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K Lokeshwar

Department of Agronomy, Sardar Vallabhbhai Patel University of Agriculture & Technology, Meerut, Uttar Pradesh, India

RK Naresh

Department of Agronomy, Sardar Vallabhbhai Patel University of Agriculture & Technology, Meerut, Uttar Pradesh, India

SP Singh

Department of Soil Science and Agricultural Science, Sardar Vallabhbhai Patel University of Agriculture & Technology, Meerut, Uttar Pradesh, India

Manisha

Depart of Agriculture Sciences, Dev Bhoomi Uttarakhand University, Dehradun, Uttarakhand, India

Rajaram Choudhary

Department of Agronomy, Sardar Vallabhbhai Patel University of Agriculture & Technology, Meerut, Uttar Pradesh, India

Mohd Shah Alam

Department of Agronomy, Sardar Vallabhbhai Patel University of Agriculture & Technology, Meerut, Uttar Pradesh, India

Himanshu T

Department of Agronomy, Sardar Vallabhbhai Patel University of Agriculture & Technology, Meerut, Uttar Pradesh, India

Corresponding Author: K Lokeshwar

Department of Agronomy, Sardar Vallabhbhai Patel University of Agriculture & Technology, Meerut, Uttar Pradesh, India

Influence of tillage practices, fertilization and straw alters on soil aggregates, organic carbon composition and microbial community in rice-wheat cropping system: A review

K Lokeshwar, RK Naresh, SP Singh, Manisha, Rajaram Choudhary, Mohd Shah Alam and Himanshu T

Abstract

In India, the average soil organic carbon (SOC) content of cultivated land is 30% less than the world average. Therefore, cultivation management-induced changes in SOC dynamics are necessary, especially in *Typic Ustochrept* soils, where the SOC stocks are limited. Soil tillage can affect the stability and formation of soil aggregates by disrupting soil structure. Frequent tillage deteriorates soil structure and weakens soil aggregates, causing them to be susceptible to decay. SOC pools, such as coarse particulate organic matter C (CPOM-C), microbial biomass carbon (MBC), and mineralizable C (C min) respond to changes in management practices and provide sensitive indication of changes in the SOC dynamics than commonly reported total soil C alone. POC reduction was mainly driven by a decrease in fine POC in topsoil, while DOC was mainly reduced in subsoil. Fine POC, LFOC and microbial biomass car be useful early indicators of changes in topsoil organic C. In contrast, LFOC and DOC are useful indicators for subsoil. Reduced proportions of fine POC, LFOC, DOC and microbial biomass to soil organic C reflected the decline in soil organic C quality caused by tillage and straw Management practices. Average SOC concentration of the control treatment was 0.54%, which increased to 0.65% in the RDF treatment and 0.82% in the RDF+FYM treatment sequestered 0.16 Mg Cha⁻¹yr⁻¹.

Relative to the control (no nutrient input CK), long-term fertilization appreciably increased SOC storage by 134, 89 and 129 kg ha⁻¹ yr⁻¹ under CF, and 418, 153 and 384 kg ha⁻¹ yr⁻¹ under organic manure plus chemical fertilizers (MCF) in plough layer soils (0–20 cm). The mineral-associated OC (MOC) pools accounted for 72, 67 and 64% of the total SOC with sequestration rates of 76, 57 and 83 kg ha⁻¹ yr⁻¹ under chemical fertilizers (CF) and 238, 118 and 156 kg ha⁻¹ yr⁻¹ under MCF, respectively. Moreover, the MOC pool displayed a saturation behavior under MCF conditions. The particulate OC (POC) accordingly constituted 27, 33 and 36% of SOC, of which Light-POC accounted for 11, 17 and 22% and Heavy-POC for 17, 16 and 15% of SOC, respectively. The sequestration rates of POC were 58, 32 and 46 kg ha⁻¹yr⁻¹ under CF, and 181, 90 and 228 kg ha⁻¹ yr⁻¹ under MCF in which Light-POC explained 59, 81 and 72% of POC under CF, and 60, 40 and 69% of POC under MCF, with Heavy-POC accounting for the balance. Compared with CK, the application of CF alone did not affect the proportions of MOC or total POC to SOC, whereas MCF application markedly reduced the proportion of MOC and increased the POC ratio, mainly in the Light-POC pool. The distribution of SOC among different pools was closely related to the distribution and stability of aggregates.

Compared to conventional tillage, conservation tillage (no-tillage coupled with straw return) increased water-stable large macro-aggregates (>2 mm) by 35.18%, small macro-aggregates (2–0.25 mm) by 33.52% and micro-aggregates by 25.10% in the topsoil (0–20 cm). The subsoil (20–40 cm) also showed the same trend. Compared to conventional tillage without straw return, large and, small macro-aggregates and micro-aggregates in conservation tillage were increased by 24.52%, 28.48% and 18.12%, respectively. Straw return also caused a significant increase in aggregate-associated C). No-tillage coupled with straw return had more total aggregate-associated C within all the aggregate fractions in the topsoil. But the different is that conventional tillage with straw return produced the highest carbon preservation capacity (CPC) of macro-aggregates and micro-aggregates in the topsoil. A considerable proportion of the SOC was found to be stocked in the small macro-aggregates under both topsoil (74.56\%) and subsoil (67.09\%).

The review study confirmed that conservation tillage with organic manure amendment not only sequestered more SOC but also significantly altered the composition of SOC, thus improving SOC quality, which is possibly related to the SOC saturation level. Thus, straw return integrated with mineral fertilization in rice- wheat croplands leads to increased SOC stocks. However, those effects of straw return are highly dependent on fertilizer management, cropping system, soil type, duration period, and the initial SOC content.

