Acta Agrophysica, 2001, 52, 133-140

INFLUENCE OF LONG-TERM ORGANIC FERTILIZATION ON THE ENZYMATIC ACTIVITY

J. Koper, A. Piotrowska

Department of Biochemistry, University of Technology and Agriculture
Bernardyńska 6, 85-029 Bydgoszcz, Poland

A b s t r a c t. Mineral and organic fertilization is one of the most important factors affecting activity of soil enzymes. It is commonly accepted that organic fertilization is more beneficial to the soil biological activity than the mineral. The objectives of this investigation were to assay C_{org}, N_{tot}, P_{tot} content and to determine urease and amylase activities in the soil fertilised for a long time with various rates of farmyard manure and slurry. Soil samples were taken from the experiment established on a typical lessivé soil. FYM was used at the following rates: 20, 40, 60, 80 t ha^{-1} fresh weight once in the rotation on the plots with potatoes, while slurry was applied in four doses corresponding to manure fresh weight. Soil samples were taken after potato harvest in the 8^{th} year after the experiment start-up from two depths: 5-15 and 25-35 cm of the soil profile. Usually, higher amounts of C_{org} and P_{tot} were found in the soil manured with FYM in comparison with the pig slurry-manured soil. These amounts increased when the doses of both fertilizers were the highest. The content of C_{org} ranged from 5.5 to 8.2 g $kg^{-1}$of soil when 80 t ha^{-1} of FYM was used. Total nitrogen ranged from 0.45 to 0.95 g $kg^{-1}$of soil, average for doses of both fertilizers, whereas total phosphorus content ranged from 0.26 to 0.48 g $kg^{-1}$of soil. The highest amylase activity was observed in the soil when FYM was used (0.48 μg starch g^{-1} 16 h^{-1}) as compared to the soil where slurry was applied (0.41 g starch μg^{-1} 16 h^{-1}). No increase of amylase activity was observed when the doses of both fertilizers were the highest. A higher urease activity was noted when FYM was used than in the case of the soil manured with slurry.A decrease of urease activity was noted in the control samples (3.0 mg NH_3 10 g^{-1}24 h^{-1}), while the urease activity increased when the doses of both fertilizers were the highest. The highest activities were noted when 80 t ha^{-1} of FYM or slurry were used.

K e y w o r d s: urease, amylase, farmyard manure, slurry, lessivé soil.

INTRODUCTION

The activity of soil enzymes is the effect of physiological processes of organic matter transformation, mainly microorganisms, which exerts a lasting influence on the soil fertility [2]. Soil hydrolytic enzymes play a special role in the transformation of soil organic compounds. Mineral and organic fertilization is one of the most important factors affecting the reaction of soil enzymes. The influence of fertilizers on the soil enzymatic activity depends on the soil type, enzyme, and application time. In a long-term experiment, effects could be caused by changes in the soil characteristics such as plant cultivation, moisture content, concentration and availability of organic and inorganic nutrients [3]. Up-to-day, unanimous opinion about the effects of various forms and rates of organic fertilization has not been reached. Usually it is said that organic fertilization is more beneficial to the soil biological activity than the mineral. Organic fertilization in general and its rate in particular stimulate development of soil microorganisms and soil enzymatic activity.

The objectives of this investigation were to assay C_{org}, N_{tot}, P_{tot} content and to determine urease and amylase activities in soil fertilised for a long time with various doses of farmyard manure and slurry.

