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Influence of manures and inorganic fertilizers on soil organic carbon and enzyme activities in rice under flooded conditions

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ABSTRACT

In a laboratory and field study, long-term application of organic manures and inorganic fertilizers in an tropical inceptisol under rice-rice cropping system at Andhra Pradesh Rice Research Institute, Maruteru, Andhra Pradesh resulted in different fractions of carbon and soil enzyme activities. Microbial biomass carbon (MBC), readily mineralizable Carbon (RMC), acid hydrolysable Carbon (AHC), water soluble carbon (WSC), permanganate oxidizable carbon (POC) were found to be higher in FYM and inorganic NPK treatments. In the field study, acid phosphatase, alkaline phosphatase, dehydrogenase and β-D-glucosidase activity of inorganic NPK + FYM recorded significantly higher activity of followed by FYM, inorganic NPK, inorganic N and control. All the enzymes recorded significantly increased activity upto panicle stage of crop growth and thereafter decreased. A two fold higher activity of acid phosphatase, four-fold of alkaline phosphates and dehydrogenase and seven fold of β-D-glucosidase was recorded at panicle initiation stage of crop growth. The enzyme activities were significantly correlated with MBC and TOC contents of soils and interrelationship between the different enzymes activities also existed in the cropped soil under flooded conditions.

Keywords: *soil organic carbon, organic manures, inorganic fertilizers, soil enzyme*

Intensification of rice cultivation to meet the demand for food by the increasing human population is imperative, especially in India where approximately 80% of rice is grown and consumed. Information on organic carbons stocks in agricultural soils is important because of the effects of Soil Organic Carbon (SOC) on climate change and on crop production. Flooded rice soil ecosystems are predominantly anaerobic and are different from upland soils in several physic-chemical and biological properties (Adhya and Rao, 2005). Decomposition of carbon substrates under anaerobic conditions of flooded soils is generally slower than in upland soil (Tate, 1979). Accordingly, growing rice especially in double cropped areas results in relatively stable soil organic carbon (SOC) levels (Cassman et al., 1995). Considerable amounts of biomass are likely to be produced phototrophically in the floodwater (Roger, 1996) and even chemoheterotrophically in the presence of inorganic electron donors and CO2 along the redox gradients in waterlogged rice soils (Revsbech *et al*., 1999). Thus, flooded soils represent a changed dynamics of microbial biomass and activity as compared to upland soils. Moreover, organic residues including green manure, animal waste and farmyard manure (FYM) are traditionally applied to rice soils in order to maintain the soil organic matter (SOM) status, to increase the levels of plant nutrients and to improve the physical, chemical and biological soil properties that directly or indirectly affect soil fertility.

Pool sizes of microbial biomass in rice soils account for only 2–4% of total C that represent an important and most labile fraction of SOM which is turned over very rapidly (Reichardt *et al.*, 1997). Microbial biomass has been assigned important roles in paddy soils as a nutrient pool, driving force of nutrient turnover and early indicator of soil/crop management (Shibahara and Inubushi, 1997). The more dynamic characteristics such as microbial biomass, soil enzyme activity and soil respiration respond very quickly to changes in crop management practices or environmental conditions than do characteristics such as total SOM (Dick, 1992; Doran *et al*., 1996). Besides

the size of microbial biomass, its functional and structural diversity has ecological relevance as well.

Soil enzyme activities can be used as an index of microbial functional diversity (Nannipieri *et al.*, 2002).Long-term effects of organic and inorganic fertilization practices on soil microbial community structure, microbial biomass (C_{mic}) dynamics and microbial activities in temperate or sub-tropical upland soils have been studied (Marschner *et al.*, 2003). However, under tropical conditions where the turnover rate of SOM is comparatively rapid (Chander *et al.*, 1977), only few studies have been conducted on SOM dynamics and soil microbial activities in relation to inorganic fertilizer or organic amendments(Goyal et al., 1999), more so under flooded condition of tropical rice soils (Shibahara and Inubushi, 1997). Our objectives in the present experiment were to evaluate the effects of long-term organic and inorganic fertilizer management practices under intensive rice cultivation on soil microbial biomass size in laboratory incubation studies and enzyme activities under field studies; to test whether a correlation exists among microbial biomass size and enzyme activities; and to determine the relationship between soil chemical properties and activity of soil enzymes in flooded rice soil.

