



How bacterial traits scale to soil organic matter pool buildup revealed by long-term maize straw mulching experiment

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ABSTRACT

No-till with maize straw mulching can enhance soil organic matter (SOM) accumulation. Soil bacterial communities, which are sensitive to habitat changes, can influence carbon (C) and nitrogen (N) cycling during SOM pool buildup, yet their temporal mediation under long-term conservation tillage remains unclear. We used 16S rRNA high-throughput sequencing to analyze bacterial traits across four maize straw mulching durations, 0 (M0), 6 (M6), 10 (M10), and 14 (M14) years in Northeast China. Compared with aboveground removal, bacteria at M6 exhibited traits of active growth and metabolism, such as higher phylogenetic diversity, increased copiotroph to oligotroph ratio (Copio/Oligo), enhanced species cross-feeding, and greater potential for biomass and carbohydrate biosynthesis efficiency. Similar trends persisted at M10, accompanied by stronger competition between Actinobacteria and other species. Till M14, bacteria further shifted toward reduced efficiency in carbohydrate biosynthesis. Correspondingly, SOM increased by 42.7 % at M10 and by 53.6 % at M14, accompanied by an increased C to N ratio, with M14 also showing higher labile C content. Such findings indicated that SOM pool buildup evolved from initial stability via bacterial turnover, to accelerated accumulation driven by bacterial transformation, and ultimately to enhanced both quantity and C availability. Structural equation modeling highlighted that temporally coupled bacterial succession, shaped by Copio/Oligo and growth strategies in response to shifting C availability, drove changes in SOM quality. Our findings suggest that bacterial traits can be effectively scaled up to understand SOM buildup under long-term maize straw mulching.

1. Introduction

Improving soil organic matter (SOM) storage in agroecosystems plays a crucial role in mitigating atmospheric carbon dioxide (CO_2) elevation and ensuring food security (Jiang et al., 2017; Xu et al., 2019). Conservational tillage practices including no-till and crop residue mulching have been widely recognized as effective measures to enlarge SOM pool capacity and improve soil nitrogen (N) supply for crop demand (Smith, 2004; Luo et al., 2018; Mitchell et al., 2018). Temporal changes of SOM quantity and quality are substantially mediated by microbial transformation under long-term maize straw mulching (Mitchell et al., 2018; Zhu et al., 2020a; Yang et al., 2022), highlighting the potential to regulate SOM pool buildup through targeted

manipulation of microbial metabolic processes. However, the adaptation of microbial growth strategies to exogenous organic matter input and the temporal dynamics of microbial traits coupling with changing SOM quantity and quality remains underexplored.

Soil bacteria are highly sensitive to environmental disturbances, and thus could be frequently used as indicators of changes in SOM cycling (Edwards et al., 2006; Zhong et al., 2018; Li et al., 2021). They exhibit vast species diversity and are characterized by various traits, such as functional group composition and co-occurrence network features. These traits can provide insights into the metabolic adaptation of bacterial community to variations in resource availability (Zhang et al., 2016; Dong et al., 2021). For example, based on functional groups linked to substrate utilization capacity, bacteria are often categorized as

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copiotroph or oligotroph (Fierer et al., 2007). Higher levels of labile carbon (C) and nutrients promote the survival of fast-growth copiotrophic bacteria, while recalcitrant substrates or nutrient limited environments favor the dominance of slow-growth oligotrophic group (Fierer et al., 2007; Orwin et al., 2018; Couso et al., 2023). A higher ratio of copiotrophic to oligotrophic bacteria indicates a labile C and nutrients-enriched environment and faster bacterial growth. Nonetheless, maintaining this condition requires continuous input of available organic resources; otherwise, it may lead to the loss of existing SOM due to accelerated bacterial proliferation, and reduced formation of bacterial-derived components due to lower C use efficiency in copiotrophic taxa (Cui et al., 2020). Therefore, investigating the succession of bacterial composition and functional groups in response for changing resource could provide insights into microbial growth strategies and their metabolic implications for SOM dynamics.

During the succession of functional groups, the soil bacterial community might also undergo changes in interactions among taxa, exhibiting distinctive co-occurrence patterns under varying resource availability. In principle, under labile C-rich conditions, bacteria could accelerate their growths, leading to intensified species interactions and competition, and faster labile organic matter turnover (Schmidt et al., 1997; McNichol et al., 2024). However, when C source becomes limited, less competitive species may be excluded, allowing bacteria with higher C utilization capabilities to dominate the network, accelerating recalcitrant component transformation (Hibbing et al., 2010; Enke et al., 2019). Nonetheless, bacteria can also cooperate or facilitate each other in decomposing complex substrates, which might favor complex resources transformation into SOM pool without threatening local biodiversity (Kong et al., 2020; Kost et al., 2023). Accordingly, the integration of bacterial co-occurrence network characteristics with community diversity and other structuring traits could offer deeper insights into the resource acquisition strategies of the community, which in turn may shape soil C availability and influence SOM quality.

