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Fungi determine increased soil organic carbon more than bacteria through their necromass inputs in conservation tillage croplands

Yali Yang [a,b,c], Hongtu Xie [a], Zhun Mao [c], Xuelian Bao [a], Hongbo He [a], Xudong Zhang [a], Chao Liang [a,*]

[a] Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang, 110164, China
[b] University of Chinese Academy of Sciences, Beijing, 100049, China
[c] Univ Montpellier, AMAP, INRAE, CIRAD, CNRS, IRD, 34000, Montpellier, France

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ABSTRACT

Stover mulching over no-till soil is regarded as a promising practice to increase soil organic carbon (SOC) in croplands against climate change. Microbial necromass is a significant source of SOC stock and unequivocally controlled by the microbial community. Yet, a complete link that spans from agricultural practices to microbial community features, to soil necromass C, and eventually to SOC is poorly understood. Here, we conducted a 10-y corn field experiment with five treatments, which included conventional tillage (CT), no-tillage without stover (NT–0), and no-tillage with low, medium, and high amounts of stover mulching (NT–low, NT–medium, and NT–high) in a Molisol of northeastern China. We investigated the stocks and changes in total SOC and its microbial necromass C along a soil depth down to 40 cm, and we evaluated how SOC dynamics and stabilization processes were associated with microbial community features. We characterized microbial community diversity and structure using 16S rRNA and internal transcribed spacer (ITS) sequencing, and we characterized microbial biomass and necromass using phospholipid fatty acid and amino sugar biomarkers. Compared with conventional tillage, no-tillage with medium and high amounts of stover mulching increased SOC stocks in the upper 0–40 cm of soil by $>$ 0.4% per year. No-tillage treatments (without and with stover) had almost no effect on the proportion of total microbial necromass C to SOC, but greatly modified the ratio of fungal necromass C to bacterial necromass C, which increased in top layers (0–5 cm) and decreased in deep layers (10–40 cm). SOC was governed mainly by fungal necromass C, which was correlated positively with fungal biomass. Fungal necromass C, not bacterial necromass C, was more closely associated with microbial community composition. Our results suggested that no-tillage with medium stover mulching was the optimal treatment to achieve the best trade-off between stover input and SOC storage. Differentiating microbial C pools from total SOC and, notably, separating fungal and bacterial necromass C pools can refine our mechanistic understanding of SOC storage as well as its association with microbial biota.

1. Introduction

Soil carbon (C) pool is the largest terrestrial C pool, and it is 2–3 times higher than the atmospheric C pool (Lal, 2004; Stockmann et al., 2013) and 3–4 times higher than the aboveground biotic C pool (Körner, 2000; Schweinle et al., 2015). A slight change in this soil C pool results in a large impact on global climate (Stockmann et al., 2013; Jackson et al., 2017). In the current context of climate change, the initiative "4 per 1000" (http://4p1000.org) has been launched to make soil a C sink by stocking 0.4% of soil organic carbon (SOC) per year as a compensation to the global emissions of greenhouse gases due to anthropogenic activity. Following the initiative, agricultural lands have an enormous potential for C storage, and the estimated rates of SOC stock can be up to 0.3–0.5 t C ha^{-1} yr^{-1} (Chenu et al., 2019; Corbeels et al., 2019). However, long-term mismanagement of agricultural soils could cause great C loss and turn croplands from C sinks to C sources (Doran and Zeiss, 2000; Lal, 2010). Therefore, it is vital to identify proper soil management practices that favor C sequestration and sustainable development in agricultural ecosystems.

No-tillage and retention of crop residues over no-tilled soil have been

* Corresponding author.
E-mail address: cliang823@gmail.com (C. Liang).

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performed widely in agricultural ecosystems as an alternative to conventional tillage management, which can limit the potential for SOC accrual or even decrease SOC stock (Kassam et al., 2018). Assessing their impact on SOC storage is necessary and has gained increasing attention in research on mitigating global change (Johnson et al., 2014; Jin et al., 2017). Existing studies debate the impacts of no-tillage and retention of residues on SOC storage (Luo et al., 2010; Turmel et al., 2015). Decomposition of plant residues, such as lignin, is a decennial scale process (Datta et al., 2017). It is often difficult to detect changes in total SOC following the implementation of new management practices in a short time (Melero et al., 2009; Liu et al., 2014a). Therefore, assessing the long-term impacts of soil practices (e.g., at the decennial scale) via controlled experiments on SOC storage is particularly meaningful (Melero et al., 2009). Although the majority of studies confirmed its positive effect on SOC storage (Turmel et al., 2015), the amount of residue that is required is seldom addressed. Residue can generate multiple ecosystem services to farmers (e.g., stock feeding and bioenergy), so optimizing the amount of residue retention is important for balancing farmers' economic benefits with our objective of C storage (Lehman et al., 2014; Turmel et al., 2015).

Most studies on residue, which included stover management, focused on their effects on the quantity of SOC storage (i.e., stock and stock changes in total SOC), but their effect on the quality of SOC is not understood fully (Johnson et al., 2014; Liu et al., 2014a; Jin et al., 2017). SOC is a structurally and chemically complex assembly composed of C pools differing in both origin and stability, i.e., the resistance of SOC to mineralization (Kohl et al., 2018). High SOC quality refers to a high relative amount of stable soil C pools, i.e., pools having high residence time during the mineralization processes (Mao et al., 2019). Although some plant compounds (e.g., lignin) are considerably stable in short-term experiments, their "quality-effect" is not always visible in long-term SOC storage (Dignac et al., 2005; Schmidt et al., 2011). Soil microbial necromass C has drawn increasing attention in C research (Simpson et al., 2007; Liang et al., 2019). In contrast to the fact that microbial biomass C usually only occupies a small proportion of total SOC, microbial necromass C may constitute over 50% of the total SOC and part of which is regarded as a long-lasting pool (Simpson et al., 2007; Liang et al., 2019). Microbial necromass C bound strongly with fine soil particles and was protected easily by chemical (interactions with soil minerals) or physical (soil aggregation) processes that resulted in a long residence time in soil (Miltner et al., 2012; Solomon et al., 2012; Samson et al., 2020; Bucka et al., 2021).

As a result, characterizing the pool size of microbial necromass C is essential for assessing soil C quality. To this end, amino sugar compounds extracted from soils can be used as a good proxy to evaluate microbial necromass C and its contribution to SOC despiting this quantification has some limitations (Appuhn and Joergensen., 2006; Liang et al., 2019). Glucosamine (GluN) in soil is mainly from fungal cell walls, and muramic acid (MurA) in soil originates uniquely from bacterial cell walls (Glaser et al., 2004). The ratio of GluN to MurA has been used as a proxy that represents the relative contribution of fungi and bacteria to total SOC (Glaser et al., 2004; Liang et al., 2016). In addition to the origin of C, soil depth is another important factor that affects quality of C stocks (Fontaine et al., 2007). For the same C pool, a deeper stock location in soil was less disturbed by the external environment (e.g., tillage or erosion) and had greater availability of mineral surface binding sites (Gross and Harrison, 2019), thus, was relatively more stable against C loss (Blanco-Canqui and Lal, 2008). Although the stock quantity of C that is dependent on soil depth is well studied in agricultural ecosystems (Syswerda et al., 2011), few data are available on the quality of C and microbial necromass C that is dependent on soil depth under different agricultural management. This highlights the importance of taking into account C origin and soil depth jointly in the assessment of SOC sequestration among different treatments of soils.

Understanding soil C storage from the view of soil microbiology is essential for improving soil practices that may greatly modify the soil microbial community (Schimel and Schaeffer, 2012; Liang et al., 2016). So far, although a number of studies have acknowledged the important role of microbial community features (e.g., biomass, diversity, and composition) in regulating soil C storage (Trivedi et al., 2013), the results on the relationships between those microbial community features and SOC have been inconsistent. For example, some studies showed that microbial biomass and composition affected SOC (Sul et al., 2013; Hao et al., 2019), whereas others showed that microbial community diversity had no direct correlation with SOC (Degrune et al., 2016). In most studies, only total SOC was used when investigating the relationship between C and microbial indicators (Trivedi et al., 2013; Li et al., 2021). However, to the best of our knowledge, how the pools of different microbial necromass C (i.e., bacterial and fungal necromass C) were associated with the microbial community remains little explored. Microorganisms mediate the dynamics of microbial necromass through their cells' uptake–biosynthesis–growth–death pathways, which result in deposition of microbial necromass C (Liang et al., 2017). Any changes in biomass, diversity, or composition of the microbial community induced by tillage and different amounts of stover retention may, therefore, influence these pathways and, consequently, alter the dynamics of soil microbial necromass C. No-tillage and straw mulching practices can modify microbial community features (Ramirez-Villanueva et al., 2015; Degrune et al., 2016; Li et al., 2021). However, a complete link that spans from agricultural practices to microbial community features and to soil necromass C is still lacking.

In this study, we aimed (i) to compare the stocks and changes in total SOC and its microbial necromass C along a soil depth down to 40 cm among five soil treatments, and (ii) to explore how the SOC storage and stabilization processes were associated with microbial community features (i.e., bacterial and fungal biomass, diversity, and composition). To do this, we performed a 10-y field experiment with corn crops in northeastern China, with five treatments of soil. The treatments included a conventional one (i.e., tillage (CT)), which is the most common practice of local farmers currently, and four alternative candidate treatments proposed to mitigate climate change that included no-tillage without stover (NT–0) and no-tillage with low, medium, and high amounts of stover (NT–low, NT–medium, and NT–high). We hypothesized that.

(H1). stover mulching on no-till soils would improve SOC quantity and fit the goal of the "4 per 1000" initiative;

(H2). stover mulching on no-till soils would improve SOC quality associated with microbial necromass C;

(H3). the changes in soil C and microbial necromass C would be explained by modified microbial community features (i.e., biomass, diversity, and composition) due to no-tillage and stover mulching.

