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Research article

Conservation tillage and wheat straw managements improve soil organic carbon sequestration via calcium-mediated microbial communities and aggregate stability in Calcaric Cambisols

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A R T I C L E I N F O

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A B S T R A C T

Conservation tillage improves soil organic carbon (SOC) management by balancing microbial decomposition and physico-chemical protection. Minerals stabilize SOC, but how tillage practices affect calcium (Ca) speciation and its role in microbial–mineral–organic matter interactions in calcareous soils remains unclear. Thus, a 22-year tillage experiment and soil incubation were conducted to investigate Ca-mediated SOC sequestration and mineralization. Conservation tillage, including no-tillage with straw mulch (NTS) and subsoil tillage with straw mulch (STS), increased SOC storage by 9.9–14.3 % at 0–20 cm soil depth by promoting organo-Ca associations, compared to conventional tillage without straw return (CTN) and reduced tillage without straw return (RTN). Moreover, NTS and STS increased aggregate stability and macroaggregate proportion, reducing total SOC mineralization, as macroaggregates had lower SOC mineralization than microaggregates. In aggregates, increased exchangeable Ca was linked to higher particle- and mineral-associated SOC and lower SOC mineralization, showing positive ($p < 0.01$) and negative ($p < 0.001$) relationships, respectively. Compared to CTN and RTN, long-term NTS and STS not only promoted the transformation of $CaCO_3$ to exchangeable Ca, but also increased microbial biomass, especially the proportion of Gram-negative and arbuscular mycorrhizal fungi. Mechanistically, higher Ca^{2+} reshaped bacterial communities and promoted the microbial by-product binding to the minerals to form stable organo-Ca complexes, which was supported by the positive correlations between Ca^{2+}, microbial composition and SOC content (PLS-PM, $p < 0.01$). Overall, conservation tillage increased Ca availability for carbon binding by mediating microbial structures, thereby promoting aggregate protection and SOC stability in Calcaric Cambisols.

1. Introduction

As the largest carbon (C) pool in terrestrial ecosystems, soil organic carbon (SOC) plays a key role in food security, ecosystem services and biogeochemical cycling (Huang et al., 2020). However, the global C storage is declining due to intensive agriculture, making SOC stabilization increasingly important for offsetting CO_2 emissions (Chen et al., 2018). Fresh organic matter is stabilized in soils by physico-chemical-biological processes, such as chemical binding to minerals via sorption, co-precipitation or complexation; physical occlusion within aggregates; and selective retention of recalcitrant molecular species (Almagro et al., 2021). It has been reported that microbial accessibility therefore determines the C stabilization rather than its chemical recalcitrance(Bayer et al., 2006). As the primary source of SOC, plant C is largely metabolized to form microbial products that partially coat soil mineral surfaces (Shabtai et al., 2023). Therefore, both mineral-mediated organic associations and aggregate physical protection should be considered in understanding SOC sequestration.

Tillage practices affect SOC stability in terms of soil aggregation, microbial communities, and mineral availability for C binding (Deiss

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et al., 2021). Conservation tillage, including no or reduced tillage with straw return, minimizes soil disturbance and enhances aggregation, thereby physically protecting plant-derived C and promoting the entrapment of microbial metabolites within small occluded micro-aggregates (Nandan et al., 2019). It also increases large aggregates proportion and helps to retain water and nutrients, in contrast to long-term intensive tillage (Gao et al., 2017). Furthermore, tillage practices can alter soil pore structure, connectivity, air-water mobility and the redox environment of minerals (Laudicina et al., 2015). Higher soil moisture and a moderate pH facilitate the formation of short-range-ordered minerals that are likely to be associated with C through cation bridges, van der Waals forces, and ligand exchange process (Eusterhues et al., 2005). In waterlogged paddy soil under no-tillage, the oxidation of ferrous ions can promote the formation of iron (Fe)- carbon complexes by reducing phenol oxidase activity and inhibiting CO_2 emission (Wang et al., 2022). However, during the transition from acidic to alkaline soils, Calcium (Ca) plays an important role in mediating SOC storage by promoting physicochemical interactions between organic compounds and minerals, including soil aggregation (Huang et al., 2019), adsorption (Shabtai et al., 2023), co-precipitation and complexation (Rowley et al., 2021). The binding interactions of Ca and C are easily affected by changes in physical and chemical conditions under tillage practices. Although calcareous soils cover more than 30 % of the Earth's surface (Wan et al., 2021), the transformation of Ca and its important role in C stabilization has received less attention under different tillage practices.

The Loess Plateau, which contains almost a third of the arable land in China, is dominated by calcareous soil (Gao et al., 2019). To mitigate SOC degradation, minimum tillage with cover crops has been identified as a promising strategy to enhance soil quality in this region (Gao et al., 2019). Different tillage systems can alter soil pore structure and the dynamics of Ca solute transport (Wan et al., 2021). During the dissolution process, driven by pH and gas partial pressure, Ca^{2+} improves cation-mediated bridging of organic matter, whereas the subsequent re-precipitation of calcium carbonate ($CaCO_3$) during soil drying promotes carbonate cementation that occludes SOC within aggregates (Ball et al., 2023). High summer rainfall accelerates Ca leaching in the Loess Plateau, while conservation tillage helps to retain minerals and nutrients by maintaining stable soil physical structures (Huang et al., 2019). $CaCO_3$ acts as an inorganic cement that binds small particles, promoting soil aggregation and structural stability under no tillage conditions (Rowley et al., 2021). Importantly, the addition of crop residues can influence the biological processes associated with Ca^{2+} formation, ultimately affecting SOC stabilization or decomposition (Adhikari et al., 2019). Under conservation tillage, organic matter inputs provide a rich substrate for microbial growth, thereby affecting microbial biomass, community composition and the by-products (Plaza et al., 2013). Moreover, root exudates that are transformed by microbial activity serve as important biological binders for aggregate formation. This biological cycle contributes to the sorption, co-precipitation and complexation between the Ca and carbon compounds derived from decomposers (Wan et al., 2021). Nevertheless, how Ca-mediated aggregate formation and microbial turnover interact to influence SOC sequestration under conservation tillage in the Loess Plateau remains poorly understood.

The conversion of plant materials to SOC depends on soil physical structure, mineralogy, and microbial processes (e.g., chemical composition, microbial activity, and microbial function) (Almagro et al., 2021). Exchangeable Ca can react with CO_2 from microbial respiration in the root zone to form carbonate, which acts as a cement to limit soil pores and decomposers (Wuddivira and Camps-Roach, 2007). Furthermore, Ca plays a key role in fungal and bacterial growth, particularly surface-adherent and biofilm-forming bacteria populations, as well as fungal lignin-degrading enzymes (Shabtai et al., 2023). The enrichment of bacteria attached to plant litter or carbon compounds can promote either SOC mineralization or enhance SOC sequestration by forming mineral-associated microbial by-products (Gargiulo et al., 2013). Microbially induced carbonate precipitation is widely applied in metal remediation (Dou et al., 2023), but its contribution to SOC storage in agricultural soils needs further study. Although mineral availability for binding C assumed to be protective, the specific role of Ca in mediating microbial communities and transferring microbial products to the persistent SOC remains unclear, especially under Ca-rich soils with active bacterial growth.

This study aims to evaluate the SOC storage under long-term conservation tillage management and to investigate the role of Ca in mediating microbial communities for SOC sequestration. We estimated carbon inputs and quantified SOC sequestration rates following a 22-year tillage experiment. We also conducted soil incubation with Ca-addition to examine SOC mineralization. Furthermore, Ca transformation, microbial biomass and communities were specifically correlated to explore mineral-microbe interactions. We hypothesis that (i) long-term conservation tillage promotes SOC storage by improving soil aggregation and physically protecting SOC from mineralization; (ii) straw return and macro-aggregation help maintain higher extractable Ca, facilitating the formation of stable organo-Ca complexes; and (iii) calcium-mediated organic–mineral bridging shifts microbial communities that are better adapted to Ca^{2+} solution chemistry, thereby regulating mineral-associated microbial biomass and enhancing SOC sequestration under conservation tillage.

2. Material and methods

2.1. Study sites and experimental design

A long-term field experiment was established in 1999 in Luoyang city (~324 m altitude, 34.80°N, 112.55°E), Henan Province, China, which located in the eastern edge of the Loess Plateau. The region has a warm temperate continental climate, characterized by an average annual temperature of 13.8 °C, rainfall of 645 mm per year, total annual evaporation of 1905 mm, a frost-free period of 236 days and a sunshine duration of 2295 h per year. The dominant cropping system is continuous winter wheat (*Triticum aestivum L.*) mono-cropping (Early October to early June). The studied soil is classified as Calcaric Cambisols according to the Food and Agriculture Organization (World reference base for soil resources 2014, 2015) with a silt loam texture (14.3 % sand, 79.8 % silt and 10.3 % clay). The initial soil pH, organic carbon (C) content, total nitrogen (N), phosphorus (P) and potassium (K) contents were 7.80, 6.94 g kg $^{-1}$, 1.12 g kg $^{-1}$, 0.69 g kg $^{-1}$ and 18.00 g kg $^{-1}$, respectively, within the 0–20 cm depth range.

The experiment was conducted as a randomized complete block design with three replications. Each plot was 10 m in length and 3 m in width, with rows spaced at intervals of 0.65 m. Consistent with previous studies from this long-term field experiment (Song et al., 2022), conservation or reduced tillage practices (NT, ST, and RT) are designed to minimize soil disturbance relative to the conventional tillage (CT) treatment, which employs moldboard or disc plowing and represents the system with maximum soil disturbance (Achankeng and Cornelis, 2023; FAO, 2012; Lu and Liao, 2017). According different straw-return practices, four tillage treatments were used in this study: (1) CTN, conventional tillage with no straw return, leaving 5–6 cm of stubble with straw and ears removed after harvest and plowing twice a year to a depth of about 20 cm in July and early October just before sowing; (2) RTN, reduced tillage with no straw return, leaving 10 cm of stubble with straw and ears removed after harvest and plowing once a year to a depth of about 20 cm in July; (3) NTS, no-tillage with straw return, 30 cm tall stubble remains with straw mulching to the field after threshing, and no plowing the whole year; and (4) STS, subsoiling with straw return, 25–30 cm tall stubble remains with straw mulching to the field after threshing, and a soil belt of 20 cm wide was sub-soiled to a depth of 30–35 cm by 60 cm intervals once a year in July. Each plot received the same chemical fertilization (150 kg N ha^{-1} as urea, 105 kg P_2O_5 ha^{-1} as calcium superphosphate, and 45 kg K_2O ha^{-1} as potassium sulphate) but no irrigation throughout the entire growing season of winter wheat in four tillage systems. Regular weddings and pest control is in line with local management.

