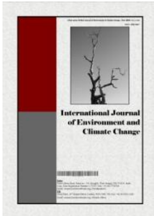

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Long-term Use of Balanced and Integrated Nutrient Management Improved Soil Aggregation and Carbon Stabilization in a Maize-wheat Cropping Sequence

Anmol Singh[1*], G. S. Dheri[1] and D. K. Benbi[1]

[1]Department of Soil Science, Punjab Agricultural University, Ludhiana, 141001, India.

Authors' contributions

This work was carried out in collaboration among all authors. Author AS managed the analyses of the study, performed the statistical analysis and wrote the first draft of the manuscript. Author GSD designed the study, wrote the protocol and managed the literature searches. Author DKB provides the literature and revise the manuscript.

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ABSTRACT

Sustainable nutrient management practices have the potential to enhance carbon (C) storage capacity of agricultural soils that may help offset increasing atmospheric CO_2 concentration. Nutrient management practices on long-term basis differentially influence aggregates and distribution of soil organic C (SOC) present within aggregates, which in time may affect C stabilization. The present study assessed the impact of long-term application of fertilizers for 44 years either alone or integration with farmyard manure (FYM) on bulk density, SOC and C pools, potassium permanganate oxidizable C ($KMnO_4$), aggregate stability and distribution of C fractions within different size aggregate under maize-wheat cropping sequence. The application of 100%NPK+FYM significantly ($P< 0.05$) improved soil aggregation and mean weight diameter (MWD). The percent of macro-aggregates (MacroA) and meso-aggregates (MesoA) was maximum in 100%NPK+FYM followed by NPK and the minimum in the control treatment. Irrespective of aggregate classes, TOC (g kg^{-1} aggregate) was maximum in 100%NPK+FYM treatment with an average of 8.42 g kg^{-1} aggregate as compared to control (5.05 g kg^{-1} aggregate). If averaged

**Corresponding author: E-mail: anmol-soil@pau.edu;*

across the treatments, TOC concentration in aggregates followed the order MacroA> MesoA>MicroA. Correspondingly, results for $KMnO_4$-C were similar in different treatments and aggregate classes. Application of FYM with inorganic fertilizers (NPK) or NPK showed a significant increase in all oxidizable organic C fractions particularly recalcitrant C fraction, which reflects the stable nature of OC as compared to very labile and labile C fractions. In general, C present in mineral fraction and large-sized aggregates (MacroA) has higher recalcitrant fractions of SOC as compared to small-sized aggregates (MesoF and MicroF). The study concluded that long-term balanced and integrated nutrient management improved soil aggregation, C distribution within aggregates, and C storage capacity of soils under maize-wheat. Carbon associated with macro aggregate and a mineral fraction has more recalcitrant C fraction compared to meso and micro aggregate fractions.

Keywords: Long-term nutrient management; water-stable aggregates; aggregate associated C; organic carbon fractions; mean weight diameter.

1. INTRODUCTION

Soil can act as a sink for atmospheric carbon (C) when appropriate management practices are used [1,2]. Estimates of the historic C loss from geologic and terrestrial pools and transfer to the atmospheric pool ranges from 40 to 90 Pg C by Houghton [3], Schimel [4]. On average, there is a net transfer of 3 ± 0.1 pg C yr^{-1} to the atmosphere from geologic and terrestrial pools and there is a potential to put back almost 500 Pg of C into terrestrial biosphere [5]. Agricultural management practices that can lead to C sequestration in soil include the application of organic manures and bio-solids, crop residue recycling, mulch farming, conversation agriculture, agroforestry systems, diversified crop rotations and elimination of fallow [6]. All these sustainable practices have the potential to enhance the C sink capability of cultivable soils [7,8]. Several studies have documented the favorable effect of balanced fertilization and integrated nutrient management on SOC [9,10,11]. Use of different fertilizers alone or in combination with organic manures add C into the soil, a part of added C is stabilized into SOC and distributed among different pools or fractions. In contrast, the simultaneous measurement of various SOC fractions i.e. TOC (total organic carbon), labile fractions, and $KMnO_4$ oxidizable C required for assessing the effects of management on C changes.

