

# CHARACTERIZATION OF SOIL ORGANIC CARBON FUNCTIONAL GROUPS AS INFLUENCED BY CONTINUOUS FERTILIZATION AND CROPPING OF FINGER MILLET (*ELEUSINE CORACANA*) – MAIZE (*ZEA MAYS* L.) CROPPING SEQUENCE USING FT-IR SPECTROSCOPY

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**Abstract.** Soil organic carbon (SOC) is a factor for soil quality changes and continuous intensive cropping may change the soil organic matter (SOM) over the years. Characterization of SOC and its functional groups show the evidence of recalcitrant nature of SOM without oxidation under long run fertilization and manuring in sandy clay loam soil. Infrared Fourier transform spectroscopy (FT-IR) measures the absorbance of infrared radiation (4000 - 400  $\text{cm}^{-1}$ ) by bonds expressing dipole moments (e.g., C-O, C=O, C=C, C-H, N-H) in functional groups that constitute SOM. Irrespective of the treatments, 100% NPK + FYM has recorded the highest transmittance intensity of hydrophilic bond of carboxyl (C=O; 1698 - 1701  $\text{cm}^{-1}$ ) carbonyl (3500 - 3200  $\text{cm}^{-1}$  and 1400  $\text{cm}^{-1}$ ), aromatic benzenes (C=C; 1650  $\text{cm}^{-1}$ ), alkenes (C-H; 2922 - 2926  $\text{cm}^{-1}$ ), S-S (500 - 400  $\text{cm}^{-1}$ ), and P=S (800 - 580  $\text{cm}^{-1}$ ) bonds. Addition of manures and mineral fertilizers have increased the SOC stock, SOC sequestration, Humic acid (HA), Fulvic acid (FA), wet ratio of HA and FA, soil microbial biomass carbon (SMBN) and soil microbial biomass nitrogen (SMBN) in the long run. The continuous application of mineral fertilizer as well as FYM over four decades have increased the more stable soil organic carbon compared to control.

**Keywords:** *humic acid, fulvic acid, soil reactive phases, Fourier transform, microbial biomass*

## Introduction

The continuous cropping system, high yielding varieties, irrigation and high analysis fertilizer naturally enhance the mining of nutrients from the soil other than externally supplied by application of fertilizers and manures. Large amount of nutrient has to be applied to soil in chemical form that might have impact on soil properties and soil productivity in the long-run intensive cultivation of crops. The long-term fertilizer experiments indicate the extent to which yield, and related parameters and the quality of ecosystem can be predicted. These are also capable of serving as an early warning system to detect problems that threaten future productivity (Berzsenyi et al., 2000).

Important soil functions depend on content and composition of clay minerals and soil organic matter (SOM). SOM is an important part in the global carbon cycle. Approximately 81% of the organic carbon that is active in the terrestrial carbon cycle is stored in soils (Paustian et al., 2000; Wattel-Koekkoek et al., 2001). SOM functional groups strongly affect sorption characteristics such as the cation exchange capacity (Gressel et al., 1995) and in turn it is reflected in soil fertility.

For characterization of SOM composition and their functional groups in cultivated soil (Kögel-Knabner, 2000), the soil needs secondary reactions and wet chemical extraction procedures for elucidating the humus formation (Grover and Baldock, 2010; Schmidt et al.,

2011) and decomposition in soil. Also avoiding the tedious chemical digestion process spectroscopic techniques such as Fourier transform infrared (FTIR) spectroscopy has been used for characterization of SOM composition. For an example, by using FTIR spectroscopy, SOM functional groups such as carboxyl (C = O) groups that are responsible for cation exchange (Celi et al., 1997) or alkyl (C–H) groups that account for wettability (Capriel et al., 1995) can be analyzed. FTIR spectra of plant and less-decomposed bog samples, which indicate a change in the molecular structure of the alkyl groups and possibly reflects a transformation of OM from simpler (plant) to more resistant aliphatic compounds in decomposed bog and fen samples (Zaccone et al., 2007).

