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Carbon Sequestration and Associated Soil Enzymatic Activities as Influenced by Long-Term Fertilizers and Manure Application under Rice-Wheat Cropping System

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Abstract: An investigation was undertaken to study 30 years fertilization impact on total soil organic carbon (TOC), C sequestration, soil enzymatic activities and yield of rice in Aquic Hapludoll under rice-wheat sequence in sub-humid and sub-tropic of India. The experiment comprised 14 treatments having N, P, K levels with or without FYM and Zn. At inception of experiment (1984) TOC was 12.18 g kg^{-1} and highest (15.64, 13.26, 11.56 g kg^{-1}) was observed with super optimal NPK (180:80:40)+Zn+FYM which was at par to optimal/100 % NPK (120:40:40)+Zn+FYM in 0-15, 15-30 and 30-60 cm soil depth. In 0-15 cm soil layer the highest and lowest C sequestration was found in 100 % NPK+Zn+FYM and 100 % N, respectively. Application of super optimal and optimal NPK in conjunction with FYM and Zn recorded maximum fluorescein diacetate activity (FDA) and β-glucosidase activity in 0-60 cm soil profile. Highest grain yield of rice found due to application of 100 % NPK+Zn+FYM. Therefore, from this experiment, it is concluded that integration of FYM and Zn with super optimal and optimal NPK are most efficient nutrient management practices in improving soil health and production of rice-wheat cropping system by sequestering more C and improving C associated enzymatic activities in soil.

Keywords: Long-term fertilization, Carbon sequestration, Fluorescein diacetate activity, β-glucosidase activity, Rice-wheat cropping system

Agricultural soil has dual nature as it also serves as a potential sink for atmospheric C as SOC, which contributes to improve productivity and quality (Rudrappa et al 2006, Kundu et al 2007). Dynamics of organic C storage in agricultural soils affects global climatic change and crop productivity (Li et al 2007). The awareness of greenhouse gas emissions and concerns about the global warming have led to an increased interest in storing and sequestering C in soils (Banger et al 2009). For enhancing the C sequestration, there is critical need to develop best nutrient management practices. Previous studies showed that C sequestration potential is influenced by nutrient management, climate and soil conditions (Chabbi et al 2009), cropping systems (Jagadamma and Lal 2010) and fertilization (Bhattacharyya et al 2007). Soil and crop management practices like crop rotation, long-term fertilization and change in land-use pattern exert a considerable impact on soil physical (Bhatt et al 2017) and biological (Yaseen et al 2017) properties *vis-à-vis* soil carbon dynamics (Yaseen et al 2022) over time.

Long-term applications of inorganic fertilizer and manure is an essential component of soil management in arable crop production systems. These practices are used primarily to increase nutrient availability to plants and crop yields in agricultural production *vis-à-vis* maintains the soil organic matter (SOM), improve soil microbial and enzymatic activities that directly or indirectly affect soil fertility (Ferreras et al 2006, Nayak et al 2007). Therefore, it becomes pertinent to study the effect of various nutrient management practices on TOC and C sequestration to store excess amount of atmospheric CO_2. The present study was carried out with hypothesis that various nutrient management practices over a period of 30 years in Aquic Hapludoll under rice-wheat cropping system might be influenced distribution of TOC, C sequestration, flourescein diacetate activity (FDA), β-glucosidase activity and yield of rice. The results of investigation would lead to identify the most efficient nutrient management practices for C sequestration in sub-humid and sub-tropic of India.

MATERIAL AND METHODS

Experimental site: A long-term fertility experiment was established in 1984 at Norman E. Borlaug Crop Research Centre of G.B. Pant University of Agriculture and Technology, Pantnagar (29° N, 79°3' E and 243.84 msl) Uttarakhand, India. Two crops per year wheat (*Triticum aestivum* L.) and rice (*Oriza sativa* L.) were cultivated. The region climatically was characterized by sub-humid and sub-tropical with hot and dry summer and cold winter. Temperatures are highest in May-June and lowest in December-January. An average total rainfall ranges from 1300-1500 mm per annum of which about 85-90 % is received from June to September. At start of experiment (1984) the silty loam soil contained 8.0 soil pH, 0.12 dS m^{-1} EC, 1.33 Mg m^{-3} bulk density, 2.1 % soil organic matter (SOM), 0.1% total nitrogen (N), 20 kg ha^{-1} available phosphorus (P) and 222 kg ha^{-1} available potassium (K).

