$N_{120}P_{90}$ (a) versus $N_{120}P_{90}$ + FYM** (b)	ADA	8.64	9.16	-0.52	0.001>p
	PDA	23.81	24.94	-1.13	0.01>p>0.001
	CA	1.96	2.39	-0.43	0.02>p>0.01
	AcPA	8.08	8.72	-0.64	0.01>p>0.001
	AlkPA	4.77	5.57	-0.80	0.001>p

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

Rejsek et al. (2012), comparing 4-years rotations, including oats and meadow, with monoculture reported positive effects of the rotation on the microbial biomass. They concluded that higher microbial biomass C and N in the 4-years rotation compared with continuous crops and soybean systems and the 2-years rotation may be due to several reasons: enhanced soil structure, greater amounts and diversity of residues produced and a higher root density under diverse crop rotations.

Other studies reported greater levels of alkaline phosphatase activity in rotations including barley-red clover compared to monoculture systems, and that soils under no-till with a corn-oats-alfalfa rotation contained the largest alkaline phosphatase activity. These results demonstrate that the enzyme activities are modulated by the C sources available in the crop residues, therefore can be an indication of the decomposition rates of the C sources in the crop residues and, thus, of its structural complexity (Aon and Colaneri, 2001).

- Soil enzymatic activities as affected by the same crop growing in different plots of the same rotation. We have to mention that, in the 4-crops rotation there were two plots (3 and 4) cropped to maize. One can see from Table 6 that each enzymatic activities gave not significantly higher values (at least at p>0.10) in plot 4 than in plot 3.

The results obtained are in a good agreement with the literature data reviewed by (Eichler et al., 2004; Martens et al., 1992). Studies have shown that enzyme activities are sensitive to the positive effects of crop rotations compared to monoculture. Cropping systems that return elevated levels of crop residues significantly increase the activities of enzymes. The increases in soil enzyme activities are not due to the addition of more enzymes with the plant residues, because free enzymes are readily decomposed or are inactivated when added to the soil environment. It is more accepted that, the observed increases in enzyme activity upon crop residues incorporation are due to the stimulation of the soil microbial biomass.

- Soil enzymatic activities as affected by different crops in the same rotation:

The 2-crops rotation. Actual and potential dehydrogenase activities measured in the maize soil exceeded not significantly (p>0.05) the corresponding activity recorded in the wheat soil. Contrarily, calatase activity and acid and alkaline phosphatase activities were not significantly higher (p>0.05) in the wheat soil than in the soil under maize.

The 3- and the 4-crops rotations. Significant ($p<0.05$ to $p<0.01$) and not significant ($p>0.05$ to $p>0.10$) differences were registered in the soil enzymatic activities depending on the type 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 9, and respectively 12, plots:

- *actual dehydrogenase activity*: maize (M.f. + FYM) > wheat (M.f. + FYM) > wheat (Mf) maize (M.f.) > maize (N_0P_0) > peas (M.f. + FYM) > wheat (N_0P_0) > peas (M.f.) > peas (N_0P_0);
- *potential hydrogenase activity*: maize (M.f. + FYM) > wheat (M.f. + FYM) > maize (M.f.) > maize (N_0P_0) > peas (M.f. + FYM) > wheat (M.f.) > peas (N_0P_0)> wheat (N_0P_0);
- *catalase activity*: maize (M.f. + FYM) > wheat (M.f + FYM) > maize (M.f.) > wheat (M.f.) > peas (M.f. + FYM) > peas (M.f.) > maize (N_0P_0) > wheat (N_0P_0) > peas (N_0P_0);
- *acid phosphatase activity*: wheat (M.f. + FYM) > peas (M.f. + FYM) > wheat (M.f) > peas (M.f.) > maize (M.f. + FYM) > maize M.f.) > peas (N_0P_0) > maize (N_0P_0)> wheat (N_0P_0);
- *alkaline phosphatase activity*: peas (M.f. + FYM) > wheat (M.f. + FYM) > maize (M.f. +FYM) > peas M.f.) > wheat (M.f.) > maize (M.f.) > peas (N_0P_0) > maize (N_0P_0) > wheat (N_0P_0);
- *actual dehydrogenase activity*: maize plot 4 (M.f. + FYM) > maize plot 3 (M.f. + FYM) > wheat (M.f. + FYM) > maize plot 4 (M.f.) > peas (M.f. + FYM) > maize plot 3 (M.f.) > wheat (Mf) > maize plot 4 (N_0P_0) > maize plot 3 (N_0P_0) > peas (M.f.) > wheat (N_0P_0) > peas (N_0P_0);
- *potential hydrogenase activity*: maize plot 4 (M.f. + FYM) > maize plot 3 (M.f. + FYM) > maize plot 4 (M.f.) > maize plot 4 (N_0P_0) > maize plot 3 (M.f.) > wheat (M.f. + FYM) > wheat (M.f.) > peas (M.f. + FYM) > peas (M.f.) > wheat (M.f.) > peas (N_0P_0) > wheat (N_0P_0);
- *catalase activity*: maize plot 3 (M.f.+ FYM) > maize plot 4 (M.f. + FYM) > wheat (M.f + FYM) > maize plot 4 (M.f.) > wheat (M.f.) > maize plot 3 (M.f.) > peas (M.f. + FYM) > peas (M.f.) > maize plot 4 (N_0P_0) > maize plot 3 (N_0P_0) > wheat (N_0P_0) > peas (N_0P_0);
- *acid phosphatase activity*: wheat (M.f) > wheat (M.f. + FYM) > maize plot 4 (M.f. + FYM) > maize plot 3 (M.f. + FYM) > peas (M.f. + FYM) > peas (M.f.) > maize plot 4 (M.f.) > maize plot 3 (M.f.) > peas (N_0P_0) > maize plot 4 (N_0P_0) > maize plot (N_0P_0) > wheat (N_0P_0);
- *alkaline phosphatase activity*: peas (M.f. + FYM) > maize plot 4 (M.f. +FYM) > maize plot 3 (M.f. +FYM) > wheat (M.f. + FYM) > peas M.f.) > wheat (M.f.) > peas (N_0P_0) > maize plot 3 (M.f.) > maize plot 4 (M.f.) > maize plot 4 (N_0P_0) > maize plot 3 (N_0P_0) > wheat (N_0P_0).

