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RESEARCH ARTICLE

Improvement of soil aggregate-associated carbon sequestration capacity after 14 years of conservation tillage

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Summary

The North China Plain is an important summer maize/winter wheat rotation area. However, over the years, continued intensive tillage has destroyed the soil aggregate accelerating the mineralization and decomposition of soil organic carbon (SOC), which plays an important role in soil quality, as increased organic carbon storage improves soil fertility and crop yields. Thus, the objective of this study was to explore the comprehensive impact of tillage methods on soil aggregates, aggregate-associated SOC, and carbon sequestration capacity under a regime of straw return. In 2002, we started a 14-year long-term tillage experiment; then in 2016–2017, we tested the following tillage methods, zero tillage (ZT), rotary tillage (RT), subsoiling (SS), and conventional tillage (CT). The results showed that in the 0–10 cm soil layer, tillage methods significantly reduced the proportion of aggregates in the order of 2–0.25 > 5–2 > 0.25–0.053 mm. Additionally, conservation tillage (i.e., SS and ZT) significantly increased the percentage of macroaggregates (0–40 cm) and their SOC content, compared to CT. Additionally, the contribution rate of macroaggregates to SOC was 17.2% and 30.6% higher under SS and ZT than under CT, respectively. Conservation tillage methods improved the carbon sequestration capacity of soil aggregates. Our study provides a theoretical basis for the development of more suitable tillage methods. Furthermore, long-term conservation tillage seemingly protected large aggregates and, SOC, whereby carbon sequestration was enhanced and soil carbon emissions were effectively reduced.

Keywords: Tillage; Conservation; Zero tillage; Aggregate; Soil carbon capacity

Introduction

Worldwide, nearly 1550 Pg of organic carbon is stored in the superficial (0–1 m) layer of the soil profile (Brahim *et al.*, 2014). Generally, total soil organic carbon (SOC) is approximately twice the atmospheric carbon pool and 2.5 times the total carbon stored in terrestrial vegetation (Schlesinger and Andrews, 2000); therefore, even small changes in SOC can cause significant changes in atmospheric CO_2 content (Brown and Lugo, 1982). Further, SOC is vital for crop production because it regulates nutrient cycling and affects soil fertility. Indeed, overall, SOC is an essential component of the healthy soil's biological, physical, and chemical regulatory functions (Al-Kaisi and Kwaw-Mensah, 2020).

Jastrow (1996) observed that 90% of farmland topsoil SOC is located in soil aggregates, which act as an intermediate link in the formation and transformation of SOC. Specifically, they protect and stabilize SOC, while their formation and structure depend on the amount of SOC (Sarker *et al.*, 2018; Somasundaram *et al.*, 2018). Studies have shown that soil aggregates can effectively retain SOC through physical encapsulation, which contributes to their long-term stability (Golchin *et al.*, 1994). Farmlands are an important part of the organic carbon pool, and agricultural production processes ensure its active transformation (Wang *et al.*, 2016). Particularly in farmlands, particle size distribution and carbon sequestration by soil aggregates are differentially affected by tillage methods. The frequent mechanical disturbance caused by farming operations destroys soil macroaggregates, exposing the original SOC protected by aggregates, and accelerating its decomposition rate (Castro Filho *et al.*, 2002; Six *et al.*, 2000).

Conservation tillage reduces the intensity and frequency of tillage, avoids soil inversion to reduce soil aggregate disruption (Aguilera *et al.*, 2013), and keeps a minimum of 30 % of the soil surface covered with residues to ensure soil conservation (Singh *et al.*, 2018). According to FAO (2015), conservation agriculture comprises cropping system based on three principles: 1) direct seeding of crops with minimal soil disturbance; 2) retention of crop residues as mulch on the soil surface, and 3) the use of crop rotations and/or intercropping. Numerous studies have shown that conservation tillage implemented to improve SOC sequestration capacity increases SOC content and promotes the formation of soil aggregates. These findings have attracted worldwide attention (Nandan *et al.*, 2019; Wang *et al.*, 2019a). Aggregates of different grain sizes play different roles in nutrient retention, supply, and transformation (Paul *et al.*, 2013). Thus, Jat *et al.* (2019) found that macroaggregates are rich conservers of organic carbon despite being highly prone to oxidation and are particularly effective in improving SOC. Further, it is especially important to increase SOC by applying the right tillage methods to retain crop residues in the soil because they promote the formation of soil macroaggregates. Andruschkewitsch *et al.* (2014) showed that conservation tillage improves the quantity of macroaggregates in the 0–5 cm topsoil layer significantly, while reducing the amount of microaggregates. In particular, zero tillage (ZT) and minimum tillage methods can reduce the interference with and the destruction of soil aggregates, thus maintaining the biological and the spatial separation of the mineralization area, compared to microbial decomposition of organic carbon. As organisms grow and develop, metabolites continue to accumulate (Horikoshi *et al.*, 1981). A large number of macroaggregates can delay the mineralization process of soil organic matter (Oberson and Joner 2005; Richardson and Simpson, 2011), thus extending the organic carbon storage cycle in aggregates and slowing down SOC flow through the soil (Barto *et al.*, 2010). However, some researchers believe that ZT and reduced tillage do not increase the soil carbon content of the whole soil profile, compared to conventional tillage (CT) (Black and Tanaka 1997; Blanco-Canqui and Lal, 2008), and that the results of experiments on increasing soil carbon content under conservation, compared with CT tillage, remain inconclusive and may be affected by multiple factors such as time interval and soil depth (Steward *et al.*, 2018; Xu *et al.*, 2016). We believe that the effects of different tillage methods on SOC have been well explained in the case of long-term experimental results. However, the dynamic effects of farming methods after long-term conservation tillage, on aggregate-related organic carbon and carbon sequestration capacity during a cropping period, await elucidation.