Experimental design: The experiment comprises of 14 fertilization treatments (Table 1) replicated 4 times in randomized block design (RBD). During *kharif* season rice (*cv*. Pant Dhan 4) was sown every year during first week of June and harvested manually during first week of November and *rabi* season wheat (*cv.* UP 2425) was sown in November and harvested during third week of April at a fixed site with the same layout plan.

Collection of soil sample: Representative soil samples from 0-15, 15-30 and 30-60 cm depth were collected after the harvest of 30th rice crop in 2014-15. The samples were divided into two parts before processing. One part was stored at 0-4°C for the assessment of enzymatic activities and other part was air dried under shade and sieved to analyze other properties of soil.

Soil analyses: Total soil organic carbon (TOC) was determined by wet oxidation followed by titration with ferrous ammonium sulphate (Walkley and Black, 1934) and bulk density by core sampler method listed by Blake (1965). The fluorescein diacetate activity (FDA) and *β*-glucosidase activity were determined by the method given by Green et al (2006) and Hayano (1973), respectively.

Data calculation: TOC stock, amount of C sequestration and C sequestration rate of profile for each of three depths was computed (Fan et al 2014).

$$\text{TOC stock (Mg C ha}^{-1}) = \text{TOC} \times D \times BD \times 10 \text{ ……..(1)}$$

Where, TOC is total soil organic carbon concentration (g C kg^{-1}), D is soil depth (m), BD is bulk density (Mg m^{-3}) and 10 is a factor to adjust unit i.e., Mg m^2 to Mg ha^{-1}

Amount of C sequestration was calculated by subtracting the values of C stock at initial year of experimentation (1984) or control from the value of C stock in investigation year (2014). Amount of C sequestration has been estimated comparing with the present level of TOC in control at 15-30 and 30-60 cm soil layers due to not available of initial data for sub-surface soil.

$$\Delta TOC = TOC_i - TOC_{presoil/control} \text{…………(2)}$$

Where, TOC_i is TOC stock in year i and $TOC_{pre\ soil/control}$ is TOC stock in 1984 or control plot

C sequestration rate was calculated according to the following equation.

$$Rseq = \frac{TOC_i - TOC\ pre\ soil}{T}$$

Where, TOC_i is Final SOC (Mg ha^{-1}), $TOC_{pre\ soil}$ is Initial SOC (Mg ha^{-1}), Rseq is C sequestration rate (Mg $ha^{-1} ha^{-1} Yr^{-1}$) and T is Time (years)

Data analysis: The data were processed using STPR software.

RESULTS AND DISCUSSION

Change in TOC, bulk density and TOC stock: TOC in 1984 was 12.18 g kg^{-1} while after 30 years of varying nutrient application ranged from 11.00 to 15.64, 9.34 to 13.26 and 7.82 to 11.56 g kg^{-1} at 0-15, 15-30 and 30-60 cm depth, respectively. A higher TOC was stored in surface soil (0-15 cm) decreasing with depth in all treatments. Maximum accumulation of TOC was observed with super optimal NPK (180:80:40) + Zn + FYM which was at par to 100% NPK (120:40:40) + Zn + FYM, while control plot soil contained the lowest value at all the three depth (Table 2). The increment in TOC under the optimal and balanced application of inorganic fertilizer alone or with FYM is ascribed to greater input of root biomass because of better crop growth along with the

Table 1. Application rates for Nitrogen (N), Phosphorous (P), Potassium (K) and Farm Yard Manure (FYM) for different treatments from 1984 to 2014

Symbol	Treatment	Inorganic fertilizer (Kg ha^{-1})			FYM (t ha^{-1})	Symbol	Treatment	Inorganic fertilizer (Kg ha^{-1})			FYM (t ha^{-1})
		N	P	K				N	P	K	
T1	Control	0	0	0	0	T8	NPK + FYM	120	40	40	5
T2	N	120	0	0	0	T9	NPK + Zn (F) + FYM	120	40	40	5
T3	NP	120	40	0	0	T10	$N_{180}P_{80}K$ + Zn (F) + FYM	180	80	40	5
T4	PK	0	40	40	0	T11	$N_{150}P_{80}K$	150	80	40	0
T5	NK	120	0	40	0	T12	$N_{180}P_{80}K$ + Zn (F)	180	80	40	0
T6	NPK	120	40	40	0	T13	$N_{180}P_{80}$ + Zn (F)	180	80	0	0
T7	NPK + Zn (F)	120	40	40	0	T14	NPK (DAP)	120	40	40	0

addition of FYM for 30 years. The TOC input over the 30 years due to an application of super optimal NPK(180:80:40) + Zn + FYM was 10393.60, 8780.8 and 8726.40 kg ha^{-1} in 0-15, 15-30 and 30-60 cm soil layer respectively. Rudrappa (2005) also reported the highest accumulation of TOC under 100% NPK + FYM while lowest in control in all the three-soil depth.