2.2. Soil sampling

Soil samples were collected in late September 2021 using a 2.64 cm diameter auger at two depths (0–10 cm and 10–20 cm) along an X-shaped pattern within each plot. Samples from the same depth were then combined to form a composite sample per plot. All collected soil samples were gently broken up and passed through a 10 mm sieve. Visible debris, including stones, plant leaves and roots were carefully removed using tweezers. The sieved soil was then divided into three portions, one portion was air-dried for determination basic soil properties; another was used to separate soil aggregate and physical fractions separation and stored at 4 °C for the determination of microbial biomass carbon (MBC) following Nyamadzawo et al. (2009); and the remaining portion was stored at a −20 °C for microbial community analysis (Zheng et al., 2023a). To calculate the bulk density, separate soil samples were taken from 0 to 10 cm and 10–20 cm soil layers using a core sampler (5 cm height, 4.5 cm diameter) and oven-dried at 105 °C for 24 h (Kan et al., 2020). Soil water content was determined after harvest t to reflect variations in soil moisture associated with wheat growth (Guan et al., 2015).

2.3. Aggregate separation and physical fractions

The 10-mm soil samples were left at room temperature until approximately 10 % moisture. Then the samples were placed on the Automatic Sieve Shaker (Analysette 8411, Shaoxing, China) with 0.25-mm and 2-mm sieves and shacked for 2 min to collect the large macroaggregates (LM: >2 mm), the small macroaggregates (SM: 0.25–2 mm), and the microaggregates (MIC: <0.25 mm). It is believed that the dry sieving method avoids the hydraulic damage and leaching of dissolved organic matter from large aggregates in wet sieving process (Sarker et al., 2018). The mean weight diameter (MWD), which serves as a measure of aggregate stability, was calculated as follows (Kasper et al., 2009).

Bulk soils (<2 mm) and different sizes of air-dried aggregates were fractionated using a combination of density and size fractionation methods following Schweizer et al. (2019). Briefly, 5 g of soil were placed into a 50 ml centrifuge tube, and 40 ml of sodium iodide (NaI) solution (density = 1.8 g cm^{-3}) was added separate soil components by density. The suspension was centrifuged at 5000 rpm for 20 min, and the supernatant was filtered through a 0.45 μm membrane under vacuum and rinsed with deionized water to obtain the light fraction, representing the free particulate organic carbon (fPOC). The remaining heavy fraction in centrifuge tube was ultrasonically dispersed using a Sonifier (12-mm probe; Ultrasonic Pulverizer, SCIENTZ, China) with an energy input of 400 J ml^{-1}. The dispersed material was then washed with deionized water and passed through the 0.053 mm sieves to separate coarse particulate organic carbon (cPOC, >53 μm) from mineral-bound organic carbon (MAOC, <53 μm). All fractions were dried at 60 °C and weighed for further analysis.

2.4. Calcium and carbon contents in bulk soil and aggregate fractions

The determination of the soil properties was carried out according to the methods described in (Hickman, 2002). Soil exchangeable Ca in the bulk soil and different aggregates was determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES, 715-ES, Varian) by extraction with 1 mol L^{-1} NaOAc (pH = 8.2) at a soil/solution ratio of 1:10 (w/v). And the $CaCO_3$ content was determined by dissolution with 1 mol L^{-1} HCl followed by back-titration of the excess acid with 0.1 mol L^{-1} NaOH. As the soil contains carbonates, inorganic carbon was removed with 1 mol L^{-1} HCl solution, and soil organic carbon (SOC) and total nitrogen were determined by dry combustion at 960 °C using an elemental analyzer (Elementar, Analysensysteme, Elementary). The calculation of SOC content, the mass contribution of aggregate-associated SOC, and the SOC content in density fractions are described in the Supplementary Methods.

2.5. Microbial communities

Soil phospholipid fatty acids (PLFAs) were extracted according to calcareous soil condition from 0 to 10 cm and 10–20 cm soil layers. All soil samples were analyzed following the protocol of (Bååth and Anderson, 2003).Total PLFA content (n mol g^{-1}) has been shown to correlate strongly with microbial biomass (Kato et al., 2005). Individual PLFAs (n mol g^{-1}) were summed to quantify the total microbial biomass in each sample. The microbial groups studied, including Gram-positive (Gram+) and Gram-negative (Gram-) bacteria, anaerobes, actinobacteria, eukaryotes, arbuscular mycorrhizal fungi (AMF), were each counted as biomass (n mol g^{-1}). Details of the biomarkers for each group can be found in (Table S1). The PLFA marker 16:1ω5 has been recommended as a biomarker for AMF were not included in the total fungal biomass calculation because it is also found in bacteria (Ball et al., 2023). The PLFA marker 18:1w9c, 18:2w6,9c, 18:3w6c have been suggested to be indicative of saprotrophic fungi (SF) (Ponder and Tadros, 2002). It is important to note that, although early consensus favored the quantified PLFA as an indicator of viable biomass (Aliasgharzad et al., 2010), recent studies have revealed that the duration of these signals is as long as days to weeks, so the presence of fractions from non-viable microorganisms should not be ignored.

2.6. Soil incubation

A 31-day laboratory soil incubation was carried out for the evaluation of OC stability in bulk soil and different aggregates. The soil amended with 10 mg Ca^{2+} g soil using an aqueous $CaCl_2$ solution, with deionized water serving as the control. Briefly, 5 g samples of bulk soil and all aggregate classes form four treatments were adjusted to 60 % water holding capacity and placed in a 50 ml sealed glass bottles (Ca addition and no addition). One week of pre-incubation at 25 °C in the dark was then performed on sealed glass bottles. After 1, 3, 6, 10, 15, 22 and 31 days of incubation, the mineralization of CO_2 concentration was determined within 24h using gas chromatography (7890A; GC system, Agilent Technologies, CA, USA). Each experimental bottle was flushed with CO_2-free air (79 % N_2, 21 % O_2) prior to the measurement of mineralization, and deionized water was added in a timely manner to keep the soil moisture at a constant level. The SOC mineralization rate was calculated as the following equation:

$$F = \frac{CD \times V \times M \times 273.15}{22.4 \times (273.15 + T) \times W \times t \times SOC} \tag{1}$$

where F is the SOC mineralization rate (mg CO_2–C g^{-1} SOC day^{-1}); CD is the CO_2 concentration (ppm) measured by Agilent 7890 A gas chromatography; V is the volume of the incubation glass bottles (0.05 L); M is the molecular mass of C (12 g mol^{-1}); 22.4 (L) is the molar volume of an ideal gas at 1 atm and 273.15 K; T is the incubated temperature (25 °C); W is the gram dry weight of incubated soil (5 g); t is the duration of CO_2 accumulation (1 day); and SOC represents the SOC concentration (g kg^{-1}). Cumulative SOC mineralization is the total CO_2 released over 31 days.

2.7. Calculation of carbon input and SOC stock

The annual C input (Ct, kg ha^{-1} yr^{-1}) was estimated as the sum of aboveground residue C input and belowground root-derived C input. The aboveground residue inputs were calculated from the measured grain yield using a grain-to-residue ratio, while belowground residue inputs were estimated from the aboveground C input based on established root-to-shoot relationships (Eghball and Maranville, 1993). Specifically, aboveground residue C input (Cs) and belowground residue C input (Cr) using the following Equations:

$$Cs = GY \times Rgs \times Fc \tag{2}$$

$$Cr = GY \times Rrs \tag{3}$$

$$Ct = \frac{\sum_1^{22}(Cs + Cr)}{22} \tag{4}$$

where GY (kg ha^{-1}) was grain yield of winter wheat form 1999–2021, Rgs (0.846) and Rrs (0.20) is the grain-to-straw ratio and root-to-straw ratio, respectively (Zhao et al., 2018), Fc (0.4) is the carbon concentration coefficient for wheat biomass (Johnson et al., 2006).

To eliminate the effect of tillage treatments on soil bulk density, SOC stocks (Mg C ha^{-1}) were calculated using the equal mass method (Lee et al., 2009), and the soil weight of the CTN treatment was used as the control. The Δ SOC stock (Mg C ha^{-1}) and SOC sequestration rate (Mg C ha^{-1} yr^{-1}) at 0–20 cm soil depth was calculated described by (Gao et al., 2019) and expressed as follows:

$$H_{add} = \frac{BD_T - BD_{CTN}}{BD_T \times 10} \tag{5}$$

$$SOS_{CTN} = SOC_{CTN} \times BD_{CTN} \times S \times H \times 10 \tag{6}$$

$$SOS_T = SOC_T \times BD_T \times S \times (H + H_{add}) \times 10 \tag{7}$$

where the subscript T represents different indicators under RTN, NTS and STS treatments. SOS, BD and SOC were the SOC stock (Mg C ha^{-1}), soil bulk density (Mg m^{-3}), and carbon concentration (g kg^{-1}) under different treatments, H (m) was the depth of the analyzed soil layer, H_{add} (m) was the depth of the soil layer to be corrected, and S is the soil area (ha).

$$\Delta\ SOC\ stock = SOS_f - SOS_i \tag{8}$$

where the SOS_f and SOS_i indicated the SOC stocks in 2021 and 1999 under different tillage practices, respectively.

$$SOC_{SR} = \Delta\ SOC\ stock/y \tag{9}$$

where the SOC_{SR} and y was the SOC sequestration rate and the duration of this experiment (22 years).

2.8. Statistical analysis

Statistical analyses of indicators under different tillage practices and aggregate fractions were conducted using the general linear model (GLM) procedure in SPSS 22.0 (IBM, USA). Analysis of variance (ANOVA) was performed, and Tukey's honestly significant difference (HSD) test applied for all post hoc comparisons at the $p < 0.05$ level. A Linear Mixed-effects Models was used to analysis the relationships among SOC content, cumulative SOC mineralization, and Ca content (Exchangeable Ca and $CaCO_3$) in bulk soil and all aggregates. Additionally, redundancy analysis (RDA) using Origin 2021 was performed to measure the correlations between Ca contents, microbial community biomass, soil physical properties and SOC contents in bulk soil and aggregates. Furthermore, Random Forest Regression Models were also used to estimate the main biochemical predictors of changes in SOC sequestration and mineralization using the "rfPermute" package (R version 4.4.0). The model can identify all relevant variables and provide good estimates of their importance in determining the classification (Speiser et al., 2019). Finally, Partial least squares path modeling (PLS-PM) was performed to reflect the complex regulatory effects of main factors on SOC sequestration under conservation tillage practices using the "plspm" package (R version 4.4.0).