Microbial growth and resource acquisition are C- and energy-consuming processes, with trade-offs determined by resource availability (Malik et al., 2020; Shao et al., 2021). For example, available resources could inspire microorganisms to invest more in central metabolic pathways to facilitate biomass yield, such as fatty acid and amino acid biosynthesis. Within the process of cellular component biosynthesis, microbial investment is directed not only toward growth but also toward the synthesis of cell maintenance/integrity materials, such as cell wall (Mitchell and Silhavy, 2019), which are widely recognized as important contributors to stable SOM pools (Liang et al., 2017; Zhu et al., 2020a; Wang et al., 2021). While in environments with abundant polymeric resources, they could invest more in complex substrate degrading pathways rather than faster biosynthesis (Malik et al., 2020). Uncovering phylogeny-based temporal patterns in bacterial metabolic potential, especially those associated with biosynthesis and degradation, could improve our ability to scale microbial dynamics to SOM buildup under long term conservation tillage.

To mitigate the degradation of black soil in Northeast China caused by conventional tillage in maize planting, no-till with maize straw mulching has been applied in this region for more than decades (Zhu et al., 2020b; Yang et al., 2022). Previous studies in this region have found that the temporal increase in SOC and the correspondingly microbial- and plant-derived components follows a non-linear pattern, with varying trends across heterogeneous components (Li et al., 2023). However, the underlying temporal response patterns of soil bacterial communities and their implications for SOM pool development remain poorly understood, highlighting a critical knowledge gap in linking microbial traits to long-term SOM buildup under conservation tillage practices. Given that maize straw contains both labile and recalcitrant components, we hypothesize that 1) in the short term, labile C in maize straw may promote rapid bacterial growth, increase copiotrophic abundance, intensify bacterial interactions, and enhance biosynthesis potentials; 2) with consecutive straw return, bacterial communities may

shift toward greater investment in resource acquisition to sustain growth, favoring oligotrophs capable of utilizing complex substrates, promoting bacterial cooperation, and limiting sustained investment in rapid growth pathways; and 3) these temporal changes in community traits may mediate the nonlinear buildup of SOM pool, with consequences for both its quantity and quality. To test these hypotheses, we employed 16S rRNA high-throughput sequencing to analyze key bacterial traits, including community diversity, functional group composition, co-occurrence network properties, and predicted metabolic pathways, across four maize straw mulching durations (0, 6, 10, and 14 years) at this study site.

2. Materials and methods

2.1. Study site and soil sampling

This study was conducted at the National Field Observed and Research Station of Shenyang Agroecosystems (41°31' N, 123°24' E), located in the center of the Liaohe Plain (Shenyang, China). The region experiences a temperate continental monsoon climate. The mean annual temperature and precipitation are 7–8 °C and 650–700 mm, respectively. The soil type in this region is classified as *Alfisol*. The soil pH before the trial was 5.38 ± 0.02. It is a monoculture cropping system with maize (*Zea mays* L.).

In 2007, a micro-plots experiment was established in the field with plot dimensions of 1.6 m × 1.3 m, and approximately 2.5 m of protection rows between plots. The plots were separated by polyvinyl chloride boards around their edges, with 35 cm underground inserting, leaving 15 cm of the board exposed above the soil surface to maintain the original soil structure. All treatments received a basal application of chemical fertilizer including 50 kg N ha⁻¹ (NH₄)₂SO₄, 30 kg P ha⁻¹ KH₂PO₄, and 58 kg K ha⁻¹ supplied by KH₂PO₄ and K₂SO₄ before maize sowing. Then, 100 and 50 kg N ha⁻¹ (NH₄)₂SO₄ were applied at the jointing and silking period, respectively. Maize straw mulching plots were set up in 2007, 2011 and 2015. Maize straw harvested from the previous season was cut into 10 cm segments and used for complete mulching in spring after seed sowing. The annual straw input was 11.6 × 10³ kg ha⁻¹ (equivalent to 5014 kg C ha⁻¹ and 96.6 kg N ha⁻¹). Each plot contained 3 rows × 4 maize crops. Soil samples were collected from these plots in May 2021. This study included four treatments under no-till practices with normal fertilization: continuous complete maize straw removal (M0), continuous complete maize straw mulching for 6 years from 2015 to 2021 (M6), 10 years from 2011 to 2021 (M10), and 14 years from 2007 to 2021 (M14), with three replications for each treatment. Using a 30 mm diameter soil auger, five random soil cores were taken from each plot at a depth of 0–10 cm and mixed thoroughly to create a representative sample for each plot. Visible roots and plant residues were removed from the soil samples before homogenization, and the soil was then passed through a 2 mm sieve. Approximately 10 g of each sample was stored at -80 °C for DNA extraction, while the remaining soils were used for analyses of other soil abiotic properties.