Several infrared absorption bands are characteristic for the molecular structure. The absorption intensity reflects the proportional amounts of functional groups. Infrared spectra of humic acids from peat, coal, lignite, lignin from straw, black earth soils, podzol and Chernozem soils are relatively parallel (Van der Marel and Beutelspacher, 1976). However, there are differences in the absorption intensity and the wave number of absorption caused by slight differences in SOM composition. Heller et al. (2015) observed the second C–H band with a maximum absorption intensity at 2860 cm<sup>-1</sup> and also an increase in the decomposition of more labile carbohydrates in contrast to less-decomposed bog and plant samples (Mudgil et al., 2012). Differences in crop yields might be explained by variations in the hydrophilic character of the SOM. The hydrophilic character of organic substances depends on the composition (type and amount) of functional groups, mainly carboxyl and hydroxyl-groups (Autorenkollektiv, 1984) and may affect the soil water storage capacity of the plough horizon.

Water repellent substances in SOM include aliphatic constituents (Capriel, 1997) and waxes (Franco et al., 2000). The amount of hydrophilic C = O groups (i.e., O and N containing hydroxyl and carboxyl groups) relative to that of hydrophobic C-H groups determines the hydrophobic character of SOM (Morrison and Boyd, 1983). The spatial arrangement of the hydrophobic components within the soil affects water affinity that influences the resistance to microbial degradation, the rate of wetting, and the adsorption processes. These properties play an essential role in the dynamics of SOM.

The weaker influence of field effects on bands as compared with C, N, and P fractions and its lower sensitivity to non-SOC field effects indicates functional group composition is more strongly associated with SOC among different fields than C, N, and P fractions (Margenot et al., 2015). The aliphatic C-H bands have positively correlated with SOC particularly labile organic C fractions (POXC, EOC, PMN and EON) and labile SOM fractions under organic management that increase SOM content (Marriott and Wander, 2006).

Measured fractions reflect processes such as mineralization (e.g., POXC, PMN) or are biomass-based measures (e.g., MBC, MBN), which is likely why these fractions are sensitive to field specific differences like nutrient management (Culman et al., 2012; Kallenbach and Grandy, 2011). Fractions like POXC and MBC are considered labile and are strongly associated (DuPont et al., 2010); yet showed different associations with bands, suggesting differences in functional group chemistry may underlie in the case of similarly labile fractions.

Long-term experiments have shown that organic matter management can increase labile C in the short-term and total soil C in the longer-term (Stevenson, 1982). The objectives of this study were to characterize Soil Organic Matter functional group with FT-IR spectroscopy under continuous cropping of finger millet (*Eleusine coracana*) - maize (*Zea mays* L.) cropping sequence.

## Materials and methods

### *Site description*

Indian Council of Agricultural Research (ICAR) has initiated the first long-term field experiment at Kanpur, India in 1905 subsequently long-term experiments were initiated at Pusa and Coimbatore in 1908 and 1909, respectively. Tamil Nadu Agricultural University initiated All India Coordinated Research Project on Long Term Fertilizer Experiment (LTFE) in 1972 at Coimbatore under irrigated conditions. Since inception, this LTFE center followed finger millet-maize-cowpea cropping sequence. From the year 2000 onwards finger millet - maize cropping system is being followed.

The present study was carried out on continuous experimentation with finger millet-maize cropping sequence over 49 years and 111 crops were raised so far. The soils of the experimental site belonged to Periyanaickenpalayam soil series and according to USDA soil taxonomy it has classified as sandy clay loam in texture and taxonomically grouped under *Vertic Ustropept*.

The experiment had ten set of treatments viz., 50% NPK (T<sub>1</sub>), 100% NPK (T<sub>2</sub>), 150% NPK (T<sub>3</sub>), 100% NPK + HW (T<sub>4</sub>), 100% NPK + Zn (T<sub>5</sub>), 100% NP (T<sub>6</sub>), 100% N (T<sub>7</sub>), 100% NPK + FYM (T<sub>8</sub>), 100% NPK (-S free) (T<sub>9</sub>) and absolute control (T<sub>10</sub>) with four replications. Out of ten treatments, four treatments were selected to study the functional group of SOM from the experiment which are absolute control, 100% recommended NPK, 100% recommended NPK + FYM @ 10 t ha<sup>-1</sup> and 100% NP alone. These four treatments showed the impact of mineral fertilizer alone including effects of sulphur (SSP as a P source), exclusion of K, mineral fertilizer along with manure 10 t ha<sup>-1</sup> of FYM and absolute control. In this cropping sequence, usually maize and finger millet crops were raised between January to May (summer period) and June-September (Monsoon period) respectively. SOC characterization was done by using the post-harvest samples of 110<sup>th</sup> Finger millet crop, which has harvested on 28.09.2020.