3. Result

3.1. Grain yield, SOC stocks and sequestration rate

22-year continuous straw mulching under subsoiling (STS) and no tillage (NTS) increased average wheat yields compared to reduced tillage and conventional tillage with straw removal (RTN and CTN), which resulted in higher C inputs from crop residues over 22 years ($p < 0.001$; Table 1). Due to the crop cultivation and soil erosion on sloping arable land in this loess soil, SOC stocks are lower than initial under CTN and RTN (Δ SOC stock). However, STS and NTS significantly reduced SOC loss and increased SOC sequestration rate compared to CTN and RTN ($p < 0.01$). In particular, the SOC contents and stock at 0–20 cm soil layer were increased by 24.2 %–29.6 % and 22.1 %–29.6 % respectively under STS and NTS in comparison to CTN and RTN.

3.2. Soil pH and physical properties

Different tillage managements affected soil properties (Table 2). The NTS treatment significantly decreased soil pH at 0–10 cm layer compared with CTN ($p < 0.05$). Moreover, soil depth and tillage exhibited significant interactive effects on soil water content ($p < 0.001$) and bulk density ($p < 0.05$). Soil water content was notably higher under STS and NTS than CTN at both soil depths ($p < 0.01$). In general, soil water content was higher in the 0–10 cm layer, whereas bulk density was lower compared with the 10–20 cm layer. In addition, NTS significantly reduced bulk density at 10–20 cm depth compared with CTN.

3.3. Aggregate-size distribution, aggregate-associated OC concentration and contribution

The mass contribution of small macroaggregates was the was the highest among all aggregate fractions at 0–20 cm depth (Table 3), accounting for 53.50 % under STS and 55.86 % under NTS, both significantly higher than under CTN and RTN (50.26–50.62 %). The large macroaggregates proportion under NTS, STS and RTN is significantly higher than CTN, with average increases of 22.1 %, 24.8 % and 15.3 % at two soil layers, respectively. Whereas the proportion of microaggregates was significantly reduced under NTS and STS compared to RTN and showed the highest value under CTN compared to the other three treatments ($p < 0.05$). Consequently, NTS and STS significantly increased MWD of aggregates by 7.4 %–25.0 % at two soil depths ($p < 0.001$, Table 2). These results showed that NTS and STS increased aggregate stability by promoting the formation of macroaggregates while decreasing microaggregates in the 0–20 cm soil layer.

The OC content was highest in small macroaggregates, and the contribution of aggregate OC to bulk soil decreased in all treatments in the order small macroaggregates > microaggregates > large macroaggregates (Table 3). Compared to CTN and RTN, both NTS and STS significantly increased the OC concentration in different aggregates in the 0–10 cm layer and only macroaggregates (LM and SM) in the 10–20 cm layer ($p < 0.001$; Table 3). Moreover, NTS and STS treatments significantly increased the OC amount in macroaggregates by 15.7–50.7 % compared to CTN and RTN across both depths. Although STS and NTS showed higher microaggregates-associated OC content compared to RTN, they reduced the OC contribution within microaggregates by 21.4–30.9 % compared to CTN at 10–20 cm layer.

3.4. Physical fractions of SOC in bulk soil and aggregates

Soil macroaggregates exhibited higher amount of particulate OC, whereas microaggregates contained the highest levels of mineral-associated OC across all treatments and depths ($p < 0.001$; Fig. 1). Across two soil depths, NTS and STS significantly increased fPOC and cPOC contents compared with CTN and RTN in bulk soil and all aggregate fractions ($p < 0.001$), except for microaggregates under CTN

Table 1
The final soil organic carbon (SOC) content, SOC stock, and the 22-year averages of wheat yield and SOC sequestration rate (SOC_{SR}) in the 0–20 cm soil layer under different tillage practices.

Treatment	Yield (kg ha^{-1})	C input (kg ha^{-1} yr^{-1})	SOC (g C kg^{-1})	SOC stock (Mg C ha^{-1})	Δ SOC stock (Mg C ha^{-1})	SOC_{SR} (Mg C ha^{-1} yr^{-1})
CTN	4764.3 b	329.3 b	6.25 b	15.15 b	−3.59 b	−0.163 b
RTN	4530.9 b	313.2 b	6.06 b	13.77 b	−4.98 b	−0.226 b
STS	5083.9 a	2108.4 a	7.78 a	18.54 a	0.31 a	0.014 a
NTS	4960.3 a	2057.1 a	8.01 a	19.45 a	0.71 a	0.032 a

Note: CTN, conventional tillage without straw return; RTN, reduced tillage without straw return; STS, subsoiling with straw return, NTS, no tillage with straw return. Different lowercase letters indicate significant differences among tillage managements within the same depth at $p < 0.05$ level.

Table 2
Soil pH, soil water content (SWC), mean weight diameter of aggregates (MWD) and bulk density (BD) and at the 0–20 cm soil depth under conventional tillage without straw return (CTN), reduced tillage without straw return (RTN), subsoiling with straw return (STS), no tillage with straw return (NTS).

Depth	Treatment	pH	SWC (%)	MWD	BD (Mg m^{-3})
0–10 cm	CTN	8.42 a	8.71 b	1.14 c	1.09 a
	RTN	8.35 a	8.91 ab	1.28 b	1.09 a
	STS	8.26 ab	9.62 a	1.52 a	1.10 a
	NTS	8.11 b	10.08 a	1.49 a	1.15 a
10–20 cm	CTN	8.34 a	8.02 b	1.52 b	1.23 a
	RTN	8.39 a	7.95 b	1.61 b	1.14 ab
	STS	8.48 a	9.43 a	1.74 a	1.17 ab
	NTS	8.52 a	8.86 a	1.79 a	1.09 b
p-value					
Tillage		0.019	<0.001	<0.001	0.009
Depth		0.063	<0.001	<0.001	0.021
Tillage*Depth		0.124	<0.001	0.108	0.043

Note: Different lowercase letters indicate significant differences among tillage managements within the same depth at $p < 0.05$ level.

at 10–20 cm, where POC remained unchanged (Fig. 1h). For MAOC, STS and NTS enhanced the values by 16.3 %–19.8 % in bulk soils and small macroaggregates, while only NTS significantly increased the values in the large macroaggregates and microaggregates compared with RTN and CTN at the 0–10 cm depth. At 10–20 cm depth, MAOC increased only under NTS in bulk soil and macroaggregates ($p < 0.05$), with no significant differences observed in microaggregates.

3.5. $CaCO_3$ and exchangeable Ca content in bulk soil and aggregates

Long-term tillage management significantly influenced the $CaCO_3$ and exchangeable Ca concentration (Fig. 2). Both $CaCO_3$ and exchangeable Ca increased in the order of microaggregates < large macroaggregates < small macroaggregates. The exchangeable Ca was higher in the 0–10 cm topsoil, whereas $CaCO_3$ was higher in the 10–20 cm subsoil. Compared to CTN and RTN, STS and NTS significantly increased the concentrations of exchangeable Ca ($p < 0.001$; Fig. 2c and d), while simultaneously decreasing $CaCO_3$ by an average of 10.5 % and 11.2 % in bulk soil and two macroaggregate classes, respectively, across two soil layers. Notably, NTS always showed the highest concentrations of exchangeable Ca than other three treatments within bulk soil and all aggregates at 0–20 cm ($p < 0.05$). Within the microaggregates, only CTN at 0–10 cm soil depth significantly reduced exchangeable Ca content compared to STS and NTS ($p < 0.05$; Fig. 2 c), and RTN at 0–20 cm depth had the highest $CaCO_3$ content.

3.6. The mineralization of SOC in bulk soil and aggregates

SOC cumulative mineralization was significantly influenced by the tillage managements ($p < 0.001$), aggregates ($p < 0.001$) and tillage × aggregates interactions ($p < 0.05$, under Ca addition) at 0–10 cm and 10–20 cm soil layer (Fig. 3). Within three aggregate sizes, the commutative mineralization decreased in the order of large macroaggregates < small macroaggregates < microaggregates in all treatments. At 0–10 cm depths, NTS decreased SOC cumulative mineralization by 14.3 %, 22.3 %, 33.2 % and 22.4 %, 27.5 %, 31.3 % compared to STS, RTN and CTN, respectively, in bulk soil and two size of macroaggregates (Fig. 3 a). However, both CTN and RTN significantly increased the SOC cumulative mineralization compared to STS and NTS in bulk soil, macroaggregates and microaggregates at two soil depths ($p < 0.001$; Fig. 3a and b).

Besides, Ca addition significantly decreased the SOC cumulative mineralization in all tillage managements after incubation, which showed a similar statistical result among four tillage managements between Ca addition and no addition ($P_T < 0.001$, Fig. 3c and d). When calculate the magnitude of the decrease, we found the reduction amount of SOC cumulative mineralization under STS and NTS (49.6 % and 50.2 %) was significantly higher than that under RTN and CTN (45.6 % and

Table 3
Aggregate size proportion and associated organic carbon (OC) content and OC contribution to bulk soil at 0–20 cm soil depth under different tillage managements.

Aggregate	Treatment	0–10 cm			10–20 cm		
		Percentage (%)	OC content (g kg^{-1} aggregate)	OC contribution (g kg^{-1} soil)	Percentage (%)	OC content (g kg^{-1} aggregate)	OC contribution (g kg^{-1} soil)
LM	CTN	11.63 Cc	6.07 Bb	0.71 Cb	18.08 Cc	6.15 Bb	1.11 Cb
	RTN	14.42 Cb	5.85 Bb	0.84 Cb	20.35 Cb	6.12 Bb	1.18 Cb
	STS	17.38 Ca	8.27 Ba	1.44 Ca	21.66 Bab	6.73 Ba	1.45 Ca
	NTS	15.71 Ca	8.94 Ba	1.40 Ca	22.09 Ba	7.14 Ba	1.58 Ca
SM	CTN	48.75 Ab	7.21 Ab	3.51 Ab	51.76 Ac	6.88 Ab	3.56 Ab
	RTN	49.12 Ab	6.81 Ab	3.35 Ab	52.11 Ac	7.03 Ab	3.80 Ab
	STS	51.83 Aa	9.22 Aa	4.78 Aa	55.16 Ab	8.09 Aa	4.54 Aa
	NTS	52.71 Aa	9.14 Aa	4.82 Aa	59.00 Aa	7.81 Aa	4.61 Aa
MIC	CTN	39.62 Ba	5.68 Bb	2.25 Ba	30.16 Ba	5.58 Bab	1.68 Ba
	RTN	36.46 Bb	6.75 Bb	2.46 Ba	26.54 Bb	5.11 Bb	1.37 Bab
	STS	30.79 Bc	8.22 Ba	2.53 Ba	22.18 Bc	5.95 Ba	1.32 Bb
	NTS	31.58 Bc	8.53 Ba	2.69 Ba	18.91 Bc	6.12 Ba	1.16 Bb

Note: CTN, conventional tillage without straw return; RTN, reduced tillage without straw return; STS, subsoiling with straw return, NTS, no tillage with straw return. BS, bulk soil; LM and SM, large and small macroaggregates; MIC, microaggregates. Abbreviations same as below. Different uppercase letters mean significant difference at $p < 0.05$ level among three aggregate size classes, and lowercase letters mean significant difference at $p < 0.05$ level among four tillage managements within the same aggregates and soil depth.