2.2. Soil chemical property analyses

SOC was determined by wet combustion with a mixture of H₂SO₄ and K₂Cr₂O₇. SOM was calculated by multiplying SOC by a conversion factor of 1.724 (Waksman and Stevens, 1930). Total N (TN) was measured using an elemental analyzer (Model CN, Vario Macro Elemental Analyzer System, GmbH, Germany). The ratio of SOC to TN (C/N) was calculated to indicate SOM quality. Ammonium N (NH₄⁺-N) and nitrate N (NO₃⁻-N) were extracted with 2 mol·L⁻¹ KCl at a soil-to-water ratio of 1:5 and then measured using a segmented flow analyzer. The C availability in soil was indicated by readily oxidizable C (ROC), which was extracted with 333 mmol·L⁻¹ KMnO₄. The colorimetric absorbance was measured using a UV spectrophotometer at a wavelength of 565 nm (Blair et al., 1995). Soil pH was determined using a pH meter (pH610, WIGGINS,

Germany) with a soil-to-water ratio of 1:2.5.

2.3. DNA extraction, PCR amplification, and high-throughput sequencing

Soil DNA extraction was performed using the OMEGA Soil DNA Kit (D5625-01) (Omega Bio-Tek, Norcross, GA, USA) from ~0.25 g frozen soil. Bacterial DNA amplification of the bacterial 16S rRNA gene V3–V4 region was performed using the forward primer 338 F (5'-ACTCC-TACGGGAGGCAGCA-3') and the reverse primer 806 R (5'-GGAC-TACHVGGGTWTCTAAT-3'). Thermal cycling was performed with an initial denaturation at 98 °C for 5 min, followed by 25 cycles of denaturation at 98 °C for 30 s, annealing at 53 °C for 30 s, and extension at 72 °C for 45 s, with a final extension of 5 min at 72 °C. PCR amplicons were purified using Vazyme VAHTSTM DNA Clean Beads (Vazyme, Nanjing, China) and quantified using the Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen, Carlsbad, CA, USA). After quantification, the amplicons were pooled in equal amounts, and subjected to pair-end 2 × 250 bp sequencing on the Illumina NovaSeq platform at Shanghai Personal Biotechnology Co., Ltd (Shanghai, China).

Bioinformatic analysis of the microbiome was performed using QIIME2 (v 2019.4). The DADA2 plug-in was used to perform quality-filtered on the raw sequences (Callahan et al., 2016). The quality-filtered sequences were clustered into non-singleton amplicon sequence variants (ASVs) by 100 % thresholds. To obtain taxonomic information for each ASV, ASVs were compared with reference sequences using the Greengenes database (v 13.8). ASVs were aligned with mafft (Katoh et al., 2002) and used to construct a phylogenetic tree with fasttree (Price et al., 2009). A total of 442958 raw 16S rRNA (V3-V4 region) sequences were obtained from 12 samples representing four maize straw mulching durations, with a range of 29932–57954 sequences per sample. After quality filtering, 178085 high-quality sequences were retained. The rarefaction curves approached saturation, suggesting adequate sequencing depth to represent bacterial diversity (Fig. S1). All samples were rarefied to 10753 sequences to standardize sampling depth. The sequences were submitted to the Sequence Read Archive (SRA) database under BioProject submission number PRJNA1181628.

Soil bacterial taxonomic and phylogenetic α diversities were estimated, with the former using the observed species and Shannon indices, and the latter using the mean pairwise distance (MPD) and mean nearest taxon distance (MNTD). Soil bacterial taxonomic and phylogenetic β diversities were estimated based on Bray-Curtis distance and β MPD, respectively. Principal coordination analysis (PCoA) was performed to visualize bacterial β diversity, and non-parametric multivariate analysis of variance (Adonis) was employed to assess the impacts of maize straw mulching durations on bacterial community structures (β diversity). Both analyses were conducted using the 'vegan package' (Oksanen et al., 2022) in R (v.4.1.2). The soil bacterial composition in this study was analyzed by relative abundance at the phylum and order levels, and bacterial functional group succession was assessed based on the relative dominance of copiotrophic and oligotrophic bacteria. In this study, we focused on analyzing bacterial phyla and orders with an average relative abundance greater than 1 %. We classified copiotrophs as including Alphaproteobacteria, Gammaproteobacteria, Firmicutes, and Bacteroidetes, while oligotrophs comprised Actinobacteria, Acidobacteria, Chloroflexi and Deltaproteobacteria, as referred to Zhao et al. (2021a) and Lupwayi et al. (2023). We then calculated the ratio of copiotrophic to oligotrophic bacteria (Copio/Oligo) to indicate the relative proportions of bacterial functional groups with different survival strategies.