MATERIALS AND METHODS

Soil was sampled from the experiment established by the IUNG in Puławy at the Experimental Station in Baborówek on a typical lessivé soil. It is a two-factor experiment with randomized sub-blocks and a control plot carried out in four replications. Mineral (NPK) fertilised plots were the control. FYM was used at the rates of: 20, 40, 60, 80 t ha^{-1} fresh weight once in the rotation on the plots with potatoes, while slurry was applied in four doses corresponding to fresh weight of manure under two plants in rotation in the proportion of 75% under potato and 25% under spring barley. Pig slurry was applied in autumn or in spring before barley sowing and potato planting. Soil samples were taken after potato harvest in the 8^{th} year after the experiment start-up, from two depths: 5-15 cm and 25-35 cm of the soil profile. The prepared material was assayed for:

- amylase activity - spectrophotomerically as described by Beck (1980) using 0.3% starch as a substrate,
- urease activity - colorimetrically according to Tabatabai and Bremner (1969) using 10% urea solution as a substrate,
- total phosphorus (P_{tot}) was isolated by the method of Mehta (after Dalal, 1977) and determined colorimetrically,
- C_{org}, N_{tot}, granulometric composition and pH in H_2O were assessed by commonly used methods.

The relationship between the investigated parameters was analysed by variance and correlation analysis with the Tukey's test.

RESULTS

The examined soil was classified as fine loamy sand and silty loamy sand. Soil samples did not differ much in their colloid content. The percentage of this fraction was 2-8 %. The loam fraction content ranged from 4 to 10%, while silty fraction ranged from 20 to 30%. Reaction of the soil samples was slightly acidic, pH in H_2O ranged from 5.1 in the control soil to 6.0 in the soil samples taken from the plots with 80 t ha^{-1} of slurry or FYM. An increase of pH in H_2O values with an increase of slurry and FYM rates was observed. There was a little difference in pH in H_2O values in the case of the same rate of slurry and FYM. Fertilization with slurry and FYM had a greater impact on the level of total N and P, C_{org} and enzymatic activity (Tables 1 and 2, Fig. 1). The C_{org} content (Table 1) ranged 5.0-8.2 g kg^{-1} in the examined soil. The content of C_{org} was significantly higher in the soil fertilised with FYM as compared to the soil samples taken from the plots with slurry. In our investigations, the amount of C_{org} increased with the increasing slurry and FYM rates (20 and 80 t ha^{-1}). The highest increase was observed when 80 t·ha^{-1} of the fertilizers were used. There was no significant difference in the C_{org} content in the 5-15 cm layer as compared with its amount in the 25-35 cm layer. The content of N_{tot} ranged 0.46-0.95 g kg^{-1} and was significantly higher in the soil fertilised with slurry as compared to the soil samples taken from the plots with FYM. It was found out that $P_{tot.}$ content and enzymatic activity depended on the kind and rates of the applied fertilisers, too. (Table 2, Fig. 1). The soil samples fertilised with mineral fertilizers (NPK) had a significantly lower P_{tot} content than the soil to which FYM and slurry was applied. The difference reached 0.23 g kg^{-1} when 80 t ha^{-1} was used. P_{tot} ranged from 0.26 to 0.49 g kg^{-1} in the soil under the present study. The soil samples taken from the plot fertilised with slurry, as compared to the soil treated with FYM, showed a slight, but significant decrease of the P_{tot}. Difference in the P_{tot} content between the highest and the lowest FYM rates ((20 and 80 t ha^{-1}) was found at the level of 59%, whereas between the same slurry doses the difference observed was only 32%.

Amylase activity was higher in the soil fertilised with FYM than in the soil treated with slurry (Table 2). The enzyme was less active in the soil with 20 t ha^{-1} of FYM and slurry (mean 0.028 g starch g^{-1} 16 h^{-1}) than in the soil treated with NPK only (0.046 g starch g^{-1} 16 h^{-1}).