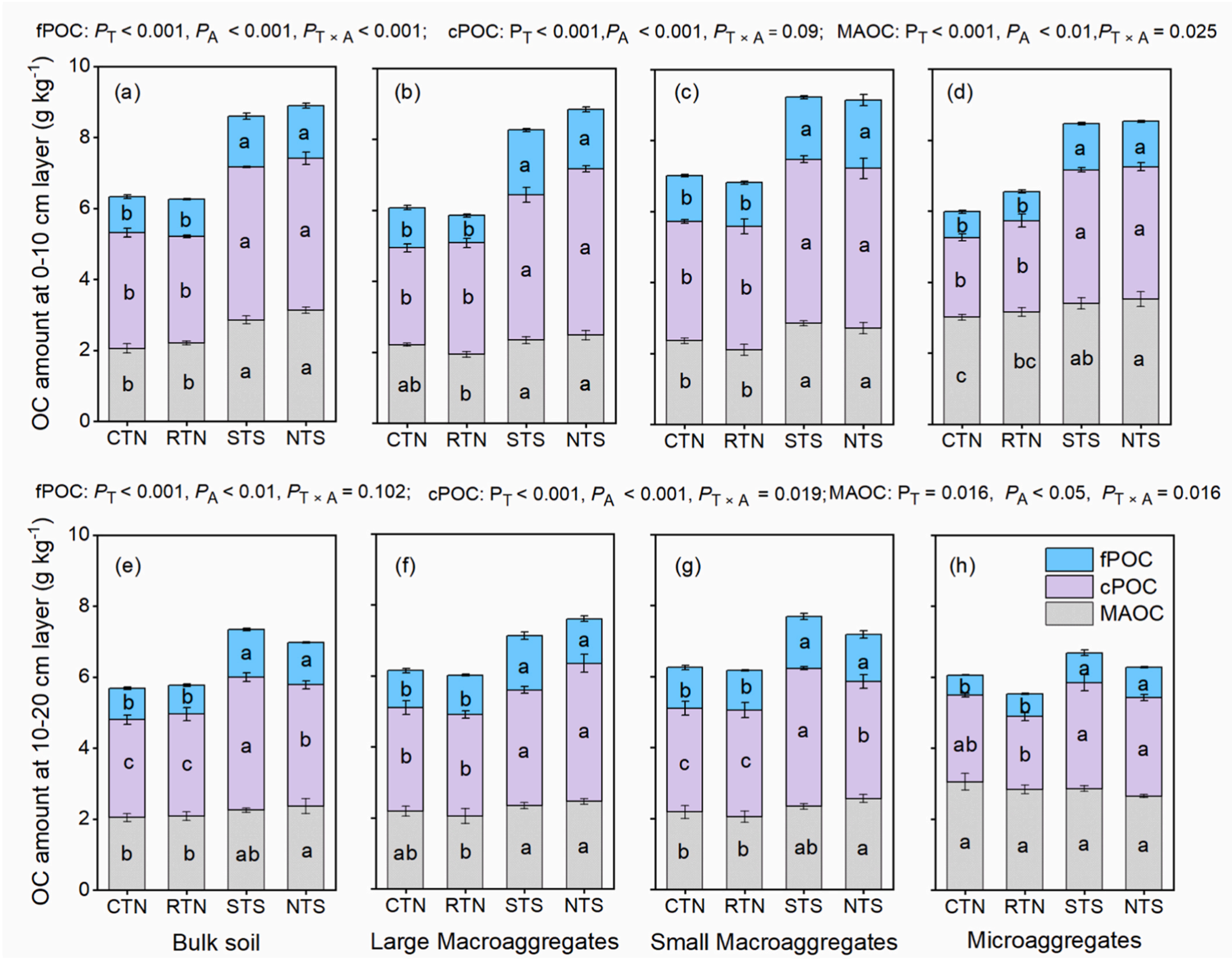

Fig. 1. The mass contribution of organic carbon (OC) in the density fractions under bulk soil and aggregates at 0–20 cm soil depth. Error bars and data and are means ± standard deviation (SD, n = 3). fPOC: free particulate OC; cPOC: coarse particulate OC; MAOC: mineral-associated OC. The T, A, and T × A are tillage managements, aggregate classes, and their interaction, respectively. Different lowercase letters indicate significant differences of four tillage managements within the same OC fractions, soil layers and aggregate classes at $p < 0.05$ level.

41.8 %) in bulk soils at 0–20 cm (Table S5). In the macroaggregates, NTS and STS reduced the SOC cumulative mineralization by 49.1 % and 48.9 %, respectively, which were both higher than in RTN and CTN (Table S5). While in microaggregates, the reduction did not differ significantly among four tillage managements.

3.7. Microbial community biomass

The microbial biomass showed similar results between two soil layers. In all treatments, the microbial biomass carbon (MBC) content was consistently higher in macroaggregates than in microaggregates (Fig. 4). Notably, compared to RTN and CTN, the MBC content under NTS and STS was significantly enhanced by 19.8–29.0 % in bulk soil and different aggregates within the 0–20 cm soil depth. In addition, NTS and STS showed significantly higher ($p < 0.001$) biomass of gram negative, gram positive, anaerobe, actinomycetes, AMF, SF, eukaryote, and fungi, and total microbial PLFAs (Fig. 5, Table S4). The ratios of different functional microbial groups were used to reveal carbon availability. Results showed that NTS and STS enhanced the AMF/SF by 22.5–46.2 % and 16.7–29.8 %, respectively, while reducing the Gram+/Gram-by 27.9–40.6 % and 25.1–28.6 % compared to CTN and RTN at two depths. The fungi/bacteria ratio was significantly higher ($p < 0.05$) under NTS and STS than CTN at 0–10 cm, whereas no significant differences were observed among four treatments at 10–20 cm.

3.8. Correlation of SOC contents, cumulative SOC mineralization and Ca contents

Both $CaCO_3$ and exchangeable Ca contents showed negative correlations with the cumulative SOC mineralization ($p < 0.001$). However, only exchangeable Ca positively correlated with SOC content ($p < 0.001$) when considering bulk soils and all aggregate fractions (Fig. 6 A and C). Furthermore, cumulative SOC mineralization was positively correlated with the fPOC to (cPOC + MAOC) ratio ($p < 0.001$) but negatively correlated with the POC ($p < 0.01$) and MAOC contents ($p < 0.001$) across bulk soils and all aggregate fractions (Fig. 6 B). Exchangeable Ca content was positively correlated with the cPOC ($p < 0.001$) and MAOC contents ($p < 0.05$) but showed no significant relationship with the fPOC to (cPOC + MAOC) ratio in bulk soils and all aggregate fractions (Fig. 6 D).

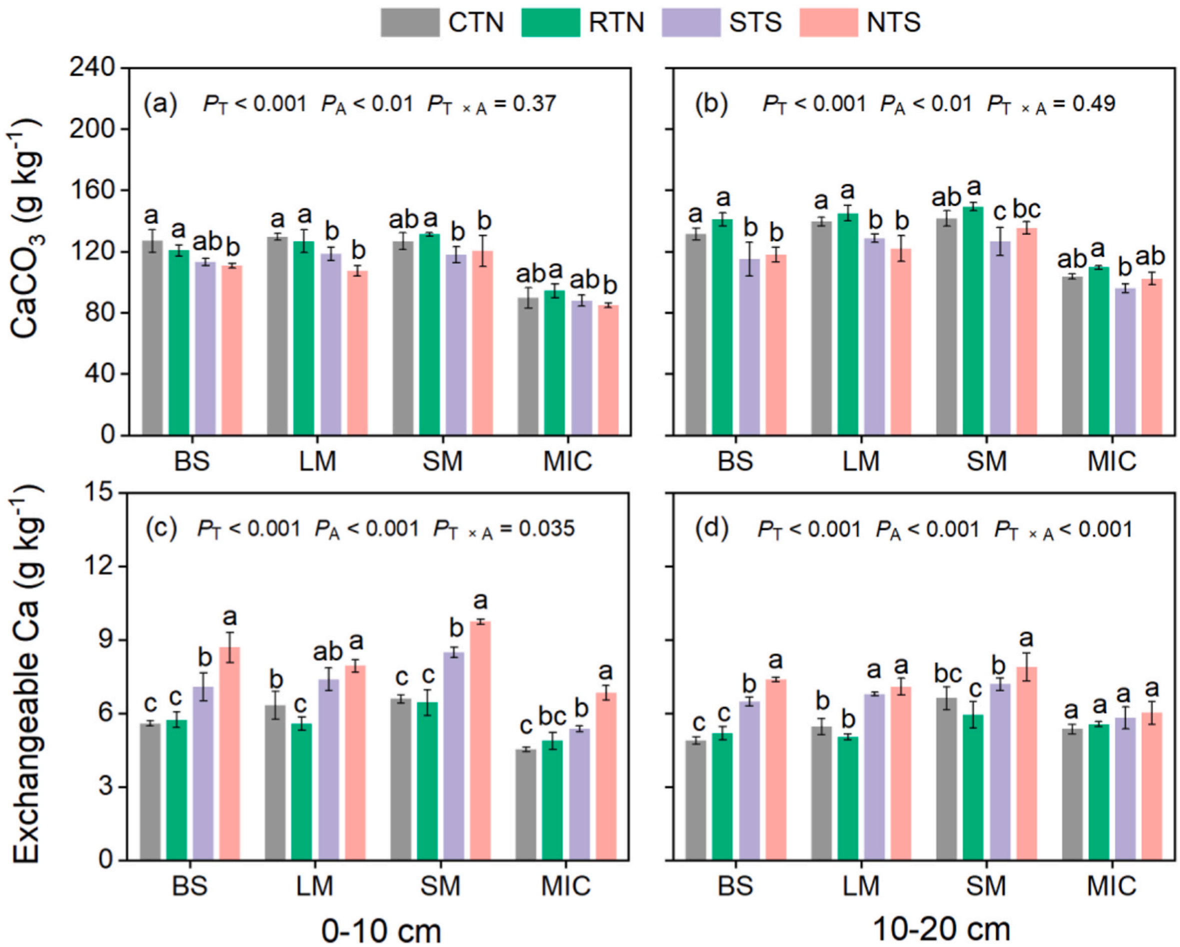

Fig. 2. The concentrations of calcium carbonate ($CaCO_3$) (a, b) and exchangeable calcium (Ca) (c, d) in bulk soil and aggregates at 0–20 cm soil depth. Error bars and data means ± SD (n = 3). The T, A, and T × A are tillage managements, aggregate classed, and their interaction. Different lowercase letters indicate significant differences of four tillage managements within same aggregate classes at $p < 0.05$ level.

