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Long term effects of organic amendments on carbon sequestration rate, soil microbial and enzymatic activities in organically managed rice-rice cropping system in inceptisols of Odisha

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Abstract
Soil organic carbon (SOC) plays an important role in sustainability of any agricultural production system as they govern most of the soil properties, and hence soil quality and health. Being a food source for soil microorganisms, they also influence microbial activity, diversity and enzymes activities. The present paper reports the results from a three years (2014-17) field experiment to elucidate the effect of soil amendments (*Dhanicha*, vermicompost and FYM) on SOC and its sequestration rate (SOCSR) and enzymes activity. Results revealed that combined application of organic amendments comprising *Dhanicha* @ 25 kg seed ha^{-1} + FYM @ 5 t ha^{-1} + Vermicompost 2 t ha^{-1} (split) in *Kharif* and FYM @ 5 t ha^{-1} + Vermicompost 2 t ha^{-1} (split) in summer significantly improved the SOC stock and sequestration rate. Soil microbial population and enzymatic activities were also better correlated with the SOC stock. Combined application of two or three organic amendments resulted in higher amount of SOC stock and SOCSR over single application, and also had better enzymatic activities and microbial population than single application. Soil enzymatic activity, which is directly related with the SOC stock, is a good indicator of changes induced by various management practices. Relative changes in the SOC stock and soil enzymes activity are more pronounced in the organic paddy soil.

Keywords: Soil organic carbon, carbon sequestration rate, microbial activity, soil enzymatic activity

Introduction
Productivity of organic rice-rice cropping system largely depends on soil organic carbon (SOC) content and microbial and enzymatic activity of the soil. Soil organic carbon (SOC) is composed of several fractions, among them some are recalcitrant and some are labile or even more labile, and hence readily lost from the soil. The relative proportion of these fractions in soil determines soil quality and its susceptibility to rapid mineralization and loss, hence it is a critical determinant of soil carbon dynamics. Mandal *et al.* [1] demonstrated that organic management of soil could have a significant impact on soil microbial diversity and soil microbiological properties such as microbial biomass carbon, microbial biomass nitrogen and respiration rate. Soil enzymes produced by microbes play key roles in the biochemical functions of organic matter decomposition and nutrient cycling which are affected by land-use management [2, 3]. Microbiological activity of soil directly influences the soil quality in general and soil fertility in particular. Soil microbiological activity or enzyme activity plays a key role in nutrient transformation because it has direct impact on soil organic matter mineralization. Among the different enzymes present in soil, dehydrogenase activity provides better correlative information regarding the biological activity and microbial population [4] hence, it is a good indicator of soil microbial diversity and activity. Transformation of organic phosphorus (P) through enzymatic reactions and immobilization of P in the biomass play an important role in P cycling and are likely to be affected by the phosphatase enzymes. Phosphatases are a broad group of enzymes that are capable of catalysing hydrolysis of esters and anhydrides of phosphoric acid, and play critical roles in P cycles [5] as evidences shows that they are correlated to P stress and plant growth. The β-glucosidase enzyme is involved in catalysing the hydrolysis and biodegradation of various glycosides present in plant debris decomposing in the ecosystem [6], which are very sensitive to changes in pH and soil management practices [7, 8, 9]. Microbial diversity and activity are largely dependent upon nature of substrate, management practices and environmental conditions.

Soil enzyme activity is considered not only as an important indicator of soil health and quality, but also reflects the real picture of soil microbial activity [10]. Soil enzymatic activities are significantly affected by the natural and anthropogenic factors, hence can be considered the good indicator of changes induced by different management practices [11]. However, the synergistic relationship between SOC and enzymes activity in the rhizosphere soil is still not clear. The present work was therefore undertaken to examine the relationship of SOC and enzymatic activities in organically managed rice-rice cropping system in Inceptisols of Odisha.

