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Microbial biomass associated with aggregates; aggregate associated carbon and soil aggregate dynamics in rice-wheat cropping system: A review

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Abstract
Aggregate-size classes may have different microbial accessibility and therefore different decomposability of aggregate-associated soil organic matter (SOM). However, processes and mechanisms of soil organic carbon (SOC) mineralisation in different aggregate-size classes, and particularly, the interaction of aggregates with tillage intensity are poorly understood. The OC and N stocks in total soils were significantly correlated with the changes in the >0.053 mm aggregates. Adoption of CA increased OC stocks in the >0.053 mm size class of aggregates and N stocks in the >0.25 mm size class but decreased OC stocks in the <0.053 mm size class and N stocks in the <0.25 mm size class. According to mean weight diameter (MWD) different treatments in regard to crop establishment technique of rice could be ranked in the order $P_1 > P_4 > P_2 > P_3$ and $T_4 > T_3 > T_1 > T_2$ in wheat strip regarding tillage practices. However, the MWD decreased drastically in lower soil depth. The increases in OC and N stocks in these aggregates accounted for 99.5 and 98.7% of the total increases, respectively, in the continuous residue retention in rice –wheat cropping system. Organic amendments increased the proportion of macro-aggregate and mean weight diameter (MWD), especially in the plow layer. The macro-aggregates accounted for 43.87% and 49.96% of the total soil weight in the straw retention (SR) and manure application (MA). The distribution patterns of soil organic C (SOC) and microbial biomass C (MBC) within aggregate sizes was affected by organic treatments. The cropping increased the stocks of OC and N in total soils at mean rates of 13.2 g OCm^{-2} yr^{-1} and 0.8 g N m^{-2} yr^{-1} at the 0–20 cm depth and of 2.4 g OCm^{-2} yr^{-1} and 0.4 g N m^{-2} yr^{-1} at the 20–40 cm depth. Microbial biomass carbon showed a seasonal pattern. It was low initially, reached its peak during the flowering stages in both rice and wheat and declined thereafter. Microbial biomass carbon was linearly related to SOC in both rice and wheat indicating that SOC could be used as a proxy for MBC. The fine (0.053–0.25 mm) intra-aggregate particulate organic C (iPOM-C), in 0.25- to 2-mm aggregates, was also higher in ZT than conventional tillage. A higher amount of macro-aggregates along with greater accumulation of particulate organic C indicates the potential of ZT for improving soil carbon over the long-term in rice-wheat rotation.

Keywords: soil organic carbon, aggregates, straw returns mode, rice- wheat cropping system

Introduction

Soil is the largest pool of terrestrial organic carbon (OC), storing approximately 1580 Gt C (three times as much as the atmosphere), and thus plays an important role in the global C cycle (Jobbágy and Jackson, 2000) [31]. Carbon sequestration by soils helps stabilize the atmospheric CO_2 content, enhance soil fertility, and improve soil quality and structure (Wiesmeier *et al*., 2014). Soil structure and soil organic matter (SOM) are the two most dynamic soil properties, and are highly sensitive to agricultural management practices (Devine *et al*., 2014) [12]. Soil structure relates to arrangement of soil particles into units called aggregates, which are commonly grouped into different size classes [mega-aggregates (>2mm), macro-aggregates (0.25–2 mm) and micro-aggregates (<0.25mm)], with different stabilities (Devine *et al*., 2014) [12]. Each aggregate-size class has been reported to contain SOM with different levels of physical protection and chemical composition, influenced further by management practices, and thus can contribute in different ways to the regulation of soil organic carbon (SOC) and nutrient mineralisation dynamics in agro-ecosystems (Von Lützow *et al*., 2007) [67].

In general, organic matter associated with micro-aggregates comprises well decomposed plant and microbial residues, that bind together with primary mineral particles via cation bridging, thus protecting SOM against microbial mineralisation

(Rabbi *et al*., 2014; Lehmann and Kleber, 2015) [53, 37]. Further, fresh plant and microbial residues may also assist in binding clay particles and micro-aggregates into macro-aggregates and mega-aggregates, thus preserving partially decaying plant residues and microbial products within the aggregates (Six *et al*., 2002) [58]. However, coarser aggregates tend to have low structural stability, and hence are more sensitive to tillage than finer aggregates (Six *et al*., 2002) [58]. Further, any organic matter, less than 2 mm (which is classified as SOM) that may still be unprotected around macro-aggregates (e.g. free particulate organic matter; fPOM) or occluded within macro-aggregates and when exposed through tillage, would be more prone to microbial decomposition than that associated with, or protected within, micro-aggregates (Six *et al*., 1999) [59].

There have been a few studies on the understanding of SOC mineralisation in aggregate classes of different sizes under different tillage intensity systems (Jacobs *et al*., 2010) [25]. In general, macro-aggregates with greater microbial accessibility and degradability of SOM showed higher SOC mineralisation than micro-aggregates (Bimüller *et al*., 2016; Cai *et al*., 2016) [7, 8]. In contrast, Rabbi *et al*. (2014) [53] found no difference in SOC mineralisation from macro-versus micro-aggregates, while other studies found greater CO_2-C production from micro-versus macro-aggregates (Sey *et al*., 2008) [57]. Thus, there is limited consensus on the pattern of changes in SOM mineralisation and nutrient release across aggregates of different sizes. The aim of this study was, therefore, to increase our understanding of how, and to what extent, different aggregate-size classes, tillage intensity systems, to influence priming of microbial biomass with aggregates; associated Carbon and soil aggregate dynamics in rice-wheat cropping system in inceptisol. We propose two broader hypotheses, i.e., the first one on the aspect of soil aggregate dynamics and the second one on the aspect of microbial biomass with aggregates. Regardless of tillage intensity, macro- and mega-versus micro-aggregates will have higher mineralisation of native SOC via positive priming following crop residue input, likely due to higher microbial activity and SOM bioavailability. A greater release of plant available nutrients, possibly from SOM- and mineral-bound nutrient reserves in soil aggregates, which may vary with soil type (e.g. greater in a SOM and clay-rich soil versus a SOM- and clay-poor soil).

Banerjee *et al*. (2006) [3] reported that the non-puddled soil organic carbon was maximum (0.76%) at 292 DAT in the FYM treatment [Fig. 1a]. This coincided with the harvesting of the first wheat crop. In this treatment SOC increased during the growth of the first wheat crop, then it slightly decreased and again it showed increasing trend during the second wheat crop. Similar trend was also observed in non-puddled no-tilled soil [Fig. 1a]. But in this case maximum value was 0.69% at 259 DAT in the green manure and 100% organic source treatments. Banerjee *et al*. (2006) [3] revealed that the puddled-tilled and puddled-no-tilled site SOC increased after transplanting and high values were observed during 364–475 days [Fig. 1b]. This increase in SOC was clearly evident in the FYM and green manure treatments. It is also evident that organic carbon content of surface soil slightly increased during the growth of the second rice crop. The maximum value of SOC was 0.81% in the FYM treatment [Fig. 1b]. Zhang *et al*. (2014) [78, 79] showed that organic amendments increased the proportion of macro-aggregate and mean weight diameter (MWD), especially in the plow layer. The macro-aggregates accounted for 43.87% and 49.96% of the total soil weight in the straw retention (SR) and manure application (MA). The distribution patterns of soil organic C (SOC) and microbial biomass C (MBC) within aggregate sizes was affected by organic treatments. In the plow layer, the SOC in MA increased by 35.5% in macro-aggregates compared with CK, and significant differences in MBC in macro-aggregates were also found among organic amendments. MWD was positively correlated with SOC, and MBC. Organic amendment, either through crop residues or manure application, enhanced soil aggregate stability through the positive effects on soil binding agents including SOC, MBC [Fig. 1c].

Banerjee *et al*. (2006) [3] revealed that the fertilizer and organic amendments had significant effect on MBC in wheat [Fig. 2a]. The maximum value of MBC was recorded in the FYM treatment (185 mg kg^{-1}) in no tilled plot followed by the green manure treatment (183 mg kg^{-1}) in tilled plot. Plots receiving crop residues showed significant increase in soil MBC compared to the control. Maximum MBC (178 mg kg^{-1}) was in the FYM treatment in tilled soil while the value was 176 mg kg^{-1} in 100% organic source treated no-tilled soil [Fig.2a]. Two conditions of rice establishment, i.e., puddled and non-pudddled direct seeded, differed in terms of trends in MBC content in soil in the rice-wheat systems. In case of puddled, transplanted rice followed by either tilled or no tilled wheat, the MBC remained unchanged during the two years of the cropping period [Fig.2a]. But in case of non-puddled, direct seeded rice followed by either tilled or no tilled wheat, there was increase in MBC [Fig. 2b]. In direct seeded rice-wheat system, though initially MBC was much lower than that of transplanted rice-wheat system, after two years of cropping MBC became on par. This suggested that puddling had initial advantage in terms of higher MBC and the non-puddled rice system had a lag phase up to 2 years to build up the microbial biomass. Bhattacharyya *et al*. (2010) [5] showed that addition of FYM with N or NPK fertilizers increased SOC and TSN contents. The overall gain in SOC in the 0- to 45-cm soil depth interval in the plots under NPK + FYM treatment over NPK was 17.18 Mg C ha^{-1} in 30 year. The rate of conversion of input C to SOC was about 19% of each additional Mg C input per hectare. SOC content in large size aggregates was greater than in smaller size aggregates, and declined with decreased aggregate size [Fig. 2c].

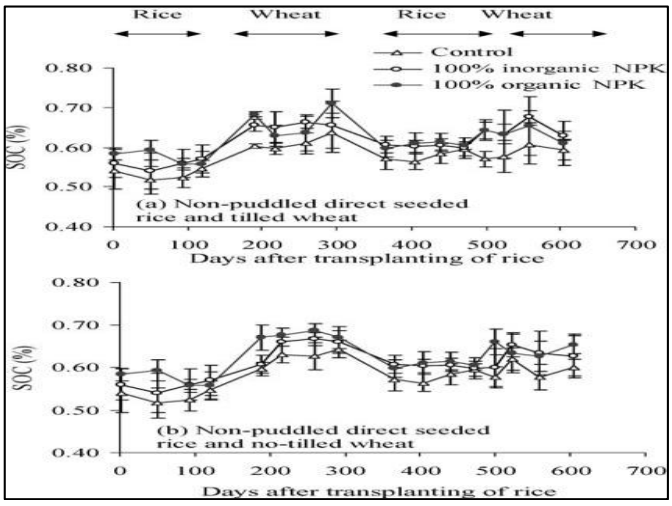

Fig 1 (a): Soil organic carbon (SOC) content in (a) non-puddled direct seeded rice and tilled wheat and (b) non-puddled direct seeded rice and no-tilled wheat

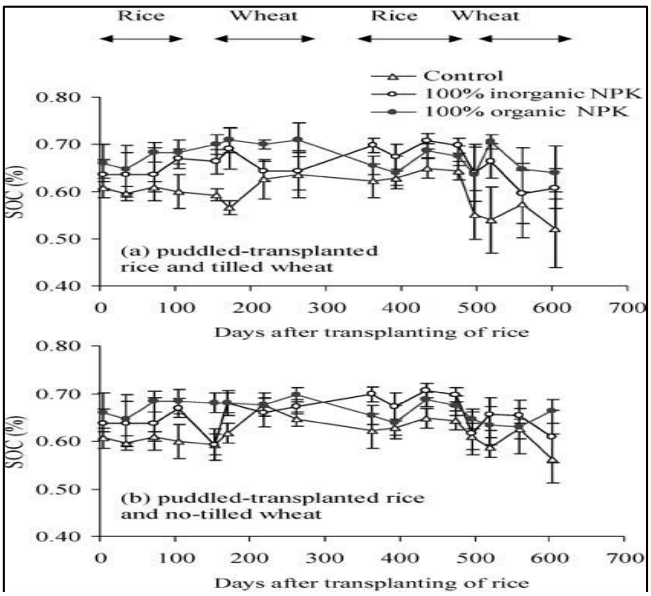

Fig 1 (b): Soil organic carbon (SOC) content in (a) puddled-transplanted rice and tilled wheat and (b) puddled-transplanted rice and no-tilled wheat

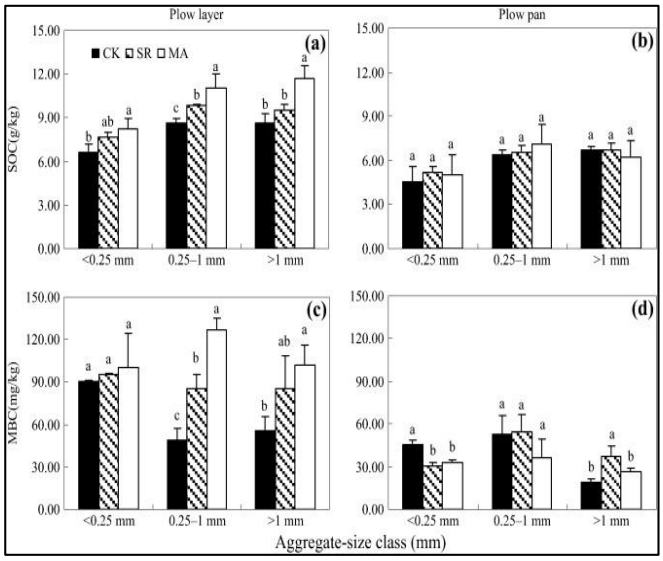

Fig 1 (c): Effects organic amendments on aggregate-associated organic C, microbial biomass C

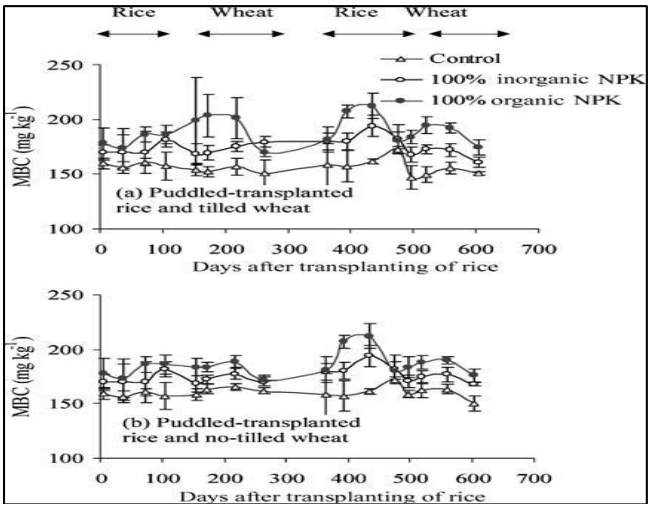

Fig 2 (a): Microbial biomass carbon (MBC) content of soil in (a) puddled-transplanted rice and tilled wheat and (b) puddle-transplanted rice and no-tilled wheat