3.9. Identification of chemical and biological factors regulating SOC sequestration and mineralization

The analysis showed that the explanation rate of soil properties and microbial communities explains 71.83 % of the variation in SOC content and 70.14 % of the variation in SOC cumulative mineralization (CMR), both in bulk soil and in different aggregates (Fig. 7a and b). MWD, microbial biomass, F/B and AMF/SF ratios positively correlated with SOC content but negatively with CMR (Fig. 7a and b). In contrast, bulk density and Gram+/Gram-ratio showed positive correlations with CMR and negative correlations with SOC content. Furthermore, exchangeable Ca content was significantly positively related to SOC sequestration and negative related to CMR in different aggregates ($p < 0.001$; Supplementary Fig. 1). Random forest analysis identified the main soil physicochemical factors influencing SOC stabilization (Fig. 7c and d), explaining 48.2 % of the variation in SOC sequestration and 65.6 % in cumulative mineralization, respectively. Specifically, exchangeable Ca, pH, bacteria, total microbial biomass, Gram- and Gram+/Gram-were significant predictors of SOC accumulation ($p < 0.01$; Fig. 7 c), while MWD, actinomycetes biomass, AMF, AMF/SF and exchangeable Ca significantly influenced SOC decomposition ($p < 0.001$; Fig. 7 d). Exchangeable Ca was the only common predictor significantly associated with SOC stabilization and mineralization, although its relative importance differed between the two processes.

PLS-PM was constructed to investigate the cascading effects of soil physio-chemical properties and microbial communities on SOC sequestration (Fig. 8). Long-term conservation tillage significantly affected Ca content and microbial composition by increasing MWD and reducing bulk density ($p < 0.05$). Furthermore, exchangeable Ca and $CaCO_3$ contents were positively related to AMF/SF and Gram-/Gram + ratios but negatively correlated to SOC mineralization ($p < 0.05$). Overall, Ca and microbial biomass significantly contributed to the final SOC sequestration by suppressing SOC mineralization ($p < 0.01$), suggesting that long-term conservation tillage regulates Ca–microbe interactions to promote SOC stabilization (see Fig. 9).

4. Discussion

4.1. Effect of long-term conservation tillage on SOC sequestration

The 22-year practices of NTS and STS enhanced the SOC stock and SOC sequestration rate ($p < 0.01$, Table 1) compared to CTN, indicating that long-term conservation tillage exerted positive feedback in reducing C loss in the Loess Plateau of China. Six et al. (2004) reported that intensive tillage induces the breakdown of macroaggregates, thereby exposing previously protected plant and microbial residues. In contrast, no tillage reduces aggregate turnover and selectively retains more chemically recalcitrant organic matter within occluded macroaggregates (Plaza et al., 2013). In this study, a synchronous response of large and small macroaggregates to tillage was consistent with their associated SOC storage (Fig. 7 A). The retained organic matter can also act as a cementing agent to help form large aggregates (Kan et al., 2020). This was reflected in the increased macroaggregate formation and MWD values under NTS and STS (Table 2). Furthermore, the positive effects of

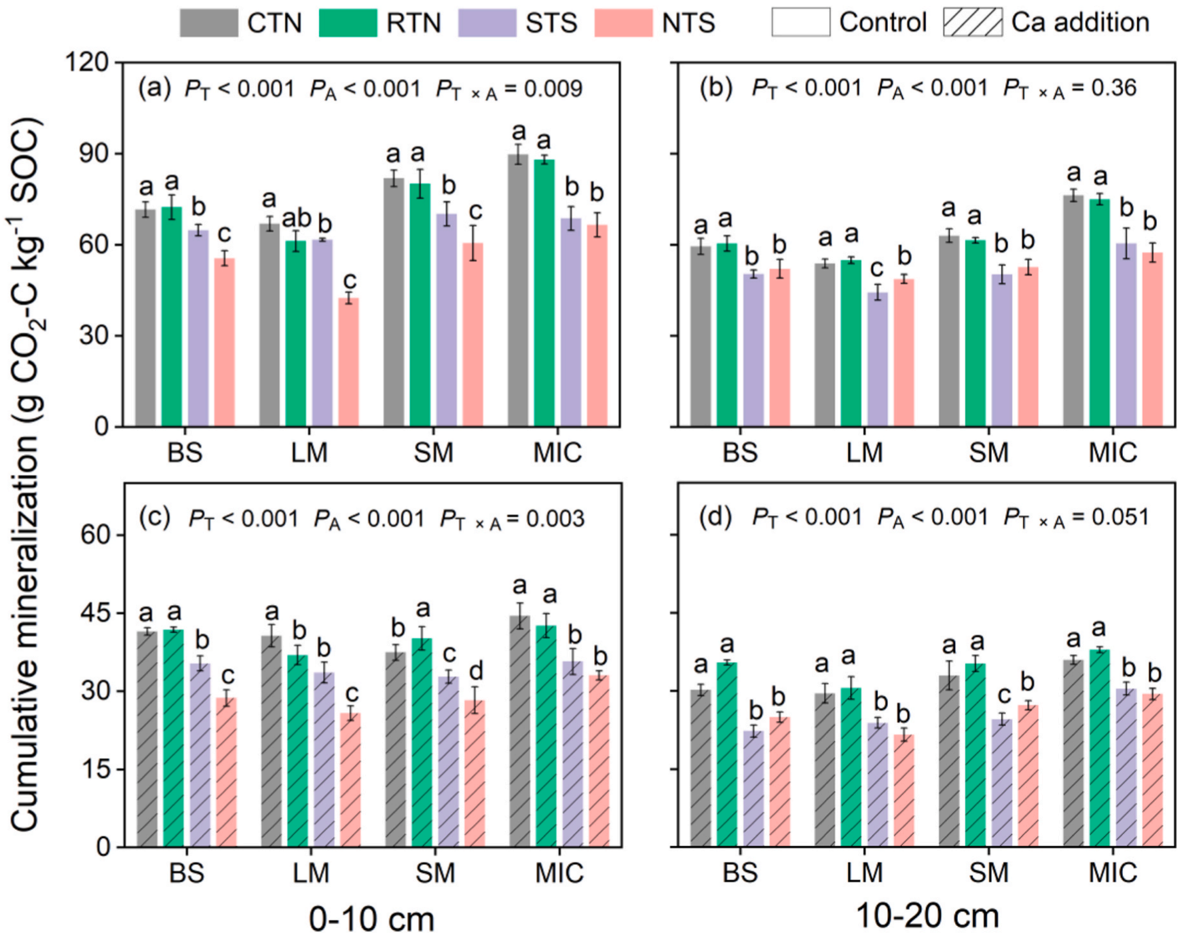

Fig. 3. Soil organic carbon cumulative mineralization under Ca addition (c, d) and no addition (a, b) in bulk soil and aggregates at 0–20 cm soil depth. Error bars and data means ± SD (n = 3). The T, A, and T × A are tillage managements, aggregate classed, and their interaction, respectively. Different lowercase letters indicate significant differences of four tillage managements within same aggregate classes at $p < 0.05$ level.

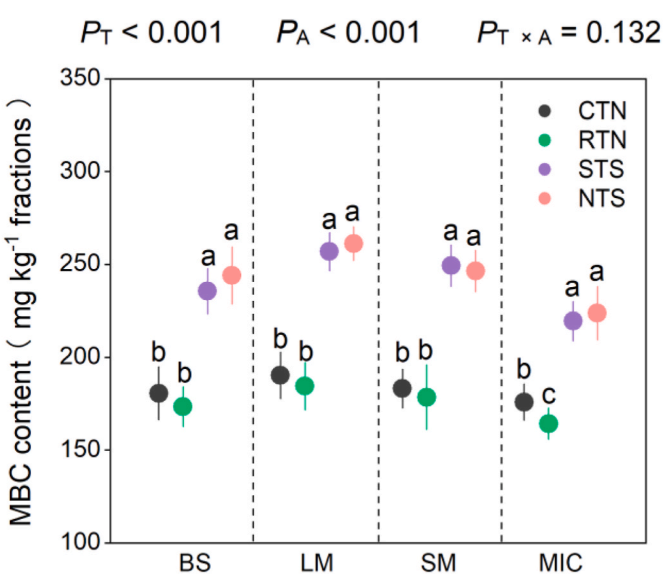

Fig. 4. Soil microbial biomass carbon content in bulk soil and soil aggregates at 0–20 cm soil depth. Error bars and data means ± SD (n = 3). The T, A, and T × A are tillage managements, aggregate classed, and their interaction, respectively. Different lowercase letters indicate significant differences of four tillage managements within same aggregate classes at $p < 0.05$ level.

NTS and STS on macro-aggregation were not limited to the 0–10 cm topsoil but also extended to the 10–20 cm subsoil, due to long term legacy impacts (Table 3, see also Liu et al., 2022). Continuous intensive tillage causes the water loss and poor connectivity in the subsoil through mechanical destruction, thereby degrading its physical structure (Shepherd et al., 2001). In this study, NTS and STS resulted in higher soil moisture and lower bulk density ($p < 0.05$; Table 2). Improved soil pore structure under conservation tillage helps retain nutrients essential for C cycling. Taken together, conservation tillage practices promote aggregate stability and soil structural integrity, which in turn physically protect SOC from microbial decomposition.