Materials and Methods

A field experiment was initiated during 2014-15 to 2016-17 with rice (*Oryza sativa* L.) cv. Lalat as the test crop in the Organic Block of Central Research Station of Odisha University of Agriculture and Technology, Bhubaneswar located at 20^0 15' N latitude and 85^0 52' E longitude and at an altitude of 25.9 m above mean sea level. The station comes under the East and South Eastern Coastal Plain Agro-climatic Zone of Odisha. The region is characterized by a sub-tropical climate with a hot and humid summer (March-June), hot and wet monsoon (late June-mid October) and a mild and dry winter (Nov.-Feb.). The soil of the experimental site was sandy loam in texture with pH 6.0. The bulk densities were 1.58 , 1.61, 1.64 , 1.66 t m^{-3} and soil organic carbon were 5.2, 4.0 , 2.8 and 1.8 g kg^{-1} for 0-15 , 15-30 , 30-45 and 45-60 cm soil depth, respectively at the start of the experiment. *Kharif* rice followed by summer rice was cultivated for nine consecutive years in a fixed site and layout with seven treatment combinations; each replicated three times. The treatments were T_1- *Dhanicha* @ 25 kg seed ha^{-1} in *Kharif* and control in summer.; T_2- *Dhanicha* @ 25 kg seed ha^{-1} + FYM 5t ha^{-1} (basal) in *Kharif* and FYM 5t ha^{-1} (basal) in summer. ; T_3- *Dhanicha* @ 25 kg seed ha^{-1} + Vermicompost 2t ha^{-1} (basal) in *Kharif* and Vermicompost 2 t ha^{-1} (basal) in summer.; T_4 - *Dhanicha* @ 25 kg seed ha^{-1} + Vermicompost 2t ha^{-1} (split) in *Kharif* and Vermicompost 2t ha^{-1} (split) in summer.; T_5- *Dhanicha* @ 25 kg seed ha^{-1} + FYM 5t ha^{-1} + Vermicompost 2t ha^{-1} (split) in *Kharif* and FYM 5t ha^{-1} + Vermicompost 2t ha^{-1} (split) in summer. T_6 - *Dhanicha* @ 25 kg seed ha^{-1} + FYM 5t ha^{-1} + Vermicompost 2t ha^{-1} (basal) in *Kharif* and FYM 5t ha^{-1} + Vermicompost 2t ha^{-1} (basal) in summer.; T_7 - *Dhanicha* @ 25 kg seed ha^{-1} + FYM 5t ha^{-1} + *Panchagavya* in *Kharif* and FYM 5t ha^{-1} + *Panchagavya* in summer.

Rice cv. Lalat (Obs 677/ IR 2071/ Vikram / WI 263) of 120 days duration (125 days in summer) with maximum yield potential of 6.8 t ha^{-1} was cultivated in both the seasons in all nine years. Square planting (25 × 25 cm) of 12 days old seedlings @ one seedling per hill were done in individual beds measuring 12 m in length and 6 m in width. Channels of 30 cm width were opened up all around the beds and water level was maintained only in the channels throughout the cropping seasons. Organic nutrient management options were adopted as per the treatments along with biodynamic formulation *'Panchagavya'*. The plots were kept moist all along. Vermicompost was applied in split as basal and at 20 DAT. Cono weeder was used thrice at 15 days interval starting from 10 DAT in order to manage weed menace. No major incidence of disease and insect pest was noticed. However, as a prophylactic measure, pot manure (5 kg cow dung + 5 litre urine + 250 g gur + 1.0 kg each of *Azadirachta indica*, *Pongamia pinnata* and *Calotropis gigantia* leaves, fermented for 15 days) was sprayed four times at 15 days interval starting from 15 DAT in both the seasons. Soil samples were collected from each plot with a post-hole auger and SOC stock was calculated from these depths using the following formula:

$$\text{SOC stock} = \sum_{1}^{n} \left(\text{Profile volume} \times \text{Bulk density} \times \text{SOC content} \right)$$

From total SOC stock, the SOC sequestration rate was calculated using the following formula [12].

