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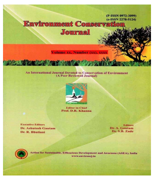

Impact of long-term fertilization on soil organic carbon dynamics and biological health of soils under Pearl millet -mustard-cowpea cropping sequence in an inceptisol of Gujarat

Bornali Borah ✉
Krishi Vigyan Kendra, Karbi Anglong, Assam Agricultural University (Assam), India.
Vinubhai Pragajibhai Ramani
College of Agriculture and Polytechnic in Agriculture, Vaso, Anand (Gujarat), India.
Dileep Kumar
Micronutrient Research Centre, Anand Agricultural University, Anand (Gujarat), India.

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ABSTRACT

The study was undertaken by utilizing an ongoing long-term experiment on continuous cropping at Anand Agricultural University that began in 1980. From 1994 onwards, a modification was made by including Farmyard Manure (FYM) treatments for studying the following objectives: long-term effect of fertility levels with and without FYM on changes in soil organic carbon pools for assessing the role of organics and chemical fertilizers on soil organic carbon buildup and their interrelationship with soil aggregate stability under the pearl millet-mustard-cowpea (F) cropping sequence. Under F1 (FYM @ 10 t/ha) and FL3 (NP application @150 percent of RDF), there was a considerable improvement in the status of Walkley and Black C (WBC), Soil Microbial Biomass Carbon (SMBC), and Total Organic Carbon (TOC) compared to the control in both depths (0-15 and 15-30 cm). Long-term manuring and fertilization practices affect aggregate development and stabilization. In all depths, the highest soil macroaggregates and microaggregates were found when FYM @ 10 t/ha and FL3 (150 percent NP) were applied. Under FYM treated plots and with the greater dose of NP (NP application @150 percent of RDF) in both the surface and sub-surface layers, the maximum water-stable aggregate expressed as mean weight diameter (MWD) was recorded. Furthermore, a significantly positive correlation was observed between SMBC and enzymatic activities (phosphatase, urease, and dehydrogenase) in both the soil depths; indicating the effect of labile C on the biological activities of soil which might be achieved by means of changes in microbial diversity of the soil.

Introduction

In the post-green revolution era, the overexploitation of natural resources to feed an exponentially rising population has posed a serious challenge to Indian agriculture systems. Some soils have lost up to 80 tonnes of carbon per hectare around the planet, with most of it being exhaled into the atmosphere (Lal, 2004). The influence of C transfer from soil to the atmosphere is important not just for the global C cycle, but also for soil's ability to produce food, fiber, fuel, and construction materials, and it is a cause of concern for future productivity. Soils contain approximately twice as much carbon (C) as the atmosphere (800 Pg C) and three times as much C as above-ground and terrestrial vegetation (500 Pg C) (Anonymous, 2015). The dynamics of the soil carbon pool affect carbon dioxide concentrations in the atmosphere, causing global climate changes (Lal, 2011). Declining soil organic C is a major issue of concern due to its vital role in maintaining beneficial physical properties of soil, such as soil porosity, aggregation, and bulk density, as well as soil water storage (Benbi *et al.,* 1998; Chenu *et al.,* 2000).

Corresponding author E-mail: *bornaliborah1993@gmail.com*
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Organic C also affects soil chemical properties such as availability and mobilization of nutrients, cation exchange, and buffering capacity along with maintaining biological activities of soil. In order to comprehend the mechanisms driving C stabilization, SOC is divided into labile and recalcitrant C pools. The labile pool is the one that fluctuates most quickly. Very labile carbon, hot water-soluble carbon, particulate organic matter-carbon (POM-C), and microbial biomass carbon are just a few examples of the several types of labile carbon that can be found in soils. Labile C is a reliable measure of variations in SOC concentration as this pool is subject to rapid alteration in response to changes in soil management (Bhattacharyya *et al.,* 2011, Dutta *et al.,* 2018). As a result, labile C may be used as an easy yet useful indicator to understand the connection between various soil management options and SOC development under a particular management strategy. The most resilient carbon pools are passive ones, which are made up of extremely stable material that has been present in the soil for hundreds or even thousands of years. Most of the humus that is physically protected in clay-humus complexes falls under this category which accounts for 60-90% of the organic matter. The colloidal characteristics of soil humus, which account for the majority of the cations and water-holding capacities added to the soil by organic matter, are most closely associated with the passive pool. For assessment of soil physical quality, the distribution, as well as stability of soil aggregates, are considered essential, emphasizing the importance of soil management on particle aggregation and disaggregation (Silva *et al.,* 2014). The aggregate stability is generally strongly correlated with SOC content by playing a vital role as the organic polymer binding agents (Haynes & Swift, 1990; Majumder *et al*., 2010) and the physical trapping of particles by fine roots and fungal hyphae (Helfrich *et al.,*2015) which promote aggregate cohesion. Additionally, fertilization and irrigation practices that boost crop productivity, biomass, and root production may indirectly enhance the system's carbon inputs and contributes to the aggregate formation (Yadav *et al*., 2017).

