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ACTIVITIES OF SOIL ENZYMES IN CORN FIELDS ENRICHED WITH MANURE

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ABSTRACT

Elevated enzyme activities in soils have been reported to be associated with enhanced contents of organic matter and total nitrogen (N). This research sought to evaluate the changes in enzymatic activities in cornfield soil enriched with organic matter. Corn field plots had been treated for 8 seasons with increasing amounts of green manure or barnyard manure (0 – 100% of 20 ton ha^{-1}) and decreasing amount of chemical fertilizers (100 – 0% of 300 kg Urea + 200 kg SP-36 + 100 kg KCl ha^{-1}). Soil samples were taken at the maximum vegetative stage and at harvest time, and analyzed for enzymatic and chemical properties. The results clearly showed that enrichment with barnyard manure significantly increased alkaline phosphatase activity. Both manures did not affect the activities of acid phosphatase, arylsulfatase and β-glucosidase. The greater effect of barnyard manure on alkaline phosphatase was closely related with increases in soil pH, available P, and microbial activities. Barnyard manure also significantly increased organic C and total N content. The activities of acid and alkaline phosphatase, arylsulfatase, and β -glucosidase showed high and significant correlations with soil pH, organic C, total N, and available P. Acid phosphatase showed a significant correlation with corn yield.

Key words: barnyard manure, chemical fertilizers, green manure

INTRODUCTION

Switching from the use of chemical fertilizers to more environmentally sound sources of plant nutrients is one of the most central issues in current agricultural system, particularly related to the use of indigenous and cheaper materials. Organic matter in the form of manure such as barnyard manure of domesticated animals and green manures are among the indigenous sources of plant nutrients available in agricultural environments. The use of these materials may lower the need for chemical fertilizers that has become very expensive. The use of these materials may also lower the emission of toxic materials such as heavy metals brought about by the application of chemical fertilizers into the soil system.

The use of organic matter in agricultural soils may, of course, change some soil properties, of which soil enzymatic property is of utmost importance. Soil enzymes are very important in accelerating the decomposition of organic matter and making available plant nutrients from organic sources in the soil system (Tabatabai, 1982; Tate III, 1987). Some accessible experimental data show that the activities of soil enzymes are well-correlated with changes in organic matter content, organic C, and total N (Nannipieri et al., 1980; Frankenberger and Dick, 1983; Baruah and Mishra, 1984; Tate III, 1984; Baligar et al., 1988; Salam et al.,1998; 1999a; 1999b). This indicates that addition of organic matter to the soil will increase soil enzyme activities, which eventually increases the decomposition of structural plant nutrients of organic materials into available nutrients. Some researchers argue that the structural C and N may increase the soil microbial population and activities responsible for the production of enzymes released into soil system (Tate III, 1984). Soil microorganisms are the most important producers of soil enzymes (Duxbury and Tate III, 1981; Ross and Cairns, 1982; Frankenberger, Jr. and Dick, 1983; Vinotha et al., 2000), probably more important than plant roots and soil mesofauna such as earthworms (Satchell and Martin, 1984).

The decreasing amounts of chemical fertilizers added into the soil system may also change the soil enzymatic properties. For example, the soluble P from P-fertilizers such as TSP, SP-36, and rock phosphates may inhibit the action of phosphatases in accelerating the detachment of phosphates from organic structures and their release into the soil solution. Some researchers report that addition of inorganic P through chemical fertilizers even decreases phosphatase activities (Pang and Kolenko, 1986; Fox and Commerford, 1992). Lower activity of phosphatases and higher soluble P in soil solution will slow the decomposition reaction and decrease the concentration of P of organic matter origin. Since the addition of organic matter usually changes the soil pH, the changes in soluble P may be lower because the activities of soil phosphatases are pH-dependent. The activities of phosphatases in general increase with soil pH at pH lower than their optimum pHs and decrease with soil pH at pH higher than their optimum pHs (Frankenberger, Jr. and Johanson, 1982; Malcolm, 1983; Rojo et al., 1990; Salam et al., 1998).

This research sought to evaluate the changes in corn-field soil enzymatic properties as affected by addition of organic matter of barnyard manure (chicken dung) and green manure (*Glyricidium* sp.) with decreasing amount of added chemical fertilizers.