Soil bacterial metabolic potentials were predicted using PICRUSt2 (Phylogenetic investigation of communities by reconstruction of unobserved states) (Douglas et al., 2020) with predictions based on MetaCyc database (<https://metacyc.org/>). Using predicted bacterial metabolic pathways, we estimated bacterial investment in biosynthesis versus acquisition for carbohydrates, aromatic compounds, and organic N. For each category, we calculated the ratio of biosynthetic to degradative

pathways to understand bacterial metabolic priorities. Specifically, for carbohydrate metabolism, we calculated the ratio of biosynthetic pathways (including glycan biosynthesis, polysaccharide biosynthesis, sugar biosynthesis, and colanic acid building blocks biosynthesis at Level 3) to degradative pathways (including polysaccharide degradation, sugar degradation, glycogen degradation II and glycogen degradation I at Level 3). For aromatic compound metabolism, we calculated the ratio of chorismate biosynthesis pathway (Level 3) to all Level 3 pathways classified under the aromatic compound degradation category at Level 2. For organic N metabolism, we assessed the ratio of biosynthesis pathways (including all Level 3 pathways categorized under amine and polyamine biosynthesis, amino acid biosynthesis, and nucleoside and nucleotide biosynthesis at Level 2) relative to degradation pathways (including all Level 3 pathways categorized under amine and polyamine degradation, amino acid degradation, and nucleoside and nucleotide degradation at Level 2).

Additionally, to assess bacterial growth investment strategies, we estimated the efficiency of bacterial-derived stable C production. This was achieved by calculating the ratio of fatty acid and lipid biosynthesis pathways (including lipid IVA biosynthesis, fatty acid biosynthesis, phospholipid biosynthesis, and superpathway of (Kdo)2-lipid A biosynthesis at Level 3) to cell structure biosynthesis pathways (lipopolysaccharide biosynthesis and cell wall biosynthesis at Level 3). A higher ratio reflects greater efficiency in bacterial fast growth but reduced efficiency in producing stable bacterial-derived C.

2.4. Network analysis

The Molecular Ecological Network Analysis (MENA) pipeline, based on stochastic matrix theory, was utilized for constructing a co-occurrence network using all soil samples (Deng et al., 2012). Subsequently, the sub-network for each soil sample was extracted with the induced-subgraph function in the 'igraph package' (Csardi and Nepusz, 2006) in R (v.4.1.2). The topological properties of each sub-network were calculated, including edges, nodes, positive edges (P), negative edges (N), the ratio of negative to positive edges (N/P), degrees, average path length, betweenness centralization, diameter, density, and modularity. Visualization of microbial co-occurrence networks was carried out using the interactive platform Gephi 0.10.1 (<https://gephi.org>). For each treatment network, we identified the shared ASVs across replicates and used them as representative nodes in the network visualization.

2.5. Statistics

Data normality and variance homogeneity were tested using the Kolmogorov Smirnov test and the Levene square test, respectively. Differences of soil C and N properties, SOM, pH, bacterial co-occurrence network topological properties and α diversity indices among treatments were identified using one-way analysis of variance (ANOVA) and the Tukey's honestly significant difference (HSD) test at a probability level of $p < 0.05$. The differences in the relative abundance of bacterial communities at the phylum and order levels between treatments, as well as differences in predicted bacterial metabolic pathways between treatments were determined in STAMP v2.1.3 using two-sided White's non-parametric t -test at a probability level of $p < 0.05$. Pearson correlations between bacterial community traits and soil physicochemical properties were tested using the 'psych package' in R (v.4.1.2), with significance determined at $p < 0.05$. Structural equation modeling (SEM) was established using AMOS 22.0 to further investigate the causal relationships between soil bacterial characteristics and soil C and N properties. A well-fitted model was indicated by an insignificant Chi-square test ($p > 0.05$), a comparative fit index (CFI) > 0.95 , a goodness-of-fit index (GFI) between 0.90 and 1, and a root mean square error of approximation (RMSEA) between 0 and 0.06. PCoA visualizations were created using the 'ggplot2 package' (Wickham, 2016) in R (v.4.1.2).