Fertilizer application has a profound impact on SOC dynamics by altering soil physical, chemical, and biological processes including soil aggregation [12]. Soil aggregation can physically protect SOC from decomposition as they entrap C within them. Physical protection has rendered through the formation of organo-mineral complexes, which reduce direct contact between the SOC and enzymes of soil microbes [13]. The SOC is stored in primary mineral fraction, peculiarly in clay [14,15]. Besides, macro-aggregates (>2 mm) having a 2:1 type clay mineralogy have a higher amount of SOC than meso (1-2 mm) and micro (<1 mm) aggregates (REF). Benbi and Senapati [9] reported that the addition of crop residue and organic manure in the rice-wheat system improves soil aggregation and results in greater C sequestration in macro-aggregates. Hence, soil aggregation can enhance SOC sequestration by preventing its losses through erosion and from mineralization by entrapment into interlayer spaces or by sorption onto clay through electrostatic forces or co-adsorption [16]. The aggregate hierarchy theories [17] along with the postulates of micro-aggregates formation within macro-aggregates by Oades [18] profusely explain the stabilization of organic C in macro-aggregates. Long-term studies conducted under temperate conditions (Rothemsted and Askow) indicate better soil aggregation and enhanced C accumulation with the application of fertilizers. Such long-term fertilizer studies under tropical conditions are limited to understand the impact of fertilizer application on soil aggregation and relative contribution of soil aggregates towards C stabilization. The present paper aimed to evaluate the effect of prolonged use of fertilizer and farmyard manure on aggregation and C fractions of soils cultivated intensively in the corn and wheat cultivation system in tropical conditions.

2. MATERIALS AND METHODS

2.1 Site Description

A field experiment on maize (*Zea mays* L.)-wheat (*Triticum aestivum* L.)-fodder cowpea (*Vigna unguiculata* L.) cropping sequence has been in progress since 1971 at the research farm of

Punjab Agricultural University, Ludhiana (30^o 54'19'' N latitude and 75^{o}47'09'' E longitude, 274 m above the mean sea level) India. Fodder cowpea was grown in the sequence until 1999 and after that its cultivation has discontinued. The experimental region has classified as semi-arid, sub-tropical with cold winters, and hot summers. The mean monthly minimum and a maximum temperature range between 18.56^{o}C and 30.57^{o}C, respectively. Annual rainfall averages 591.6 mm and ~80% of it has received during monsoon i.e. months of June to September.

2.2 Description of the Experiment

The experiment has laid out in a randomized block design (RBD) with three replicates having a plot size of 12 m × 15 m. Three treatments viz. i) control- no fertilizer application, ii) NPK – application of university recommended rates of fertilizer N, P and K, and iii) INM- integrated nutrient management involving the use of fertilizer NPK along with farmyard manure (FYM @ 10 Mg ha^{-1}) selected. The recommended NPK rates from 1971-72 to 1998-99 were 150 kg N-32.7 kg P and 31.1 kg K ha^{-1} to both maize and wheat and 20 kg N, 17.5 kg P and 16.6 kg K ha^{-1} to cowpea. After discontinuation of cowpea in 1999, the fertilizer N, P, and K rates for both maize and wheat changed to 120, 26.5, and 25 kg ha^{-1}, respectively. The dose of fertilizer (NPK) and FYM applied annually to one cropping cycle of maize-wheat. Annually FYM was used before the sowing of each maize crop. Fertilizer N, P, and K were applied through urea (46% N), single superphosphate (16% P_2O_5) and muriate of potash (60% K_2O), respectively. Maize was grown during monsoon season (May to September) and wheat during the winter season (November to April). In maize, one-third of N and the entire quantity of P and K drilled at the time of sowing, and one-third of N top-dressed at the knee-high stage and the remaining one-third at the pre-tasseling stage. In wheat, half of N, whole P, and K drilled at the time of sowing, and half of N was top-dressed at the time of first irrigation. The soil has intensively plowed using one harrowing and two cultivators and one planked to prepare the final seedbed. Tractor operated drill has applied for the sowing of wheat and dibbling methods has used for the sowing of maize on flat seedbed. Crops have irrigated using both canal and borewell water at critical crop growth stages. All other agronomic practices followed as per the package and practices for crops of Punjab as recommended by Punjab Agricultural University, Ludhiana and Punjab, India.

2.3 Soil Sampling and Analysis

Surface soil samples (0-15 cm depth) have collected after wheat harvest in May 2016. A large piece of soil clod was obtained from each plot and let it break naturally by dropping on a hard floor from knee height. The broken small clods have used for aggregate size analysis. Soil bulk density (D_b) of surface soil (0-15 cm) has determined by the core method. Metallic cores (10 cm inner diameter and 13 cm length) were used to collect soil samples for D_b. The fresh soil samples were initially weighed and then dried at a temperature of 105°C for 24 hr to determine the moisture and D_b were calculated as per the procedure outlined by Grossman and Reinsch [19]. The collected soil samples were air-dried in the shade and a small portion of it was passed through 2 mm sieve for organic carbon (OC) analysis of bulk soil and the rest of the samples were processed for subsequent analysis of aggregate size distribution and aggregate associated organic carbon.

