

Linking soil microbial community traits and organic carbon accumulation rate under long-term conservation tillage practices

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ABSTRACT

Conservation tillage practices may stimulate soil organic carbon (SOC) storage in croplands by reducing soil disturbance and increasing inputs of crop residue. Although the effect of tillage practices on soil C dynamics is mediated by soil microbes, the relation between microbial community traits and SOC accumulation rate (SAR) remains unclear. In this study, we investigated the effect of tillage management on soil properties and microbial community traits (i.e. diversity, composition and keystone taxa) in a long-term (17 years) field experiment. Our experiment was located in a spring maize field with a sandy loam soil (Calcaric-Fluvic Cambisol) in northwest China and included three tillage practices: 1) CT-RR, conventional tillage with residue removed; 2) RT-RI, reduced tillage with residue incorporated; and 3) NT-RM, no-tillage with residue mulched. We sampled soil at 0–10 cm and 10–25 cm to assess the relation between bacterial and fungal community traits and SAR. We found that the initial and current of average SOC stocks was 19 and 28 Mg ha⁻¹ at 0–25 cm, respectively. RT-RI and NT-RM increased SOC accumulation at 0–10 cm by 116% and 131% compared with CT-RR, respectively, and RT-RI increased SOC accumulation at 10–25 cm by 159%. Changes in co-occurrence network analysis revealed that NT-RM resulted in a stable bacterial network, whereas both RT-RI and NT-RM produced a stable fungal network relative to CT-RR. Microbial diversity and keystone taxa correlated positively with SAR under conservation tillage practices. Using a Zi-Pi plot, we identified several keystone OTUs, five of which (i.e. *Cytophagales*, *JG30-KF-CM45*, *Sphingobacteriales*, *Thelephorales* and *Pleosporales*) showed a positive correlation with SAR. Our results suggest a strong link between microbial community traits and SOC accumulation rate under conservation tillage practices, and provide insights into the contribution of microbial traits to the sustainability of agroecosystems.

1. Introduction

Soil organic carbon (SOC) plays a major part in sustaining soil fertility (Oldfield et al., 2019) and soil biological health (Alhameid et al., 2019). However, through several mechanisms, agriculture cultivable cropping has caused a reduction in SOC stocks around the world (Lal, 2004). Firstly, soil disturbance during tillage and other management operations reduces the physical protection of soil carbon (C) through breaking macroaggregates into microaggregates (Six et al., 2000; Du et al., 2015). The exposed SOC is more easily accessible to

microorganisms, resulting in decreasing SOC stocks (Razafimbelo et al., 2008). Secondly, cultivated land is used to maintain close to neutral soil pH levels and prevent water accumulation, thereby stimulating microbial oxidation of SOC. Finally, cropland is generally covered with vegetation for a short time relative to natural ecosystems and part of the vegetation is removed through harvest, causing lower soil C input rates (Guo et al., 2017). The resulting decline in soil C stocks reduces soil quality and adds to anthropogenic CO₂ emissions (Li et al., 2019). These issues prompted researches into management practices that slow down or reverse soil C losses in croplands (Post and Kwon, 2000; Smith, 2016).

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By minimizing soil disturbance and retaining crop residue, conservation tillage practices may increase soil C stocks (e.g. Chavarria et al., 2018). However, soil C gains under these management practices varies widely between studies, ranging from positive (Jha et al., 2020; Li et al., 2021a) to negligible (Niu et al., 2019; Gao et al., 2021). These inconsistent effects on SOC content may be due to soil microorganisms, which are involved in the formation, transformation and decomposition of SOC (Fan and Liang, 2015; Kallenbach et al., 2016). However, the relationship between soil microbial communities and SOC change is still not fully understood, limiting our ability to predict soil C storage in terrestrial ecosystems, including croplands (Wieder et al., 2015). Thus, their relationship needs further study (Malik et al., 2016; Anthony et al., 2020).

Previous studies assessed the relation between soil C storage and various soil microbial community traits (Li et al., 2021b; Duan et al., 2021), including diversity, composition and keystone taxa (e.g. Fontaine and Barot, 2005; Kallenbach et al., 2016; Zheng et al., 2021). Li et al. (2020a) found that microbial diversity is positively correlated with SOC stocks and total N under no-till management, suggesting that more diverse soil microbial communities may be conducive to soil C storage. However, the impact of tillage practices on soil microbial diversity differs among previous studies. For instance, Legrand et al. (2018) reported that bacterial diversity was more responsive to tillage disturbance than fungal diversity, whereas Wang et al. (2020) found the opposite. As microbial diversity is linked to C transformation rates (Wertz et al., 2006; Wagg et al., 2014) and nutrient availability (Maron et al., 2018), it plays a crucial role in predicting ecosystem functioning and soil C storage potential under different management practices.

Besides microbial diversity, the composition of the soil microbial community also affects soil C dynamics. In this context, microbial taxa are usually divided according to their functions and the difference between soil bacteria and fungi is especially important (Waldrop et al., 2000; Lin et al., 2020). Fungi have a greater capability to degrade lignin, cellulose, and other recalcitrant organic C (Crowther et al., 2012), whereas bacteria generally prefer labile organic matter such as sugars and fats (Paterson et al., 2008; Zheng et al., 2018). These preferences are reflected in the extracellular enzymes released by either microbial group; bacteria are the main producers of cellulase (e.g. β -1,4-glucosidases, β -1,4-xylanases and β -1,4-D-cellulohydrolases) targeting labile C pools, whereas fungi are the main producers of non-specific ligninase (e.g. peroxidases and polyphenol oxidases) depolymerizing recalcitrant C pools (Chen et al., 2020a). Recent evidence suggests that soil C stocks across tillage practices are correlated with the abundance of bacterial groups, but not fungal groups (e.g. Sun et al., 2018). Whereas a recent meta-analysis showed that conservation tillage practices do not affect fungal-to-bacterial biomass ratios on average (Chen et al., 2020b). Hydbom and Olsson (2021) found conservation tillage had a positive effect on arbuscular mycorrhizal fungi but not on saprotrophic fungi and bacteria. This suggests that treatment effects are context dependent, and underline the need to study the relation between SOC and microbial composition across a range of management practices and environmental conditions.

Recent studies have used network analyses (Weiss et al., 2016) to identify taxonomic levels and keystone microbial groups in soils (e.g. Lupatini et al., 2014). These analyses suggest that keystone microbial groups are affected by tillage frequency and straw management, with possible consequences for soil C dynamics. For instance, Banerjee et al. (2016) found that straw amendment favor keystone taxa such as *Acidobacteria*, *Frateuria*, and *Gemmimonas* in bacteria and *Chaetomium*, *Cephalotheca*, and *Fusarium* in fungi, which had strong associations with organic matter decomposition rates. Lin et al. (2019) identified *Thermogemmatisporales* as the most important keystone taxa across various straw and manure treatments and found that this taxon decreased with an increase in SOC content. Li et al. (2021b) indicated that several bacterial taxa (e.g. *Acidobacteria* and *Bacteroidetes*) were closely related to turnover of specific organic components during straw decomposition

under different tillage practices. Using a similar approach, several studies linked specific fungal and bacterial taxa to organic matter decomposition and transformation in agricultural soil (Banerjee et al., 2016; Li et al., 2017). These preferences of bacteria and fungi for specific residue compounds may affect long-term soil C dynamics (Frey et al., 2003; Fontaine et al., 2011). However, the role of keystone taxa in SOC accumulation itself remains to be explored.

The Loess Plateau, a semi-arid region in northwest China, suffers from severe soil erosion and soils in this area are not conducive to soil C sequestration (Zhang et al., 2014a). In addition, conventional tillage practices have caused a decline in SOC stocks across the Loess Plateau (Chen et al., 2009; Hou et al., 2013). Conservation tillage practices have often been proposed to stimulate soil C sequestration in this area (Zhang et al., 2014b; Wang et al., 2018), but the relation between soil microbial traits (i.e. diversity, composition and keystone taxa) and SOC accumulation is still unclear. Thus, our objectives in this study were: (i) to evaluate the impact of tillage managements on the soil environment (e.g. C and N properties), microbial community traits, microbial activity, and SOC accumulation rate (SAR); (ii) to identify keystone taxa and assess the links between soil microbial community traits and SAR.

