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Long-Term Minimum Tillage and Straw Retention Promote Macroaggregate Formation, Carbon and Nitrogen Sequestration under Wheat-Maize Rotation in Northern China

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Abstract: Conservation tillage is believed to promote soil aggregate stability, carbon (C) and nitrogen (N) sequestration, but the underlying mechanisms remain unclear. In this study, soil samples from an 18-year experiment including conventional tillage with straw removal (CT), deep scarification with straw mulching (DS), and no-tillage with straw mulching (NT) were used to obtain different fractions based on a comprehensive wet-sieving method of aggregate and particle size. The results showed that NT and DS increased soil organic carbon (SOC) and N by 9.3–16.4% and 10.8–25.8%, respectively, in addition to increasing the weight proportion of macroaggregates and the contribution of macroaggregate-associated C and N to total SOC and N. The C change in the total POM accounted for 77.4% and 79.9% of the total SOC increase by NT and DS, while the MAOM only accounted for 29.2% and 25.2%, respectively. Meanwhile, microaggregates-within-macroaggregates accounted for 96.9% and 90.5% of the SOC increase by NT and DS, respectively. The total SOC and N were positively correlated with the C and N of the macroaggregates and subfractions. In conclusion, the formation of macroaggregates drives soil C and N sequestration under conservation tillage, and POM and mM were important functional pools in this process.

Keywords: carbon sequestration mechanism; conservation tillage; straw mulching; aggregate-associated C and N; mineral-associated organic matter

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1. Introduction

Promoting soil organic carbon (SOC) sequestration through different paths can effectively mitigate atmospheric greenhouse gas emissions because SOC is the largest carbon (C) reservoir in the terrestrial ecosystem [1]. According to the "4 per 1000" initiative, increasing the amount of C stored in the soil by 4‰ per year can effectively slow the increase in carbon dioxide (CO_2) in the atmosphere, thereby helping to combat climate change [2]. Additionally, SOC sequestration has been promoted to improve soil fertility and ensure food security [3]. The SOC sequestration potential mainly depends on the balance of C input and output [4,5]. After exogenous C enters the soil, it degrades continuously under the action of microorganisms and is sequestrated by the soil under the physical protection of aggregates, where it also undergoes chemical or physicochemical binding to soil minerals [6]. During this process, the structure and composition of the soil organic matter (SOM) change constantly, while microbial residues and metabolites also continuously participate in the turnover process [7]. The SOC is decomposed by microorganisms to release CO_2, which is the primary source of SOC output. Environmental conditions, soil properties, and farmland management can all affect the input-output balance of SOM, thereby influencing SOC changes [8,9].

Conservation tillage methods, including minimal tillage, no tillage, and straw mulching, originated in the 1930s as a series of farmland management technologies that could increase crop yield and improve the ecological environment of farmland. A previous study showed that conventional tillage disturbs soil aggregates, resulting in the SOC in the aggregates being exposed, thereby leading to a reduction in SOC content [10]. However, conservation tillage techniques, such as no tillage and minimal tillage, can reduce the disturbance and destruction of soil aggregates, reduce soil water evaporation and soil surface temperature, reduce the decomposition rate of SOC by microorganisms, and prolong the retention time of SOC in aggregates [11,12]. Studies have found that no tillage can increase the SOC concentration in the soil surface by improving the stability of aggregates compared with traditional tillage, increasing the proportion of large aggregates, and decreasing the SOC mineralization of aggregates of different particle sizes [6,13]. Deep scarification can effectively plow the bottom soil layer without overturning the topsoil layer, thus deepening the topsoil layer and significantly increasing the proportion of macroaggregates [14].

Soil aggregates are the basic units of soil structure and are closely related to soil SOC sequestration [15]. Soil aggregates provide physical protection for SOC by encapsulating SOM and regulating the flow of oxygen and water inside and outside the soil, thus influencing the microbial mineralization and decomposition of SOC [16]. The size, quantity, and stability of soil aggregates are sensitive to changes in SOC and are often used to assess SOC changes in agricultural systems [17]. In addition, SOC is an important factor affecting the stability of aggregates, as it plays the role of a binding agent during the formation of aggregates. Many studies have shown that an increase in SOC content can promote the formation of aggregates and improve the stability of aggregates [18,19]. The SOC and aggregates depend on and influence each other, resulting in beneficial effects on soil structure and C sequestration. As the understanding of soil aggregate turnover and C sequestration mechanisms has improved, aggregate fractionation has been combined with other methods such as particle size fractionation [20]. In this way, aggregates with different particle sizes are further separated into different components, such as microaggregates enclosed by macroaggregates, particulate organic matter (POM), and mineral-associated organic matter (MAOM) enclosed by aggregates [21]. The quantification of these fractions and their responses to management practices will improve our understanding of SOC sequestration mechanisms and assist in the evaluation of sustainable management practices. However, changes in soil aggregates and SOM occur slowly. Evaluating the effects of conservation tillage on soil aggregates and aggregate-associated C using long-term experiments is important for elucidating the mechanisms by which conservation tillage affects SOC changes [22].

Based on the long-term experiment of the China Arid Agriculture Experimental Station, including three tillage models of conventional tillage plus straw removal (CT), deep scarification plus straw mulching (DS), and no tillage plus straw mulching (NT), the aims of this study were as follows: (1) to evaluate the changes in soil aggregate composition, aggregate-associated C and N, and C:N ratio; (2) to quantify the contribution of SOC in different aggregates and subfractions, especially microaggregates with macroaggregate (mM) and POM, to total SOC and N changes; and (3) to study the relationship between these C and N pools in different aggregates.

2. Materials and Methods

2.1. Experimental Site

The field experiment was carried out at the Luoyang Academy of Agriculture and Forestry Sciences (112°29′25″ E, 34°38′27″ N), located in the west of China's Henan

Province, which has a monsoon climate. The average annual precipitation of the experimental site is 600 mm, with 70% of the annual rainfall occurring from June to September. The annual evaporation and average temperature are 1872.1 mm and 13.7 °C, respectively. The cropping system is rainfed wheat–maize rotation in meadow-cinnamon soil [23]. The textural class is clay loam, according to the USDA (United States Department of Agriculture), and other properties of the 0–20 cm soil layer are shown in Table 1.