MATERIALS AND METHODS

The study was conducted at the experimental farm of the Andhra Pradesh Rice Research and Regional Agricultural Research Station, Maruteru, A.P., India, mean annual temperature is 27.2°C. Annual precipitation is about 1200 mmyr^{-1}of which 75–80% is received during June to October. The soil of the farm area has been developed from the deltaic sediments of Godavari River. The soil is an Inceptisol with clay loam texture and the physic-chemical properties of the experimental soils are presented in Table 1.

The field experiment on intensive rice cropping was established in 1989 to assess the long-term impact of both organic and inorganic fertilizers on different soil physicochemical properties and crop yield. Wet season (June-October) rice was grown under rainfed conditions. Farm Yard Manure (FYM) at 5Mg ha^{-1} was applied before every wet and dry (November-February) seasons. The field was ploughed thoroughly and flooded 2–3 days before transplanting for puddling and levelling. Rice plants (25- days old seedlings of cv. MTU-1061 was transplanted at a spacing of 20 cm x10 cm with two seedlings per hill in the field plots. The experiment was laid out in a randomized block design with three replicates each. There were five treatments, viz. (i) Control, (ii) inorganic N fertilizer @ 90 kg ha^{-1} (iii) Inorganic fertilizer [N+P+K (90:60:60 kg ha^{-1})], (iv) FYM @ 10 Mg ha^{-1} and (iv) FYM (5Mg ha^{-1}yr^{-1})+ Inorganic fertilizer [N+P+K (90:60:90 kg ha^{-1})]. Water was maintained at 2 cm depth during vegetative and 5 cm depth during reproductive stage of the crop until ripening and was drained 10 days before harvest. The crop was given recommended agronomic practices and harvested at maturity.

Soil samples were collected before the start of the wet season crop during 2009 at a depth of 0–15 cm from the soil surface from five different places within individual replicated plots and mixed together to prepare a composite sample for the plot. These were collected and analyzed for microbial biomass and soil enzyme activity. Immediately after sampling, excess water was allowed to drain off, visible root fragments and stones removed manually and transferred to the laboratory for analyses. Moisture content of individual samples was

Table 1. Physico-chemical properties of the soils of long term trial at APRRI & RARS, Maruteru

Treatments	pH	EC (dS m^{-1})	TOC (mg kg^{-1})	Avail - N (kg ha^{-1})	Avail - P_2O_5 (kg ha^{-1})	Avail - K_2O (kg ha^{-1})
Control	6.14	0.47	7.4	273	20.35	265
Inorganic N fertilizer @ 90 kg ha^{-1}	6.21	0.65	8.1	297	21.72	305
Inorganic NPK 90:60:60 kg ha^{-1}	6.33	0.86	10.4	314	67.03	318
FYM @ 10 Mg ha^{-1}	6.25	0.78	13.7	339	88.29	374
Inorganic NPK 90:60:60 kg ha^{-1} +FYM @ 5 Mg ha^{-1}	6.40	0.82	12.5	355	108.20	412

determined Gravimetrically in 10 g portions after drying at 105°C for 48 h.

Laboratory soil incubation studies were carried out from the above five treatments to estimate the different fractions of soil organic carbon. Soil microbial biomass-C was measured by modified chloroform fumigation–extraction method with fumigation at atmospheric pressure (Witt *et al.,* 2000). Soil samples, 35 g on an oven-dry basis (48 h at 105°C), were weighed into 500 ml glass Schott bottles and fumigated by adding 2ml of ethanol-free chloroform directly onto the soil. Microbial biomass C was estimated by extracting the fumigated soil with 0.5 M K_2SO_4 and extractable C determined by modified dichromate digestion of soil extract (Vance *et al.,* 1987). Water soluble carbon was measured by the procedure of Redl *et.al.* (1990). Acid hydrolysable carbohydrates were estimated by Cheshire and Mundle (1966).Permanganate Extractable Carbon was determined by procedure of Blair *et al,* (1995).