2. Materials and methods

2.1. Site description and experimental design

A long-term field experiment was established in 2003 at the Dryland Farming Experimental Station, which is located in Shou Yang, Shanxi Province ($113^{\circ}10' E$, $37^{\circ}90' N$, 1100 m. ASL), on the Loess Plateau in northeast China. The site has a continental monsoon climate, with mean annual evaporation of 1700–1800 mm (Wang et al., 2019), mean annual precipitation of 483 mm, and a mean annual temperature of 7.4 °C (Li et al., 2021c). Fig. S1 showed the detailed rainfall and mean daily temperature data. The site has a sandy loam cinnamon soil, classified as a Calcaric-Fluvic Cambisol (Wang et al., 2011). Initial soil properties before the establishment of the experiment are reported in Table S1. Spring maize, a crop grown on over 50% of the cropland area of the Loess plateau (Wang et al., 1999), is grown at the site in a one-crop-per-year system.

The experiment consists of 9 plots, measuring $5 \times 5 m^2$ each. Each plot received one of three management practices, which was applied in triplicate in a randomized complete block design: (1) CT-RR: conventional tillage with residue removal. These plots were plowed after harvesting (in October) and before seeding (in April) to ~ 25 cm depth with a moldboard plow. Maize stalks were removed after harvesting prior to tillage operations. (2) RT-RI: reduced tillage with residue incorporation. These plots were plowed once per year to ~ 25 cm depth with a plough after harvesting. Maize straws were retained and incorporated in the soil with tillage operations. (3) NT-RM: no-tillage with residue mulching. These plots were not tilled. Maize stalks were mulched after harvesting. In April of each year, a no till-planter was used for seeding and fertilizing. For the purpose of this study, RT-RI and NT-RM were both considered conservation tillage practices. Spring maize was sown at a rate of $30 kg ha^{-1}$ at the end of April and the crop was harvested in October. The row and plant spacings were 60 cm and 30 cm. Each plot received $105 kg N ha^{-1} yr^{-1}$ (urea) and $105 kg P_2O_5 ha^{-1} yr^{-1}$ (calcium superphosphate). Table S2 and Fig. S2 summarized the major field management operations for each treatment.

2.2. Soil sample collection

Soil samples were obtained at harvest 2019 (17 years after experimental plots were established). We collected five soil cores per plot at 0–10 cm and 10–25 cm; the depth of 25 cm corresponds to the maximum tillage depth in the RT-RI and CT-RR plots. The samples were bulked per soil layer, cleaned of roots, ground, and sieved (2 mm). Soil samples for high-throughput sequencing were stored at $-80^{\circ}C$. Samples for other

soil physicochemical analyses were divided into two parts. One part was air-dried at room temperature for SOC and total soil N (TN) analyses. The other part was kept field-moist at 4 °C, and was analyzed for microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), ammonium nitrogen (NH_4^+ -N), nitrate nitrogen content (NO_3^- -N) and enzyme activities. Historical soil samples were taken in 2003 using the same approach outlined above. These samples were air-dried and stored prior to analyses.

2.3. Soil properties

MBC and MBN were determined by fumigation extraction and calculated using conversion factors of 0.45 for MBC and 0.54 for MBN (Vance et al., 1987). NH_4^+ -N and NO_3^- -N contents were extracted using 2.0 M KCl at a ratio of 1:5 (w/v), filtered with quantitative filters, and measured using a continuous flow Autoanalyzer (Autoanalyzer 3, SEAL, Germany) (Lin et al., 2018). Samples for TN and SOC analyses were air-dried, passed through a 0.15-mm mesh size and treated with 1.0 M HCl to remove carbonates. The samples were then analyzed using an elemental analyzer (Vario Macro C/N, Elementar, Germany). Soil temperature was recorded using a geothermometer and soil moisture was measured gravimetrically. The SOC stocks (Lee et al., 2009; Modak et al., 2019) was calculated as follows:

$$\text{SOC stocks}(\text{Mg ha}^{-1}) = \left[\frac{\text{SOC}(\%)}{100} \right] \times \text{bulk density}(\text{Mg m}^{-3}) \times \text{soil depth}(m) \times 10000(\text{m}^2 \text{ ha}^{-1})$$

Soil bulk density, SOC concentrations (%) and SOC stocks (Mg ha^{-1}) for the initial and final sampling year were shown in Fig. S4. We used these data to calculate SAR, i.e. the average change in SOC stocks per year (Bhattacharyya et al., 2015) after 17 years of experimental treatments:

$$\text{SAR} (\text{Mg ha}^{-1} \text{yr}^{-1}) = (\text{SOC}_{\text{final}} - \text{SOC}_{\text{initial}}) / \text{experiment duration (yr)}$$

where $\text{SOC}_{\text{final}}$ and $\text{SOC}_{\text{initial}}$ indicated the SOC stocks in 2019 and 2003, respectively.

2.4. Soil microbiological properties

2.4.1. Enzyme activity

Soil microbial activity was assessed based on the activities of cellulase and ligninase. Cellulase activity, associated with labile-C-cycling, was represented by β -1,4-glucosidase (BG), cellobiohydrolase (CBH), β -1,4-xylanidase (BXYL). Ligninase activity, associated with recalcitrant-C-cycling, was represented by polyphenol oxidase (PPO) and peroxidase (PER). Microbial preference for various C sources was quantitatively characterized with the ratio of ligninase to cellulase activity (Yang et al., 2019). All enzyme activities were analyzed by a microplate fluorometer (Scientific Fluoroskan Ascent FL, Thermo) and different substrates (Table S3) according to DeForest (2009), German et al. (2011) and Ai et al. (2012).

2.4.2. High throughput sequencing analyses

The 0.5 g frozen soil were used to extract DNA with a Power Soil DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA). The purity and quality of the extracted DNA were checked on 1% agarose gels using a NanoDrop spectrophotometer (Thermo Scientific). The DNA was used for quantitative analysis and 16 S and ITS gene amplicon sequencing. The PCR was carried out on a Master cycler Gradient (Eppendorf, Germany) using 25 μl reaction volumes with 30 ng of template DNA, containing 12.5 μl 2xTaq Plus Master Mix, 1 μl Forward Primer (5 μM), 1 μl Reverse Primer (5 μM), 3 μl BSA (2 ng μl^{-1}), and 7.5 μl ddH₂O.

The V3-V4 hypervariable region of bacterial 16 S rRNA gene was amplified with the primers 338 F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806 R (5'-GGACTACNGGTATCTAAT-3') (Caporaso et al., 2012;

Derakhshani et al., 2016). The ITS region of fungi 18 S rRNA gene was amplified with the primers ITS1F/ITS2R (CTTGGTCATTAGAGGAAGTAA/TGCGTTCTTCATCGATGC) (Luan et al., 2015). DNA amplification and sequencing were performed by Beijing Auwigene Tech, Ltd. (Beijing, China) using the MiSeq sequencing platform (Illumina, Inc., San Diego, CA, USA).