$$\text{SOC sequestration rate} = \frac{\text{Increase in SOC stock due to treatments over the initial stock}}{\text{Number of years of experimentation}}$$

For determination of microbiological parameters *viz.*, microbial population and enzymatic activity, surface soil samples were collected after each crop cycle and were kept in the deep freeze (at -2 °C). The SOC content in soil was determined by wet oxidation method of Walkley and Black [13]. Soil enzymatic activity (urease, fluorecein diacetate, dehydrogenase, acid phosphatase and β-glucosidase activity) was determined following procedure laid down by Page *et al*. [14]. The data so obtained for each observation were analyzed using analysis of variance technique for randomized block design as described by Gomez and Gomez [15]. Correlation study was carried out to find out the relationships of microbial and enzymatic activities with SOC.

Results and Discussion

The effect of organic soil amendments (Dhanicha, FYM and vermicompost) on SOC and soil enzymes were significant. It was observed that combined application of *Dhanicha* @ 25 kg seed ha^{-1} + FYM @ 5 t ha^{-1} + Vermicompost 2 t ha^{-1} (basal) in *Kharif* and FYM + Vermicompost 2 t ha^{-1} (basal) in summer for consecutive three years significantly improved the TOC from 50.30 to 56.54 t ha^{-1} (T_6) *i.e.* 12.4 % higher over single application of *Dhanicha* @ 25 kg seed ha^{-1} in *Kharif* and control in summer (T_1). However, sequestration rate exhibited 37.2 % higher values over the same period (Table 1). It is due to the fact that, continuous and combined application of organic amendments for the entire experiment period improved the TOC content in soil and there by the sequestration rate. Beside this, it may be attributed to enhanced crop growth which in turn, resulted in increased below- ground organic residues (*e.g.*, root biomass, rhizo-deposition, root exudates etc.), and thus raised the soil organic matter (SOM) status. Improvement of SOM also adds to the carbon fractions that can be easily oxidized by strong oxidizing agent in presence of acids. This might be the possible reason for improvement of SOC and sequestration rate in the soil under this study. Moharana *et al*. [16] observed that the SOC was considerably greater in soils receiving more of organic amendments than from single source. In this study, the combination of two to three organic amendments enhanced the accumulation of SOC, which is consistent with other studies [17, 18]. Dheri G. S. and Nazir G. [19] and Sharma *et al*. [3] also showed that application of nutrients and manure maintained or increased SOC content in long-term fertility experiments in India. The higher values of SOC in T_6 may be attributed to balanced and combined application of two or three organic sources of nutrients. There was significant (Table 1) and progressive increase in microbial population (Bacteria, Fungi and Actinomycetes) was noticed after each crop cycleover the base year (2014) in the treatment receiving *Dhanicha* @ 25 kg seed ha^{-1} + FYM + Vermicompost 2t ha^{-1} (split) in *Kharif* and FYM + Vermicompost 2t ha^{-1} (split) in summer (T_5) as more and more organic amendments were added continuously than through single source (*Dhanicha* @ 25 kg seed ha^{-1}) *i.e.* T_1 . Highest microbial population was observed in T_5 followed by T_6, T_4 and T_7 and the lowest in case of control (T_1). This might be due to addition of more and more organic substrate through two or three sources than single one which might have improved the soil condition for microbial proliferation and enzymatic activity and also triggered up the microbial population [20]. The higher microbial population and enzymatic activity in T_5 might be due to higher underground plant biomass [21]. Soil enzymes (dehydrogenase, β-glucosidase, urease, FDA and phosphatases) activities followed the similar trend with respect to addition of two or three organic soil amendments (T_5) than sole one (T_1). Maximum activity was observed in T_5 which was at par with T_6. There was non-significant difference between T_3 and T_2 and lowest activity was observed in control (T_1) (Table 2) indicating that enough and favourable substrate present in this soil which triggered the microbial growth. Higher SOC and organic substrate represents high microbial activity which results in high enzymatic activity [10]. It is well known that dehydrogenase activity is the function of microbial population and their activity [22]. Saha *et al.* [23] observed that organic manure application increased soil dehydrogenase activity significantly. Application of organic nutrient through two or three sources improved the organic matter status of soils, which enhanced dehydrogenase activity. Kunito *et al.* [24] attributed the positive correlation between enzyme activities and MBC to an indirect effect of the increased SOC. Acid phosphatase activity was significantly affected by the soil amendments and the highest values were observed in T_5 followed by T_6, T_4, T_3, T_7, T_2 and T_1. The results revealed that the highest value of this enzyme was associated with the optimum resources management. The treatment T_5 is the combination of *Dhanicha* @ 25 kg seed ha^{-1} + FYM + Vermicompost 2 t ha^{-1} (split) in *Kharif* and FYM + Vermicompost 2 t ha^{-1} (split) DAT in summer which maximized the crop growth and nutrient requirement. Majumdar *et al.* [18] also reported that combined application organic amendments increase the growth in maize. Besides this, the microbial activity was also highest in this combination leading possibly to P stress in the soil, thereby increasing the phosphates released by the microorganism to counteract the deficiency and make P available for the crops. Increase in the microbial activity leads to higher soil enzymatic activity and therefore the management practices that induce P stress may also affect the secretion of these enzymes in the ecosystem and presence of easily decomposable organic substances and high microbial activity in rhizosphere soil [25]. Allmaras *et al.* [26] describe that important source of organic substrate is the below ground rhizodeposits in the rhizosphere. Root exudates and rhizodeposition are the substrate for higher microbial population and enzymatic activity which lead to high microbial growth. All the soil enzymes activity under study was significantly higher in T_5 as compared to T_1 both year and treatment wise (Table 2). It might be due to the fact that rhizodeposition which is easily decomposable organic substances are available as food source enhanced the microbial activity [27]. Increase in microbial population and organic carbon leads to high microbial activity and soil enzymes activity in soil. It was also reported by many that in rhizosphere root exudation and rhizodeposition increased the biological activities which directly and indirectly enhanced the enzyme activity in soil [28, 29]. Correlation analysis indicated that SOC, microbial and enzymatic activities were highly correlated ($P=0.05$) with each other (Table 3 and Fig. 1- 8) indicating existence of favourable soil environment and suitable substrate availability for microbial growth and their proliferation [23].