### *Experimental details*

Raised bed nursery was prepared and finger millet (CO 13) was sown, and the seedlings were transplanted with 30 cm X 10 cm spacing in main field. As per the treatment structure, twenty days before the transplanting of crop farmyard manure @ 10 t ha<sup>-1</sup> was applied uniformly over the main field. Hundred percent-recommended doses of N, P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O @ 90:45:17.5 kg ha<sup>-1</sup> respectively was applied basally in the main field.

### *Soil sampling*

Core sampler was used to collect the undisturbed soil core from 0-15 cm depth from four treatments with five replications for bulk samples. Bulk samples were mixed thoroughly and sub sampling (standard soil sampling method – quartering) was adopted for SOC functional group analysis through FTIR. For soil biochemical properties, humic acid and fulvic acid analysis the soils were collected from all the ten treatments along with four replications. The collected soil samples were air dried in room temperature and the clods were broken by wooden mallets and sieved with 2 mm mesh so that samples were free of roots or plant debris.

### ***Functional groups of SOM by Fourier Transform Infrared Spectroscopy (FT-IR)***

FTIR spectrometers are mostly used for measurements in the mid and near IR regions. For the mid-IR region, 2-25  $\mu\text{m}$  ( $5000\text{-}400\text{ cm}^{-1}$ ), the most common source is a silicon carbide element heated to about 1200 K. The output is similar to a blackbody. Shorter wavelengths of the near-IR, 1-2.5  $\mu\text{m}$  ( $10000\text{-}4000\text{ cm}^{-1}$ ), require a higher temperature source, typically a tungsten-halogen lamp. The long wavelength output of these is limited to about 5  $\mu\text{m}$  ( $2000\text{ cm}^{-1}$ ) by the absorption of the quartz envelope. For the far-IR, especially at wavelengths beyond 50  $\mu\text{m}$  ( $200\text{ cm}^{-1}$ ) a mercury discharge lamp gives higher output than a thermal source.

The air-dried soil samples were ground and sieved through a 2 mm sieve for further analysis. For FTIR analysis, the sample (300 mg) was mixed with 900 mg KBr (FTIR grade 99%) and ground in an agate mortar. The homogenous mixture was transferred into a diffuse reflectance cup without any pressure and leveled with a microscope glass slide. The FTIR spectra of the samples were measured on FTIR spectrometer. Soil microbial biomass C (SMB-C) and N (SMB-N) was estimated by chloroform-fumigation-extraction methods (Vance et al., 1987; Jenkinson, 1988) and SOC was estimated by Walkley and Black (1934) through chromic acid wet digestion.

The SOC stock was calculated by using the following formulae in terms of Mg C ha<sup>-1</sup>:

$$\text{SOC stock (Mg C ha}^{-1}\text{)} = (D \times \frac{BD}{100} \times \text{OC} \times 10^4 \text{ m}^{-2} \text{ ha}^{-1}) / 100 \quad (\text{Eq.1})$$

where D is the Depth of soil (cm) layer; B is the Bulk density ( $\text{g cm}^{-3}$ ); OC is the organic carbon in  $\text{g kg}^{-1}$ ; SOC sequestration ( $\text{kg ha}^{-1}$ ) = SOC stock in last year – SOC stock in initial year.

### ***Fractionation of humic acid and fulvic acid***

For analysis of humic substance, 10 g of air-dried soil was taken in a 200 ml propylene flask and 100 ml of 0.1 N NaOH solution was added to soil and kept for shaking at 24 h with room temperature. Then the sample was undergone centrifuging at 10,000 rpm for 10 min to separate the dark colored supernatant solution from the soil and again 50 ml of distilled water was added to the residual soil for collecting supernatant and this process was repeated until the supernatants obtained were clear or uncolored.

The alkaline extract was acidified by washing with 2N HCl (pH 2) then it was allowed to stand for 24 h at room temperature then the soluble material (fulvic acid) was separated from the coagulates (Humic Acid) by centrifugation. Both fractions were dried by rotary evaporator at about 40 °C.