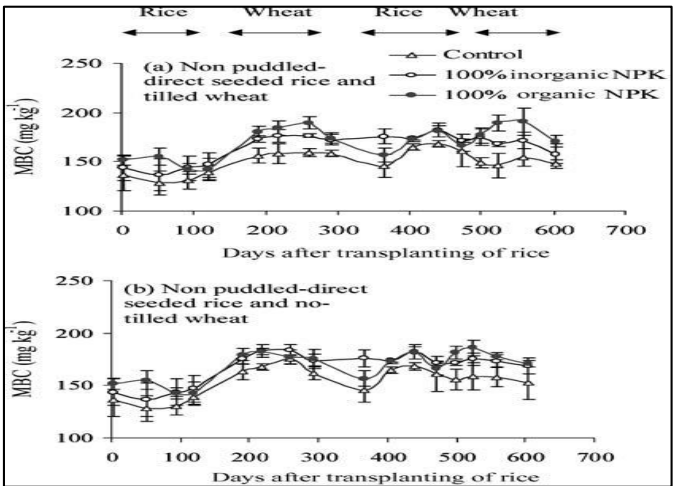

Fig 2 (b): Microbial biomass carbon (MBC) content of soil in (a) non-puddled direct seeded rice and tilled wheat and (b) nonpuddled direct seeded rice and no-tilled wheat

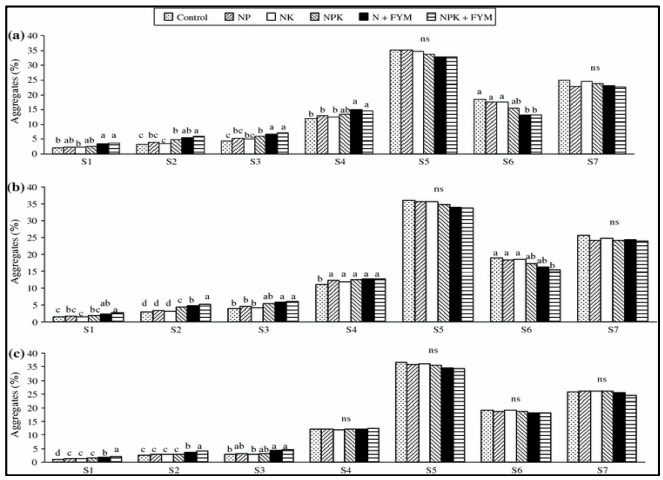

Fig 2 (c): Effects of fertilization on carbon and nitrogen sequestration and aggregate associated carbon

Dou *et al*. (2016) [15] reported that the iPOM stored the largest C fraction of the total SOC pool across all the aggregate sizes in the fertilized soils, which accounted for 80.79–90.32% in >2000 µm and 250–53 µm aggregates, and as well as accounted for 49.59–63.89% in 2000–250 µm aggregates [Fig.3a]. The mSOM accounted for the smallest fraction (1.54–4.92%) of total organic C in >2000 µm aggregates, whereas the LF accounted for the smallest fraction (4.10–6.74%) of total organic C in 250–53 µm aggregates [Fig.3a]. The greatest SOC storage in the LF, iPOM, and mSOM of all the aggregates was found in MNPK-treated soils [Fig.3a]. Additionally, inorganic N and NPK fertilizers significantly increased the SOC storage in mSOM of all the aggregates. Moreover, inorganic fertilizers increased the LF in >2000 µm aggregates but decreased it in 250–53 µm aggregates. The higher C: N ratios occurred in LF while the lower C: N ratios occurred in mSOM among soil density fractions across all fertilization treatments [Fig.3a]. Yagüe *et al*. (2012) [76] also found that additionally, the LF of SOM, as an early and sensitive indicator of the response to the long-term effects of agricultural practices, indicated that improvement of SOM in MNPK-treated soils may be first ascribed to a decline of C/N ratios in LF (Hai *et al*., 2010) [20].

In the wheat season, SOM of TS treatment significantly higher than that of CM and HS treatments at a depth of 20 cm. Straw return significantly increased wheat SOM at a depth of

30 cm [Fig.3b]. The significantly difference of ASOM was only found at a depth of 20 cm with the highest value occurred in the HS and TS treatments in rice and wheat season, respectively [Fig.3b]. Straw return had significantly increased soil NO_3^-–N leaching at a depth of 10 cm, whereas significantlydecreased soil NO_3^-–N leaching at depths of 30cm and 90cm in the rice season [Fig.3c]. Furthermore, significant interactions were found between the observation date and straw return treatment, except for the depth of 90 cm. In wheat season, HS and TS showed significantly decreased soil NO_3^-–N leaching at depth of 90 cm, compared with CM [Fig.3c]. The significant interaction for soil NO_3^-–N leaching was only found at depth of 30 cm between observation date and straw return treatment. Organic amendments are often shown to increase soil nitrogen retention and reduce N leaching (Wang *et al*., 2017) [70]. First, the moderate increase in the N use efficiency may be associated with higher reduction rate of N leaching. The promotion of crop N uptake is critical to reduce N pollution in agro ecosystems due to minimizing surplus soil N (Xia *et al*., 2014) [73]. Second, large quantity of straw return may strongly physically absorb N and alter N spatial distribution in the soil profile. Wheat straw carries negative charges and shows good adsorptive capability for urea-N (Wang *et al*., 2007) [68]. Otherwise, straw fixed part of the NO_3^-–N and released organic acids during its decomposition and inhibited the transformation of $NH4^+$–N to NO_3^-–N.

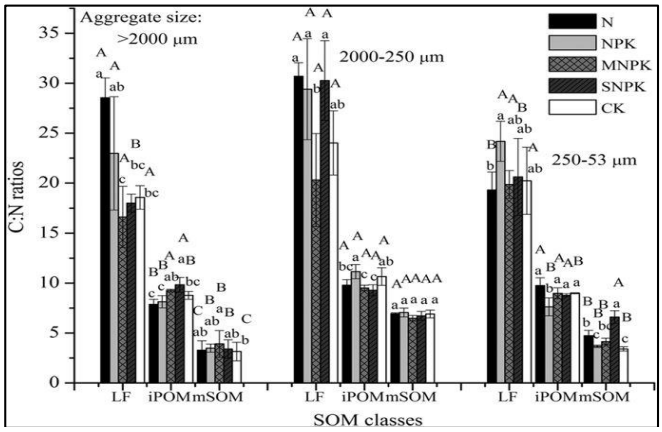

Fig 3 (a): C: N ratios of LF, iPOM and mSOM of aggregate size classes separated from the soils (0–20 cm) under long-term fertilization.

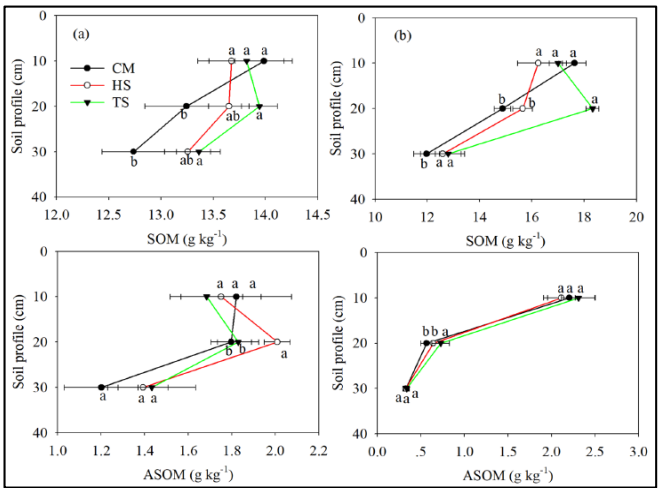

Fig 3 (b): Arithmetic means of SOM and ASOM concentrations under the experimental treatments in rice (**a,c**) and wheat (**b,d**) season.

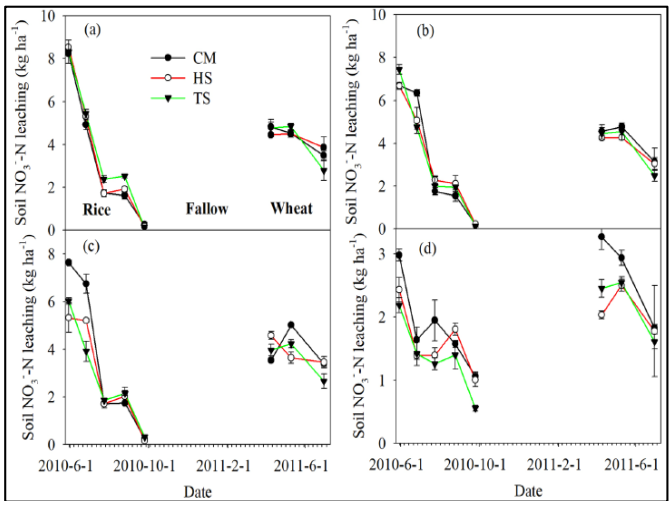

Fig 3(c): Variation of soil NO_3^-–N leaching at various soil depths under the experimental treatments

Gathala *et al*. (2017) [17] reported that the bulk density (D_b) increased with an increase in depth. At the 0–5-cm and 6–10-cm soil depths, D_b was higher in T_5 (ZT-DSR (–S,–WR)/ZT-DSW (–RR)) than in T_2 (CT-PTR (+S,+WR)/ZT-DSW (+RR)), whereas it was not significantly different from the rest of the treatments except T_4 (CT-DSR (+S,+WR)/ZT-DSW (+RR)) at 6–10 cm depth [Fig.4a]. The difference in Db between CT-DSR and ZT-DSR was not significant at 0–5 cm and 6–10 cm soil depths, whereas, at both 11–15 cm and 16–20 cm soil depths, T_3 and T4 had 4–5% higher D_b than T_5 and T_6 [Fig.4a]. Tripathi *et al*. (2007) [66] reported that puddling is known to increase D_b in soil immediately below the plow layer due to (i) destruction of soil aggregates The greater D_b in 15-30 cm layer of the CT treatment indicates the development of a compacted "hard pan" beneath tillage depth, caused by the compacting and shearing action of tillage implements (Dolan *et al*., 2006); (ii) filling of macro-pores with finer soil particles, which ultimately reduces the porosity; and (iii) direct physical compaction caused by implements. Puddling provides favourable conditions for soil compaction and reducing percolation losses, which decreases with a decrease in moisture content (Gathala *et al*., 2011] [16]. Treatment $_6$ (ZT-DSR (+S+WR)/ZT-DSW (+RR)) attained significantly higher water-stable macro-aggregates (8.0–4.75 mm and 4.0–2.0 mm size) than those of the rest of the treatments (T_1–T_5). Aggregates of less than 1.0 mm in size, though, tend to be in greater proportion with puddling or dry tillage, and there were no significant differences among treatments [Fig.4b]. Zhang *et al*. (2008) [77] reported that zero tillage with residue mulching had the lowest proportion of smaller WSA (<0.83 mm diam.) and greatest proportion of larger (>12.7 mm diam.) aggregates, compared to corresponding aggregates in conventional tillage without residue, indicating improvement in soil structure when tillage was omitted and crop residues were retained Malhi *et al*. (2011) [39]. Irrespective of treatment, SPR increased with the increase in depth up to 20 cm. In surface soil (5 cm depth), SPR was significantly higher in T_5 [ZT-DSR (–S, –WR)/ZT-DSW (-RR)] compared to the other treatments. At 10-cm depth, SPR was significantly lower in T4 (CTDSR (+S, +R)/ZT-DSW (+RR)) than in T_5 (ZT-DSR (–S, WR)/ZTDSW (–RR)), whereas the rest of the treatments did not differ from either T_4 or T_5 [Fig.4c]. Mohanty *et al*. (2007) [47] reported that ZT and residue management had a positive effect on soil physical parameters, notably soil aggregation, D_b, SPR, and infiltration rate. In medium-textured soils, the critical

mechanical impedance for wheat root development is around 1.75 to 2.00 MPa (Taylor *et al*., 1966) [64].

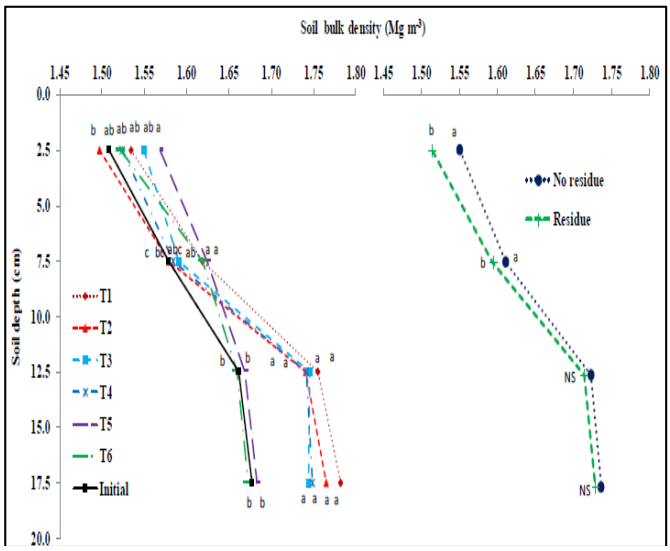

Fig 4(a): Effect of different tillage/crop establishment and residue retention on soil bulk density at wheat harvest after two cycles of rice-wheat cropping. T_1, CT-PTR (–S, –WR)/ZT-DSW (–RR); T_2, CT-PTR (+S, +WR)/ZT-DSW (+RR); T_3, CT-DSR (–S,–WR)/ZT-DSW (–RR); T_4, CT-DSR (+S,+WR)/ZT-DSW (+RR); T_5, ZT-DSR (–S,–WR)/ZT-DSW (–RR); T_6, ZT-DSR (+S,+WR)/ZT-DSW (+RR)

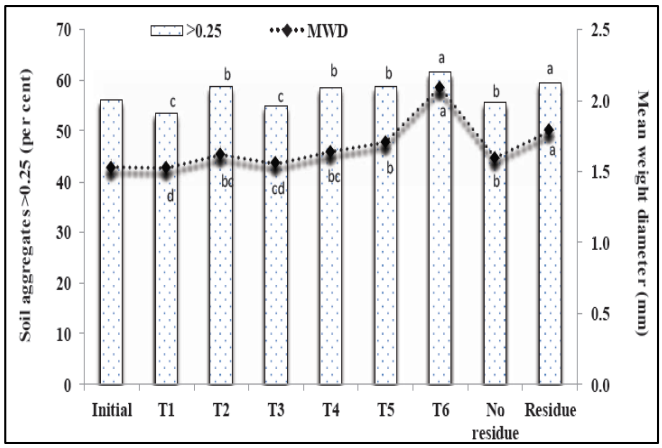

Fig 4(b): Effect of different tillage/crop establishment and residue retention on soil aggregates (>0.25 mm) and mean weight diameter (mm) at wheat harvest after two crop cycles of rice-wheat cropping.