Keywords: Tillage management, carbon fractions, soil organic carbon

Introduction

Soil organic carbon (SOC) is an important soil component that plays a crucial role in soil fertility (Brar *et al.*, 2013) ^[11], environmental protection (Ghosh *et al.*, 2018) ^[21] and sustainable agricultural development (Li *et al.*, 2018) ^[30]. It has therefore been regarded as the foundation of soil quality and function (Brar *et al.* 2013) ^[11] Farmland SOC sequestration is closely related to the reduction of CO₂ emissions (Poulton *et al.* 2018) ^[39].

the enhancement of soil fertilization the maintenance of soil structure (Sainju et al., 2009) [41] and the promotion of microbial diversity (Fonte et al., 2012; Bhattacharyya et al., 2018) ^[20, 8] among other items. Hence, it is the decisive factor affecting the quality of cultivated land and crop yield (Brar et al., 2013; Hassan et al., 2016) [11, 25]. However, the SOC content in Chinese farmland soil is generally low (Chen et al., 2017) ^[13, 14] which is lower than the world average by more than 30% and that of Europe by more than 50%. Organic C in Indian soils was estimated as 23.4-27.1 Pg (Dadhwal and Nayak, 1993) ^[16]. Chhabra et al. (2003) ^[12] estimated the organic C pool of Indian forest soils as 6.8 Pg C in top 1 m, using estimated SOC densities and Remote Sensing based area of forest types. Another attempt to estimate SOC stock was made by Gupta and Rao (1994)^[24] who reported an SOC stock in Indian soils of 24.3 Pg for soil depths ranging from surface to an average depth of 44-186 cm. However, the first comprehensive report of SOC stock in India, was carried out by Bhattacharyya et al. (2000)^[6] who estimated 9.5 Pg SOC at a depth of 0–0.3 m. Soil type could be one of the important parameters that regulate organic C status of the soil. The major portion of SOC is retained through clay-organic matter interactions indicating the importance of the inorganic part of the soil as substrate to bind the organic carbon (Manna et al 2005) ^[33]. Therefore, the improvement of the SOC content of cultivated soil has been a topic of great concern in the field of agricultural science. In addition to the influence of natural factors such as regional weather and soil conditions (Gonçalves *et al.*, 2017) $^{[22]}$ the variation in the agriculture SOC stock is most strongly affected by human activities (Ghosh et al., 2018; Liang et al., 2012) [21, 31]. The effect of management practices on farmland SOC content has been extensively investigated, and most studies have indicated that conservation farming measures (e.g.) no-tillage, application of organic fertilizer, and straw return) not only increase the agriculture SOC stock (Liu and Zhou, 2017) [32] but also improve crop yield (Bai et al., 2016; He et al., 2018) [4, 26]. These measures mainly increase farmland SOC content by increasing SOC input and improving soil aggregate retention (Arai et al., 2013)^[3]. However, some studies have suggested that although no-tillage and straw return are beneficial to SOC accumulation, they may also reduce crop yield (Tian et al., 2016) [48]. Moreover, currently, organic fertilizers have mostly been applied to orchards and vegetable plots that are economically intensive. In contrast, they have rarely been applied to field crops and thus have made little contribution to field carbon inputs (Li et al., 2011). Therefore, these studies raise new questions. Firstly, among various farming management measures, which measure is most effective in retaining SOC, and which aggregate distribution relationship is involved? Secondly, is straw return more effective than organic fertilizer application in retaining SOC and how can carbon sequestration capacity is quantitatively represented? Thirdly, do no-tillage and straw return truly lower crop yield? No-tillage coupled with straw-returning and nitrogen (N) fertilizer application helps to improve soil aggregation (Cowie et al., 2014; Angers and EriksenHamel, 2008) [15, 2]. It also decreases soil disturbance and reduces the activity of the soil microbial community that results in SOC decomposition, favoring the formation of large macro-aggregates (LMA)associated OC (Six et al., 2010; Bhattacharyya et al., 2012) ^[45, 7]. Conventional tillage destroyed LMAs in the soil (Abid and Lal, 2008; Six et al., 2004)^[1, 46]. In contrast, long-term

no-tillage drastically reduced the rate of macro-aggregate (MA) turnover and resulted in the formation of stable microaggregates (MIs), favoring C stabilization and sequestration (Jiang et al., 2010; Blancocanqui and Lal, 2004)^[27, 10] The LMAs formed under no-tillage management had higher SOC contents and increased numbers of macro-pores, resulting in higher water infiltration and better aeration when compared with soils that were richer in MIs (Jiang et al., 2011)^[28]. LMAs also physically protect the labile soil OC from enzymatic and microbial attack (Nakajima et al., 2016; Bhattacharyya *et al.*, 2012) ^[36, 7]. Therefore, the accumulation of soil OC could be achieved by establishing management practices that increase the proportion of soil MAs (Blanco-Canqui et al., 2007)^[9]. Moreover, long-term application of chemical fertilizers either alone or in combination with organic manure could increase total SOC, but the distribution of sequestered OC into the C pools or the roles of OC pools in stabilizing SOC may differ between these two fertilization regimes due to the variations in their C input and soil environmental conditions; and these differences may subsequently further alter the SOC quality.

Different types of soil carbon

Song *et al.* (2019) ^[47] reported that the average topsoil contents of total carbon (TC), SOC and labile organic carbon (LOC) were 12.72 gkg⁻¹, 11.01 gkg⁻¹, and 7.13 gkg⁻¹, respectively, under the three no-tillage treatments (T_7 , T_8 , and T_9). These mean values were significantly higher than those under the conventional tillage treatments (T_1 , T_2 and T_3), which had average TC, SOC, and LOC contents of 9.87 gkg⁻¹, 8.56 gkg⁻¹, and 5.20 gkg⁻¹, respectively. The average contents of the three carbon types under rotary tillage were between those under conventional tillage and those under no-tillage. The contents of TC and SOC were significantly lower than those under no-tillage. The subsoil contents of TC, SOC, and LOC under conventional tillage were 9.42 gkg⁻¹, 7.60 gkg⁻¹, and 5.97 gkg⁻¹, respectively, which were 17.16%, 4.25% and 16.83% higher than those under no-tillage.