2. Materials and methods

2.1. Study sites and collection of samples

The experiment was set up in 2007 in the Lishu Conservation Tillage Research and Development Station of the Institute of Applied Ecology, Chinese Academy of Sciences (43°31'87"N, 124°24'10"E), which is located in Jilin Province, northeastern China. The mean annual temperature and precipitation were approximately 6.9 °C and 614 mm, respectively.

A randomized complete block design was performed with different amounts of corn stover over croplands of corn (*Zea mays* subsp. *mays*) (i.e., one of the major cereals in northern China). The total amount of corn stover that remained at harvest per year was 7.5 t ha^{-1}. The stover mulching managements were repeated every 3 y as an entire mulching circle. The experiment lasted 10 y and contained three entire mulching cycles. We only evaluated the initial (t0) and final (t10) status of SOC storage and focused on the final result of continuous input of the stover

mulching treatment. The five treatments were as follows:

(i) conventional tillage with plow and no stover mulching (CT);
(ii) no-tillage with no stover mulching (NT–0);
(iii) no-tillage with a low amount of stover (NT–low): in each mulching cycle, 7.5 t ha^{-1} stover was mulched in the first year, but no mulching in the next 2 y, which was equivalent to 2.5 t ha^{-1} per year;
(iv) no-tillage with a medium amount of stover (NT–medium): in each mulching cycle, 7.5 t ha^{-1} stover was mulched in the first 2 y, but no mulching in the third year, which was equivalent to 5.0 t ha^{-1} per year;
(v) no-tillage with a high amount of stover (NT–high): in each mulching cycle, 7.5 t ha^{-1} stover was mulched every year, which was equivalent to 7.5 t ha^{-1} per year.

Each treatment contained three replicates, and the total number of plots was 15. Plots from different treatments were mixed randomly and arranged with a distance of 2.0 m between plots. The size of each plot was 8.7 m × 30.0 m. The plots shared the same topography, geology, seeding, irrigation (natural precipitation), weeding, and pesticide regimes. All plots received the same fertilizer amounts: N 240 kg ha^{-1} y^{-1}, P_2O_5 110 kg ha^{-1} y^{-1}, and K_2O 110 kg ha^{-1} y^{-1}. All the plots share the same soil characteristics, and especially, the SOC in different plots was not significantly different at the beginning of the experiment (Table S1).

Before the experiment, fields had been cultivated with corn using conventional tillage, where the soil was plowed, and all the stover had been removed for >30 y. The cultivation of corn in spring 2007 was regarded as the beginning of the experiment (t0). The harvested corn stover residuals were left in the field as the mulch for the next year, and the first stover mulching event occurred in April 2008. Soil samples were taken in April 2007 and April 2017 to measure the SOC stocks at the beginning (t0) and end (t10) of the experiment, respectively. These samples were taken by a probe of 4.18 cm in diameter, belonging to four soil depths (0–5 cm, 5–10 cm, 10–20 cm, and 20–40 cm). For the samples taken in 2017, some sieved soil samples (2 mm) were stored at −80 °C for extracting bacterial and fungal DNA, some at −20 °C for analyzing microbial PLFA, some at 4 °C for dissolved organic carbon (DOC) analyses, and some at room temperature for quantifying SOC, soil water content (WC), total nitrogen (TN), and amino sugar analyses.

2.2. Soil physicochemical properties

Soil samples for measuring bulk density and water content at t10 were taken using a stainless steel ring, and then they were dried at 105 °C (>24 h). Bulk density (in g cm^{-1}) was calculated by dividing soil dry weight by ring volume. WC (in %) at t10 was calculated by the weight loss of soil after drying at 105 °C for 24 h divided by the weight of fresh soil. The soil was classified as Mollisol with a clay loam texture (IWG, 2007). Proportions of sand, silt, and clay in the soil were 24.81%, 47.65%, and 27.54%, respectively, based on samples collected in a depth of 0–20 cm.

Soil pH was measured using a standard pH electrode (soil: water = 1:2.5). DOC at t10 was extracted using ultrapure water at a ratio of 1:5 (weight/volume), extracts were passed through a 0.45 μm filter, and then measured using a Multi N/C 3000 (Analytik Jena, Jena, Germany). TN (g kg^{-1}) at t10 was determined by combustion gas chromatography with air-dried soil (sieved to 0.15 mm) (EL III elemental analyzer; Elementar Analysensysteme GmbH, Hanau, Germany). SOC content (g C kg^{-1}) at t0 and t10 was measured by potassium dichromate oxidation (Walkley and Black, 1934). SOC stock (t C ha^{-1}) in a soil depth of 0–40 cm was calculated by the equivalent soil mass (ESM) method (von Haden et al., 2020). Changes in SOC stock (t C ha^{-1} y^{-1}) was calculated as the stock difference standardized over the entire experimental period (t10-t0):

$$\text{SOC stock change} = \frac{\text{SOC stock}_{t10} - \text{SOC stock}_{t0}}{t10 - t0} \quad (1)$$

Stover efficiency was represented by SOC stock change per unit stover amount:

$$\text{stover efficiency} = \frac{\text{SOC stock change}}{\text{stover amount}} \quad (2)$$

2.3. Phospholipid fatty acid analysis (PLFA) for soil microbial biomass

Soil microbial biomass was assessed using PLFA analysis that was described by Bossio et al. (1998). PLFAs were extracted from freeze-dried soil (4 g for soils in 0–5 cm and 5–10 cm depths; 8 g for soils in 10–20 cm and 20–40 cm depths) and extracted by a chloroform/methanol/phosphorus-buffer with a ratio of 1:2:0.8. Phospholipids were separated by a solid phase extraction (SPE) column (Supelco Inc., Bellefonte, PA). The phospholipids were transformed into fatty acid methyl esters using methanolysis methyl esterification. The methyl esters were analyzed by an Agilent 7890B GC (Agilent Technologies, Santa Clara, CA, USA) using MIDI peak identification software (version 4.5; MIDI Inc., Newark, DE). We used an internal standard peak (19:0) that converted peak areas to μg or nmol lipid biomass g^{-1} soil. Microbial lipid biomass (nmol g^{-1} soil) was calculated as the sum of all identified peaks (detectable at > 0.5%).

Microbial functional composition was classified by biomarker PLFAs as follows: gram-positive (G^+) bacteria (15:0iso, 15:0anteiso, 15:1isoω6c, 16:0iso, 17:0iso, and 17:0anteiso), gram-negative (G^-) bacteria (16:1ω7c, 16:1ω9c, 17:1ω8c, 18:1ω5c, 18:1ω7c, 21:1ω3c, 17:0cyclow7c, and 19:0cyclow7c), actinomycetes (10Me16:0, 10Me17:0, 10MeC17:1ω7c, 10Me18:0, and 10MeC18:1ω7c), and fungi (18:1ω9c and 18:2ω6c). The sum of the G^+, G^-, and non-specific bacteria (14:0, 15:0, 15:0DMA, 16:0, 17:0, 18:0, and 20:0) was used for total bacteria (nmol g^{-1} soil) (Liang et al., 2016).

2.4. 16S rRNA and ITS gene sequencing for microbial community structure

Total genomic DNA was extracted from 5 g of each homogenized soil sample that was purified using the Powersoil DNA Isolation Kit (MoBio, Carlsbad, CA, USA). The V3–V4 region of bacterial 16S rRNA gene and region 1 of fungal internal transcribed spacer (ITS) were amplified by primer sets 338F/806R and ITS1/ITS2, respectively. The 16S rRNA PCR mixtures were as follows: 2.5 μl of 10 × Pyrobest Buffer, 2 μl of 2.5 mM dNTPs, 1 μl of each primer (10 μM), 0.4 U of Pyrobest DNA Polymerase (TaKaRa), 15 ng of template DNA, and 18.5 μl ddH_2O. The following conditions were used: initial denaturation at 95 °C for 5 min, followed by 25 cycles of 95 °C for 30 s, 55 °C for 30 s, 72 °C for 30 s, and a final extraction at 72 °C for 10 min. The PCR mixtures of ITS consisted of 1 μl of each primer (5 μM), 3 μl BSA (2 ng μl^{-1}), 12.5 μl of 2 × Taq Plus Master Mix (Vazyme, China), 30 ng of template DNA, and 7.5 μl ddH_2O. The conditions used were 94 °C for 5 min, followed by 32 cycles of 94 °C for 30 s, 55 °C for 30 s, 72 °C for 60 s, and a final extraction at 72 °C for 7 min. PCR products were subjected to high-throughput sequencing using the Illumina Miseq PE300 sequencing platform (Illumina, Inc., San Diego, CA, USA).

The raw sequence reads were trimmed initially using the QIIME package (Quantitative Insights Into Microbial Ecology) (version 1.2.1). Sequences that met all three of these criteria were kept: (1) sequences with precise primers and barcodes, (2) sequences with a quality score >20, and (3) sequences >120 bp of fungi and >200 bp of bacteria in length. The algorithm for the sequence database search called Usearch was then used to filter out sequences that were erroneous or chimeric (Edgar, 2010). The remaining high-quality sequences were clustered into operational taxonomic units (OTUs) at a 97% similarity level using UPARSE (Edgar, 2013). 16S rRNA sequences were aligned and classified

against the SILVA123 SSU bacterial reference database (Quast et al., 2013). ITS sequence alignments used reference database Unite 7.0 (Kõljalg et al., 2013). The richness estimator (Chao1) and alpha-diversity index (Shannon) of each sample were calculated using QIIME 1.8.