The enzymatic analysis was estimated from the rhizopshere soils of the field experiment at critical stages of crop growth Dehydrogenase activity was determined by reduction of tri-phenyl tetrazolium chloride (TTC) (Chendrayan *et al.,* 1980). â-D-Glucosidase (EC 3.2.1.21) activity was assayed following the method of Eivazi and Tabatabai (1988). Acid phosphatase (EC 3.1.3.2) and alkaline phosphatase (EC 3.1.3.1) activity was measured following the method of Eivaji and Tabatabai (1977).

All data was recalculated on the basis of oven-dry soil weight and was analysed using two way ANOVA considering main treatments and assay time at specific periods of crop growth and individual character datasets were statistically analysed and mean comparison between treatments was established by Duncan's multiple range test as furnished in Gomez and Gomez (1984).

RESULTS AND DISCUSSION

Microbial biomass-C (C_{mic}) (Table 2) content was highest in plots receiving both FYM and inorganic fertilizer NPK and lowest in unamended control plots. C_{mic} content followed the order FYM + inorganic fertilizer NPK > inorganic fertilizer > inorganic N >control. The C_{mic} content increased by 33%, 29%, 31% and 38% in inorganic nitrogen and inorganic fertilizer (N+P+K), FYM and FYM + inorganic fertilizer (N+P+K) treatments, respectively, over that of unamended control. Similarly, readily mineralizable Carbon (RMC), acid hydrolysable Carbon (AHC), water soluble carbon (WSC), permanganate oxidizable carbon (POC) were estimated in the same soils and found that highest values were found in FYM + inorganic fertilizer NPK > inorganic fertilizer > inorganic N >control.

Microbial biomass-C (C_{mic}), the most labile fraction of SOM in rice soils, accounted for only 2–3.5% of total organic C (Corg). In the present study, the C_{mic} content was lowest in the control plots possibly because of high stress due to inadequate nutrient supply, lack of fertilizer application and lower amounts of rhizodeposition (root exudates and root biomass). An increase in Cmic content in tropical soil following application of FYM has been reported earlier (Dhull *et al.,* 2004). Addition of inorganic fertilizer with FYM enhanced the microbial biomass and could be due to

Table 2. Different pools of carbon in an alluvial soil of rice-rice cropping system in a long term trial.

Treatments	Organic carbon (g kg^{-1})	MBC (µg g^{-1} dry soil)	RMC (µg g^{-1} dry soil)	WSC (mg kg^{-1} dry soil)	AHC (mg kg^{-1} dry soil)	POSC (mg kg^{-1} of dry soil)
Control	7.4	108.40	46.24	21.81	235.15	721.48
Inorganic N fertilizer @ 90 kg ha^{-1}	8.1	144.63	72.40	58.24	276.60	807.30
Inorganic NPK 90:60:60 kg ha^{-1}	10.4	176.20	86.40	96.50	301.82	830.82
FYM @ 10 Mg ha^{-1}	13.7	209.28	101.20	123.42	420.14	847.15
Inorganic NPK 90:60:60 kg ha^{-1} + FYM @ 5 Mg ha^{-1}	12.5	250.78	119.28	141.68	440.385	888.66

MBC - , RMC - readily mineralizable caron, WSC - water soluble carbon, AHC - acid hydrolysable carbon, POSC - permanganate oxidizable soluble carbon

adequate availability of both C and N to the soil microbial pool. Integrated use of chemical fertilizers and organic matter results in the production of more Cmic in soil compared to their single application (Leita *et al.,* 1999). Similarly, the RMC and POC were significantly higher in the treatment received combined application of chemical fertilizer and FYM (Changming Yang *et.al.* 2005).

In the field study, the activities of acid phosphatase, alkaline phosphatase, dehydrogenase and β-D-glucosidase activity were significantly influenced by the application of organic manures and chemical fertilizers and stages of crop growth. (Table 3 to 6). All the enzymes showed significantly maximum activity at panicle initiation stage of crop growth and decreased at harvest. Through the cropping period, the treatment inorganic NPK + FYM recorded significantly higher activity of all the enzymes followed by FYM, inorganic NPK, inorganic N and control. Highest activity of two fold increase of acid phosphatase, four-fold of alkaline phosphates and dehydrogenase and seven fold of β-D-glucosidase was recorded at panicle initiation stage of crop growth.