In addition to fertilizer application, one of the most significant factors affecting SOC content in soil is the cropping system (Bhutiani and Ahamad 2019; Bhardwaj *et al.,* 2020; Ruhela *et al.,* 2022). Continuous cereal farming could affect productivity, carbon pools, and soil health. Adding organics and legumes to cropping systems enhances soil aggregation and boosts C and N sequestration, according to research (Bandyopadhyay, *et al.,* 2010). Bajra, or pearl millet, and mustard are important food grain crops in India, and they are primarily grown in semi-arid tropic regions with sandy loam soils. Pearl millet is grown on 7.12 million hectares and produces 8.06 million tonnes of grain with the country's productivity of 1132 kg/ha. In Gujarat, the total planted area is 4.31 lakh acres with a total productivity of 9.31 lakh hectares tonnes, with a yield of 2158 kg/ha (Anonymous, 2018). In India's tropical and subtropical climates, cowpea is a common green fodder legume. However, due to increased soil temperature and increased soil erosion issues, respectively, the SOC pools are lower in the tropical and sub-temperate regions of India compared to temperate soils worldwide (Kumar *et al*., 2013; Nianpeng *et al*., 2013; Dixit *et al.,* 2019). Several research highlighted that nutrient management strategies have the ability to alter the C storage capacity of agricultural soils depending on climate, soil conditions, and cropping systems (Bhattacharyya *et al*., 2011; Dutta *et al.,* 2018; Ghosh *et al.,* 2019; Dixit *et al*., 2019). Therefore, it is necessary to determine and implement the optimal management strategies to maintain or raise SOC levels, especially in those areas where production systems are naturally poor in soil fertility (Manna *et al*., 2013; Dixit *et al*. 2019).

Long-term fertilizer experiments (LTFEs) are essential for evaluating how chemical fertilizers and organic additives affect the soil carbon buildup by the transformation of SOC into different pools, which is important for quantifying soil quality and system sustainability. Keeping all of the aforementioned, the current study was designed to investigate the effect of continuous organic and inorganic fertilizer applications on changes in soil organic carbon fractions, aggregate stability, and enzymatic activities during the pearl millet-mustard-cowpea (F) cropping sequence.

Material and Methods

The Micronutrient Research Project (ICAR) started the long-term experiment in 1980 at Anand

Agricultural University in Anand, Gujarat, which was located at $22^0 35'$ North latitude and $72^0 55'$East longitude with an elevation of 45.1 meters above mean sea level on loamy sand soil (81.10 percent sand, 12.20 percent silt and 6.70 percent clay). Later in 1994, the experiment was changed to a Randomized Block Design with two Factors to investigate the effect of Farmyard manure in the maintaining of soil fertility and productivity at various levels of fertility during intense cropping. Under the pearl millet (*kharif*) -mustard (rabi) - cowpea (F) (summer) cropping sequence, FYM treatments were applied in half of the replications. The experiment contained four fertility levels and two levels of FYM, namely F0 (no-FYM) and F1 (FYM @ 10 t/ha) to be delivered to the *kharif* (pearl millet) crop once a year. The eight fertilizer treatments included in the experiment were: F_0FL_0: Control, $F_0 FL_1$:50 % NPK (50% RDF), $F_0 FL_2$: 100 % NPK (100% RDF), F_0 FL_3 :150 % NPK (150% RDF), F_1 FL_0: FYM 10 t/ ha, F_1 FL_1: FYM 10 t/ha+ 50 % NPK (50% RDF), F_1 FL_2: FYM 10 t/ha+ 100 % NPK (100% RDF), F_1 FL_3: FYM 10 t/ha+ 150 % NP (150% RDF). The recommended doses of N-P fertilizer for pearl millet: 80 kg N/ha, 40 kg P_2O_5/ha, Mustard:50 kg N/ha, 50 kg P_2O_5/ha and Cowpea: 25 kg N/ha, 50 kg P_2O_5/ha were applied. The FYM was given before *kharif* season @ 10 t/ha to F_1 treatment. The basal dose of Nitrogen fertilizer was applied in the form of urea as per recommendation. The plow furrow was treated with the required amount of P_2O_5 via diammonium phosphate. The remaining Nitrogen was added in two splits to pearl millet and one split to mustard. The experimental site's soil reaction was moderately alkaline (pH 8.35), and the EC was normal (0.16 dS/m). The highest monthly average rainfall (312 mm) has been received during the growing season of bajra, with a well-distributed pattern.