Table 1. The properties of 0–20 cm soil before the experiment.

Mechanical Composition (%)			Bulk Density (g/cm^3)	pH	SOM (g/kg)	Total N (g/kg)	Alkali-Hydrolysable N (mg/kg)	Olsen-P (mg/kg)	NH_4OAc-K (mg/kg)
Sand	Silt	Clay							
30.2	41.6	28.2	1.53	7.3	15.8	0.95	62.7	3.58	138.3

2.2. Experimental Design

The tillage experiment began in October 2004. Three different tillage systems, namely CT, DS, and NT, were chosen in this study. Each treatment was repeated three times and arranged in random blocks. For the CT (ploughing plus straw removal), the soil was deeply plowed to a depth of 0.25–0.30 m before sowing wheat, but no tillage was conducted during the maize growing season. The straw and stubble of both the wheat and maize were removed from the field. For the DS (deep scarification plus straw mulching), the subsoil was scarified to a depth of 0.30–0.40 m with the subsoiler when wheat was sowed, but no tillage was conducted during the maize growing season. The type of the subsoiler is Zhongxin3S-1.0, 3-pointed mounted with tractor with a working width of 100 cm. Five legs are used for subsoiling, of which three are in the front and two are in the back. For the NT (no tillage plus straw mulching), no tillage was conducted during either the wheat or maize seasons. The straw and stubble of the pre-season crops in the NT and DS were all returned to the field by covering in the rows [23].

2.3. Soil Sampling, Aggregate Fractionation, and C and N Measurements

Three soil samples were collected randomly from each plot in June 2022 from the 0–20 cm soil layer using a tubular drill. The soil samples collected from the same plot were thoroughly mixed to form a composite sample. The mixed soil samples were air-dried, and then visible plants, root materials, and pebbles were removed. The soil samples were gently broken apart by hand along the soil texture to pass through an 8 mm sieve. The air-dried soil samples were used for aggregate analysis via a wet-sieving method [24] (Figure 1).

A total of 50.0 g of air-dried bulk soil that had been sieved to pass through an 8 mm sieve was weighed and placed on top of a 0.25 mm sieve. The sieves were placed into a clean plastic basin, and deionized water was added to cover 2 cm of the soil. After the soil sample was immersed for 5 min, the sieves were then gently shaken up and down 50 times for 2 min at a constant rate [25]. All the effluent from the plastic basin was then gently poured over a 0.053 mm sieve and sieved as described above to obtain three different aggregate size fractions: a macroaggregate fraction (>0.25 mm), microaggregate fraction (0.053–0.25 mm), and silt + clay fraction (<0.053 mm). The aggregates remaining on the sieve were washed with deionized water into a pre-weighed petri dish and then placed in an oven to dry at 50 °C until a constant weight. The effluent in the plastic basin was placed into a beaker, and the supernatant was poured off and dried at 50 °C until a constant weight was achieved.

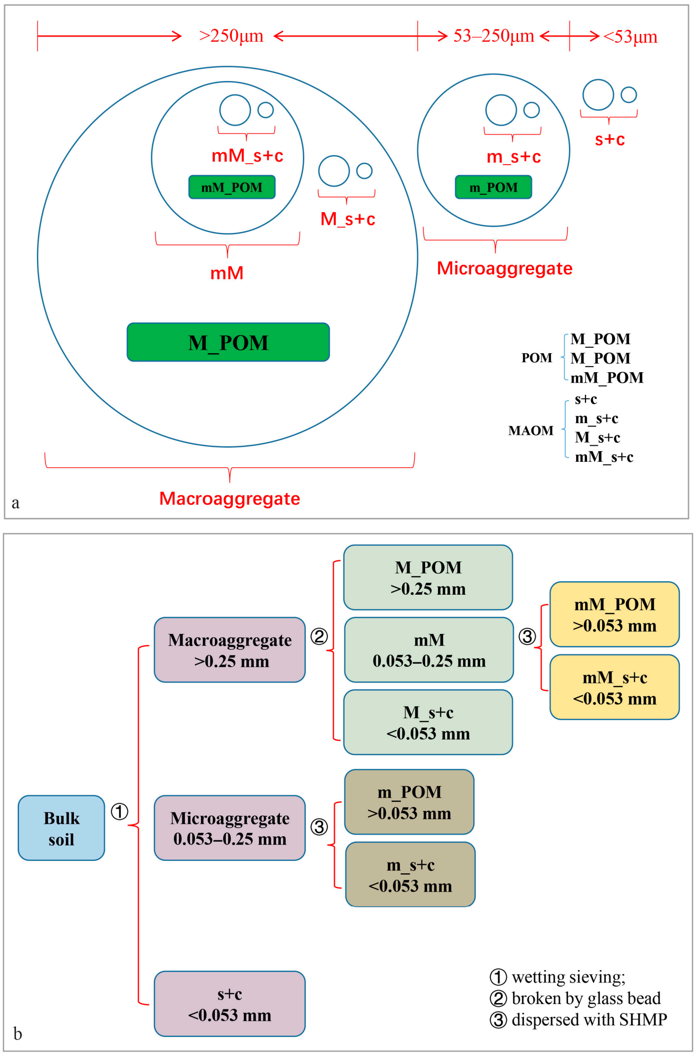

Figure 1. Conceptual diagram of aggregate structure (**a**) and fractionation scheme (**b**). POM: particulate organic matter; MAOM: mineral-associated organic matter; silt and clay; mM: microaggregate within macroaggregate; m_POM: POM in microaggregate; m_s+c: silt and clay in microaggregate; M_POM: POM in macroaggregate; M_s+c: silt and clay in macroaggregate; mM_POM: POM in mM; mM_s+c: silt and clay in mM.