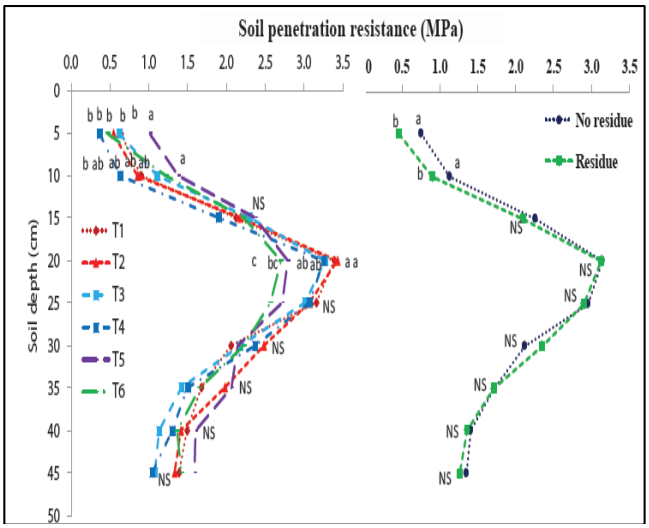

Fig 4(c): Effect of different tillage/crop establishment and residue retention on soil aggregates (>0.25 mm) and mean weight diameter (mm) at wheat harvest after two crop cycles of rice-wheat cropping.

Zhang *et al*. (2017) [82] revealed that MSAs >5 mm had a range of 18.82% in the T2 treatment to 42.02% in the T_5 treatment and were significantly higher in the T5 treatment than in the other treatments except T_4. The MSAs >5 mm of T3 increased 7.04%, 13.87%, 6.09%, T_4 increased 10.86%, 17.69%, 9.91% and T5 increased 16.37%, 23.20%, 15.42%, respectively, compared to treatments T_1, T_2 and T_6. The highest proportion of MSAs 5-2 mm was observed in the T_6 treatment compared with the other treatments, whereas the lowest was found in the T_2 treatment [Fig. 5a]. Huang *et al*. (2010) [23] observed a significantly higher percentage of macro0aggregates and greater SOC concentrations in the bulk soils and >2 mm aggregate fraction after combined application of manure and chemical fertilizer.

The varying distribution of SOC concentrations in the MSAs depended on the application of fertilizer types [Fig 5b]. The highest SOC concentration in MSAs > 5 mm, MSAs 5-2 mm, MSAs 2-1 mm and MSAs 1.0-0.5 mm was all observed in treatment T_5 and was significantly higher than that in the other treatments. Adversely, the T_5 treatment showed significantly lower SOC concentration in MSAs 0.50-0.25 mm and MSAs <0.25 mm compared with other treatments. At a soil depth of 0-20 cm, the SOC stock in MSAs > 5 mm, MSAs 5-2 mm, MSAs 2-1 mm and MSAs 1.0-0.5 mm significantly increased in treatment T_5, while the SOC stock in MSAs 0.50-0.25 mm and MSAs <0.25 mm significantly decreased, compared with other treatments [Fig.5b]. Application of N fertilizer improved the SOC status, while organic carbon added resulted in significantly higher SOC and TN concentrations in both bulk soil and aggregates (Benbi and Senapati, 2010) [4]. Jia *et al*. (2018) [26] reported that compared with NF, the OM treatment significantly increased soil organic carbon (SOC), water-soluble carbon (WSC), total nitrogen, microbial biomass carbon and nitrogen (MBC and MBN), and significantly decreased MBC:MBN ratios thus improving the soil quality of abandoned farmland [Fig.5c]. Organic fertilizers also provide a stable source of organic carbon and nitrogen for the growth of soil microorganisms thereby increasing MBC and MBN (Mandal *et al*., 2007) [40].

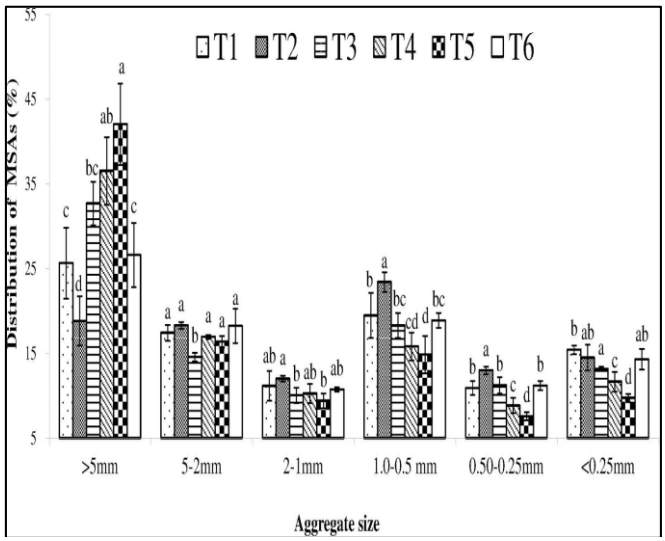

Fig 5 (a): Distribution of soil mechanical stable aggregates (MSAs) under different treatments

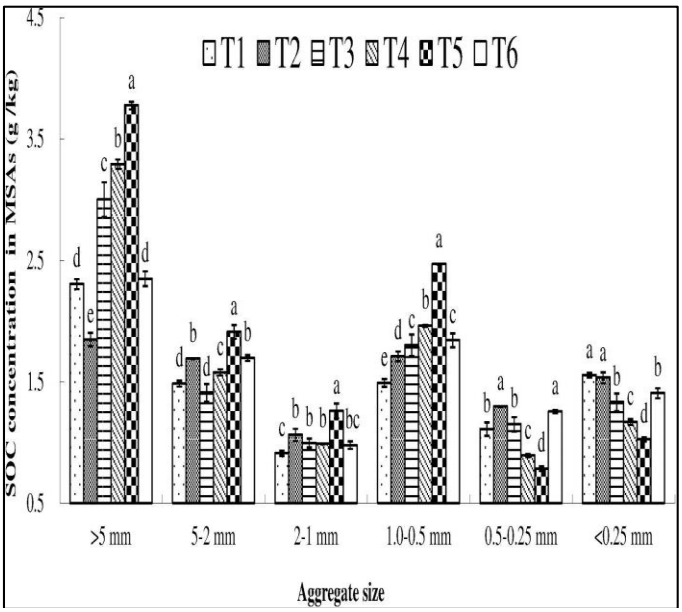

Fig 5(b): SOC concentration in MSAs under different treatments.

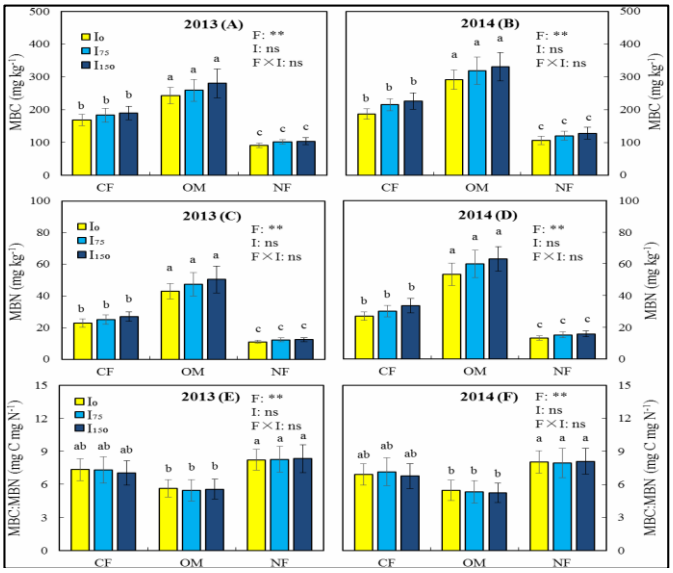

Fig 5(c): Effects of different treatments on microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), and the MBC: MBN ratio

Chu *et al*. (2016) [10] reported that the OC and N stocks in total soils were significantly correlated with the changes in the >0.053 mm aggregates. OC stocks in the >0.053 mm size class of aggregates and N stocks in the >0.25 mm size class but decreased OC stocks in the <0.053 mm size class and N stocks in the <0.25 mm size class [Fig.6a]. The increases in the OC and N stocks associated with the >0.25 mm aggregate size class accounted for more than 97% of the total increases in the continuous wheat and the legume-grain rotation systems. The aggregate distribution changed significantly at the 20–40 cm depth mainly due to the activities of the plant roots in the deeper soils. Plant roots can significantly accelerate the aggregation of soil particles (Six *et al*., 2004) [60]. The cropping increased the stocks of OC and N in total soils at mean rates of 13.2 g OCm^{-2} yr^{-1} and 0.8 g N m^{-2} yr^{-1} at the 0–20 cm depth and of 2.4 g OCm^{-2} yr^{-1} and 0.4 g N m^{-2} yr^{-1} at the 20–40 cm depth. The continuous alfalfa system had the largest increases at the 0–20 cm depth. The stocks of OC and N in this system increased by 45 and 36%, respectively, (with recovery rates of 31.1 OCm^{-2} yr^{-1} and 2.4 g N m^{-2} yr^{-1}) at the 0–20 cm depth and by 5 and 6%, respectively, (with recovery rates of 3.0 OCm^{-2} yr^{-1} and 0.03 g N m^{-2} yr^{-1}) at the 20–40 cm depth [Fig.6b]. Averaged across the cropping systems, the OC and N stocks associated with each size class were 12–67 and 13–63% higher, respectively, at the 0–20 cm depth than at the 20–40 cm depth. The stocks of OC and N in the <0.053 mm size class were 1.4–2.3 and 1.5–2.4 times higher, respectively, than the stocks in the other size classes [Fig.6c]. The continuous wheat system led to 9–35 and 4–28% increases in the OC and N stocks, respectively, in the >0.053 mm size class but to no changes in the <0.053 mm size class at the 0–20 cm depth. At the 20–40 cm depth, this system led to 10 and 12% decreases in the OC and N stocks in the 0.25–2mm size class and to 19 and 25% decreases in the 0.053–0.25mm size class, respectively, but to 93 and 87% increases in the >2 mm size class and to 10 and 8% increases in the <0.053 mm size class, respectively [Fig.6c]. The C/N ratios averaged across all cropping systems were relatively higher in the >0.053 mm size class than the <0.053 mm size class at both depths. For example, the average C/N ratios in the >2, 0.25–2 and 0.053–0.25mm size classes were 3–7% higher at the 0–20 cm depth and 1–6% higher at the 20–40 cm depth than in the <0.053 mm size class [Fig.6c].

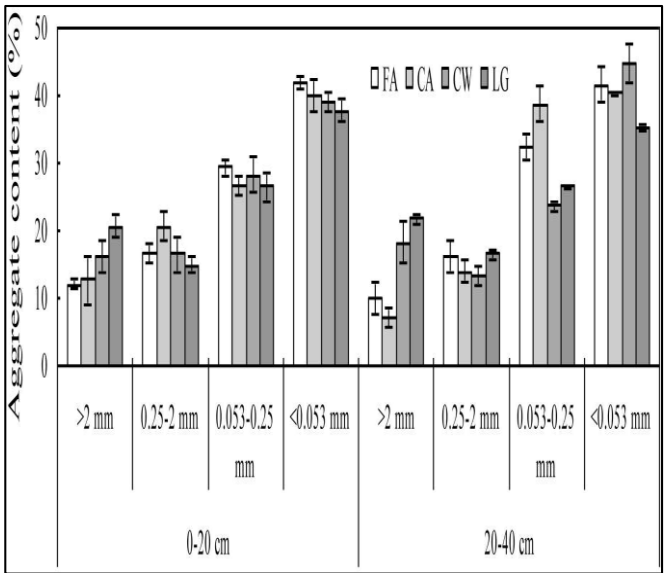

Fig 6(a): The effects of cropping systems on the distribution of aggregate size classes at the 0-20 and 20-40 cm depths

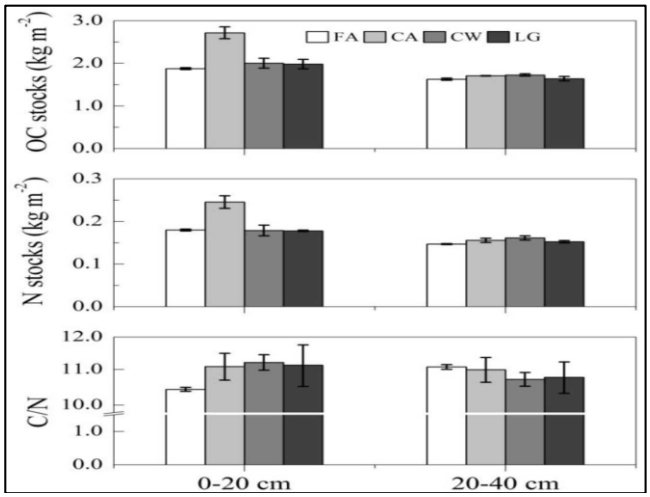

Fig 6(b): The effects of cropping systems on the OC and N stocks and C/N ratios in total soils at the 0-20 and 20-40 cm depths

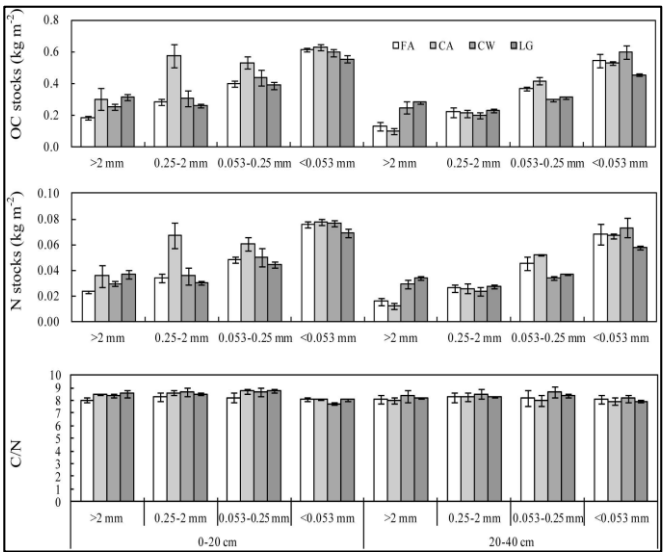

Fig 6c): The effects of cropping systems on OC and N stocks and C/N ratios in aggregate size classes at the 0-20 and 20-40 cm depths

Huang *et al*. (2016) [24] revealed that an application of compost plus chemical fertilizer (NPKM) increased the SOC content more efficiently than other treatments. The specific carbon mineralization rate (SCMR, rate per unit SOC) increased in the following order: micro-aggregate < macro-aggregate < silt and clay, suggesting that SOC in the micro-aggregates is more stable than in the silt and clay fraction. Compared with chemical fertilization alone (NPK), NPKM not only significantly improved soil aggregation but also efficiently activated the iron oxides as indicated by an increase in the aggregate mean-weight diameter (MWD [Fig.7a]. Dhaliwal *et al*. (2018) [13] revealed that the SOC concentration decreased with soil depth, the decrease was higher (89.6%) in soils under maize-wheat than in soils under agro-horticulture (81.3%) and agroforestry (77.8%). The mean SOC concentration decreased with the size of the dry stable aggregates (DSA) and water stable aggregates (WSA). In DSA, the mean SOC concentration was 58.06 and 24.2% higher in large and small macro-aggregates than in micro-aggregates respectively; in WSA it was 295.6 and 226.08% higher in large and small macro-aggregates than in micro-aggregates respectively in surface soil layer. The mean SOC concentration in surface soil was higher in DSA (0.79%) and WSA (0.63%) as compared to bulk soil (0.52%) [Fig.7b]. Niu *et al*. (2017) [50] also found that the microbial biomass carbon and contents of the biological crusts were higher than those of the physical crust. There was a decreasing trend in all the microbial biomass carbon contents of the five crust and layers samples with depth. The microbial biomass carbon contents of the moss crust (crust 3) and underlying soil layers were higher than those of the algal crust and underlying soil layers at the same depth [Fig.7c].