2.5. Measurement of amino sugar for soil microbial necromass C

Three types of amino sugars, which included glucosamine (GluN), galactosamine (GalN), and muramic acid (MurA), were determined according to the protocol of Zhang and Amelung (1996). Air-dried soil samples (<0.25 mm) that contained >0.3 mg N were hydrolyzed with 6 M HCl at 105 °C for 8 h after adding 100 μl myoinositol (internal standard). The solution was filtered, adjusted to pH 6.6–6.8, and centrifuged for 10 min at 3000 rpm. The supernatant solution was dried at 52 °C under vacuum on a rotary evaporator, and 5 ml absolute methanol was added to extract amino sugars from the residue. Amino sugars were transferred to a vial and dried with N_2 at 45 °C. The dried residue was re-dissolved using 1 ml deionized water, 100 μl standard N-methylglucamine, and lyophilized. Derivatization reagents (32 mg ml^{-1} hydroxylamine hydrochloride and 40 mg ml^{-1} 4-dimethylamino--pyridine in pyridine-methanol with 4:1) were added, and the solution was kept at 75–80 °C for 35 min. After being cooled to room temperature, 1 ml acetic anhydride was added and reheated at 75–80 °C for 25 min. After acetylation, 1.5 ml of dichloromethane and 1 ml of 1 M HCl were added in sequence. Excessive anhydride was reacted with 1 M HCl and removed carefully with the water in HCL using a pipette. Then, the sample was washed three times with 1 ml deionized water to remove residual anhydride thoroughly. The remaining organic phase was dried using N_2 gas at 45 °C. The dried amino sugar derivatives were dissolved in ethyl acetate-hexane (1:1) and then analyzed on an Agilent 7890B GC (Agilent Technologies, Santa Clara, CA, USA) that was equipped with a HP-5 column (30 m length × 0.25 mm diameter × 0.25 μm thickness) and flame ionization detector. The contents of individual amino sugars were calculated based on the internal standard. We used amino sugar-C contents by normalizing their molecular masses as a proxy to calculate bacterial necromass C and fungal necromass C contents (μg g^{-1} soil) using the following equations, following Appuhn and Joergensen (2006) and Liang et al. (2019):

$$\text{fungal necromass C} = \left(\frac{\text{GluN}}{179.17} - 2 \times \frac{\text{MurA}}{251.23}\right) \times 179.17 \times 9 \quad (3)$$

$$\text{bacterial necromass C} = \text{MurA} \times 45 \quad (4)$$

For each of the four treatments with no-tillage, we estimated the changes in microbial necromass C content (ΔMNS) and in microbial biomass (ΔPLFA) from the control treatment, which could be either the conventional tillage treatment (CT, as the control of NT–0) or no-tillage with no stover mulching (NT–0; as the control of NT–low, NT–medium and NT–high). Then, we calculated the ratio of ΔMNS to ΔPLFA, to assess the changes in necromass C per unit change in microbial biomass due to no-tillage or stover mulching, as follows:

$$\frac{\Delta\text{MNS}}{\Delta\text{PLFA}} = \begin{cases} \dfrac{\text{MNS}_{\text{NT}-0} - \text{MNS}_{\text{CT}}}{\text{PLFA}_{\text{NT}-0} - \text{PLFA}_{\text{CT}}} & \text{, when ref. is CT} \\ \dfrac{\text{MNS}_{\text{NT}-X} - \text{MNS}_{\text{NT}-0}}{\text{PLFA}_{\text{NT}-X} - \text{PLFA}_{\text{NT}-0}} & \text{, when ref. is NT–0} \end{cases} \quad (5)$$

where the subscript *X* in Eq. (5) refers to 0, low, medium, or high stover mulching. When there was no significant biomass change, (i.e., ΔPLFA is too small and had no significant difference from 0), the ratio was NA (not applicable).

2.6. Date processing and statistical analyses

In this study, we relied on metrics of SOC stock and stock change to assess the performance of each treatment with regard to our objectives for C sequestration. When studying the mechanisms by which microbial community features mediated SOC under treatments, we relied on metrics for SOC content instead of stock metrics to minimize the variability brought by soil bulk density.

For each treatment, we first compared its change in SOC stock with zero to verify if the field was a C sink or C source; then, we compared its change in SOC stock with the minimum stock change that the field should reach if the "4 per 1000" objective was fulfilled. The minimum stock change of "4 per 1000" objective was estimated as follows:

$$\text{SOC stock}_{\text{4 per 1000 objective at t10}} = \frac{\text{SOC stock}_{\text{t0}} \times (1 + 0.4\%)^{10} - \text{SOC stock}_{\text{t0}}}{10} \quad (6)$$

We used analysis of variance (ANOVA) to test the responses of SOC stock, change in SOC stock, SOC, amino sugar, and PLFA content to treatments within each soil layer or with all the soil layers pooled. We used the Least Significant Difference (LSD) test to make pairwise comparisons for any significance among different modalities of treatment. The above analyses were performed using SPSS19 statistical software (SPSS Inc., Chicago, IL, USA). We performed non-metric multidimensional scaling (NMDS) based on the Bray-Curtis distance to depict changes in microbial community structure among treatments and soil layers (beta-diversity). Permutational multivariate analysis of variance (PERMANOVA) was used to test the dissimilarity of microbial structure among five treatments with the help of *vegan* package (Oksanen et al., 2007) in R. 4.0.2 (R Core Team, 2020). We performed analyses of indicator species to identify the characteristic bacterial or fungal genera in each treatment using the *indicspecies* package of R. 4.0.2. Indicator value (*IndVal*) was used to evaluate how strongly individual species were associated with a particular treatment and was calculated following De Cáceres et al. (2010). Higher *IndVal* reflected that the species was detected more easily in a particular treatment.

We performed constrained redundancy analyses (RDA) and Monte-Carlo permutation tests to examine the relationships between soil physicochemical properties and soil microbial community using the *vegan* package in R. 4.0.2. We also performed Pearson's correlations to evaluate the relationships between microbial necromass C with microbial community features using the *psych* package (Revelle, 2017) in R. 4.0.2.

Path analysis was performed to evaluate direct and indirect links among the soil microbial community, microbial necromass C, and SOC under different treatments using AMOS software (AMOS 17.0.2 student version; Amos Development, Crawfordville, FL, U.S.A.). To simplify the model and to highlight the primary mechanism, we focused on the MCP within the bacterial and fungal communities in path analysis. A maximum likelihood estimation method was chosen to fit the measured data to the model. The fit of the resulting model was evaluated using *p*-values, χ^2-values, a goodness-of-fit index (GFI), and the root mean square error of approximation (RMSEA). All the above tests were based on a significance level of $p < 0.05$.

3. Results

3.1. Total SOC stock and temporal changes in SOC

At the beginning of the experiment (t0), no significant difference in SOC content was found among samples from total soil depth (0–40 cm) (ANOVA, $p > 0.05$) and from each soil layer (ANOVA, $p > 0.05$; Table S1). At the end of the experiment (t10), the effect of treatment was significant in SOC stock down to 40 cm in depth (Fig. 1a). At t10, when stover mulching was absent (NT–0), total SOC stock in 0–40 cm had not increased compared with CT (Fig. 1a). SOC changes with stover mulching over no-tilled soil (NT–low, NT–medium, and NT–high) were higher than zero, but only NT–medium and NT–high attained the goal of the "4 per 1000" initiative (i.e., 0.28 t C ha^{-1} yr^{-1}) (Fig. 1b). The C

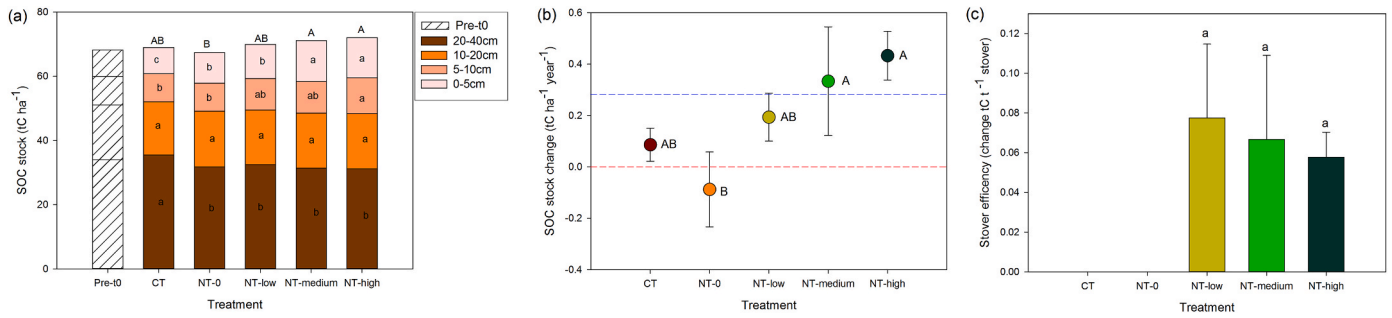

Fig. 1. The soil organic carbon (SOC) stock (0–40 cm) of all five treatments (a) at t10 and average SOC stock at t0, (b) average change in SOC stock of the five treatments from t0 to t10, and (c) efficiency of stover to increase SOC of the three stover mulching treatments. Bars in (a) represent means ($n = 3$); circles or bars in (b) and (c) represent means ± standard errors ($n = 3$). Different lowercase letters indicate significant differences (LSD test) between treatments in each soil layer. Different uppercase letters in (a) and (b) indicate significant differences (LSD test) between treatments of total SOC stock (0–40 cm) and SOC stock change, respectively. All significant differences are at the $p < 0.05$ level. Pre-t0: pre-beginning of the experiment (t0), and t10 is the end of the experiment. CT: conventional tillage; NT–0: no-tillage with no corn stover mulching; NT–low: no-tillage with a low stover mulching amount equivalent to 2.5 t ha^{-1} per year; NT–medium: no-tillage with a medium stover mulching amount equivalent to 5.0 t ha^{-1} per year; NT–high: no-tillage with a high stover mulching amount equivalent to 7.5 t ha^{-1} per year.

increase in soil was 0.33 t C ha^{-1} yr^{-1} (i.e., 0.49%) for NT–medium and 0.43 t C ha^{-1} yr^{-1} (i.e., 0.64%) for NT–high. The stover efficiency indicated by SOC changes per t stover was moderate with the medium stover mulching treatment (NT–medium), but there was no significant difference among the three stover mulching treatments (Fig. 1c).