MATERIALS AND METHODS

Study site and experimental design

Soil samples were taken from experimental plots designed for a long-term evaluation set up in Tamanbogo experimental farm at Purbolinggo, East Lampung. Experimental treatments were arranged in randomized block design with three replicates. Treatments were Control = no manure and chemical fertilizers, 0% M = 100% chemical fertilizers, 50% GM or 50% BM = 50% green manure (*Glyricidium* sp.) or 50% barnyard manure (chicken dung) and 50% chemical fertilizers, 75% GM or 75% BM = 75% green manure or 75% barnyard manure and 25% chemical fertilizers, and 100% GM or 100% BM = 100% green manure or 100% barnyard manure. A 100% barnyard manure or green manure was equivalent to 20 ton ha^{-1}. A 100% chemicals fertilizers was equivalent to 300 kg Urea + 200 kg SP-36 + 100 kg KCl ha^{-1}. Selected properties of barnyard and green manures are listed below (Table 1).

Table 1. Properties of barnyard manure and green manure.

Manure	pH	C/N	C	N	P	Na	K	Ca	Mg	WC
						mg kg^{-1}				(%)
1. BM	7.01	5.83	81.0	13.9	19.8	0.80	8.80	75.4	4.60	77.3
2. GM	5.77	3.27	115	35.0	2.00	1.70	20.6	6.00	2.70	62.9

Note: BM = barnyard manure; GM = green manure; after Afriyani (2003)

The experimental plots were consecutively planted with different crops and left to fallow between seasons from the time it was set up in March 2001 (Table 2).

Table 2. Cropping history of experimental field.

Season	Time of Planting	Crop Plants	Fallow period
1	March 2001	Corn var Bisma	
2	August 2001	Corn var Bisma	2 weeks
3	November 2001	Upland paddy var Limboto	1 month
4	April 2002	Upland paddy var Limboto	3 months
5	November 2002	Upland paddy var Limboto	1 month
6	April 2003	Corn var Bisma	1 season*
7	-	-	
8	April 2004	*Corn var Bisma*	

* from August 2003 – April 2004

During the very first season, treatments were applied after plowing and plotting. Each plot measured 6 m x 3 m, 0.8 m between plots and 1 m between blocks. Organic matter was mixed to 20-cm depth. In the 8th season, the experimental plots were plowed and treatment materials were applied to 20-cm depth. Chemical fertilizers were injected beside corn seedlings which had a planting distance of 25 cm x 25 cm. SP-36 and KCl were applied once at the beginning of corn growth (7 DAP, days after planting). Urea was applied twice, at the beginning of corn growth (7 DAP) and at 30 DAP.

Soil samples were taken twice from three random sites in each experimental plot. The first sampling was at maximum vegetative stage (t-1 = 60 DAP) and the second sampling was at the harvest time (t-2). Soil samples were taken from the corn plant rooting zones. After a thorough mixing, one part of the soil samples was stored in a cold room for soil enzymatic analyses and another part was air-dried for determination of pH, organic C, total N, and available P.

Analyses included soil enzymatic properties (acid and alkaline phosphatases, arylsulfatase, and β-glucosidase) using modified Tabatabai method (Tabatabai, 1982); some soil chemical properties (soil pH using pH-electrode, organic C using Walkey and Black method, total N using Kjeldahl method, and available P using Bray I method) and soil microbial biomass C using chloroform fumigation-extraction (CFE) method (Wu et al., 1990). Data were analyzed using ANOVA. Differences among treatments were analyzed with Least Significant Difference (LSD) at the 5% level.

Soil Enzymatic assay

Phosphatase Activity. After stopping the soil microbial activities with toluene, 4 mL of a modified universal buffer (MUB), pH 6.5 (for acid phosphatase measurement) or pH 11 (for alkaline phosphatae) and 1 mL of 0.025 ρ- nitrophenyl phosphatase (ρ-NPP) dissolved in MUB solution with the corresponding pH were added to 1 g of soil sample and incubated at 3 ^{o}C for 60 min. The enzymatic reaction was stopped by the addition of 2 ml of 0.5 M NaOH solution, followed by 0.5 ml of 0.5 M $CaCl_2$ to extract ρ- nitrophenol. The concentration of ρ- nitrophenol was determined with a Shimadzu UV-2200 UV-VIS Recording Spectrophotometer at 400 rpm for 5 min. Controls were made in the same way, although the substrate was added before the $CaCl_2$ and NaOH (Tabatabai and Bremner, 1969).

β-glucosidase Activity. The activity *β-glucosidase* was determined by the above method but ρ- nitrophenyl β- D- glucopyranoside (ρ- NGP) was added as a substrate instead of ρ- nitrophenol (ρ-NPP), and a MUB solution pH 6 was employed. The rest of the method was the same as phosphatase activity but to stop the reaction, tris-hydroxymethyl aminomethane (THAM) was used, as suggested by Tabatabai (1982).