Following the harvest of cowpea (2018), soil samples were collected from two depths, 0-15 and 15-30 cm, and were prepared by mixing samples collected randomly from four spots in each plot. For storing the soil samples were air-dried and passed through a 2 mm sieve and then kept in polythene bags for further analysis of the physicochemical properties. For the biological parameter analysis, collected fresh soil samples from both depths were immediately transferred to the refrigerator, where they were kept at 4°C.

***Analysis of Physico-chemical parameters**:*

Soil pH and EC were measured by using a soil water suspension ratio of 1:2.5. (Jackson, 1979).

The procedure used for aggregate analysis was Modified Yoder's wet sieving method (Yoder, 1936). To calculate the mean weight diameter (MWD), oven dry weight of the fractions of the aggregates retained on each sieve was determined using the formula:

$$\text{MWD} \quad = \sum_{i=1}^{i=n}(\text{Xi Wi}) \,/\, \sum_{i=1}^{i=n}(\text{Wi})$$

Where, X_i = Mean opening of the sieve (i.e. 0.05, 0.175, 0.375, 0.75, 1.5, 3.5 mm for 0.1, 0.1-0.25, 0.25-0.5, 0.5-1.0, 1-2, 2-5 mm size classes, respectively); W_i = Weight of retained aggregates (g) and n = Number of size classes.

Large macroaggregates were classified as soil held between 4 and 2 mm, whereas small macroaggregates were classified as soil retained between 2 mm and 250 µm. Small micro aggregates were defined as soils held between 250 µm and 53 µm, whereas silt and clay fractions were defined as soils passing through 53 µm sieve and retained on the pan. However, macro-aggregates include both large and small macroaggregates.

Organic Carbon Fractions and total organic carbon:

The Organic carbon in soil was determined by Walkley and Black (1934) rapid titration method.

The fumigation extraction method was used to determine the SMBC (Vance *et al.,* 1987). In 100-mL capacity beakers, two portions of moist soil, each containing 50 g oven-dry soil, were weighed. One portion was used as a control and was immediately extracted with 0.5 M K_2SO_4. Another portion was fumigated. For 30 minutes, the fumigated soil samples were extracted with 200 mL 0.5 M K_2SO_4. Organic carbon in soil extracts was evaluated using dichromate oxidation, and SMBC was calculated.

Hot Water-soluble C (HWSC) was determined using the method described by Mc Gill *et al.,* (1986). Five grams of soil were centrifuged for 30 minutes at 10,000 rpm with 10 ml of distilled water. A portion of the supernatant was filtered. The filtrate was then treated with 5 ml of 0.07 N $K_2Cr_2O_7$, 10 ml of 98 percent H_2SO_4, and 5 mL of 88 percent H_3PO_3, and digested for 30 minutes at 150^{0}C. After cooling, the samples were titrated with 0.01 N ferrous ammonium sulphate in 0.4 N H_2SO_4 using diphenylamine as an indicator.

TOC content of the soils was determined by the method of Yeomas and Bremmer (1989) after treating the soil with dilute acid. In this method, one gram of soil was oxidized with 5 mL of 1*N* $K_2Cr_2O_7$ and 7.5 mL concentrated H_2SO_4 utilizing external heat (150 ^{0}C for 30 minutes). The tubes were removed from the block digester and cooled down for 15 minutes before being filled with water to a capacity of 50 mL. The contents were determined by titrating with ferrous ammonium sulphate (0.2 N) to a bright green endpoint using diphenylamine as an indicator. To compensate for the loss of $K_2Cr_2O_7$ due to boiling, two controls were included: one boiled and one unboiled. TOC was calculated as

$$\text{TOC (\%)} = \text{A (N of F. A. S.)} \times (0.003) \times \frac{100}{\text{Sample weight (g)}}$$

Where, A= (BC- S) × (UC - BC) / UC) + (BC –S)
BC = Boiled control
UC = Unboiled control
S = F. A. S. used in sample titration

Organic carbon stock

The following formula was utilized to calculate the Soil organic carbon stock:

C stock (Mg/ha) = [Total organic carbon (%)/100] × bulk density (Mg/m^3) × depth (m) ×10,000 (m^2/ha).

Enzymatic activity of soil:

Acid and alkaline phosphates activity in soil were assayed by the method described by Tabatabai and Bremnar (1969).