Throughout the soil profile, NT–0 increased SOC stock in the uppermost soil layer (0–5 cm), but decreased it in the deepest soil layer (20–40 cm; Fig. 1a). Increasing stover over no-tilled soil increased SOC stock, with significant effects found for medium and high amounts of stover in the 0–20 cm soil layer compared with conventional tillage ($p < 0.05$, Fig. 1a). Such an increase in total SOC was largely contributed by those in the top soil layers (0–5 cm and 5–10 cm; Fig. 1a).

3.2. Microbial necromass C

Bacterial, fungal, and total microbial necromass C stocks in 0–40 cm in depth were little affected by tillage and stover mulching (Table S3). All the no-tillage treatments with stover mulching increased bacterial, fungal, and total microbial necromass C stock in the uppermost soil layer (0–5 cm), but the treatments decreased fungal necromass C in the deepest soil layer (20–40 cm) (Table S3). No-tillage treatments increased bacterial necromass C stock in the 10–20 cm soil layer compared with conventional tillage (Table S3).

No-tillage and stover mulching had a significant effect on the content of total amino sugars and resultant microbial necromass C in the uppermost (0–5 cm) and deepest soil layers (20–40 cm) (Figs. S2 and S3).

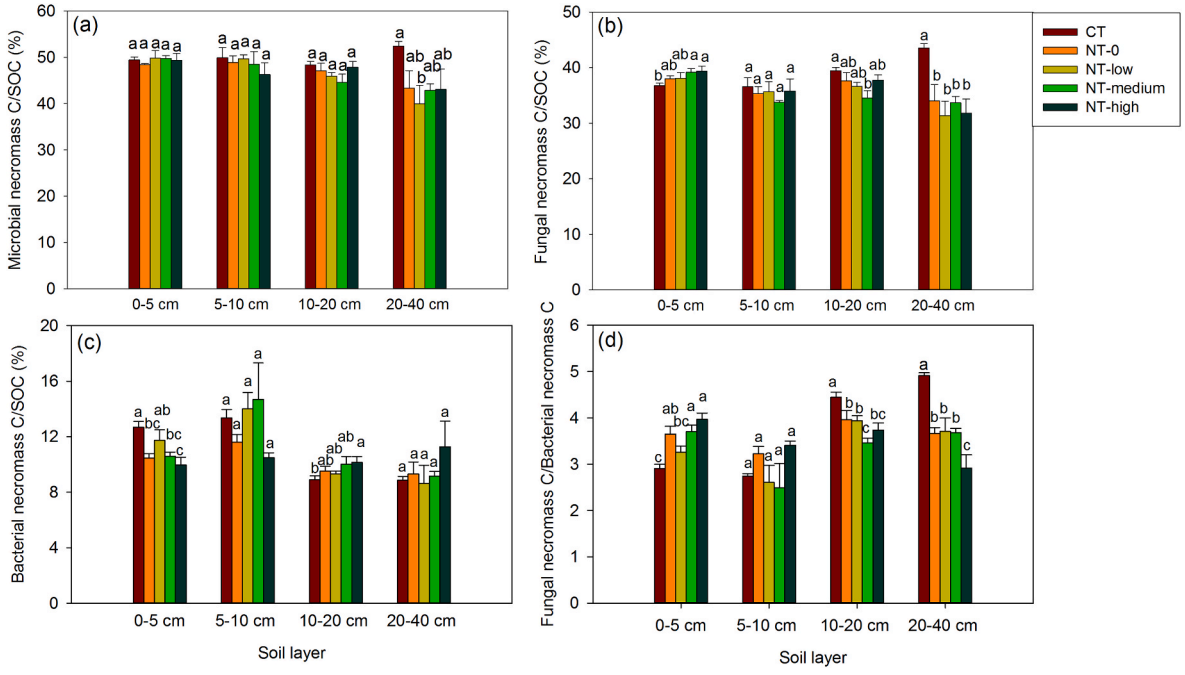

Fig. 2. The contribution of (a) total microbial necromass carbon (C) to soil organic carbon (SOC), (b) fungal necromass C to SOC, (c) bacterial necromass C to SOC, and (d) the ratio of fungal and bacterial necromass C of all five treatments along soil depth. Bars represent means ± standard errors ($n = 3$); different letters indicate significant differences between treatments in each soil layer (LSD test, $p < 0.05$). CT: conventional tillage; NT–0: no-tillage with no corn stover mulching; NT–low: no-tillage with a low stover mulching amount equivalent to 2.5 t ha^{-1} per year; NT–medium: no-tillage with a medium stover mulching amount equivalent to 5.0 t ha^{-1} per year; NT–high: no-tillage with a high stover mulching amount equivalent to 7.5 t ha^{-1} per year.

No-tillage treatments were more similar to each other: compared with CT, they all contained higher GluN, GalN, MurA, higher total amino sugar contents, and higher fungal, bacterial, and total microbial necromass C in 0–5 cm and 5–10 cm, but they exhibited lower values of the same metrics in 10–20 cm and 20–40 cm (Figs. S2 and S3). No-tillage with medium and high amounts of stover mulching (NT–medium and NT–high) increased GluN, GalN, total amino sugars, and fungal and total microbial necromass C significantly compared with low amounts of stover mulching (NT–low) in the 0–5 cm soil layer (Figs. S2 and S3).

The proportion of total microbial necromass C to SOC reached about 50% and was generally not significant among all the treatments for all the soil layers (Fig. 2a). Fungal necromass C occupied up to 30–40% of the total SOC and was approximately three times higher than bacterial necromass C (Fig. 2b and c). When no-tillage and stover mulching treatments were applied, the proportion of fungal necromass C to SOC increased, but the proportion of bacterial necromass C to SOC decreased in the uppermost soil layer (0–5 cm; Fig. 2b and c). That pattern was the opposite in the deepest soil layer (20–40 cm; Fig. 2b and c).

Soil management modified the ratio of fungal necromass C to bacterial necromass C significantly, especially between conventional and no-tillage. Compared with conventional tillage, that ratio was higher in no-tillage treatments in the uppermost soil layer (0–5 cm), but lower in deeper layers (10–20 cm and 20–40 cm) (Fig. 2d). A higher amount of stover increased the ratio of fungal necromass C to bacterial necromass C in the 0–5 cm layer, but decreased it in deeper layers (e.g., 10–20 cm and 20–40 cm; Fig. 2d).

3.3. Microbial community features

Microbial community features include microbial biomass, alpha-diversity, and composition of the taxa. No-tillage and stover mulching increased biomass of G^- and G^+ bacteria, fungal biomass, and total

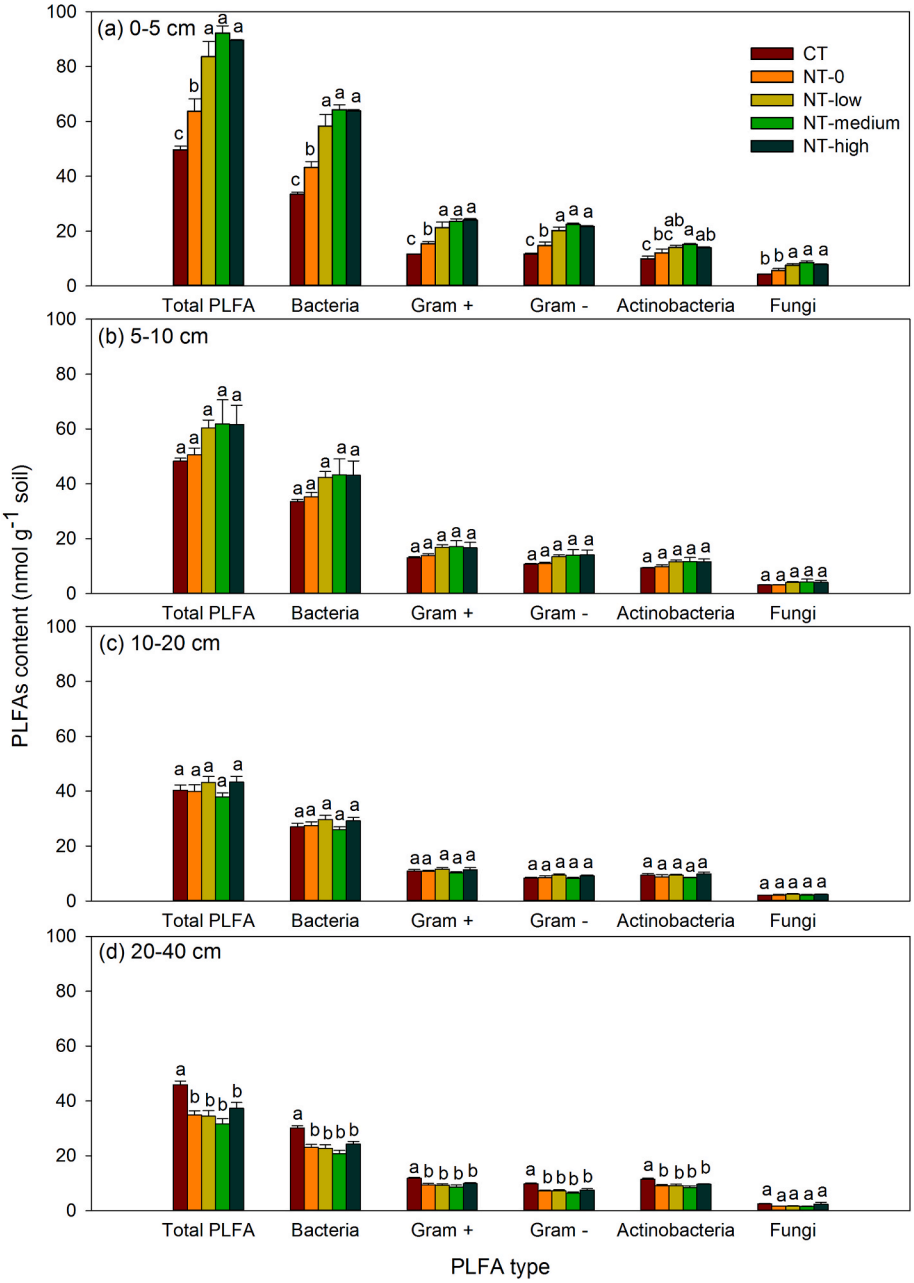

Fig. 3. The total and different types of phospholipid fatty acid analysis (PLFA) contents of all five treatments based on soil depth. Bars represent means ± standard errors ($n = 3$); different letters indicate significant differences between treatments in each soil layer (LSD test, $p < 0.05$). CT: conventional tillage; NT–0: no-tillage with no corn stover mulching; NT–low: no-tillage with a low stover mulching amount equivalent to 2.5 t ha^{-1} per year; NT–medium: no-tillage with a medium stover mulching amount equivalent to 5.0 t ha^{-1} per year; NT–high: no-tillage with a high stover mulching amount equivalent to 7.5 t ha^{-1} per year.