3. Results

3.1. Soil chemical properties

Compared to M0, SOM increased significantly at M10, rising from 24.11 to 34.41 g kg⁻¹ soil, and further reached 37.03 g kg⁻¹ soil at M14 (Table 1). The content of TN increased significantly from 1.40 to 1.70 g kg⁻¹ soil at M10, with a further significant rise to 1.92 g kg⁻¹ soil at M14 (Table 1). The C/N ratio significantly increased at both M10 (11.72) and M14 (11.17) compared to M0 (10.01) (Table 1). Soil ROC significantly increased at M14 compared to M0 from 1.93 to 3.87 g kg⁻¹ soil (Table 1). Conversely, NO₃⁻ and NH₄⁺ concentrations were significantly lower at M6 relative to M0, and remained at reduced levels thereafter (Table 1). Soil pH ranged from 4.12 to 4.45 and, compared to straw removal, was not affected by maize straw mulching duration (Table 1).

3.2. Soil bacterial diversity and composition

For soil bacterial α diversity, taxonomic indices did not show significant differences among treatments (Fig. 1A & B), while phylogenetic indices exhibited significant changes over time (Fig. 1C & D). Specifically, compared to M0, soil bacterial MPD was significantly higher at M10 and M14 (Fig. 1C), and MNTD was significantly higher at M6 and M10, but potentially declined to near original levels at M14 (Fig. 1D). Regarding soil bacterial β diversity, maize straw duration significantly affected both taxonomic (Fig. 1E) and phylogenetic structures (Fig. 1F).

For community composition at the phylum level, bacteria underwent a significant succession after 6 years of straw mulching, shifting towards Proteobacteria and Acidobacteria at the cost of Actinobacteria (Fig. 2A). Moreover, Gemmatimonadetes and WPS-2 significantly increased at M10 and M14, respectively, while Chloroflexi and Gemmatimonadetes significantly decreased at M14 compared to M6 and M10, respectively (Fig. 2A). At the order level, the community shifted mainly toward Rhizobiales, Micropepsales, and Acidobacterales at the cost of Micrococcaceae after 6 years of straw mulching (Fig. 2B). Finally, the ratio of copiotrophs to oligotrophs (Copio/Oligo) significantly increased over time from 0.35 at M0 to 0.54 at M6, 0.69 at M10, and reaching 0.78 at M14 (Fig. 2C).

3.3. Soil bacterial co-occurrence networks

Compared to M0, bacterial network properties including edges, degree, average path length, betweenness centralization, diameter and negative edges significantly increased, while network modularity significantly decreased at M6, M10, and M14 (Table 2). Moreover, compared to M0, bacterial positive edges were significantly higher at M6 and M10, the ratio of negative to positive edges (N/P) was significantly higher at M6 and M14, and network density was significantly higher at M14 (Table 2). Bacterial co-occurrence network became increasingly complex following maize straw mulching (Fig. 3). At M6, M10, and M14, notable high-degree nodes emerged from Proteobacteria and Acidobacteria, and from Actinobacteria at M10 and M14 (Fig. 3). While the former showed more positive connections with other species,

the latter exhibited more negative connections (Fig. 3).

3.4. Soil bacterial metabolic predictions

We found that several metabolic pathways related to Biosynthesis, Degradation/Utilization/Assimilation, and Generation of Precursor Metabolite and Energy were significantly influenced by the duration of maize straw mulching (Fig. S3A). We further analyzed trends in the predicted bacterial metabolic investment in biosynthesis versus acquisition of carbohydrates (Fig. 4A-C), aromatic compounds (Fig. S3B), and organic N (Fig. S3C). Only the ratio of pathways involved in carbohydrate biosynthesis versus degradation showed significant temporal variation, increasing from M0 to M6, remaining stable at M10, and declining to initial levels by M14 (Fig. 4C). Moreover, after 6 years of straw mulching, bacterial relative investment on fatty acid and lipid biosynthesis relative to cell structure biosynthesis significantly increased and then gradually stabilized (Fig. 4F), driven by a significant rise in fatty acid and lipid biosynthesis (Fig. 4D), while no notable increase occurred in cell structure biosynthesis (Fig. 4E).

3.5. Relationships among soil chemical and microbial properties

To reveal the relationship between soil bacterial community change and SOM pool buildup, we constructed correlation analysis and a SEM. The correlation analysis indicated that soil C and N properties were more strongly associated with most of the bacterial traits (Fig. S2). The SEM results further showed that soil ROC responded positively to maize straw mulching duration, increasing Copio/Oligo bacteria, which in turn directly affected the C/N ratio (Fig. 5). Additionally, Copio/Oligo bacteria indirectly impacted C/N ratio by enhancing the ratio of fatty acid and lipid biosynthesis/cell structure biosynthesis, which positively responded to mulching duration (Fig. 5).