The oven-dried macroaggregates (10.0 g) were placed on the top of a 0.25 mm sieve (with 50 glass beads of 4 mm diameter) and immersed in deionized water in the same manner for 5 min. The sieve was shaken slowly and evenly, while a steady and gentle flow of water was passed over the sieve to ensure that the macroaggregates that had broken were immediately flushed onto the 0.053 mm sieve. After all the macroaggregates were broken, the 0.053 mm sieve was moved up and down at a constant speed to obtain water-stable microaggregates. Three different aggregate fractions were obtained: free POM in macroaggregates (>0.25 mm, M_POM), microaggregates encapsulated within macroaggregates (0.053–0.25 mm, mM), and silt and clay fractions encapsulated within macroaggregates (<0.053 mm, M_s+c). All aggregate fractions were placed in an oven at 50 °C [26].

Five grams of the oven-dried microaggregate fractions were weighed into a tripod flask, to which 30 mL of 0.5% sodium hexametaphosphate (SHMP) was added. The samples were shaken on a shaker at 112 oscillations per minute for 18 h. The dispersed suspension was passed through a 0.053 mm sieve, and the material remaining on the sieve was washed with deionized water and dried to a constant weight at 50 °C. Two aggregate fractions were obtained: POM encapsulated with microaggregates (>0.053 mm, m_POM) and silt and clay fractions encapsulated within microaggregates (<0.053 mm, m_s+c). In the same way, the POM (mM_POM) and silt + clay fractions (mM_s+c) of the microaggregates wrapped in macroaggregates were obtained [27].

The bulk soils and dried aggregate fractions were ground to pass through a 0.02 mm sieve. Calcium carbonate was removed from all fractions, and the organic C and total N concentrations were measured using a fully automatic CNS element analyzer. The total POM was calculated by the sum of m_POM, M_POM, and mM_POM, while MAOM was calculated by the sum of s+c, m_s+c, M_s+c, and mM_s+c.

2.4. Date Analysis

Soil C storage was estimated according to Equation (1) [28]:

$$\text{SOCS} = \text{BD} \times \text{SOC} \times \text{D} \times 0.1 \tag{1}$$

where SOCS is soil organic C storage (t/ha), BD is bulk density (g/cm^3), and D is soil depth (cm). The estimation of soil TN storage (STNS) is consistent with SOCS with replacing SOC by soil TN.

The mean weight diameter (MWD) and geometric weight diameter (GWD) of the aggregates were estimated according to Equations (2) and (3) [29]:

$$\text{MWD} = \sum_{i=1}^{n} (\overline{x_i} \times w_i) \tag{2}$$

$$\text{GMD} = \exp\left[\frac{\sum_{i=1}^{n} w_i \ln x_i}{\sum_{i=1}^{n} w_i}\right] \tag{3}$$

In the equations, $\overline{x_i}$ is the mean diameter of aggregate fractions with different sizes (mm), w_i is the percentage of each aggregate weight to the total sample weight (%), and n is the number of fractions sieved per sample.

The contribution of organic C of each fraction to the total SOC of the sample was estimated according to Equation (4) [30]:

$$\text{SOC}_{i-\text{contribution}} = \frac{(\text{SOC}_{i-\text{concentration}} \times W_i)}{\sum_{i=1}^{n} (\text{SOC}_{i-\text{concentration}} \times W_i)} \times 100\% \tag{4}$$

where $\text{SOC}_{i=\text{contribution}}$ is the contribution of the organic C of each aggregate fraction to the total organic C of the bulk sample (%), $\text{SOC}_{i=\text{concentration}}$ is the organic C concentration of the aggregate fraction (g/kg aggregate), and w_i is the percentage of each aggregate weight to the total sample weight (%). The equation used to calculate $\text{STN}_{i-\text{contribution}}$ is consistent with that used to calculate $\text{SOC}_{i=\text{concentration}}$.

2.5. Statistical Analysis

Mean differences were compared using Duncan's multiple comparison test at $p < 0.05$. The analysis of variance and the graphs were performed in software of IBM SPSS 26.0 and Origin 2022. A structural equation model (SEM) was constructed using IBM SPSS Amos 24.0 to explore the influence paths of C and N in different fractions on total SOC and N contents [23]. The structural calibration of the SEM was evaluated according to the relative Chi-square value (χ^2/df), goodness of fit index (GFI), and root mean square error of approximation (RMSEA). The Mantel test was used to determine the relationship between soil C and N and soil aggregate stability. The SEM and Mantel test were conducted in R software (https://www.r-project.org/ accessed on 19 July 2024).

3. Results

3.1. Soil C, N Content, and Sotrage in Bulk Soil

After 18 years, the NT and DS exhibited an increase in SOC content and soil organic C storage (SOCS) in the 0–20 cm layer compared with the CT ($p < 0.05$, Figure 2). Similarly, NT showed an increase in soil TN content and soil total nitrogen storage (STNS) in the 0–20 cm layer ($p < 0.05$). However, there were similar levels between the DS and CT.

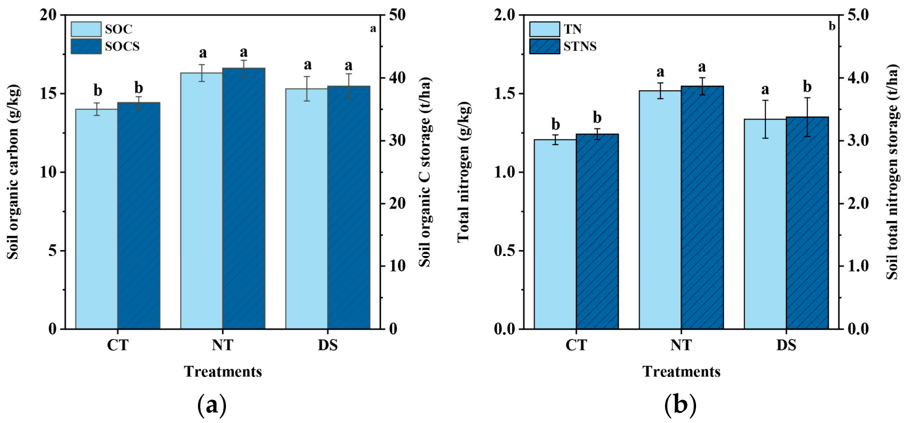

Figure 2. Soil organic carbon (SOC) and soil organic C storage (SOCS) (**a**), total nitrogen (TN) and soil total nitrogen storage (STNS) (**b**) of the 0–20 cm soil layer under different tillage treatments. Different lowercase letters represent significant differences among the different treatments at $p < 0.05$ level. Error bar represents the standard error of the mean. CT: conventional tillage with straw removal; DS: deep scarification with straw mulching; NT: no tillage with straw mulching.