Moharana *et al*. (2012) [48] revealed that the soil MBC regulates all SOM transformations and is considered to be the chief component of the active SOM pool. It is evident that the MBC contents in both surface and sub-surface soil were significantly higher in plots receiving FYM + NPK and FYM treated plots compared to NPK fertilizer and unfertilized control plots [Fig.8a]. The values of MBC in surface soil varied from 155 mg kg^{-1} in unfertilized control plot to 273 mg kg^{-1} in integrated nutrient use of FYM + NPK plots, respectively; while it varied from 113 mg kg^{-1} (control) to 156 mg kg^{-1} (FYM + NPK) in subsurface soil. Kandeler *et al*. (1999) [33] reported that FYM (30 Mg ha^{-1}, applied every second year) doubled the microbial biomass under spring barley. Manjaiah and Singh (2001) [41] reported an increase of MBC by a factor of three after a combined application of FYM and mineral N-fertilizer in a semiarid Cambisol. The higher microbial biomass in FYM + NPK might be both due to higher below ground plant residues as well as added FYM (Grego *et al*., 1998) [18]. This observation is consistent with that of Hopkins and Shiel (1996) [22], who reported that the MBC was greater in soils receiving annual additions of FYM for nearly 100 years in addition to inorganic NPK.

Bhattacharyya *et al*. (2012) [6] reported that the plots under CT–NT and NT–CT had similar LF–C in that depth layer. On average, the iPOM– C concentration in all sand free aggregate size classes (except the coarse iPOM in the 250 to 2000 μm aggregate size) was higher in the NT–NT plots than CT–CT plots in the 0- to 5-cm soil layer [Fig.8b]. The NT–NT plots had about 12 and 28% higher iPOM–C concentration in the coarse and fine macro-aggregates, respectively, than CT–CT plots. Tillage had no effect on iPOM–C concentration within the coarse iPOM in the 250 to 2000 μm aggregate size class in that depth layer. Irrespective of tillage, the iPOM–C concentration was higher in fine (53–250 μm) than coarse (250–2000 μm) fractions in the 0- to 5-cm soil layer. Plots under NT–CT and CT–NT had similar iPOM–C concentrations within different aggregate sizes in the 0- to 5-cm soil layer (Fig. 4a). Between NT–NT and CT–NT plots, iPOM–C concentration in the coarse macro-aggregate fraction (>2000 μm) under NT–NT plots was significantly higher than CT–NT plots. Like LF–C, the differences in iPOM–C due to tillage management were insignificant in the 5- to 15-cm soil layer [Fig. 8b]. Six *et al*. (2000), suggesting the fact that slower macro-aggregate turnover in the continuous NT compared with continuous CT leads to micro-aggregate formation within macro-aggregates formed around fine iPOM and to a long-term stabilization of SOC occluded within these micro-aggregates.

Tillage induced changes in the light fraction C (LF–C) was distinguishable in the 0- to 5-cm soil layer only [Fig.8c] and as the differences were insignificant in the 5- to 15- cm soil layer, the data are not shown. On average, NT–NT plots contained significantly higher LF–C in all aggregate size classes (>2000-, 250–2000- and 53–250 μm) compared with CT–CT plots [Fig.8c]. The seasonal tillage alteration (plots under CT–NT and NT–CT) also had significantly higher LF–C than continuous CT system (CT–CT plots) in all aggregate size classes, except in the largest aggregate fraction [Fig.8c].

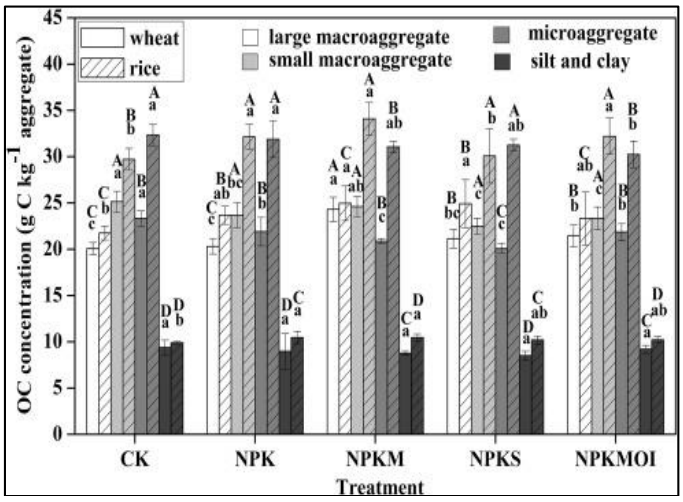

Fig 7(a): Integrated nutrient effects on soil aggregate-associated organic carbon in a rice–wheat cropping system

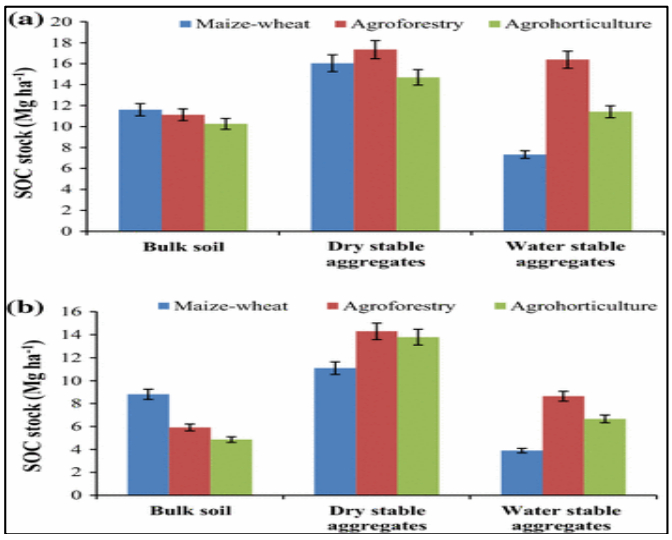

Fig 7(b): Soil organic carbon stock in relation to aggregate size

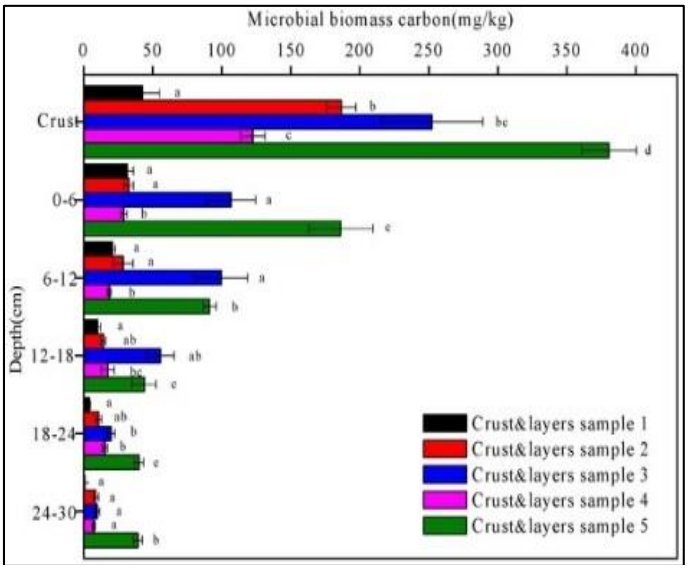

Fig 7(c): The microbial biomass carbon contents of the five crust and layers samples at different depths

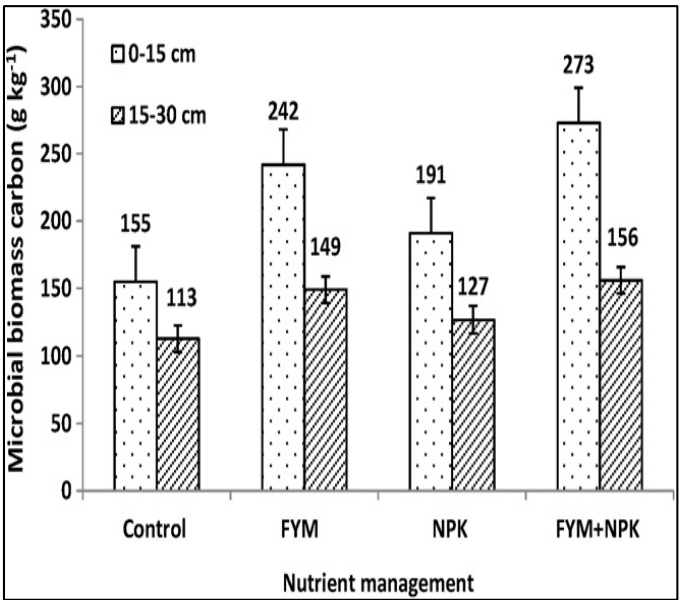

Fig 8 (a): Changes in microbial biomass carbon as influenced by application of FYM and fertilizers after wheat grown in a 6-year-old pearl millet–wheat cropping system

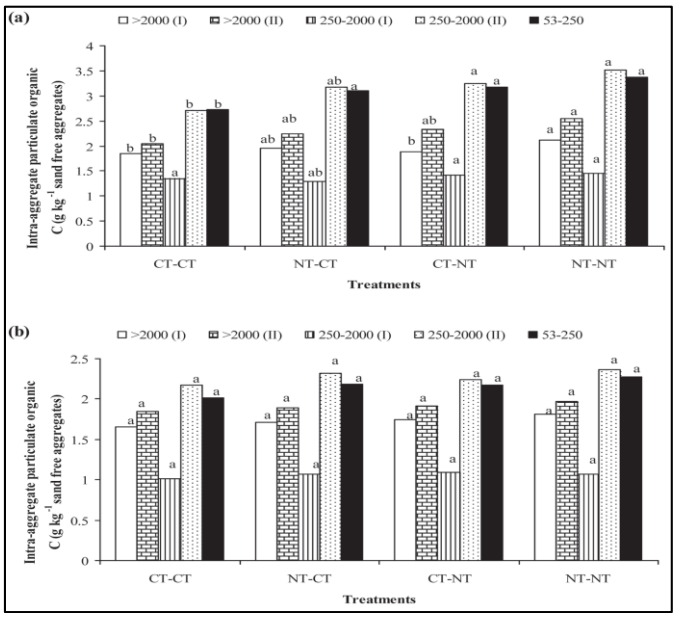

Fig 8(b): Intra-aggregate particulate organic matter–carbon (iPOM–C; g kg^{-1} of sand-free aggregates) in aggregate-size fractions at the (a) 0- to 5-cm and (b) 5- to 15-cm soil layers as affected by tillage practices

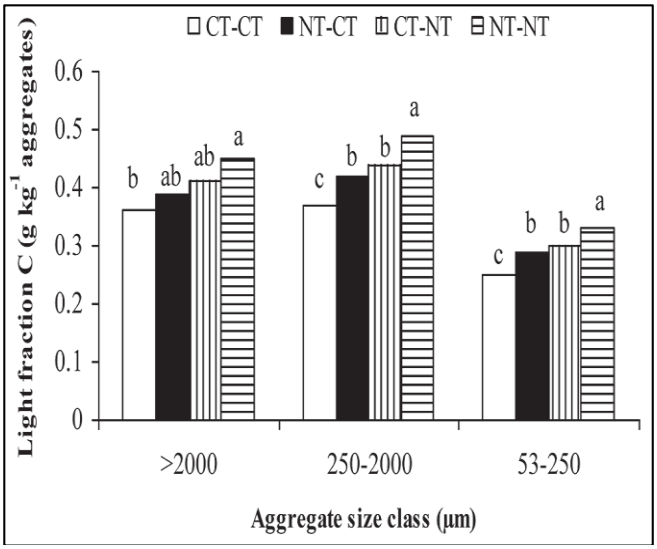

Fig 8(c): Light fraction C in aggregate size fractions of large (>2000 µm) and small (250–2000 µm) macro=aggregates and micro-aggregates (53–250 µm) in the 0- to 5-cm soil layer as affected by conservation tillage practices

Jiang *et al.* (2011) [28-30] also found that the bacterial biomass C ranged from 106 to 464mgC kg^{-1} soil, and exhibited a fluctuating pattern with aggregate size, but was highest for 1–2mm and 0.053–0.25mm aggregates [Fig.9a]. For whole soil, bacterial biomass was 52% and 73% higher under NT than CT and FPF, respectively. Bacterial biomass exhibited a fluctuating pattern corresponding to different aggregate sizes. The highest bacterial biomass was observed in the 2.0–1.0mmand 0.25–0.053 mm fractions for all tillage regimes. Few tillage effects were observed in macro-aggregates >1.0mm, with the exception of CT promoting the highest bacterial biomass in aggregates >4.76mm. However, tillage effects were very evident in aggregates <1.0mm. Under 1mm, bacterial biomass was significantly highest under NT and lowest under FPF. For aggregates <1.0mm, bacterial biomass under NT was 54% higher than under CT, which was 104% higher than for FPF [Fig. 9a]. Whole soil, fungal biomass was

43% and 84% higher under NT than CT and FPF, respectively. Fungal biomass ranged from 81 to 736 mg Ckg^{-1} soil, and was significantly higher for macro-aggregates >1.0mm (445, 624 and 424 mg Ckg^{-1} soil for CT, NT, and FPF, respectively) than the three micro-fractions <1.0mm (109, 230 and 108 mg C kg^{-1} soil for CT, NT, and FPF, respectively). The average fungal biomass for macro-aggregates >1mm (498mg C kg^{-1} soil) was 3.3 times higher than in micro-fractions <1mm (149 mg Ckg^{-1} soil), regardless of tillage regime. Fungal biomass significantly increased from >4.76mmto the 2.0–4.76 mm fractions for all tillage regimes, then it decreased with decreasing aggregate size until the 0.25–1.0 mm fraction. Then fungal biomass remained unchanged in fractions <0.25 mm. For all aggregate size fractions except <0.053mm, fungal biomass was higher for NT than other tillage regimes [Fig.9b]. Qualitative differences in microbial communities between micro-aggregates and macro-aggregates are likely related to substrate availability and pore-size distribution [Fig.9c]. The major determinants of C and nutrient turnover in soils are thought to be physical protection of substrates and the spatial distribution of microorganisms (primary decomposers) and micro-faunal predators (secondary decomposers) among pore sizes (Ladd *et al*., 1993) [36]. The distribution of pore sizes among aggregates and the associated microbial community provides a theoretical basis (Hattori, 1988) [21] for the change in the distribution of microbial biomass among aggregate sizes. However, soil microbial biomass change associated with aggregates was different than the response for whole soil. Soil total microbial biomass C, N and fungal biomass were significantly higher under NT than CT and FPF in all aggregate sizes, whereas bacterial biomass did not show significant differences in macro-aggregates (>1.0mm) for all tillage regimes. Soil total microbial biomass was mainly concentrated within macro aggregates, and tillage initiated a shift from soil macro aggregates to micro-aggregates and individual particles, leading to a decrease of 67% in macro-aggregates (Jiang *et al*., 2011) [28-30].