Fertilizer application with different doses alone or in conjunction with FYM did not influence the bulk density of soil significantly in 0-60 cm soil profile (Table 3). The decrease in bulk density over the years with fertilizer and manure application can be ascribed to the addition of root and plant biomass *vis-à-vis* conversion of some micro-pores into macro-pores due to cementing action of organic acids and polysaccharides formed during the decomposition of organic residues.

After 30 years, TOC stock in 0-60 cm soil profile greatly enhanced to 94.18 Mg C ha^{-1} due to application of 100 % NPK+ Zn + FYM and 106.30 Mg C ha^{-1}in super optimal NPK (180:80:40) + Zn + FYM treatment. In 1984 the initial value TOC stock in 0-15 cm soil layer was 24.30 Mg C ha^{-1}. The highest TOC stock in 0-15 cm layer was 30.28 Mg C ha^{-1} due to 100 % NPK + Zn + FYM, followed by super optimal NPK (180:80:40) + Zn + FYM, while in 15-30 and 30-60 cm soil layers greatest (26.45 and 49.59 Mg C ha^{-1}) TOC stock registered under super optimal NPK(180:80:40) + Zn + FYM and lowest (23.27, 20.59 and 37.30 Mg C ha^{-1}) found in control in all three depths respectively (Table 2). These results are comparable with other workers (Pathak et al 2011, Kumara et al 2014) where TOC stock was higher in treatments which were receiving integrated use of organic and inorganic fertilizers against sole use of chemical fertilizers and control. The development in TOC stock with nutrient management was due to the increased level of organic carbon in the soil. This must be because of direct application of FYM and addition of root biomass, root exudates and crop residues by rice and wheat over a period of 30 years. Similar results were observed by Benbi et al (2012) and Srinivasarao et al (2012).

Amount of C sequestered and C sequestration rate: In 0-15 cm soil layer after 30th crop of rice, all the treatments comprising fertilizers at varying levels with or without FYM increased the amount of C sequestered over the initial TOC stock (24.30 Mg C ha^{-1} in 1984) by 1.40 to 5.98 Mg C ha^{-1}. However, in control, it decreased by 1.03 Mg C ha^{-1}. The highest amount of C sequestered (5.98 Mg C ha^{-1}) was due to application of 100 % NPK + Zn + FYM, which was at par to super optimal NPK(180:80:40)+ Zn + FYM and lowest in 100 % N with only 1.40 Mg C ha^{-1} (Table 2). In 15-30 cm and 30-60 cm soil layer, amount of C sequestered showed a significant effect of different fertilizer treatments either alone or in combination with FYM over 100 % N. Amount of C sequestered ranged from 2.14 to 5.86 Mg C ha^{-1}and 1.10 to 12.29 Mg C ha^{-1} in 100 % N and super optimal NPK

Table 2. Total soil organic carbon (TOC) and C sequestration in the different horizon of rice soils under long-term fertilization

Treatment	TOC (g kg^{-1})			TOC stock (Mg C ha^{-1})			Amount of C sequestered (Mg C ha^{-1})			C sequestration rate (Mg C ha^{-1} yr^{-1})		
	0-15 cm	15-30 cm	30-60 cm	0-15 cm	15-30 cm	30-60 cm	0-15 cm	15-30 cm	30-60 cm	0-15 cm	15-30 cm	30-60 cm
Pre-Soil	12.18	-	-	24.30	-	-	-	-	-	-	-	-
T1	11.00	9.34	7.82	23.27	20.59	37.3	-1.03	-	-	-0.03	-	-
T2	12.24	10.31	8.05	25.7	22.73	38.4	1.40	2.14	1.1	0.05	0.07	0.04
T3	13.15	10.88	8.50	27.22	23.66	40.04	2.92	3.07	2.74	0.10	0.1	0.09
T4	12.92	10.65	8.39	26.94	23.32	39.52	2.64	2.73	2.22	0.09	0.09	0.07
T5	12.58	10.54	8.16	26.42	23.08	38.68	2.12	2.49	1.38	0.07	0.08	0.05
T6	13.94	11.56	8.76	28.65	24.8	40.73	4.35	4.21	3.43	0.15	0.14	0.11
T7	14.28	12.24	9.52	29.2	25.89	43.7	4.90	5.3	6.4	0.16	0.18	0.21
T8	14.96	12.81	10.54	29.4	26.13	45.85	5.10	5.54	8.55	0.17	0.18	0.29
T9	15.53	12.92	10.88	30.28	26.16	47.33	5.98	5.57	10.03	0.20	0.19	0.34
T10	15.64	13.26	11.56	30.26	26.45	49.59	5.96	5.86	12.29	0.20	0.20	0.41
T11	14.35	12.47	9.86	28.84	26.19	44.67	4.54	5.6	7.37	0.15	0.19	0.25
T12	13.96	11.90	9.18	28.48	25.53	42.47	4.18	4.94	5.17	0.14	0.16	0.17
T13	14.42	12.58	10.09	28.77	26.23	45.41	4.47	5.64	8.11	0.15	0.19	0.27
T14	13.26	11.22	8.73	27.45	24.4	40.86	3.14	3.81	3.56	0.11	0.13	0.19
CD (p=0.05)	1.37	1.22	1.17	NS	NS	NS	0.18	0.33	0.44	0.01	0.01	0.02