Soil dehydrogenase activity has been used as a parameter to study biological activity of soil (Nannipieri *et al*., 2002) and is an important indicator of microbial activity in flooded soils (Chendrayan *et al.,* 1980). In the present study, lowest dehydrogenase activity measured after harvest can be attributed to oxidation status of the soil as water was drained at maturity. Higher dehydrogenase activity in FYM applied plots might be due to higher organic matter content and relatively higher C_{mic} (Wlodarczyk *et al.*, 2002).

Our study revealed that crop growth stages also affected soil dehydrogenase activity. b-Glucosidase is widely abundant, and is synthesized by soil microorganisms in response to the presence of suitable substrate (Turner *et al.,* 2002). In the present study, β-D-glucosidase activity was influenced by the crop growth stages. Among the different treatments, combined application of FYM and inorganic fertilizer exhibited highest amount of b-glucosidase activity. Acid phosphatase and alkaline phosphatase activities were also found to be relatively higher in FYM and inorganic NPK fertilizer treatment. Pulford and Tabatabai (1988) reported that addition of organic amendments resulted in an increase in acid alkaline phosphatase activity in submerged soils.

Soil enzyme activities were also strongly influenced by the long-term application of FYM and inorganic fertilizer, as evidenced by highly significant F-values (P 0.001) for the treatment effects on enzyme activities (Table 7). β-D-glucosidase exhibited higher variability in activity among replicates with coefficient of variability higher than those remaining enzymes**.** β-D-glucosidase activity ranged from 5.20 µg p-

Table 3. Acid Phosphatase activity in submerged rice soil as influenced by organic and inorganic treatments and stages of crop growth during wet season 2009.

Treatment	Initial	Max. Tillering	PI stage	Harvest stage	Mean
		(µ g of 4-nitrophenol released /g soil /hr)			
Control	22.03	43.28	62.11	32.82	40.06
Inorganic N fertilizer @ 90 kg ha^{-1}	34.61	61.60	76.36	40.05	53.15
Inorganic NPK 90:60:60 kg ha^{-1}	58.62	58.62	129.53	52.42	74.80
FYM @ 10 Mg ha^{-1}	62.72	94.85	102.43	46.53	76.63
Inorganic NPK 90:60:60 kg ha^{-1} + FYM @ 5 Mg ha^{-1}	73.78	121.47	156.37	72.37	106.00
Mean	50.35	75.96	105.36	48.84	
		S.Em+	C.D.		
Incubations		0.50	1.43		
Treatments		0.56	1.60		
Incubations x Treatments		1.12	3.21		

Table 4. Alkaline Phosphatase activity in submerged rice soil as influenced by organic and inorganic treatments and stages of crop growth during wet season 2009.

Treatment	Initial	Max. Tillering	PI stage	Harvest stage	Mean
		(µ g of 4-nitrophenol released /g soil /hr)			
Control	7.32	19.42	45.18	16.84	22.19
Inorganic N fertilizer @ 90 kg ha^{-1}	15.24	21.26	56.25	22.82	28.89
Inorganic NPK 90:60:60 kg ha^{-1}	29.36	43.15	82.14	49.77	51.17
FYM @ 10 Mg ha^{-1}	27.31	48.51	94.64	40.68	52.79
Inorganic NPK 90:60:60 kg ha^{-1} + FYM @ 5 Mg ha^{-1}	33.03	59.84	128.37	66.17	71.85
Mean 22.45	38.44	81.37	39.26		
	S.Em+	C.D.			
Incubations		0.45	1.28		
Treatments		0.50	1.43		
Incubations x Treatments		1.00	2.86		

Table 5. Dehydrogenase activity in submerged rice soil as influenced by organic and inorganic treatments and stages of crop growth during wet season 2009

Treatment	Initial	Max. Tillering Stage	PI stage	Harvest stage	Mean
		(m g of TPF produced /g soil /day)			
Control	14.06	24.67	42.55	21.72	25.75
Inorganic N fertilizer @ 90 kg ha^{-1}	21.65	32.02	66.57	32.21	38.11
Inorganic NPK 90:60:60 kg ha^{-1}	28.26	52.56	105.56	47.24	58.40
FYM @ 10 Mg ha^{-1}	28.47	47.68	66.25	43.81	46.55
Inorganic NPK 90:60:60 kg ha^{-1} + FYM @ 5 Mg ha^{-1}	34.79	68.23	136.14	51.72	72.72
Mean	25.44	45.03	83.41	39.34	
	S.Em+	C.D.			
Incubations		0.53	1.49		
Treatments		0.58	1.66		
Incubations x Treatments		1.16	3.33		