microbial biomass in the uppermost soil layer (0–5 cm) and decreased them (except for fungal biomass) in the deepest layer (20–40 cm; Fig. 3). The two alpha-diversity indicators (i.e., richness (Chao1) and diversity (Shannon)) showed different responses to treatments. Compared with CT, no-tillage and stover mulching had no significant effect on bacterial and fungal diversity (Shannon), and no-tillage and stover mulching decreased bacterial richness (Chao1) in the uppermost soil layer, but increased fungal richness (Chao1) for all soil layers (Table S4). Beta-diversity represented by NMDS showed significant differences (PERMANOVA, $p < 0.05$) in composition of bacterial communities among soil depths, but not among treatments (Figs. S4a and S4b); composition of fungal communities showed significant differences (PERMANOVA, $p < 0.05$) among both soil depths and treatments (Figs. S4c and S4d). When NMDS was performed per soil layer, composition of bacterial communities was significantly different (PERMANOVA, $p < 0.05$) among treatments in both the uppermost and deepest layers (Figs. S5a and S5c).

The responses of the microbial phyla to soil practices in the top and deep layers were markedly different (Fig. 4). In the uppermost layer (0–5 cm), no-tillage was correlated negatively with bacterial phyla Nitrospirae and Choroflexi (Fig. 4a), and fungal class Saccharomycetes (Fig. 4b). Stover amount was correlated positively with bacterial phyla Proteobacteria and Bacteroidetes (Fig. 4a), and fungal classes Glomeromycetes and Tremellomycetes (Fig. 4b), but correlated negatively with bacterial phyla Chloroflexi and Firmicutes (Fig. 4a), and fungal classes Spizellomycetes and Dothideomycetes (Fig. 4b). At the genus level, we did indicator analysis. The genera whose *IndVal* had a significant difference among treatments were defined as indicator genera, and these indicator genera belonged to the treatment with the highest *IndVal*. There were more indicator genera with *IndVal* values that were significantly different for fungi (39 indicator genera) than for bacteria (18 indicators) in the uppermost soil layer (Figs. S6a and S6b). For example, *Curvularia*, *Hymenobacter*, *Penicillium* in CT, *Acremonium* in NT–0, and *Vishniacozyma* in NT–high were fungal indicators (Figs. S6a and 6b). In the deepest soil layer (20–40 cm), no-tillage was correlated positively with bacterial phyla Nitrospirae, but negatively correlated with Bacteroidetes (Fig. 4c) and fungal class Archaeorhizomycetes (Fig. 4d). Stover amount was correlated positively with bacterial phyla Chloroflexi (Fig. 4c), fungal class Pezizomycetes and Ascomycota cls Incertae sedis (Fig. 4d), but correlated negatively with fungal class Kickxellomycetes (Fig. 4d). There were approximately twice as many indicator genera for fungi (28 indicator genera) as for bacteria (15 indicator genera) in the deepest soil layer (Figs. S6c and S6d). For example, *Archaeorhizomyces* and *Ceroophora* in CT, *Cladophialophora* in NT–high, and *Oidiodendron* in NT–medium were fungal indicators (Figs. S6c and 6d).

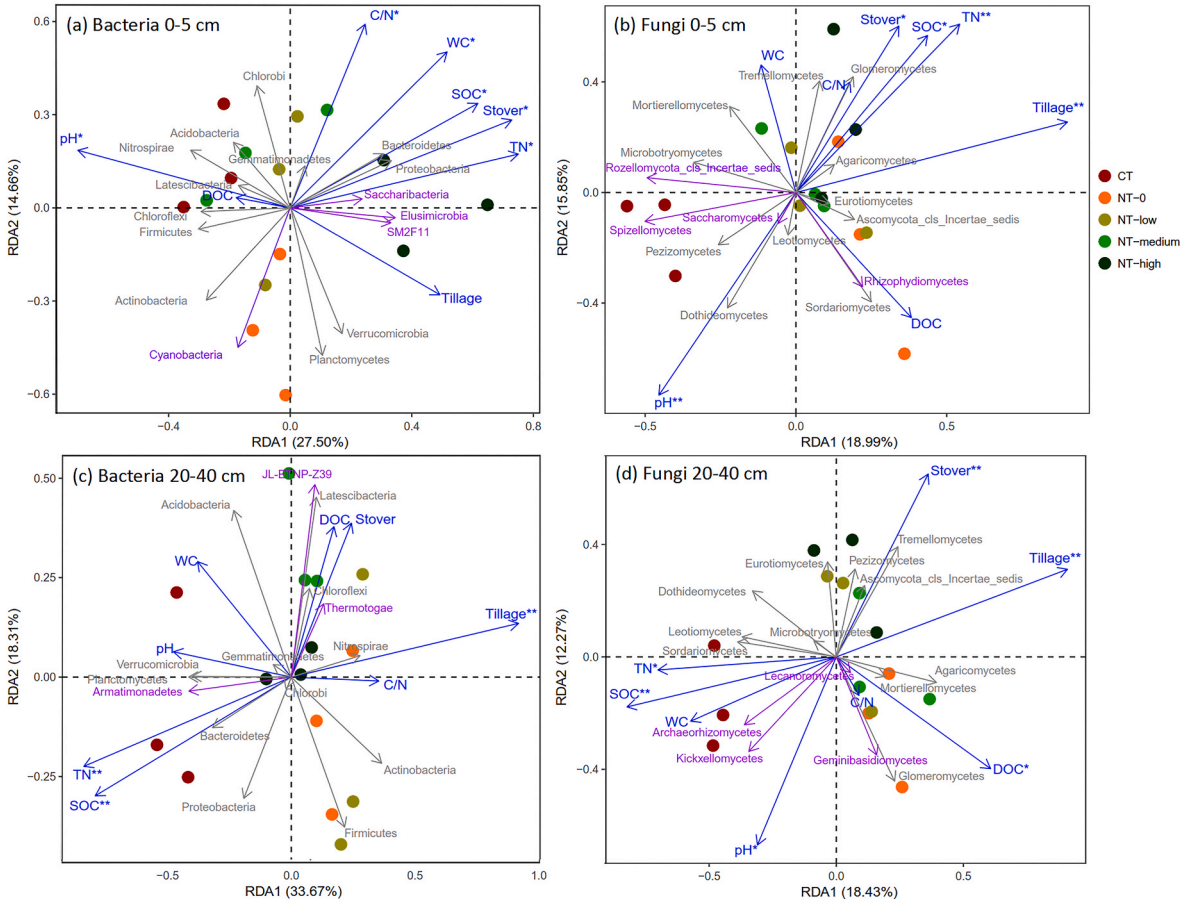

Fig. 4. Constrained redundancy analysis (RDA) of the soil physicochemical properties and soil microbial communities, which include (a) bacterial dominant phyla in 0–5 cm soil depth, (b) fungal classes in 0–5 cm soil depth, (c) bacterial phyla in 20–40 cm soil depth, and (d) fungal classes in 20–40 cm soil depth. Only dominant taxa with relative abundance at 0.01% are included. Colors of arrows: gray represents common taxa in both top (0–5 cm) and deep (20–40 cm) soil layers; purple represents specialist taxa that are found only in the top or deep soil layer; red represents soil physicochemical properties and soil management treatments. Soil physicochemical and management variables with significant effects on dominant taxa were analyzed by a Monte Carlo test with 999 permutations: $*p < 0.05$, $**p < 0.01$. Abbreviations: soil organic carbon content (SOC), total nitrogen content (TN), SOC to total nitrogen ratio (C/N), soil dissolved organic carbon content (DOC), and soil water content (WC). CT: conventional tillage; NT–0: no-tillage with no corn stover mulching; NT–low: no-tillage with a low stover mulching amount equivalent to 2.5 t ha^{-1} per year; NT–medium: no-tillage with a medium stover mulching amount equivalent to 5.0 t ha^{-1} per year; NT–high: no-tillage with a high stover mulching amount equivalent to 7.5 t ha^{-1} per year. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

3.4. Relationships between microbial community features and SOC

Total SOC was governed mainly by fungal necromass C rather than bacterial necromass C, and this phenomenon was found in both soil layers (0–5 cm and 20–40 cm) (Fig. 5). Both fungal and bacterial necromass C were associated with their own biomass in the 0–5 cm soil layer (Fig. 5a and b), whereas only fungal necromass C was associated with its biomass in the 20–40 cm soil layer (Fig. 5c and d). In addition, fungal necromass C was more correlated with microbial community features than was bacterial necromass C (Figs. 5 and 6 and S7).