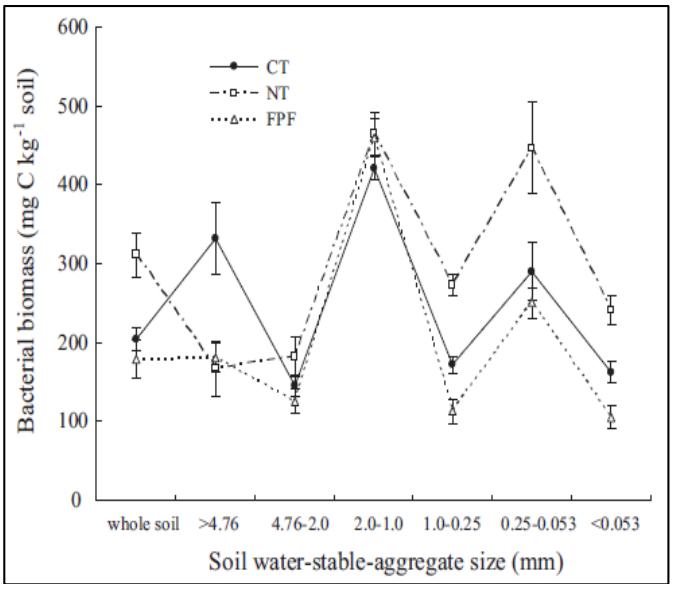

Fig 9(a): Distribution of bacterial biomass within soil aggregates under different tillage management (CT, conventional tillage; NT, combines ridge with no tillage; FPF, flooded paddy field).

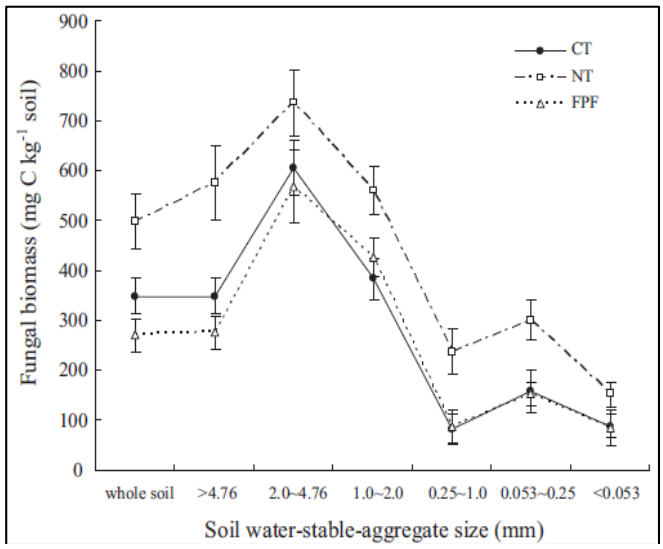

Fig 9(b): Distribution of fungal biomass within soil aggregates under different tillage management

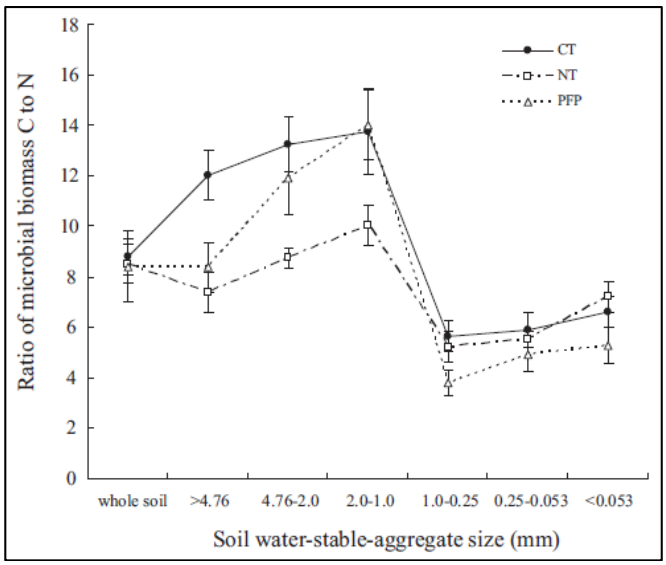

Fig 9(c): Ratio of microbial biomass C to N within soil aggregates under different tillage management

Zhao *et al*. (2018) [81] reported that the relative to the control, the proportion of large and small macro-aggregates in the 0–20 cm soil layer increased the most in MR-WR (32% and 24%), followed by MR (22% and 13%), and WR (11% and 10%). Straw return significantly increased the SOC content in each soil aggregate size class relative to no straw return [Fig.10a]. The order of SOC fractions with respect to SOC content was mSOM > fine iPOM > coarse iPOM > free LF. Straw return significantly increased the C stock in iPOM and mSOM relative to the control. Coarse iPOM was the most sensitive indicator of C change and mSOM was the main form of SOC under long-term straw return [Fig.10b]. The SOC content in each soil aggregate size class in the 0–20 cm layer significantly increased in the straw return treatments compared with no straw return [Fig.10a]. Moreover, the SOC content of each aggregate class in the 0–20 cm layer was significantly higher than that in the 20–40 cm layer. Increases in the SOC content of aggregate fractions were highest in MRWR, followed by MR, and finally WR [Fig.10a]. Higher OC content of micro-aggregates due to straw return may be beneficial to long-term SOC sequestration because micro-aggregates have a longer turnover time and higher stability relative to macro-aggregates (Qiao *et al*., 2015) [52]. The

carbon content of soil aggregates was much lower in the 20–40 cm layer than in the 0–20 cm layer because the field machinery used mainly distributed straw within the topsoil. Straw return treatments, particularly MR-WR, increased the proportions of mSOM and fine iPOM within small macro-aggregates and micro-aggregates, especially in the 0–20 cm layer [Fig.10b]. The carbon content of iPOM was much lower at 20–40 cm than at 0–20 cm [Fig.10b]. Fine particulate OC of small macro-aggregates tended to increase with increasing straw input in the 0–20 cm layer [Fig.10b], indicating that increased straw input is conducive to the formation of micro-aggregates due to the positive role of intra-POM on the formation and stability of micro-aggregates (Six and Paustian, 2014) [61]. All three straw return treatments (MR-WR, MR and WR) largely improved the SOC stock in each aggregate fraction in the 0–20 cm depth; increases were highest in MR-WR, followed by MR, and finally WR [Fig.10c]. In the 20–40 cm layer, the SOC stock of small macro-aggregates significantly increased in MR-WR, but the SOC stock in the silt plus clay fraction decreased relative to other three treatments. Samahadthai *et al.* (2010) [54] reported that lower quality residues enhanced the free LF pool, whereas higher quality residues promote heavy SOM fractions.

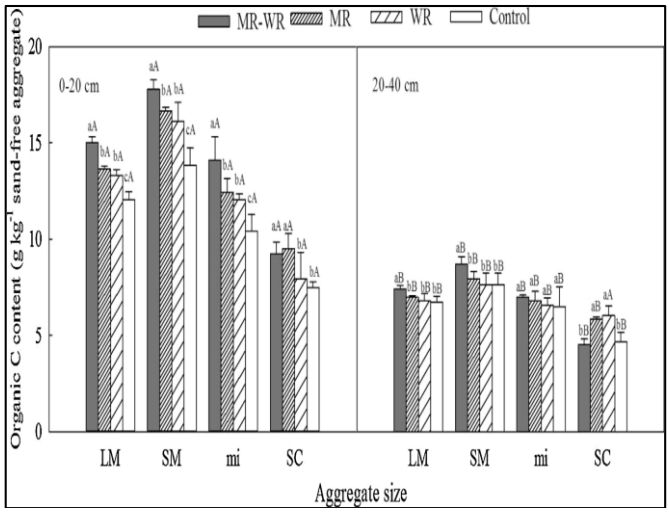

Fig 10(a): Organic C content (g kg^{-1} aggregate) of aggregates: LM, SM, mi, and SC in the 0–20 cm and 20–40 cm soil layers under MR-WR, MR, WR, and Control.

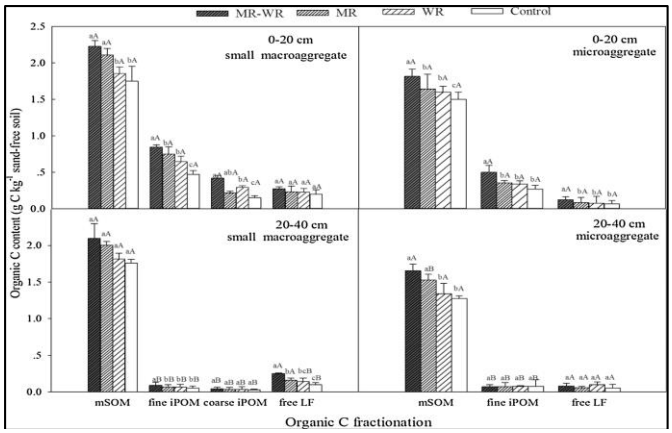

Fig 10(b): Organic C content (g kg^{-1} soil) of the SOC fractions: coarse iPOM, fine iPOM, mSOM, and free LF of small macro-aggregates and micro-aggregates in the 0–20 cm and 20–40 cm soil layers under MR-WR, MR, and WR.

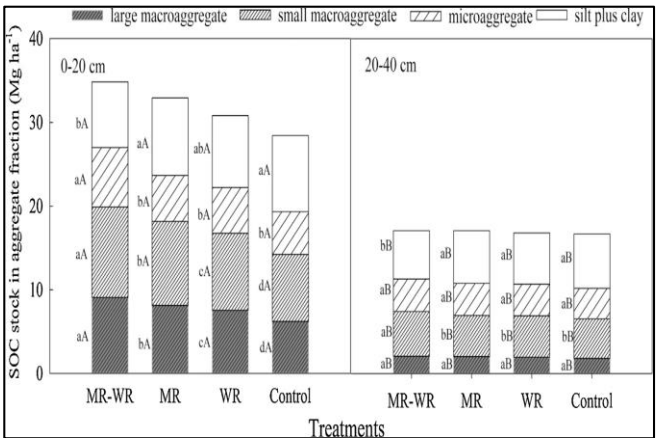

Fig 10(c): SOC stock of aggregate fractions (Mg ha^{-1}): large macro-aggregates, small macro-aggregates, micro-aggregates, and silt plus clay in the 0–20 cm and20–40 cm soil layers under MR-WR, MR, WR, and Control.

Xiao *et al*. (2016) [74] showed that crop fields remarkably decreased the SOC and TN concentrations in aggregates [Fig.11a]. Land had the highest SOC and TN concentrations in the three sizes of aggregates and bulk soil, followed by prescribed-burning land and fuel-wood shrub land, and pasture and maize field had relatively lower SOC and TN concentrations [Fig.11a]. Intra-aggregate water is vaporized when burning takes place, and the increased pressure causes the internal bonds to rupture, leading to aggregate breakdown (Albalasmeh *et al*., 2013) [2]. Moreover, maize field had the lowest amount of large macro-aggregates. Physical disturbance resulting from tillage might be responsible for breakage of the large aggregates (Cates *et al*., 2016) [9].

Large macro-aggregates comprised the major SOC and TN pools regardless of the land uses [Fig. 11b]. They contained 18.35–44.59 g Ckg^{-1} soil and 1.51–2.99 g Nkg^{-1} soil, accounting for 58.76–82.54% of SOC and 59.68–81.92% of TN. Further, the micro-aggregates had the lowest SOC and TN pool regardless of land uses. In general, our results showed that SOC and TN stocks in large macro-aggregates significantly decreased when the natural vegetation was converted to other land uses. In small macro-aggregates, the SOC and TN stocks in prescribed-burning land were higher by 8.96 g kg^{-1} and 0.68 g kg^{-1} than those in the enclosure land, respectively. The SOC and TN stocks in micro-aggregates were not remarkably different across the land uses [Fig.11b].

MBC in aggregates and bulk soil in other land uses decreased compared with that in enclosure land [Fig.11c]. Conversion of natural system to other land uses decreased MBC and increased C_{mic}: C_{org} ratios in aggregates. The extent of the response to land uses of SOC and TN concentrations was similar whereas that of MBC and C_{mic}: C_{org} ratios differed across the three aggregate sizes. Further, the SOC concentrations were significantly higher in macro-aggregates than micro-aggregates; the MBC and C_{mic}: C_{org} ratios were highest in small macro-aggregates [Fig.11c]. However, no significant differences in the C_{mic}: C_{org} ratios in large macro-aggregates were identified for all land uses. Among the three aggregates, small macro-aggregates had the highest C_{mic}: C_{org} ratios, followed by micro-aggregates; large macro-aggregates had the lowest ratio. Land uses have great impacts on microbial activity (MBC and C_{mic}: C_{org} ratio) in small macro-aggregates and micro-aggregates, but not in large macro-aggregates. This is mainly because the large radius of large aggregates could limit the O_2 concentration and gas diffusion

required by microbes (Gupta and Germida, 2015; Jiang *et al*., 2011) [28-30]. Thus, large macro-aggregates might diminish the impacts of land uses and facilitate the maintenance of a stable microbial biomass.