Arylsulfatase Activity. Arylsufatase activity was measured by the following method. A 1 g aliquot of soil sample (< 2mm, oven dry equivalent) was put into a 50-mL Erlenmeyer flask. The microbial activity was stopped by the addition of 0.25 mL toluene, followed by 4 mL acetate buffer 0.5 M (pH 5.8) and 1 mL ρ- nitrophenyl sulfate solution 0.025 M (ca. 3.5 mg of ρ- nitrophenol equivalent). After gentle swirling, the mixture was incubated for 60 min. A 1 mL aliquot of 0.5 M $CaCl_2$ and 4 mL aliquot of 0.5 M NaOH solution were then added. The ρ- nitrophenol concentration in the solution phase was determined with aspectrophotometer at 400 nm wavelength after filtering through a Whatman No. 42 paper (Tabatabai, 1982). Activity of each enzyme was determined at 30^{o}C.

RESULTS AND DISCUSSION

Changes in Soil Chemical and Biological Properties

Addition of organic matter (barnyard manure and green manure) into the soil system was observed to significantly change the soil chemical (Table 3) and biological properties (Table 4).

Table 3. Changes in some soil chemical characteristics as affected by enrichment with organic matter.

Treatment	pH		Organic C		Total N		Available P	
	Maximum Vegetative	Harvest Time	Maximum Vegetative	Harvest Time	Maximum Vegetative	Harvest Time	Maximum Vegetative	Harvest Time
				g kg^{-1}			mg kg^{-1}	
Control	4.66 ab	4.63 b	10.2 a	10.2 a	1.2 a	1.2 a	20.1 a	15.4 a
0% M	4.54 a	4.37 a	13.3 b	13.2 b	1.6 bc	1.2 a	59.8 d	64.7 b
50% GM	4.61 ab	4.60 b	12.6 b	12.4 b	1.7 c	1.3 b	49.2 c	49.1 ab
75% GM	4.75 b	4.59 b	12.3 b	12.1 b	1.5 b	1.3 b	38.6 b	38.6 ab
100% GM	4.73 b	4.79 c	12.8 b	12.6 b	1.6 bc	1.3 b	30.0 b	27.0 ab
50% BM	6.16 c	6.33 d	17.3 d	16.3 c	2.2 e	1.6 c	170 e	228 c
75% BM	6.54 d	6.37 d	15.1 c	15.0 c	1.9 d	1.7 d	213 f	270 d
100% BM	6.55 d	6.61 e	19.7 e	19.5 d	2.5 f	1.9 e	241 g	300 d

Table 4. Changes in soil microbial biomass C as affected by enrichment with organic matter.

Treatment	Maximum Vegetative	Harvest time
	mg CO_2-C kg^{-1}	
Control	22 a	81 a
0% M	154 b	210 c
50% GM	61 a	141 b
75% GM	161 b	149 b
100% GM	190 b	154 b
50% BM	290 c	293 d
75% BM	368 d	268 d
100% BM	583 e	412 e

Barnyard manure increased significantly the soil pH, organic C, total N, and available P (Table 3). Green manure, however, increased significantly only the soil pH to a lower extent and significantly decreased the soil available P. Sampling time (maximum vegetative period and harvest time) did not change the soil chemical properties except the available P in barnyard-manure treated plots; available P in soils sampled at harvest time was significantly greater that those sampled at maximum vegetative period.

The greater effect of barnyard manure compared to green manure in affecting the soil chemical properties was associated with the greater population and activities of microorganisms in barnyard treatment plots. Microbial population was more active in barnyard manure treated plots. Observations confirmed that microbial population activities increased with barnyard manure treatment and, conversely, decreased with green manure treatment (Table 4).

Application of barnyard manure increased the soil pH; higher than with green manure both exclusively or in combination with inorganic fertilizer (Table 3). The increases in soil pH were probably caused by the fact that barnyard manures contained high Ca (7.5 %) (Table 1). Calcium is a liming cation that can increase soil pH. The same results have been reported by Simex et al. (1999); soil pH was increased by the addition of organic plus inorganic fertilizer applied in conjunction with lime, but was decreased in the absence of liming. Green manure showed a lower Ca content (0.6 %).

The soil organic C and total N contents were all higher in all fertilized treatments compared to the control treatment (Table 3). The greatest amounts of both organic C and total N were observed in soils treated with barnyard manure and the least amount organic C and total N were in unfertilized treatments, except for total N, lowest plots fertilized with inorganic fertilizer. The increases in soil C were probably due to the combined effects of C addition in the manure and increased plant productivity as a result of both manure and organic fertilizer additions. Soil organic C and total N provide a measure of soil organic matter level. In this study, the aboveground crop biomass was removed and not incorporated into the soil. The increase in soil organic matter with the application of inorganic fertilizer was probably because of the greater input of root biomass due to better crop growth (Goyal et al., 1992).