The results showed that exchangeable Ca was strongly positively correlated with total SOC, POC and MAOC content ($p < 0.001$; Fig. 6C and D). Chemical binding between soil mineral particles and organic material plays crucial roles in SOC stabilization (Witzgall et al., 2021). In this Calcaric Cambisols, NTS and STS reduced the $CaCO_3$ and increased the exchangeable Ca contents compared to CTN ($p < 0.05$; Fig. 4). This may result from the dissolution and transformation of the $CaCO_3$ into exchangeable Ca facilitated by soil water movement (Huang et al., 2019). Ca^{2+} released through hydrolysis of $CaCO_3$ can adsorb onto organic matter, contributing to SOC stabilization (Adhikari et al., 2019). The higher water content under NTS and STS ($p < 0.01$; Table 2) may be responsible for this transformation and the higher MAOC accumulation ($p < 0.05$; Fig. 1). In addition, exchangeable Ca contributes to SOC sequestration by forming organo-mineral complexes in both the inner and outer spheres (Rowley et al., 2018). Ca–organo associations, driven by ligand exchange, direct cation bridging, hydrogen bonding and van

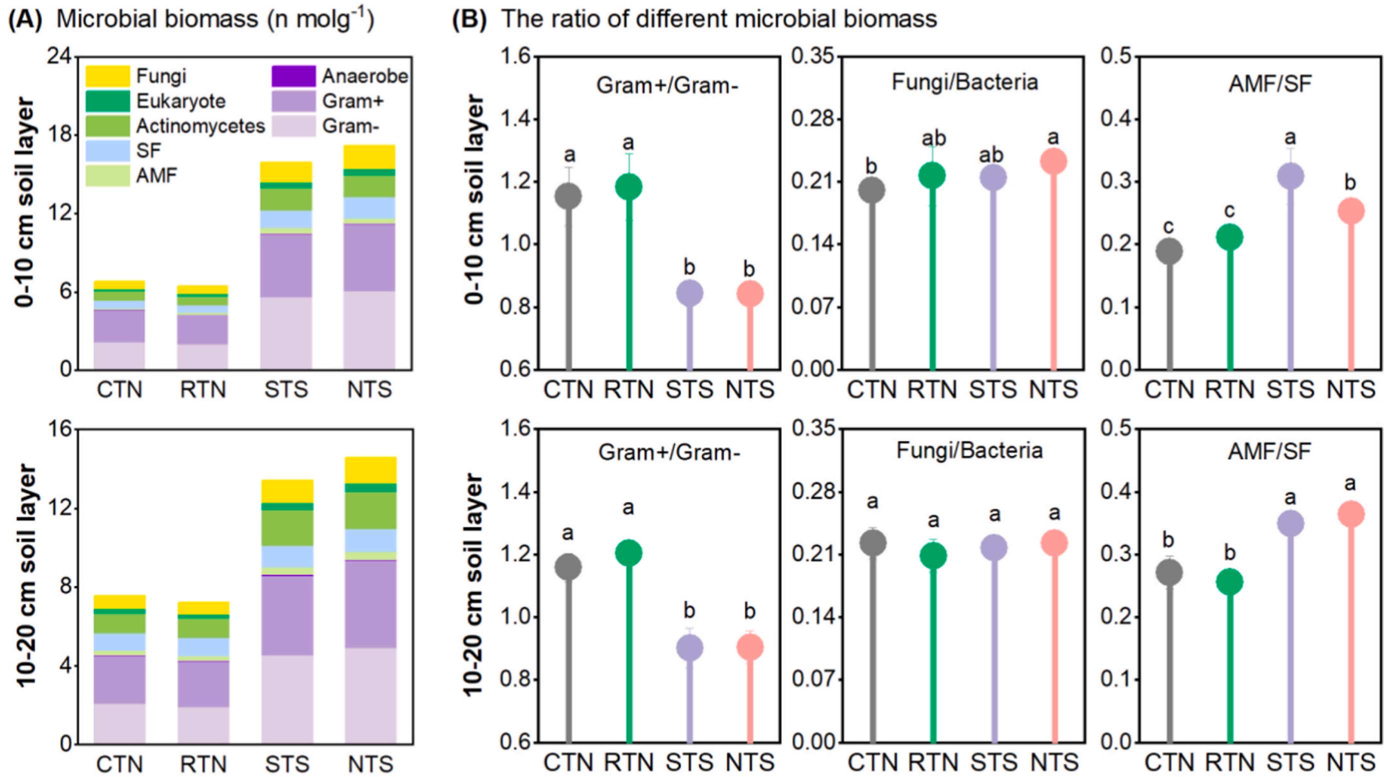

Fig. 5. Microbial biomass contents of Gram-, Gram+, anaerobe, actinomycetes, AMF, Eukaryote and Fungi, SF, and the ratio of different microbial under four tillage practices at 0–20 cm soil depth. Gram+: gram-positive bacteria; Gram-: gram-negative bacteria; AMF: arbuscular mycorrhizal fungi; SF: saprotrophic fungi; AMF/SF: the ratio of arbuscular mycorrhizal fungi and saprotrophic fungi. Different lowercase letters indicate significant differences among management practices at 0–20 cm, respectively, according to one-way ANOVA ($p < 0.05$). Bars indicate mean values ± standard error (n = 3).

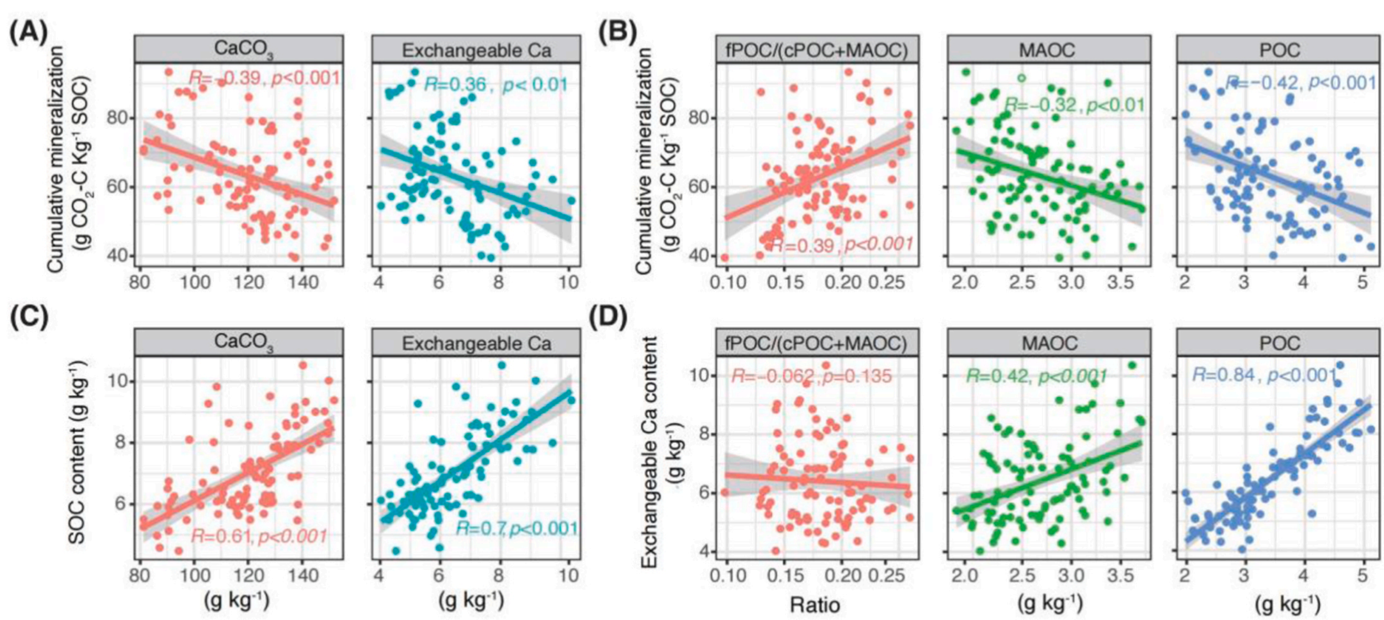

Fig. 6. The correlation of different indicators in bulk soils and all aggregate fractions. The relationship among cumulative soil organic carbon (OC) mineralization with $CaCO_3$ contents and exchangeable Ca contents (A); among cumulative SOC mineralization with fPOC/(cPOC + MAOC) ratio, POC and MAOC contents (B); among SOC contents with $CaCO_3$ contents and exchangeable Ca contents (C); among exchangeable Ca contents with fPOC/(cPOC + MAOC) ratio, cPOC and MAOC contents (D). fPOC: free particulate OC; cPOC: coarse particulate OC; MAOC: mineral-associated OC. Asterisks indicates statistical significance: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, n = 96.

der Waals forces, can selectively adsorb some carboxyl functional groups or strongly chelate microbial residues (Rowley et al., 2021). In this long-term NTS and STS system, microbial biomass carbon was significantly higher in bulk soil and macroaggregates (Fig. 4). Small molecule degradation products generated during active microbial metabolism may be selectively adsorbed by Ca^{2+} (Feng et al., 2016). On