2.4.3. Network construction and analyses

The co-occurrence network was constructed using the Molecular Ecological Network Analyses Pipeline (MENA, <http://ieg2.ou.edu/MENA/main.cgi>) (Zhou et al., 2010; Deng et al., 2012). The network of fungi and bacteria was built separately for each of the three management treatments. Because the difference in microbial community composition between soil depths is small relative to the difference between treatments (Table S6), our network analysis combined the data from both soil depths in each treatment. This approach resulted in six replicates per treatment, i.e. the appropriate sample requirement for network analysis (Deng et al., 2012; Zheng et al., 2018; Wang et al., 2021). To improve network reliability, only OTUs that were present in > 50% of all samples (He et al., 2017) with average relative abundances > 0.01% (Shi et al., 2020) were retained. Network topological properties such as average degree, geodesic distance, modularity and clustering coefficient were obtained; the use of these network topological properties can be found in Deng et al. (2012). The average degree describes the average number of neighbors per node; geodesic distance is the shortest path length to connect any two nodes; modularity denotes the extent that a network is divided into modules; the clustering coefficient represents the extent to which neighbors of a node in a network tend to cluster together (Shi et al., 2016). The topological role of each node was determined using the among-module connectivity (P_i) and the within-module connectivity (Z_i) (Guimerà and Nunes Amaral, 2005). The threshold values of P_i and Z_i for assorting network nodes are 0.62 and 2.5, respectively. Module hubs and connectors have been suggested to be as putative keystone taxa (Deng et al., 2012; Shi et al., 2016). Gephi (version 0.9.2) was used to visualize the co-occurrence network.

2.5. Statistical analyses

We assigned raw reads to different samples based on the barcodes and trimmed the primers by Illumina Analysis Pipeline Version 2.6. The split sequences were merged using FLASH (Magoc and Salzberg, 2011), after low-quality sequences (reads \leq 230 bp or quality scores < 20) were removed using QIIME v. 1.9.1 (Caporaso et al., 2010). Finally, sequences were clustered into operational taxonomic units (OTUs) at a similarity level of 97% using UPARSE version 7.0 (http://www.drive5.com/usearch/manual/uparseotu_algo.html) (Edgar, 2013). We classified all sequences in taxonomic groups through the Ribosomal Database Project Classifier tool (Wang et al., 2007). In total, 528858 bacterial and 417996 fungal sequences from 18 soil samples were respectively grouped into 5517 and 1803 OTUs (Table S4). For each sample, OTU was normalized to the same least sequences for alpha diversity analyses.

Prior to statistical analysis, we tested data on soil properties, SOC accumulation rate, microbial activity and sequences for homogeneity of variance by a Levene's test. Then, the effects of treatments and soil depths on soil properties, microbial activity and α -diversity were analyzed by a two-way ANOVA using SAS v8.0 software (SAS Institute Inc., USA). To assess the variation in microbial composition between treatments and depths, the similarities in OTU composition were assessed by principal coordinate analysis (PCoA) based on the Bray-Curtis dissimilarity matrix using the 'vegan' package using R software version 3.6.2 (<http://www.R-project.org>). Simultaneously, a nonparametric permutational multivariate analysis of variance (R vegan ADONIS) (Anderson, 2001) with 999 permutations was carried out to test for significant differences among sample groups based on Bray-Curtis distance. A Mantel test was used to analyse the correlations between SOC accumulation rate and microbial community traits based

on Pearson's product-moment using the 'vegan' package. Among microbial community traits, microbial diversity parameters, including Observed species, Chao1, PD whole tree and Shannon diversity index, were assessed by QIIME; microbial composition contains total taxa at the OTU level; microbial keystone OTUs are these putative taxa by the co-occurrence network. For both fungi and bacteria, relationships between keystone taxa and microbial activity and SAR were analyzed by Spearman's correlation at a significance level of 0.05. The data analysis and corrplots were performed using R packages "ggplot2", "vegan", "corrplot", and "Hmisc".

3. Results

3.1. Soil properties and SAR

Soil properties differed among the three treatments (Table 1). Compared to CT-RR, both RT-RI and NT-RM significantly increased SOC and MBC contents at 0–10 cm, whereas only RT-RI increased SOC and MBC contents at 10–25 cm. The TN contents were similar for the three treatments at 0–10 cm, and the highest TN contents were observed under RT-RI at 10–25 cm. Soil NO₃⁻-N concentrations also differed significantly among treatments at both soil depths and ranged from high to low as follows: RT-RI > CT-RR > NT-RM. NT-RM increased soil NH₄⁺-N concentrations at both depths relative to RT-RI and CT-RR. Moreover, the soil temperature of RT-RI and NT-RM were lower than CT-RR at both soil depths, especially under NT-RM (Fig. S3).

We found that SAR was significantly greater under RT-RI (+116%) and NT-RM (+131%) compared with CT-RR at 0–10 cm. At 10–25 cm, RT-RI increased SAR relative to both other treatments (Table 1). In addition, SAR was significantly higher at 10–25 cm than at 0–10 cm for RT-RI treatment.

3.2. Soil enzyme activity

Soil enzyme activity varied strongly between treatments ($p < .05$) and soil depths ($p < .01$, except for PPO) (Table S5). At 0–10 cm, RT-RI increased the activity of PPO relative to CT-RR, and NT-RM increased the activities of all enzymes included in our analysis relative to CT-RR (Fig. 1). In the 10–25 cm layer, NT-RM increased the activities of CBH and BXYL and decreased the activity of PER relative to CT-RR. NT-RM decreased the ratio of ligninase to cellulase activity at both depths compared to CT-RR and RT-RI.

3.3. Soil microbial diversity, composition, and co-occurrence networks

The community alpha diversity was evaluated based on four common indexes (i.e. Observed species, Chao1, PD whole tree, and Shannon)

(Table 2). At 0–10 cm, NT-RM increased the alpha diversity of both the bacterial and fungal communities relative to CT-RR and RT-RI. However, at 10–25 cm, the alpha diversity of the bacterial community was lower under NT-RM compared with CT-RR and RT-RI. Tillage practices did not affect alpha diversity indicators of the fungal community at 10–25 cm, except for the Shannon index being lower for RT-RI than for CT-RR and NT-RM. A principal coordinate analysis (PCoA) indicated that both the bacterial (Fig. 2B) and fungal (Fig. 2D) community composition could be well separated by treatments. Overall, this analysis explained 47% and 54% variation in bacterial and fungal communities, respectively. Moreover, an Adonis analysis showed that the total difference among treatments (bacteria $p = .001$, fungi $p = .036$) was greater than that between two soil depths (bacteria $p = .008$, fungi $p = .331$) (Table S6). Specifically, the abundance of *Proteobacteria* increased under NT-RM compared with CT-RR and RT-RI at 0–10 cm (Fig. S5), whereas the abundance of *Ascomycota* significantly increased under RT-RI compared with CT-RR and NT-RM at both soil depths (Fig. S6). Management practices did not affect the abundance of phyla *Acidobacteria*, *Chloroflexi*, and *Mortierellomycota* at either soil depth.

Bacterial and fungal networks at the OTU level were generated for each treatment (Table S7). The six networks are regarded as modular given that all the modularity is above 0.4 (Newman, 2006). For bacterial networks, RT-RI and NT-RM increased the total nodes (+46% and +14%), links (+196% and +54%), average connectivity (+103% and +36%), and average path length (+922% and +121%) compared with CT-RR, indicating that RT-RI and NT-RM resulted in a more complex co-occurrence network. A large proportion of bacterial interactions under NT-RM consisted of negative links. From the Zi-Pi plot, we found 6 bacterial nodes sinking into "module hubs" and 4 nodes sinking into "connectors" under RT-RI (Fig. 4A). Members from *Actinobacteria*, *Acidobacteria*, *Bacteroidetes*, *Chloroflexi*, *Gemmimonadetes*, and *Proteobacteria* were identified as keystone bacterial taxa.

For fungal networks, the total nodes, links, average connectivity, and average path length showed a decreasing trend under RT-RI and an increasing trend under NT-RM compared with CT-RR (Table S7). Fungal interactions were connected through more negative links under RT-RI (55%) and NT-RM (49%) than under CT-RR (47%). Using a Zi-Pi plot, we classified 2 and 4 fungal nodes as "module hubs" and 5 and 0 nodes as "connectors" under CT-RR and NT-RM, respectively (Fig. 4B). Members from *Ascomycota*, *Basidiomycota*, *Glomeromycota*, *Mortierellomycota*, and *Rozellomycota* were identified as keystone fungal taxa. Table 3 further summarized more details of these hubs and connectors.