### ***Welt ratio (E4/E6 value of HA and FA)***

To know the degree of humification, the alkali soluble fraction of humic acid and fraction was determined by using the ratio of optical absorbance at 465 and 665 nm. Each sample of 10 mg HA and FA samples were dried at 60 °C and dissolved by 25 ml of 0.05 N NaHCO<sub>3</sub> (Mangrich et al., 2000) and the absorbance of HA and FA were measured at 465 nm and 665 nm.

$$\text{E4 / E6 ratio} = \frac{\text{Optical absorbance at 465 nm}}{\text{Optical absorbance at 665 nm}} \quad (\text{Eq.2})$$

## Statistical analysis

The experimental design was randomized block design and to compare the differences in measured soil attribute between treatments and replications one way ANOVA was used and the significant differences between the treatments were tested by using Duncan's multiple-range test (DMRT) for comparison of means at  $P < 0.05$  significance level. The normalized FTIR spectral data are used to derive the spectral images by using Origin Pro 8.5 software packages.

## Results

### FT-IR transmittance spectra

The FTIR spectra of the soil samples in different treatments under long term fertilizer experiment are given in *Table 1*. The band wavelength of  $1081\text{ cm}^{-1}$  shows C-O vibration of C-O-C group of cellulose (Grube et al., 2006) and  $1400\text{ cm}^{-1}$  for carboxylic and carbonylic groups (Parker, 1971). Similarly,  $1540\text{ cm}^{-1}$  is due to C = C (Cocozza et al., 2003) and  $1600\text{ - }1613\text{ cm}^{-1}$  for C-C conjugated with C = O or  $\text{COO}^-$  (Niemeyer et al., 1992; Cocozza et al., 2003) and  $1698\text{ - }1701\text{ cm}^{-1}$  stands for C = O (carboxylic, cyclic and acyclic aldehydes and ketones) (Gondar et al., 2005). Aliphatic C-H (methyl and methylene) groups were resulted from the  $2922\text{ - }2926\text{ cm}^{-1}$  wave band (Niemeyer et al., 1992; Cocozza et al., 2003) and O-H group (including alcohols (R-OH), Phenols, water molecules (strong stretching) are from  $3500\text{ - }3200\text{ cm}^{-1}$  waveband (Cocozza et al., 2003) (*Fig. 1*).

**Table 1.** Soil organic carbon functional groups in various fertilizer management practices under finger millet–maize cropping sequence

Control	100% NP	100% NPK	100% NPK + FYM
OH group (including alcohols (R-OH), phenols, water molecules (low conc)	OH group (including alcohols (R-OH), phenols, water molecules (high conc)	OH group (including alcohols (R-OH), phenols, water molecules (medium stretching)	OH group (including alcohols (R-OH), phenols, water molecules (strong stretching)
Carbonyl (RCOR), carboxylic (RCOOH)	Carbonyl (RCOR), carboxylic (RCOOH)	Carbonyl (RCOR), carboxylic (RCOOH)	C = O (carboxylic acids), amide (RCONR <sub>2</sub> )
C-H aromatic (meta disubstituted benzenes - weak), vinyl (Cis substituted alkenes)	C-H aromatic (meta disubstituted benzenes), vinyl (Cis substituted alkenes)	P-O (phosphorus oxide)	C = O (carboxylates salts), amino acid, zwitterions
Alkyl halogen (C-Br, C-F, C-I, Si-Cl)	S - S (disulphide bond)	C-H aromatic (meta disubstituted benzenes), vinyl (monosubstituted alkenes)	C = O (aliphatic amines)
Peroxides (R-O-O-R)	P-O-P	P = S (P-S-S-P)	C-H (cis disubstituted alkenes)
	Peroxides (R-O-O-R)	Halogenes (C-Cl, C-F, CF)	C-H (aromatic – meta disubstituted benzenes) strong
	P = S (phosphorothioate)		P = S (phosphorothioate)
			S – S (disulphide bond)
			Halogen (C-Cl, RCF <sub>3</sub> )

### Control

Transmittance spectra of control treatment showed the presence of OH group (including alcohols (R-OH), Phenols, water molecules (Low Conc), Carbonyl (RCOR),