Aggregate size distribution was determined by wet sieving [20]. A Yoder assembly fitted with a nest of sieves of pore diameter of 2.0, 1.0, 0.5, 0.25 and 0.053 mm was used for separating the soil aggregate classes. Air-dried soil samples were broken apart by giving gentle strokes with a rubber hammer. The one portion of samples (50g) retained on 4.75 mm sieve after passing through an 8 mm sieve was placed on the top (2.0 mm sieve) of a nest of sieves. Four nests of sieves were placed in different drums filled with water such that the water level in the drum just touched the base of the 2 mm sieve and samples were allowed to slake for 15 min. The four nests of sieves oscillated simultaneously for 30 min at 30 vertical oscillation per min in drums. The amount of soil retained on the sieve was dried at 105^{o}C for 24 h to determine the mean weight diameter (MWD) and water-stable aggregates (WSA). Similarly, another portion of samples (200 g) retained on 4.75 mm sieve was oscillated in drums to collect the maximum weight of different aggregates and these aggregates were dried at 40°C to determine aggregate-associated OC. Total WSA were grouped into three main aggregate size classes viz. MacroA (>2.0 mm), MesoA (2.0-0.25 mm) and MicroA (0.25 mm - 0.053 mm). The MWD was calculated using the following relationship [21]:

$$MWD = \frac{\sum_{i=1}^{n} XiWi}{\sum_{i=1}^{n} Wi}$$

Where 'n' = number of fractions, X_i = mean diameter (mm) of the sieve size class, and W_i= weight of soil (g) retained on the i^{th} sieve. Similarly, a mineral fraction (MinF <0.053mm) collected from the base of each drum after siphoning off the water was air-dried at 40^0C to determine C fractions associated with them.

Aggregates and MinF were analyzed for organic C fractions of different oxidisability by modified Walkley and Black [22] method using acid-aqueous solutions of 12 *N*, 18 *N* and 24 *N* H_2SO_4 [23].

Based on oxidizability, SOC was categorized into the following 4 fractions:

- Fraction 1 (Very labile) = Organic C oxidizable under 12 *N* H_2SO_4
- Fraction 2 (Labile) = Difference in oxidizable organic C extracted between 18 *N* and 12 *N* H_2SO_4 (18 *N*-12 *N* H_2SO_4)
- Fraction 3 (Less Labile) = Difference in oxidizable organic C extracted between 24 *N* and 18 *N* H_2SO_4
- Fraction 4 (Recalcitrant): Residual organic C after reaction with 24 *N* H_2SO_4 when compared with the total organic carbon (TOC-24 *N* H_2SO_4)

Total organic carbon (TOC) was determined using dry combustion method as mentioned by Ball, [24] and $KMnO_4$-C was determined by potassium permanganate oxidation (0.33 *M* $KMnO_4$) method as described by Blair [25].

2.4 Statistical Analysis

Data on the aggregate size distribution and C concentration were statistically analyzed using the Generalized Linear Model (GLM). The treatment means were compared by Duncan's Multiple Range Test (DMRT) using SAS software version 9.3.

3. RESULTS AND DISCUSSION

3.1 Bulk Density and Mean Weight Diameter

Long-term use of inorganic fertilizers either alone or in combination with FYM resulted in an insignificant decrease in soil Db (Fig. 1). Long-term use of NPK and NPK+FYM decreased soil Db by 6% and 10%, respectively compared to unfertilized control. Whereas, results for mean weight diameter (MWD) were vice-versa for the long-term use of inorganic fertilizers either alone or in combination with FYM as compared to results for bulk density (Fig. 1). The MWD was significantly higher in 100%NPK+FYM application (0.40 mm) as compared to control treatment (0.18 mm).