According to Pearson's correlation analysis, more bacterial phyla and fungal classes had a significant relationship with fungal necromass C in both soil layers (Fig. 6). Actinobacteria and Nitrospirae were correlated negatively with bacterial and fungal necromass C in both soil layers, although most were only significant with fungal necromass C (Fig. 6a and c). Proteobacteria, Bacteroidetes, and Kickxellomycetes were correlated positively with fungal necromass C in a depth of 20–40 cm (Fig. 6c and d). Fungal necromass C was also correlated with more genera (Fig. S7). For example, in the surface layer (0–5 cm), *Pontibacter* and *Gaiella* were correlated negatively with bacterial and fungal necromass C (Fig. S7a), and *Curvularia* and *Acremonium* were correlated negatively with bacterial and fungal necromass C (Fig. S7b). In the deepest layer (20–40 cm), *Parafilimonas*, *Acremonium*, *Archaeorhizomyces*, *Cercophora*, *Escovopsis*, *Isaria*, *Lepraria*, *Neoidriella*, and *Ramicandelaber* were correlated positively with fungal necromass C (Figs. S7c and S7d).

Although microbial living biomass (presented by total PLFA) correlated positively with necromass C, their increments were not totally the same. All the stover mulching treatments increased PLFA contents significantly, but only medium and high amounts of stover mulching increased microbial necromass C significantly (Fig. 7a). To assess the ability of increased living biomass to contribute to the necromass C accumulation, we calculated the ratio of ΔMNS to ΔPLFA. The ratio of ΔMNS to ΔPLFA was 216.50 for NT–0 compared with CT in the 0–5 cm layer. When compared with NT–0, the ratios of ΔMNS to ΔPLFA in the 0–5 cm layer were 163.65, 313.41, and 239.26 for NT–low, NT–medium, and NT–high, respectively (Fig. 7a). The ratios of ΔMNS to ΔPLFA was 516.38 for NT–0 compared with CT in the 20–40 cm layer. The ratio of ΔMNS to ΔPLFA in the 20–40 cm layer for all the stover mulching treatments were NA, because there was no significant biomass and necromass C change produced by stover mulching (Fig. 7b). The ratios of fungi to bacteria for biomass and necromass C exhibited fairly distinct trends. The ratio of fungal biomass to bacterial biomass represented by PLFA did not change between treatments, whereas such a ratio for necromass C was affected significantly by tillage and stover mulching (Fig. 7c and d).

4. Discussion

4.1. Tillage and stover mulching affected SOC quantity

Through a 10-y field experiment with controlled treatments, we assessed the impact of tillage and corn stover mulching on SOC storage. Compared with conventional tillage, we confirmed the positive effect of stover mulching in enhancing SOC stock, which validated our hypothesis (H1).

Soil disturbance due to plowing in the tillage process is usually considered a primary cause of SOC loss (Baker et al., 2007). Yet, in this

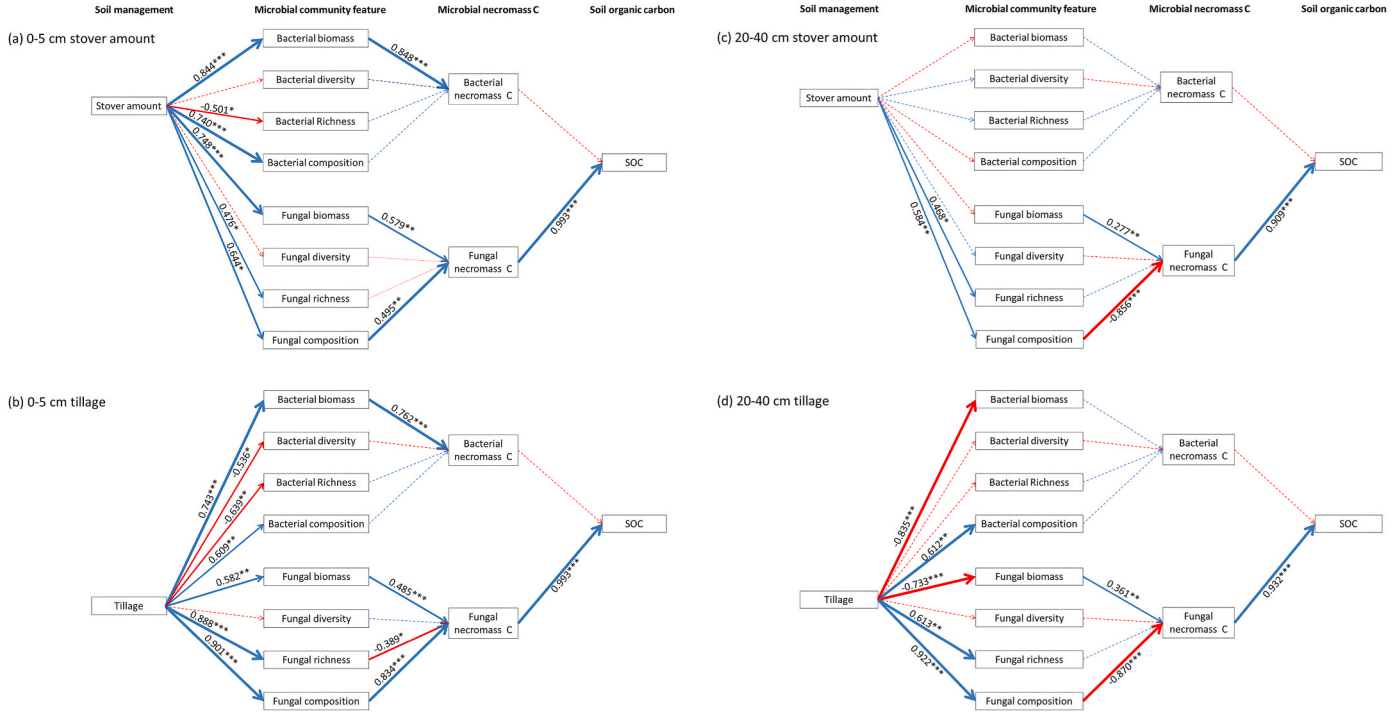

Fig. 5. Path analysis of the hypothesized causal relationships among soil management treatments, microbial community features, microbial necromass carbon (C), and soil organic carbon (SOC) contents for the effect of stover amount in soil depths of (a) 0–5 cm and (c) 20–40 cm and for the effect of tillage in soil depths of (b) 0–5 cm and (d) 20–40 cm. Model fits: for (a), $\chi^2 = 46.11$, $p = 0.10$, GFI = 0.86, RMSEA = 0.15; (b), $\chi^2 = 46.86$, $p = 0.09$, GFI = 0.96, RMSEA = 0.16; (c), $\chi^2 = 37.82$, $p = 0.06$, GFI = 0.95, RMSEA = 0.18; (d), $\chi^2 = 46.28$, $p = 0.08$, GFI = 0.95, RMSEA = 0.16. Blue and red lines represent positive and negative paths, respectively. Solid and dashed lines indicate significant ($p < 0.05$) and non-significant effects, respectively. The width of solid lines indicates the strength of significant standardized path coefficients ($p < 0.05$). Values beside solid lines refer to standardized path coefficients. Significance codes: ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$. Bacterial and fungal biomasses are represented by the phospholipid fatty acid (PLFA) contents; bacterial and fungal richness is represented by the Chao1 index; bacterial and fungal diversity is represented by the Shannon index; bacterial and fungal community composition is represented by the first axis of the non-metric multidimensional scaling (NMDS) based on Bray-Curtis distance. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

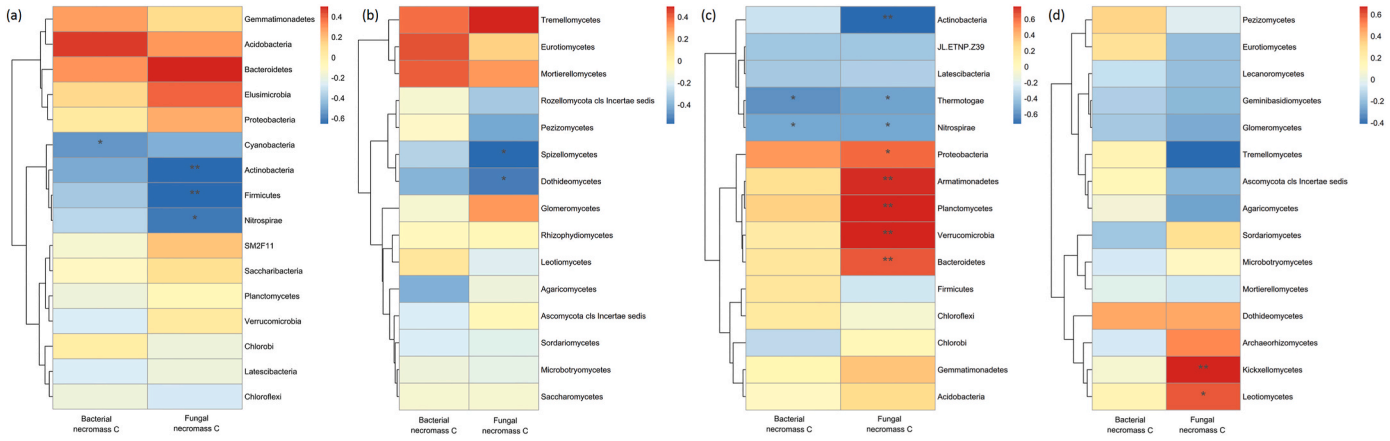

Fig. 6. Heatmap of Pearson's correlations between bacterial/fungal necromass carbon (C) content and relative abundance of (a) bacterial phylum in 0–5 cm, (b) fungi class in 0–5 cm, (c) bacteria phylum in 20–40 cm, and (d) fungi class in 20–40 cm. Phyla or class with relative abundance >0.1% are included. Significance codes: $*p < 0.05$; $**p < 0.01$.