Changes in Soil Enzymatic Properties

Changes in the activities of soil enzymes as affected by increasing manure and decreasing chemical fertilizers are shown in Table 5.

Table 5. Changes in soil enzymatic activities as affected by enrichment with organic matter.

Treatment	Acid Phosphatase		Alk. Phosphatase		Arylsulfatase		*β*-Glucosidase	
	Maximum Vegetative	Harvest Time	Maximum Vegetative	Harvest Time	Maximum Vegetative	Harvest Time	Maximum Vegetative	Harvest Time
	microgram p-nitrophenol g^{-1} h^{-1}							
Control	266 a	371 a	43.1 a	50.9 a	1380 a	1420 a	208 a	189 b
0% M	216 a	543 a	46.4 a	39.8 a	1480 a	1370 a	200 a	228 c
50% GM	221 a	627 a	38.7 a	42.0 a	1380 a	1440 a	216 a	230 c
75% GM	304 a	560 a	52.0 a	46.5 a	1380 a	1440 a	230 a	231 c
100% GM	293 a	632 a	56.5 a	47.6 a	1320 a	1520 a	205 a	234 c
50% BM	366 a	693 a	83.1 b	189 b	1460 a	1310 a	208 a	164 a
75% BM	305 a	777 a	170 c	218 c	1410 a	1400 a	230 a	331 d
100% BM	321 a	738 a	257 d	239 d	1510 a	1500 a	357 a	225 c

Note: Different characters in one column indicates a significant difference by LSD= 0.05.

Dick (1992) has noted that numerous researchers have found that soil enzyme activity increases with the addition of organic material. However, our experiment showed that both barnyard manure and green manure did not give consistent effect on the activities of acid phosphatase, arylsulfatase, and β-glucosidase, except for the alkaline phosphatase activity which was observed to increase significantly with increasing barnyard manure treatment. Time of sampling (maximum vegetative period and harvest time) generally did not affect the soil enzymatic properties, except for acid phosphatase, which was higher at harvest time, and closely associated with the more available P observed at harvest time (Table 3).

Activities of acid and alkaline phosphatases, arylsulfatase, and β-glucosidase at maximum vegetative period were all significantly well-correlated with soil pH, organic C, total N, and available P. At harvest time, only acid and alkaline phosphatase activities were significantly well-correlated with soil pH, organic C, total N, and available P. Only acid phosphatase activity was well correlated with corn yield (Table 6).

Table 6. Coefficient correlations between soil enzymatic activities and selected chemical properties and plant yield.

Properties	Acid Phosphatase		Alk. Phosphatase		Arylsulfatase		β-Glucosidase	
	t-1	t-2	t-1	t-2	t-1	t-2	t-1	t-2
Soil pH	0.71**	0.77**	0.87**	0.99**	0.57*	- 0.14	0.60*	0.18
Organic C	0.61*	0.79**	0.83**	0.88**	0.76**	- 0.02	0.72**	0.07
Total N	0.58*	0.87**	0.83**	0.97**	0.68**	0.03	0.72*	0.25
Avail P	0.59*	0.80**	0.90**	0.98**	0.69**	- 0.19	0.67**	0.28
Yield	-	0.87**	-	0.43	-	- 0.02	-	0.40

Note: *significant at 0.05 and **at 0.01

All plots showed similar values of β-glucosidase activities in the maximum vegetative phase (T1), although at harvest time (T2) different results were observed. β-glucosidase activity was higher in plots with barnyard manure than the other plots. The lowest activities of β-glucosidase was observed in the control plot.

Inorganic fertilizer and barnyard manures significantly affected soil microbial biomass carbon (Table 4). Microbial biomass C was greatest with the barnyard manure amendment and lowest in unfertilized soil. Soil microbial biomass and soil enzyme activities show a more quick response to the changes in soil management practices compared to total soil organic matter (Dick, 1992; Doran et al., 1996). The increased levels of microbial biomass in the barnyard manure treatment (Table 4) reflect high annual inputs of organic matter in the form of barnyard manure. Several studies have been focused on the shifts in microbial population and activity in soil as related to the change in C inputs. In some studies these were attributed to amount and diversity of organic inputs (Powlson et al., 1987). The microbial biomass and extracellular enzyme activity in assessment of soil quality were established by the essential role of soil microorganisms in nutrient cycling within agriculture ecosystems (Rice et al., 1986).

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