3.4. Correlations between SAR and soil microbial community traits

A Mantel test revealed that bacterial diversity and keystone OTUs were all highly correlated with SAR under NT-RM (Table 4). Specifically, OTU_1760 (*Cytophagales*), OTU_4685 (*JG30-KF-CM45*) and

Table 1

Soil C and N properties under three treatments following a 17-year field trial. CT-RR: conventional tillage with residue removal; RT-RI: reduced tillage with residue incorporation; NT-RM: no-tillage with residue mulching. SOC: Soil organic carbon; TN: total nitrogen; NH₄⁺-N : ammonium nitrogen; NO₃⁻-N : nitrate nitrogen; MBC: microbial biomass carbon; MBN: microbial biomass nitrogen; SAR: SOC accumulation rate.

Soil layer (cm)	Treatments	SOC g kg ⁻¹	TN g kg ⁻¹	NH ₄ ⁺ -N mg kg ⁻¹	NO ₃ ⁻ -N mg kg ⁻¹	MBC mg kg ⁻¹	MBN mg kg ⁻¹	SAR Mg C ha ⁻¹ yr ⁻¹
0–10	CT-RR	16.85(0.68)b	1.03(0.07)a	7.04(0.16)b	1.79(0.03)b	66.84(1.04)c	12.50(0.64)b	0.25(0.09)b
	RT-RI	19.91(0.00)a	1.15(0.04)a	6.28(0.28)b	9.08(0.90)a	91.02(1.31)b	15.18(0.28)a	0.54(0.02)a
	NT-RM	19.99(0.21)a	1.13(0.04)a	9.37(0.30)a	0.45(0.05)b	158.74(10.09)a	13.87(0.18)ab	0.58(0.02)a
10–25	CT-RR	16.50(0.40)b	0.92(0.02)b	6.29(0.55)ab	5.77(0.17)b*	61.66(3.58)b	8.26(0.18)b*	0.41(0.03)b
	RT-RI	19.98(0.91)a	1.19(0.07)a	5.31(0.22)b	12.69(0.64)a*	115.82(7.01)a*	15.41(0.80)a	1.07(0.16)a*
	NT-RM	16.24(0.44)b*	0.91(0.03)b*	7.07(0.12)a*	0.29(0.07)c	58.22(3.14)b*	15.40(0.49)a*	0.33(0.05)b*
Treatment		$p < .001$	$p < .01$	$p < .001$	$p < .001$	$p < .001$	$p < .001$	$p < .001$
Depth		$p < .01$	$p < .05$	$p < .001$	$p < .001$	$p < .001$	ns	$p < .05$
Treatment×Depth		$p < .01$	ns	ns	$p < .01$	$p < .001$	$p < .001$	$p < .001$

Note: The data represent the mean (standard error); n = 3. Different lowercase letters (a-c) indicate significant differences among three treatments within the same depth at the level of $p < .05$; * indicates significant differences between depths within the same treatment.

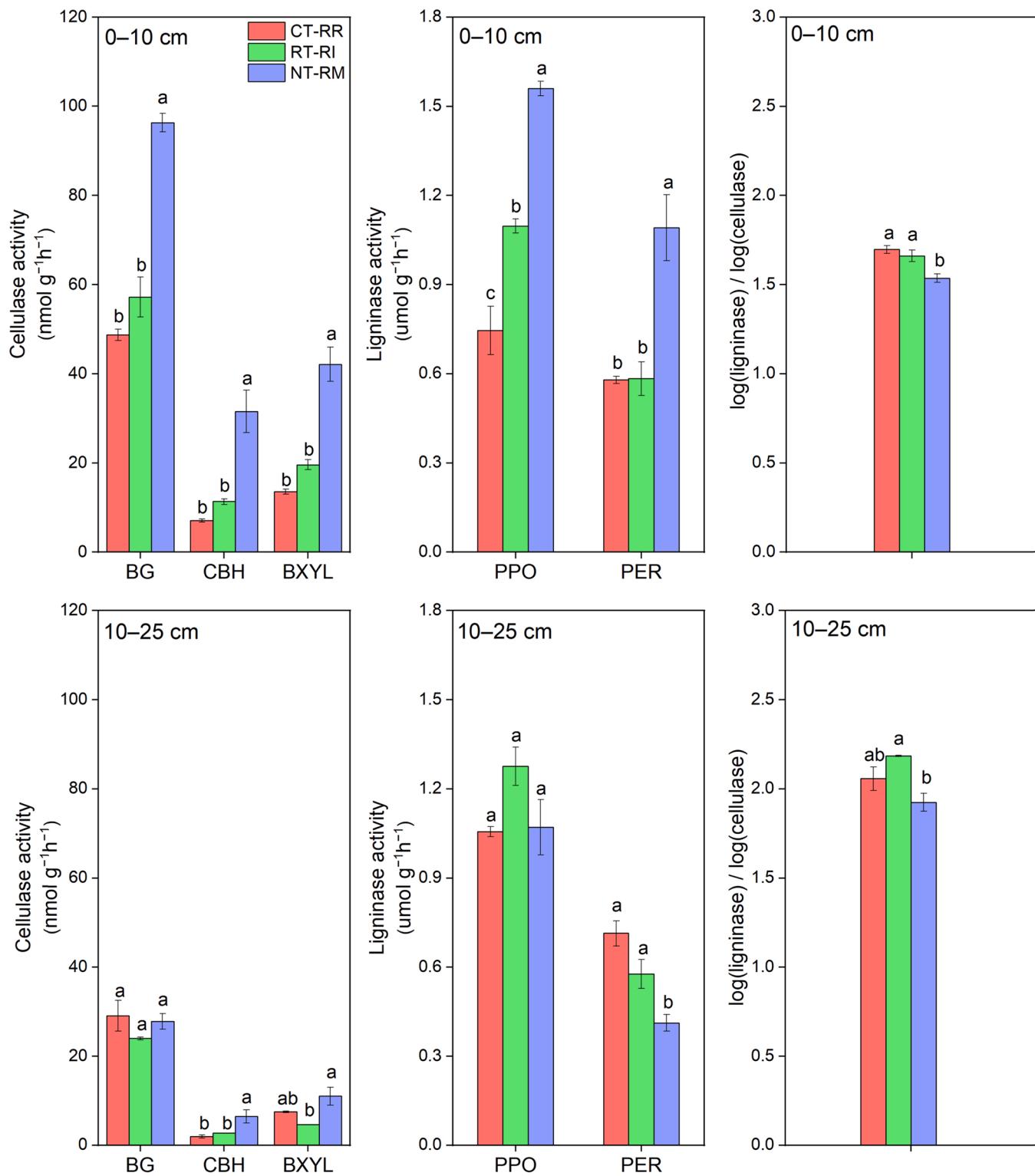


Fig. 1. Activities of soil cellulases (BG, CBH and BXYL) and ligninases (PPO and PER) affected by three treatments at two soil depths. Results are based on one-way ANOVAs that compared activities among three treatments between two soil depths. Mean \pm SE (standard error) are shown ($n = 3$). Different lowercase letters indicate significant differences among the three treatments. BG: β -1,4-glucosidase; CBH: cellobiohydrolase; BXYL: β -1,4-xylanidase; PPO: polyphenol oxidase; PER: peroxidase.

OTU_940 (*Sphingobacteriales*) were significantly correlated with SAR at 0–10 cm or 10–25 cm (Fig. 5A, B). Fungal keystone OTUs were significantly correlated with SAR under both RT-RI and NT-RM (Table 4). Most fungal OTUs were not significantly correlated with SAR. OTU_131 (*Thelephorales*) was significantly correlated with SAR at 10–25 cm.

However, OUT_836 (*Pleosporales*) correlated negatively with SAR (Fig. 5C) in the 0–10 cm layer, and correlated positively with SAR in the 10–25 cm layer (Fig. 5D).

Table 2

Alpha-diversity indices of bacteria and fungi at two soil depths as affected by three treatments.