4. Discussion

4.1. Temporal response of soil bacterial structuring under consecutive maize straw mulching

Compared with aboveground removal, maize straw mulching resulted in notable shifts in soil bacterial structuring indices, driving community succession and evolution. Specifically, after 6 years of maize straw mulching, both phylogenetic diversity indices of MNTD and MPD exhibited an upward trend (Fig. 1C & D), indicating that early-stage crop residue mulching created more ecological niches for bacterial communities. Such habitat not only alleviated resource limitations for certain species in short-term, but also facilitated the proliferation of distantly-related species, potentially enhancing interspecies resource competition (Mayfield and Levine, 2010; Zhang et al., 2019; Wahdan et al., 2023).

Building upon the observed diversity patterns, the bacterial community underwent a significant succession. The increase in Proteobacteria was primarily driven by Rhizobiales and Micropepsales (Fig. 2B), both being categorized as the copiotrophic class Alphaproteobacteria, with Rhizobiales capable of N₂ fixation (Kappler et al., 2012; Padda

Table 1

Soil physicochemical properties at four maize straw mulching durations.

Treatment	SOC g·kg ⁻¹	SOM g·kg ⁻¹	TN g·kg ⁻¹	ROC g·kg ⁻¹	NO ₃ ⁻ mg·kg ⁻¹	NH ₄ ⁺ mg·kg ⁻¹	C/N	pH
M0	13.98 ± 0.18b	24.11 ± 0.32b	1.40 ± 0.02c	1.93 ± 0.05b	65.90 ± 4.98a	27.99 ± 3.62a	10.01 ± 0.20b	4.34 ± 0.08ab
M6	14.88 ± 0.81b	25.65 ± 1.39b	1.37 ± 0.06c	2.63 ± 0.32b	22.40 ± 1.17c	11.21 ± 2.38b	10.88 ± 0.09ab	4.45 ± 0.06a
M10	19.96 ± 1.02a	34.41 ± 1.75a	1.70 ± 0.07b	3.03 ± 0.29ab	47.68 ± 5.98ab	17.23 ± 1.89ab	11.72 ± 0.13a	4.19 ± 0.08ab
M14	21.48 ± 0.75a	37.03 ± 1.30a	1.92 ± 0.01a	3.87 ± 0.27a	37.86 ± 6.25bc	7.10 ± 1.20b	11.17 ± 0.32a	4.12 ± 0.03b

SOC, soil organic carbon; SOM, soil organic matter; TN, total nitrogen; ROC, readily oxidizable carbon; NH₄⁺, ammonium; NO₃⁻, nitrate; C/N, the ratio of SOC to TN. Data were presented as mean ± standard errors. Different lowercase letters in the same column indicate that mean values are significantly different ($p < 0.05$). M0, M6, M10, and M14 represent complete maize straw mulching durations of 0, 6, 10 and 14 years, respectively.