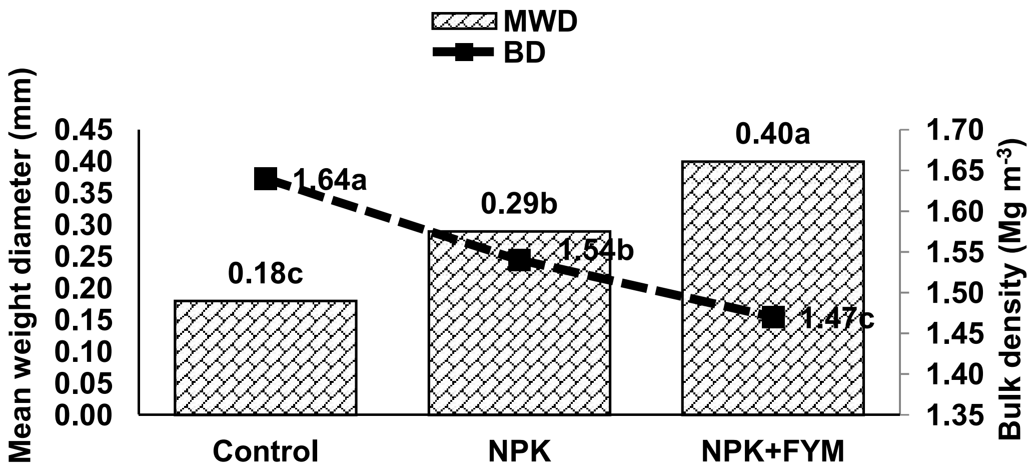
Long-term application of fertilizer and FYM resulted in a significant decrease in D_b and an increase in MWD. These effects could be attributed to improved soil aggregation under NPK and FYM treatments. Application of inorganic and organic fertilizers results in greater root-derived and external C input to the soil [26, 27,28]. The organic acids and polysaccharides released during the decomposition of root biomass and organic residues act as a cementing agent resulting in better aggregation (Ref). All these factors help in increasing the aggregate size, hence the MWD [29]. The improved MWD results in higher porosity, and mass per unit volume has decreased, i.e. lower D_b. Improvement of MWD and decline in D_b results in better soil aggregation, which helps in better crop growth. Our results for MWD were in agreement with Su et al [30], who reported that MWD of aggregates was 0.48 mm higher for treatments under FYM than for treatments lacking FYM in the wheat-wheat-maize cropping system. Higher soil aggregation in maize-based system might be due to the gel-like root exudates of maize released to the soil creating a more stable soil structure around the roots [31].

3.2 Soil Organic Carbon and $KMnO_4$-C in Bulk Soil

The application of balanced fertilizers accumulated 34.2% more soil TOC compared to unfertilized control (Fig. 2). A maximum and significant buildup of TOC (70.3%) was observed under the integrated use of fertilizer NPK and FYM. $KMnO_4$-C was more sensitive to fertilizer application and showed more considerable improvement than TOC. $KMnO_4$-C improved by 43.6% and 59.45% under NPK and NPK+FYM, respectively over control. $KMnO_4$-C comprised 33 to 37% of TOC under different treatments (Fig. 2).

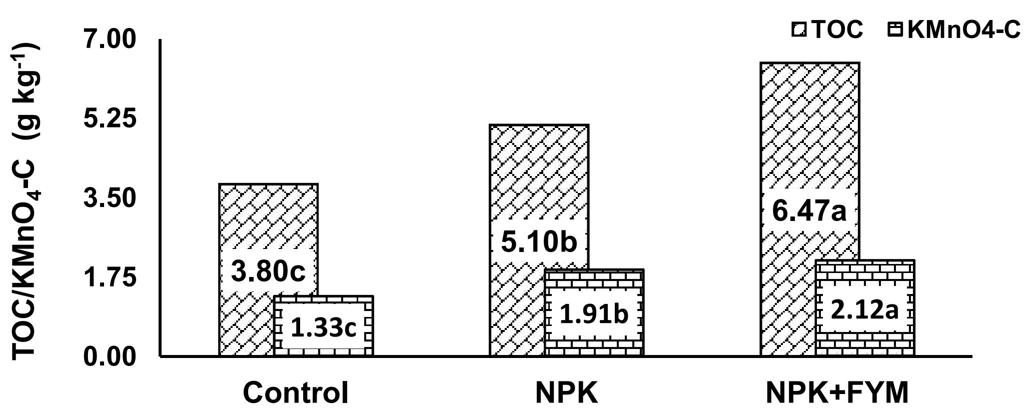
Higher $KMnO_4$-C concentration in fertilized plots may be attributed to increased crop root and shoot biomass having higher microbial activity and secretion of root exudates (rich in C compounds) which are easily oxidized [32].

Kumar et al [33] also reported higher $KMnO_4$-C with the application of fertilizers compared to control in the maize-wheat cropping system. Significantly higher $KMnO_4$-C in soil with fertilizers and FYM application in maize-wheat and rice-wheat cropping systems had been reported earlier [34,12]. Improvement of TOC under long-term application of balanced fertilizer and INM under sub-tropical conditions indicates that the adoption of improved fertilizer recommendations in intensive maize-wheat cropping systems has the potential to improve C status of soil which was low in antecedent C level.