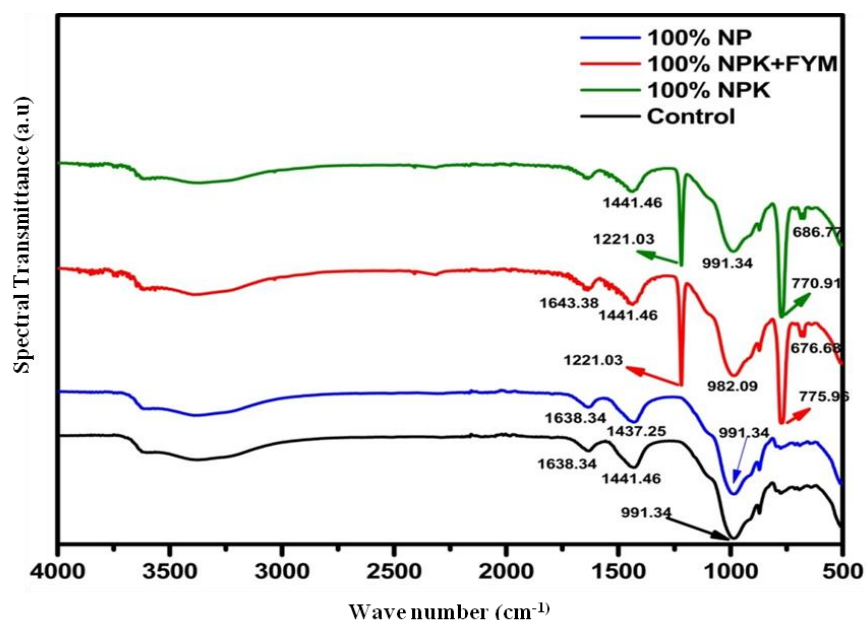
Carboxylic (RCOOH), C-H aromatic (meta disubstituted benzenes - Weak), Vinyl (Cis substituted alkenes), Alkyl Halogen (C -Br, C-F, C-I, Si- Cl) and Peroxides (R-O-O-R) functional groups in soil.

#### 100% NPK

The transmittance spectrum for the soils with 100% NPK treatment showed the presence of OH group (including alcohols (R-OH), Phenols, water molecules (medium stretching), Carbonyl (RCOR), Carboxylic (RCOOH), P-O (Phosphorus Oxide), C-H aromatic (meta disubstituted benzenes), Vinyl (monosubstituted alkenes), Halogens (C-Cl, C-F, CF) and P = S (P-S-S-P) (Phosphorothioate).

#### 100%NPK + FYM

Over the years, application of mineral fertilizer along with FYM treatment contained strong stretching OH groups (including alcohols (R-OH), Phenols, water molecules, C = O (Carboxylic Acids), Amide (RCONR<sub>2</sub>), C = O (Carboxylates (salts), amino acid, Zwitterions, C = O (Aliphatic Amines), C-H (Cis disubstituted alkenes), C-H (Aromatic - meta disubstituted Benzenes) strong P = S (Phosphorothioate), S - S (Disulphide Bond) and Halogen (C-Cl, RCF<sub>3</sub>) functional groups.



**Figure 1.** Spectral transmittance pattern of different treatments by using FT-IR spectroscopy

#### 100%NP

Omission of K and application of NP mineral fertilizer showed the presence of OH group (including alcohols (R-OH), Phenols, water molecules (High Conc), Carbonyl (RCOR), Carboxylic (RCOOH), C-H aromatic (meta disubstituted benzenes), Vinyl (Cis substituted alkenes), S - S (Disulphide Bond), P-O-P and Peroxides (R-O-O-R) and P = S (Phosphorothioate).

### SOC stock and carbon sequestration/depletion

Over the four decades, application of inorganic and organic nutrients showed significant buildup of SOC stock and carbon sequestration except in control. The highest net carbon sequestration was recorded in greater extent under INM over 47 years (6342 kg ha<sup>-1</sup>) followed by application of 150%NPK (5550 kg ha<sup>-1</sup>) and also the SOC stock was registered as high in INM (133.3 Mg C ha<sup>-1</sup>) followed by 150% NPK (108.2 Mg C ha<sup>-1</sup>) (Fig. 2).