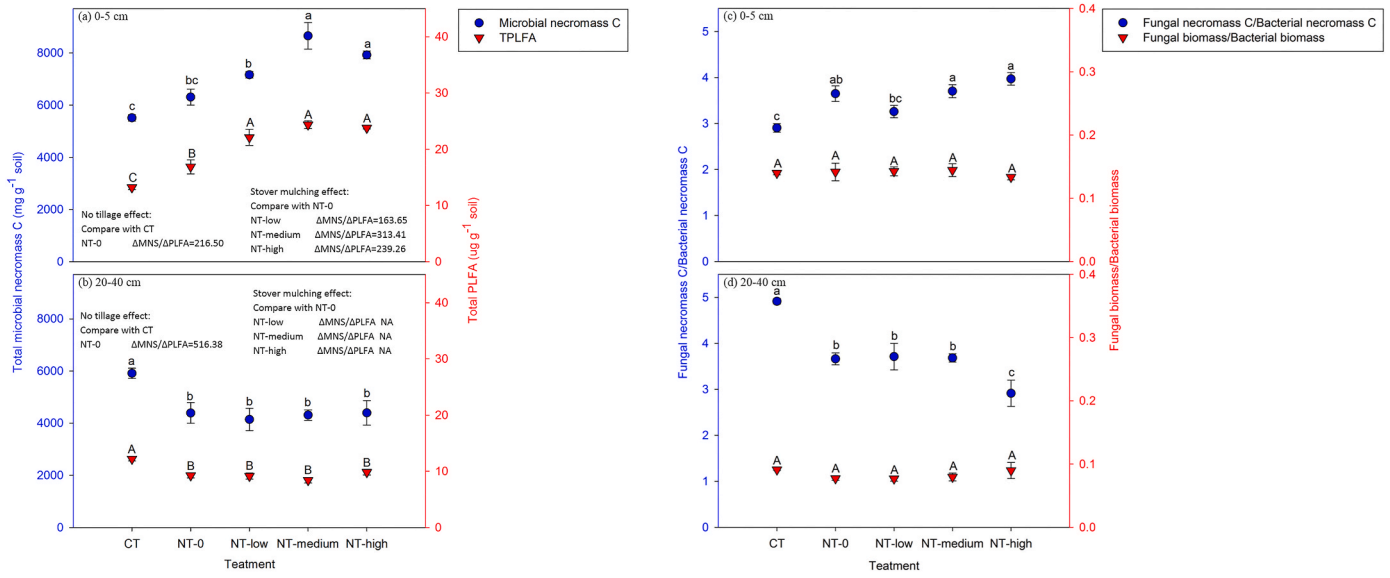

Fig. 7. Contents of total microbial necromass (MNS) carbon (C) and total phospholipid fatty acid (PLFA) in the five treatments for soil depths of (a) 0–5 cm and (b) 20–40 cm. The ratio of fungal necromass carbon (C) to bacterial necromass C and ratio of fungal biomass to bacterial biomass in the five treatments in soil depths of (c) 0–5 cm and (d) 20–40 cm. In (a) and (b) plots, blue circles represent the MNS content (with the y-axis on the left), and red triangles represent total PLFA content (with the y-axis on the right). In (c) and (d) plots, blue circles represent the ratio of fungal necromass C to bacterial necromass C (with the y-axis on the left), and red triangles represent the ratio of fungal biomass to bacterial biomass (with the y-axis on the right). The ratio of fungal biomass to bacterial biomass is represented by their PLFA ratio. Symbols represent means ± standard errors ($n = 3$). Different letters indicate significant differences (LSD test, $p < 0.05$). CT: conventional tillage; NT–0: no-tillage with no corn stover mulching; NT–low: no-tillage with a low stover mulching amount equivalent to 2.5 t ha^{-1} per year; NT–medium: no-tillage with a medium stover mulching amount equivalent to 5.0 t ha^{-1} per year; NT–high: no-tillage with a high stover mulching amount equivalent to 7.5 t ha^{-1} per year. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

study, the soils subjected to conventional tillage at t10 did not lose C significantly compared with those at t0 (Fig. 1b). Plowing in conventional tillage might move crop residues and surface soil C into deeper soil layers (Blanco-Canqui and Lal, 2008), where C would be protected better. At the same time, plowing can also loosen soil, which may promote root growth of crops in deeper soil layers and, thereby, increase C input through root turnover (Baker et al., 2007; Luo et al., 2010). Without stover mulching, no-tillage alone (NT–0) did not improve SOC storage at t10 compared with either conventional tillage or the initial stage (t0) (Fig. 1a and b). This was due to the offset effect between the gain of SOC in top soil layers and the loss of SOC from deep soil layers. Compared with conventional tillage, no-tillage reduced soil disturbance and degradation of stable aggregates, and it diminished SOC loss on the surface (Six et al., 2000). At the same time, no-tillage increased soil strength and limited both root penetration and vertical mobilization of C and, therefore, reduced the C supply to deeper soil layers (Blanco--Canqui and Lal, 2008; Luo et al., 2010).

Our results evidenced the positive effect of stover mulching on total SOC compared to no mulching treatments, and this is in line with meta-studies such as Liu et al. (2014a) and Xu et al. (2019). Stover mulching can provide more favorable water, nutrients, and thermal conditions favoring microbial growth (Luo et al., 2010; Turmel et al., 2015), which is a prerequisite for microbe-related SOC accumulation. Further, we also found that increasing stover amount enhanced SOC stock and made the soil a C sink. In previous studies, stover amounts were usually studied to verify if the loss of C was caused by soil degradation and overuse could be compensated to zero by stover (Johnson et al., 2014; Jin et al., 2017), but rarely did they compare the stover-related C input with the goal of

the "4 per 1000" initiative. Here, we confirmed quantitatively that the goal of the "4 per 1000" initiative was met when the mulching amount was medium or high. Furthermore, medium stover achieved the best trade-off between farmers' economic benefits from stover (e.g., bioenergy and stock feeding) and the goal of C storage required by the "4 per 1000" initiative. Our study provides clear evidence that optimizing the amount of stover mulching reconciled the multiple ecosystem services brought by stover.

4.2. Tillage and stover mulching affected SOC quality

To assess if SOC quality was improved by no-tillage and stover mulching or not, we checked (i) the quantity and the proportion of microbial necromass C (Fig. 2a and S3) and (ii) the relative contribution of fungal necromass C in the microbial necromass C compared with bacterial necromass C (Fig. 2d). The use of these multiple criteria to quantify microbial necromass C associated with SOC sequestration provided an examination of our hypothesis H2.

Similar to the case of total SOC, all the no-tillage treatments promoted microbial necromass C in terms of both stock and content, although this mostly occurred in the uppermost soil layer of 0–5 cm (Table S3). No-tillage decreased soil disturbance and, thus, favored fungal hyphae growth in the top soil layers (Six et al., 2006). Compared with CT (plowing), the microbial community under no-tillage was better adapted to assimilate recalcitrant parts of plant residues which can be efficiently used by soil fungi and produced fungal residues (Bai et al., 2013). Stover mulching provided more available substrates for bacteria and fungi and then boosted the production and accumulation of microbial residues (Ding et al., 2011). Indeed, we found a pronounced increase in total microbial biomass by 13.96–39.90 nmol g^{-1} soil in the no-tillage treatments, which supported such an argument.

Counter to our expectation, the relative contribution of total microbial necromass C to SOC remained stable and did not change among treatments, especially among treatments with stover mulching (Fig. 2a). This may be due to that the C of particulate organic matter (POM) from plant residues also increased with stover mulching (Liu et al., 2014b; Samson et al., 2020), which caused the ratio of microbial necromass C to total SOC to be stable.

In this study, no-tillage and increasing stover mulching increased the ratio of fungal necromass C to bacterial necromass C in the top soil layers (0–5 cm). Guggenberger et al. (1999) and Liu et al. (2019) also found that no-tillage and retention of plant residues increased the ratio of fungal residues to bacterial residues in the surface layer. Ding et al. (2011) and Zhang et al. (2014) found that such an increase diminished with increasing soil depth. Yet, most previous studies focused on top soil layers (0–20 cm), which may have resulted in a strong bias for the effect of tillage. In our study, we investigated down to the 20–40 cm layer and found the ratio dropped drastically by 25% from conventional tillage to no-tillage and by 20% from NT–0 to NT–high; this highlights the importance of investigating SOC in deep soils, which may be more susceptible to treatment than top soils. The increased ratio of fungal: bacterial necromass C in the treatments in the top soil layers may be explained by the minimal soil disturbance brought by no-tillage. Further, fungal hyphae growth could be greatly favored by added stover at the soil surface to form a fungi-associated soil-litter interface (Six et al., 2006). The decreased ratio of fungal:bacterial necromass C from CT to no-tillage treatments in deeper soil layers may be attributed to the more favored deep root growth and mycorrhization in the tillage treatment (Baker et al., 2007; Luo et al., 2010).

Overall, although no-tillage and stover mulching clearly enhanced C sequestration for top soils, their role for deep soils was largely less, even negative. Understanding the vertical patterns of fungal and bacterial necromass C, their sum compared with total SOC, and their relative sizes are, therefore, critical steps for a comprehensive and fair assessment of C storage.

4.3. Tillage and stover mulching affected microbial community features

In the uppermost soil layer (0–5 cm), no-tillage and stover mulching had significant effects on microbial community features. No-tillage and stover mulching increased bacterial and fungal biomass (Fig. 3). Compared with CT, no-tillage treatments had the opposite effect on bacterial and fungal alpha-diversity (Chao 1) by increasing fungal diversity, but decreasing bacterial diversity (Table S4). In the literature, such an effect of no-tillage and stover/straw mulching was highly variable and was positive, null, or negative for both bacterial and fungal alpha-diversity indicators (Degrune et al., 2016; Zhang et al., 2018; Li et al., 2020). These studies differed in abiotic conditions, crop species, management, thickness of soil layers, and experimental duration, which made it difficult to compare their results (Zhang et al., 2018; Li et al., 2020).

