

Effects of Long Term Application of Organic and Mineral Fertilizers on Soil Enzymes

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Long term productivity and conservation of soils is critical for sustaining agricultural ecosystems. The specific objective of the work reported was to determine the effects of long term application of organic and mineral fertilizers on soil enzyme activity as an index of soil biology and biochemistry. 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 an eroded soil submitted to a complex fertilization experiment. Results showed that addition of mineral fertilizers to organic (green manure and farmyard manure) fertilizers led to a significant increase in each activity because of increased plant biomass production which upon incorporation stimulates soil biological activity. The enzymatic indicators of soil quality calculated from the values of enzymatic activities depending on the kind of fertilizers showed that by the determination of enzymatic activities valuable information can be obtained regarding fertility status of soils. A weak positive correlation between enzymatic indicators of soil quality and maize yield was established. The yield data demonstrate the superiority of farmyard manure which provided greater stability in crop production. Substantial improvement in soil biological activity due to application of organic fertilizers with mineral fertilizers contribute in maintaining the productivity and soil health.

Keywords: dehydrogenase, catalase, phosphatase, green manure, farmyard manure

In the effort to achieve sustainable agricultural production while maintaining and preserving the environment, it is crucial that soil biological health be improved or maintained [1-6]. Agricultural practices that improve soil quality and agricultural sustainability have been receiving more attention [7]. Inorganic fertilizer, especially N, P, and K, not only serve to maintain, but their application directly or indirectly causes changes in chemical, physical and biological properties of the soil. These changes, in the long term, are believed to have significant influences on the quality and productive capacity of soils [8].

Soil enzymes are the biological catalysts of innumerable reactions in soils. Although some enzymes (e.g. dehydrogenase) are found only in viable cells, most soil enzymes can also exist as exoenzymes secreted by microorganisms or as enzymes originating from microbial debris and plant residue that are stabilized in complexes of clay minerals and humic colloids [9]. Since it is difficult to extract enzymes from soils, enzymes are studied indirectly by measuring their activity via assays. Nevertheless, studying soil enzyme activities provides insight into biochemical processes in soils and is sensitive as a biological index [10].

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 [12], animal and green manures [13], and tillage [14].

In soil exposed to degradative processes, its biological properties are affected firstly, and so productive capacity diminishes. Early indicators of ecosystem stress may function as the sensors, whose perturbation may provide a

sensitive warning of soil degradation [15]. Soil enzyme activities are useful candidate sensors since they integrate information about the microbial status and soil physical and chemical conditions. Soil enzyme production as result of microbial metabolism is a sensitive indicator of soil microbial activity [16].

With increasing awareness the soil is a non-renewable resource that can no longer be taken for granted, it is imperative to identify agriculture systems that conserve the soil resource while maintaining a high level of agricultural production. Long term field treatments provide means of investigating the influences of organic and inorganic fertilizers on soil characteristics. The objective of this research was to investigate these effects in relation to soil enzyme activity as an index of soil biological and biochemical parameters. Three key soil enzymes involved in intracellular metabolism of microorganisms (actual and potential dehydrogenase and catalase activities) and two soil enzymes involved in phosphorus metabolism (acid and alkaline phosphatase activities) were chosen for the study.

Experimental part

Materials and methods

The soil that was selected for this study is located at the Agricultural Research and Development Station in Oradea (Bihar County) because of its extensive agricultural practices.

The experiment started in 1999. The experimental field occupying 9600 m², was divided into plots and subplots for comparative study of mineral (NP) and organic fertilization. The plots cultivated with maize, have received organic amendments: green manure at rates of 47.8 t/ha lupinus

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(P1), 29.9 t/ha vetch + oat + ryegrass (P2), 3.97 t/ha lupinus + oat (P3), 23.9 t/ha lupinus + rape + oat (P4) and 50t/ha farmyard manure (P5), excepting, a maize plot (P0) which received mineral fertilizers instead of organic fertilizers. Each plot consisted of two subplots representing the no-mineral fertilized and mineral fertilized variants. The mineral fertilized subplots were annually NP fertilized at rates of 120 kg of N/ha and 90 kg of P/ha. The plots and subplots were installed in three repetitions.

In July 2017, soil was sampled from the 0-20 cm depth of the plots. The soil samples were allowed to air-dry, then ground and passed through a 2 mm sieve, and finally used for enzymological analyses. Three enzymatic activities (actual and potential dehydrogenase and catalase) were determined according to the methods described in [17]. Dehydrogenase activities were expressed in mg of triphenylformazan (TPF) produced (from 2,3,5-triphenyltetrazolium chloride, TTC) by 10 g soil in 24 h, whereas catalase activity was recorded as mg H₂O₂ decomposed by 1 g of soil in 1 h. Two activities (acid and alkaline phosphatase) were assayed according to the methods of [17,18]. Phosphatase activities are expressed in mg phenol/g soil/2 h. The activity values were submitted to statistical evaluation by the two-way-t-test [19].

