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Root traits determine the effects of organic amendments on soil aggregate organic carbon by regulating soil microbial multitrophic networks

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ABSTRACT

Crop root traits are known to influence soil properties that underpin agroecosystem functions, including soil aggregates, soil microbial properties, and soil organic carbon (SOC). However, the relationships among root traits, soil microbes, and organic carbon (OC) in soil under organic amendments remain poorly understood, particularly at the aggregate scale. Here, a 3-month rhizo-box experiment was conducted to investigate the impacts of organic amendments on SOC accumulation under long-term conventional and conservation tillage, with and without organic amendments. This experiment included five treatments: no fertilizer (Conv), chemical fertilizer (Conv_F), pig manure (Conv_M), and vermicompost (Conv_V) under conventional tillage and no fertilizer under conservation tillage (Cons). Compared with the Conv and Conv_F treatments, the organic amendment (Conv_M and Conv_V) and Cons treatments significantly increased root surface area and volume. Compared with the Conv treatment, the organic amendment treatments significantly increased the proportion of macroaggregates (0.25–2 mm) and the SOC content. Additionally, the organic amendment treatments significantly increased the concentrations of mineral-associated and iron-bound OC in macroaggregates and their contributions to SOC. The organic amendment treatments significantly increased the microbial network complexity and reduced the proportion of negative within-trophic associations in the microbial network of each aggregate size fraction. The partial least squares path model analysis indicated that with organic amendments, root traits regulated stable OC pools and thereby influenced aggregate-associated OC accumulation in soil by affecting microbial properties (*e.g.*, microbial associations and network complexity). Our findings highlight the potential role of root traits in regulating the accumulation of OC in agricultural soils, which contributes to increased soil carbon sequestration potential and sustainable agriculture.

Key Words: microbial association, microbial taxa, network complexity, soil aggregates, soil organic carbon

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INTRODUCTION

Soil organic carbon (SOC) is essential for maintaining soil fertility, soil function, and world food security in agricultural ecosystems (Hao *et al*., 2022). Approximately half of the global agricultural land is estimated to be moderately or severely affected by soil degradation (mainly due to a reduction in SOC stocks) (Ma S H *et al*., 2024). In recent years, numerous field management practices have been suggested to increase the SOC content in agricultural soils, with organic amendments (*e.g*., straw, manure, vermicompost, and biochar) shown to be effective (Wang *et al*., 2021; Hao *et al*., 2022). For example, long-term straw return increases the SOC content both directly and indirectly by influencing rhizodeposition (Song *et al*., 2022). Manure application increased carbon (C) sequestration by 17.7% (Du *et al*., 2020) and was positively correlated with SOC in deep soil layers (Bai *et al*., 2023). Furthermore, vermicompost application offset reductions in SOC caused by deep tillage and even increased the overall SOC content (Ding *et al*., 2021). Given that the SOC stocks in croplands represent more than 10% of total SOC stocks in terrestrial ecosystems and are far from saturated (Bai *et al*., 2023; Ma S H *et al*., 2024), it is vital to investigate the impacts of organic amendments on SOC accumulation for improving the health of cropland soils and mitigating climate change.

In agricultural ecosystems, changes in field management practices (*e.g*., the application of organic amendments) induce changes in crop root traits, including root length, surface area, and specific root length (Wang J Q *et al*., 2023). These changes in root traits may subsequently influence the rhizosphere environment and shape the rhizosphere soil

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microbial community (Ni and Su, 2024). As a zone of intense plant root-soil interactions, the rhizosphere plays a pivotal role in the acquisition of resources, such as water and nutrients, which are crucial for crop production and SOC accumulation (Philippot *et al.*, 2013; Kuzyakov and Razavi, 2019). Additionally, as a significant microbial hotspot, rhizosphere soil provides a suitable habitat for numerous microbial taxa and is influenced by external environmental factors and root exudates (Kuzyakov and Razavi, 2019). Rhizosphere soil microorganisms are essential for crop growth and health, tolerance to stresses, pathogen control, and soil nutrient cycling (Philippot *et al.*, 2013). Furthermore, compared with those in bulk soil, the interactions among rhizosphere soil microorganisms are more complex (Zhang R F *et al.*, 2017), potentially supporting a broad array of ecological functions. Therefore, it is essential to understand how root traits respond to organic amendments and the relationships of roots with rhizosphere soil microorganisms for increasing crop productivity and SOC accumulation.

Individual populations of microbial species in rhizosphere soil do not exist in isolation; instead, through interactions such as competition and predation, they form a complex soil micro-food web that enables message transfer and substance exchange (Zhang *et al.*, 2024). In this web, bacteria and fungi represent the base trophic level, whereas protists occupy a higher trophic level (Yao *et al.*, 2024). Interactions between multiple trophic levels can increase the production of microbial biomass and residues, thereby promoting SOC accumulation (Gunina and Kuzyakov, 2015; Yao *et al.*, 2024). In recent years, microbial co-occurrence network analysis has been employed to explore microbial associations and network complexity (Qiu *et al.*, 2021; Khatri-Chhetri *et al.*, 2024; Ma L *et al.*, 2024; Zhang *et al.*, 2024). Negative associations between bacteria and fungi (*e.g.*, potential cross-kingdom competition) in the cross-kingdom co-occurrence network can regulate SOC by influencing bacterial diversity (Chen *et al.*, 2024). The ratio of negative to positive associations in the microbial multitrophic network is negatively correlated with the SOC content (Li *et al.*, 2024). Additionally, topological features (*e.g.*, edge, node, average degree, and average path length) of microbial networks are commonly used to assess network complexity (Jiao *et al.*, 2022b). The complexity of microbial networks is a major and direct driver of soil multifunctionality and serves as an important predictor of SOC content in agricultural soils (Wang C *et al.*, 2023; Wang X *et al.*, 2023). Despite the growing interest in studying cross-kingdom microbial associations, research focusing on these associations within multitrophic networks remains limited, especially in soil aggregates.