A considerable increase of amylases activity (0.065 g starch g^{-1} 16 h^{-1}) was observed for 40 t ha^{-1} of FYM and 60 t ha^{-1} of slurry. Enzymatic activity was less

Table 1. Organic carbon and total nitrogen content in the investigated soil (g kg^{-1})

Kind of manure	Dose ($t\ ha^{-1}$)	Organic carbon			Total nitrogen		
		Depth of sampling (cm)					
		5-15	25-35	$\bar{x}$	5-15	25-35	$\bar{x}$
NPK		5.00	4.85	4.93	0.48	0.46	0.47
FYM	20	5.45	5.63	5.54	0.51	0.46	0.52
	40	6.83	6.90	6.86	0.58	0.49	0.54
	60	7.53	7.69	7.61	0.67	0.53	0.60
	80	8.23	8.13	8.18	0.76	0.64	0.70
	$\bar{x}$	7.01	7.09	7.05	0.63	0.55	0.59
Pig slurry	20	5.79	5.58	5.69	0.53	0.51	0.52
	40	6.21	6.26	6.24	0.64	0.58	0.61
	60	6.75	6.68	6.71	0.72	0.64	0.68
	80	7.28	7.37	7.33	0.95	0.71	0.83
	$\bar{x}$	6.51	6.47	6.49	0.71	0.61	0.66
$LSD_{0.05}$	I		0.12			0.038	
	II		0.22			0.017	
	III		n.s.			0.018	
Interaction	I/II II/I	0.32		0.24	0.037		0.046
$LSD_{0.05}$	II/III III/II	n.s.		n.s.	0.025		0.022
	I/III III/I	n.s.		n.s.	0.040		0.048

n.s. - not significant.

pronounced at higher FYM rates (60 and 80 t ha^{-1}). There was a significant difference between amylase activity in the 5-15 cm layer as compared to the soil taken from the 25-35 cm layer. Urease activity was usually slightly higher in the soil samples taken from the plots treated with pig slurry (3.7 mg NH_3 10 g^{-1}24 h^{-1}) as compared to the soil samples supplemented with FYM (3.5 mg NH_3 10 g^{-1}24 h^{-1}). The lowest enzyme activity (3.0 mg NH_3 10 g^{-1}24 h^{-1}) was found in the samples fertilised with NPK only. A considerable increase in the urease activity was observed at increased rates of FYM and slurry. Differences in the activity of the soil samples treated with the highest and the lowest FYM rates (20 and 80 t ha^{-1}) was 16%. It was much lower at the same slurry rates 7.7%.

Correlation coefficients relating to enzyme activity and soil properties are shown in Table 3. Urease activity was positively correlated with C_{org} and N_{tot} content, but there was no significant relation between C and N and amylase activity, or between activities of both enzymes.

Table 2. Amylase and urease activity in the investigated soil

Kind of manure	Dose ($t ha^{-1}$)	Amylase (μg starch g^{-1} $16 h^{-1}$)			Urease (mg NH_3 10 g^{-1} $24 h^{-1}$)		
		Depth of sampling (cm)					
		5-15	25-35	$\bar{x}$	5-15	25-35	$\bar{x}$
NPK		0.048	0.045	0.046	3.1	2.9	3.0
FYM	20	0.033	0.020	0.027	3.7	3.4	3.6
	40	0.076	0.053	0.065	3.9	3.4	3.7
	60	0.046	0.040	0.043	3.7	3.5	3.6
	80	0.056	0.050	0.053	4.5	3.3	3.9
	$\bar{x}$	0.053	0.041	0.047	4.0	3.4	3.7
Pig slurry	20	0.032	0.025	0.029	3.2	3.0	3.1
	40	0.039	0.036	0.038	3.5	3.3	3.4
	60	0.050	0.044	0.049	3.6	3.6	3.6
	80	0.049	0.041	0.045	3.9	3.5	3.7
	$\bar{x}$	0.043	0.037	0.040	3.6	3.4	3.5
$LSD_{0.05}$	I		0.009			0.24	
	II		0.005			0.07	
	III		0.002			0.11	
Interaction $LSD_{0.05}$	I/II II/I	0.012		0.013	0.16		0.24
	II/III III/II	0.003		n.s.	0.15		0.12
	I/III III/I	0.005		0.008	0.24		0.29

n.s. - not significant.