Depths (cm)	Treatments	Bacteria				Fungi			
		Observed species	Chao1	PD whole tree	Shannon	Observed species	Chao1	PD whole tree	Shannon
0–10	CT-RR	2722.63(12.21) b	3301.65(11.30) b	212.01(1.39) b	9.82(0.02) b	480.97(21.94) b	641.53(39.15) b	95.11(5.01) b	6.03(0.31) b
		2690.60(51.70) b	3368.53(73.83) b	211.94(4.01) b	9.77(0.03) b	427.60(15.61) b	579.25(25.79) b	84.89(1.93) b	5.45(0.17) b
		3053.27(39.89) a	3721.39(72.42) a	238.80(2.69) a	10.11(0.02) a	710.00(10.26) a	864.44(27.65) a	131.71(2.58) a	6.96(0.04) a
	RT-RI	2732.47(37.30) a	3286.96(36.67) a	206.58(3.24) a	9.79(0.02) a	596.97(87.76) a*	734.50(75.63) a*	103.86(10.32) a	6.51(0.11) a
		2661.90(43.76) a	3238.90(67.28) ab	202.59(2.63) ab	9.82(0.04) a	509.63(30.35) a	640.53(30.99) a	91.59(3.31) a	5.46(0.17) b
		2516.43(18.25) b*	3077.78(20.38) b*	195.28(1.78) b*	9.62(0.06) b*	606.30(79.34) a	741.60(66.77) a*	105.46(11.86) a	6.57(0.29) a
Treatment (T)		p < .05	p < .01	p < .001	ns	p < .001	p < .001	p < .01	p < .001
Depth(D)		p < .01	ns	p < .05	p < .001	ns	ns	ns	ns
T × D		p < .001	p < .001	p < .001	p < .001	p < .01	p < .01	p < .05	ns

Note: Different lowercase letters indicate significant differences among three treatments within the same depth ($p < .05$). * indicates significant differences between two depths within the same tillage treatment.

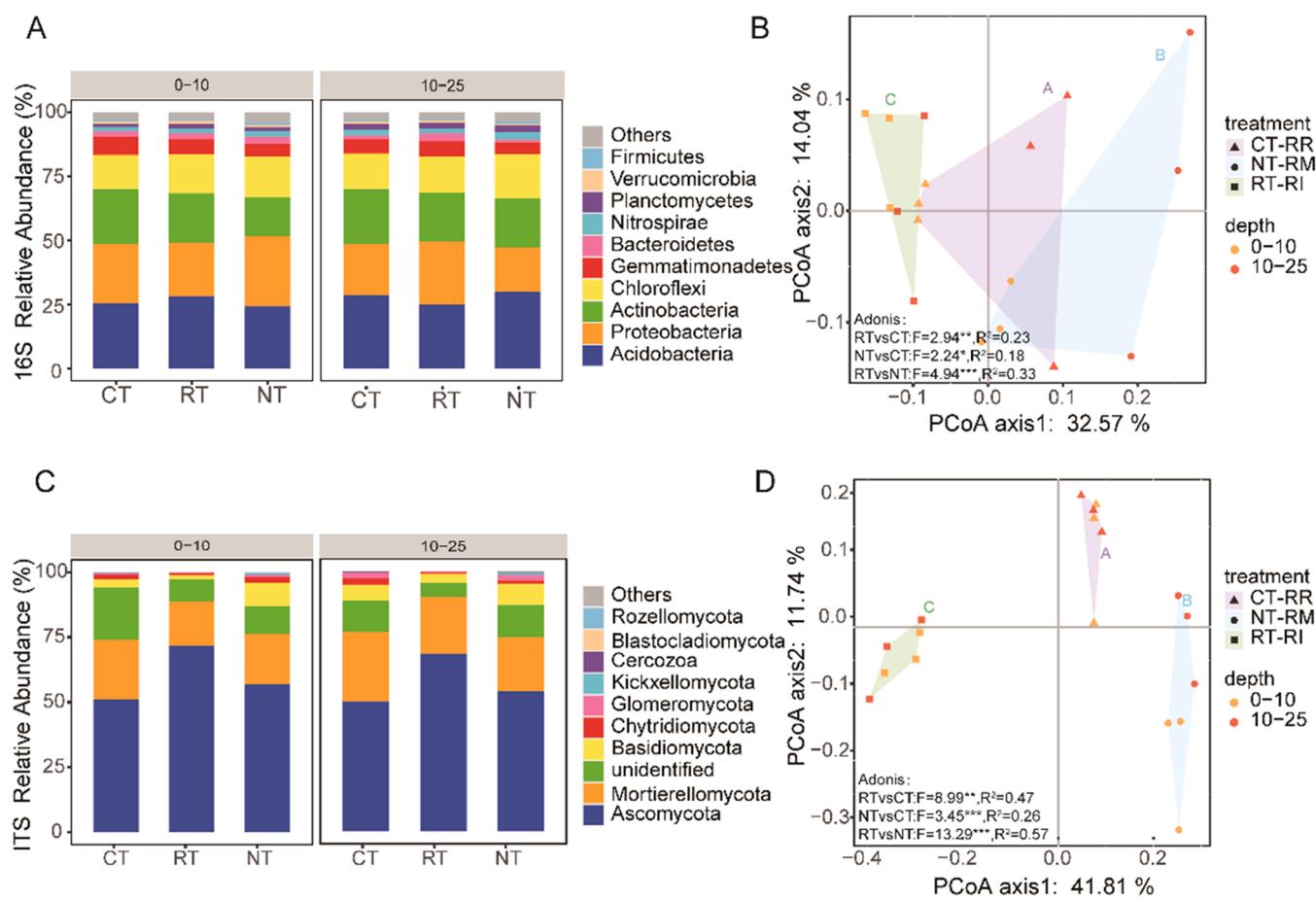


Fig. 2. The top 10 relative abundances of the bacterial (A) and fungal (C) communities structure at the phylum based on Illumina sequencing of the 16 S rRNA gene and the ITS region from three treatments at two soil depths. The data represent the mean values ($n = 3$). And the bacterial (B) and fungal (D) communities composition were assessed by principal coordinate analysis (PCoA, based on the Bray-Curtis distance) and Adonis (based on permutational MANOVA). For the single treatment, A (light purple), B (light blue), and C (light green) indicate the CT-RR, NT-RM, and RT-RI treatments, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