Results and discussions

Physical and chemical analyses are specified in table 1. They were determined in the Laboratory of Pedology from Agricultural Research and Development Station, Oradea, as recommended by [20].

Results of the enzymatic analyses are presented in figures 1-5.

Soil enzymatic activities as affected by fertilization

Addition of mineral fertilizers to organic fertilizers increases soil enzymatic activities. Each activity was higher in the mineral fertilized maize subplot than in the no-mineral fertilized maize subplot. The differences were significant ($p < 0.01$), excepting potential dehydrogenase activity and

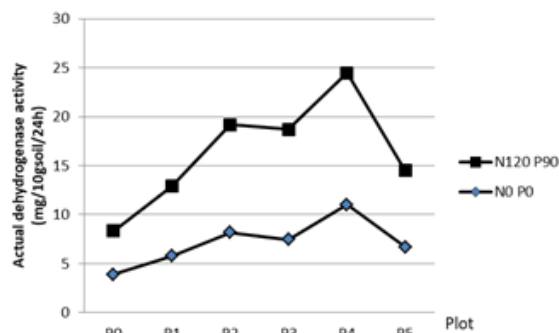


Fig.1. Actual dehydrogenase activity in no-mineral fertilized (N_0P_0) and in mineral fertilized ($N_{120}P_{90}$) soil

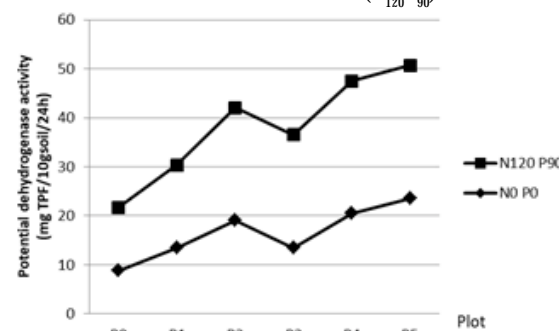


Fig. 2. Potential dehydrogenase activity in no-mineral fertilized (N_0P_0) and in mineral fertilized ($N_{120}P_{90}$) soil

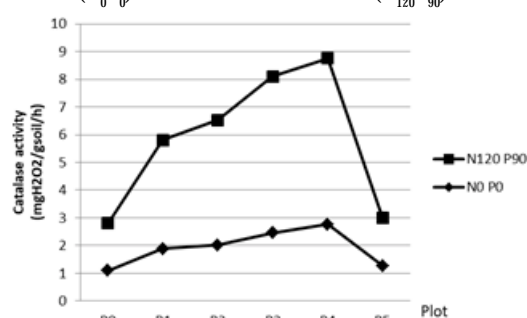


Fig. 3. Catalase activity in no-mineral fertilized (N_0P_0) and in mineral fertilized ($N_{120}P_{90}$) soil

Physical and chemical variables	Plots*					
	P0	P1	P2	P3	P4	P5
Particle size %	55.80	55.72	59.80	58.41	60.62	68.30
Bulk density (g/cm ³)	1.44	1.41	1.40	1.39	1.38	1.34
Penetration resistance (kg/cm ²)	25.60	20.60	20.10	20.50	19.50	15.80
Conductivity(mm/h)	13.89	15.97	16.19	16.91	16.57	20.61
pH	6.34	6.25	6.31	6.30	6.33	6.46
P(ppm)	34.1	37.4	38.7	38.6	38.6	42.5
K(ppm)	209.2	208.2	213.1	214	214	226.2

*P0 - Unfertilized; P1- Lupinus; P2-Vetch + oat + ryegrass; P3- Lupinus + oat; P4 - Lupinus + rape + oat; P5 - Farmyard-manured.