3.2. Size Distribution of the Soil Aggregates

Long-term tillage influenced the distribution of aggregates and subfractions (Figure 3a). The proportion of macroaggregates (>0.25 mm) and subfractions (M_POM, M_s+c, mM_POM, mm_s+c) was higher in the NT than in the CT ($p < 0.05$). In contrast, the proportion of microaggregates and fractions (m_POM and m_s+c) and silt + clay in the NT were lower than in the CT. A similar trend was also observed for the size distribution of soil aggregates in the DS, but the difference between DS and CT was not significant. Conservation tillage affected the MWD and GWD of the soil aggregates in the sequence of NT > DS > CT (Figure 3b).

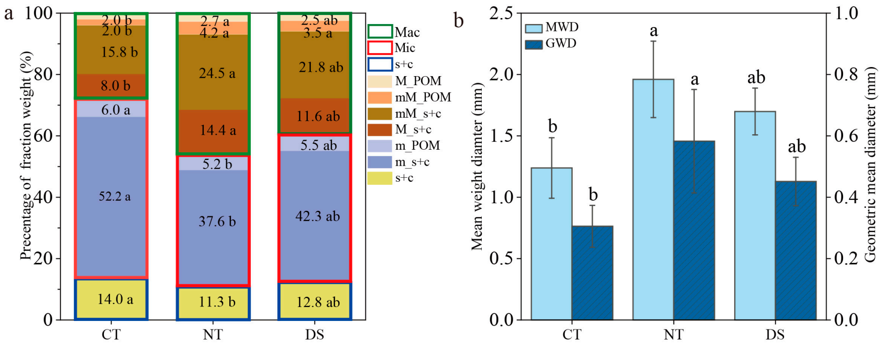

Figure 3. Soil aggregate distribution (**a**), mean weight diameter (MWD) and Geometric mean diameter (GMD) (**b**) for different tillage treatments. Different lowercase letters represent significant differences among the different treatments at $p < 0.05$ level. Error bar represents the standard error of the mean. CT: conventional tillage with straw removal; DS: deep scarification with straw mulching; NT: no tillage with straw mulching; Mac: macroaggregate; Mic: microaggregate; s+c: free silt and clay; M_POM: POM in macroaggregate; mM_POM: POM in microaggregate within macroaggregate; mM_s+c: silt and clay in microaggregate within macroaggregate; M_s+c: silt and clay in macroaggregate; m_POM: POM in microaggregate; m_s+c: silt and clay in microaggregate.

3.3. C, N Concentration, and C:N Ratio in Aggregate Fractions S

The C concentration for different aggregates increased with the increase in aggregate size (Figure 4a). With the exception of the silt + clay of DS, the C concentrations of the other soil aggregates with different sizes were higher in NT and DS than in the CT ($p < 0.05$). When the microaggregates were divided into m_s+c and m_POM, there was no significant difference in C concentration between the different treatments, although the C concentration of m_POM in NT and DS was higher than in the CT (Figure 4b). Similar to the macroaggregates, the C concentrations of the fractions (M_s+c, M_m, M_POM) of NT and DS were higher than those of CT ($p < 0.05$; Figure 4c). There was no difference in the C concentration of mM_s+c among the different treatments, while the C concentration of mM_POM under conservation tillage (NT and DS) was higher than the CT ($p < 0.05$; Figure 4d). Comparing the C concentrations of the different fractions (Figure 4a–d), it was found that the C concentrations of M_POM and mM_POM were higher ($p < 0.05$) than that of m_POM, and the C concentration of mM was higher than that of the microaggregates; however, there was no substantial difference between different s+c fractions.

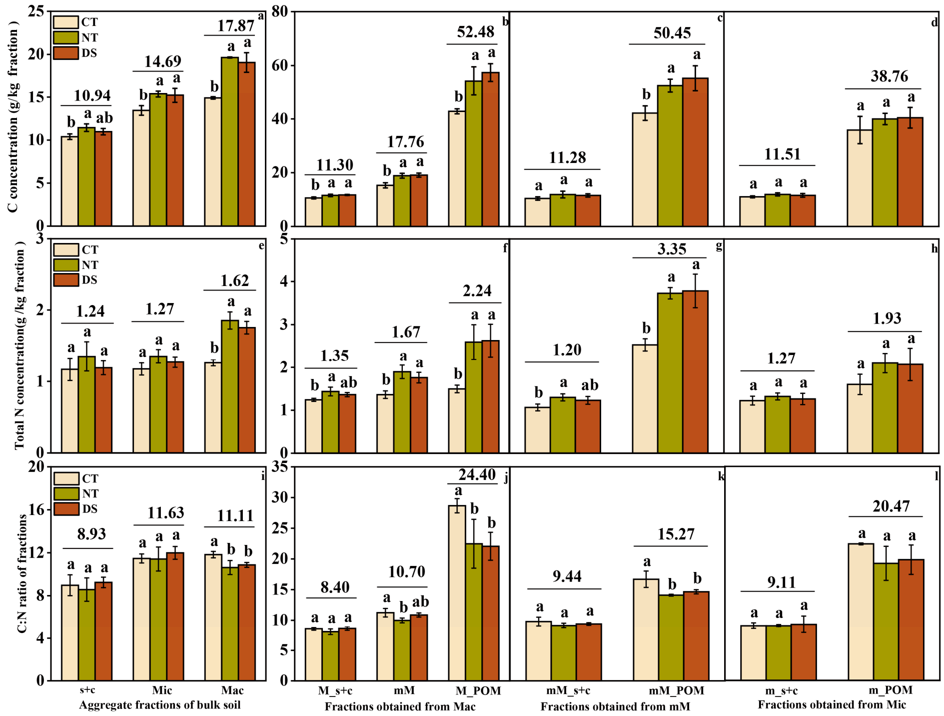

Figure 4. Carbon (**a**–**d**), nitrogen (**e**–**h**) concentration (g/kg fraction), and C:N ratio (**i**–**l**) in different fractions under different tillage practices. Different lowercase letters represent significant differences among the different treatments at $p < 0.05$ level. Error bar represents the standard error of the mean. CT: conventional tillage with straw removal; DS: deep scarification with straw mulching; NT: no tillage with straw mulching; Mac: macroaggregate; Mic: microaggregate; s+c: free silt and clay; m_POM: POM in microaggregate; m_s+c: silt and clay in microaggregate; M_s+c: silt and clay in macroaggregate; mM: microaggregate within macroaggregate; M_POM: POM in macroaggregate; mM_POM: POM in mM; mM_s+c: silt and clay in mM.