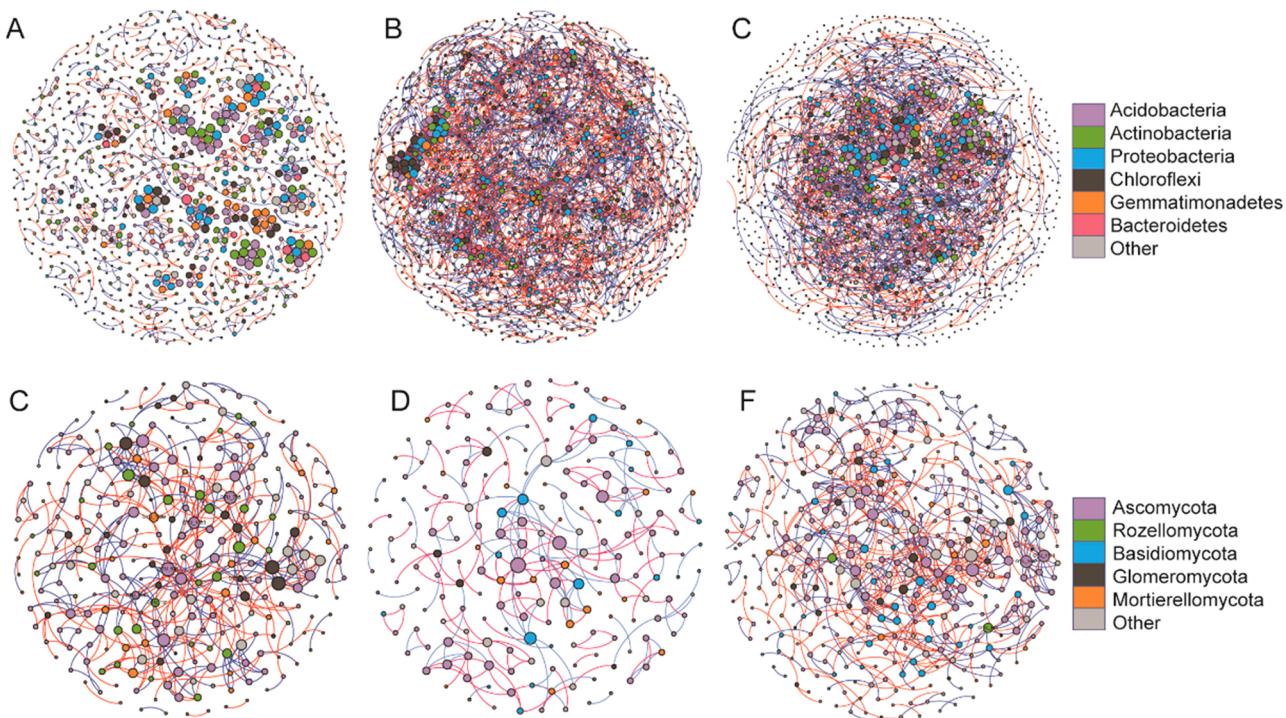


Fig. 3. Co-occurrence networks of the bacterial (A-C) communities and the fungal (D-F) communities under CT-RR, RT-RI, and NT-RM treatments, respectively. The node size is equivalent to the relative abundance. The line between each pair of nodes represents strong positive (blue) or negative (red) interaction. Only the average relative abundances $> 0.01\%$ and the occurrence $> 50\%$ in all samples were included in the analysis. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

4. Discussion

4.1. SAR in response to tillage practices

Soil C accumulation reflects the net balance between organic matter inputs (e.g. crop residue and rhizodeposition) and soil C decomposition by soil microbes (e.g. Meng et al., 2017). Compared with CT-RR, RT-RI increased SAR at both soil depths, and NT-RM increased SAR at 0–10 cm (Table 1). These findings corroborate numerous studies suggesting that conservation tillage practices with residue retention stimulate SOC sequestration compared to conventional tillage without residue retention, especially in the topsoil (e.g. Zhao et al., 2015; Modak et al., 2019). Conventional management practices usually increased SOC decomposition due to high-frequency soil disturbance and decreased soil C input through residue removal (Huggins et al., 2007). The minor positive SAR values in the CT-RR treatment likely reflect the change in management practices throughout the experiment relative to previous management, e.g. changes in planting density or fertilization rates, both of which affect plant growth and thus soil C input (Zanatta et al., 2007; Stewart et al., 2017; Fiorini et al., 2020).

Higher SAR was induced by RT-RI than NT-RM in the 10–25 cm layer, probably because residue input under RT-RI was mixed throughout the soil profile by tillage operations, thereby stimulating C and N accumulation in the subsoil. Similarly, SOC, TN, NO_3^- -N and MBC contents all showed the same trend as SAR among the three treatments at 10–25 cm (Table 1). Previous research at our site showed that RT-RI increased yields relative to NT-RM (Li et al., 2020b), suggesting that RT-RI may also have stimulated soil C accumulation by increasing plant growth and soil C input. Finally, no-till practice may stimulate soil C storage by increasing soil aggregate stability (Guo et al., 2020). Indeed, NT-RM improved soil aggregate stability at our site (Li et al., 2020b) and in a global meta-analysis (Liu et al., 2021) presumably by increasing microbial biomass and their activity (Kandeler and Murer, 1993). However, the increase in aggregate stability is most pronounced in the

topsoil (Wright and Hons, 2005; Zhang et al., 2018a), suggesting this mechanism has little impact on soil C storage at lower soil depths.

4.2. The effects of tillage practices on microbial community traits

We found that 17 years of tillage practices significantly affected both bacterial and fungal diversity (NT-RM $>$ RT-RI = CT-RR) at 0–10 cm (Table 2; Fig. 2). These results likely reflect a difference in substrate availability; under NT-RM, soil C input in the 0–10 cm layer was higher than under both RT-RI, where similar amounts of residue were distributed across a larger soil depth range through tillage operations, and under CT-RR, where residue was removed. In addition, NT-RM increased soil moisture at our site relative to RT-RI and CT-RR throughout several years (Li et al., 2020b), which in turn may promote soil microbial diversity (Maestre et al., 2015). Finally, slightly lower soil temperatures under NT-RM and RT-RI relative to CT-RR may also have an impact, as soil temperatures closely affect the diversity of microbial communities (Sabri et al., 2018).

Understanding which microbial taxa are associated with the degradation of crop residue may improve predictions of soil C accumulation (Zhang et al., 2015, 2018b). In our study, NT-RM increased *Proteobacteria*, which prefer nutrient-rich environments, in the surface layer compared to the other two treatments (Fig. S5). This response may be driven by *Alphaproteobacteria* that is important for cellulose hydrolyzation (Pankratov et al., 2006). Indeed, NT-RM increased both the relative abundance of *Alphaproteobacteria* and the cellulase activity relative to CT-RR and RT-RI (Fig. 1 and Table S8).

The increase in the abundance of *Actinobacteria* under CT-RR relative to RT-RI and NT-RM (Fig. S5) was in line with Ramirez-Villanueva et al. (2015), who also found that *Actinobacteria* showed higher relative abundance under conventional practices than conservation agriculture. This result is likely related to the fact that conservation tillage practices increased labile C concentrations, which reduces the abundance of *Actinobacteria* (Fierer et al., 2007). Moreover, RT-RI and NT-RM

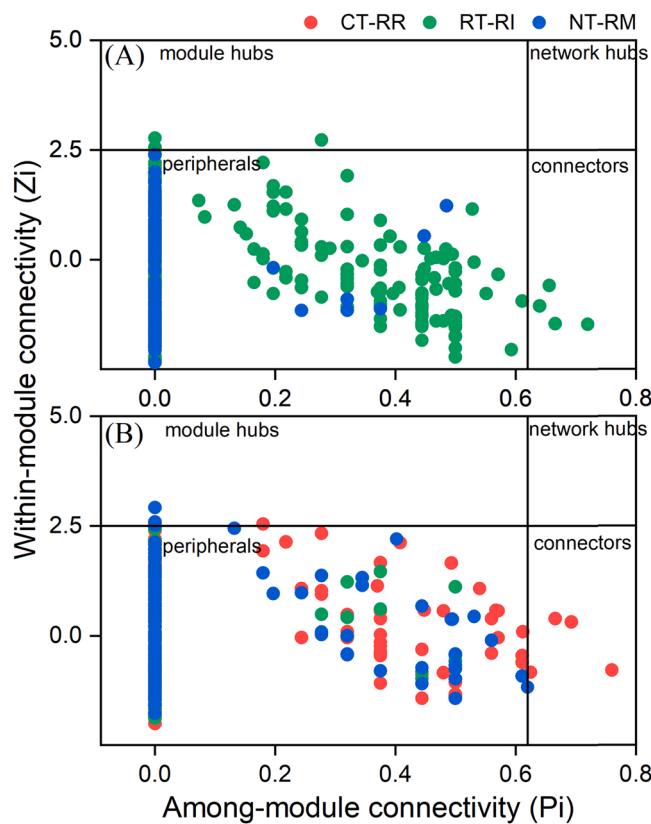


Fig. 4. Zi-Pi plots showing the distribution of OTUs based on their topological roles in bacterial (A) and fungal (B) networks. The threshold values of Zi and Pi for categorizing OTUs were 2.5 and 0.62, respectively. Nodes are defined as peripherals ($\text{Pi} \leq 0.62$, $\text{Zi} \leq 2.5$), module hubs ($\text{Pi} \leq 0.62$, $\text{Zi} > 2.5$), connectors ($\text{Pi} > 0.62$, $\text{Zi} \leq 2.5$) and network hubs ($\text{Pi} > 0.62$, $\text{Zi} > 2.5$). CT-RR: conventional tillage with residue removal; RT-RI: reduced tillage with residue incorporation; NT-RM: no-tillage with residue mulching.

significantly enhanced the relative abundance of *Bacteroidetes* and *Planctomycetes*. These results are supported by recent studies showing that the abundance of *Bacteroidetes* increased with straw return (Hao et al., 2019) and that *Planctomycetes* acted as slow-growing decomposers of soil organic matter (Kulichevskaya et al., 2012).