In addition to the influence of tillage, straw return and organic fertilizer also led to variations in soil carbon content among the different treatments. The average top soil TC contents under straw return (T₂, T₅ and T₈) and organic fertilizer (T₃, T₆ and T₉) were 16.83% and 19.78% higher than those under chemical fertilizer only (T₁, T₄ and T₇), with F = 6.852. Similar results were observed for the SOC and LOC. The average subsoil contents of TC, SOC, and LOC under straw return were 8.82 gkg⁻¹, 7.61 gkg⁻¹, and 5.68 gkg⁻¹, respectively. The corresponding values under organic fertilizer were 9.33 gkg⁻¹, 8.15 gkg⁻¹, and 5.67 gkg⁻¹, respectively. The sub soil TC and SOC contents under straw return were significantly higher than those under chemical fertilizer only, but no significant differences were observed in the LOC content.

Sainju *et al.* (2009) ^[41] reported that differences in tillage and cropping sequences among treatments resulted in variations in SOC, STN, POC, and PON concentrations in whole-soil and aggregates (Fig. 1a and 1b). Concentrations were lower in STW-F than in other treatments in almost all aggregate-size classes at both depths. This was probably a result of increased tillage frequency, followed by reduced amount of crop residue returned to the soil. While plots were not tilled in NTCW or tilled once a year in the spring at planting in STCW, plots in STW-F were tilled three to four times a year for seeding and

controlling weeds. Compared with no-tillage in the continuous spring wheat system, tillage primarily reduced SOC and STN in the <0.25-mm size class at 0 to 5 cm, POC in the 4.75- to 2.00-mm size class at 0 to 5 cm and 5 to 20 cm, and PON in the 2.00- to 0.25-mm size class at 5 to 20 cm. This suggests that tillage mineralizes slow fractions of C and N in micro-aggregates in the surface soil and intermediate fractions in macro-aggregates in both surface and subsurface soils. The mean annualized amount of crop residue returned to the soil from 1984 to 2004 was 2.31 Mg ha⁻¹ in STW-F compared with 3.58 to 3.91 Mg ha⁻¹ in other treatments (Sainju *et al.*, 2007a) ^[40]. Reduced amounts of crop residue returned to the soil due to an absence of crops during fallow also probably reduced SOC, STN, POC, and PON in whole-soil and aggregates in STW-F. Furthermore, greater soil

temperature and water contents during fallow than during cropping probably enhanced the mineralization of residue and soil organic matter (Naresh *et al.*, 2018) ^[37], thereby lowering SOC, STN, POC, and PON levels in STW-F.

Greater SOC, STN, POC, and PON were also observed in NTCW and STCW than in FSTW-B/P in the <2.00-mm size class at 0 to 5 cm (Fig. 1a and 1b). This indicates that reduced tillage, followed by a change in the cropping system from continuous spring wheat to spring wheat-barley/pea probably increased organic C and N concentrations in small macro-aggregates and micro-aggregates at the surface soil. While residue quantity is directly related with SOC and STN in whole-soil and aggregates, residue quality, such as C: N ratio can influence SOC and STN by altering the decomposition of residue in the soil.



Fig. 1a: Effects of tillage and cropping sequence on dryland soil organic C (SOC) and total N (STN) concentrations in aggregates at the 0- to 5and 5- to 20-cm depths. NTCW, no-tilled continuous spring wheat; STCW, spring-tilled continuous spring wheat; and STW-F, spring-tilled spring wheat-fallow [Source: Sainju *et al.*, 2009] ^[41].



Fig 1b: Effects of tillage and cropping sequence on dryland soil particulate organic C and N (POC and PON) concentrations in aggregates at the 0- to 5- and 5- to 20-cm depths [Source: Sainju *et al.*, 2009] ^[41].

Zhang *et al.* (2020) ^[49] revealed that long-term tillage, straw, and fertilization management could significantly affect soil aggregate stability in both surface and subsurface layers. Aggregate stability decreased with the depth, as indicated by the mean weight diameter (MWD) and the geometric mean diameter (GWD) (Fig.2a and 2b). In the surface layer, the treatment with straw-returning increased the MWD and GWD of soil aggregates by 16–50% and 14.67–70.88%, respectively, compared with the treatment without straw (CT₁-N₀-P₀-Straw₀, CT₂-N₁-P₂-Straw₀, and NT-N₂-P₁-Straw₀). Long-term applications of N and P fertilizer without straw did not significantly affect soil aggregate stability, as indicated by the similar MWD values in both surface and subsurface

layers. However, the GWD of $CT_1-N_0-P_0$ -Straw₀ and $CT_2-N_1-P_2$ -Straw₀ treatments did not decrease at 20–40 cm depth soil (Fig.2b). Meng *et al.* (2014) ^[34] reported that straw-returning caused lower mineralization of native SOM and thus facilitated increased LMA formation, leading to higher MWD. This may be attributed to the fact that no-tillage decreased soil disturbance, facilitating the protection of soil organic matter from microbial degradation, which in turn favored the generation of physically stable LMAs and Mas, and increase the soil stability (Sarker *et al.*, 2018) ^[42]. The N and P fertilizer had no significant effect on MWD in the soil, but when increased the amount of N or P fertilizer, the MWD reduced in both layers (Fig.2a).



Fig 2a: The mean weight diameter (MWD) of different tillage management and fertilization at 0–20 and 20–40 cm and 20–40 cm [Source: Zhang *et al.*, 2020]^[49]



Fig 2b: The geometric mean diameter (GWD) of different tillage management and fertilization at 0–20 and 20–40 cm [Source: Zhang *et al.*, 2020]^[49]

Dey et al. (2016) ^[17] reported that in the surface (0-15 cm) soil, DSR+BM-ZTW, DSR+BM-ZTW+RR, MBR+DSR-ZTW-ZTMB and MBR+DSR-ZTW+RR-ZTMB had 15.6, 31.0, 62.1 and 70.6 per cent more POM-C, respectively compared with TPR-CTW (Fig. 3a). Compared with DSR-ZTW+RR, POM-C under DSR+BM-ZTW +RR and MBR+DSR-ZTW+RR-ZTMB was higher by 21.5 and 58.2 per cent, respectively (Fig. 3a) owing to addition of extra biomass in the latter options. Zero tillage only in wheat or addition of rice residue alone did not contribute much to the POM-C compared to conventional practice. Maximum improvement in POM-C was there due to mung bean residue retention compared to others. Among the single residue DSR-ZTW systems, retention of mung bean residue resulted in the highest POM-C, which was 52.7 per cent higher than DSR-ZTW without any residue retention. Out of the two double residue systems, MBR+DSR-ZTW+ RR-ZTMB had 30.2 per cent higher POM-C compared with DSR+BM-ZTW+RR (Fig. 3a). In the sub-surface soil layer, all treatments had similar

POMC except under MBR+DSR-ZTW+RR-ZTMB, which registered 23.3 per cent higher POM-C compared with that under TPR-CTW (Fig. 3a). The DSR+BMZTW+ RR, MBR+DSR-ZTW-ZTMB and MBR+DSR-ZTW+ RR-ZTMB had 42.6, 50.4 and 66.5 per cent more POM-N in surface soil as compared to TPR-CTW (Fig. 3b).