(180:80:40)+ Zn + FYM through 15-30 cm and 30-60 cm soil layer, respectively. This enhanced level of C sequestration with the addition of FYM + inorganic fertilizers might be due to better crop growth with higher roots biomass production, higher return of left over surface residues and slower breakdown or constant mineralization of added organic residues resulting in more SOC accumulation (Mandal et al 2007). Higher C sequestration in a 33-year-old rice-wheat system due to application of FYM and the cropping system has greater capacity to sequester C because of high C input through enhanced productivity (Kukal et al 2009).

After 30 years, with regard to initial status of carbon i.e., 1984, the rate of C sequestration in 0-15 cm soil layer showed a statistically significant differences among various treatments and it ranged from 0.05 to 0.20 Mg C ha^{-1} yr^{-1}. In contrast a negative rate (-0.03 Mg C ha^{-1}yr^{-1}) was observed in control wherein no extraneous nutrients were provided. The highest C sequestration rate was observed due to application of 100 % NPK+ Zn + FYM and super optimal NPK(180:80:40) + Zn + FYM much lower with 100 % N. The rate of C sequestration varied from 0.07 to 0.20 Mg C ha^{-1}yr^{-1} and 0.04 to 0.41 Mg C ha^{-1}yr^{-1} in 15-30 cm and 30-60 cm soil layer with the application of various fertilizer with or without FYM (Table 2). The increase in C sequestration rate with the addition of FYM and inorganic fertilizers might be due to better crop growth, higher root biomass and higher return of left over surface plant residues in soil. Pathak et al. (2011) observed rate of C sequestration in the NPK + FYM treatment was 0.33 Mg C ha^{-1} yr^{-1} whereas in the NPK treatment was 0.16 Mg C ha^{-1} yr^{-1}.

Soil enzymes and yield trends: The hydrolysis of FDA was increased significantly under various fertilizer combinations with or without FYM in all the soil layers of profile up to 60 cm (Table 3). In 0-15 cm layer hydrolysis of FDA ranged from 110.28 to 290.73 µg fluorescein h^{-1} g^{-1} dry soil, highest being produced due to super optimal NPK(180:80:40)+ Zn + FYM which was at par with 100 % NPK + Zn + FYM and 100 % NPK + FYM and lowest in control. In 15-30 and 30-60 cm soil layer, the greatest value of hydrolysis of FDA to the tune 250.70 and 105.71 µg fluorescein h^{-1} g^{-1} was measured with super optimal NPK(180:80:40)+ Zn + FYM followed 100 % NPK + Zn + FYM and lowest (60.15 and 15.03 µg fluorescein h^{-1} g^{-1}) in control. The FDA enhanced under balanced application of inorganic fertilizer alone or in conjunction with FYM, which might be due to the higher levels of organic matter, coupled with the presence of metabolically active micro-organisms (Taylor et al 2002). The adherence of enzymes to the colloids of the FYM also increase the rate of fluorescein diacetate hydrolysis under FYM treated plots (Nannipieri et al 2003).