Table 1: Soil organic carbon, soil organic carbon sequestration rate and microbial population as influenced by long term organic nutrient management in rice-rice cropping system

Treatment	Total SOC (t ha^{-1})	SOCSR (t ha^{-1} year^{-1})	Bacteria (×10^7 CFU g^{-1}soil)		Fungi (×10^4 CFU g^{-1}soil)		Actinomycetes (×10^6 CFU g^{-1}soil)		Total Microbial population (×10^6 CFU g^{-1}soil)	
			2014	2017	2014	2017	2014	2017	2014	2017
T_1	50.30	1.88	10.26	18.45	8.45	11.28	9.14	18.45	111.82	203.06
T_2	51.24	1.99	12.61	21.42	9.62	12.25	9.63	20.42	135.83	234.74
T_3	51.94	2.07	14.32	21.46	10.29	12.36	10.44	19.63	153.74	234.35
T_4	52.23	2.10	15.25	23.65	10.84	13.28	12.29	22.54	164.90	259.17
T_5	56.14	2.53	17.86	29.82	12.67	14.78	15.15	28.56	193.88	326.91
T_6	56.54	2.58	17.54	26.24	11.56	13.46	13.17	26.94	188.69	289.47
T_7	52.38	2.11	12.28	22.26	9.28	11.45	10.52	21.26	133.41	243.97
SEm (±)	0.971	0.058	0.17	1.214	0.39	0.47	0.65	0.6	3.33	12.48
CD (0.05)	2.98	0.18	0.52	3.74	1.23	1.46	1.99	1.86	10.26	38.45

Initial Total SOC (2008) -33.35 t ha^{-1}

Table 2: Enzymatic activity as influenced by long term organic nutrient management in rice-rice cropping system