It is evident from these orders that each of the 9, and respectively 12 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 Kiss et al. (1975). Briefly, by taking the maximum mean value of each activity as 100%, we calculated the relative (percentage) activities. The sum of the relative activities is the enzymatic indicator that is considered as an index of the biological quality of the soil in a given plot.

Tables 7 and 8 show that the first two positions are occupied by plots under the cereals (wheat, maize) which received mineral fertilizers and farmyard manure. The unfertilized soil under the legume plot (peas) occupying the last position can be considered as the least enzyme-active soil.

Table 7. Enzymatic indicators of soil quality in plots of the 3-crops rotation

Position	Plot*	Enzymatic indicator of soil quality
1	Minerally fertilized (M.f.) + FYM wheat	483.47
2	M.f. + FYM maize	474.30
3	M.f. wheat	449.96
4	M.f. + FYM peas	444.48
5	M.f. maize	443.39
6	M.f. peas	411.11
7	Unfertilised maize	393.57
8	Unfertilised wheat	377.07
9	Unfertilised peas	370.52

*FYM – farmyard-manured.

Table 8. Enzymatic indicators of soil quality in plots of the 4-crops rotation

Position	Plot*	Enzymatic indicator of soil quality
1	Minerally fertilized (M.f.) + FYM wheat	482.16
2	M.f. + FYM maize (plot 4)	479.77
3	M.f. + FYM maize (plot 3)	478.92
4	M.f. + FYM peas	451.41
5	M.f. wheat	432.29
6	M.f. maize (plot 4)	426.23
7	M.f. maize (plot 3)	420.66
8	M.f. peas	410.53
9	Unfertilised maize (plot 4)	395.21
10	Unfertilised maize (plot 3)	388.36
11	Unfertilised wheat	371.52
12	Unfertilised peas	371.47

*FYM – farmyard-manured.

The effect of fertilization on the enzymatic activities in soil

Table 6 shows that each of the five enzymatic activities was found to be significantly higher (at least at $p<0.02$), in the plots which received farmyard manure than in the mineral fertilized and unfertilized plots. In the plots that received mineral fertilizers, actual and potential dehydrogenase, catalase and alkaline phosphatase activities were significantly higher (at least at $p<0.01$), while acid phosphatase activity was nor significantly higher ($p>0.05$) than in the unfertilised plots.

In general, management practices that increase inputs of organic residue, increase biological activity. Addition of farmyard manure (FYM) usually increases microbial biomass and soil enzyme activities (Parham et al., 2002) over soils that have not received any organic or inorganic fertilizers. However when comparisons have been made between soils amended with FYM or inorganic fertilizers, there have been mixed results which vary with cropping system and biological index (Lemanowicz et al., 2014).

Use of inorganic fertilizer can increase the plant biomass production, which in turn increases the amount of residue returned to the soil and stimulates biological activity. In addition, the research which compared the effect of applications of animal manure and varying rates of N fertilizer on soil enzymes indicates that management practices that minimize organic inputs diminish the potential for enzymatic activity, which is likely to affect the ability of the soil to cycle and provide nutrients for plant growth (Dick et al., 1992).

CONCLUSIONS

Soil microorganisms and soil enzymes not only play an active role in influencing soil fertility as a result of their involvement in the cycle of nutrients, which are required for plant growth, but also are sensitive biological indicators for soil quality evaluation, sensitively reflecting changes in soil environment.

A better knowledge of changes in soil microbial biomass and soil enzyme activities would allow better understanding of the effect of a disturbance on soil community functions. Because some soil enzymes respond to sudden disturbances of the soil system, they can effectively aid developing land management practices.

Understanding of soil enzymes activity is a critical factor in assuring that soil remains healthy for an integrated biological assessment of soil, due to their crucial role in several biological activities, their ease of measurement and their rapid response to changes in soil management.

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