Table 6. β-D-Glucosidase activity in submerged rice soil as influenced by organic and inorganic treatments and stages of crop growth during wet season 2009

Treatment	Initial	Max. Tillering Stage	PI stage	Harvest stage	Mean
		µ g of 4-nitrophenol released /g soil /hr)			
Control	2.52	6.13	8.94	3.24	5.21
Inorganic N fertilizer @ 90 kg ha^{-1}	3.63	8.17	14.41	6.75	8.24
Inorganic NPK 90:60:60 kg ha^{-1}	5.47	10.22	32.54	8.45	14.17
FYM @ 10 Mg ha^{-1}	4.60	9.44	18.90	7.64	10.15
Inorganic NPK 90:60:60 kg ha^{-1} + FYM @ 5 Mg ha^{-1}	6.96	11.22	52.40	10.24	20.20
Mean	4.64	9.04	25.44	7.26	
	S.Em+	C.D.			
Incubations		0.22	0.64		
Treatments		0.25	0.71		
Incubations x Treatments		0.49	1.41		

nitrophenol g^{-1} soil h^{-1} in the control plots to 20.20 µg p-nitrophenol g^{-1} soil h^{-1} in the plots receiving FYM + inorganic fertilizer NPK (Table 6).

In the present study, the enzyme activities were significantly correlated with pH, TOC and MBC contents of soils (Table 8) and interrelationship between the different enzymes activities also existed in the cropped soil under flooded conditions (Table 9).

Enzyme activities of soils are usually correlated with their Corg contents (Taylor *et al.,* 2002). Higher levels of Corg stimulate microbial activity, and therefore enzyme synthesis. In the present study the Cmic was significantly and highly correlated with Corg (0.985**). In addition, the higher organic matter levels in the FYM treatments may provide a more favorable environment

Table 7. Coefficients of variability for soil enzyme activities and F values for effects of FYM amendment on soil enzyme activities.

Soil enzyme	Coefficients of variability (%)	F values*
Dehydrogenase	4.2	140.88
β-D-glucosidase	7.4	248.56
Acid phosphatase	2.8	163.06
Alkaline phosphatase	3.8	103.58

for the accumulation of enzymes in the soil matrix, since soil organic constituents are thought to be important in forming stable complexes with free enzymes (Marx *et al.,* 2005). The importance of Corg in nutrient cycling was evident from the fact that Cmic as well as the enzyme activity quantified in the present study showed positive relation with Corg showed statistically

Table 8. Correlation matrix between soil enzyme activities and selected soil properties following long term application of FYM and inorganic fertilizer and planted to rice under flooded condition

	pH	TOC	MBC
β-D-glucosidase	0.989**	0.831	0.882*
Acid phosphatase	0.863	0.830	0.839
Alkaline phosphatase	0.940*	0..967**	0.975**
Dehydrogenase	0.998**	0.853	0.902*
MBC	0.879*	0.985**	
TOC	0.829		

* Significant at P < 0.05, ** Significant at P < 0.01

Table 9. Correlation matrix between soil enzyme activities in an alluvial soil following long-term amendments of FYM and inorganic fertilizers and planted to rice (MTU-1061) under flooded condition (N=5)

	Dehydro-genase	β-D-glucosidase	Acid phosphatase
β-D-glucosidase	0.958*		
Acid phosphatase	0.964**	0.935*	
Alkaline phosphatase	0.954*	0.918*	0.993**

* Significant at P < 0.05, ** Significant at P < 0.01

significant positive correlation with dehydrogenase, b-glucosidase and alkaline phosphatases.

The study reveals that long term application of FYM and inorganic fertilizers NPK causes a significant build up of Cmic and soil enzyme activities. It also provides information on soil microbial biomass dynamics and biocatalytic activities as influenced by organic and inorganic fertilization and continued cropping on a long-term basis under flooded conditions. The results further demonstrated that microbial biomass and soil enzyme activity is sensitive in discriminating between FYM and inorganic fertilizer application on a long-term basis. Soil microbial biomass and enzymatic properties were also closely related with the C inputs and crop growth stages.

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