Treatment	Dehydrogenase Activity (DA) µg TPF g^{-1} soil hr^{-1}		Urease Activity (UA) µg NH_4N g^{-1} soil 2 hr $^{-1}$		FDA (µg Fluorescein g^{-1} h^{-1})		β-glucosidase (µg PNGg^{-1} d^{-1})		Acid phosphatase Activity (APA) µg PNPg^{-1} soil hr $^{-1}$		Alkaline phosphatase Activity (APA) µg PNP g^{-1} hr $^{-1}$	
	2014 (Initial)	2017	2014 (Initial)	2017	2014 (Initial)	2017	2014 (Initial)	2017	2014 (Initial)	2017	2014 (Initial)	2017
T_1	0.21	0.3	66.25	77.12	4.26	6.78	32.46	47.62	0.143	0.162	0.015	0.02
T_2	0.24	0.32	67.84	79.34	5.12	6.71	37.28	50.63	0.142	0.173	0.014	0.022
T_3	0.23	0.32	68.73	80.26	5.46	6.56	42.82	56.56	0.156	0.178	0.016	0.024
T_4	0.24	0.33	69.45	81.86	5.41	6.98	44.68	57.24	0.164	0.179	0.017	0.024
T_5	0.26	0.36	71.62	84.79	6.75	8.46	46.16	69.72	0.172	0.184	0.018	0.025
T_6	0.26	0.35	70.23	83.16	6.62	8.12	45.11	66.29	0.171	0.182	0.017	0.024
T_7	0.23	0.32	67.81	79.54	4.96	6.41	44.42	53.5	0.167	0.176	0.014	0.021
SEm (±)	0.003	0.006	0.604	0.675	0.058	0.058	0.416	1.263	0.001	0.001	0.000	0.001
CD (0.05)	0.01	0.02	1.86	2.08	0.18	0.18	1.28	3.89	0.003	0.004	0.001	0.002

Table 3: Correlation coefficients of soil organic carbon (SOC) with the soil enzymes and microbial population

Parameters	DA	UA	FDA	β-glucosidase	APA	Alk.PA	Bacteria	Fungi	Actinomycetes
SOC	0.95**	0.91**	0.90**	0.96**	0.81**	0.71**	0.92**	0.92**	0.97**

** indicate significance at *P = 0.05*

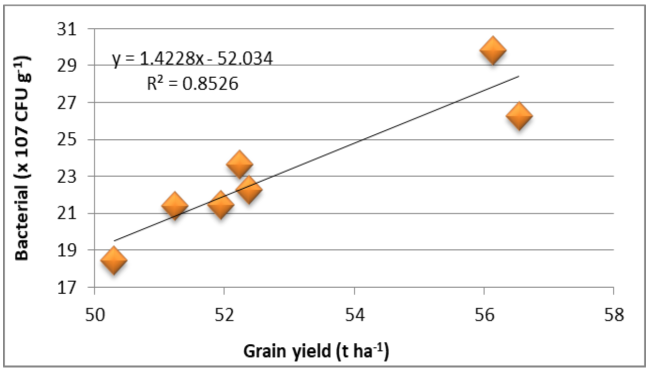

Fig 1: Correlation of soil organic carbon (SOC) with total heterotrophic bacteria