The N concentration of different fractions under different treatments was almost consistent with the trend in C concentration (Figure 4e–h). Compared with the CT, the N concentration of the different fractions was increased by the conservation tillage, especially in the macroaggregates and their subfractions (Figure 4e–g). In contrast to the C concentration, there was little difference in the N concentration of the microaggregates and silt + clay fractions (Figure 4e), and the N concentration of mM_POM (3.35 g/kg fraction, Figure 4g) was higher than that of M_POM (2.24 g/kg fraction, Figure 4f). Compared with the s+c fractions, POM was more sensitive to tillage.

The macroaggregate and microaggregate fractions showed a higher C:N ratio than the silt + clay fractions, and conservation tillage decreased the C:N ratio of the macroaggregates compared to the CT ($p < 0.05$; Figure 4i). The C:N ratio of the different POM fractions was as follows: M_POM > m_POM > mM_POM, and the C:N ratios of different POM fractions for the NT and DS were lower than for CT ($p < 0.05$; Figure 4j–l). The different MAOM fractions were in the order of mM_s+c > m_s+c > s+c > M_s+c. Compared with POM, the difference in the C:N ratio of the MAOM fractions was small, and no significant difference was found between the different treatments (Figure 4j–l).

3.4. Partitioning Proportion of C and N in Each Fraction

The contribution of C and N in different fractions to total SOC and N was altered by different tillage practices ($p < 0.05$; Figure 5a–c). The NT and DS increased the proportion of C (53.1% and 46.3%) and N (53.7% and 47.5%) in the macroaggregates compared with the CT (30.7% for C and 29.2% for N) and also increased the C and N proportion of the subfractions, i.e., M_POM, mM (including mM_POM and mM_s+c), and M_s+c. In contrast, the proportion of C and N in the microaggregates (including m_POM and m_s+c) and free s+c to total SOC and N were reduced by the NT and DS. In total, mM contributed 18.9–33.7% and 19.1–35.0% of total C and N, respectively, and POM contributed 28.0–37.1% and 14.1–23.1%.

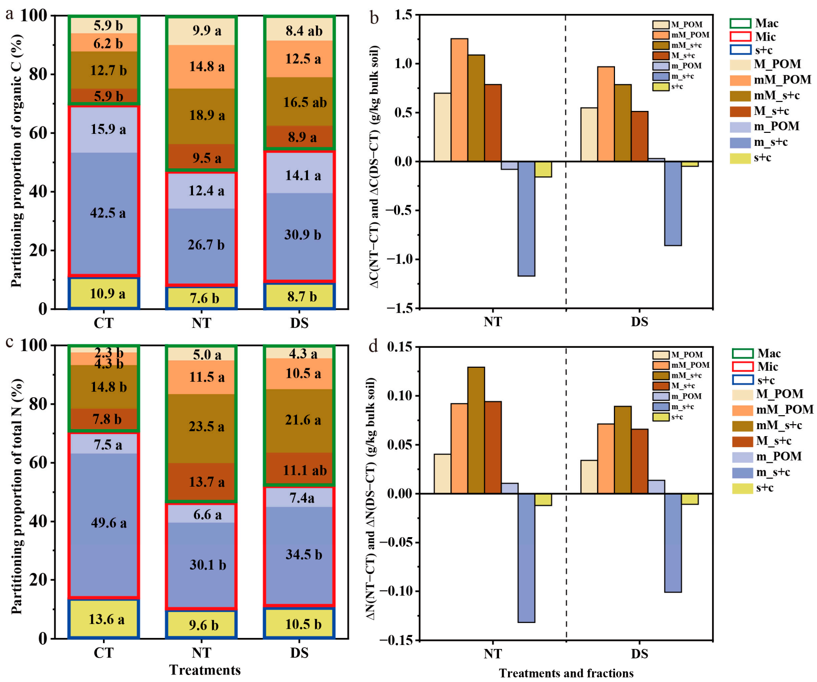

Figure 5. Contributions of SOC (**a**) and N (**c**) of different fractions to total SOC and N, and C (**b**) and N (**d**) change in different fractions for the NT and DS compared to the CT. Different lowercase letters represent significant differences among the different treatments at $p < 0.05$ level. Error bar represents the standard error of the mean. CT: conventional tillage with straw removal; DS: deep scarification with straw mulching; NT: no tillage with straw mulching; Mac: macroaggregate; Mic: microaggregate; s+c: free silt and clay; M_POM: POM in macroaggregate; mM_POM: POM in microaggregate within macroaggregate; mM_s+c: silt and clay in microaggregate within macroaggregate; M_s+c: silt and clay in macroaggregate; m_POM: POM in microaggregate; m_s+c: silt and clay in microaggregate.

The cumulative C and N content of the different fractions under tillage was consistent with the total SOC and N content. Compared with CT, the increase in C and N for the NT and DS was mainly due to the increase in C and N of the fractions within macroaggregates, while the changes in the C and N of the m_s+c and free s+c fractions for the NT and DS were decreased compared to the CT, particularly that of m_s+c (Figure 5c,d). Compared with CT, the C change in POM (sum of M_POM, mM_POM, m_POM) contributed 77.4% and 79.9% of the total SOC increase by NT and DS, and 64.2% and 73.3% of the total N increase. However, mM contributed 96.9% and 90.5% of the total C change by NT and DS, respectively, as well as 99.4% and 98.9% of total N change.