Soil aggregates, as fundamental units of soil structure, play a pivotal role in SOC accumulation and are influenced by management practices such as addition of organic amendments (Zhang *et al.*, 2021). Soil aggregates have high spatial heterogeneity, which significantly impacts the community composition, diversity, and association of soil microorganisms (Han *et al.*, 2021). For example, the spatial distribution of protists within soil aggregates largely depends on the size of soil pores (Liao *et al.*, 2021), with macroaggregates and silt + clay hosting greater relative abundances of bacterivores and more intense microbial associations compared with microaggregates (Liao *et al.*, 2024). Furthermore, the size distribution of soil aggregates directly influences the spatial distribution of SOC; macroaggregates generally contain more organic C (OC) than do microaggregates (Zhang X F *et al.*, 2017). Recently, the roles of particulate OC (POC) and mineral-associated OC (MAOC) in soil aggregates and their contributions to SOC have attracted considerable attention (Zhang *et al.*, 2023). Mineral-associated OC, which includes significant components such as calcium-bound OC (CaOC) and iron-bound OC (FeOC), forms a stable OC pool with a longer turnover time than does POC (Lavallee *et al.*, 2020). These stable OC pools are essential for SOC sequestration. Therefore, it is crucial to understand the interactions among root traits, rhizosphere soil microbes, and SOC at soil aggregate level for sustaining agricultural ecosystems.

In this study, we conducted a rhizo-box experiment to investigate the effects of organic amendments on root traits and aggregate-associated OC in rhizosphere soil and determine the roles of root traits and rhizosphere soil microbial multitrophic associations and network complexity in aggregate-associated OC. However, the confined space of rhizo-boxes limits their ability to replicate the complex interactions found in natural soil environments, which may exacerbate the pot boundary effect and lead to diverse results (Mašková and Klimeš, 2020). Nevertheless, rhizo-boxes remain valuable tools for investigating plant root-soil interactions. We hypothesized that 1) organic amendments improve root traits and increase the concentrations of soil aggregate-associated OC; 2) organic amendments increase the proportion of positive cross-trophic (and/or within-trophic) associations and network complexity in microbial multitrophic networks within soil aggregates, as improved soil structure and resources may facilitate cooperation between microbes; and 3) root traits influence soil aggregate-associated OC accumulation by affecting microbial associations.

MATERIALS AND METHODS

Site description and sample collection

A long-term field experiment on tillage practices was initiated in 2007 in Lishu County, Siping City, Jilin Province, China (43°20′ N, 124°15′ E). The region features a temperate subhumid continental monsoon climate, with a mean annual precipitation and temperature of 614 mm and 6.9 °C, respectively. According to USDA Soil Taxonomy, the soil is categorized as a Mollisol. The initial basic soil properties are as follows: 11.3 g kg^{-1} SOC, 1.2 g kg^{-1} total nitrogen, and pH 7.1. The cropping system consisted of single-season spring maize. In this study, we selected two treatments: conventional tillage and conservation tillage. Conventional tillage involves removing maize straw from the field after maize harvest and rotary plowing to 25–30 cm before maize is planted in the following year. Conservation tillage involves no-tillage with 100% maize straw mulching. The quantity of maize straw mulch was 7 500 kg ha^{-1}. The annual application rates of fertilizers were 240 kg N ha^{-1}, 110 kg K_2O ha^{-1}, and 110 kg P_2O_5 ha^{-1}. In 2022, we collected soil samples from the plots in each replicate of the two treatments. As described in previous studies (Saffari *et al*., 2020; Saedi *et al*., 2021), these samples were then passed through a 4-mm sieve for subsequent rhizo-box experiment, ensuring minimal damage to the soil aggregate structure. Soil properties are shown in Table SI (see Supplementary Material for Table SI).

Rhizo-box experiment

A rhizo-box experiment was conducted to assess the effects of organic amendments on aggregate-associated OC in rhizosphere soil and the legacy effects of long-term conservation tillage. The rhizo-box experiment consisted of five treatments with four replicates: no fertilizer (Conv), chemical fertilizer (Conv_F), pig manure (Conv_M), and vermicompost (Conv_V) under conventional tillage and no fertilizer under conservation tillage (Cons). For the organic amendment treatments (Conv_M and Conv_V), an equivalent amount of C (3 000 kg C ha^{-1}) was applied (Mehmood *et al*., 2020). The chemical fertilizer applied in the treatments was consistent with that used in the long-term field experiment, except that no chemical fertilizer was applied in the Conv and Cons treatments. The details of the composition and dose for each treatment are provided in Table SII (see Supplementary Material for Table SII). In accordance with prior studies (Gao *et al*., 2019; Tang *et al*., 2019), we used a rhizo-box (16 cm × 12 cm × 26 cm) to collect rhizosphere soil (Fig. S1, see Supplementary Material for Fig. S1). The rhizo-box was partitioned into three zones using nylon mesh (300-mesh pore size) to prevent the interspersion of root hairs, with the central 5 cm designated the rhizosphere zone (Gao *et al*., 2019). Each rhizo-box was filled with 5 kg of soil at a bulk density of 1.2 g cm^{-3}. Soil moisture was adjusted to approximately 60% of the water-holding capacity and consistently maintained at this level throughout the experiment (Zhou *et al*., 2019). In the organic amendment treatments, organic amendments were thoroughly mixed with the soil prior to filling the rhizo-boxes. All the rhizo-boxes were positioned outdoors, and maize was cultivated in them under natural field conditions. This approach allowed the maize to receive natural sunlight, closely simulating the light conditions typically experienced by maize in its growth environment. After three months of maize cultivation, we collected the samples of both maize plants and the soil from the rhizosphere zone.

Maize growth and root morphological analysis

Aboveground maize plant samples were oven-dried at 105 °C for 30 min, followed by drying at 65 °C to a constant weight to determine shoot biomass after plant height and stem diameter were recorded (Tang *et al*., 2019). The root samples were washed with water, and WinRHIZO software (Regent Instrument Inc., Canada) was used to scan and analyze the following root traits: length, volume, average diameter, and surface area. The root samples were subsequently dried to a constant weight to determine root biomass. The specific root length was calculated by dividing the total root length by root biomass (Xu *et al*., 2021). The total biomass is the sum of shoot biomass and root biomass.

Soil aggregates and aggregate-associated OC

Soil aggregates were classified into macroaggregates (0.25–2 mm), microaggregates (0.053–0.25 mm), and silt + clay ($<$ 0.053 mm) using a wet-sieving method (Duan *et al*., 2021; Liao *et al*., 2024). In brief, fresh soil that had passed through an 8-mm sieve was placed on the top layer of a series of sieves (from top to bottom: 2-, 0.25-, and 0.053-mm sieves) and submerged in deionized water for 5 min, followed by 2 min of agitation with an up-and-down motion (3 cm amplitude) at a rate of 30 times min^{-1}. Notably, $>$ 2 mm aggregates were not obtained in this study; thus, only three fractions were analyzed. Each aggregate fraction was divided into two subsamples: one was dried at 50 °C for subsequent physicochemical property measurements, and the other was stored at -80 °C for microbial community analysis.