Averaged values in bars by different alphabets, differ significantly (p = 0.05) by Duncan's Multiple Range Test (DMRT)

Fig. 1. Effect of long-term integrated nutrient management on mean weight diameter and bulk density of surface soil (0-15 cm) under maize-wheat system



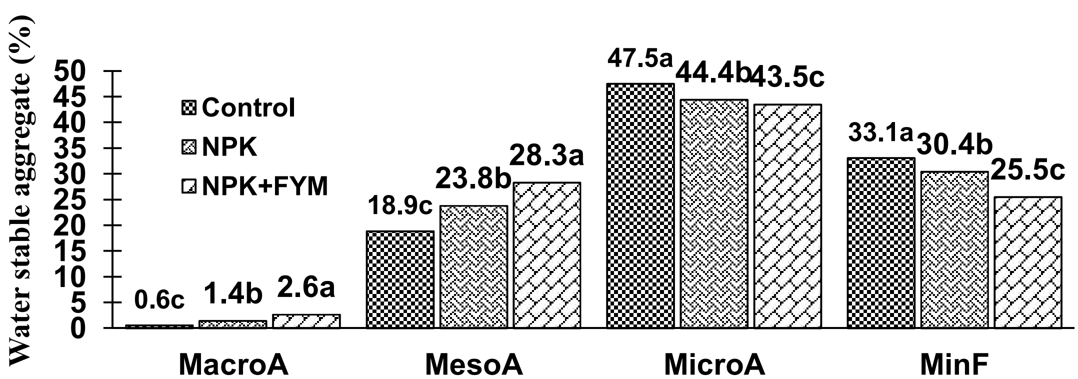
Averaged values in bars by different alphabets, differ significantly (p = 0.05) by Duncan's Multiple Range Test (DMRT)

Fig. 2. Effect of long-term use of fertilizer and FYM on total organic carbon (TOC) and $KMnO_4$-C of surface soil (0-15 cm) under maize-wheat system

3.3 Water Stable Aggregates

Continuous use of fertilizers and FYM significantly influenced water-stable aggregates and their distribution among different size classes. Macro and Meso aggregates were highest (30.9%) under conjoint application of NPK and FYM and lowest (19.5%) in control plots (Fig. 3). The contribution of MicroA in TWSA was maximum (43.5% to 47.5%) followed by MesoA (18.9% to 28.3%) of TWSA and the proportion of MacroA was at least (0.52% to 2.64%). When evaluated across fertilizer treatments, the application of NPK+FYM increased the occurrence of MacroA and MesoA by 312% and 49.7% respectively, as compared to control. Similarly, the use of NPK increased the presence of MacroA and MesoA by 119% and 25.9% respectively, over control. On the contrary, the proportions of MicroA fraction was significantly lower under the application of NPK +FYM (43.5%) as compared to control (47.5%). A similar trend as that of MicroA fraction was noticed in MinF i.e. the application of NPK +FYM (25.5%) has lower MinF as compared to control (31.1%).

Several theories regarding the improvement of macro-aggregates with the application of organic manures were projected [17,35,36]. Cheshire [37] and Martin [38] proposed the theory that polysaccharides and organic acids were released during the microbial breakdown of organic material, which leads to the stabilization of soil macroaggregates. Freshly added organic residues act as a site of nucleation for fungal and other microbial growth because polysaccharides and organic acids produced from their decomposition had lower diffusivity from the site of production, resulting in the cementation of residues and soil particles into macro-aggregates [34,39,40]. In our study, the favorable effect of FYM may also be attributed to the theories mentioned earlier. Our results depicting the positive impact of FYM incorporation on the formation of macro-aggregates are similar to those reported by other authors in several studies [41,42,43,44]. Benbi et al. [43] reported that there was a significant increase in WSA of all the sizes (>2, 1-2, 0.5-1, 0.25-0.5 and 0.11-0.25mm) and TWSA was significantly increased by 12% over control with the incorporation of FYM for 20 years under a maize-wheat-fodder cowpea cropping system. Elliot [45] revealed that aggregation of soil particles was improved with the application of organic materials into the soil. Although the present study was conducted under intensive cultivation conditions, still maize-wheat cropping system received balanced or INM fertilizer applications promote soil aggregation hence soil physical conditions. Perhaps, the reading of Tisdall and Oades [17] and Oades [18], mentioned above explain why there are a higher microA and MinF under control treatment. Under control, polysaccharides and other binding agents are missing which are released during the decomposition of organic matter and hence MicroA and MinF are unable to bind together in MacroA.