Therefore, we hypothesized that after long-term conservation tillage, soil aggregates and carbon sequestration capacity show dynamic changes during the crop growth period due to the differential influence of different tillage methods. To test this hypothesis, we examined (1) the effects of different tillage methods on organic carbon in soil aggregates and (2) the effects of tillage methods on the contribution rate to total SOC and the capacity for carbon sequestration by aggregates of different particle sizes. The results will provide a sound theoretical basis for the development of more suitable tillage methods to improve the carbon sequestration capacity of soil aggregates in the North China Plain (NCP).

Materials and Methods

Site description

A long-term pilot experiment based on different tillage methods began in 2002 at the Experimental agronomy station(36°09′30.78″-36°09′27.59″N 117°09′13.79″-117°09′12.02″E),

Table 1. Initial characteristics of the main soil physicochemical properties in the 0–20 cm soil layer

Physical properties	Chemical properties
Sand (%) 37	SOC (g kg^{-1}) 10.87
Silt (%) 48	TN (g kg^{-1}) 1.1
Clay (%) 19	TP (g kg^{-1}) 8.89
SBD (g cm^{-3}) 1.4	TK (g kg^{-1}) 2.79
	AN (mg kg^{-1}) 108.8
	AP (mg kg^{-1}) 0.79
	AK (mg kg^{-1}) 41.32
	pH 6.22

SBD-bulk density, SOC-soil organic carbon, TN-total nitrogen, TP-total phosphorus, TK-total potassium, AN-available nitrogen, AP-available phosphorus, AK-available potassium.

Shandong Agricultural University, in the North China Plain (NCP), and the data were used in the present experiment, which was conducted from October 2016 to October 2017. The climate at the study site is semidry temperate, with annual average temperature of 15.02 °C and an annual average precipitation of 786.2 mm. Basic soil physical and chemical properties in the 0–20 cm topsoil layer are shown in Table 1.

Experimental design

Summer maize/winter wheat rotation cropping is common practice across the region. For the experiment described herein, straw comprising all crop residues remaining after harvest was pulverized and returned to the field. Straw was managed twice: once before tillage methods were implemented in mid-October, and once before summer maize was sown in mid-June. Four tillage methods, namely ZT (zero tillage, tillage depth 0 cm), RT (rotary tillage, tillage depth 10 cm), SS (subsoiling, tillage depth 40 cm), and CT (conventional tillage, tillage depth 20 cm), were included. RT consisted of 60 blades (Guangming Model® 1GQN-200, Jiangsu, China), while SS consisted of five shovels (Haofeng Model® 1SF–200, Henan, China) to a depth of 40 cm.

Treatment within each experimental plot was 15 m × 4 m, and each area was duplicated three times. The experimental plots were planted with summer maize variety Zhengdan 958; at a population density of 7.5×10^4 plants/ha with plant and row spacings of 22.2 and 60 cm, respectively. Fertilization included potassium chloride 180 kg ha^{-1} ($K_2O \geq 60\%$), urea 225 kg N ha^{-1} ($N \geq 46.2\%$), and superphosphate 180 kg ha^{-1} ($P_2O_5 \geq 12\%$). All tillage treatments were watered with 60 mm during the bell mouth stage of summer maize and fertilized with 100 kg N ha^{-1} ($\geq 46.2\%$) fertilizer. The four tillage methods tested were performed once a year before wheat sowing, and summer maize was sown directly by a no-tillage seeder, a multi-functional machine that can finish sowing, rolling, fertilization, and pressing at the same time; experimental operations are summarized in Table 2.

Measured variables and methods

Soil samples were collected at jointing stage (JS), anthesis stage (AS), grain filling stage (FS), and maturity stage (MS) of summer maize. Undisturbed arable soil was sampled at 0–10, 10–20, and 20–40 cm depths using a 10 cm diameter ring cutter according to the five-point sampling method in each of the three replicate plots. Five randomly selected soil cores were taken and mixed into a composite sample, for total of 45 number of samples for each tillage treatment. Soil aggregates were separated by the wet sieve method (Yoder 1936). One hundred grams of air-dried soil was placed on the top layer of the aggregate analyzer (Tuopuyunnong Model® TPF-100, Zhejiang, China) sieve-cover, which comprised a vertical series of sieves with mesh sizes of 5, 2, 0.25, and 0.053 mm from top to bottom. Deionized water was added slowly to the soil; the range