The OC in the soil macroaggregate and microaggregate samples was separated into POC and MAOC using a previously described method (Lugato *et al*., 2021). A soil aggregate sample (5 g) was placed in 0.5% (weight:volume) sodium hexametaphosphate solution and shaken at 200 r min^{-1} for 18 h. Subsequently, POC ($>$ 0.053 mm) and MAOC ($<$ 0.053 mm) were obtained by passing the sample through a 0.053-mm sieve. The OC content was determined using the $K_2Cr_2O_7$ titration method (Sun *et al*., 2020). In accordance with the citrate-bicarbonate-dithionite (CBD) method, the FeOC was calculated as the difference in OC between the soil residue extracted with CBD solution and that extracted with NaCl solution (Fang *et al*., 2019). The CaOC content was determined using a total OC analyzer (Elementar, Germany) after extraction with 0.5 mol L^{-1} Na_2SO_4 (Feng *et al*., 2022).

DNA extraction and sequencing

Total microbial DNA from the soil aggregate samples was extracted using a FastDNA SPIN kit for soil (MP Biomedicals, USA). Analysis of soil microbial communities, including those of bacteria, fungi, and protists, was conducted using 16S rRNA and 18S rRNA gene amplicon sequencing with the primer pairs 515F/907R and 528F/706R, respectively (Jiao *et al*., 2022b). After sequencing on an Illumina NovaSeq 6000 platform, the raw sequences were quality-screened using the QIIME pipeline to produce high-quality clean tags, which were then clustered into operational taxonomic units (OTUs) at 97% similarity with the UPARSE pipeline. The representative sequences of bacterial OTUs were subsequently classified according to the SILVA database (release 138), and the representative sequences of fungi and protists were classified according to the Protist Ribosomal Reference database. We manually removed sequences belonging to Metazoa. To remove random effects, the OTUs with sequences present in at least 4 of the 60 samples were retained for subsequent analysis. Finally, to ensure an equivalent sequencing depth, the OTU tables for bacteria, fungi, and protists were rarefied to 38 165, 27 598, and 6 446 sequences, respectively. The sequencing data can be accessed in the National Center for Biotechnology Information (NCBI) Sequence Read Archive under accession number PRJNA1120632.

Statistical analysis

Statistical differences among fertilization regimes within the same soil aggregates were assessed using one-way analysis of variance performed with SPSS 25.0 software (IBM, USA). To identify the OTUs that varied with fertilization (indicator species) at the aggregate scale, we conducted indicator species analyses using the "indicspecies" package in R (version 4.1.2) (Fig. S2, see Supplementary Material for Fig. S2) (Fan *et al*., 2023). Based on a previous study (Yao *et al*., 2024), we identified functionally important (FI) taxa related to soil C cycle. Specifically, we used the means of the min-max standardized values of aggregate-associated OC, POC, MAOC, CaOC, and FeOC to characterize the function of soil C cycle. We then employed Spearman's correlation analysis to examine the correlations between the function of soil C cycle and the OTUs from these indicator species. Microbial taxa with the correlations at $P < 0.05$ were considered FI taxa. We further analyzed the compositions of these FI taxa groups (Fig. S3, see Supplementary Material for Fig. S3). Based on Spearman's correlation analysis, we constructed multitrophic networks for different soil aggregates using indicator OTUs with correlation coefficient $|r| > 0.8$ and $P < 0.05$ (adjusted by the Benjamini-Hochberg procedure) using the "WGCNA" package in R (version 4.1.2). These networks were visualized with the "igraph" package in R (version 4.1.2). Using the methodologies described in previous studies (Jiao *et al*., 2022b; Wang X *et al*., 2023), we extracted the topological features of each subnetwork and assessed microbial network complexity using the "igraph" package in R (version 4.1.2). In brief, the topological features (degree, node, edge, betweenness centrality, average path length (APL), and clustering coefficient) were normalized and then averaged. Notably, microbial network complexity is inversely related to APL; thus, the APL values were inverted before they were integrated into the network complexity calculation. To identify topologically important (TI) taxa, the within- (Zi) and among-module connectivity (Pi) plots were created (Fig. S4, see Supplementary Material for Fig. S4). According to a previous study (Yao *et al*., 2024), the OTUs classified as network hubs ($Zi > 2.5$, $Pi > 0.62$), module hubs ($Zi > 2.5$, $Pi \leqslant 0.62$), and connectors ($Zi \leqslant 2.5$, $Pi > 0.62$) were designated TI taxa. The taxa that qualified as both FI and TI were deemed functionally and topologically important (FTI) taxa.

Given the minimal sample size requirements of the partial least squares path model (PLS-PM) approach (Yan *et al*., 2023; Han *et al*., 2024), we employed PLS-PMs to explore the relationships among root traits, microbial properties, and aggregate-associated OC using the "plspm" package in R (version 4.1.2). Before the PLS-PM analysis, an *a priori* model was constructed based on the hypothesis that root traits influence SOC fractions and thus OC accumulation by influencing microbial properties (Fig. S5, see Supplementary Material for Fig. S5). The PLS-PM performance was assessed using the goodness of fit (GOF) index.

RESULTS

Maize root traits and physiological status

Maize root traits and physiological status were significantly altered by fertilization (Fig. 1). Specifically, compared with Conv and Conv_F, Conv_V and Conv_M significantly increased root volume and root surface area (Fig. 1a). Additionally, compared with Conv_F, Conv_V and Conv_M resulted in a significant increase in root diameter, and Conv_M significantly reduced specific root length (Fig. 1a). Furthermore, maize plants treated with organic amendments presented significantly greater stem diameter, root biomass, shoot biomass, and total biomass than those in Conv, and Conv_M significantly increased plant height (Fig. 1b). Overall, Cons favored more root and plant growth than did Conv, particularly in terms of root volume, root surface area, root diameter, and biomass (Fig. 1).