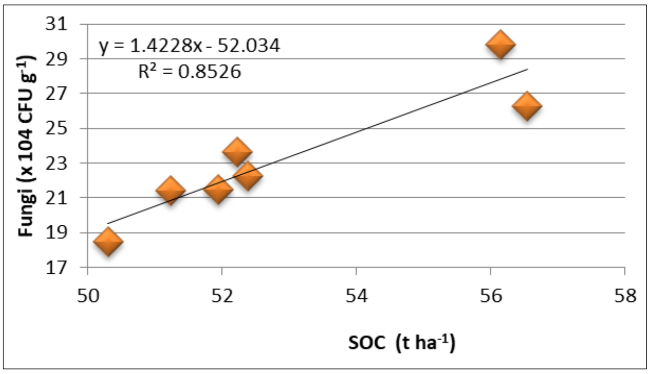

Fig 2: Correlation of soil organic carbon (SOC) with soil fungi

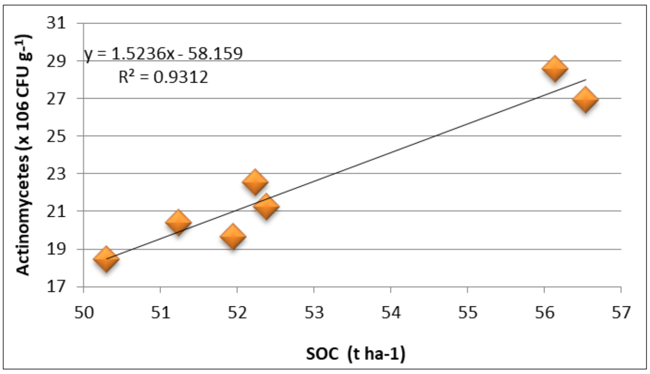

Fig 3: Correlation of soil organic carbon (SOC) with soil actinomycetes

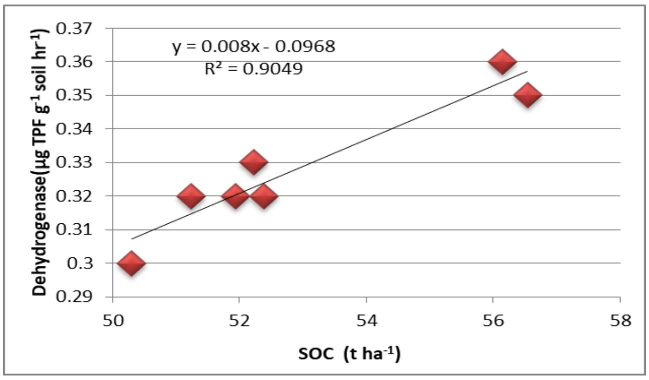

Fig 4: Correlation of soil organic carbon (SOC) with dehydrogenase activity

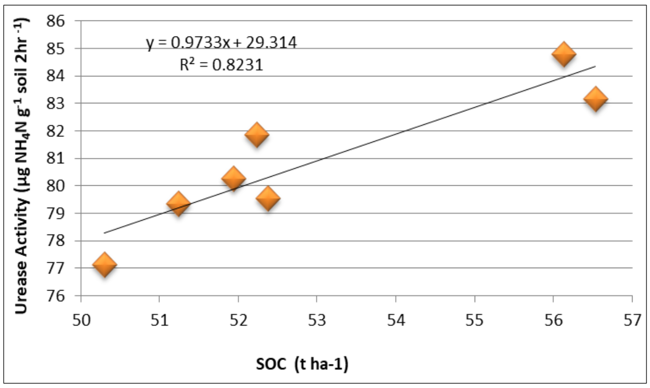

Fig 6: Correlation of soil organic carbon (SOC) with fluorscein diacetate activity

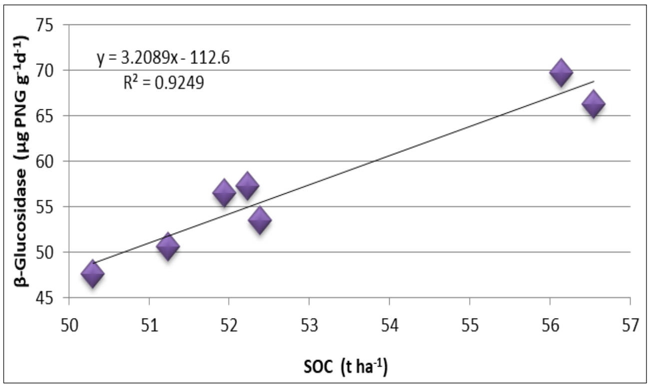

Fig 8: Correlation of soil organic carbon (SOC) with acid phosphatase activity

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