Table 1
DIFFERENT
PHYSICAL AND
CHEMICAL
VARIABLES (0-20
CM) AFFECTED
BY MINERAL
FERTILIZERS AND
MANURE
TREATMENT

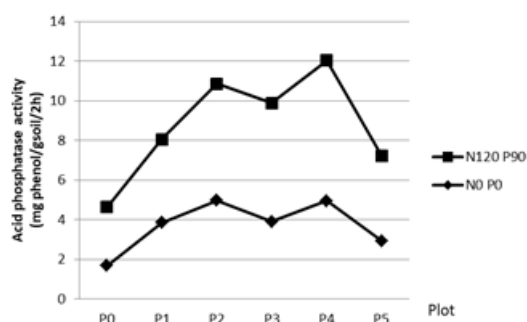


Fig. 4. Acid phosphatase activity in no-mineral fertilized (N_0P_0) and in mineral fertilized ($N_{120}P_{90}$) soil

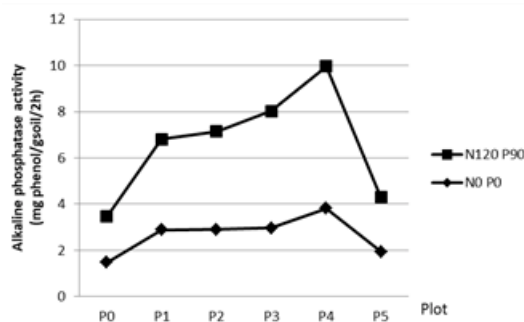


Fig. 5. Alkaline phosphatase activity in no-mineral fertilized (N_0P_0) and in mineral fertilized ($N_{120}P_{90}$) soil

acid phosphatase activity which were insignificant higher ($p > 0.05$) in the mineral fertilized subplot than in the no-mineral fertilized subplot.

Although soil organic fertilizers such as animal and green manures increase the soil enzymatic activities, the effect of the green manure was much more pronounced. The main influence of fertilization was the production of increased crop residues, root exudates, and root biomass, providing additional substrate for soil microorganisms and improving the environment for microbial growth.

Many investigations have shown that long term management of plant nutrients and organic amendments does affect soil biological properties. In general, management practices that increase inputs of organic residue, plant or animal manures, increase biological activity. Exemplifying this effect is reported [21]; it was found that manure application increased dehydrogenase, acid and alkaline phosphatase activities significantly. In the study it was observed that both acid and alkaline phosphatase activities were negatively influenced by chemical fertilizer treatment. The results suggest that application of manure treatment directly or indirectly influences the enzyme activity and in turn regulates nutrient transformation.

Other studies [22] compared the effects of application of green manure, animal manure and varying rates of N fertilizer on six soil enzymes and showed that the form of N added to soils is important for the enzymes involved in N cycling. Organic amendments (manure and pea vine) stimulated activity but increasing rates of inorganic N decreased activity of these enzymes [23]. For the other enzymes tested which are not in the N cycle, these relationships were not noted. Thus, this research 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. In addition, inorganic fertilizer amendments can selectively affect only certain biochemical processes.

Enzymatic indicators of soil quality

Significant and insignificant differences were registered in the soil enzymatic activities depending on the kind of enzymatic activity and the type of fertilization. Based on these differences the following decreasing orders of the enzymatic activities could be established in the soil of the six plots (table 2).

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 [24]. 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.

Tables 3 and 4 show that the first three positions are occupied by these plots in which actual dehydrogenase activity, potential dehydrogenase activity, catalase activity and acid and alkaline phosphatase activities were the highest. The soil that had not received organic and mineral fertilizers occupying the last position can be considered as the least enzyme-active soil.

The results obtained are in good concordance with the literature data [25-27]. Studies have shown that soil properties based on biological and biochemical activities, such as soil enzymes, have been shown to respond to small changes in soil conditions, thus providing information sensitive to subtle alterations of soil quality. Therefore, soil enzyme activities have been suggested as suitable indicators of soil quality because of their intimate relationship with soil biology, ease of measurement and rapid response to change in soil management.

Table 2
HIERARCHY OF THE PLOTS BASED ON THE ENZYMATIC ACTIVITIES

Soil enzymes*		ADA		PDA		CA		AcPA		AlkPA	
Chemical fertilization		N ₀ P ₀	N ₁₂₀ P ₉₀	N ₀ P ₀	N ₁₂₀ P ₉₀	N ₀ P ₀	N ₁₂₀ P ₉₀	N ₀ P ₀	N ₁₂₀ P ₉₀	N ₀ P ₀	N ₁₂₀ P ₉₀
Hierarchy of the plots	1	P4	P4	P5	P5	P4	P4	P2	P4	P4	P4
	2	P2	P3	P4	P4	P3	P3	P4	P3	P3	P3
	3	P3	P2	P2	P3	P2	P2	P3	P2	P2	P2
	4	P5	P5	P3	P2	P1	P1	P1	P5	P4	P1
	5	P1	P1	P1	P1	P5	P5	P5	P1	P5	P5
	6	P0	P0	P0	P0	P0	P0	P0	P0	P0	P0