Averaged values in bars by different alphabets, differ significantly (p = 0.05) by Duncan's Multiple Range Test (DMRT)

Fig. 3. Distribution of water stable aggregate (%) in surface soil (0-15cm) under long-term integrated nutrient management in maize-wheat system

Table 1. Distribution of carbon fractions in different aggregate fractions (g kg-1 of aggregate) and bulk soil (g kg-1 soil) as influenced by long-term application of fertilizer

Treatment	MacoA	MesoA	MicroA	MinF	Bulk Soil
			Very labile C fraction		
Control	0.98c	0.75c	0.37c	0.29c	1.15c
NPK	1.88b	1.49b	1.15b	1.09b	1.89b
NPK+FYM	2.60a	2.31a	1.67a	1.61a	2.62a
	Labile C fraction				
Control	0.72c	0.53c	0.24c	0.24c	1.01b
NPK	1.37b	1.24b	0.86b	0.87b	1.08b
NPK+FYM	2.34a	1.89a	1.74a	1.74a	1.56a
	Less labile C fraction				
Control	2.87c	2.23b	1.32b	1.32b	0.78b
NPK	3.13b	2.86a	2.07a	2.07a	1.29a
NPK+FYM	3.37a	3.08a	2.42a	2.33a	1.40a
	Recalcitrant C fraction				
Control	1.29b	1.66c	0.97a	3.25a	0.87a
NPK	2.67a	2.07b	1.42a	3.77a	0.84a
NPK+FYM	3.28a	2.39a	1.34a	4.34a	0.89a

#Averaged values within a column, succeeded by different alphabets, differ significantly (p = 0.05) by Duncan's Multiple Range Test (DMRT)

3.4 Aggregate-associated Carbon

3.4.1 Oxidizable organic carbon fractions

The very labile fraction of SOC was significantly higher under NPK and NPK+FYM over control (Table 1). MacroA had a higher concentration of very labile C fraction than MesoA and MicroA. The MinF exhibited the lowest concentration of very labile C. Integrated nutrient management (INM) significantly improved very labile C fraction over NPK and control. Across the aggregate classes, INM increased very labile C fraction by 212% over control and 46% over NPK treatment. Irrespective of treatments, all the aggregate classes had higher value for very labile C fraction than MinF. Among aggregate sizes, the very labile fraction is more elevated in MacroA as compared to MicroA fraction. Similar effects of fertilizer application on labile C fraction were observed for different aggregate size classes.

The concentration of C in less labile C fraction was higher compared to other fractions. MacroA contained a maximum amount of less labile C fraction compared to MesoA, MicroA, and MinF across the fertilizer treatments. The effect of INM and NPK on less labile C fractions in MesoA, MicroA, and MinF was statistically at par, differ from control. Long-term application of fertilizer did not influence recalcitrant C fraction associated with MicroA and MinF. However, the effect was significant for MacroA and MesoA. The recalcitrant C fraction under NPK+FYM (irrespective of aggregate fractions) was higher by 78.6 and 14.0% over control and NPK treatment, respectively.

3.4.2 Total organic carbon

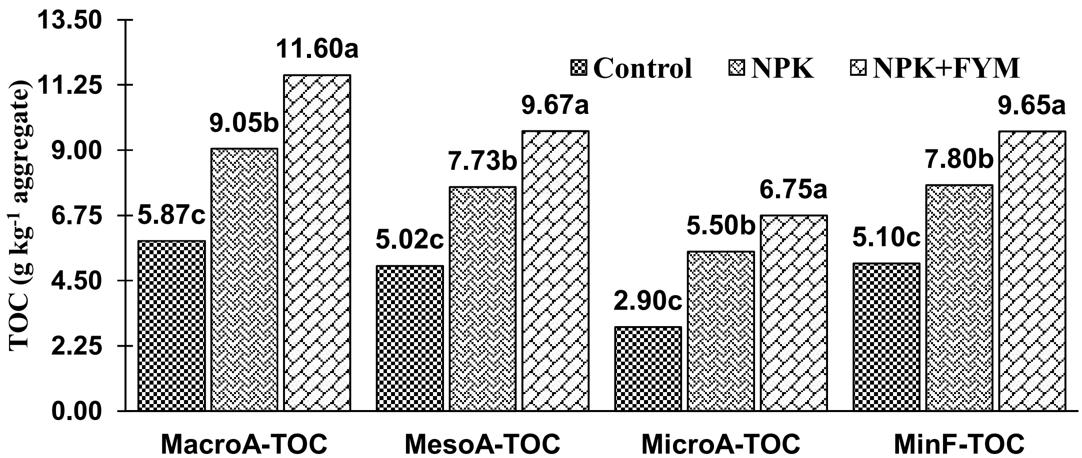
Total organic carbon was significantly higher in both NPK and NPK+FYM application over control, in all aggregate classes (Fig. 4). In NPK +FYM and NPK treatment TOC (g kg^{-1} aggregate fraction) was higher and ranged between 11.6-6.75 g kg^{-1} aggregate fraction and 9.05-5.50 g kg^{-1} aggregate fraction, respectively as compared to control, i.e. 2.90-5.87 g kg^{-1} aggregate fraction. Averaged across the aggregate class application of FYM and NPK resulted in significantly higher TOC by 99.6% and 59.3%, respectively compared to control.