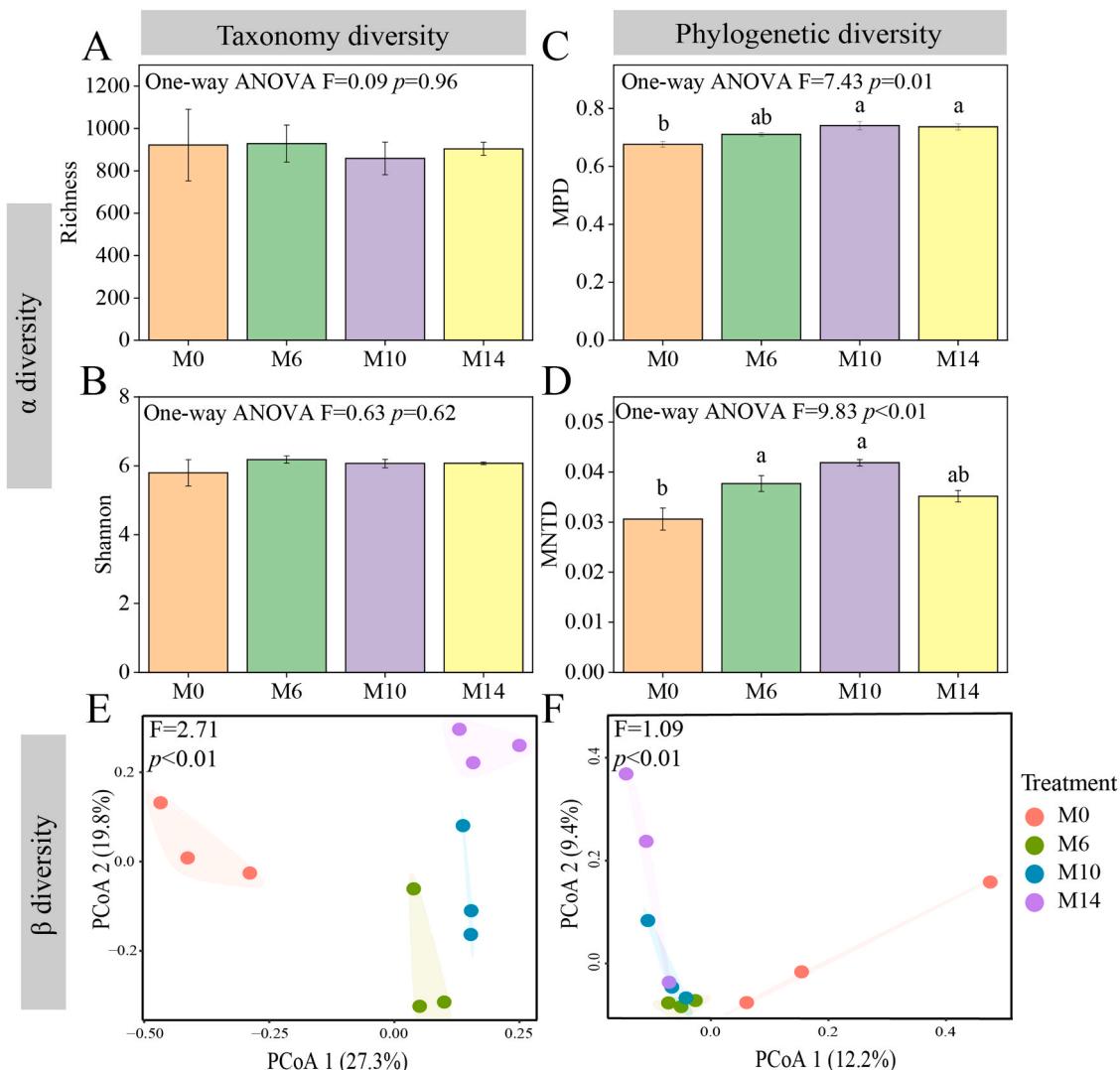


Fig. 1. Soil bacterial α - (A-D) and β - (E-F) diversities. In panels (A) to (D), error bars represent standard error ($n = 3$). Different lowercase letters indicate significant differences among the four durations within each panel ($p < 0.05$). MPD, mean pairwise distance; MNTD, mean nearest phylogenetic taxon distance. In panels (E) and (F), principal coordinates analysis (PCoA) was used to visualize soil bacterial β diversities based on Bray-Curtis distance (E) and β MPD (F), respectively. M0, M6, M10, and M14 represent complete maize straw mulching durations of 0, 6, 10 and 14 years, respectively.

et al., 2022; Cui et al., 2023). These groups likely accelerated the turnover of soil inorganic nutrients and maize straw-derived labile C (Saifuddin et al., 2019; Shao et al., 2021), as evidenced by the decrease in inorganic N and subtle accumulation of ROC by M6 (Table 1). However, when maize straw mulching was extended till 14 years, the relative abundance of these dominant groups remained relatively stable (Fig. 2B). Such lack of further increase suggested that, with continued input of maize residues, more partially-decomposed residue C may have been selectively incorporated into SOM pool, contributing to SOM accumulation and a more pronounced increase in ROC after 10–14 years of treatment (Table 1; Li et al., 2023; Yang et al., 2024). Furthermore, the proliferation of N₂-fixing bacteria (Rhizobiales) likely enhanced microbial contributions to soil N, as reflected in the significant increase in TN till the 10th year (Table 1). For the other two oligotrophic groups contributing to the community succession, the increase of Acidobacteria was primarily driven by the order Acidobacterales while the decrease of Actinobacteria was mainly caused by the order Micrococcales (Fig. 2B). Micrococcales have stronger decomposition abilities, primarily participating in nutrient cycling under stressful conditions (Tian et al., 2023). Comparatively, Acidobacterales are less effective at breaking down high lignin-to-N ratio substrates but capable utilizing hemicellulose,

cellulose and organic acids during crop residue decomposition (Gu et al., 2022; Gonçalves et al., 2024). Thereby, the return of maize straw may alleviate habitat stress by increasing substrate availability, which was less favorable for the proliferation of Actinobacteria. Nevertheless, microbes displayed a preference for polysaccharides over lignin in this field (Li et al., 2023; Fu et al., 2024), a process likely closely linked to the increased abundance and functional role of Acidobacteria.