Table 2. Specific operation schemes for field testing design

Treatment	Tillage depth	Specific operation scheme
CT	20 cm	Maize mechanical harvesting → Straw returning to the field (full amount) → Conventional tillage → Wheat sowing → Wheat mechanical harvesting → Straw returning to the field (full amount) → Direct seeding of summer maize no-tillage planter.
SS	40 cm	Maize mechanical harvesting → Straw returning to the field (full amount) → Subsoiling tillage → Wheat sowing → Wheat mechanical harvesting → Straw returning to the field (full amount) → Direct seeding of summer maize no-tillage planter.
RT	10 cm	Maize mechanical harvesting → Straw returning to the field (full amount) → Rotary tillage → Wheat sowing → Wheat mechanical harvesting → Straw returning to the field (full amount) → Direct seeding of summer maize tillage planter.
ZT	0 cm	Maize mechanical harvesting → Straw returning to the field (full amount) → No tillage → Wheat sowing → Wheat mechanical harvesting → Straw returning to the field (full amount) → Direct seeding of summer maize no-tillage planter.

ZT-zero tillage, RT-rotary tillage, SS-subsoiling, and CT-conventional tillage.

of the aggregate analyzer was adjusted to 20 times/min and the soil was soaked for 10 min after sieving for 3 min, and three duplicate soil samples were set for each composite soil sample. Soil aggregates < 0.053 mm were silt-clay and were not retained. Soil aggregates from different levels of the screen layer were collected in aluminum boxes, air-dried to a constant weight, and weighed (Madari *et al.*, 2005). The organic carbon content of the soil aggregates was determined by the potassium dichromate external heating method (Bao 2000).

The contribution rate of each particle aggregate to soil total organic carbon (F) was calculated as per eq. 1 (Xu *et al.*, 2018):

$$F = \frac{c \times m}{C} \tag{1}$$

where c is the organic carbon content in the aggregate, m is the aggregate mass (g), and C is the total SOC content (g kg^{-1}).

The carbon sequestration capacity of each aggregate was calculated as per eq. 2 (Wang *et al.*, 2018):

$$\mathrm{CFC} = \frac{\mathrm{MACi} \times \mathrm{MAi}}{100} \tag{2}$$

where CFC is the carbon sequestration capacity of each aggregate (g kg^{-1}), MAC_{i} is the organic carbon content of each aggregate particle size (g kg^{-1}), and MA_i is the aggregate mass (g).

Carbon input

After harvest, wheat and maize crop residues to be returned to the soil included straw, stubble, and roots. Carbon input for each different straw returning treatment was calculated using equations 3, 4, and 5:

$$C_{straw} = R_{straw} \times Y_{straw} \times OC_{plant} \tag{3}$$

$$C_{stubble} = R_{stubble} \times Y_{stra} \times OC_{plant} \tag{4}$$

$$C_{roots} = R_{routs} \times \left(Y_{straw} + Y_{grain}\right) \times OC_{plant} \tag{5}$$

where C_{straw}, $\mathrm{C}_{stubble}$, C_{roots} are carbon inputs (t ha^{-1}) from wheat or maize stalks, stubble, and roots, respectively; R_{straw} (%) is the ratio of returned straw to total straw biomass; $\mathrm{R}_{stubble}$ (%) is the ratio of the residue to total straw biomass, and the values for wheat and maize are 26% and 3%, respectively (Wang *et al.*, 2015); R_{roots}(%) is the ratio of below-ground biomass to aboveground biomass, that is, 24% and 29%, for wheat and maize, respectively (Bolinder *et al.*, 2007); Y_{straw} and Y_{grain} are straw biomass and grain yield (t ha^{-1}); OC_{plant} is the carbon content of the aboveground crop biomass, that is, 0.399 and 0.444 kg kg^{-1}or Chinese wheat and maize, respectively (Zhang *et al.*, 2010).

Statistical analyses

Data were processed and statistically analyzed using Excel 2016 (Chicago, USA). All statistical analyses were performed using SPSS for Windows software v. 19. Multiple comparisons ($\alpha = 0.05$) were made among different treatments using the least significant difference (LSD), and Pearson's correlation method was used to analyze the correlations between variables.

Results and Discussion

Percentage of soil aggregates

Soil aggregate stability can be used to evaluate the soil structure dynamics (He *et al.*, 2021). Generally, aggregates with a particle size greater than 0.25 mm are called macroaggregates, while the smaller particles are called microaggregates. Their respective contents, in the 0–10 cm topsoil layer, differed significantly ($p < 0.05$) under different tillage treatments and decreased in the order of 2–0.25 > 5–2 > 0.25–0.053 mm (Figure 1a). Compared with CT, the aggregate content size 5–2 mm under aggregate SS increased significantly by 15.0% at the FS stage, while it increased significantly ($p < 0.05$) by 13.6% at the MS stage under ZT. Compared with CT, macroaggregates content under ZT and SS in the 0–10 cm topsoil layer increased by 5.5% and 4.8%, respectively, while conversely the soil microaggregates content decreased. The 5–2 mm number of aggregates was significantly higher by 15% in ZT- and SS-treated plots than in CT-treated plots, at FS; furthermore, the same number was significantly higher by 13.6% under ZT than CT at the MS stage. Thus, our results have shown that SS and ZT can significantly ($p < 0.05$) increase macroaggregates content in the surface. This result is consistent with those obtained by previously reported protective tillage measures in that both effectively reduced the damage to soil aggregates under mechanical disturbance to varying extents, thereby increasing the macroaggregate content of the topsoil layer (Briar *et al.*, 2011). Straw returned to the field provides carbon material for the formation of macroaggregates, which increases macroaggregate contents and improves surface soil structure (Carter, 1992). Studies have shown that, in northern China, conservation tillage, especially SS and ZT, reduces soil bulk density by enhancing the activity of the crop root system and increasing the activity of root microorganisms (He *et al.*, 2019), thus contributing to the formation of soil aggregates and the transformation of microaggregates into macroaggregates.