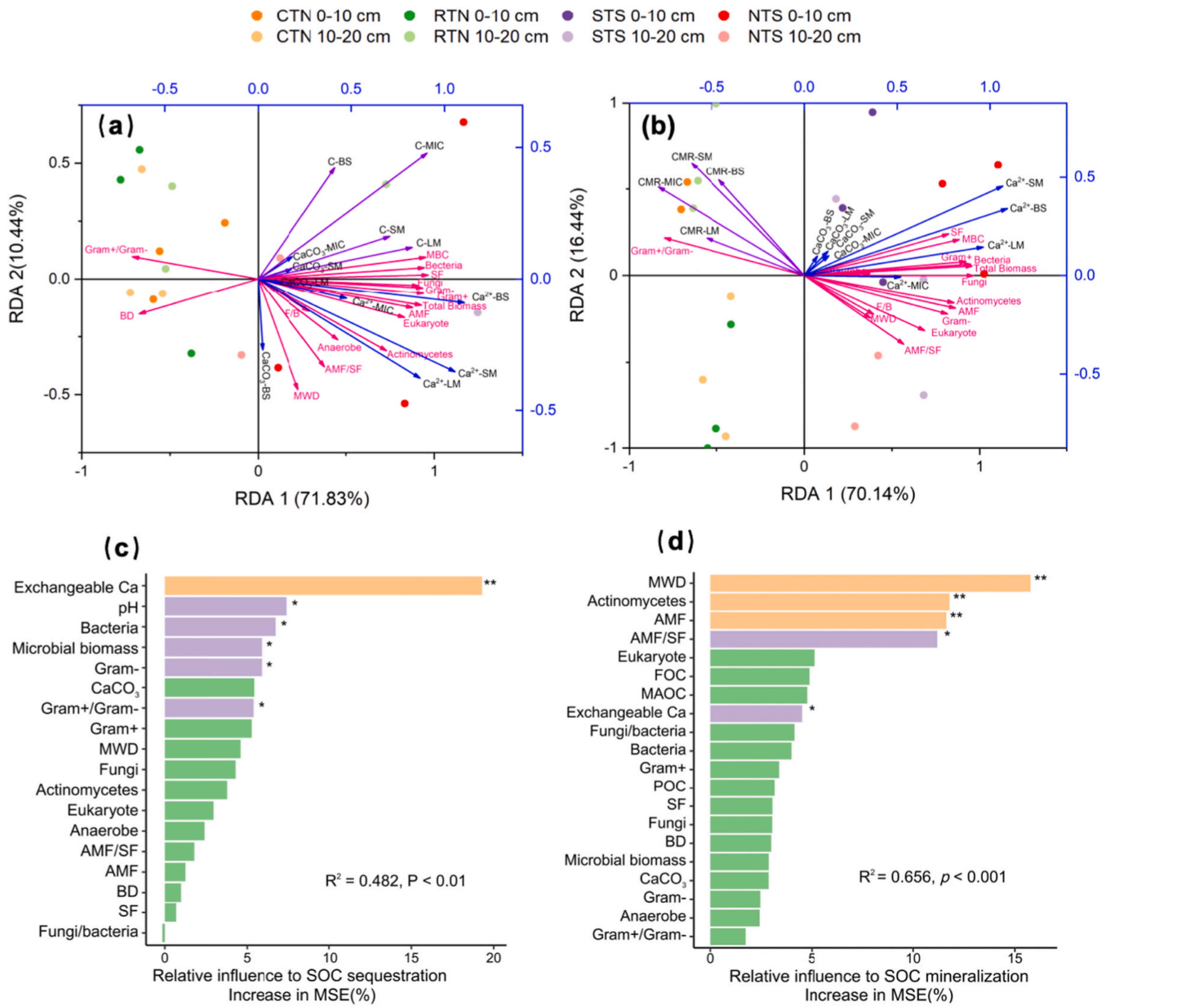

Fig. 7. Redundancy analysis (RDA) for the relative contribution of soil physio-chemical properties and microbial communities to SOC sequestration (a) and cumulative mineralization (b). The pink, blue and purple arrows indicate microbial PLFAs groups, soil properties, and SOC content in bulk soil and aggregates, respectively. The random forest analysis to predict the key factors of various soil physio-chemical and microbial characteristics that influence SOC sequestration (c) and cumulative mineralization (d) (*$p < 0.05$, **$p < 0.01$). BS: bulk soil; LM and SM: large and small macroaggregates; MIC: microaggregates; FOC: light fraction OC; POC: particulate OC; MAOC: mineral-associated OC; MWD: mean weight diameter; BD: bulk density; Ca^{2+}: exchangeable calcium (Ca); Gram+: gram-positive bacteria; Gram-: gram-negative bacteria; AMF: arbuscular mycorrhizal fungi; SF: saprotrophic fungi; AMF/SF: the ratio of arbuscular mycorrhizal fungi and saprotrophic fungi.

the other side, the chemical sorption of SOC by reactive minerals could be encapsulated within macroaggregates and further physically protected SOC (Zhu et al., 2022). This can be proved by the positive relationship between MWD, MAOC, Ca contents and microbial biomass (Supplementary Fig. 1). Moreover, the rich adhesive Ca-mucilage matrices act as a binding agent for soil particles, enhancing aggregate stability and SOC occlusion (Wuddivira and Camps-Roach, 2007). Based on the results, we can reasonably infer that long-term SOC persistence under conservation tillage is driven by the synergistic effects of chemical organo-Ca associations and physical soil aggregation.

In general, the exchangeable Ca and microbial biomass are major contributors to total SOC sequestration (Fig. 8). The positive effects of straw mulching under STS and NTS cannot be ignored. The addition of plant litter not only introduces multiple mineral elements, but also provides a rich nutrient source for microorganisms (Peng et al., 2016). This process subsequently decomposes organic molecules into soil particles to form large aggregates (Kasper et al., 2009). In this work, both NTS and STS have similarly higher MWD and SOC storage than CTN and RTN, largely due to the difference in straw management. More importantly, straw mulching protects the surface soil from frequent freezing and thawing, maintaining a relatively stable physio-chemical environment (Zhou et al., 2022). The higher total soil porosity (Table S3) and soil water content under NTS and STS at 0–20 cm is conducive to nutrient recycling and replenishment to the subsoil (Table 2). Improved nutrient availability also enhances aggregate physical structure and counteracts the subsoil compaction that may occur under no-till (Sarker et al., 2018). Although Arvidsson et al. (2014) reported that straw mulching and no tillage would result in inability of OC to replenish the subsoil and yield loss, our results proved equal effective improvement in crop yields between NTS and STS compared to CTN (Table 1). One reason may be the long-term infiltration and efficient transformation of straw litter in the soil profile (Kan et al., 2020). Another may be the relatively high potential for SOC sequestration in the low-carbon soils, as they are still a long way from nutrient saturation (Wang et al., 2020). Therefore, 22 years legacy of straw input offsets deficiency of bottom nutrients under NTS when compared to STS. Overall, long-term organic inputs and efficient straw conversion under conservation tillage improved SOC sequestration.

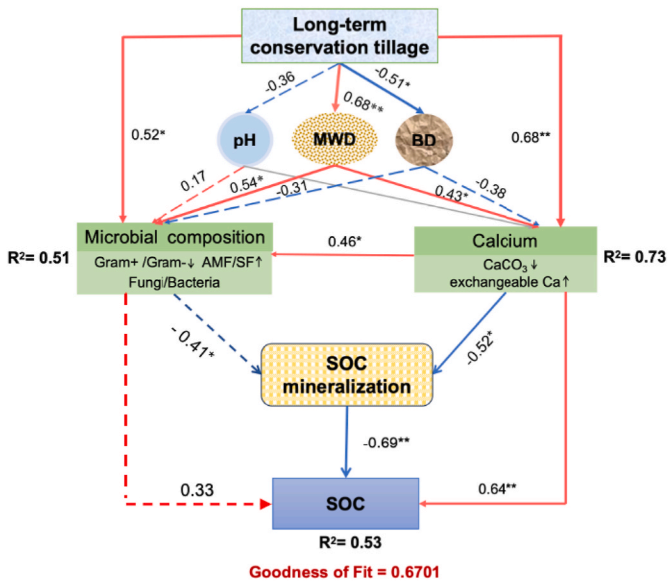

Fig. 8. The influence of long-term conservation tillage on soil properties, calcium contents, microbial characteristics and SOC sequestration using the partial least squares path model (PLS-PM) analysis. The widths of the arrows represent the path coefficients, where red and blue indicate statistically positive and negative influences (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$), respectively. Grey dashed arrows denote non-significant relationships between variables. R^2: the explained variability. MWD: mean weight diameter; BD: bulk density; Ca: calcium; AMF: arbuscular mycorrhizal fungi; SF: saprotrophic fungi; AMF/SF: the ratio of arbuscular mycorrhizal fungi and saprotrophic fungi; F/B: the ratio of fungi and bacteria.

4.2. Aggregate-associated SOC mineralization mediated by Ca and microbes

Cumulative SOC mineralization was lower ($p < 0.05$; Fig. 3) in large and small macroaggregates (>250 mm) than in microaggregates (<250 mm). Consistently, a short soil incubation soil incubation test demonstrated that SOC mineralization is accelerated when large macroaggregates are broken down into microaggregates (Li et al., 2023). This was partly attributed to the physical occlusion of the macroaggregates, which limits the microorganisms and enzymes access to the substrate (Liu et al., 2022). We also observed a higher C/N ratio and a lower DOC/SOC ratio within macroaggregates than in microaggregates (Supplementary Fig. 2). A higher C/N may inhibit microbial growth and enzyme activity due to limited nitrogen availability (Witzgall et al., 2021), while a lower DOC percentage represents organic matter with more complex molecular structures which is difficult for decomposers to utilize (Li et al., 2022). In this study, macroaggregates accounted for 74.02–74.82 % under NTS and STS, significantly higher than CTN and RTN ($p < 0.001$; Table 3). The encapsulation of colloidal organic matter on the mineral surface in microaggregates within occluded macroaggregates increases the tortuosity of the pore network, which plays an important role in limiting microbial entry and attack (Zhang et al., 2023). In contrast, the free microaggregates were no longer protected by undisturbed soil, and free silt and clay fractions tended to adsorb labile carbohydrate and polysaccharide compounds that are highly susceptible to decomposers (Schweizer et al., 2019). Thus, conservation tillage (NTS and STS) reduced the total SOC decomposition due to a reduced mineralization contribution from macroaggregates.

Cumulative SOC mineralization exhibited a significant negative correlation with the soil $CaCO_3$ and exchangeable Ca contents when considering bulk soil and all aggregate fractions ($p < 0.01$; Fig. 6A), suggesting that $CaCO_3$ and Ca^{2+} likely contribute to the stabilization of aggregate-associated OC in this calcareous soil. Consistently, $CaCO_3$ and exchangeable Ca contents were higher in macroaggregates than in microaggregates ($p < 0.05$; Fig. 2). These findings align with those of Rowley et al. (2021), who reported that calcium fertilization enhances aggregate stability and suppresses SOC mineralization, supporting incubation results (Fig. 3C and D). At the microscopic level, however, the availability of the mineral to bind plant or microbial metabolites plays an important role in the persistence of the SOC (Plaza et al., 2013). A positive correlation was observed between fPOC to (cPOC and MAOC) ratio and cumulative SOC mineralization ($p < 0.001$; Fig. 6 B), while MAOC and cPOC showed significant positive relationships with exchangeable Ca^{2+} contents ($p < 0.001$; Fig. 6 D). In the latest study, Shabtai et al. (2023) suggested that it is the association between Ca^{2+} and microbial byproducts reduced respiration rather than physical protection by litter occlusion.