Soil aggregate distributions and OC fractions in soil aggregates

Organic amendments significantly affected the distributions of soil aggregates (Fig. S6, see Supplementary Material

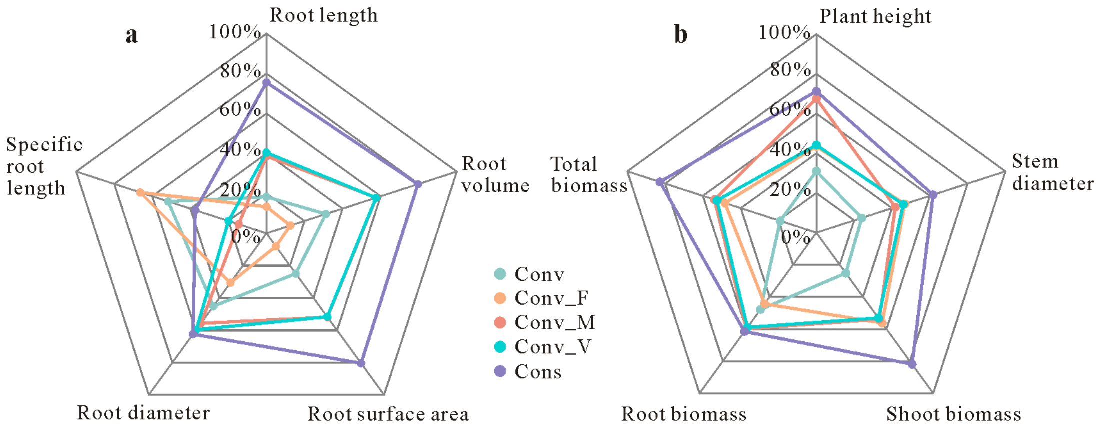

Fig. 1 Maize root traits (a) and physiological status (b) under different fertilization treatments. Conv = no fertilizer under conventional tillage; Conv_F = chemical fertilizer under conventional tillage; Conv_M = pig manure under conventional tillage; Conv_V = vermicompost under conventional tillage; Cons = no fertilizer under conservation tillage.

for Fig. S6). In brief, Conv_V, Conv_M, and Cons significantly increased the proportion of macroaggregates and the mean weight diameter of soil aggregates relative to those in Conv and Conv_F. Additionally, organic amendments significantly increased the SOC content by increasing the proportion of macroaggregate-associated OC to 60.65% (Conv_M) and 55.65% (Conv_V) (Fig. 2). Within macroaggregates, organic amendments increased not only the proportion of MAOC to 18.17% (Conv_M) and 18.19% (Conv_V), but also the proportion of FeOC to 10.73% (Conv_M) and 9.93% (Conv_V) and increased their concentrations compared with those in Conv and Conv_F (Figs. 2 and S7, see Supplementary Material for Fig. S7). Similar patterns were observed for Cons (Figs. 2 and S7). Furthermore, there were significant increases in both the proportion and concentration of POC in macroaggregates in Conv_M compared with Conv (Figs. 2 and S7). Additionally, compared with Conv, Conv_V and Conv_M increased the concentrations of MAOC and FeOC in microaggregates and the concentration of FeOC in silt + clay (Fig. S7).

Microbial community structure and co-occurrence networks within soil aggregates

Fertilization and aggregate size significantly affected the community structures of bacteria, fungi, and protists (Fig. S8a–c, see Supplementary Material for Fig. S8). Additionally, Proteobacteria, Acidobacteriota, and Actinobacteriota were the predominant phyla among soil bacteria (Fig. S8d); Ascomycota and Mucoromycota dominated among soil fungi (Fig. S8e); and Cercozoa and Ciliophora were the most prevalent among soil protists (Fig. S8f).

We constructed multitrophic co-occurrence networks at the aggregate scale to estimate the potential associations between soil microorganisms (Fig. 3). Overall, the numbers of edges and nodes contained in the multitrophic co-occurrence network increased with decreasing soil aggregate size (Fig. 3a–c). To clarify the effects of fertilization regimes on microbial network complexity, we analyzed the topological features of the subnetworks and calculated the network complexity (Fig. 3d). Compared with Conv and Conv_F, Conv_V, Conv_M, and Cons significantly increased microbial network complexity of each aggregate fraction. Additionally, we found no changes in the proportions of overall within-trophic associations (bacteria-bacteria, bacteria-fungi, fungi-fungi, and protists-protists links) or overall cross-trophic associations (bacteria-protists and fungi-protists links) in the macroaggregate multitrophic co-occurrence network under different fertilization regimes (Fig. 3e). However, in the co-occurrence networks of microaggregates and silt + clay, compared with Conv and Conv_F, Conv_M significantly increased the proportion of overall within-trophic associations and decreased the proportion of overall cross-trophic associations. Further analyses revealed that regarding the within-trophic associations, compared with Conv and Conv_F, Conv_V and Conv_M significantly increased the proportion of positive associations and decreased the proportion of negative associations in the co-occurrence networks of each aggregate fraction (Fig. 3f). Additionally, similar to the within-trophic associations, Conv_V, Conv_M, and Cons tended to reduce the proportion of negative cross-trophic associations in the microbial co-occurrence networks of each aggregate fraction (Fig. 3g).

Compositions of FTI taxa within soil aggregates

The fertilization regime significantly altered microbial compositions of FTI taxa within soil aggregates (Fig. 4). Specifically, for bacterial FTI taxa in macroaggregates, compared with Conv, Conv_F significantly increased the relative abundances of Nitrospirota, Myxococcota, and Chloroflexi (Fig. 4a). Compared with Conv, Cons significantly increased the relative abundances of Proteobacteria, Gemmatimonadota, and Actinobateriota. For eukaryotic FTI taxa in