Under SS and ZT treatments, the 5–2 mm aggregates content in the 10–20 cm soil layer in SS treatment increased significantly ($p < 0.05$) by 18.18% (Figure 1b) and by 13.63% respectively, compared with CT, at MS stages. Consistently, the number of 5–2 mm aggregates was significantly higher in ZT than that in CT at AS, FS, and MS growth stages by 15%, 31.25, and 18.18%, respectively. Similarly, it was significantly higher in SS than in CT by 10%, 25%, and 13.64%, respectively. Working at the experimental station of the Instituto Agronomico, Castro Filho *et al.* (2002) showed that ZT allows more organic matter to be stored in the soil surface, which is conducive to the formation of macroaggregates. In turn, Wang *et al.* (2019b) conducted a field tillage experiment in the south-eastern Loess Plateau of China in 2007 and showed that SS increased the diversity of soil microbial communities, promoted the formation of macroaggregates, and allowed greater organic matter fixation activity.

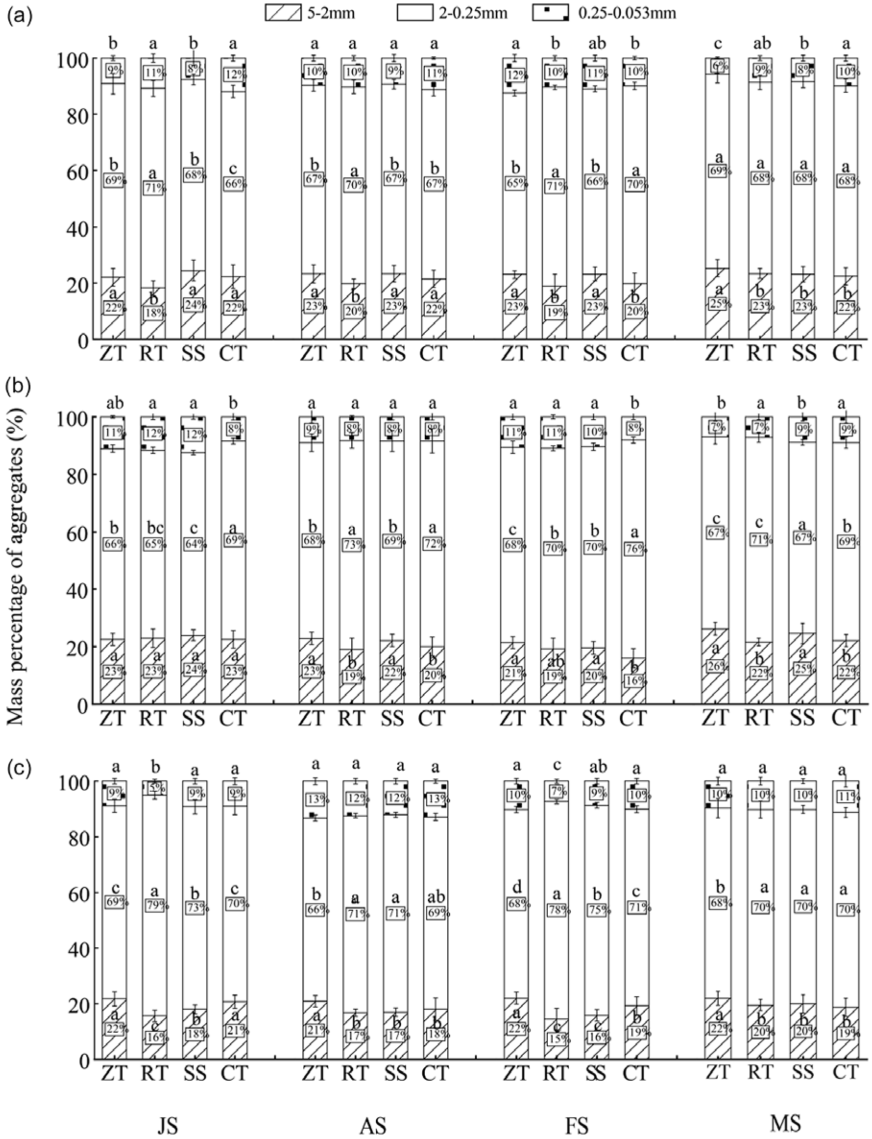

Figure 1. Percentage of mass of soil water aggregates in 0–10 cm (a), 10–20 cm, (b) and 20–40 cm (c) soil layer of maize. The vertical error bar represents the standard error of the mean. ZT-zero tillage, RT-rotary tillage, SS-subsoiling, and CT-conventional tillage. The abscissa is the four growth periods of summer maize. JS-jointing stage; AS-anthesis stage; FS-filling stage; MS-maturation stage and the ordinate is the content percentage of aggregates.