With regards to fungi, RT-RI increased *Ascomycota* at both soil depths relative to CT-RR and NT-RM, whereas *Basidiomycota* was significantly enriched under NT-RM in the surface layer (Fig. S6), implying that these dominant fungal phylum taxa have some functional overlap in the organic substrate decomposition process (Sun et al., 2018). Indeed, *Ascomycota* and *Basidiomycota* are the predominant saprotrophic fungi in agricultural ecosystems (Salo et al., 2019). We speculate that by lowering soil temperatures and increasing C contents, RT-RI produced environmental conditions that allow *Ascomycota* to better utilize easily degradable substrates (Table 1 and Fig. S3). This explanation is in line with Wang et al. (2016), who found that dense populations of *Ascomycota* appeared under conservation tillage and mainly affected crop residue decomposition. In addition, soil tillage under CT-RR and RT-RI generally increases soil porosity and soil oxygen content (e.g. Stępniewski and Stępniewska, 2009). These changes may suppress the growth of *Basidiomycota*, which usually degrade lignin and cellulose under anaerobic conditions (De Boer et al., 2005).

Long-term changes in tillage practices cause a shift in microbial co-occurrence networks at our site. The bacterial network under conservation management (i.e. RT-RI and NT-RM) and the fungal network under NT-RM were more complex, with more links and higher average connectivity relative to CT-RR (Fig. 3). This could be due to the increase in soil C and N pools and decomposition rates supported by the increase of microbial biomass, providing more interactions among microbial taxa (Zhou et al., 2020). These negative interactions possibly indicate competition for nutrient resources (Ghoul and Miti, 2016), and they increase the stability of networks (de Vries et al., 2018). Thus, our results suggest that NT-RM promotes a stable network in the bacterial community, whereas both RT-RI and NT-RM built up a stable network in the fungal community (Table S7).

4.3. Linking microbial community traits and SOC accumulation

Bacterial and fungal diversity were significantly and positively correlated with SAR under NT-RM and RT-RI, respectively (Table 4). Generally, the addition of residue under conservation tillage practices increases soil C and N contents (Giambalvo et al., 2018) and microbial metabolic activity (Zhang et al., 2016), thereby increasing soil microbial diversity. However, microbial diversity does not just respond to increased C input, it may also drive soil C accumulation. For instance, by increasing microbial diversity, conservation tillage can stimulate the availability of nutrients, especially N (Table 1). This in turn may

Table 3

The keystone taxa identified as module hubs and connectors in the soil bacterial and fungal networks.

Category	OTU	Role	Phylum	Order	Degree	Pi	Zi	Betweenness centrality	Clustering coefficient	Treatments
Bacteria	OTU_110	Module hub	<i>Gemmatimonadetes</i>	<i>Gemmatimonadales</i>	12	0	2.772	26634.13	0.424	RT-RI
	OTU_940	Module hub	<i>Bacteroidetes</i>	<i>Sphingobacteriales</i>	12	0	2.772	26634.13	0.424	RT-RI
	OTU_3254	Module hub	<i>Actinobacteria</i>	<i>Micromonosporales</i>	8	0	2.557	5308.89	0.321	RT-RI
	OTU_3654	Module hub	<i>Actinobacteria</i>	<i>Acidimicrobiales</i>	8	0	2.557	5308.89	0.321	RT-RI
	OTU_4353	Module hub	<i>Chloroflexi</i>	<i>Chloroflexales</i>	6	0.277	2.729	22312.12	0.133	RT-RI
	OTU_4685	Module hub	<i>Chloroflexi</i>	<i>JG30-KF-CM45</i>	12	0	2.722	26634.13	0.424	RT-RI
	OTU_142	Connectors	<i>Acidobacteria</i>	<i>unidentified</i>	8	0.656	-0.589	42896.48	0.142	RT-RI
	OTU_245	Connectors	<i>Acidobacteria</i>	<i>unidentified</i>	3	0.666	-1.459	16697.55	0	RT-RI
	OTU_1760	Connectors	<i>Bacteroidetes</i>	<i>Cytophagales</i>	5	0.64	-1.05	5342.09	0.2	RT-RI
	OTU_5175	Connectors	<i>Proteobacteria</i>	<i>Sphingomonadales</i>	5	0.72	-1.468	37629.18	0.1	RT-RI
Fungi	OTU_735	Module hub	<i>Ascomycota</i>	<i>Sordariales</i>	10	0.18	2.545	5026.86	0.177	CT-RR
	OTU_1350	Module hub	<i>Ascomycota</i>	<i>Hypocreales</i>	9	0	2.545	1678.35	0.305	CT-RR
	OTU_1615	Module hub	<i>Ascomycota</i>	<i>Sordariales</i>	8	0	2.587	2100	0.392	NT-RM
	OTU_940	Module hub	<i>Rozellomycota</i>	<i>unidentified</i>	10	0	2.517	2221	0.377	NT-RM
	OTU_1537	Module hub	<i>Ascomycota</i>	<i>Coniochaetales</i>	13	0	2.921	2832.23	0.371	NT-RM
	OTU_1113	Module hub	<i>Ascomycota</i>	<i>Hypocreales</i>	13	0	2.921	2832.23	0.371	NT-RM
	OTU_131	Connectors	<i>Basidiomycota</i>	<i>Thelephorales</i>	6	0.666	0.392	545.63	0.533	CT-RR
	OTU_596	Connectors	<i>Ascomycota</i>	<i>Oryngales</i>	6	0.666	0.392	545.63	0.533	CT-RR
	OTU_836	Connectors	<i>Ascomycota</i>	<i>Pleosporales</i>	11	0.76	-0.791	7361.49	0.109	CT-RR
	OTU_1817	Connectors	<i>Glomeromycota</i>	<i>Diversisporales</i>	4	0.625	-0.83	2109.16	0	CT-RR
	OTU_1499	Connectors	<i>Mortierellomycota</i>	<i>Mortierellales</i>	7	0.693	0.316	1916.99	0.19	CT-RR

Table 4

Mantel test correlations between soil accumulation rate (SAR) and soil microbial community traits (diversity, composition and keystone OTUs).

Factor	Treatment	Bacteria						Fungi					
		Diversity		Composition		Keystone OTUs		Diversity		Composition		Keystone OTUs	
		r	p	r	p	r	p	r	p	r	p	r	p
SAR	CT-RR	-0.280	0.824	0.561	0.050	0.761	0.024	-0.091	0.615	0.512	0.056	0.561	0.059
	RT-RI	0.123	0.307	0.140	0.311	0.340	0.131	0.599	0.036	0.138	0.311	0.625	0.033
	NT-RM	0.732	0.026	0.346	0.128	0.617	0.030	0.225	0.183	0.573	0.040	0.641	0.031

Notes: Similarity matrices of SAR and soil microbial traits were calculated by Euclidean distance and Bray-Curtis distance, respectively. The r values shown are correlation coefficients using the “Pearson” method, p values in boldface type indicate significant effects ($p < .05$).