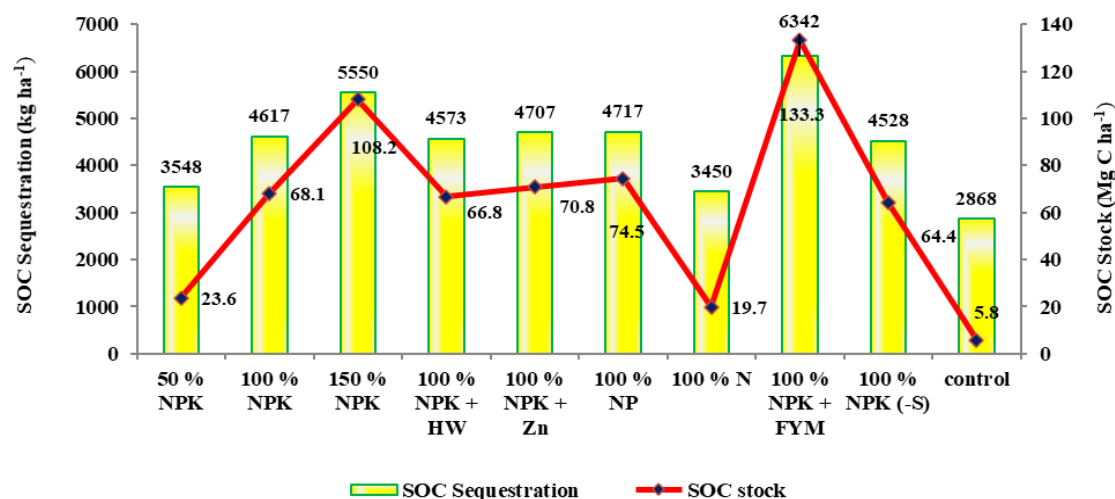


Figure 2. Soil organic carbon (SOC) sequestration (kg ha<sup>-1</sup>) and SOC stock (Mg C ha<sup>-1</sup>)

### Changes of biochemical properties (SOC, SMBC and SMBN)

Biochemical changes are one of the nutrient transformations in soil and a pool of nutrients for crop growth. Continuous application of manure along with fertilizers (INM or T<sub>8</sub>) led to increase in ( $P < 0.05$ ) SOC (%), SMBC and SMBN compared to control which was ranged from 0.490 – 0.749%, 195 – 319 and 19.90 – 47.16 mg kg<sup>-1</sup> respectively in finger millet (Table 2).

### Humic acid (HA) and fulvic acid (FA) fractions

SOC stability in soil was characterized by quantification of humic acid (HA) and fulvic acid (FA) properties further the spectroscopic studies has been performed in fraction of HA and FA fractions with the optical absorbance of 465 and 665 nm (E<sub>4</sub>/E<sub>6</sub> or Welt ratio) (Table 3).

Humic and fulvic acid fractions were significantly higher in the treatment of FYM @10 t ha<sup>-1</sup> along with 100% NPK followed by 150% NPK and the lowest values were found in control treatment. Application of FYM @10 t ha<sup>-1</sup> was recorded the highest HA of 0.79 and FA of 3.93% in 100% NPK + FYM 10 t ha<sup>-1</sup> and the lowest HA of 0.10 and FA of 0.78% in control treatment in continuous addition of manures and fertilizers with intensive cropping.

Welt ratio (E<sub>4</sub>/E<sub>6</sub>) significantly implied by addition of manures and fertilizers and the lowest E<sub>4</sub>/E<sub>6</sub> values of 3.85 and 6.54 and the highest value of 4.73 and 7.93 was observed in humic and fulvic acid respectively.

**Table 2.** Soil biochemical changes by continuous fertilization and intensive cropping under finger millet–maize cropping sequence

Treatments	Soil organic carbon (%)	Soil biomass carbon (mg kg <sup>-1</sup> )	Soil biomass nitrogen (mg kg <sup>-1</sup> )
50% NPK	0.559	281	30.76
100% NPK	0.59	300	42.29
150% NPK	0.652	317	43.82
100% NPK + HW	0.61	294	34.23
100% NPK + Zn	0.61	293	38.63
100% NP	0.588	284	28.93
100% N	0.571	188	22.16
100% NPK + FYM	0.74	319	47.16
100% NPK (-S)	0.613	295	37.15
Control	0.49	195	19.9
SEd	0.013	5.92	0.939
CD (p = 0.05)	0.028	10.08	1.59

**Table 3.** Soil organic fraction (SOC) by continuous fertilization and intensive cropping under finger millet–maize cropping sequence