The content of 2–0.25 mm aggregates content in the 20–40 cm soil layer was increased by 12.86% and 5.63% at JS and FS, respectively, under SS treatment compared with that under the CT treatment. Consistently, the 5–2 mm aggregates contents increased by 16.67%, 15.79%, and 15.79% under the ZT treatment at AS, FS, and MS (Figure 1c), respectively, relative to CT. This showed that SS and ZT significantly ($p < 0.05$) increased the percentage content of macroaggregates, while reducing the percentage content of microaggregates. Compared with the 0–20 cm topsoil layer, the percentage content of macroaggregates in the 20–40 cm soil layer decreased, whereas that of microaggregates increased, indicating that the surface soil was more conducive to the formation of microaggregates than to that of macroaggregates, likely because the amount of stubble in the surface soil is larger than that in the deeper soil, which further increases the proportion of macroaggregates in the surface soil. From the perspective of tillage methods, CT often disturbs soil aggregates and may lead to the loss of large C-rich aggregates and an increase in microaggregates lacking C (Six *et al.*, 2000). Relative to CT, the microaggregates were gradually encapsulated by clay particles and microbial products under ZT (Six *et al.*, 1998), which significantly improved the turnover rate of macroaggregates (Six *et al.*, 1999).

Soil aggregate-associated organic carbon

Organic carbon content of the soil aggregates showed a decreasing trend with increasing soil depth (Figure 2). Organic carbon content of water-stable aggregates in the 0–10, 10–20, and 20–40 cm soil layers was 6.59–12.82, 6.19–11.36, and 5.67–10.65 g kg^{-1}, respectively. Furthermore, aggregate organic carbon content decreased as aggregate size decreased. Thus, in the 0–10 cm topsoil layer, organic carbon content of 5–2, 2–0.25, and 0.25–0.053 mm aggregates was 9.46–12.82, 7.22–10.81, and 6.59–10.71 g kg^{-1}, respectively; meanwhile in the 10–20 cm soil layer, organic carbon was 8.65–11.36, 7.35–9.55, and 6.19–8.55 g kg^{-1}, respectively and in the 20–40 cm soil layer, it was 7.95–1.65, 6.50–8.72, and 5.67–7.17 g kg^{-1}, respectively. Aggregate organic carbon content initially increased and then decreased with growth, and generally reached a maximum value during AS. From the perspective of soil depth, aggregate organic carbon content in the surface soil was significantly($p < 0.05$) higher than that in the deeper soil layers studied; thus, soil depth significantly affected soil aggregate carbon content (Table 3), presumably, mainly because the newly imported external organic matter first accumulated in the surface soil, and then the gradient in organic carbon content between the surface layer and the lower layer acted as the driving force for carbon infiltrate deeper into the soil (Gupta Choudhury *et al.*, 2014). Consistently, as the soil depth increases the soil aggregate-associated SOC content generally decreased due to low organic input levels, and SOC content increased with increasing soil aggregate size (Wu *et al.*, 2019). From the perspective of tillage method, aggregate organic carbon content in the 0–40 cm soil layer increased in the order SS > ZT > RT > CT ($p < 0.05$), similar to total carbon input (Table 4), which reached 246.34, 241.46, 260.37, and 251.22 t ha^{-1} under ZT, RT, SS, and CT, respectively.

Clearly, conservation tillage increases aggregate organic carbon content, presumably, mainly, because conservation tillage reduces soil cultivation intensity, which in turn reduces SOC decomposition rate, therefore promoting an increase in SOC content (Six *et al.*, 2004). Conversely, CT soil disturbance by the machinery accelerates organic carbon mineralization, thereby reducing organic carbon content (Al-Kaisi and Yin, 2005). In our experiment, compared with CT, the ZT treatment effectively improved the soil capacity for accumulation of organic carbon by not only protecting soil aggregates from decomposition (Singh *et al.*, 2020), but by stimulating further isolation of organic carbon in soil aggregates retained from plant residues as well (Alvarez and Alvarez, 2000; Six *et al.*, 1999). The SS treatment disrupted the compacted hardpan layer without damaging the surface soil structure that benefits soil carbon storage and growth of the crop root system. Moreover, subsoiling increased soil permeability and water retention, which indirectly increased root growth and thus increased crop root exudates, and protected soil aggregates. Additionally, the resulting increase in root secretions enhanced microbial nourishment and promoted rapid decomposition of straw, along with the increase in SOC accumulation (Cai *et al.*, 2014; Jin *et al.*, 2007).