After 30th cycle of rice, in surface and sub-surface soil layers (0-15 cm, 15-30 cm) the *β*-glucosidase activity increased significantly in all treatments over control (Table 3). Highest *β*-glucosidase activity of 231.07 µg PNP g^{-1} soil h^{-1} in 0-15 cm layer was assessed in soil from super optimal NPK (180:80:40)+ Zn + FYM, which was at par with 100 % NPK +

Table 3. Bulk density and enzymatic activities in the different horizon of rice soils under long term fertilization

Treatment	B.D. (Mg m^{-3})			Fluorescein diacetate activity (µg fluorescein g^{-1}h^{-1})			*β*-glucosidase activity (µg PNP g^{-1} h^{-1})			Grain yield (Kg ha^{-1})
	0-15 cm	15-30 cm	30-60 cm	0-15 cm	15-30 cm	30-60 cm	0-15 cm	15-30 cm	30-60 cm	
T1	1.41	1.47	1.59	110.28	60.15	15.03	50.32	44.73	12.80	2118
T2	1.40	1.47	1.59	149.88	68.13	25.08	64.29	61.50	13.73	3024
T3	1.38	1.45	1.57	173.33	90.23	44.13	87.59	68.02	19.32	5102
T4	1.39	1.46	1.57	170.43	80.20	35.10	80.48	65.22	15.59	3080
T5	1.40	1.46	1.58	150.38	70.18	28.33	78.61	62.50	14.66	2917
T6	1.37	1.43	1.55	190.48	110.28	55.15	101.57	88.52	23.04	5399
T7	1.35	1.41	1.53	209.51	130.33	65.18	104.50	99.84	27.70	5696
T8	1.31	1.36	1.45	270.68	210.45	99.23	215.25	120.20	33.30	6809
T9	1.30	1.35	1.45	280.70	219.50	101.25	228.29	149.01	37.02	6825
T10	1.29	1.33	1.43	290.73	250.70	105.71	231.07	191.95	44.48	6781
T11	1.34	1.40	1.51	210.53	160.40	70.81	108.08	90.88	28.64	6094
T12	1.36	1.43	1.54	205.50	125.30	57.71	102.50	97.84	25.84	5714
T13	1.33	1.39	1.50	240.60	170.43	75.20	137.91	117.41	30.50	6292
T14	1.38	1.45	1.56	180.45	100.25	45.13	100.63	81.07	21.18	5472
CD (p=0.05)	NS	NS	NS	20.78	15.18	6.85	13.02	11.02	4.39	403

Zn + FYM. In 15-30 and 30-60 cm soil layer the highest *β*-glucosidase activity of 191.95 and 44.48 µg PNP g^{-1} soil h^{-1} under super optimal NPK (180:80:40) + Zn + FYM, while lowest (44.73 and 12.80 µg PNP g^{-1} soil h^{-1}) in control. The glucosidase enzyme activity increases with the use of FYM along with fertilizer, resulting in high availability of C in the soil and improves the microbial population. Similar results were reported by Zhang et al. (2010). Srinivas et al (2015) also observed highest activity of β-glucosidase due to combined application of FYM and mineral fertilizers. Similarly, higher β-glucosidase activity in organic amended soils as compared to that of the soils with mineral fertilization and control has been reported (Gopinath et al 2007).

Grain yield of rice varied from 2118 to 6825 kg ha^{-1} with the application of various fertilizer with or without FYM, respectively and effects produced due to different combinations of fertilizer application were statistically significant in all treatments over control. Maximum grain yield (6825 kg ha^{-1}) was with 100 % NPK +Zn + FYM which was at par with 100 % NPK + FYM and super optimal NPK (180:80:40) + Zn + FYM and minimum in control (Table 3). The increase in grain yield due to addition of organics through FYM may be attributed to supply of N, P, K and micronutrients in addition to the recommended dose of fertilizers and increase in total N, P and K uptake. An application of FYM also acts a source of substrate for microbial metabolism resulting in enhanced microbial activity and subsequently nutrient transformations resulting in enhanced plant growth and yield. Thind *et al*. (2016) reported increase in N, P and K uptake to the tune of 29 to 32 %, 29 to 33 % and 27 to 49% in wheat due to FYM which increased grain and straw yield. The integrated supply of nutrients found beneficial in recording higher grain and straw yield of rice and wheat. The results are in close conformity with the findings of others (Saini et al 2005, Kundu et al 2008).

CONCLUSIONS

The continuous application of balanced inorganic fertilizer alone and with FYM for 30 years significantly improved TOC, C sequestration, fluorescein diacetate (FDA) activity, *β*-glucosidase activity and grain yield compared to control and imbalanced fertilizer application under rice-wheat sequence in sub-humid and sub-tropic of India. This study concludes that application of super optimal NPK (180:80:40) + Zn + FYM and 100 % NPK + Zn + FYM was most promising and efficient nutrient management practices for improving TOC stock, C sequestration, associated enzymatic activities and crop yield. This practice should be recommended to farmers for improving soil health, reducing cost of production and improving agricultural economy.

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