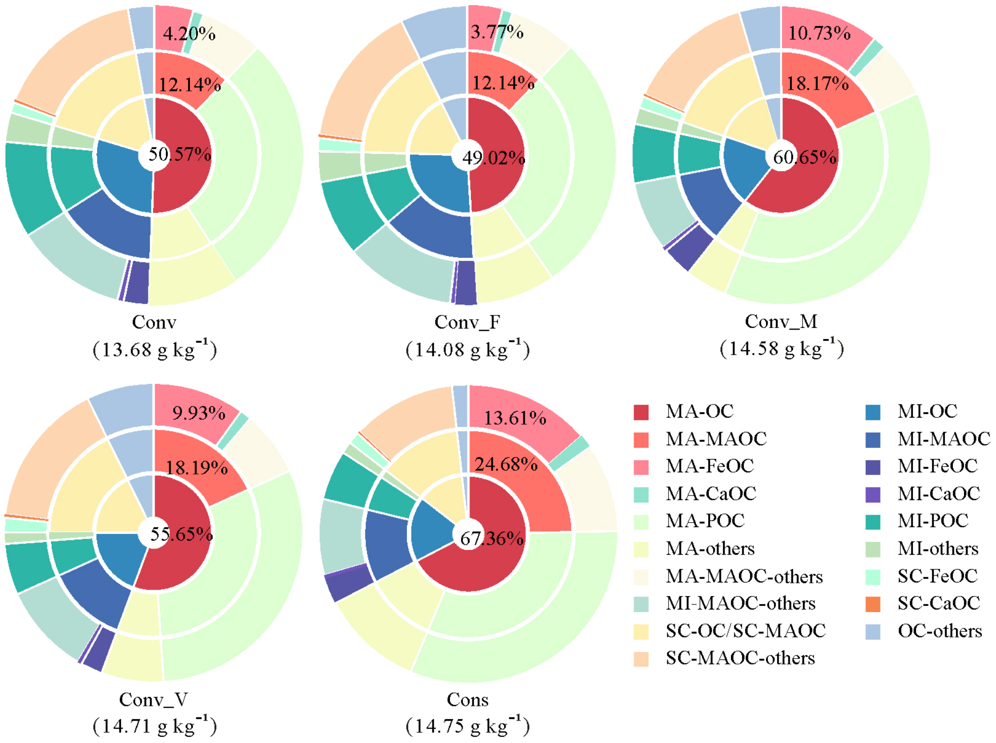

Fig. 2 Distributions of organic C (OC) in soil macroaggregates (MA), microaggregates (MI), silt + clay (SC), and other aggregates (MA-OC, MI-OC, SC-OC, and OC-others, respectively) in soil organic C (SOC) under five fertilization treatments of Conv, Conv_F, Conv_M, Conv_V, and Cons. The data in parentheses are the SOC content in each treatment. See Fig. 1 for the detailed descriptions of the abbreviations for treatments. OC-others = SOC − MA-OC − MI-OC − SC-OC; MAOC = mineral-associated OC; FeOC = iron-bound OC; CaOC = calcium-bound OC; POC = particulate OC; MA-others = MA-OC − MA-MAOC − MA-POC; MI-others = MI-OC − MI-MAOC − MI-POC; SC-OC = SC-MAOC; MA-MAOC-others = MA-MAOC − MA-FeOC − MA-CaOC; MI-MAOC-others = MI-MAOC − MI-FeOC − MI-CaOC; SC-MAOC-others = SC-MAOC − SC-FeOC − SC-CaOC.

macroaggregates, Conv_M and Cons significantly decreased the relative abundances of Ascomycota and Basidiomycota compared with Conv, with Cons significantly increasing the relative abundances of Cercozoa and Ciliophora. For bacterial FTI taxa in microaggregates, compared with Conv, Conv_M and Cons significantly increased the relative abundances of Actinobacteriota and Planctomycetota, whereas Conv_F significantly increased the relative abundance of Nitrospirota (Fig. 4b); all the other treatments significantly decreased the relative abundance of Basidiomycota compared with Conv, with Conv_M and Cons also significantly reducing the relative abundance of Ascomycota. Additionally, the fertilization regime affected mainly the bacterial FTI taxa in silt + clay (Fig. 4c). Compared with Conv, Conv_V and Conv_M significantly increased the relative abundance of Actinobacteriota, and Conv_V also significantly increased the relative abundance of Proteobacteria.

Relationships between root traits and soil microbial properties and aggregate-associated OC

The PLS-PM analysis was employed to determine the linkages between root traits, microbial properties, and aggregate-associated OC at the aggregate scale. In macroaggregates and microaggregates, the stable OC pools had a significantly direct effect on aggregate-associated OC (path coefficients = 0.765 and 0.763, respectively) (Fig. 5a, b). These findings suggest that root traits indirectly regulate aggregate-associated OC accumulation mainly by influencing stable OC pools. Specifically, root traits affected the accumulation of stable OC pools by directly influencing FTI taxa and microbial network properties (Fig. 5a, b). In both macroaggregates and microaggregates, the standardized total effects of root traits on aggregate-associated OC were 0.631 and 0.537, respectively, whereas those of microbial network properties were 0.421 and 0.386, respectively (Fig. 5c). Unlike in macroaggregates and microaggregates, there was a significantly direct effect of microbial network properties (path coefficient = 0.534) on the aggregate-associated OC in silt + clay (Fig. S9a, see Supplementary Material for Fig. S9). The standardized total effect of microbial network properties on the aggregate-associated OC in silt + clay was 0.443 (Fig. S9b). The linear relationship between aggregate-associated OC and SOC showed that SOC increased with increasing macroaggregate- and microaggregate-associated OC but not with silt + clay-associated OC (Fig. 5d).

DISCUSSION

Effects of organic amendments on crop growth and soil aggregate size distribution