Carbon sequestration capacity of aggregates

There were significant differences in carbon sequestration capacity of soil aggregates of different particle size (Figure 3a); being highest for particles 2–0.25 mm in size, followed by 5–2 mm particles, and finally by the 0.25–0.053 mm particles. Carbon sequestration capacity of soil aggregates was determined by organic carbon content and soil aggregate mass. It was highest for 2–0.25 mm

Table 3. The ANOVA for tillage, soil layer, particle size on aggregate-associated organic carbon, aggregates carbon sequestration capacity, and aggregates total organic carbon contribution rate

Difference source	AOC ($g{\cdot}kg^{-1}$)	ACSC ($g{\cdot}kg^{-1}$)	ATOCC (%)
Tillage (T)	68.12***	15.69***	15.69***
Soil layer (L)	68.78***	14.59***	14.59***
Particle size (S)	502.15***	1929.04***	1929.04***
T × L	3.40**	1.32^{ns}	1.32^{ns}
T × S	8.14***	5.78***	5.78***
L × S	7.30***	11.45***	11.45***
T × L × S	7.44***	2.53**	2.53**

AOC-aggregate-associated organic carbon content, ACSC-aggregate carbon sequestration capacity, ATOCC-aggregate total organic carbon contribution rate. **Significant at the 0.01 level; *** Significant at the 0.001 level.

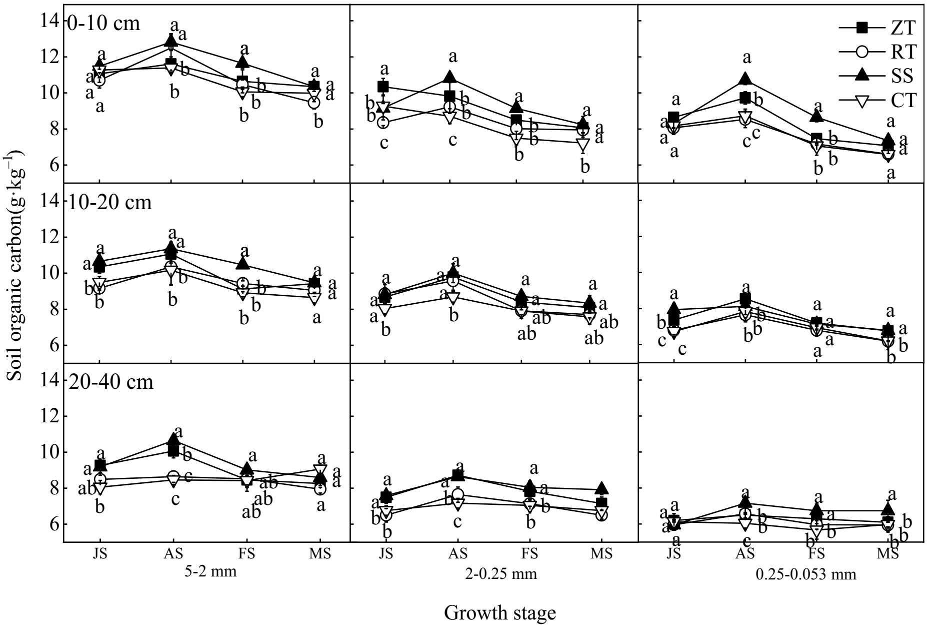

Figure 2. Dynamic changes of organic carbon in aggregates. The vertical error bar represents the standard error of the mean. ZT-zero tillage, RT-rotary tillage, SS-subsoiling ,and CT-conventional tillage. The abscissa is the four growth stages of summer maize, JS-jointing stage; AS-anthesis stage; FS-filling stage; MS-maturation stage, and the ordinate is the organic carbon content of aggregates.

particle aggregates, which were the particles with the largest mass percentage. These results showed that higher SOC content in macroaggregates is consistent with those reported by Wang *et al.* (2019c) and Zheng *et al.* (2022). Compared to CT, in the 0–40 cm soil layer, macro-aggregate carbon sequestration capacity in SS and ZT plots increased by 13.3% and 10.1% at MS, respectively. In terms of tillage method, SS showed carbon sequestration capacity, followed by ZT, RT, and finally CT. Therefore, we conclude that ZT is an effective approach to improve SOC sequestration capacity. (Bessam and Mrabet, 2003; Blanco-Canqui and Lal, 2008; Das *et al.*, 2014). Consistently, a nine-year long-term experiment by Modak *et al.* (2020) showed that ZT can keep crop residues on the soil surface and reduce wind and rain erosion of aggregates. Further, the combination of ZT and crop residues return improved the physical protection of the soil, which may be one of the reasons for the high carbon content of macroaggregates. At

Table 4. Carbon input accumulation during 2002–2017 under the different tillage and straw returning treatments

	ZT(t/ha)	RT(t/ha)	SS(t/ha)	CT(t/ha)
Wheat	110.98	111.59	117.68	112.20
Maize	135.36	129.87	142.69	139.02
Total carbon input	246.34	241.46	260.37	251.22

the same time, earthworms are more active under the ZT treatment and may also enhance carbon sequestration in aggregates under ZT (Arai *et al.*, 2013). Application of conservation tillage improved the carbon sequestration capacity of the soil macroaggregates, consistently with results reported by Xu *et al.* (2013).