Under CA practices, slow macro-aggregate turnover in ZT allowed time for the formation of POM from recent cropderived organic matter and subsequent encapsulation of this POM by mineral particles and microbial byproducts to form stable aggregates containing young crop-derived C. Again, increasing supply of crop derived organic matter under double residue plots (DSR+BM-ZTW+RR and MBR+DSR-ZTW+RR-ZTMB) maintained the continuity of this process and thus resulted in higher portion of SOC and N complexes or encapsulated within POM, especially in the 0-15 cm soil layer. In contrast, the turnover of macro-aggregates in CT is faster, providing less opportunity for formation of POM and stable aggregates (Six et al., 2000)^[44].



Fig 3a: Changes in POM associated C as an effect of tillage, residue retention and crop establishment practices [Source: Dey et al., 2016] [17]



Fig 3b: Changes in POM associated N as an effect of tillage, residue retention and crop establishment practices [Source: Dey et al., 2016] [17]

Aggregate size distribution

Song et al. (2019)^[47] also found that the topsoil contents of large macro-aggregates (>2 mm), small macro-aggregates (2-0.25 mm), and micro-aggregates (0.25-0.053 mm) were approximately 10%, 50% and 20%, respectively. The subsoil contents of the three size aggregates had similar distribution trends but were lower than those of the topsoil. No-tillage and straw return caused a significant increase in the contents of macro-aggregates and micro-aggregates, especially in the topsoil. No-tillage (T7, T8 and T9) increased the numbers of large macro-aggregates (11.25%) and small macro-aggregates (9.45%) compared to those under conventional tillage (T₁, T₂, and T_3). A similar trend was observed in the subsoil. Under the same tillage, the order of large and small macro-aggregate contents in the topsoil and subsoil was as follows: straw return >organic fertilizer >single application of chemical fertilizer. In particular, more large macro-aggregates were observed under no-tillage than under straw return (T₈), and these values were 6.76% and 28.68% higher than those under the single application of fertilizer (T_7) in the topsoil and subsoil, respectively.

Zhang *et al.* (2020) ^[49] observed that the treatments, SC fractions (<0.053 mm) were predominant, accounting for 32–56% of the mass of the 0–20 cm layer (Fig. 4a). LMAs were the smallest fractions, accounting for 4–12% of the mass of the bulk soil at 0–20-cm depth. The mass of LMAs was not significantly affected by the tillage method, mineral fertilizer, and straw (Fig. 4a). However, no-tillage increased LMA mass by 55% at 0–20 cm depth, compared with conventional tillage, (Fig. 4a). The application rates of straw increased the mass of LMA, MA, and MI fractions, and decreased the SC fractions, but had no significant effects on statistically. Additionally, different amounts of N and P fertilization did not significantly influence the mass distribution of MA, MI, and SC fractions (Fig.4a). Similarly, at the 20–40 cm layer,

SC fractions were the predominant fractions among all the size aggregates, accounting for 41-55% of the bulk soil. The LMAs were the smallest fraction (Fig. 4a). No-tillage increased the mass of the LMAs by 71% at the 20-40 cm depth, while the mass of the SC fractions and MIs decreased, compared with the conventional tillage method (Fig. 4a). The no straw-returning treatments (CT₁-N₀-P₀-Straw₀, CT₂-N₁-P₂-Straw₀, and NT-N₂-P₁-Straw₀) had a lower mass of LMAs and MAs (Fig. 4a). Guo et al., (2019) [23] who found that straw acts as an exogenous C source contributing to OC sequestration, and the increased OC content of LMAs, because the LMAs have higher water infiltration and better aeration, while also having higher stability (Jiang et al., 2011) ^[28]. However, only the application of N and P fertilizers or no fertilizer (CT₁-N₀-P₀-Straw₀, CT₂-N₁-P₂-Straw₀, and NT-N₂-P₁-Straw₀) increased the mass and associated OC content of SC fractions, compared with other straw treatments (Fig. 4a). It is also showed that no-tillage method would become meaningless without straw application.

Sainju et al. (2009) [41] observed that No-till increased aggregate proportion compared with tilled treatments in the continuous spring wheat system in the 4.75- to 2.00-mm size class at 0 to 5 cm and in the 2.00- to 0.25-mm size class at 5 to 20 cm (Fig.4b). This resulted in subsequent increases in aggregate proportions in smaller aggregates. No-till increases soil aggregation by reducing soil disturbance and increasing soil organic matter content and the growth of fungi that bind the soil particles and micro-aggregates together. An increase in aggregate proportion was also observed in the fallow treatment in the 2.00- to 0.25-mm size class at 5 to 20 cm, whose reasons were not known. Since aggregates <0.84-mm size class are prone to wind erosion in semi-arid drylands increased proportion of aggregates >2.00-mm size class in notill compared with conventional till might reduce the risk of soil erosion.



Fig 4a: Mass distribution of four different size aggregates (>2, 0.25–2, 0.053–0.25, and <0.053 mm) under tillage and fertilization treatments from 0–20 and 20–40 cm [Source: Zhang *et al.*, 2020]^[49]



Fig 4b: Effects of tillage and cropping sequence on dryland soil aggregate-size distribution at the 0- to 5- and 5- to 20-cm depths. NTCW, notilled continuous spring wheat; STCW, spring-tilled continuous spring wheat; and STW-F, spring-tilled spring wheat-fallow [Source: Sainju *et al.*, 2009] ^[41].