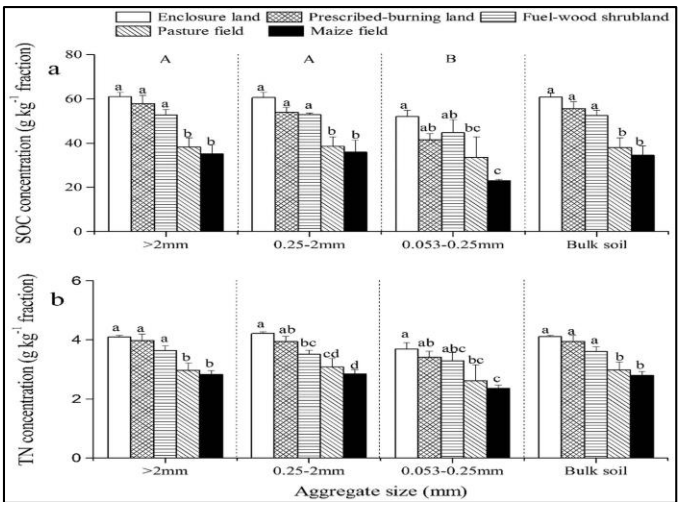

Fig 11 (a): Soil organic carbon (SOC) (a) and total nitrogen (TN) (b) concentrations in the three sizes of soil aggregates and in bulk soil of different land uses

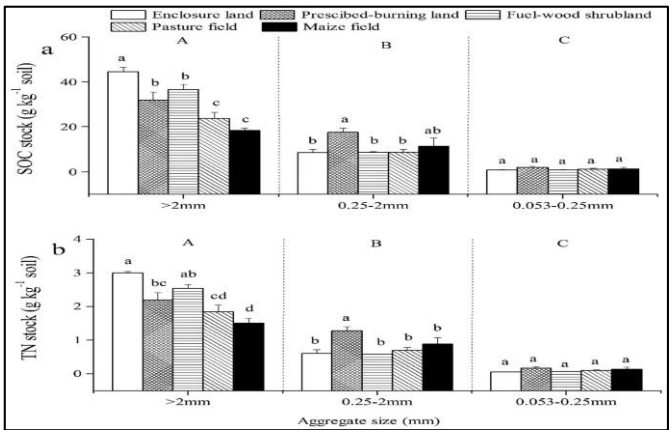

Fig 11 (b): Soil organic carbon (SOC) (a) and total nitrogen (TN) (b) stocks in the three sizes of soil aggregates and in bulk soil of different land uses.

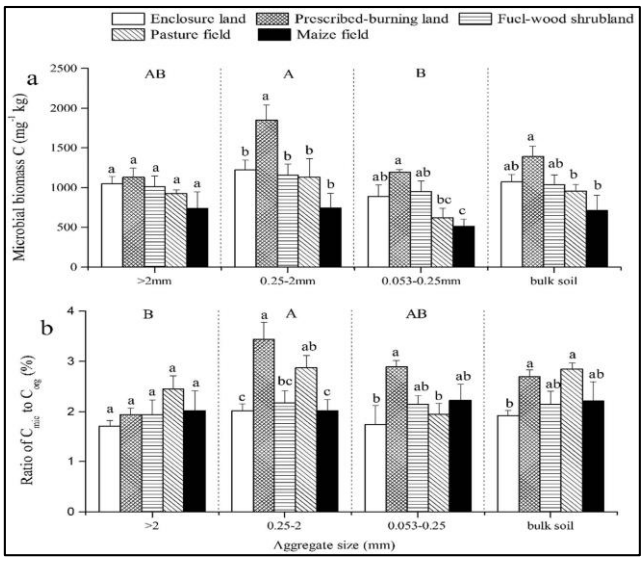

Fig 11(c): Microbial biomass carbon (MBC) (a) and the C_{mic}: C_{org} ratios (b) of the three sizes of soil aggregates and bulk soil of different land uses.

Huang *et al*. (2016) [24] revealed that the specific carbon mineralization rate (SCMR, rate per unit SOC) increased in the following order: micro-aggregate < macro-aggregate < silt and clay, suggesting that SOC in the micro-aggregates is more stable than in the silt and clay fraction [Fig. 12a]. Kong *et al*. (2004) [34] found a strong linear relationship (r^2 = 0.70) between SOC sequestration and cumulative C input, with a residue-C conversion to SOC rate of 7.6%. This linear relationship suggests that these soils have not reached an upper limit of C sequestration. In addition, C shifted from the <53-µm fraction in low C input systems to the large and small macro-aggregates in high C input systems. A majority of the accumulation of SOC due to additional C inputs was preferentially sequestered in the micro-aggregates-within-small-macro-aggregates (mM) [Fig.12b].

Retention of rice residues alone in DSR-ZTW increased SMB-C and SMB-N to the extent of 11 and 35 per cent respectively compared to conventional TPR-CTW in the surface soil [Fig.12c]. Brown manuring in DSR-ZTW had 13 and 34 per cent greater SMB-C and SMB-N than that under TPR-CTW. Among all the three single residue retention plots, retention of mungbean residue in DSR-ZTW (MBR+DSR-ZTW-ZTMB) had most beneficial effect on SMB, thus registering an increase of 15 and 44 per cent higher SMB-C and SMB-N compared to conventional practice (TPR-CTW). Double residue retention showed further improvement in SMB. Plots under DSR+BM-ZTW+RR had 23 and 46 per cent more SMB-C and SMB-N, respectively compared with TPR-CTW, whereas MBR+DSR-ZTW+RR-ZTMB showed an increase of 18 and 61 per cent, in the same order [Fig.12c]. Spedding *et al*. (2004) [63], wherein positive effect of residue retention on SMB-C and SMB-N was more pronounced than that of reduced tillage

Das *et al*. (2014) [11] revealed that the addition of organic substrates significantly improved soil organic C contents, but the type and source of inputs had different impacts [Fig.13a]. A larger amount of C in soil was achieved through green manuring in rice, although the C input through green manuring was much lower (63.9 Mg ha^{-1}). Similarly, SPM referred to a greater amount of addition of C (68.9 Mg ha^{-1}) compared to NPK + Zn + S and FYM addition in rice, while the resultant SOC (8.2 g kg^{-1}) was close to NPK + Zn + S (7.5 g kg^{-1}) and less than FYM (10.2g kg^{-1}). Addition of green manure in rice followed by FYM in wheat resulted in greater SOC content (16.4gkg^{-1}) than either green manuring or FYM addition in rice. Straw incorporation in both rice and wheat crops indicated a large amount of organic matter (154.4 Mg C ha^{-1}) addition to soil, which was higher than any other treatment. However, the corresponding SOC content was lower (14.7gkg^{-1}) [Fig.13a]. Tensile strength of aggregates decreased and friability increased through organic inputs, with a maximum effect under green gram residue (rice)-farmyard manure (wheat) substitution [Fig.13b]. Higher macro-aggregates in the crop residue- and farmyard manure-treated soils resulted in a higher aggregate mean weight diameter, which also had higher soil organic C contents. The bulk soil organic C had a strong relation with the mean weight diameter of aggregates, but the soil organic C content in all aggregate fractions was not necessarily effective for aggregate stability [Fig.13b]. Aggregate strength decreases with increase in SOC, initially at a faster rate and then gradually. The tensile strength was the highest in the unfertilized plots. Due to the low amount of SOC, the air-dry aggregates from the unfertilized plots had increased internal friction between the particles upon drying [Fig.13b]. Organic matter helps in

particle orientation to form aggregates and also reduces the amount of non-complexes clay for cementation upon drying of the aggregates (Schjonning *et al*., 2012) [56]. The soil organic C content in large macro-aggregates (2-8 mm) had a significant positive effect on aggregate stability, although a reverse effect was observed for aggregates <0.25 mm. Partial substitution of nitrogen by organic substrates improved aggregate properties and the soil organic C content in bulk soil and aggregate fractions, although the relative effect varied with the source and amount of the organic inputs [Fig.13b].

Mitran *et al*. (2018) [46] reported that the fine micro aggregated (0.05-0.25 mm) and silt plus clay associated organic carbon collectively contributes towards the micro aggregated organic carbon. All the organic amendments have similar effects C distribution in aggregate fraction. The fine micro aggregated organic carbon contributed 58.7% of total micro aggregated organic carbon in the experimental soils under different treatments [Fig.13c]. The organic carbon content in the fine micro aggregates increased by 72% in T6 (50% NPK plus 50% N through FYM), 34.8% in both T8 (50% NPK plus 50% N through PS) and T10 (50% NPK plus 50% N through GM) respectively over control. (Majumder *et al*., 2008; Naresh *et al*., 2017) [38] also found that the differences in macro aggregated carbon and micro aggregated carbon would be governed by interplay of several factors including climate, soil characteristics, substrate, biochemistry, C loading and associated environment.

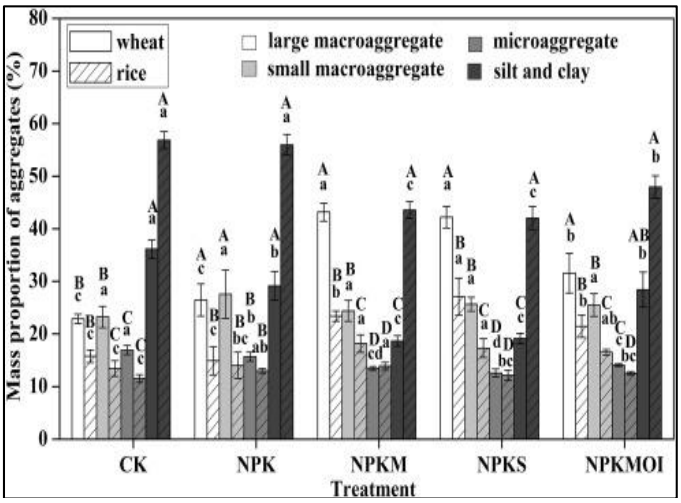

Fig 12(a): Soil aggregate-associated organic carbon in a rice–wheat cropping system

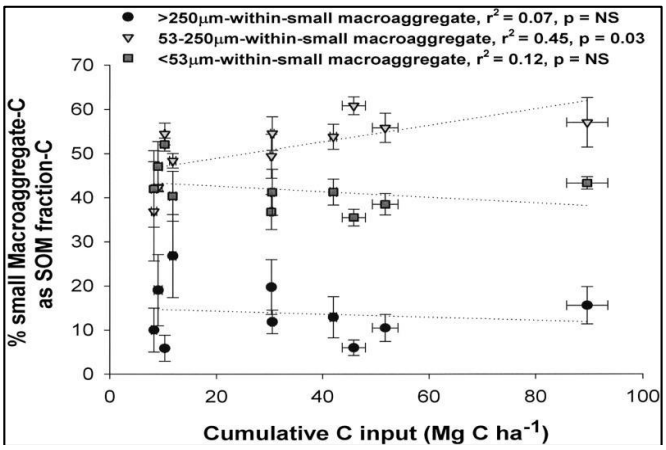

Fig 12 (b): Relationship between Carbon Input, Aggregation, and Soil Organic Carbon Stabilization

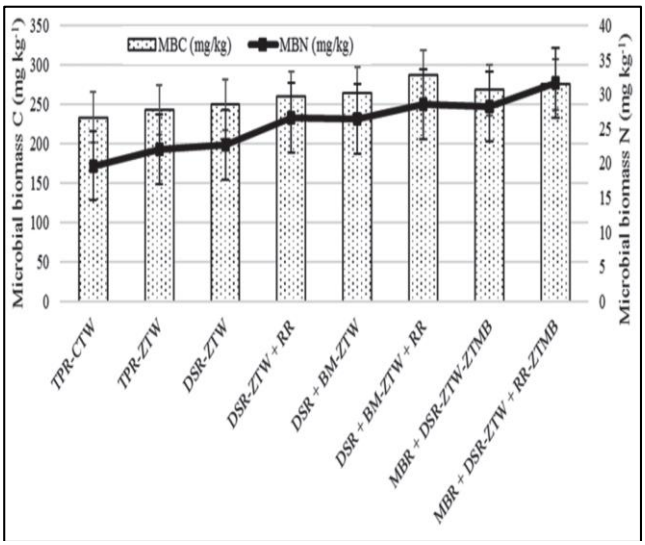

Fig 12(c): Changes in SMB-C and SMB-N and N in the surface 0-15 cm soil depth as an effect of tillage, crop residues and crop establishment

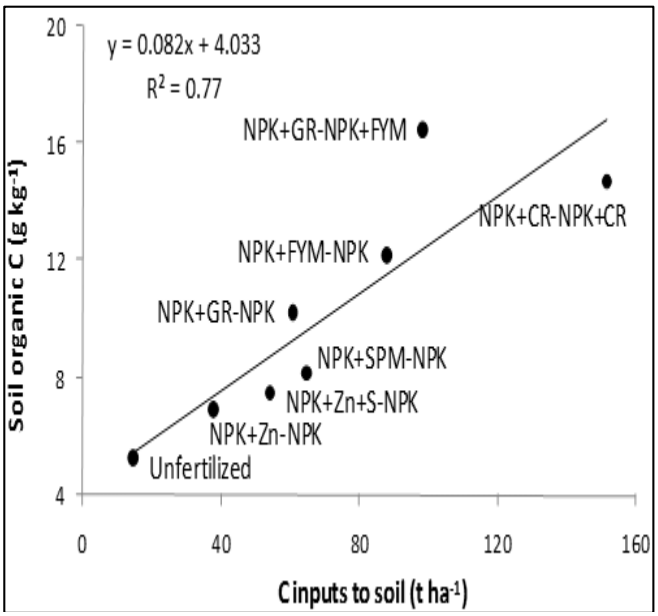

Fig 13 (a): Effect of the source and amount of organic inputs on the soil organic carbon content in the 0-15 cm layer of a sandy loam soil under the rice-wheat rotation

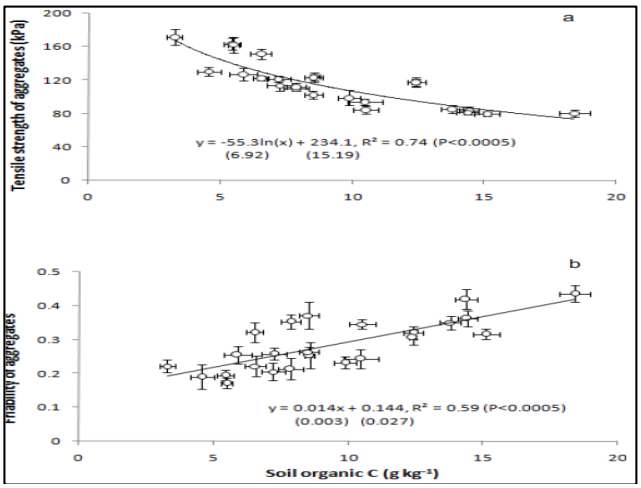

Fig 13 (b): Relations between: – tensile strength and b – friability of aggregates and soil organic carbon