Treatments	HA (%)	FA (%)	E4/E6 (HA)	E4/E6 (FA)
50% NPK	0.30 g	2.78 d	4.30 c	7.75 b
100% NPK	0.67 bc	3.35 b	4.10 de	6.98 de
150% NPK	0.70 b	3.42 b	4.05 e	6.80 f
100% NPK + HW	0.63 cd	3.36 b	4.16 d	7.03 cd
100% NPK + Zn	0.66 bc	3.40 b	4.28 c	6.84 ef
100% NP	0.43 f	3.00 c	4.51 b	7.71 b
100% N	0.52 e	2.41 e	4.59 b	7.82 ab
100% NPK + FYM	0.79 a	3.93 a	3.85 f	6.54 g
100% NPK (-S)	0.61 d	3.30 b	4.14 de	7.16 c
Control	0.10 h	0.78 f	4.73 a	7.93 a
SEd	0.01	0.05	0.04	0.07
CD (p = 0.05)	0.02	0.11	0.10	0.15

## Discussion

### Characterization of soil organic matter functional groups by FTIR spectroscopy

#### Control

Continuous cropping and omission of mineral fertilizer and organic manure could have reduced the organic carbon and carbon sequestration (*Fig. 1*). In control treatment it was maintained with no mineral fertilizer and manure, the contribution of root biomass may be the reason for the presence of weak C-H aromatic and peroxide functional groups. Sometimes presence of peroxide functional groups acts as catalyst in the decomposition of organic matter, which might have resulted in the lower organic carbon content (0.49%),



and carbon sequestration (2868 kg ha<sup>-1</sup>). Positive correlation (which is modest) is between the proportions of soil C in aromatics and carbonyls functional groups in cultivated, non-cultivated, grass cultivated and forest whole soils (Mahieu et al., 1999). Similarly, Rumpel et al. (2006) who reported that in soil of tropics, OM accumulation in the soil is low as high temperatures and moist conditions cause high litter decomposition and mineralization rates, resulting in low levels of OM accumulating in the soil. Similarly, Heller et al. (2015) who suggested that more aerobic conditions in topsoil peats resulted in larger rates of mineralization and causes a considerable loss of C under long-term arable farming.

#### *100% NPK*

As compared to the control treatment, it contained phosphate and aromatic carbon functional groups excluding peroxides. This might have attributed to the presence of higher humic acid (0.67%) and fulvic acid (3.35%) coupled with higher organic carbon (0.59%) and total carbon stock (4617 kg ha<sup>-1</sup>). This was in line with the findings of Jasmund and Lagaly (1993) who revealed that, the CEC active part of SOM is able to interact with clay mineral particles building up organo mineral complexes using polyvalent cation as a bridge. This stabilization is the cause that fertilization needs a longer time to affect SOM composition in soil. SOC functional groups identification, the C-H and C = O absorption bands, which are specific for organic matter in soil (Heller et al., 2015). The increasing ratio of C to O functional group (Margenot et al., 2015) is thought to be associated with greater recalcitrance of SOM (Ding et al., 2002).

#### *100% NPK + FYM*

Application of 100% NPK along with FYM was found favorable for increased content of humic acid (0.77%) and fulvic acid (3.93%) which might have contributed to the presence of amide (Margenota et al., 2015) and functional groups containing sulphur as compared to other treatments. Continuous additions of FYM have resulted in higher organic carbon content (0.74%) and carbon stock (6342 kg ha<sup>-1</sup>) and humic acid. Humic substances coated on clay mineral have many types of reactive functional groups present as a reactive surface. Sulfur nucleophiles can react with some of those functional sites, incorporating sulfur into humic substances. Thus, sulfhydryl (thiol) and polysulfide functionalities are from the reactions involving hydrogen sulfide (HS<sup>-</sup>) and polysulfide ions, respectively. Thiol groups can react in a variety of ways. When a thiol group is added across an unsaturated bond, an organic sulfide bond has to be formed.

Adding a thiol group to an unsaturated bond represents a diagenetic mechanism for introducing a sulfide bond in humic substances. However, compounds containing sulfide bonds are common in organisms (for example, the amino acid methionine), and therefore, the sulfide bond also could be of biological origin (Vairavamurthy et al., 1987). This was supported by the findings of Wattel-Koekkoek et al. (2001) who found that the higher stability of OM from the smectitic soil is caused by its interaction to cation bridges, and its content of aromatic components. Also supported by Autorenkollektiv (1984) who stated that, hydrophilic character organic substances depends on the composition (type and amount) of functional groups, mainly carboxyl and hydroxyl-groups and may affect the soil water storage capacity, CEC and stability of SOM in the plough horizon. Persson and Axe (2005) and Heller et al. (2015) reported that the large C-H/C = O ratio might

have protected the C-H containing SOM against microbial decomposition because of the adsorption of heavy metal especially Fe.