3.5. Relationship among C and N in Bulk Soil and Fractions

Correlation analysis showed that there were always significant and positive correlations between C and N in both the bulk soil and the same fraction. The total C and N values of the bulk soil were positively ($p < 0.05$) correlated with those in the macroaggregates and associated subfractions (M_s+c, M_POM, mM, mM_s+c, and mM_POM) but negatively correlated with those in the microaggregates and silt + clay fractions. The C and N in the macroaggregates and associated subfractions were negatively correlated with microaggregates and free s+c. However, there was no significant correlation between the C and N content of m_POM and other fractions, except for the correlation between the C content of m_POM and microaggregates and the s+c fraction (Figure 6). Overall, the soil C and N in bulk soil and aggregate fractions showed the significant correlation with MWD and GWD, except for the N content of s+c, m_POM, and M_POM fractions (Mantel's r > 0.2, $p < 0.05$). In the SEM, the predictors accounted for 80% and 93% of variations in SOC and TN across different long-term tillage practices (Figure 7). The SOC was affected by MAOM-C, whereas the TN was impacted by the N content of MAOM and Mic. The C and N contents of MOAM were mainly influenced by POM or Mac. Similar relationships were observed for the C and N of Mac, mM, and Mic, with Mac affecting mM and the latter affecting Mic. This indicated that mM played a link role in the turnover of macroaggregates and microaggregates.

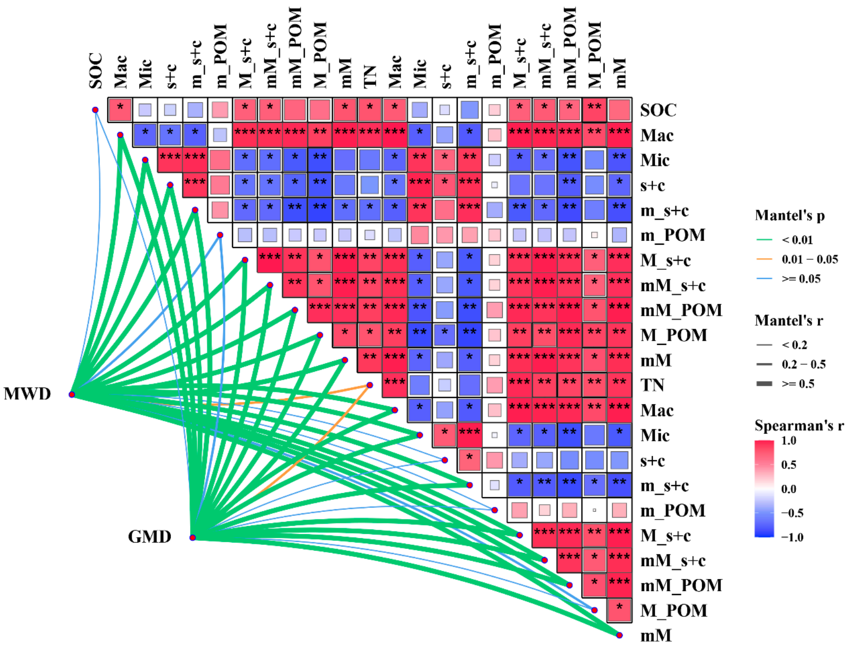

Figure 6. Relationship among soil C and N in bulk soil and aggregate fractions and their relationship with MWD and GWD. Pairwise comparisons of the soil C and N in bulk soil and aggregate fractions are displayed with a color gradient denoting Pearson's correlation coefficient. Edge width and color correspond to Mantel's r and Mantel's p, respectively. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Mac: macroaggregate; Mic: microaggregate; s+c: free silt and clay fractions; m_POM: POM in microaggregate; m_s+c: silt and clay in microaggregate; M_s+c: silt and clay in macroaggregate; mM: microaggregate within macroaggregate; M_POM: POM in macroaggregate; mM_POM: POM in mM; mM_s+c: silt and clay in mM.

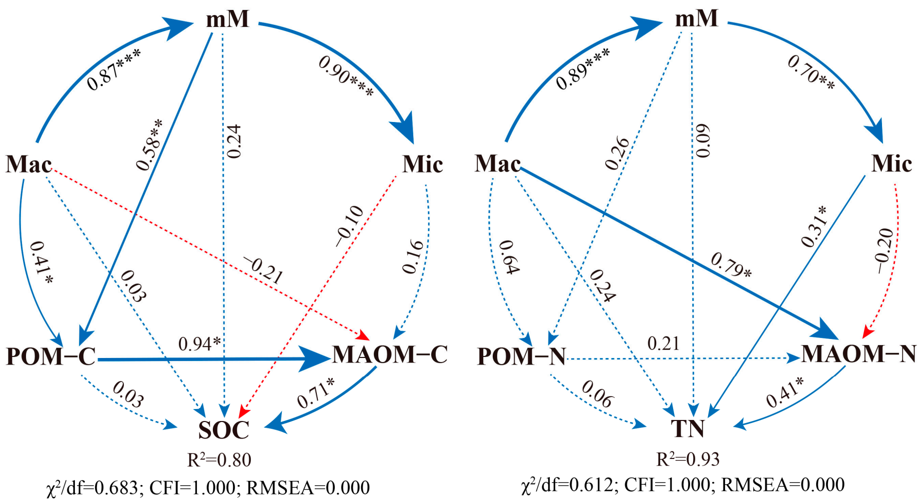

Figure 7. Structural equation model (SEM) for soil C and N in bulk soil and aggregate fractions. The numbers adjacent to arrow lines are standardized coefficients that show the variances explained by the variables. The width of the line is proportional to the strength of factor loading. Blue and red solid line indicated the positive and negative effect, respectively. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Blue and red dashed line indicated similar level. Mac: macroaggregate; Mic: microaggregate; mM: microaggregate within macroaggregate; POM: particulate organic matter; MAOM: mineral-associated organic matter.