Ou *et al.*, (2016) ^[38] reported that tillage systems obviously affected the distribution of soil aggregates with different sizes (Fig. 5a). The proportion of the >2 mm aggregate fraction in NT+S was 7.1% higher than that in NT-S in the 0.00-0.05 m layer. There was no significant difference in the total amount of all the aggregate fractions between NT+S and NT-S in both the 0.05-0.20 and 0.20-0.30 m layers. NT+S and NT-S showed higher proportions of >2 mm aggregate and lower proportions of <0.053 mm aggregate compared to the MP system for the 0.00-0.20 m layer. The proportion of >0.25 mm macro-aggregate was significantly higher in MP+S than in MP-S in most cases, but the proportion of <0.053 mm aggregate was 11.5-20.5% lower in MP+S than in MP-S for all the soil layers.

Sheng *et al.* (2020) ^[43] also found that Compared with the initial soil in 1985, the weight percentages of >2 mm large macro-aggregate and 0.25-2.0 mm small macro-aggregate dramatically increased from 24.7% to 52.2% in grassland, but the percentages of 0.053-0.25 mm micro-aggregate and <0.053 m silt-clay fraction decreased. On the contrary, 31-year cropping and bare-land caused decreases in >0.25 mm macro-aggregates and increases in <0.25 mm aggregates, with

larger changes in bare-land than in cropland soil (Fig. 5b).31year grassland significantly increased the OC concentration in all aggregate fractions, with the mean aggregate associated OC (g kg⁻¹ aggregate) from 31.6 to 44.7, and higher increments were observed in 0.25-2.0 mm and 0.053-0.25 mm aggregate fractions, the increment was lowest in the silt-clay unit (Fig. 5b). Converting cropland to bare-land caused substantial depletion of macro-aggregates and their associated OC concentrations. The percentage of macro-aggregate (%) and mean aggregate associated OC (g kg^{-1} aggregate) significantly decreased from 34.8 to 13.6 and from 31.6 to 26.2, respectively. Bare-land only caused significant decrease of the OC concentration within 0.25-2 mm macro-aggregate compared with soil in 1985. Cropland caused no significant changes of OC concentrations in all aggregate sizes, being higher in macro- and micro-aggregates, and lower in the siltclay unit compared with soil in 1985. The aggregateassociated OC concentrations in all aggregates were significantly higher in grassland, followed by cropland, and lowest in bare-land, significantly higher OC was also found in >0.25 mm macro-aggregate in cropland than that in bare-land (Fig. 5b).



Fig 5a: Distribution (%) of water-stable aggregates with different sizes in different soil layers as influenced by tillage treatments. (a) 0.00-0.05 m; (b) 0.05-0.20 m; (c) 0.20-0.30 m. MP-S: moldboard plow without straw; MP+S: moldboard plow with straw; NT-S: no-tillage without straw; NT+S: no-tillage with straw [Source: Ou *et al.*, 2016] ^[38]



Fig 5b: Soil aggregate distribution (a) and organic carbon concentrations in aggregates (b) in soil in 1985 and different land use treatments [Source: Sheng *et al.*, 2020] ^[43].

Naresh *et al.* (2016) ^[37] reported that compared to the conventional tillage treatments, zero tillage and furrow irrigated raised beds treatments had significantly higher amount of total aggregate associated carbon within all the aggregate size classes in surface soil depth. In the 0–5 cm layer of soil with residue retention the organic C content in the large macro-aggregates was greater (av.12.3%) than in soil where residue was removed (av.8.8%), except in the T₆ and T₇ treatment where it was similar(9.3%)

to treatments without residues and in the T_2 and T_3 (11.8%) where it was similar to treatments with residues. In the small macro-aggregates, the greatest organic C was found for

treatment T₄ (av.13.4%), while the lowest organic C was found in soil without residues cultivated (av.6.3%). Residue management had a significant effect on C content in large and small macro-aggregates. In sub-surface soil layer, treatment (T₉) resulted in 11.8% higher total soil aggregated carbon as compared with wheat in zero tillage without residue retained treatment (T₁). In surface soil, the maximum (13.5%) and minimum (4.3%) proportion of total aggregated carbon was retained with >2 mm and <0.053 mm size fractions, respectively. Similarly, in the sub-surface layer >2mm size particles occluded highest proportion (12.0%) of total aggregated carbon followed by 0.25 - 2.0 mm, 0.053– 0.25 mm and <0.053 containing 9.4% 5.9% and 3.7%, respectively. Conservation tillage (both ZT and FIRB) caused 35.5%, 28.1%, 17.9% and 10.5% higher accumulation of SOC >2mm, 0.25 -2.0 mm, 0.053–0.25mm and <0.053size particles, respectively, than conventional tillage treatments (T₉). Wheat seeding on wide raised beds with residue retention (T₈) had the highest capability to hold the organic carbon on surface (10.73g kg⁻¹ soil aggregates) and retained least amount of SOC in sub-surface (7.13g kg⁻¹ soil aggregates) soil.

Memon *et al.* (2018) ^[35] also found that the values in Fig.6a presenting the results for the mean impact of crop straw incorporation on the main soil quality variables, SOM and TN concentration, at various simple depths (0–30 cm). The average SOM content in 2016–2017 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 g/kg) in the RTsi₆₀,

RTsi₁₀₀, CTsi₁₀₀, CTsi₆₀, RTsi₃₀, and CTsi₃₀ treatments, respectively, compared to RT ns (21.10 g/kg) and CT ns (20.61 g/kg). The SOM difference between $CTsi_{60}$ and $CTsi_{100}$ was non- significant in the 0–30 cm soil profile depth. Moreover, SOM in the topsoil (0–10 cm) was higher in RTsi₆₀ (26.31 g/kg) and CTsi₆₀ (24.51 g/kg) under RT and CT, respectively.