Ca may also induce shifts in microbial community structure by favoring surface-colonizing bacterial taxa (Dou et al., 2023). Oligotrophic species, characterized by slower growth rates and higher substrate use efficiency, often dominate undisturbed soils (Hu et al., 2023). We found a higher MBC in macroaggregates compared to microaggregates ($p <$

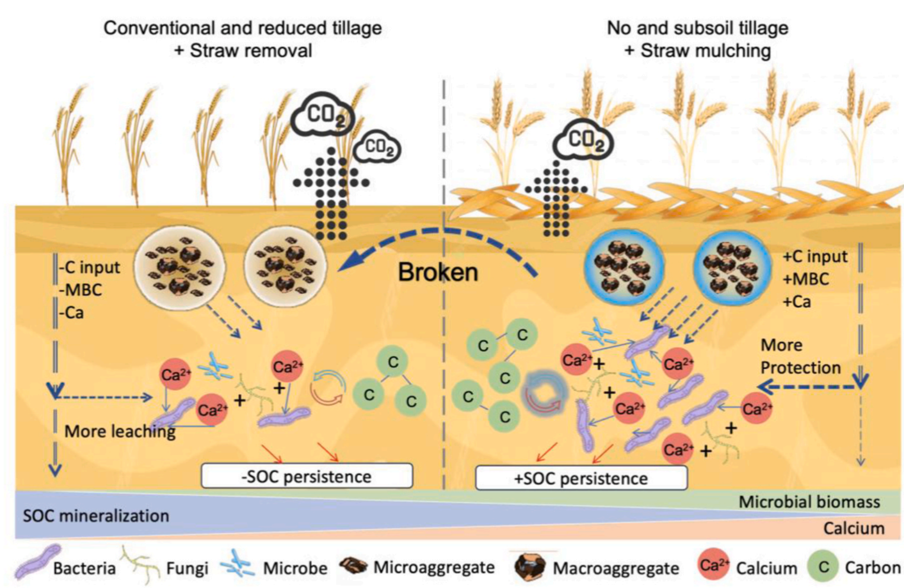

Fig. 9. Conceptual map illustrating the effects of different tillage practices on Ca-mediated SOC stabilization in Calcaric Cambisols of the Chinese Loess Plateau.

0.001; Fig. 4). SOC mineralization in both bulk soil and aggregate fractions was positively correlated with the AMF/SF ratio and negatively correlated with the Gram+/Gram-ratio ($p < 0.05$; Supplementary Fig. S1). According to the Fanin et al. (2019), the Gram+/Gram-ratio serves as an effective indicator of carbon source availability, with Gram^{+} bacteria preferentially utilizing older, more stable carbon, and Gram-bacteria favoring labile substrates (Zheng et al., 2023a). SF groups, typically copiotrophic, contribute to rapid carbon turnover, while a higher AMF/SF ratio and lower SF abundance promote the stabilization of recalcitrant organic matter (Hu et al., 2023). In this study, both NTS and STS reduced SOC mineralization while increasing AMF and SF biomass compared to CTN (Fig. 3; Fig. 4). Overall, the reduced mineralization under NTS and STS can be attributed to Ca-mediated organo-mineral interactions, reduced microbial respiration in the altered surface-attached populations, and increased stabilization of microbial metabolites.

4.3. Microbial community composition contributes to the SOC formation

NTS and STS increased both microbial biomass and SOC sequestration, with the biomass of Gram-, Gram+, Anaerobe, Actinomycetes, AMF, SF, Eukaryote, and Fungi showing a positive relationship with SOC accrual ($p < 0.01$; Supplementary Fig. 1). Degradation and conversion to straw by more abundant microorganisms results in the accumulation of stable or labile C metabolites (Feng et al., 2016). However, bacterial biomass, rather than fungal biomass, emerged as a significant predictor of SOC content, particularly the Gram-biomass ($p < 0.05$; Fig. 7 c). This is because fungi are more sensitive to tillage in croplands, which may disrupt mycelium and contribute to bacteria occupy ecological niches (Wang et al., 2018). Compared to fungi, bacteria were reported to be more favorable to proliferation when high Ca was added (Mehra et al., 2019). Consistent with our study sites, in arid-calcic soils with low organic matter, microbes may preferentially obtain energy through chemosynthetic processes or alternate metabolic pathways that favor carbonate precipitation (Ball et al., 2023). This implies that active microbial growth under reduced tillage and straw return provides abundant substrates for carbonate precipitation or cation bridging, supporting microbial-mineral interactions that stabilize SOC.

The Gram-, Gram+ and Actinomycetes were three dominant bacterial groups, together accounting for more than 70 % of the total biomass (Fig. 5). NTS and STS increased ($p < 0.001$) the biomass of these bacteria while simultaneously enhancing Ca^{2+} contents. Actinomycetes are considered in some studies to be oligotrophic, stress-resistant bacteria, and occur mainly in the soil as spores and hyphae (Liu et al., 2023). Direct Ca addition has been reported to promote surface-adhering bacterial taxa like Actinomycetota, Nocardioides, and some surface motile populations with slower growth rates (Feng et al., 2016). Our results also showed that the bacterial Gram+/Gram-ratio was negatively correlated with soil Ca^{2+} content (Fig. 7a and b). Fanin et al. (2019) suggested that the ratio of Gram+/Gram-could be an effective indicator of the availability of C. The Gram + prefers to utilize older and stable C sources, whereas the Gram-prefers fresh detritus or simple organic compounds (Zheng et al., 2023b). In fact, both NTS and STS increased plant-derived labile organic matter by returning crop residues to the field. Shabtai et al. (2023) stated that Gram-populations generally turn over faster after the input of exogenous substances, this process may contribute more to persistence SOC than Gram+. In this study, higher exchangeable Ca under NTS and STS directly contributed to the total SOC accumulation ($R^2 = 0.482$; Fig. 7c), and Gram-was the best bacterial predictor of SOC content ($p < 0.05$; Fig. 7c). It is reasonable to speculate that Ca may shift microbial communities to produce more by-products, thereby promoting the sequestration of adhesion proteins and cellular polymers on mineral surfaces for long-term SOC stabilization.

Interestingly, the MWD, Actinomycetes, AMF and AMF/SF ratio were the significant predictors of the cumulative SOC mineralization ($p <$ 0.01; Fig. 7 d). All these indicators are positively correlated with SOC sequestration and negatively correlated with cumulative SOC mineralization ($p < 0.01$; Supplementary Fig. 1). The AMF and SF are generally recognized as two major functional subgroups of the fungal community (Zheng et al., 2023a). AMF can produce cyst proteins that affect the adherence of soil particles, and SF promote the growth of collagen or collagenous tissues through excretions that aid in aggregate formation (Agnihotri et al., 2022). As the most significant contributor to SOC sequestration (Fig. 7c), exchangeable Ca^{2+} demonstrated a positive relationship with AMF/SF ratio (Fig. 7a). As Ca is a crucial component of fungal cell walls, it can affect the growth and composition of the fungal community (Shabtai et al., 2023). Furthermore, fungal necromass was reported as a significant contributor to SOC stabilization under conservation agriculture (Sae-Tun et al., 2022). This suggests that Ca-mediated organic bridging may explain the accumulation of mineral-associated fungal biomass under conservation tillage.

4.4. Conservation tillage practices promote SOC stabilization through mineral-organic-microbial interactions

In this Calcaric Cambisols, CTN and RTN promoted SOC mineralization by destroying large aggregates, whereas conservation tillage practices (NTS and STS) favored the sequestration and stability of aggregate-associated OC. This process is further influenced by the low carbon inputs from wheat straw under CTN and RTN, greater SOC mineralization and Ca deficiency, which reduces Ca-mediated organo-mineral associations (Wan et al., 2021). In addition, NTS had higher crop yields and lower soil pH compared to CTN (Table 1; Table 2). Generally, massive root exudation in the soil can release hydrogen ions to slightly acidify the soil (Jilling et al., 2021). This change of soil chemical environment favors the formation of exchangeable Ca, and higher plant growth leads to continuous carbon input and maintaining microbial biomass and calcium in the root zone where carbon is stabilized (Ball et al., 2023). More importantly, the higher Ca content shifts microbial community composition, causing some beneficial populations to transform the litter and strengthen microbial-mineral interactions that lead to the formation of stable organo-Ca complexes.

Based on our results (Fig. 8), lower pH (path coefficient = −0.21) and higher MWD (path coefficient = 0.85; $p < 0.001$) contributed to increased Ca^{2+} levels, providing favorable sites for microbial colonization and the adsorption of litter-derived conversion products. Furthermore, elevated levels of exchangeable Ca ions may lead to changes in microbial life strategies, thereby inducing association with surface-dwelling populations (Hu et al., 2023). In addition, Ca-driven mineral-organic complexes with highly recalcitrant molecular structures can reduce microbial substrate access (Huang et al., 2019). Taken together, small changes in Ca levels can affect SOC cycling and persistence through direct cation bridging or biotic modulation of microbial behavior.

Our study revealed a previously overlooked role for Ca-regulated microbial biochemical mechanisms in SOC sequestration. Although the processes of Ca-driven biotic control of SOC cycling depend on soil type, our findings demonstrate that Ca availability under conservation tillage enhances the conversion of organic inputs into persistent SOC. This provides an opportunity to apply Ca-rich amendments to optimize carbon management and soil quality in agroecosystems (Dou et al., 2023). For example, fertilizers such as gypsum and lime are often added to improve soil structure and enhance fertility to ensure crop yield (Adhikari et al., 2019). A deeper understanding of the minerals that mediate biotic and abiotic processes in SOC turnover will aid in restoring climate-adapted soils (Shabtai et al., 2023). However, further research should quantify in detail the contribution of mineral-regulated microbial by-products to the stable carbon pool to unlock the SOC sequestration potential. Likewise, a wider variety of mineral types with different crop residues needs to be comprehensively studied to better predict the mineral-microbial associated global C cycle.

5. Conclusions

This study demonstrates that 22-year conservation tillage practices (NTS and STS) increased SOC stocks and stability through organo-Ca associations, providing positive feedback for higher crop production compared to CTN and RTN. The macroaggregates sequestered more stable carbon than microaggregates due to lower SOC mineralization. In addition, Ca mediates aggregate-associated OC persistence partly by direct cation bridging to limit decomposition, and more importantly, by shifting microbial communities towards oligotrophic populations. In combination with wheat straw mulch, conservation tillage increased microbial growth and created a slightly low pH, high moisture soil environment, thereby promoting the conversion of exchangeable Ca. Bacterial rather than fungal communities behave more adaptively to changing exchangeable Ca levels. This interaction effect contributes positively to particle- and mineral-associated OC sequestration. Furthermore, Ca-associated microbial biomass can also be physically protected within macroaggregates because of the higher aggregate stability (MWD) under conservation tillage. Through comprehensive analysis, we highlighted the role of Ca availability in driving the microbial biomass and communities that promote stable associations between microbial by-products and minerals in Calcaric Cambisols. Overall, long-term conservation tillage contributed to SOC sequestration on the Loess Plateau of China by altering Ca-mediated microbial growth and soil aggregation and thus may provide a practical strategy for managing the global C cycle.

CRediT authorship contribution statement

Zixuan Han: Writing – review & editing, Writing – original draft, Data curation, Conceptualization. **Shengping Li:** Supervision. **Aurore Degré:** Writing – review & editing, Conceptualization. **Huizhou Gao:** Methodology. **Fengjun Zheng:** Methodology. **Xiaojun Song:** Software. **Angyuan Jia:** Visualization. **Xueping Wu:** Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvman.2025.128182.

Data availability

Data will be made available on request.

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