TOC was higher in MacroA than other aggregate classes and MinF. The TOC (g kg^{-1} aggregate fraction) concentration in the MacroA fractions varied from 5.87-11.6 g kg^{-1} aggregate fraction. The corresponding results for MesoA and MicroA were 5.02-9.67 g kg^{-1} aggregate fraction and 2.90-6.75 g kg^{-1} aggregate fraction, respectively (Fig. 4). When averaged across treatments, TOC concentration (g kg^{-1} aggregate fraction) decreased by 15.4% and 42.9% as aggregate size decreased from MacroA to MesoA and MicroA, respectively.

Application of FYM along with the recommended dose of NPK increased TOC concentration in different aggregate fractions as compared to control. It may be attributed to the fact that the decomposition of organic matter results in the secretion of various organic acids and polysaccharides which binds roots, hyphae, and mineral particles into micro-aggregates and these micro-aggregates further binds to form C rich macro-aggregates. This type of C is physically protected within macro-aggregates and is known as intra-aggregate particulate organic matter, which is recalcitrant. Incorporation of this iPOM in macro-aggregates is the result of the humification of organic matter added to the soil through FYM, hence the addition of FYM leads to increased concentration of TOC. Puget [38] also reported that the higher TOC content in macro-aggregates might be attributed to the lower microbial decay of SOM and its direct role in the stability of macro-aggregates that leads to macro-aggregates enriched with C being able to withstand slaking. Benbi and Senapati [9] reported that the application of organic matter enhanced C content in various aggregate fractions and the enhancement was greatest in plots receiving both rice straw and FYM each year. Similarly Monreal [35] reported that OC content was higher in aggregate fraction >0.25mm (23-26 g kg^{-1}) as compared to aggregate fraction of <0.05mm (16.5-19.4 g kg^{-1}). A considerable amount of C was associated with MinF indicate the importance of organometallic complexes in C stabilization. Moreover, the occluded C associated with macro-aggregates may be subjected to breakdown due to a change in cultural practices and land use. Hence, more studies are needed to understand the relative stability of C and mechanism accumulating OC in intensively cultivated soil under a tropical climate.

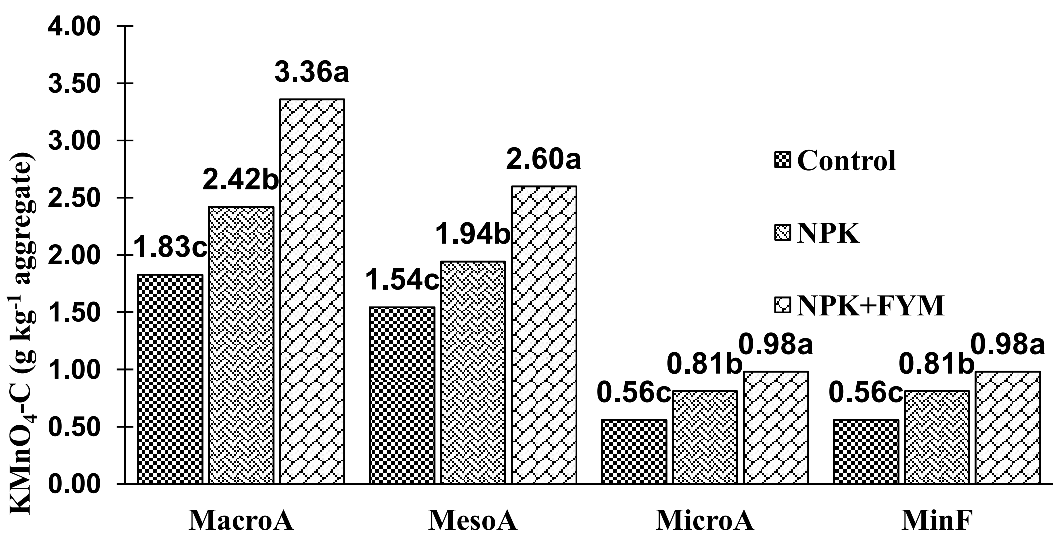
3.4.3 KMnO4-C

$KMnO_4$-C was significantly higher in both inorganic and FYM application over the control, irrespective of different aggregate classes (Fig. 5). $KMnO_4$-C (g kg^{-1} aggregate fraction) was highest in NPK +FYM treatment (0.98-3.36 g kg^{-1} aggregate fraction) followed by NPK (0.81-2.42 g kg^{-1} aggregate fraction) and minimum in control (0.56-1.83 g kg^{-1} aggregate fraction).