In this study, we found that organic amendments and

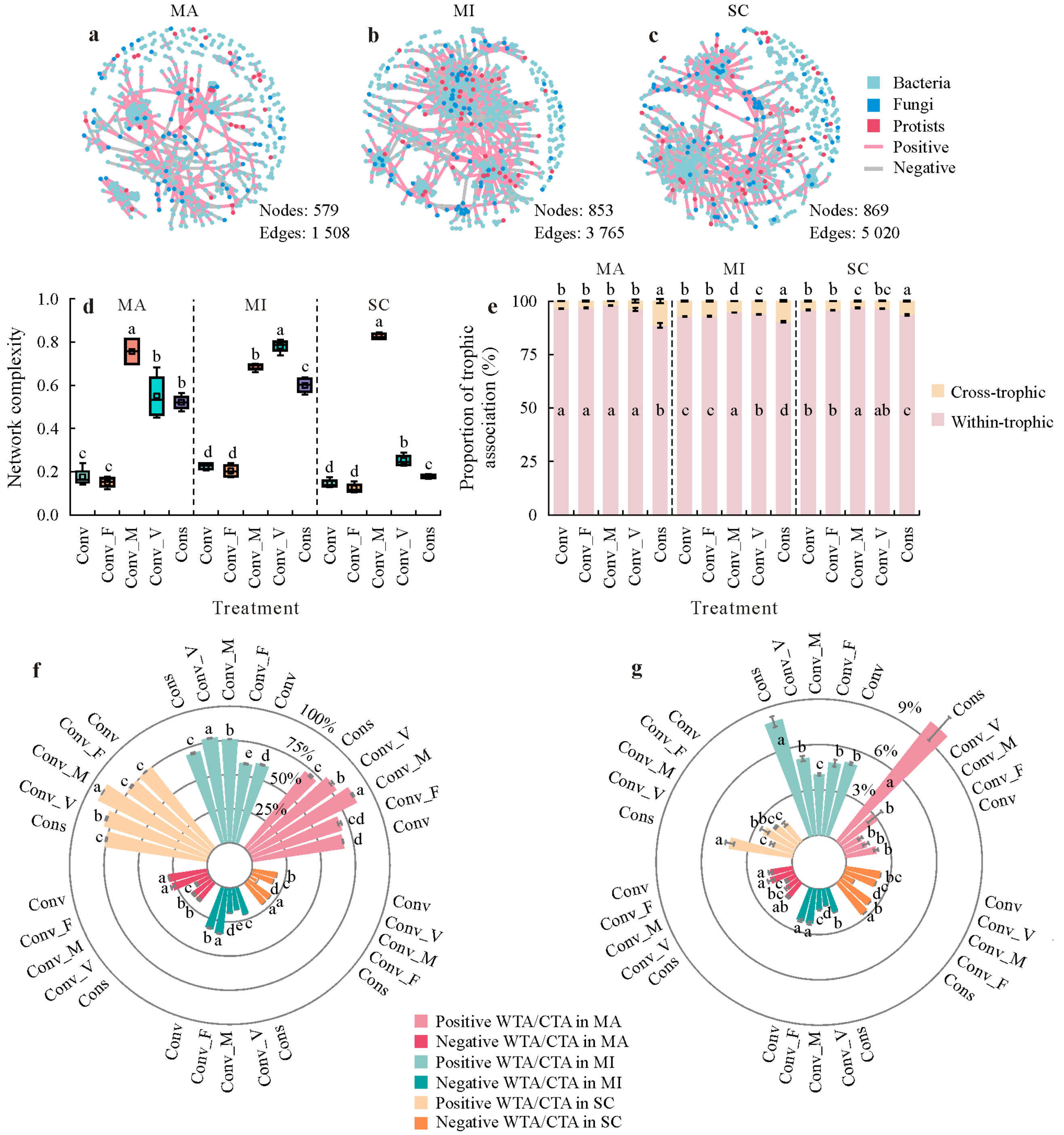

Fig. 3 Co-occurrence networks within soil macroaggregates (MA) (a), microaggregates (MI) (b), and silt + clay (SC) (c) and microbial network complexity (d), proportions of trophic associations (e), and positive and negative within-trophic associations (WTA) (f) and cross-trophic associations (CTA) (g) within soil aggregates under different treatments. Vertical bars indicate standard errors of the means ($n = 4$). Different letters indicate significant differences at $P < 0.05$ between treatments for a given aggregate fraction. See Fig. 1 for the detailed descriptions of the abbreviations for treatments.

Cons favored changes in root traits, such as increased root surface area, root volume, and root biomass (Fig. 1). These findings align with our first hypothesis and underscore the potential legacy effects of long-term conservation tillage in supporting maize growth. With high soil nutrient availability, for example, through the application of organic amendments, an increase in belowground C allocation to plant root systems occurs (Lu *et al.*, 2023), which in turn promotes root development and increases root surface area (Amendola *et al.*, 2017). This expands the contact area between root system and soil, facilitating the absorption of nutrients and water (Han *et al.*, 2023) and thereby increasing root biomass. Additionally, some physiologically active substances (*e.g.*, amino acids) from organic amendments penetrate the root

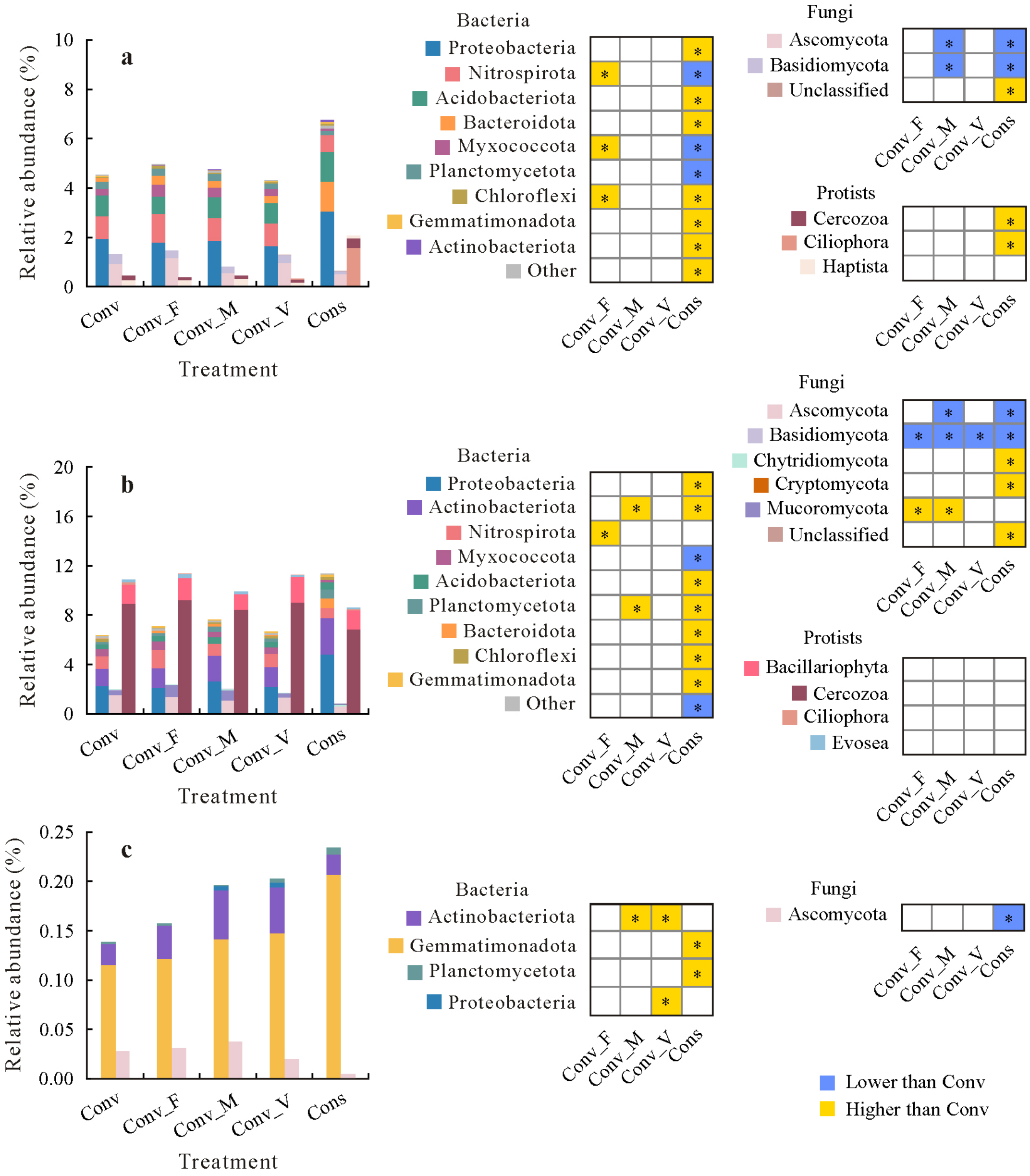

Fig. 4 Microbial compositions of functionally and topologically important (FTI) taxa within soil macroaggregates (a), microaggregates (b), and silt + clay (c) under different treatments. Asterisk * indicates significant differences between Conv and other treatments at $P < 0.05$. See Fig. 1 for the detailed descriptions of the abbreviations for treatments.

zone, thereby promoting root growth (Wang J Q *et al*., 2023). The findings of this study were derived from a rhizo-box experiment. Although widely used, the spatial constraints within rhizo-boxes can alter root competition and growth behavior compared with those in natural soil environments, potentially affecting the generalizability of these findings to real-world conditions (Mašková and Klimeš, 2020).