For beta-diversity, no-tillage and stover mulching had significant effects on bacterial and fungal structure (Fig. S5). We found that stover mulching increased copiotrophic bacteria (e.g., Proteobacteria and Bacteroidetes), but decreased oligotrophic bacteria (e.g., Firmicutes and Chloroflexi) in the top soil layers compared with NT–0 (Fig. 4a). That may be because stover mulching raised nutrient availability (Shao et al., 2016). Previous studies have confirmed that copiotrophic bacteria were dominant under the condition of high nutrient availability, whereas oligotrophic bacteria were more abundant in soils with low nutrient availability (Sul et al., 2013; Trivedi et al., 2013).

Stover mulch increased the relative abundance of fungal class Glomeromycetes (Fig. 4b). Schüßler et al. (2001) found that Glomeromycetes formed arbuscular mycorrhizae with the plant roots. We also found stover mulching decreased the relative abundance of fungal class Dothideomycetes. This may benefit crop health and productivity because several common plant pathogens that infect almost all major monocotyledon and dicotyledon crops are in the class Dothideomycetes (Goodwin and Kema, 2009). Stover mulch and no-tillage affected more fungal genera than bacterial genera (Fig. S6). However, because our knowledge of the function of these genera and their interactions still remain poor, the impact of such a shift in microbial taxonomic composition on soil functioning and services is largely unknown.

In deeper soil layers (e.g., 20–40 cm), we found distinct, even sometimes opposite, responses of microbial community features to treatments compared with those in top soil layers (e.g., 0–5 cm). For example, tillage had a greater effect on microbial community features than stover mulching (Fig. 5c and d). No-tillage decreased bacterial and fungal biomass in 20–40 cm (Fig. 3). This was because no-tillage increased soil strength and bulk density (Luo et al., 2010), resulted in fewer roots or less pores, and decreased available oxygen for microorganisms growth in deep soil layers (Erktan et al., 2020). For bacterial composition: no-tillage resulted in more oligotrophic bacteria in 20–40 cm than conventional tillage (Fig. 4c) which was consistent with the lower SOC content in 20–40 cm (Fig. S1). According to other studies, the possible reason is that the quantity of nutrients brought by roots was less in deep soil under no-tillage treatments (Baker et al., 2007; Luo et al., 2010). Hence, oligotrophic bacteria may have been more abundant under low nutrient conditions (Fierer et al., 2007; Sul et al., 2013), which would explain such a phenomenon. Compared with bacteria, fungal richness (Chao 1) and composition in 20–40 cm were more similar to those in 0–5 cm (Figs. 4 and S4). This may be explained by fungal morphology in soil because fungi can form hyphal networks that extend farther than several centimeters in length (Carlile, 1995), which would reduce the disparity in fungal diversity among soil depths.

Overall, our results revealed the complex impacts of no-tillage and stover mulching on bacterial and fungal features among soil depths, which suggested distinct microbiological mechanisms in shallow and deep soils. Future studies should take into account the vertical distribution of microbial community features when assessing the effect of soil management on soil biota.

4.4. Mechanism of no-tillage and stover mulching that affected SOC through microbial community features

We confirmed our H3 that the soil C content and microbial necromass C were associated with microbial community features (biomass, diversity, and composition) due to the no-tillage and stover mulching. In this study, SOC content was governed mainly by fungal necromass C rather than bacterial necromass C, and fungal necromass C was more closely related to microbial community features (Figs. 5 and 6 and S7).

Despite the distinct difference in contribution to necromass C between bacteria and fungi, both bacteria and fungi biomass were related closely to the absolute content of their necromass C (Fig. 5a). This was consistent with Chen et al. (2020), whose experiment was similar to ours in terms of climate and soil type, although they studied the effect of land use and mineral fertilization. In another experiment performed by He et al. (2020) in a warmer climate (subtropical cropland), bacterial necromass was correlated positively with bacterial biomass, but fungal necromass had no relation with fungal biomass. Through an iterative process of cell generation, growth, and death, microorganisms contributed directly to SOC as necromass (Miltner et al., 2012; Liang et al., 2017). Ideally, greater microbial biomass produced more cells, which resulted in more microbial necromass (Liang et al., 2016). Such a process of C accumulation is altered by the physical and chemical properties of soils that vary among climates, which leads to different results.

Previous studies have used the ratio of fungal:bacterial biomass to represent their relative contributions to SOC accumulation (Malik et al., 2016; Kumar and Ghoshal, 2017). Our results do not support the appropriateness of using such a proxy because we found no relationship between the ratio of fungal:bacterial biomass and any necromass C indicators, which included both relative necromass C content and ratio of fungal:bacterial necromass C (Fig. 7). Our results were also well supported by van Groenigen et al. (2010), who worked in an agroecosystem with tillage treatments. The absence of the relationship suggested that fungal:bacterial biomass and fungal:bacterial necromass C ratios reflected fairly different aspects in the entire microbial process: the former represented their proliferation-mortality dynamics, and the latter represented their accumulation-mineralization dynamics. Both dynamics did not necessarily share the same drivers, which rendered different patterns and a low correlation level.

The disparity in biomass between bacteria and fungi is dependent on food (e.g., litter and plant residues), nutrient availability, and their life strategies (Zhou et al., 2018). According to a recent meta-analysis, both fungal and bacterial biomasses clearly benefited from no-tillage and mulching with plant residues, but fungal:bacterial biomass was not affected by no-tillage and plant residues (Chen et al., 2020). Regarding necromass C, the difference in the resistance of residues between bacteria and fungi should be the primary driver of the fungal:bacterial ratio. Bacterial residues had a faster turnover rate (Kindler et al., 2006; Zheng et al., 2021) because they can be reutilized directly by microbes after primarily decomposed to muropeptides (300–2000 Da) (Hu et al., 2020), but fungal residues had a much longer turnover time (Schweigert et al., 2015) and contributed more to SOC stabilization over the long term (Six et al., 2006). Further, fungal residues interacted more easily with clay minerals through chemical adsorption to the mineral matrix (Guggenberger et al., 1999; Six et al., 2006), and they were more likely to be protected physically by binding the microaggregates into macroaggregates against mineralization (Simpson et al., 2004; Veloso et al., 2020). Therefore, directly quantifying the fungal:bacterial necromass C ratio, rather than their biomass ratio, is key to estimating their relative contribution to soil C storage.

In addition to the primary mechanisms revealed by path analysis, there were complex relationships between microbial taxa and necromass C (e.g., bacterial taxa with fungal necromass C, fungal taxa with bacterial necromass C). Specifically, more microbial taxa related to fungal necromass C were detected than bacterial necromass C, both at the phylum and genus levels (Figs. 6 and S7). We found that Actinobacteria and Nitrospirae were correlated negatively with fungal and bacterial necromass C (Fig. 6). A similar result for Actinobacteria was found, which may be because Actinobacteria are known to thrive under low nutrient conditions and can uptake microbial necromass for growth (Apostel et al., 2018). Proteobacteria and Bacteroidetes were correlated positively with fungal necromass C in 20–40 cm. We speculate that the possible reason was that fungi use fast-growing copiotrophic bacteria and their products to grow and to promote fungal residues ultimately. According to a ^{13}C label experiment of Zheng et al. (2021), bacterial biomass was utilized by fungi first, then G^+ bacteria and arbuscular mycorrhizal fungi (AMF), and the proportion of ^{13}C label from bacterial biomass in fungi gradually increased with time. More significant relationships were shown at the genus level. For example, *Curvularia, Bipolaris*, *Plenodomus,* and *Volutella,* which contains many plant pathogens (Manamgoda et al., 2012, 2014; Cannon et al., 2012; Gai et al., 2016) in the uppermost soil layer (0–5 cm), were indicators of CT and correlated negatively with fungal necromass C. In the deepest soil layer (20–40 cm), *Acremonium*, which is one of the largest and most complex genera of hyphomycetes (Giraldo et al., 2017) and *Archaeorhizomyces* which has saprotrophic potential (Rosling et al., 2011), were correlated positively with fungal necromass C. At present, the functions of these microbial taxa derived from sequencing data are not yet understood fully, and what these relationships imply in terms of soil functioning is unknown and needs to be explored and verified in further studies.

Overall, we demonstrated the associated links from tillage management to microbial community features to C-related soil functioning. Microbial lipids (PLFA) reflect the living status of the microbial community (Bossio et al., 1998), but microbial residues (necromass C) reflect the legacy results of microbial metabolism on SOC storage (Liang et al., 2017). The ratio of ΔMNS to ΔPLFA was used to assess the contributions of per unit microbial biomass (i.e., relative change induced by no-tillage or stover mulching) to necromass C. This showed the ability of microorganisms to convert the current increased living biomass into longer-term residual C through MCP. By explicitly representing the ratio between change in microbial living biomass and change in necromass compared with the control (CT or NT–0), this index provides a process-based understanding of microbial contributions to soil C cycling. We found that the increased microbial biomass under medium stover mulching (NT–medium) had the highest microbial residue accumulation among all the stover mulching treatments (Fig. 7a). This suggested that using a medium amount of stover with no-tillage seems to have the most potential to translate increased living biomass to necromass, so it is the optimal management approach for deposition of microbial-derived C.

5. Conclusion

With a 10-y experiment of no-tillage and stover mulching in a Mollisol, we found that no-tillage with a medium or a high amount of stover mulching were efficient measures to enhance SOC storage and meet the goal of “4 per 1000” initiative, therefore, is a recommended practice. Further, by comparing the efficiency of no-tillage and stover mulching to produce microbial necromass C, we concluded that a medium amount of stover achieved the best trade-off between stover input and the goal of C storage. No-tillage treatments increased the ratio of fungal to bacterial necromass C in the top soil layer, but decreased it in the deep soil layer. We found that fungal necromass C, rather than bacterial necromass C, played a dominant role in the accumulation of SOC, and this process was mediated mainly by fungal biomass and community structure. These results may guide us to manipulate microbial community features more efficiently, using soil inoculation as an example, to better favor SOC storage. By bridging a fundamental understanding of SOC and microbial community features with applied soil management practices, our study provides a methodological framework to improve climate mitigation and sustainable development in croplands.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.soilbio.2022.108587.

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