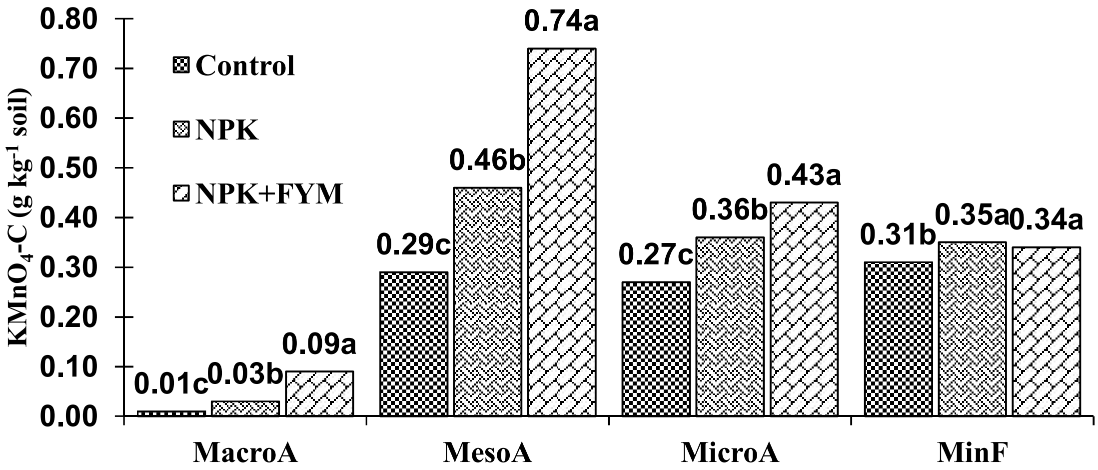
Averaged values in bars by different alphabets, differ significantly (p = 0.05) by Duncan's Multiple Range Test (DMRT)

Fig. 4. Distribution of total organic carbon (TOC) in soil aggregates as influenced by integrated nutrient management under maize-wheat system



Averaged values in bars by different alphabets, differ significantly (p = 0.05) by Duncan's Multiple Range Test (DMRT)

Fig. 5. Distribution of $KMnO_4$-C in soil aggregate fractions under integrated nutrient management in maize-wheat system



Averaged values in bars by different alphabets, differ significantly (p =0.05) by Duncan's Multiple Range Test (DMRT)

Fig. 6. Relative concentration of $KMnO_4$-C in aggregate fractions as influenced by integrated nutrient management in maize-wheat system

In all the treatments, $KMnO_4$-C was higher in MacroA than other aggregate classes and MinF. The $KMnO_4$-C (g kg^{-1} aggregate fraction) concentration in the MacroA fractions varied from 1.83-3.36 g kg^{-1} aggregate fraction. The corresponding results in MesoA and MicroA were 1.54-2.60 and 0.56-0.98 g kg^{-1} aggregate fraction, respectively (Fig. 5). On the contrary, if the relative contribution of aggregates towards bulk soil was considered for $KMnO_4$-C (g kg^{-1}soil), it ranges maximum in MesoA (0.29-0.74 g kg^{-1} aggregate fraction) followed by MicroA (0.27-0.47 g kg^{-1} aggregate fraction) and minimum in MacroA (0.01-0.09 g kg^{-1} aggregate fraction) (Fig. 6).

Tobiašová [46] similarly conducted a study on labile carbon content in different aggregate sizes and results revealed that macro- aggregates having size 0.25–1 mm had a maximum fraction of labile C especially labile C oxidizable with KMnO4. The aggregate fraction of size 0.5–1 mm was in a negative correlation with labile C fraction and appears to be a crucial indicator of changes in the ecosystems. This may be attributed to the fact that TOC content was in a direct relationship with the labile fraction. Yang et al. [47] and Xue et al. [48] recorded a high positive correlation between TOC and labile carbon ($r = 0.901$; $P < 0.01$) in soil. Hence, higher TOC in MacroA and MesoA is responsible for higher KMnO4 oxidizable labile carbon in them.

5. CONCLUSION

Application of fertilizers and farmyard manure for 44 years in maize-wheat sequence resulted in lower bulk density and higher mean weight diameter, total organic carbon, $KMnO_4$-C, and water stale aggregates. Aggregate associated C decreased with a decrease in aggregate size, which indicates a higher stabilization of C within MacroA. The higher amount of C as reflected in TOC and recalcitrant C fraction indicates better protection of C in the MacroA as compared to MicroA. Balanced and INM practice of fertilizer application for a prolonged period has the potential to improve soil aggregation and C stability in a maize-wheat system under sub-tropical conditions.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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