4. Discussion

4.1. Coupling of SOM Improvement and Aggregate Stability under Conservation Tillage

Soil aggregate structure and SOM are key indexes of soil quality and fertility. In this study, the long-term conservation tillage promoted SOC and N sequestration and increased the proportion of macroaggregates but lowered the proportion of microaggregate and silt + clay fractions. This is consistent with many other studies [31,32]. Meanwhile, conservation tillage increased the contribution of C in the macroaggregates to total SOC. This indicated that conservation tillage promoted the formation of macroaggregates, which was conducive to soil C and N accumulation. The protection of SOM by aggregates is an important mechanism for SOM stability, and the formation of macroaggregates from microaggregates, silt, and clay fractions will promote the stability and accumulation of SOM [33,34]. On the other hand, the enrichment of SOM is conducive to the soil agglomeration process and, thus, promotes the formation of large-sized aggregates, as SOM is the cementing material of aggregates [35,36]. The C and N contents in the bulk soil were positively correlated with the C and N contents in the macroaggregates and sub-fractions. The quantitative analysis proved that the turnover of SOM and aggregates is a coupled process, and conservation tillage improved this process (Figure 8).

Macroaggregates develop mainly from small-sized aggregates and mineral silt and clay, which are intimately mixed and combined by binding agents such as fungal hyphae, extracellular polysaccharides, and occluded POM [33]. Therefore, it was further found that all subfractions wrapped in macroaggregates (M_POM, M_s+c, mM, mM_POM, and mM_s+c) increased to different degrees with the increase in the proportion of macroaggregates, and the m_POM and m_s+c fractions wrapped in the microaggregates similarly decreased with the decrease in the proportion of microaggregates. This change in the soil mass resulted in an increase in the contribution of macroaggregates to total C and N but a decrease in the microaggregate and silt + clay fractions under conservation tillage, despite an increase in C and N concentrations in all fractions. This suggests that the overall distribution pattern of soil C and N in aggregates is more determined by the distribution of soil aggregate matrix rather than the concentration of C and N in different aggregates [33].

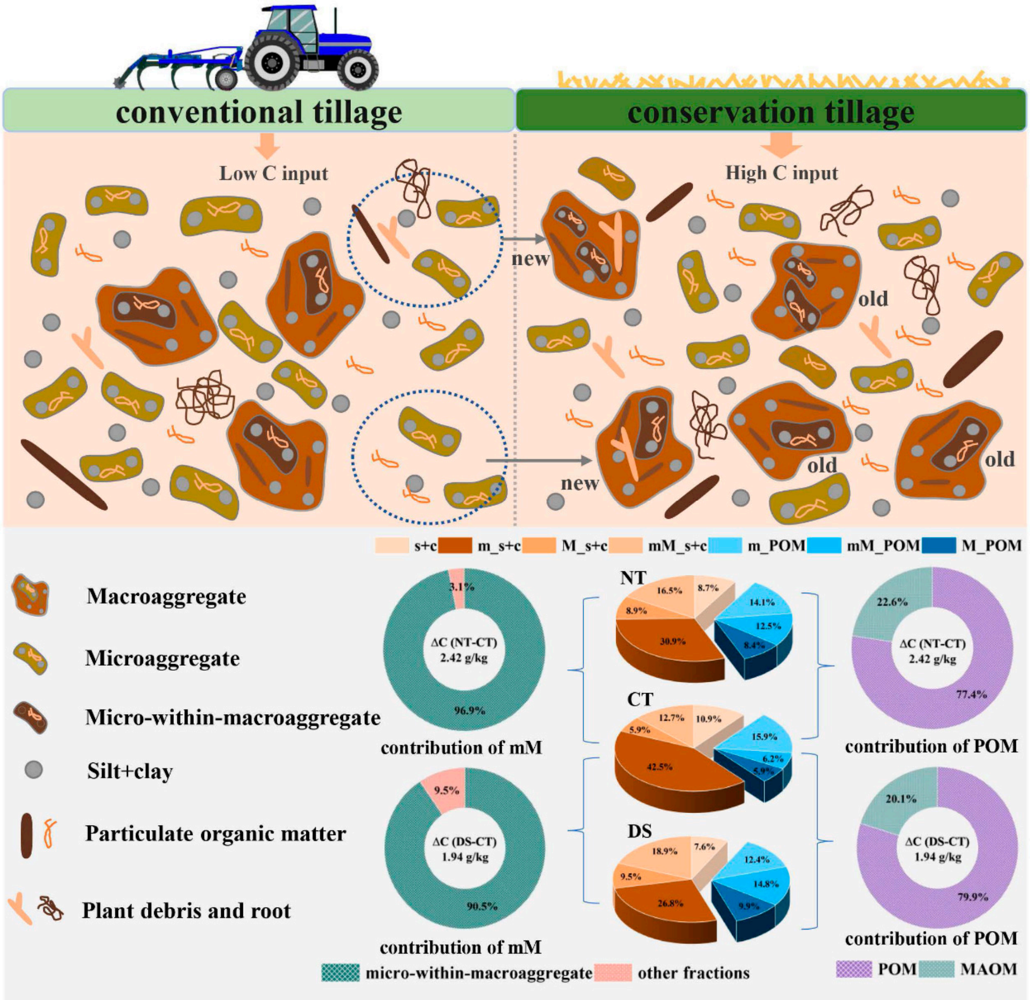

Figure 8. The schematic diagram of C and N sequestrations under long-term conservation tillage. CT: conventional tillage with straw removal; DS: deep scarification with straw mulching; NT: no tillage with straw mulching; POM: particulate organic matter; MAOM: mineral-associated organic matter; mM: microaggregate within macroaggregate; s+c: free silt and clay fractions; m_s+c: silt and clay in microaggregate; M_s+c: silt and clay in macroaggregate; mM_s+c: silt and clay in mM; m_POM: POM in microaggregate; M_POM: POM in macroaggregate; mM_POM: POM in mM.