Urease activity in soil samples was estimated according to the "determination of urea remaining" methodology given by Tabatabai and Bremnar (1972) and expressed as µg urea hydrolyzed/g/h.

Soil dehydrogenase activity was determined by the method described by Cassida *et al.,* (1964) and results were expressed as µg TPF formed/ha/g of soil.

Results and Discussion

The application of FYM @ 10 t/ha over no FYM (F0) enhanced soil WBC by 26.3 percent in the 0-15 cm depth and 6.0 percent in the 15-30 cm depth, according to data in Table.1. Long-term application of FYM led to enhanced root development, which resulted in the concentration of organic residue in the soil and decomposition may have added to the organic carbon content of the soil. Ghosh *et al.,* (2018) also concluded that the Walkley-Black C (oxidizable organic C) concentration of surface soils decreased by 51%relative to the initial value when a wheat-based cropping system was used for 44 years without fertilization. However, the balanced fertilization (NPK) sustained or even increased the Walkley-Black C concentration. The amount of oxidizable organic C in the surface soil layer was higher in the NPK, 150 percent NPK, and NPK + FYM treatments compared to the unfertilized control plots by up to 103, 116, and 141 percent, respectively. In the subsurface soil layer, a similar pattern was observed.

In both the surface and subsurface depths, the HWSC status improved significantly with the treatment FYM 10 t/ha compared to the control. The improvement was 35.4 percent in the Surface depth, while subsurface depth was improved by 38.0 percent. In the manure-treated plots, the freshly humified organic carbon from FYM addition may have sustained a higher amount of HWSC than in the control plots (Liang *et al.,* 2012). In this investigation, soil microbial biomass carbon was considerably higher in the FYM treated plots (290.18 and 255.69 µg/g) in both surface and subsurface soil. This result is in agreement with numerous earlier observations (Chakraborty *et al.*, 2011; Marschner *et al.,* 2003). The presence of fast metabolizable C and N in FYM had the greatest impact on microbial biomass carbon levels, and increased root biomass and root exudates led to improved crop production in manure-treated plots (Benbi *et al.,* 2015). TOC increased by 31.6 percent on the surface and 18.2 percent on the subsurface under FYM (10 t/ha). The amount of TOC dropped as depth was increased, and the difference in TOC between fertilization treatments was more noticeable at 0-15 cm depths than at 15-30 cm depths. The SOC stock under FYM @ 10 t/ha(F1) was greater by 28.1 and 15.84 percent over control for profile depths of 0-15 and 15-30 cm, respectively. The rise in carbon content could be related to the addition of organic manure, which increases SOC stock.

Table 1: Long-term effect of FYM and fertilizer application on different carbon fractions in two soil depths after harvest of cowpea in a cropping sequence

Treatments	Soil Microbial Biomass C (µg/g)		Walkley & Black C (g/kg)		Water Soluble C (mg/kg)		Total Organic Carbon (g/kg)		Soil Organic Carbon stock (Mg/ha)	
	0-15 cm	15-30 cm	0-15 cm	15-30 cm	0-15 cm	15-30 cm	0-15 cm	15-30 cm	0-15 cm	15-30 cm
FYM levels										
F$_0$ (Without FYM)	270	242	3.65	3.13	26.02	23.04	5.62	4.83	11.63	10.1
F$_1$ (FYM 10 t ha^{-1})	290	256	4.61	3.32	35.24	31.84	7.40	5.71	14.90	11.7
S.Em ±	5.4	3.4	0.073	0.029	0.568	0.435	0.073	0.109	0.19	0.31
C.D.(P=0.05)	16	10	0.22	0.08	1.72	1.31	0.22	0.32	0.57	0.94
Fertility levels										
FL$_0$ (Control)	243	221	3.66	3.15	16.51	19.84	5.48	4.86	10.51	10.13
FL$_1$ (50 % NP)	271	246	3.95	3.20	22.26	24.92	6.37	5.06	11.80	10.85
FL$_2$ (100 % NP)	286	256	4.20	3.24	25.66	28.86	6.96	5.30	12.57	11.12
FL$_3$ (150 % NP)	320	273	4.71	3.29	31.46	36.78	7.24	5.86	12.99	11.49
S.Em ±	7.6	4.8	0.103	0.041	0.803	0.615	0.103	0.153	0.17	0.44
C.D.(P=0.05)	23	14	0.31	NS	2.43	1.86	0.31	0.46	0.31	1.33
Mean	280	249	4.13	3.22	30.63	27.44	6.51	5.27	13.26	10.90
F x FL	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
CV %	6.67	4.70	6.12	3.09	6.42	5.49	3.88	7.13	4.90	9.88