DISCUSSION

Comparison of the effects of mineral and organic fertilization has been the subject of many long-term experiments [2]. Fertilisation with FYM and slurry has a great impact on the level of C_{org}, N_{tot} and P_{tot} and enzymatic activity. The highest C_{org} content in the soil taken from the plots with FYM as compared to the soil taken from under slurry fertilisation is in agreement with the findings of Mazur *et al.* [7]. The latter author showed a significant increase of C_{org} content in the soil fertilised with FYM. It decreased (by 10-30%) when different rates of slurry were used.

In general, higher enzymatic activity was observed in the soil treated with FYM as compared to slurry and NPK fertilization only. This is in agreement with the data reported by Khan [4] who observed higher urease activity (by about 15%) in the soil samples treated with manure than in the soil with NPK fertilization only. Higher difference in the soil urease activity was found by Kucharski *et al.* [2].

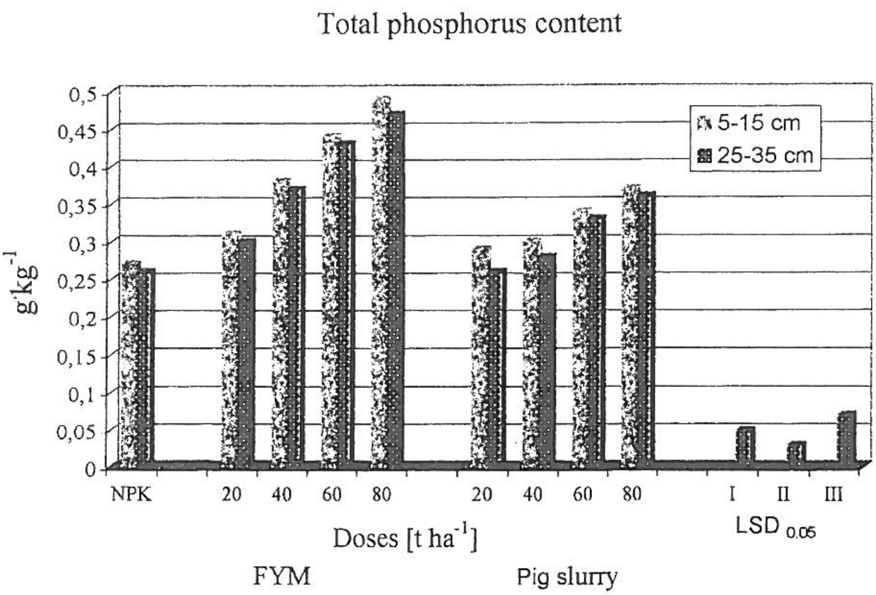

Fig. 1. Total phosphorus content in the investigated soil.

In the soil fertilised with FYM, he found 50% higher urease activity than in the soil supplemented with NPK only. Urease activity is usually positively correlated with some soil biological and chemical properties. For example, it was correlated with C-microbial (r=0.84-0.97***) and N-microbial (r=0.66-0.80**) [6] or C_{org} (r=0.83**) and N_{tot} (r=0.69**) [1]. In our investigations, the lowest coefficients were found (Table 3).

Agricultural application of pig slurry for waste disposal and improving soil quality is of extreme practical interest considering environmental issues related to this residue. In the field experiment [8] carried out to determine the effects of pig slurry soil supplements on the oxidoreductase and hydrolase activity, an increase of urease activity from 90 to 150 m^3 ha^{-1} was noted. While the lowest rate of

Table 3. Correlation coefficients ($P_{0.05}$) between soil parameters

Parameters	C_{org}:N_{tot}	C_{org}:P_{tot}	C_{org}:Urease	C_{org}:Amylases
FYM	0.524*	0.409	0.564*	0.161
Slurry	0.612*	0.482	0.570*	0.495
	N_{tot}:P_{tot}	N_{tot}:Urease	N_{tot}:Amylases	Urease:Amylases
FYM	0.436	0.610*	0.102	0.359
Slurry	0.373	0.597*	0.370	0.287

slurry had no impact when compared to the control. In our experiment, lower rates of pig slurry (20 and 40 t ha^{-1}) caused a higher decrease of amylase activity than the application NPK only. We observed soil amylase decrease in the soil samples treated with 80 t ha^{-1} slurry dose, suggesting that pig slurry released some inhibitors of enzyme activity to the soil.