Soil aggregates are important indicators of soil structure and function and influence nutrient cycling (Zhang *et al*., 2023). In this study, compared with Conv and Conv_F, Conv_V and Conv_M increased the proportion of macroaggregates (Fig. S6a). This increase may be attributed to the improvements in root traits facilitated by organic amendments. Plant roots are crucial for promoting the formation and stability of soil aggregates (Li *et al*., 2020). For example, good root traits (*e.g*., increased root surface area and root volume) can contribute to the formation of soil macroaggregates through entanglement, the release of greater amounts of root exudates, and greater belowground C allocation (acting as organic binding agents) (Hudek *et al*., 2022; Lu *et al*., 2023; Ni and Su, 2024). Additionally, organic amendments increase C and nitrogen inputs, which may stimulate soil microbial activity, such as the growth of fungal hyphae (Luan *et al*., 2021). Similarly, fungal hyphae exhibit some degree of soil entanglement that favors the formation of aggregates (Wang *et al*., 2018).

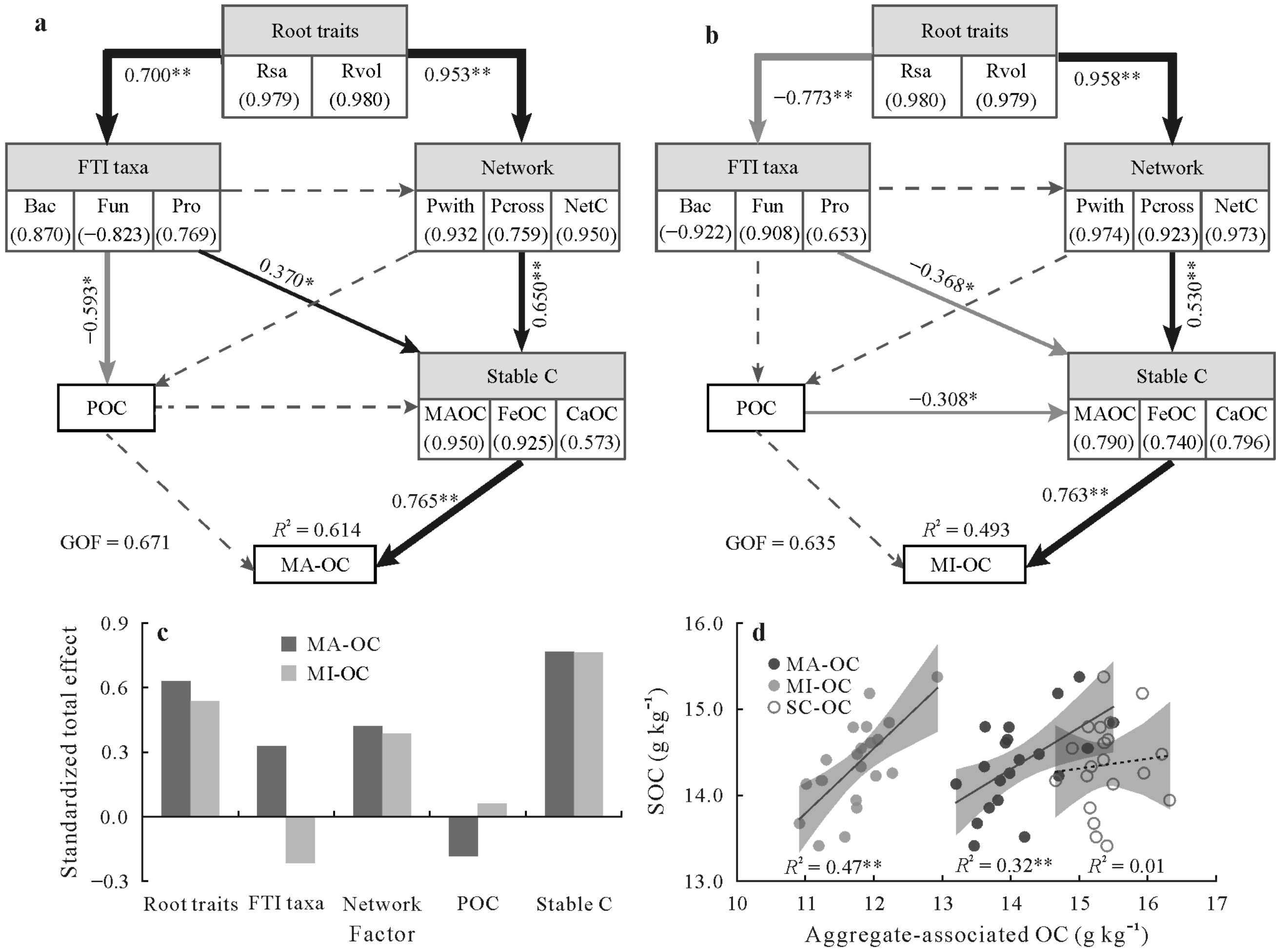

Fig. 5 Partial least squares path models (PLS-PMs) illustrating direct and indirect effects of root traits and soil functionally and topologically important (FTI) taxa, microbial network properties (Network), and stable organic C pools (Stable C) on soil macroaggregate (MA, a)- and microaggregate (MI, b)-associated organic C (OC) accumulation, standardized total effects of each factor on soil aggregate-associated OC (c), and relationships between soil organic C (SOC) and aggregate-associated OC (d). The black and gray arrows with path coefficients represent significantly positive and negative relationships, respectively. The dotted lines denote non-significance. The data in parentheses are the loadings of variables. Asterisks * and ** indicate significances at $P < 0.05$ and $P < 0.01$, respectively. The shaded areas around the lines indicate the 95% confidence intervals. Rsa = root surface area; Rvol = root volume; Bac = relative abundance of bacterial FTI taxa; Fun = relative abundance of fungal FTI taxa; Pro = relative abundance of protozoan FTI taxa; Pwith = proportion of positive within-trophic associations/overall within-trophic associations; Pcross = proportion of positive cross-trophic associations/overall cross-trophic associations; NetC = microbial network complexity; MAOC = mineral-associated OC; CaOC = calcium-bound OC; FeOC = iron-bound OC; POC = particulate OC; MA-OC = MA-associated OC; MI-OC = MI-associated OC; SC-OC = silt + clay-associated OC; GOF = good of fitness.