Table 2: Long-term effect of FYM and fertilizer application on the size distribution of soil aggregate and mean weight diameter (MWD) of in two different soil depths after harvest of cowpea in a cropping sequence

Treatments	Macroaggregates (g 100 g^{-1})		Microaggregates (g 100 g^{-1})		Silt + clay-sized fraction (g 100 g^{-1})		MWD (mm)	
	0-15 cm	15-30 cm	0-15 cm	15-30 cm	0-15 cm	15-30 cm	0-15 cm	15-30 cm
FYM levels								
F$_0$ (Without FYM)	19.56	18.44	41.48	43.79	37.10	36.46	0.63	0.46
F$_1$ (FYM 10 t ha^{-1})	22.92	21.30	45.64	49.20	29.10	28.63	0.77	0.59
S.Em ±	0.643	0.641	0.803	1.552	1.048	1.217	0.022	0.019
C.D.(P=0.05)	1.95	1.94	2.43	4.70	3.18	3.69	0.07	0.05
Fertility levels								
FL$_0$ (Control)	15.99	14.86	32.59	33.97	45.48	41.39	0.45	0.41
FL$_1$ (50 % NP)	19.51	19.50	41.23	43.09	35.60	37.00	0.61	0.59
FL$_2$ (100 % NP)	22.63	21.25	47.93	52.42	28.00	29.42	0.79	0.63
FL$_3$ (150 % NP)	25.83	23.89	51.00	56.74	22.83	22.36	0.94	0.74
S.Em ±	0.910	0.906	1.135	2.194	1.482	1.722	0.031	0.027
C.D.(P=0.05)	2.76	2.75	3.44	6.65	4.49	5.22	0.10	0.08
Mean	21.24	19.87	43.56	46.49	33.10	32.54	0.70	0.52
F x FL	NS	NS	NS	NS	NS	NS	NS	NS
CV %	10.49	11.17	6.44	11.56	11.01	12.96	10.98	12.23

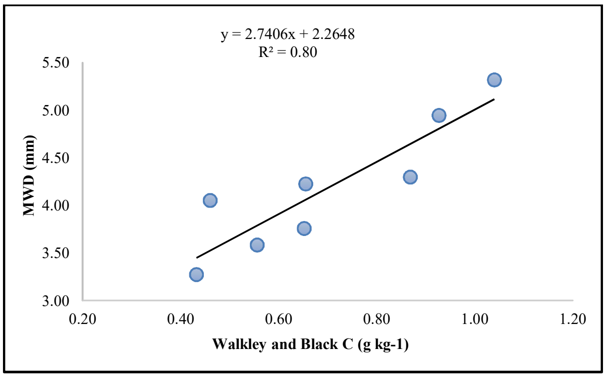

Figure 1: Soil organic carbon (Walkley and Black carbon) relationship with MWD at 0-15 cm soil depth

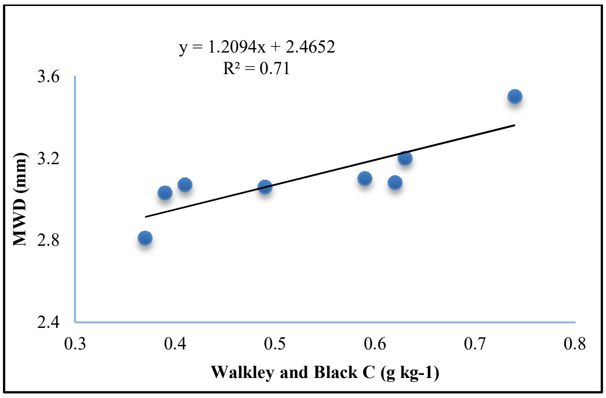

Figure 2: Soil organic carbon (Walkley and Black carbon) relationship with MWD at 15-30 cm soil depth

Among the different fertility levels, FL3 (NP application @ 150 percent RDF) had the highest WBC (4.71 g/kg) for surface soil, but the influence of fertility levels on changes in WBC at subsurface depths of the soil profile was non-significant. The changes in OC concentration caused by graded fertilizer application could be related to differences in organic matter quantity and rate of oxidation by microorganisms (Peacock *et al.,* 2001; Zhang *et al.,* 2010). The significantly highest values for HWSC were recorder with NP application @ 150 % RDF i.e. FL_3 in 0-15 cm (31.46 mg/ kg) and 15-30 cm (36.78 mg/ kg), respectively. The highest TOC accumulation was seen with NP @ 150 percent of RDF, while the control plot had the lowest TOC. In both soil depths, 150 percent RDF (NP) significantly enhanced TOC content in soil compared to the control and 50 percent NP. Increased fertilizer dose raises TOC content considerably over control regardless of depth. Furthermore, FL3 (NP application @ 150 percent RDF) had the highest SOC stock of fertility doses. Over the control, the SOC stock increased by 23.6 at 0-15 cm depth layer and 13.42 percent at15-30 cm depth, respectively in the highest fertility level i.e. FL3 (NP application @ 150 percent RDF). Fertilizer additions contributed to carbon accumulation, mostly by increasing root biomass; crop leftovers eventually resulted in carbon accumulation over time (Kundu *et al.,* 2007, Ghosh *et al.,* 2012).