Amylase activity is one of the polysaccharidases found in the soil. Hydrolytic decompositions of polysaccharides and the subsequent mineralization of the products have a special significance in the biological carbon cycle [5]. Amylase activity increases with organic matter content and may also be correlated with the cation-exchange capacity. Amylases activity was correlated positively with the rate of CO_2 production (r = 0.57**), but it was negatively correlated with urease activity (r = -0.37*) [10]. Earlier, the same author [9] found positive correlation coefficients between amylase activity and the C_{org} content (r= 0.78**-0.83**) and N_{tot} (r= 0.80**-0.86**).

CONCLUSIONS

1. A long-term FYM and pig slurry application has been the basic factor which caused changes in the soil chemical parameters and enzymatic activity.

2. Organic carbon and total phosphorus content was higher in the soil samples taken from the plots manured with FYM as compared to the soil fertilised with pig slurry Total nitrogen was slightly higher in the soil samples fertilised with slurry, than in the soil with FYM doeses. These amounts increased with increasing rates of both fertilizers.

3. Application of FYM increased activity of soil urease and amylases. Urease activity increased with the use of both fertilizers, while amylase activity decreased with high fertiliser rates, suggesting their negative effect on the soil biochemical properties.

4. Enzymatic activity and the content of the studied elements was higher in the soil samples taken from the depth of 5-15 cm as compared to the samples from the depth of 25-35 cm.

5. Variation analysis demonstrated a significant interaction between C, N and P content, enzymatic activity and the kind and rates of organic fertilizers. Correlation analysis showed a very significant relationship between the investigated parameters, which proved a significant differentiation in the amount of the determined elements and enzyme activities in the investigated soil.

REFERENCES

1.**Baligar V.C., Stanley T.E., Wright R.J.:** Commun. Soil Sci. Plant Anal., 22, 315-322, 1991.
2.**Blecharczyk A., Skrzypczak G., Małecka I.:** Effect of long-term organic and mineral fertilization on chemical properties and enzymatic activity of soil. [In:] Proc. Int. Symp. "Long-term static fertilizer experiments". (Eds K. Mazur, J. Filipek, B. Mazur). Agricultural University of Cracow, 167-176, 1993.
3.**Gianfreda L., Bollag J.M.:** Influence of natural and antropogenic factors on enzyme activity in soil. In: Soil Biochemistry (Eds G. Stotzky, J.M. Bollag). Marcel Dekker, New York, Basel, Hong Kong, 9, 123-193, 1996.
4.**Khan S.U.:** Soil Biol. Biochem., 2, 137-139. 1970.
5.**Kiss S., Dragan-Bularda M.:** Influence of enzyme substrates on microbial production of polysaccharidases in soil. In: Fourth Symposium on soil biology (Eds M.P. Nemes, S.Kiss *et al.*) Rumunian National society of Soil Science,Cluj-Napoca, 29-51, 1977.
6.**Klose S., Tabatabai M.A.:** Soil Biol. Biochem., 31, 205-211, 1999.
7.**Mazur T., Sądej W.:** Roczn. Glebozn., 40, 1, 147-153, 1989.
8.**Plaza C., Garcia-Gil J.C., Soler-Rovira P., Polo A.:** Effects of agricultural application of pig slurry on enzyme activities. [In:] Enzymes in the Environment: Activity, Ecology and Applications (Ed. R.P. Dick). Granada, Spain, 131, 1999.
9.**Ross D.J.:** New Zealand J. Sci., 26, 339-346, 1983.
10.**Ross D.J., Speir T.W., Cowling J.C., Whale K.N.:** Aust. J. Soil Res., 22, 319-330, 1984.