Effects of organic amendments on soil microbial network complexity and microbial associations at aggregate scale

Consistent with our second hypothesis, compared with Conv and Conv_F, Conv_V, Conv_M, and Cons significantly increased microbial network complexity of each aggregate fraction (Fig. 3). This finding aligns with those of previous studies (Zhou *et al.*, 2019; Li *et al.*, 2022). Microbial communities form complex networks through interactions such as competition, commensalism, and predation (Banerjee *et al.*, 2016; Khatri-Chhetri *et al.*, 2024). In agricultural ecosystems, the complexity of soil microbial networks increases with increasing resource availability, notably with increasing soil fertility (Qiu *et al.*, 2021). In this study, organic amendments and legacy effects of long-term conservation tillage enriched soil nutrient resources, as exemplified by increased SOC (Fig. 2). This enrichment likely supported more microbial ecological niches and influenced microbiome growth strategies, thereby fostering stronger associations among microbes and augmenting the complexity of microbial networks (Zhou *et al.*, 2019; Yang *et al.*, 2023). Additionally, increased microbial network complexity may also be related to root traits, such as increased root surface area and biomass (Fig. 1). These changes may have contributed to the diversity and abundance of root exudates, thereby recruiting specific microbial groups, altering microbial community structure, and strengthening microbial associations (Yang *et al.*, 2023).

Positive and negative associations in multitrophic networks suggest potential cooperative or competitive relationships among microorganisms, although they may not always accurately mirror actual species interactions (Zhang *et al.*, 2024). In this study, organic amendments significantly reduced the proportions of negative within- and across-trophic associations and increased the proportion of positive within-trophic associations in the multitrophic network of soil aggregates (Fig. 3). These findings suggest that organic amendments may promote cooperative behaviors among microorganisms while reducing competitive interactions. This effect is likely due to organic amendments being rich in nutrients and substrates, which create a conducive microenvironment that supports microbial survival and growth (Yang *et al.*, 2023), thereby alleviating competition within the microbial network (Ratzke *et al.*, 2020). Additionally, the increase in the proportion of positive within-trophic associations due to organic amendments may be explained by the dynamics of microbial decomposition, as outlined by the r- and K-strategy theories. When organic amendments are introduced into soil, fast-growing bacteria serve as the primary decomposers (Kuzyakov, 2010). As the supply of readily decomposable resources diminishes, some fast-growing bacteria might die, and slow-growing fungi might emerge as the dominant decomposers (Han *et al.*, 2024). During this transition, bacterial residues are reused by fungi, which are capable of breaking down more recalcitrant organic matter (Zheng *et al.*, 2023), thereby releasing substrates that are readily available for symbiotic utilization by other bacteria (Jiao *et al.*, 2022a).

Potential mechanisms of aggregate-associated OC accumulation

It is widely acknowledged that plant root-microbe interactions are pivotal in soil C cycling (Wan *et al.*, 2021). In support of our third hypothesis, root traits influenced stable OC pools by affecting microbial properties such as the relative abundance of FTI taxa and the microbial network, thereby facilitating the accumulation of aggregate-associated OC (Fig. 5). This observation is consistent with a previous study (Wan *et al.*, 2021). Root traits are major factors controlling soil C inputs and can influence the release of 20%–80% of total photosynthetic products as root exudates (Finzi *et al.*, 2015; Guyonnet *et al.*, 2018). For example, the roots with larger surface areas can release substantial amounts of labile C resources (Guyonnet *et al.*, 2018; Li *et al.*, 2023). Given that soil microorganisms are often C-limited, this influx of C stimulates the growth and activity of specific microbes (Wan *et al.*, 2022), impacting their relative abundance, associations, and network complexity. Additionally, the close association of plant roots with soil microbes in the rhizosphere means that root traits can directly affect the composition and complexity of microbial communities (Spitzer *et al.*, 2021).

In soil micro-food webs, soil microorganisms, their potential trophic associations, and network complexity play crucial roles in nutrient cycling (Yao *et al.*, 2024). The relative abundance of FTI taxa and microbial network properties significantly influenced the accumulation of stable OC pools in this study (Fig. 5). Additionally, a negative correlation was observed between the relative abundance of fungal FTI taxa and stable OC pools (Fig. 5). This may be attributed to the low soil nutrient availability, which likely led to an increase in the relative abundance of fungi, particularly those taxa that specialize in degrading stable OC pools (Jiao *et al.*, 2020; Zhao *et al.*, 2023). Moreover, positive microbial associations are essential for the accumulation of stable OC pools (Fig. 5). Typically, these associations are self-interested in nature (Palmer and Foster, 2022). The application of organic amendments or increased amounts of root exudates due to improved root traits can provide essential nutrients to soil microorganisms, potentially accelerating synergistic microbial metabolic processes (Slatyer *et al.*, 2013; Letten, 2021). These increases in resource acquisition and transformation efficiency subsequently affect the accumulation of SOC. Moreover, an increase in nutrients leads to an increase in the number of microorganisms at the basal trophic level, which, through bottom-up effects, results in a corresponding increase in their predators and thus enhances cross-trophic associations (Geisen *et al.*, 2021; Zhu *et al.*, 2024). This process may promote energy flow and nutrient cycling within the soil micro-food web (Li *et al.*, 2024), favoring the formation of microbial necromass and consequently increasing the accumulation of stable OC pools. Additionally, microbial network complexity favored the accumulation of stable OC pools in this study (Fig. 5). An increase in microbial network complexity enhances synergistic decomposition and metabolite exchange (van der Heijden *et al.*, 2016; Rozmo *et al.*, 2022), thereby improving resource use efficiency and then promoting OC accumulation (Yang *et al.*, 2023).

CONCLUSIONS

The present study revealed that organic amendments and the legacy effects of long-term conservation tillage enhanced maize root traits and growth, soil aggregation, and SOC accumulation. Moreover, they also increased microbial network complexity of each soil aggregate fraction. Root traits such as root surface area and root volume influenced the accumulation of stable OC pools by modulating microbial associations and network complexity. This influenced the accumulation of OC in macro- and microaggregates. In conclusion, we suggest that organic amendments and the enduring effects of long-term conservation tillage promote SOC accumulation at the aggregate level through changes in root traits and soil microbial properties.

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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SUPPLEMENTARY MATERIAL

Supplementary material for this article can be found in the online version.

CONTRIBUTION OF AUTHORS

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