Soil macroaggregates and microaggregates were significantly higher under FYM application @ 10 t/ha, with an increase of 17.1% and 10% over unfertilized control plots in the surface soil, and 15.5 and 12.2% increases in the sub-surface soil, respectively. FL3 (150 percent NP) was observed to contribute significantly to the development of macro and microaggregates in both depths over control and rest of fertility levels. For subsurface layers, however, this treatment impact was comparable to FL2 (100 percent NP). The silt + clay-sized fractions of aggregates decreased significantly in FYM and fertilizer-treated plots compared to the untreated control. There are some primary mechanisms for the formation and stabilization of soil macroaggregate under integrated nutrient management. The by-products of FYM decomposition, glue compounds generated by roots, fungal hyphae, and polysaccharides' action as a binding agent to bind microaggregates together to form soil macroaggregates are among them (Liao *et al.,* 2006 and Tripathi *et al.,* 2014). In FYM-treated plots, the formation of micro and macroaggregates by soil clay particles could result in very low silt + clay fractions. Organic fertilizer application over time increases SOC content and macroaggregate proportion (Saha *et al*., 2014).

In both soil layers, manure and a super-optimal fertilizer dose significantly increased water-stable aggregate (WSA). Significantly, FYM treated plots had 22.2 and 28.2 percent higher MWD in the surface and subsurface layers, respectively than control plots. Under NP application @ 150 percent RDF (FL3), the increase in MWD for surface and sub-surface layers was 108.8 and 80.4 percent over control, respectively. The continuous application of chemical fertilizers also showed improvement in the MWD in the long run indicating the role played by phosphate ions in bindings soil particles or due

Table 3: Effect of FYM and fertilizer application on microbial count in in two different soil depths after harvest of cowpea in a cropping sequence

Treatments	**Bacteria (CFU g^{-1} x 10^8)**		**Actinomycetes (CFU g^{-1} x 10^1)**		**Fungi (CFU g^{-1} x 10^3)**	
	0-15 cm	**15-30 cm**	**0-15 cm**	**15-30 cm**	**0-15 cm**	**15-30 cm**
FYM levels						
F_0 (Without FYM)	**146**	**100**	**83**	**57**	**5**	**4**
F_1 (FYM 10 t ha^{-1})	**182**	**111**	**103**	**62**	**7**	**5**
S.Em ±	5.2	3.2	2.7	1.6	0.2	0.2
C.D.(P=0.05)	15.9	9.8	8.3	5.0	0.6	0.5
Fertility levels						
FL_0 (Control)	**106**	**93**	**63**	**44**	**4**	**3**
FL_1 (50 % NP)	**139**	**101**	**88**	**57**	**5**	**4**
FL_2 (100 % NP)	**184**	**108**	**104**	**67**	**6**	**5**
FL_3 (150 % NP)	**227**	**118**	**118**	**70**	**9**	**7**
S.Em ±	7.4	4.5	3.8	2.3	0.3	0.3
C.D.(P=0.05)	22.4	13.8	11.7	7.1	0.8	0.8
Mean	**164**	**105**	**93**	**60**	**6**	**4**
F x FL (Interaction)	NS	NS	NS	NS	NS	NS
CV %	**11.07**	**10.66**	**10.19**	**9.60**	**11.86**	**13.90**

***Significant at P 0.05; NS- Non-Significant at P > 0.05**

Table 4: Long-term effect of FYM and fertilizer application on soil enzymatic activities in two different soil depths after harvest of cowpea in a cropping sequence