Aggregate total organic carbon contribution rate

The contribution of soil aggregate-related organic carbon to total SOC differed significantly among aggregate particle sizes. Specifically, it was highest for the 2–0.25 mm particle size (Figure 3b). Further, in the 0–40 cm soil layer, the contribution rate of 5–2 mm aggregates in the ZT treatment was significantly higher by 17.2%, than that in CT. Overall, the contribution of soil aggregate-related organic carbon to total SOC decreased in the order of ZT > SS > RT > CT, with significant ($p < 0.05$) differences. The contribution rate of 2–0.25 mm granular aggregates in the SS treatment was 30.6% higher than that of CT and it decreased in the order of SS > ZT > RT > CT ($p = 0.12$). Compared with CT, the SS and ZT treatments increased the contribution rate of total SOC, probably because these tillage treatments imply less disturbance to the soil, which is conducive to the process of soil aggregation and slows down the decomposition of soil organic matter. Therefore, in the process of soil aggregation, this leads to more sequestration of soil organic carbon in soil aggregates. Therefore, the contribution rate of organic carbon improved in the surface soil, plow bottom, bottom layer aggregates. Finally, the carbon sequestration capacity of the soil was enhanced (Ankrom, 2009; Tian *et al.*, 2014). The contribution of different tillage methods to the total organic carbon in macroaggregates was significant in the 0–40 cm soil layer ($p < 0.05$), and the highest was found under SS, followed by ZT and RT, while the lowest was recorded for CT. Tillage, soil depth, and particle size have a highly significant relationship with aggregates' total organic carbon contribution rate (Table 3). The interaction of tillage, soil depth, and particle size had a significant effect on the contribution rate of total organic carbon.

The reason may be that continuous ZT reduced the exchange capacity between the deep soil and the surface soil, resulting in the accumulation of organic matter in the surface soil; in turn, this may have reduced the supply of nutrients in the lower layer (Zhou *et al.*, 2007), along with organic carbon content in deeper soil profile (Li *et al.*, 2006). The SS treatment allowed for full reaction of soil nutrients, and it accelerated the decomposition of organic matter and helped increase the organic carbon content in the deeper soil layers (Hernanz *et al.*, 2002). Compared with CT, the organic carbon contribution rates in SS and ZT both increased. Indeed, our results unequivocally that, compared with CT, conservation tillage was more effective in increasing the contribution rate of macroaggregates (0–40 cm) to total soil organic carbon content.

Correlations among the aggregates and SOC pools

CT correlation results showed that the number of macroaggregates was significantly and positively correlated with aggregate-associated SOC, aggregate carbon sequestration capacity, and aggregates' total organic carbon contribution rate (Table 5). In contrast, microaggregates were negatively correlated with aggregate-associated SOC, aggregates carbon sequestration capacity, and aggregates total organic carbon contribution rate. Additionally, there was a significant positive

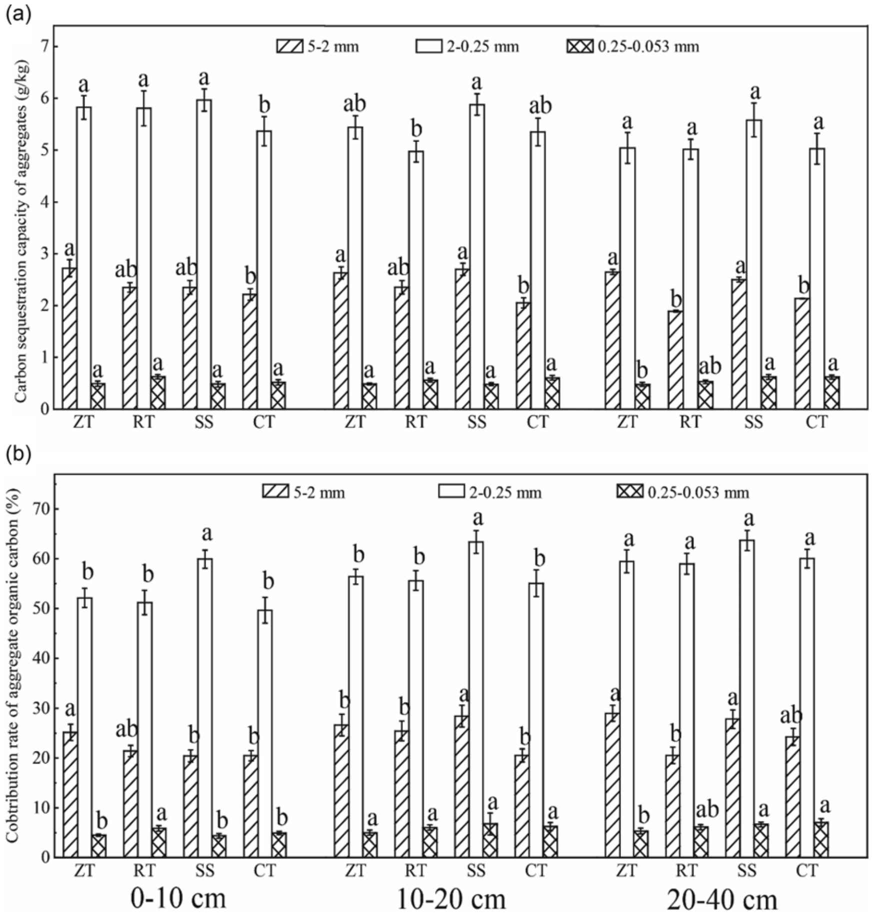

Figure 3. Changes of carbon sequestration capacity of grain aggregates under different tillage methods (a); changes of soil organic carbon by different tillage methods and aggregates (b). The abscissa is the four tillage methods, and the ordinate is the carbon sequestration capacity of aggregates. ZT-zero tillage, RT-rotary tillage, SS-subsoiling, and CT-conventional tillage. The different letters in the picture indicate that they are significantly different in different particle size.