Conservation tillage improved soil C, N, and aggregate stability in several ways. First, under conservation tillage, long-term straw return by mulching to the field increased the input of soil organic materials, which entered into aggregates of different sizes, continuously increased the soil C and N of all aggregate fractions, and enhanced the diversity and activity of microorganisms [23]. This exogenous organic matter and microbial residues and metabolites constitute important cementation materials of aggregate formation and promote the agglomeration of small-sized fractions (microaggregates, silt, and clay) into large-sized aggregates [36]. Second, conservation tillage enhanced crop productivity and biomass by reducing soil water volatilization, improving water use efficiency, and enhancing the root growth environment, especially under rainfed farmland conditions, thereby increasing soil C input from the root system and rhizosphere secretions [21,37]. Third, traditional tillage causes severe soil disturbance, resulting in the fragmentation of large-sized aggregates and a decline in aggregate stability, while conservation tillage reduces soil disturbance [6,38]. The disturbance intensity affected the turnover of aggregates, and the smaller the disturbance to the soil, the more favorable the formation of large-sized aggregates [39]. In addition, straw mulching under conservation tillage reduced the direct impact of rainfall on the soil, reduced the damage to aggregates, and promoted the protection of SOM.

4.2. Microaggregates within Macroaggregates as a Diagnostic Fraction for SOM Change and Turnover

The microaggregates enclosed by macroaggregates are important functional fractions for C sequestration in soil and can be used for the early detection of changes in soil C arising from changes in soil management [40]. In this study, mM only accounted for 17.8–28.7% of bulk soil weight and contributed 18.9–33.7% of total SOC. Importantly, the C increase in mM under the NT and DS contributed 96.9% and 90.5% of the change in total SOC and 99.4% and 99.0% of soil bulk N change, respectively (Figure 8). This was consistent with the results of previous studies, supporting the viewpoint that mM is an important diagnostic fraction and measurement pool in soil C sequestration [41].

The mM fraction might play an indicative role in the turnover process of soil aggregates and SOM by connecting microaggregates and macroaggregates as a "bridge" between soil aggregates and C and N turnover. Under conservation tillage conditions, the increased SOM input and reduced perturbation promoted the formation of macroaggregates from small-sized fractions. With the gradual decomposition of M_POM, some macroaggregates disintegrated, and the mM fraction was released as free microaggregates. The microaggregates interacted again with mycelia, polysaccharides, roots, and microorganisms to form new macroaggregates and participate in the next turnover [42]. Reduced soil disturbance under conservation tillage resulted in a slower turnover of macroaggregates, which enhanced the protective effect of mM on SOM.

The C:N ratio in the macroaggregates and microaggregates was higher than that in the silt + clay fractions in this study, which indicates that a higher proportion of new exogenous organic materials preferentially entered into large-sized aggregates or that exogenous organic materials formed new aggregates with larger sizes from the silt and clay fractions and microaggregates [33,35]. Additionally, the C:N ratio of the mM fraction was lower than that of the free microaggregates, meaning a higher degree of SOM in the mM fraction. The possible reasons were that the free microaggregates evolved into more exogenous fresh organic materials with high C:N ratios than mM. Furthermore, during the turnover of aggregates and SOM within the macroaggregates, the POM in the macroaggregates was degraded by microorganisms to a certain extent and, together with the decreased C:N ratio, then became a component of mM [36,40]. Therefore, under long-term stable management conditions, the C:N ratio of mM would gradually tend to be lower than that of free microaggregates.

4.3. Quantifying the Contribution of POM to Soil C and N Sequestration under Conservation Tillage

SOM mainly exists in soil in two states of particulate organic matter (POM, articulate organic residues mostly of plant origin) and mineral-associated organic matter (MAOM; OM adhering to mineral surfaces) [35]. Combined with aggregate fractionation, POM can be further divided into M_POM, mM_POM, and m_POM, while MAOM can be divided into free s+c, m_s+c, mM_s+c, and m_s+c. In this study, the C and N of the silt and clay fractions (MAOM) in different aggregates maintained relatively stable levels, but the C and N of POM showed great differences among fractions and were very sensitive to tillage management. The POM-C fraction accounted for only 28–37% of total SOC, but the change in C in the three POM fractions under the NT and DS accounted for 77% and 80% of total SOC increase compared with CT, while C changes in the MAOM fractions only accounted for 20–23% of total SOC increase (Figure 8). A similar trend was observed for the distribution of N in POM and MOAM and their response to different tillage practices. The results are consistent with previous studies and indicated that POM components played an crucial role in soil C and N sequestration and turnover under conservation tillage conditions [43,44].

The C:N ratio of different POM fractions was in the order of M_POM > m_POM > mM-POM, suggesting different degradation degrees of POM among the aggregate fractions. When the organic materials were placed into the soil, more preferentially entered the macroaggregates as M_POM, and then the POM fraction was fractionated into microaggregates within macroaggregates as mM_POM under the action of microorganisms, making its C:N ratio lower than that of M_POM. The SOC concentration of different POM fractions was in the order of M_POM > mM_POM > m_POM, but the N concentration was mM_POM > M_POM > m_POM. This might be caused by the different decomposition abilities of microorganisms to organic matter at different locations or to the difference in POM sources in these three fractions [45,46]. However, the specific reasons need to be further explored. Although the C and N concentrations of all MAOM fractions were less sensitive to tillage than POM, it determined the total SOC change on a long-term scale, reflecting its important role in the C stability and sequestration potential [7,44].

5. Conclusions

The 18-year minimum tillage with straw mulching increased soil C and N storage and stimulated the formation of macroaggregates from small-sized fractions. Therefore, the long-term adoption of conservation tillage could improve the sustainability of farmlands by increasing aggregate stability and C and N sequestration. Conservation tillage increased the proportion of weight of mM fraction and its C and N contents, revealing the importance of mM as a diagnostic and measurable fraction in the formation and turnover of soil aggregates. POM was more sensitive to tillage management than MAOM and played a dominant role in soil C and N sequestration by conservation tillage. However, as the receiver of other C and N pools such as POM and macroaggregates, the SOC and N sequestration potential might still depend on MAOM under long-term conditions.

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Abbreviations

CT: conventional tillage; DS: deep scarification; NT: no-tillage; POM: particulate organic matter; MAOM: mineral-associated organic matter; Mac: macroaggregate; Mic: microaggregate; s+c: silt and clay; mM: microaggregate within macroaggregate; m_POM: POM in microaggregate; m_s+c: silt and clay in microaggregate; M_POM: POM in macroaggregate; M_s+c: silt and clay in macroaggregate; mM_POM: POM in mM; mM_s+c: silt and clay in mM.

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