Treatments	**Alkaline Phosphatase (µg pNP g-1 hr-1)**		**Acid Phosphatase (µg pNP g-1 hr-1)**		**Dehydrogenase (µg TPF g-1 hr-1)**		**Urease (µg urea g-1 hr-1)**	
	0-15 cm	**15-30 cm**	**0-15 cm**	**15-30 cm**	**0-15 cm**	**15-30 cm**	**0-15 cm**	**15-30 cm**
FYM levels								
F0 (Without FYM)	**37.82**	**25.34**	**7.47**	**7.34**	**24.97**	**23.32**	**170.29**	**131.01**
F1 (FYM 10 t ha-1)	**41.67**	**27.70**	**11.64**	**8.03**	**26.73**	**26.01**	**190.32**	**147.72**
S.Em ±	1.146	0.748	0.278	0.184	0.548	0.820	4.789	2.643
C.D.(P=0.05)	3.47	2.27	0.84	0.56	1.67	2.48	14.52	8.01
Fertility levels								
FL0 (Control)	**34.23**	**22.30**	**8.42**	**7.06**	**21.37**	**20.21**	**151.61**	**114.40**
FL 1 (50 % NPK)	**39.97**	**25.79**	**9.18**	**7.45**	**24.11**	**24.02**	**173.60**	**136.84**
FL 2 (100 % NPK)	**40.60**	**28.16**	**9.63**	**7.93**	**26.08**	**26.14**	**182.22**	**149.28**
FL 3 (150 % NPK)	**44.16**	**29.82**	**10.99**	**8.31**	**31.84**	**28.28**	**213.78**	**156.72**
S.Em ±	1.620	1.058	0.393	0.261	0.774	1.160	6.773	3.738
C.D.(P=0.05)	4.91	3.21	1.193	0.79	2.34	3.52	20.54	11.34
Mean	**39.74**	**26.52**	**9.55**	**7.68**	**25.85**	**24.66**	**180.30**	**139.36**
F x FL(Interaction)	NS	NS	NS	NS	NS	NS	NS	NS
CV %	**9.99**	**9.77**	**10.08**	**8.31**	**7.34**	**11.52**	**9.20**	**6.57**

may be due to a greater amount of organic residues produced due to balanced fertilization (Bandyopadhyay *et al.*, 2010). Our findings also revealed a strong and positive relationship between SOC and MWD (Fig. 1 & 2).The soil microbial activities have been significantly affected by long-term manuring in the surface as well as subsurface depths (Table 3). With the long-term application of FYM, the increase in the bacterial, fungal and actinomycetes population were to the tune of 24.8, 52.6 and 23.0 percent for the 0-15 while 11.0, 27.0 and 10.2 percent for 15-30 cm depths, respectively. Among the fertility doses, FL_3 (150 % NP) showed a significantly highest population of bacteria, fungi and actinomycetes and the lowest were recorded under control. Again, for 15-30 cm depth the maximum population of microbes was recorded under the same fertility level i.e. NP application @ 150 % RDF. However, it was at par with NP application @ 100 % RDF for bacteria and actinomycetes.

For the enzymatic activity (Table 4) it has been observed that irrespective of depths the alkaline phosphatase activity was higher than acid phosphatase. Again, FYM application @ 10 t/ha improved the alkaline phosphatase and acid phosphatase activities to the tune of 10.1, 9.3 for surface and 55.8, 9.4 percent for subsurface layers, respectively over the control (F_0). The application of 150% NP resulted in higher phosphatase enzyme activity as compared to control and rest of fertility levels which was increased to the tune of 29.0 percent over the control for 0-15 cm depths. In the case of subsurface depth (15-30 cm), maximum activity for Alkaline and acid phosphatase was found in treatment FL_3 (150 % NP) which was at par with FL_2 (100 % NP). Regardless of treatment, higher enzyme activity was observed in the surface soil layer than in the subsurface soil. The significantly higher alkaline and acid phosphatase activities in the organically treated plots could be attributed to increased microbial activity and diversity of phosphate solubilizing bacteria as a result of organic matter input over the period. These results are similar to the findings of Kanchikerimath and Singh (2001); Mandal *et al.,* (2007), and Manna *et al.,* (2007). Similarly, long-term application of FYM @ 10 t/ha significantly increased dehydrogenase and urease activity by 7.0, 11.5, and 11.8,12.7 percent over control in the surface and subsurface layers, respectively. The beneficial effects of FYM on dehydrogenase activity might be due to the more easily decomposable components of crop residues on the metabolism of soil microorganisms and the increase in microbial growth with the addition of carbon source, which is in conformity with the findings of Manna *et al.,* (2005) and Mandal *et al.,*(2007). The graded levels of fertility enhanced dehydrogenase and urease enzyme activity by 40.9, 39.9, and 41.0, 36.9 percent under higher fertility levels (FL_3) over FL_0 in the surface and subsurface layers, respectively. However, in the subsurface, FL_3 was at par with FL_2. The results are like the findings of Bhatt *et al.,* (2016) as they reported that, fertilizer treatment with 100% NPK and 150 % NPK were comparable and significantly superior to 50 % NPK for dehydrogenase activity of soil. Different soil organic carbon fractions were found to be positively and significantly correlated with soil microbial populations and enzymatic activities (Table 5).