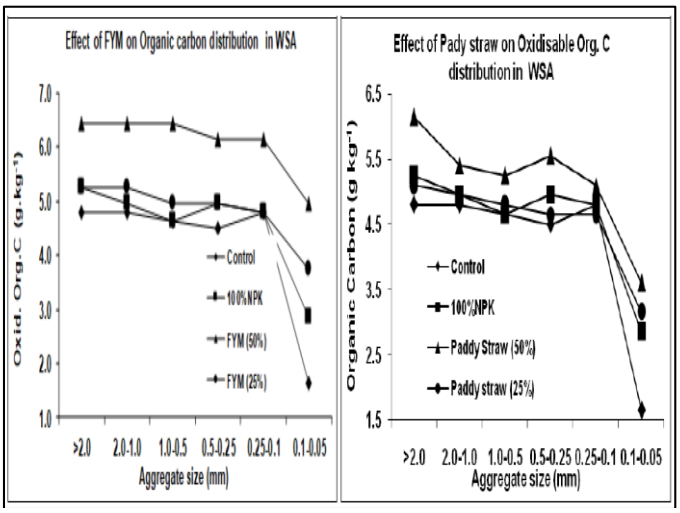

Fig 13(c): Distribution of Organic Carbon within water stable aggregates under various treatments

Pei *et al*. (2015) [51] also found that the total organic carbon content and $\delta^{13}C$ value clearly increased with the addition of straw retention [Fig.14a], and were significantly affected by time, soil type, fertility, and straw amendment. There existed a gradual decline for total organic carbon content and $\delta^{13}C$ in the treatment with straw during the experimental period; meanwhile, a period of rapid decrease occurred during 0 to 2 months [Fig.14a]. Total organic carbon content in the treatment with straw during the experimental period was in the order of high fertility Phaeozem > high fertility Luvisol > low fertility Phaeozem > low fertility Luvisol, while $\delta^{13}C$ was in the order of low fertility Luvisol > low fertility Phaeozem > high fertility Luvisol > high fertility Phaeozem. Moreover, total organic carbon content and $\delta^{13}C$ value in the treatment without straw during the experimental period varied a little [Fig.14a]. The order of total organic carbon content also showed that high fertility Phaeozem > high fertility Luvisol > low fertility Phaeozem > low fertility Luvisol, while the $\delta^{13}C$ followed the order of low fertility Luvisol > high fertility Luvisol > high fertility Phaeozem > low fertility Phaeozem. There were significant interactions between two, three or four factors, but there were no interactions on total organic carbon content between soil type and maize amendment; among time, soil type and maize amendment; among time, fertility and maize amendment; among soil type, fertility and maize amendment; and among four factors. The addition of straw generally increases total organic carbon content, whereas the amount of remaining maize straw decreases over time due to decomposition (Kuang *et al*., 2010) [35]. Different soil types and fertility levels influence the decomposition and accumulation of straw due to the differences in soil characteristics and nutrient conditions (Jiang *et al*., 2007) [27].

Memon *et al*. (2018) [43] revealed that the average SOM content significantly increased by 3.08% to 17.07% under all residue-incorporated treatments. Plots without straw incorporation showed a decreased SOM content (1.69–3.97%) compared with pre-treatment values under reduced and conventional tillage methods. However, the SOM content was higher (25.12, 24.06, 23.83, 23.80, 22.41, and 22.12 gkg^{-1}) in the RT_{si60}, RT_{si100}, CT_{si100}, CT_{si60}, RT_{si30}, and CT_{si30} treatments, respectively, compared to RT_{ns} (21.10 gkg^{-1}) and CT_{ns} (20.61 gkg^{-1}). The SOM difference between CT_{si60} and CT_{si100} was non- significant in the 0–30 cm soil profile depth. Moreover, SOM in the topsoil (0–10 cm) was higher in RT_{si60} (26.31 gkg^{-1}) and CT_{si60} (24.51 gkg^{-1}) under RT and CT, respectively [Fig.14b]. Minimal tillage or RT generally increased the SOM of the plow layer (Wang *et al*., 2015) under rice-wheat cropping (RWC) system, because RT can reduce soil disturbance and promote root growth in the topsoil, thus enhancing soil aggregate stability (Mathew *et al*., 2012) [42] and increasing SOM in the soil profile. Kabiri *et al*. (2016 [32] and Muhammad *et al*. (2017) [49] reported that water-logged conditions in paddy fields generate anaerobic environment and reduce the rate of SOM mineralization by limiting the microbial population and their growth; also, Zhou *et al*. (2014) [83] demonstrated that wheat straw incorporation is the best approach to maximize C accumulation and reduce atmospheric carbon, which improves the physical conditions of the soil and subsequently enhances the root development for crop production. Yadav *et al*. (2017) [75] and Mi *et al*. (2016) [44] concluded that SOM significantly improved with the incorporation of straw into the soil profile at depths of 0–40 cm under RWR system. The average TN content increased in the range of 1.17 to 14.51% in the soil profile (0–30 cm) compared with the pre-analysed value. The mean maximum TN (0.981 gkg^{-1}) was found with RT_{si60}, and the lowest (0.848 gkg^{-1}) was found under CT_{ns} after two rice crop growing cycles. Moreover, a considerable positive variation in TN was recorded at 10–20 cm in RT_{si60} (1.051 gkg^{-1}), which was higher than CT_{si60} (0.926 gkg^{-1}) treatments under RT and CT methods [Fig.14c]. Wienhold *et al*. (1999) [71] found higher TN content under conservation tilling than conventionally tilled soil in continuous spring wheat cropping. Zhang *et al*. (2015) [80] demonstrated that SOC storage was significantly higher by 29.7% and 17.7% at depths of 0–10 and 10–20 cm, respectively, when incorporating different amounts of residue.

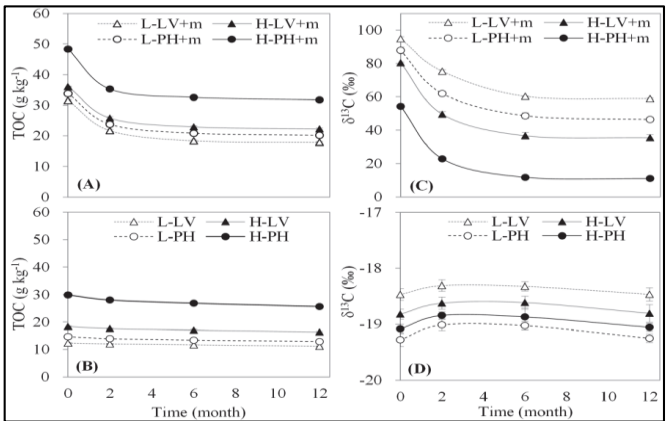

Fig 14 (a): Dynamic patterns of total organic carbon content (TOC) and $\delta^{13}C$ signature

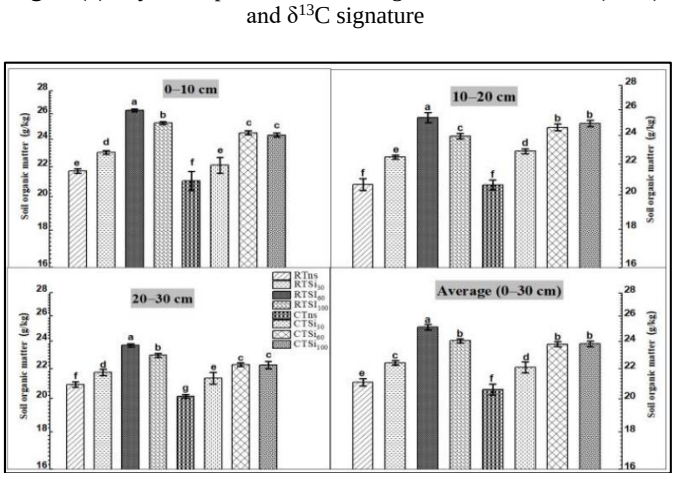

Fig 14(b): Depth-wise distribution of mean soil organic matter (SOM) under each treatment

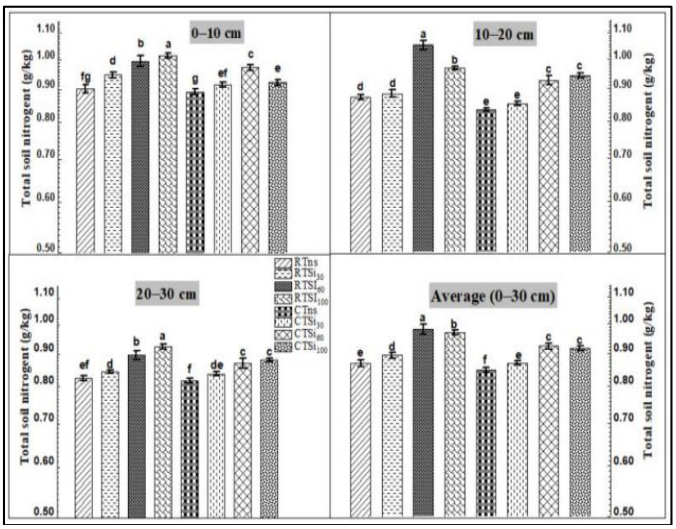

Fig 14(c): Changes Mean total soil nitrogen at different soil depths

Zhu *et al*. (2014) [84] reported that the different treatments significantly affected the contents of soil TOC and labile organic C fractions, where PD generally had the highest contents of TOC, DOC, MBC and EOC at the three soil depths. Crop straw return treatments (PR, PW, PD, RR, RW, RD) had consistently higher amount of TOC and labile organic C fractions at the three soil depths than without crop straw return treatments (PN, RN). Moreover, PN had significantly lower TOC, DOC, MBC and EOC at 0–7 cm and 7– 14 cm, and RN had the lowest TOC and MBC at 14–21 cm compared to other treatments [Fig.15a]. PD had the highest content of soil TOC at all the three soil depths [Fig.15a]. The reason might be that plowing tillage made the soil and straw in the plow layer turned over quarterly, which increased the stability of the TOC content at each soil layer (Zhang *et al*., 2011). In addition, the rice and wheat straw were both returned under PD treatment from 2009, plowing tillage made much SOM enter into the soil and accumulate (Song *et al*., 2008) [62]. However, Tian *et al*. (2010) [65] found that rotary tillage with straw return had higher SOC than plowing tillage with straw return at 0–10 cm soil depth in wheat field.

Sarkar *et al*. (2018) [55] reported that the cumulative amount of native SOC mineralisation was significantly higher in the residue-amended than the control aggregates [Fig.15b]. The amount of native SOC mineralised across the residue-amended aggregates was in the order of macro- ≥ micro- > mega-aggregates in both soils [Fig.15b]. Across the tillage treatments, native SOC mineralisation was in the order of CT ≥ RT > NT in the Luvisol, and CT = NT in the Vertisol. The pattern of total organic C (TOC) mineralisation, i.e. the combined residue-C and native SOC mineralisation was similar to native SOC mineralisation across all the treatments and aggregate-size classes [Fig.15b]. Aggregate-size classes, with a hierarchy of smaller to higher structural units, are likely to have different aggregate stability and SOM bioavailability and consequently microbial activity (Zhang *et al*., 2014) [78, 79], which may further vary with tillage intensity.

Guan *et al*. (2018) also found that the macro-aggregate-associated organic C had the highest content (6.38–7.32 g kg^{-1}) in all three treatments, followed by free micro-aggregates (4.57–5.89 g kg^{-1}) and non-aggregated silt + clay fractions (3.25–4.97 g kg^{-1}) [Fig.15c]. Moreover, M-silt + clay fractions, Fm silt + clay fractions and mM-silt + clay fractions exhibited the highest organic C contents in macro-aggregates, free micro-aggregates and mM, respectively. Compared with CK, WW and YW had no significant effect on macro-aggregates-associated organic C content, whereas C associated with non-aggregated silt + clay fractions significantly increased by 52.9% in WW and 46.8% in YW. Free micro-aggregate- associated C was not significantly different in WW than in CK but was 22.4% lower in YW than in CK. Moreover, a significant decrease in the organic C content of Fm-POM (49.4%) and Fm-silt + clay fractions (16.9%) was detected in YW compared with CK.