Furthermore, the soil TN concentrations were significantly variable among different straw incorporation management options and tillage practices (Fig. 6b). Consequently, TN content in all experimental treatments demonstrated an increasing trend, except for CT ns. 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-analyzed value. The mean maximum TN (0.981 g/kg) was found with RTsi₆₀, and the lowest (0.848 g/kg) 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 RTsi₆₀ (1.051 g/kg), which was higher thanCTsi₆₀ (0.926 g/kg) treatments under RT and CT methods.



Fig 6a: Depth-wise distribution of mean soil organic matter (SOM) under each treatment. RT ns: RT without straw incorporation, RTsi₃₀: RT with straw incorporation (SI) at 30%, RTsi₆₀: RT with SI at 60%, RTsi₁₀₀: RT with SI at 100%, CT ns: CT without straw incorporation, CTsi₃₀: CT with SI at 30%, CTsi₆₀: CT with SI at 60%, and CTsi100: CT with SI at 100% [Source: Memon *et al.*, 2018]^[35]



Fig 6b: Changes Mean total soil nitrogen at different soil depths under all experimental treatments in 2016 and 2017 [Source: Memon *et al.*, 2018]^[35]

Organic carbon in soil aggregates

Song *et al.* (2019) ^[47] observed that the aggregate-associated C content within varied aggregate sizes was significantly higher in the topsoil than in the subsoil. The order was as follows: small macro-aggregates >micro-aggregates >large macro-aggregates, with average values of 25.14 gkg⁻¹, 23.34 gkg⁻¹, and 20.54 g kg⁻¹, respectively. In contrast to the topsoil, the variation in aggregate -associated C in the sub-soil was smaller between the different aggregate sizes. The average contents under the different treatments were from 10.42–11.77 g kg⁻¹.

In contrast to the topsoil, the aggregate-associated C contents in the subsoil showed the trend of conventional tillage >rotary tillage >no-tillage. Without straw return and organic fertilizer, the average contents of aggregate-associated C were 11.60 gkg⁻¹, 10.83 gkg⁻¹ and 10.33 gkg⁻¹, respectively, under the T₁, T₄, and T₇ treatments. T₁ was significantly greater than T₄ and T₇. Under the same tillage, the application of organic fertilizer and straw return increased the content of aggregateassociated C in the subsoil. Under conventional tillage, the average aggregate-associated C contents of the large and small macro-aggregates and micro-aggregates under T₁, T₂, and T₃ were 11.60 gkg⁻¹, 12.14 g kg⁻¹and 12.33 g kg⁻¹, respectively. These values under organic fertilizer and straw return were significantly higher than those under single chemical fertilizer.

Ou *et al.* (2016) ^[38] reported that the aggregate-associated SOC concentration in different soil layers was influenced by tillage systems (Fig. 7a). In the 0.00-0.05 m layer, SOC concentration in macro-aggregates showed the order of NT+S>MP+S = NT-S>MP-S, whereas the NT system was superior to the MP system. However, the NT system significantly reduced the SOC concentration in the 2.00-0.25 mm fraction in the 0.05-0.20 m layer. A similar trend was observed in the 0.25-0.053 mm fraction in the 0.20-0.30 m layer. Across all the soil layers, there was no difference in the <0.053 mm fraction between NT-S and MP-S, as well as between NT+S and MP+S, indicating that the NT system did

not affect the SOC concentration in the silt+clay fraction. In average across the soil layers, the SOC concentration in the macro-aggregate was increased by 13.5% in MP+S, 4.4% in NT-S and 19.3% in NT+S, and those in the micro-aggregate (<0.25 mm) were increased by 6.1% in MP+S and 7.0% in NT+S compared to MP-S. For all the soil layers, the SOC concentration in all the aggregate size classes was increased with straw incorporation, by 20.0, 3.8 and 5.7% under the MP system, and 20.2, 6.3 and 8.8% under the NT system.

Sheng et al. (2020) [43] observed that the OC concentrations of different density and humic fractions differed obviously among soil aggregate hierarchy and land use patterns. Specifically, within all aggregate fractions, the OC concentrations of all density and humic fractions were highest in grassland, followed by cropland and bare-land. Also, the OC concentration showed no significant differences in cropland and bare-land soils among aggregates, with the exception of higher OC of free light (fLF) and occluded light (oLF) fractions within micro-aggregate in cropland than those in bare-land soil. The free light (fLF) and occluded light (oLF) fractions contained obviously higher OC concentrations than those in heavy fraction (HF) and humic fractions (Fig. 7b). Among all the field treatments, the OC concentrations of density fractions were highest within 0.25-2 mm aggregates, and then decreased with the size of the aggregates, with the <0.053 mm silt-clay unit having the lowest OC concentrations (Fig. 7b). The OC concentrations of humic fractions within aggregates did not show the same changing treads as density fractions after 31-year land use changes.

There were no significant differences of OC concentration of fulvic acids (FA) and humic acids (HA) among field soils, with the exception of higher OC concentrations of FA in >2 mm and 0.25–0.053 mm aggregate fractions in grassland than in cropland. The OC concentration of humin (HU) showed similar changes as HF, significantly higher OC concentrations were found within 0.25–2 mm and 0.053–0.25 mm aggregates in grassland (Fig. 7b).



Fig 7a: Aggregate-associated SOC concentration in different layer intervals as influenced by tillage treatments. (a) 0.00-0.05 m;(b) 0.05-0.20 m; (c) 0.20-0.30 m. MP-S: mouldboard plow without straw; MP+S: moldboard plow with straw; NT-S: no-tillage without straw; NT+S: no-tillage with straw [Source: Ou *et al.*, 2016)^[38]



Fig 7b: Organic carbon concentrations of density (**a**–**c**) and humic fractions (d–f) within soil aggregate fractions after 31-year different land use patterns [Source: Sheng *et al.*, 2020] ^[43].