correlation among aggregate-associated SOC, aggregates carbon sequestration capacity, and aggregates total organic carbon contribution rate, indicating that conservation tillage significantly increased the distribution and quantity of macroaggregates and improved the aggregate-associated SOC, thereby increasing SOC content.

According to Six *et al.* (2000), tillage practices can damage soil aggregates. Tillage and frequent mechanical disturbances will have a great impact on soil aggregates, thus triggering the transformation of macroaggregates into microaggregates (Qian *et al.*, 2018). In this study, there was a significant and positive correlation between macroaggregates and aggregate-associated SOC, whereas microaggregates showed the opposite trend (Table 5). Increasing the proportion of macroaggregates is important for increasing SOC content (Al-Kaisi *et al.*, 2014).

Consistently, Jastrow (1996) showed that the larger the aggregate particle size, the larger the carbon content in the aggregates. Among the four tillage methods tested herein, ZT improved the aggregation ability of macroaggregates, which supports the results of Fernández *et al.* 2010), the main reason being that ZT minimizes soil disturbance and plays a positive role in macroaggregate formation (Sheehy *et al.*, 2015). Consistently, Song *et al.* (2016) found that macroaggregates were formed by organic matter cementation, and the increase in the number of

Table 5. Correlation analysis of soil aggregate, aggregate-associated organic carbon, aggregates carbon sequestration capacity, and aggregates total organic carbon contribution rate

	1	2	3	4	5	6	7	8	9	10	11
1Macro-aggregates											
2Micro-aggregates	0.26										
3AOC_1	0.36*	−0.45**									
4AOC_2	0.38*	−0.55**	0.61***								
5AOC_3	0.57***	−0.36*	0.74***	0.79***							
6$ACSC_1$	0.57***	−0.35*	0.75***	0.64***	−0.65***						
7$ACSC_2$	0.55***	−0.41*	0.66***	0.94***	0.87***	0.58***					
8$ACSC_3$	0.63***	−0.52***	0.01	0.09	0.24	0.07	0.10				
9$ATOCC_1$	0.57***	−0.35*	0.75***	0.64***	0.65***	1.00***	0.58***	0.07			
10$ATOCC_2$	0.55***	−0.41*	0.66***	0.94***	0.87***	0.07	1.00***	0.10	0.58***		
11$ATOCC_3$	0.63***	−0.52***	0.01	0.09	0.24	1.00***	0.10	1.00***	0.07	0.10	

AOC_1-organic carbon content of 5–2 mm size aggregate-associated, AOC_2-organic carbon content of 2–0.25 mm size aggregate-associated, AOC_3-organic carbon content of 0.25–0.053 mm size aggregate-associated. $ACSC_1$-carbon sequestration capacity of 5–2 mm size aggregate, $ACSC_2$-carbon sequestration capacity of 2–0.25 mm size aggregate, $ACSC_3$-carbon sequestration capacity of 0.25–0.053 mm size aggregate. $ATOCC_1$-total organic carbon contribution rate of 5–2 mm size aggregate, $ATOCC_2$-total organic carbon contribution rate of 2–0.25 mm size aggregate, $ATOCC_3$-total organic carbon contribution rate of 0.25–0.053 mm size aggregate. *Significant at the 0.05 level; **Significant at the 0.01 level; *** Significant at the 0.001 level.

macroaggregates under ZT treatment protected unstable C from microbial attack, which may also be an important reason to explain the observed ZT-induced increase in SOC. The SS treatment reduced the extent of soil cultivation and changed the spatial position of, such that straw and soil could be thoroughly mixed, and improved the number of aggregates (Zhang *et al.*, 2019). Conversely, CT brings about severe soil disturbance, exposing SOC to the air, which in turn exacerbates SOC oxidation and decomposition (Luo *et al.*, 2010), leading to the decomposition of soil aggregates and the loss of carbon associated with aggregates.

Conclusion

The results of the long-term conservation tillage experiment reported herein showed that the SS and ZT treatments significantly increased macroaggregates and aggregate-associated SOC content. Thus, these conservation tillage methods were effective in improving carbon sequestration capacity and total organic carbon contribution rate of soil aggregates in the 0–40 cm soil layer during the critical growth period of summer maize. Therefore, both SS and ZT were effective tillage management strategies in the cropping areas of the NCP. This means that long-term conservation tillage, especially SS and ZT, may protect large soil aggregates, and SOC and, consequently, contribute significantly to the achievement of greater carbon sequestration and reduced soil carbon emissions. Therefore, conservation tillage should be promoted in the future.

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