Table 5: Correlation between total organic C (TOC, g kg^{-1}), Walkley & Black C (g kg^{-1}), microbial biomass C (MBC, g kg^{-1}), microbial count and enzymatic activity at 0-15 cm depth

.

0-15 cm	**TOC**	**WBC**	**SMBC**	**Bacteria**	**Fungi**	**Actinomycetes**	**Alkaline P**	**Acid P**	**Dehydrogenase**	**Urease**
TOC	1									
WBC	0.961**	1								
SMBC	0.785*	0.814*	1							
Bacteria	0.828*	0.832*	0.970**	1						
Fungi	0.788*	0.852**	0.983**	0.933**	1					
Actinomycetes	0.882**	0.871**	0.942**	0.953**	0.936**	1				
Alkaline P	0.859**	0.879**	0.923**	0.873**	0.944**	0.943**	1			
Acid P	0.944**	0.961**	0.943**	0.693**	0.735*	0.718*	0.778*	1		
Dehydrogenase	0.694**	0.776*	0.954**	0.871**	0.943**	0.914**	0.871**	0.592	1	
Urease	0.831*	0.885**	0.946**	0.929**	0.970**	0.966**	0.939**	0.739*	0.972**	1

Table 6: Correlation between total organic C (TOC, g kg^{-1}), Walkley & Black C (g kg^{-1}), microbial biomass C (MBC, g kg^{-1}), microbial count and enzymatic activity at 15-30 cm depth

15-30 cm	TOC	WBC	SMBC	bacteria	fungi	Actinomycetes	alkaline p	acid p	dehydrogenase	urease
TOC	1									
WBC	0.854**	1								
SMBC	0.790*	0.720*	1							
Bacteria	0.880**	0.839*	0.960 **	1						
Fungi	0.733*	0.780*	0.956**	0.945**	1					
Actinomycetes	0.692*	0.700**	0.933**	0.906**	0.936**	1				
Alkaline p	0.683*	0.789**	0.914**	0.896**	0.944**	0.943**	1			
Acid p	0.909**	0.857**	0.929**	0.990**	0.735*	0.895*	0.868*	1		
Dehydrogenase	0.741*	0.805*	0.910**	0.940**	0.943**	0.948**	0.988**	0.916**	1	
Urease	0.777*	0.821**	0.944**	0.924**	0.970**	0.9072**	0.978**	0.919**	0.990**	1

**. significant at the 0.01 level   *. significant at the 0.05 level

Significantly the highest correlation (Table 6) was observed between SMBC and microbial populations (bacteria, fungi, actinomycete; R^2 = 0.97, 0.98 and 0.94 for surface and R^2 = 0.96, 0.95 and 0.93 for sub-surface, respectively). Similarly, SMBC revealed a positive and significantly the highest correlation with soil enzymatic activity.For all of the above-mentioned parameters, the interaction effect of FYM and fertility levels has been found to be non-significant for both the surface and subsurface layers.

Conclusion

After 15 years of long-term fertilization on the pearl millet-mustard-cowpea cropping system, the soil carbon buildup in different labile fractions from the surface and sub-surface depths was assessed. The results reveal that long-term cultivation with NP + FYM treatments increased SOC and its selected labile fractions (WBC, HWSC, and SMBC) over no fertilization (control) and treatments with a solitary application of inorganic fertilizers. Such augmentation of SOC and almost all its labile fractions were higher with the application of FYM @10 t/ha + 150 % NP in the system. The significantly higher accumulation of TOC as well as SOC stock with higher doses of inorganic fertilizer conjugated with FYM also signifies the contribution of inorganic fertilizer in oil C buildup. Again, soil aggregate stability and biological activity were also significantly higher under the integrated application of FYM @10 t/ha + 150 % NP in the cropping system. The labile C fractions and activities of soil enzymes and microbes were predominated in the near-surface soil layers but declined significantly as depth increased, which could be due more to biomass accumulation in the surface soil layer. Moreover, the significant positive correlations of labile C fractions to microbial and biological activity also indicate the crucial role played by these fractions for regulating biological health of soil. Although these overall advantages may be viewed as an opportunity to improve soil quality and productivity, there is still a need to evaluate and control any negative consequences associated with the long-term use of organic amendments, such as excessive nutrient accumulation or leaching and phytotoxicity.

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Conflict of interest

The authors declare that they have no conflict of interest.

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