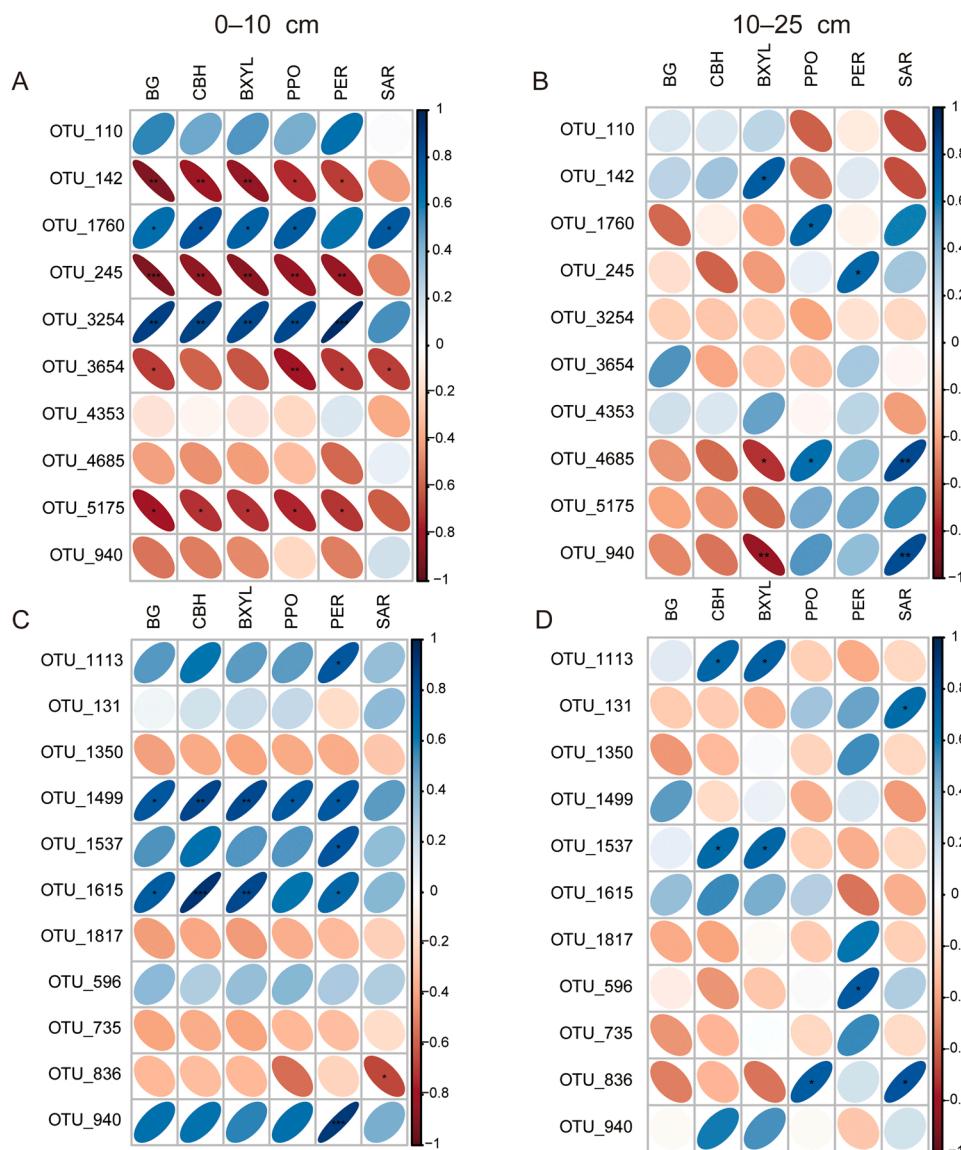


Fig. 5. Correlations between microbial activity and the relative abundance of keystone taxa (A and B for bacterial OTUs; C and D for fungal OTUs) at different layers (0–10 cm and 10–25 cm) of three treatments. Blue panels indicate a positive correlation and red panels indicate a negative correlation. The smaller the panel area, the larger the r-value. * indicates significant correlations (**p < .001; **p < .01; *p < .05). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

stimulate soil C storage by promoting plant growth and soil C input (e.g. Lu et al., 2011; Huang et al., 2020). Moreover, high N availability can promote soil C storage by reducing the activity of ligninase, a key enzyme for the degradation of recalcitrant C (e.g. Macdonald et al., 2018). Finally, the increase in microbial diversity and MBC may stimulate the secretion of microbial byproducts and the formation of microbial necromass, which contributes to the stable soil C pool (Prommer et al., 2020). These compounds might also stimulate soil aggregate formation, which in turn promote soil C accumulation (Wiesmeier et al.,

2019).

Keystone taxa play distinct roles in the soil microbial network (Berry and Widder, 2014) and some microbial taxa functions are highly correlated with soil C dynamics (Zhan et al., 2018). Indeed, our result also suggested a significant correlation between bacterial and fungal keystone OTUs and SAR (Table 4). Our result showed that OTU_1760 (*Cytophagales*) had a significant positive correlation with SOC accumulation at 0–10 cm (Fig. 5). *Cytophagales* belong to r-selected microbes (Wu et al., 2021). R-strategists consume labile substrates by releasing

extracellular enzymes, which stimulate microbial biomass formation and turnover (Fierer et al., 2012). Thus, the significant correlation possibly reflects microbial necromass accumulation contributing to long-term SOC storage, consistent with Kallenbach et al. (2016) who showed that microbial necromass is an important constituent of stable SOC. Both OTU_4685 (*JG30-KF-CM45*) and OTU_940 (*Sphingobacteriales*) correlated positively with SAR at 10–25 cm (Fig. 5). Previous research suggested that both these bacterial OTUs assimilate root exudates (Haichar et al., 2008; He et al., 2021) and incorporate plant-derived C (Hernández et al., 2015). Thus, the positive correlation may indicate that these taxa were involved in the stabilization of C derived from plant roots.

As for fungal keystone taxa, our result suggests that OTU_836 (*Pleosporales*), belonging to the *Ascomycota* phylum, was negatively and positively correlated with SAR at 0–10 cm and 10–25 cm (Fig. 5), respectively. These responses might reflect different levels of root biomass and rhizosphere C in the topsoils relative to subsoils. Indeed, Guo et al. (2021) found that maize root biomass in the topsoil is lower than that of the subsoil. Wang et al. (2017) suggested that *Pleosporales* are susceptible to rhizosphere influence by directly using root exudate C resources. Thus, we speculate that *Pleosporales* have more ability to transform rhizosphere C to SOC at 10–25 cm than 0–10 cm, thereby contributing to SAR.

Our study aimed to increase understanding on the role of the microbe in stimulating soil C storage and improving other ecosystem services. Indeed, the composition of soil microbial communities is increasingly recognized as important for C cycle processes (e.g. Denef et al., 2009; Zhang et al., 2013). Insights in soil microbial community functions are also gradually adopted as a tool to improve agroecosystem sustainability, e.g. by soil reciprocal transplantation and other methods (Liang et al., 2020). Unfortunately, our experiment does not allow us to distinguish between the various mechanisms through which microbes may promote soil C storage, i.e. by stimulating plant growth, creating stable forms of soil C through microbial turnover, and promoting soil aggregation. Future research might be able to quantify the effect of shifts in the microbial communities on these various mechanisms of soil C storage. For instance, the role of key taxa in soil C accumulation may be explored by combining lab incubation studies in the absence of plants with field experiments. Further insights may also be gained by quantifying the relation between microbial community composition, diversity and SAR at different resolution levels (e.g. within soil pores and within aggregate size classes), and by assessing the role of bacteria-fungi interactions in affecting soil C sequestration. Clearly, the role of soil microbial traits in determining C storage in agricultural soils cannot be ignored.

5. Conclusions

In summary, our study showed that RT-RI and NT-RM significantly increased SAR in the 0–10 cm soil layer relative to CT-RR, likely due to a combination of reduced soil disturbance and increased input of crop residue. Network analysis suggested that NT-RM resulted in a more stable bacterial network compared to conventional practices, and both RT-RI and NT-RM produced a more stable fungal network. Moreover, several bacterial and fungal keystone taxa and microbial diversity correlated positively to SOC accumulation rates. Together, these results suggest that RT-RI is a promising practice to improve C sequestration and soil health and highlight the importance of the relationship between microbial community traits and SOC accumulation under long-term conservation tillage in semi-arid areas.

Declaration of Competing Interest

The authors declare that there are no competing interests

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.still.2022.105360.

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