Bandyopadhyay *et al.* (2010) ^[5] reported that the physical fractions of the four different aggregated C pools distributed in MacAC as well as in MicAC by 49.8 and 50.2% (Fig. 8a). Native soil and organics showed higher values of both MacAC (50.7and 50.2%) and MicAC (49.3 and 49.8%). On average, the CMacAC and MesAC pools showed 49.5 and 50.5% of total MacAC, while CMicAC and 'silt+clay' C represented 25.5 and 74.5% of total MicAC, respectively, (Fig. 8a). The NPK+FYM, NPK+PS and NPK+GM treatments contributed CMacAC, MesAC, CMicAC and 'silt+clay' C by 27.2, 26.2 and23.2%, 24.7, 24.5 and 24.5%,

13.1, 12.3 and 12.4% and 35.0, 37.0 and 39.9%, respectively of total aggregated C (Fig. 8a) where NPK+GM produced more MicAC (52.3%) than NPK+FYM (48.1%) and NPK+PS (49.3%) treatments, respectively. Moreover, the aggregated C (<2.0 mm) was found to be distributed among MesAC, CMicAC and 'silt+clay' C with a ratio of ~1.9:1: 2.8 (Fig. 8b), contributing 33.4, 17.5 and 49.1%, respectively. Fig. 8b depicts that all the treatments, except the native soil, retained the equal amount of MesAC and about 50% C was entrapped in 'silt+clay' sized fraction. The MicAC made up 2/3rd of the total aggregated C(<2.0 mm).



Fig 8a: Allocation of C in different aggregate size classes under different treatments at0–0.45 m depth; CMacAC = coarse macro-aggregated C (N₂ mm); MesAC = meso-aggregated C (0.25–2 mm); CMicAC = coarse micro-aggregated C (0.05–0.25 mm); silt+clay C (b 0.05 mm) [Source: Bandyopadhyay *et al.*, 2010] ^[5]



Fig 8b: Distribution of C in aggregates sized <2.0 mm under different treatments at0–0.45m depth; MesAC = meso-aggregated C (0.25–2 mm); CMicAC = coarse micro-aggregated C (0.05–0.25mm); silt+clay C (b0.05mm) [Source: Bandyopadhyay *et al.*, 2010]^[5]

Microbiological Composition

Sainju et al. (2009) ^[41] revealed that soil organic C and N fractions, PCM, PNM, MBC, and MBN were lower in STW-F than in other treatments in whole-soil and aggregate-size classes, especially at 0 to 5 cm (Fig. 9a and 9b). This suggests that fallowing reduced microbial biomass and activities and N mineralization compared with annual cropping probably by reducing the substrate levels (SOC and STN) due to reduced crop residue C and N inputs. In contrast, PCM and MBC were greater in NTCW or STCW than in FSTWB/P in the <2.00mm size class at 0 to 5 cm, probably a result of difference in the quality of crop residue input. Pea residue in FSTW-B/P has lower C: N ratio that results in rapid mineralization of residue and lower microbial biomass and activities than spring wheat and barley (Sainju et al., 2007a) ^[40]. Similarly, PCM, MBC, and MBN were greater in tilled than in no-tilled treatments in the continuous wheat system in the 4.75- to 2.00-mm size class. This was probably due to stimulation of

microbial biomass and activities as a result of incorporation of fresh crop residue due to tillage, as large macro-aggregates are formed from small macro-aggregates and microaggregates. On the other hand, PNM was greater in FSTW-B/P than in NTCW or STW-F in the <0.25-mmclass size at 0to 5-cm and <2.00-mm size class at 5 to 20 cm (Fig. 9a), indicating that greater N concentration or lower C/N ratio in pea than in spring wheat or barley probably increased N mineralization in small macro-aggregates, micro-aggregates, and silt and clay fractions. Elliott, (1986) [19] observed similar observations and suggested that organic matter in macroaggregates is labile and subjected to rapid mineralization when aggregates are disrupted. In contrast, greater MBC at 0 to 5 cm or greater MBC and MBN at 5 to 20cm in the <0.25 mm than in the 4.75- to 2.00-mm size class (Fig. 9b) suggests that micro-aggregates probably protect microbial biomass from predators.



Fig 9a: Effects of tillage and cropping sequence on dryland soil potential C and N mineralization (PCN and PNM) concentrations in aggregates at 0- to 5- and 5- to 20-cm depths [Source: Sainju *et al.*, 2009] ^[41].



Fig 9b: Effects of tillage and cropping sequence on dryland soil microbial biomass C and N (MBC and MBN) concentrations in aggregates at the 0- to 5- and 5- to 20-cm depths [Source: Sainju *et al.*, 2009] ^[41].

Dhaliwal et al. (2021)^[18] reported that the PMN ranged from 10.3 to 13.7 mg kg⁻¹ 7d⁻¹ in D₁ and from 7.2 to 10.6 mg kg⁻¹ 7d⁻¹ in D₂. Among different treatments, PMN was significantly greater in treatment T₃ as compared with the other treatments and was lowest in treatment T₅. The soil MBC varied from 116.3 to 132.8 mg kg⁻¹ in D₁ and from 42.7 to 56.6 mg kg⁻¹ in D₂. Application of chemical fertilizers with FYM enhanced the MBC content over their initial levels which were reported to be 82.9 and 65.4 mg kg^{-1} in D_1 and D₂, respectively. Among different treatments, T₃ treatment resulted in maximum content of MBC followed by treatments T_2 , T_4 , T_1 and T_5 , respectively. The MBN showed a similar trend as MBC and it ranged from 42.7 mg kg⁻¹ in T5 to 56.6 mg kg⁻¹ in T₃ in D₁ and from 36.8 mg kg⁻¹ in T₅ to 44.7 mg kg^{-1} in T₃ in D₂. The addition of chemical fertilizers with FYM improved the CO₂-C content to a significant extent in all treatments over its initial levels, which were reported to be 1.8 and 0.8 mg kg⁻¹ 10d⁻¹ in D₁ and D₂, respectively. It was found maximum in treatment T_3 (4.9 mg kg⁻¹ 10d⁻¹) and showed non-significant variation with treatments T_2 (4.4 mg $kg^{-1} 10d^{-1}$) and T_4 (4.1 mg $kg^{-1} 10d^{-1}$) and lowest variation in T_1 (3.7 mg kg⁻¹10d⁻¹) in D₁. In D₂, it was highest in treatment T3 (3.7 mg kg⁻¹ 10d⁻¹) and showed non-significant variation with treatments T_2 (2.9 mg kg⁻¹ 10d⁻¹) and lowest in T_5 (2.1 $mg kg^{-1} 10d^{-1}$).