### *100% NP*

Absence of potassium application would have resulted in the less stable organo-metal complex containing peroxide functional groups. When comparing the treatments, control and omission of K plots observed the peroxide functional groups and absence of carboxylates. This may be because of the less stable aggregates by weak interaction of cation bridges with clay organo-metal complexes leading to easily soluble OM. This was in association with the findings of Wattel-Koekkoek et al. (2001) who argued that higher stability of OM from smectite is caused by interaction to cation bridges and its content of aromatic components also Kaiser and Ellerbrock (2005) stated that the solubility of SOM is related to a certain degree to the stability of SOM components.

### ***Biochemical changes in continuous addition of chemical fertilizers and organic amendments on soil***

Combined application of mineral fertilizer along with organic amendments would help to increase the plant biomass yield and increases the carbon input to soil which is a main factor for higher SOC (0.74%). Also, addition of FYM with mineral fertilizer produces the cationic bridges with the functional groups, which leads to reduced SOM solubilization or oxidation. These SOM provide a better soil environment for proliferation of soil microbial population which would increase the SMBC (319 mg kg<sup>-1</sup>) and SMBN (47.16 mg kg<sup>-1</sup>). Even though, mineral fertilizer alone also gave higher plant biomass that could increase the SOC (0.652%), SMBC (317 mg kg<sup>-1</sup>) and SMBN (43.82 mg kg<sup>-1</sup>) but when compared the manure with mineral fertilizer, these mineral fertilizer treatments (50% NPK, 100% NPK and 150% NPK) decreased the SOM and other microbial biomass like SMBC and SMBN (Li et al., 2008). Because application of mineral fertilizers alone did not improve soil physical properties like aggregate stability and low bulk density, which may directly have influenced the soil biological as well as physico-chemical properties. Though application of organic amendments, provide some extents of major and micronutrients and carbon inputs to soil. This was in agreement with the findings of Jiang et al. (2014) who stated that greater SOM was observed by the application of organics + fertilizers treatments than either organics or fertilizers alone, which may also be attributed to increased root growth associated with additional C inputs from roots (Luo et al., 2015).

### ***Humic acid and fulvic acid fractions***

By using FYM @ 10 t ha<sup>-1</sup> has added quantum of nutrients as well as organic matter to the soil since, observed the highest HA (0.79) and FA (3.93%) in 100% NPK + FYM 10 t ha<sup>-1</sup> (Marinari et al., 2007) and the lowest was in (HA of 0.10 and FA of 0.78%) control treatment.

Spectroscopic analysis of humic fractions of HA and FA, the lowest w/e ratio of 3.85 and 6.54 and the highest value of 4.73 and 7.93 was observed in humic and fulvic acid respectively. Here, the w/e ratio was less than 5 showing the high degree of humification (Freppaz et al., 2002) and well decomposed organic matter with high molecular weight aromatic molecules in their structure (Duval, 1993) and low molecular weight have high w/e ratio (Chen et al., 1977) of fulvic acid. In addition, Peat soil showed selective

enrichment of more recalcitrant compounds that could be traced by their alkyl-C groups with NMR spectroscopy (Leifeld et al., 2012).

## Conclusion

The results of this investigation indicated that differences in fertilizer applications viz., mineral fertilizers alone and along with FYM in finger millet – maize cropping sequence over a long period (over 48 years) lead to differences in the amount and functional composition of SOM in swell shrink soil (*Vertic Ustropept*). Among the different treatments such as 100% NPK, 100% NPK + FYM, 100% NP and control, INM treatment (100% NPK + FYM) observed the P = S (Phosphorothioate), S-S (Disulphide Bond), aromatic C- H functional group were present. This may be due to high carbon stability by the way of high degree of humification and high weight aromatic molecules and formation of clay metal ion complexes through cationic bridges of polyvalent cations. Control and 100% NP treatments observed the peroxide and strong stretching of hydroxyl functional group. It showed the easily soluble and oxidizable OM which lead to less carbon stock. The results suggest that application of mineral fertilizers along with FYM increases the carbon stock and sequestration by the presence of more recalcitrant SOM, CEC and nutrient availability over the years of cropping in smectitic clay. According to the present studies, it is strongly recommended for future studies to identify and quantify the SOC functional groups by practicing various nutrient management in the long run.

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