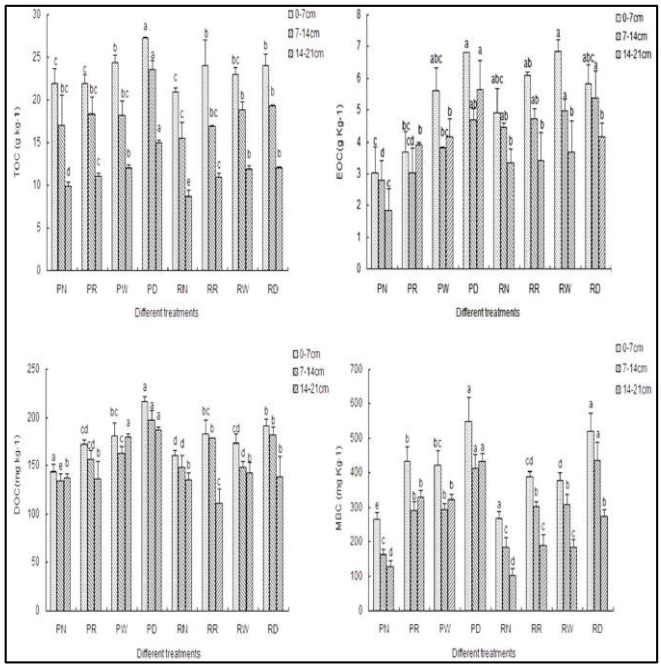

Fig 15(a): Effects of eight treatments on soil TOC, EOC, DOC and MBC contents at three depths

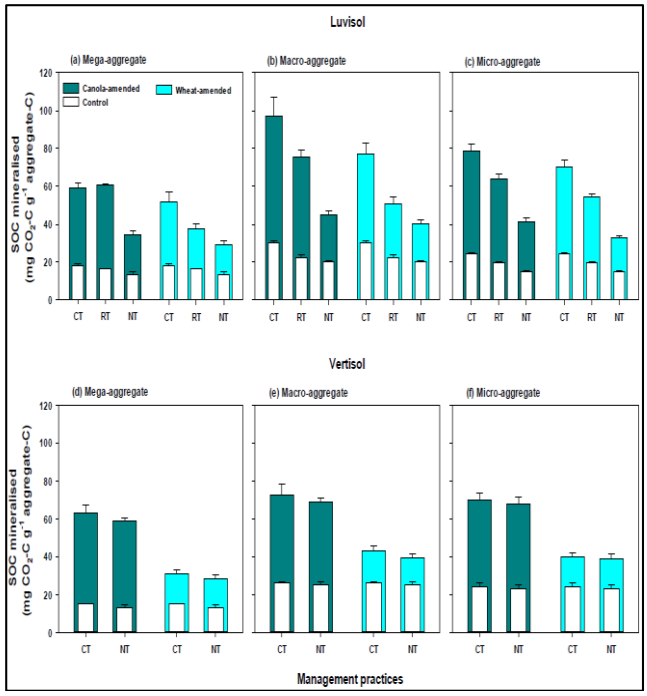

Fig 15 (b): Cumulative amount (mg CO_2-C g^{-1} aggregate-C) of native soil organic carbon (SOC) mineralised from the non-amended control

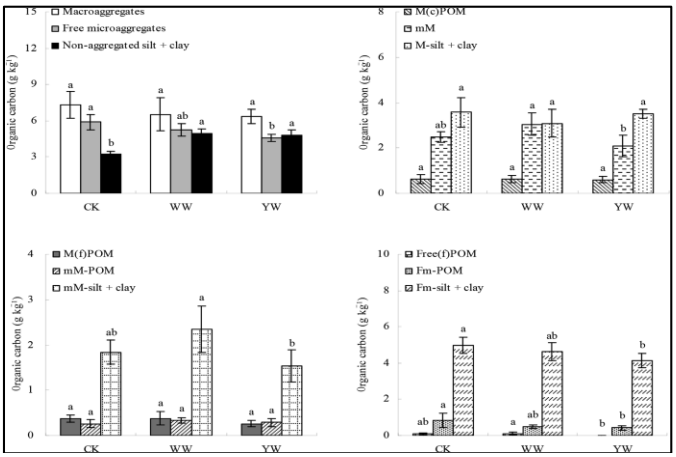

Fig 15(c): Organic carbon contents of hierarchical aggregates in soil under different treatments

Zou *et al*. (2018) revealed that the depth by rotation interaction, crop rotation significantly increased large macro-aggregates and depth was synergistic to rotation's effect [Fig.16a]. These results indicate that rotation and manure application practices can improve soil structure and macro-aggregates but varied with soil depth increment. Nitrogen fertilizer management and rotation all significantly affected whole-soil and small macro-aggregate and micro-aggregate associated SOC, while fertilizer and rotation had no significant effect on SOC in large macro-aggregates and the silt-clay size class fraction [Fig.16b]. With increasing soil increment, the whole soil, micro-aggregates, and silt-clay fraction associated SOC stocks increased accordingly. Fertilizer management and rotation significantly affected all whole-soil and all aggregates associated SOC stocks. The interaction of depth by rotation significantly affected whole-soil, large macro-aggregate, and small macro-aggregate associated SOC stocks. The interaction of depth by fertilizer significantly affected whole-soil SOC stocks. At 0–10 cm, manure amendment significantly increased SOC stock in the whole soil for mono-cropping, while at 10–20 cm manure amendment significantly increased the whole soil SOC stock only in mono-cropping [Fig.16c].

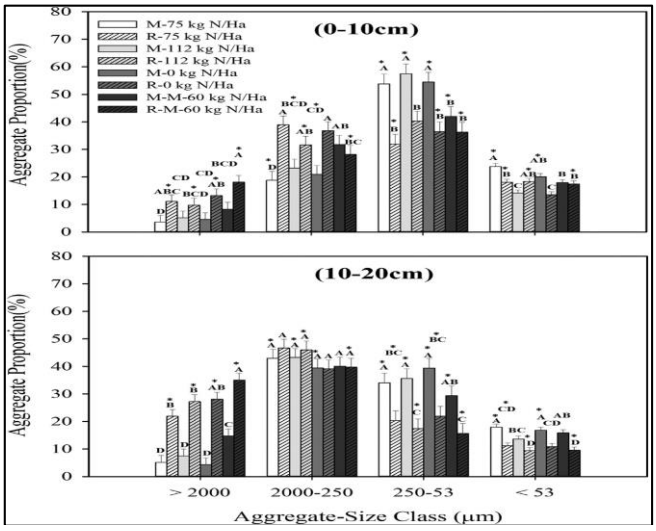

Fig. 16 (a): The proportions of soil in large macro-aggregates, small macro-aggregates, micro-aggregates, and the silt-clay size class percentage due to the rotation by fertilizer interactions at the 0–10 cm (upper) and 10–20 cm (lower) soil layers.

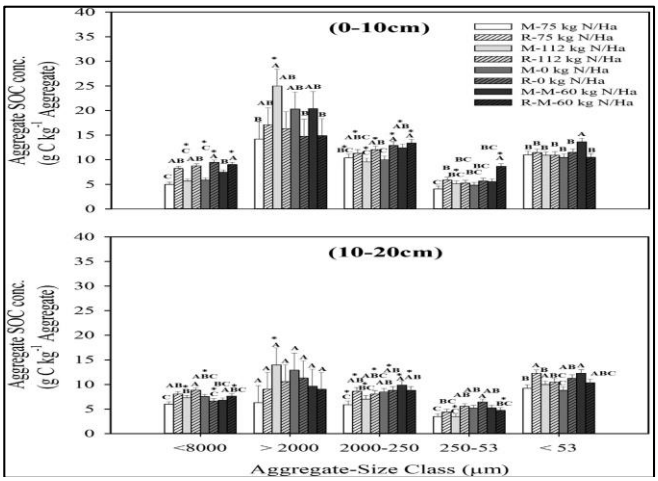

Fig 16(b): The SOC concentrations in the silt-clay size grade, micro-aggregates, small macro-aggregates, large macro-aggregates, and whole-soil, for the rotation with fertilizer interactions in the 0 to 10 cm (upper) and 10 to 20 cm (lower) depths.

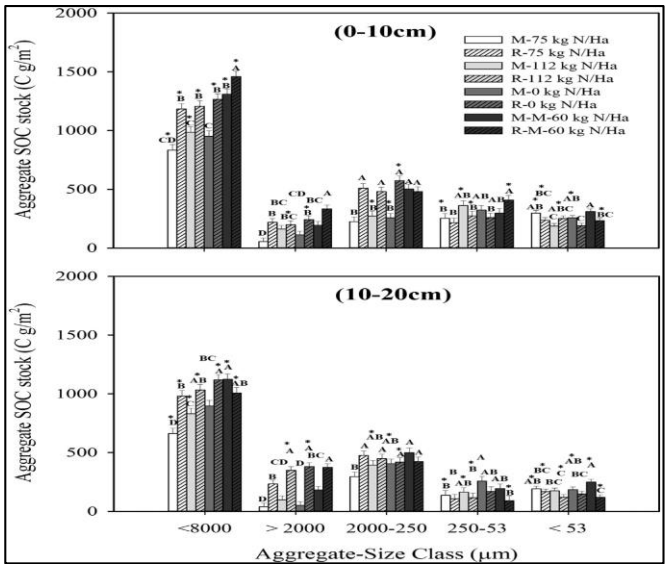

Fig 16(c): The SOC stocks (SOCS) in the silt-clay size grade, micro-aggregates, small macro-aggregates, large macro-aggregates, and whole-soil, for the rotation with fertilizer interactions in the upper and lower soil layers at depths of 0 to 10 cm and 10 to 20 cm.

Mikha and Rice, (2004) [45] reported that the aggregate associated labile C was significantly affected by tillage, N source, and aggregate size fractions. Aggregate labile C [Fig.17a] was significantly greater with NT than CT and with M than F, except for the 20- to 53-µm aggregates where tillage and N source had no significant effect on labile C and N [Fig.17a]. Aggregate labile N was significantly greater for aggregates >2000 with NT than CT and at aggregates >2000-µm and 53- to 250-µm diam. with M than F [Fig.17a]. In general, aggregate labile C and N were significantly greater with macro-aggregates than micro-aggregates. The masses of total C and N were significantly associated with aggregate 250- to 2000-µm NT and M, while total C and N were significantly associated with aggregates 53- to 250- and 250- to 2000-µm diam. for CT and F, respectively [Fig.17b]. Labile C was significantly associated with NT and Min the 250- to 2000-µm aggregate [Fig.17c]. Aggregate labile N was significantly associated with macro-aggregates (>2000- and 250- to 2000-µm diam.) in NT and with aggregates 53- to 250-µm diam. for CT [Fig.17c]. Nitrogen was greater with M than F treatment in aggregate-size classes 53 to 250 and

>2000 while labile C was greater in all aggregate-size classes except for those of 20 to 53 µm [Fig.17c].

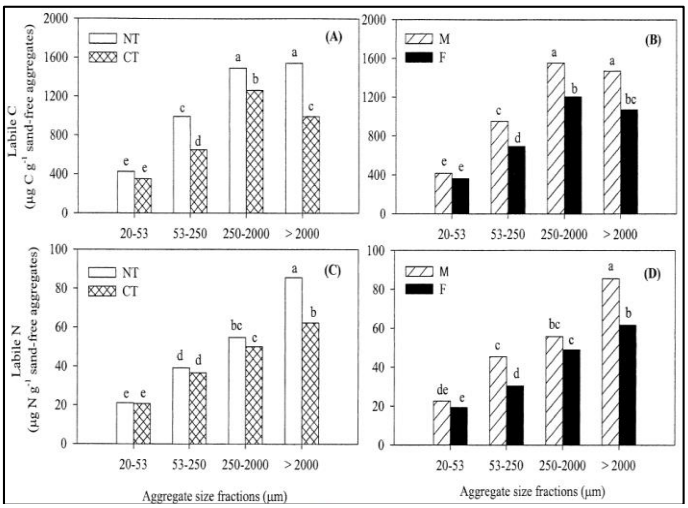

Fig 17 (a): Labile C and N after 28 d of incubation (µg g^{-1}; normalized to sand-free basis) in water-stable aggregates

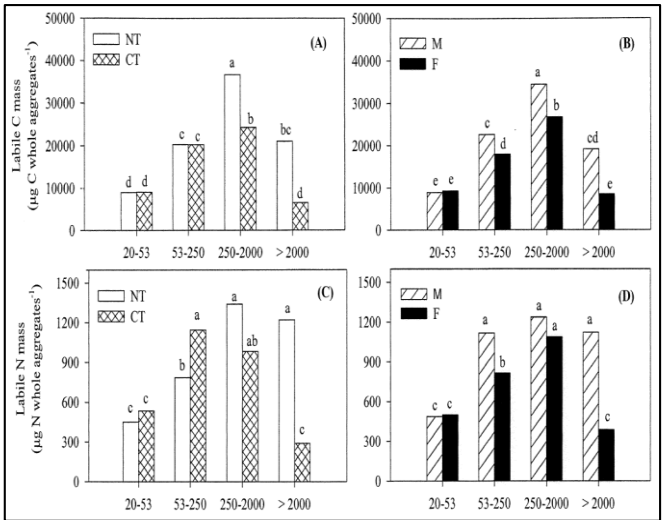

Fig 17(b): Labile C and N mass after 28 d of incubation (µg whole $aggregate^{-1}$; normalized to sand-free basis) in water-stable aggregates

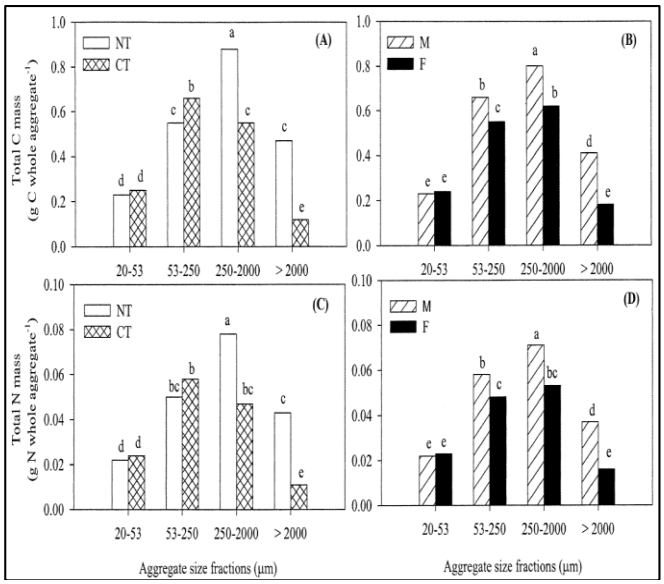

Fig 17 (c): Total C and N masses (g whole $aggregate^{-1}$; normalized to sand-free basis) in water-stable aggregates

Conclusion

Effect of aggregate size was relatively small on the priming of aggregate associated carbon and soil aggregate dynamics compared with tillage intensity and soil type. In general, the soil aggregate dynamics was greater in the macro- versus mega- and micro-aggregates under ZT or RT versus CT systems in the Inceptisol. A range of biological (e.g. C mineralisation, microbial activity) and chemical mechanisms could operate simultaneously to release considerable plant available nutrients from the soil aggregate reserves in residue-amended farming systems. Organic C associated with free micro-aggregates increased whereas organic C associated with non-aggregated silt + clay fractions significantly decreased. For hierarchically organized soil aggregates, non-aggregated silt- and clay-sized fractions noticeably decreased, which resulted in MWD and GMD of water-stable aggregates improved under conservation agriculture (CA) practices. The results suggested that adoption of CA affects total soil C stocks but positively impacted WSOC, carboxyl C and non-aggregated silt- and clay-associated C and positively impacted phenol C, free micro-aggregates-associated C and aggregate stability. Greater proportion of labile C and N and mineral–associated organic matter in water-stable aggregates were associated with macro-aggregates than micro-aggregates as affected by aggregate associated carbon. Manure addition and NT not only increased labile C in macro-aggregates, but also significantly increased labile C in aggregates at 53 to 250µm in diameter. Increasing labile C in micro-aggregate (53–250µm) indicated that NT and M addition improved microbial biomass associated with aggregates. No-tillage and M treatments significantly increased total C and N and the formation of macro-aggregates. Conventional tillage in comparison with NT significantly reduced macro-aggregates with a significant redistribution of aggregates - into micro-aggregates. Aggregate protected labile C and N were significantly greater for macro-aggregates, (>2000 and 250–2000 µm) than – micro-aggregates (53–250 and 20–53 µm) and greater for M than F indicating physical protection of labile C within macro-aggregates. No -tillage and M a lone each significantly increased soil aggregation and aggregate-associated C and N; however, NT and M together further improved soil aggregation and aggregate-protected C and N.

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