Dey *et al.* (2016) ^[17] observed that 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. 10a). 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 mung bean residue in DSR-ZTW (MBR+DSR-ZTW-ZTMB) had most beneficial effect on SMB, thus registering an increase of 15 and 44 percent 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. 10a).

Zheng et al. (2019)^[50] reported that compared with the CK treatment, SMBC in the C and S treatments significantly increased at each growth stage. Moreover, compared with the NPK treatment, SMBC in the CNPK treatment increased by 11.6% and 12.9% at the transplanting and heading stages, yet decreased by 15.9% at the tillering stage; SMBC in the SNPK treatment increased by 37.1%, 16.5% and 18.4% at the tillering, heading and maturity stages, respectively, yet decreased by 25.2% at the transplanting stage. In addition, SMBC in the CNPK treatment was significantly lower than that in the SNPK treatment at the tillering and maturity stages, while the opposite trend was exhibited at the transplanting stage. During the whole growth stage, SMBC firstly decreased from transplanting stage to tillering stage, and then increased at the heading and maturity stages (Fig. 10b). Obvious differences were also observed in SMBN among different treatments at each growth stage (Fig. 10c). Compared with the CK treatment, SMBN in the C treatment decreased by 30.2%, 50.3% and 54.8% at the transplanting, tillering and maturity stages, respectively; SMBN in the S treatment decreased by 61.1% and 58.6% at the transplanting and tillering stages, respectively. Moreover, compared with the NPK treatment, SMBN in the CNPK and SNPK treatments significantly increased at each growth stage. Additionally, SMBN in the CNPK treatment was significantly lower than that in the SNPK treatment at both the heading and maturity stages, while an opposite trend was exhibited at the transplanting stage (Fig. 10c). During the whole growth stage, SMBN in CK, NPK, CNPK, and SNPK treatments decreased from transplanting stage to the maturity stage while SMBN in the C and S treatments firstly decreased, then increased, and finally decreased. Compared with transplanting stage, SMBN significantly decreased at the tillering, heading, and maturity stages.



Fig 10a: 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 [Source: Dey *et al.*, 2016]^[17]



Fig 10b: The SMBC of different fertilization treatments at different growth stages [Source: Zheng et al., 2019] [50]



Fig 10c: The SMBN of different fertilization treatments at different growth stage [Source: Zheng et al., 2019]^[50]

Conclusions

The review study shows that no-tillage and straw return in rice-wheat cropping rotation systems is an effective management practice for the formation and stability of soil aggregates. This practice has shown potential to increase the number of water-stable macro-aggregates and microaggregates in both the topsoil and subsoil compared to that under conventional tillage. However, straw return and application of organic fertilizer increased the cumulative carbon input and increased the aggregate-associated C content. Moreover, straw return is a better option for improving CPC than application of organic fertilizer in ricewheat cropping rotations. Higher SOC stocks were observed in the subsoil under straw return with conventional tillage than under straw return with no-tillage. This suggests that the benefits of no-tillage in sequestering SOC are concentrated mostly to the topsoil, while conventional tillage is more conducive to SOC accumulation in the subsoil.

Across the management practices evaluated in the review study, tillage had the greatest effect on SOC and its various fractions (CPOM-C, and C $_{\rm min})$ in the surface (0–15 cm) soil with positive results observed with conservation tillage practices compared with conventional tillage. SOC concentration in surface soil (0-15 cm) and SOC storage of the profile (0-30 cm) were slightly increased by the long term fertilizer treatments, but they were sharply increased by the manure and straw amendment (VC, 50% RDF+ VC @ 5tha-1 and 100% RDF+ VC @ 5tha-1). OC and microbial biomasses in the macro-aggregates are more sensitive to manure amendment than in the micro-aggregates. Manure amendment benefited soil structure, increased C sequestration, microbial activities, andmost likely soil fertility. No tillage can increase soilaggregations and favorably influence SOC accretion. Effects of crop residue addition are often observed when they are integrated with reduced tillage systems or with improved nutrient management. The rate of new soil OC increase ranged from 110.18 to 28.17 g m⁻² yr⁻¹in the early (~10 year) and later stages (~160 year), respectively. It took about 30 years for the amount of new soil OC to reach the same level as old OC in 0-20 cm of soil after farmland conversion. This suggests that organic matter in soils under rice-wheat systems could be easily lost through decomposition if the existing land use is altered. Most of the soil C pools, except water-soluble C, were correlated though the amount extracted by different methods varied considerably, suggesting that each method enumerated a different fraction of TOC.

No-tillage system promoted large macro-aggregation in both the 0.00-0.05 and 0.05-0.20 m layers. Accordingly, associated SOC concentration and SOC stock within the >0.25 mm macro-aggregate fraction also significantly increased in the 0.00-0.05 m layer in the NT system, while those within the 2.00-0.25 mm aggregate fraction were significantly reduced in the 0.05-0.20 m layer under the NT system. The review study may be stated that the adoption of the NT system that increases SOC stock in the 0.00-0.05 m soil profile might be dependent on the macro-aggregate fractions and the high amount of associated SOC. Increased tillage frequency followed by a change in the cropping sequence from continuous spring wheat to spring wheat also reduced SOC, STN, POC, PON, PCM, and MBC in FSTWB/P than in NTCW or STCW in the <2.00 mm aggregate-size class. In contrast, PNM and NO₃-N were greater in FSTW-B/P than in NTCW or FSTCW in the <2.00-mm size class. Except MBC, most of C and N fractions were greater in macro-aggregates than in micro-aggregates. Reduced tillage with annual cropping can enhance soil aggregation, C and N sequestration, and microbial biomass and activities compared with the conventional STW-F system. Because of greater aggregate proportion and intermediate SOC and STN levels between large macro-aggregate sand micro-aggregates, C and N sequestration can be mainly enhanced in small macroaggregates by using these management practices.

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