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

Position	Plot	Enzymatic indicator of soil quality
1	Lupinus + rape + oat	335.5
2	Vetch + oat + ryegrass	281.5
3	Lupinus + oat	249.0
4	Farmyard-manure	230.0
5	Lupinus	224.5
6	Unfertilized	127.0

Table 3
ENZYMATIC INDICATORS OF SOIL QUALITY IN PLOTS WHICH RECEIVED ORGANIC FERTILIZERS

Position	Plot	Enzymatic indicator of soil quality
1	Lupinus + rape + oat	499
2	Lupinus + oat	429
3	Vetch + oat + ryegrass	394
4	Lupinus	303.5
5	Farmyard-manure	286
6	Mineral fertilized maize	191

Table 4
ENZYMATIC INDICATOR OF SOIL QUALITY IN PLOTS WHICH RECEIVED MINERAL AND ORGANIC FERTILIZERS

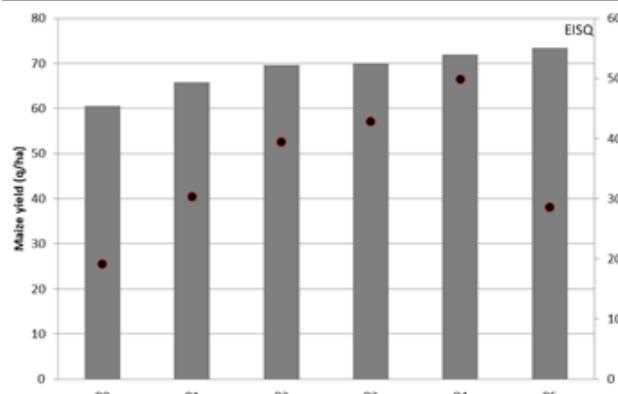


Fig. 6. Correlation between enzymatic indicator of soil quality (●) and maize yield in no-mineral fertilized soil

The effect of fertilization on the maize yield

Grain yields of maize were significantly influenced by fertilization (figs. 6 and 7). All the fertilized treatments improved maize yields relative to unfertilized control (P0).

Application of organic (green manure and farmyard manure) fertilizers with mineral nutrients ($N_{120}P_{90}$) yielded 8.75 to 26.21 times more than no-fertilized soil. The yield data clearly demonstrate the superiority of farmyard manure, which provided greater stability in crop production. This is attributed to the maintenance and improvement of soil nutrient status as well as soil biological activity especially, soil enzymatic activity. Also one can see from figures a weak positive correlation ($r=+0.307$ and $r=+0.212$, respectively) between enzymatic indicators of soil quality and maize yield.

It should be emphasized that the repeated applications of organic fertilizers (green manure and farmyard manure) with mineral fertilizers (NP) have substantial effects on the activities of enzymes which are considered indicators of the global and respiratory activity of soil (dehydrogenase and catalase) and on the phosphatase activities which are related to the P cycle in soil.

Conclusions

Measurements of these enzymatic activities have been used to establish the indices of soil biological fertility because of their utmost sensitivity to management practices. Management practices that minimize the addition of organic matter to soils may reduce enzyme activities and thus affect the ability of soils to supply nutrients needed for plant growth. Therefore, changes in enzyme activities could alter the availability of nutrients for uptake, and these changes are potentially sensitive indicators of soil quality.

A better knowledge of changes in soil enzyme activities would allow better understanding of the disturbance effect on soil functions. Because soil enzymes respond to sudden disturbances of the soil system, they can effectively aid

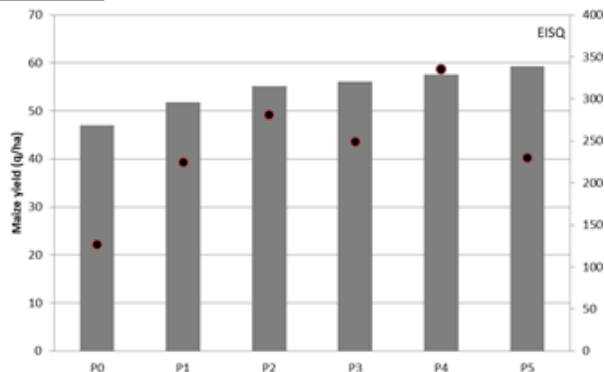


Fig. 7. Correlation between enzymatic indicator of soil quality (●) and maize yield in mineral fertilized soil

developing sustainable land management practices. Improvement of soil biological activity through application of organic amendments along with inorganic nutrients resulted in substantial increase in crop yield, which can be sustainable for years.

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Manuscript received: 5.03.2018