While dominant bacterial succession mainly occurred within the first 6 years of maize straw return, changes in some other bacterial groups appeared over a longer timescale. With the prolonged duration of maize straw mulching to 10 years, the increased relative abundance of Gemmatimonadetes, known for its diverse metabolic capabilities in decomposing complex organic substrates (Zheng et al., 2022), inferred a greater accumulation of various C substrates within bacterial habitats, as consistent with the significant increase in SOC at M10 (Table 1). By the 14th year of maize straw mulching, the enhancement of WPS-2, recognized for its strong potential for utilizing recalcitrant C in C-rich environments (Fan et al., 2019; Sheremet et al., 2020), corresponded to the significant rise in the C/N ratio at M14 (Table 1). This suggested that while straw mulching provided abundant C for copiotrophic bacteria, the soil bacterial community may have developed functional groups

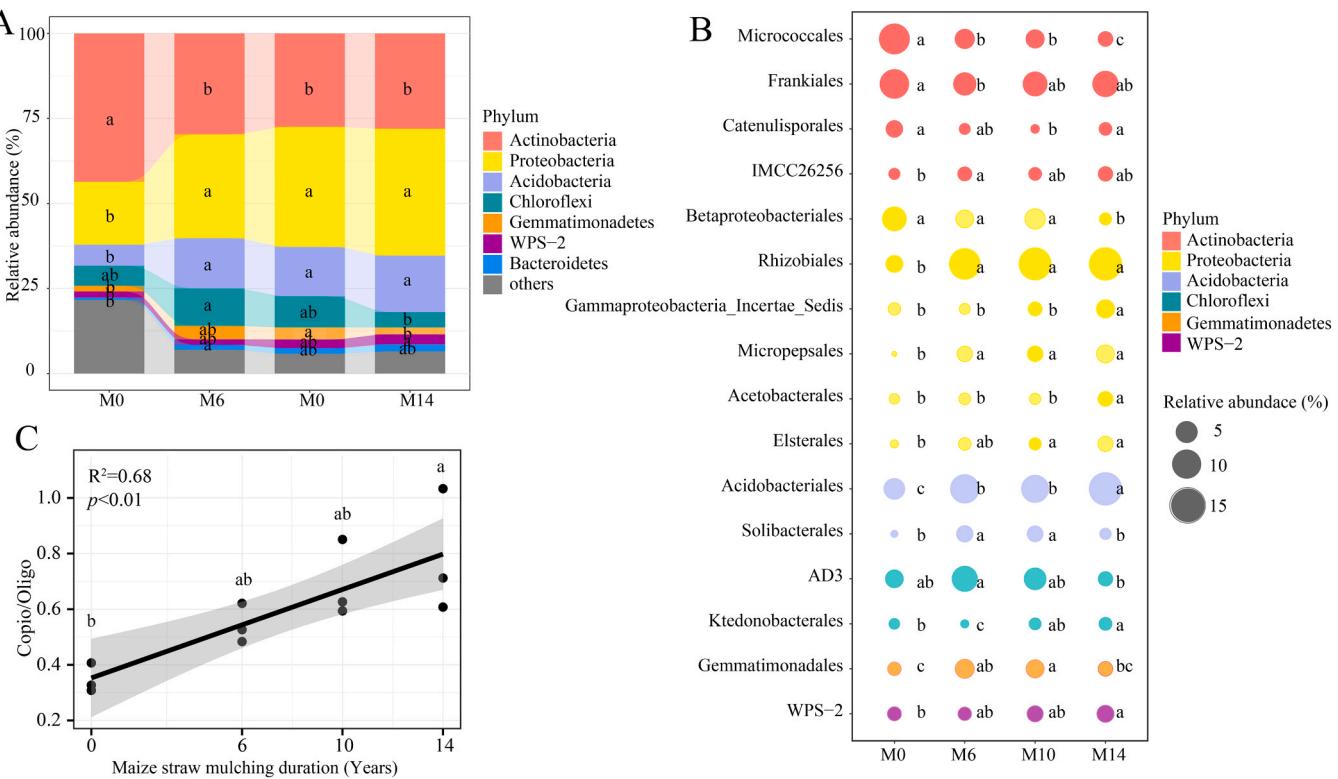


Fig. 2. Relative abundance of bacteria at the phylum (A) and order (B) levels, and changing ratio of copiotrophic (Copio) to oligotrophic (Oligo) bacteria over time (C). In panel (B), bacterial orders that show significant differences between treatments are presented. The size of each circle represents the relative abundance of the corresponding bacterial order, while the color indicates its associated phylum. Panel (C) shows the relationship between the Copio/Oligo ratio and the duration of maize straw mulching, with the R-square and p-value provided. Different lowercase letters in panels (A), (B) and (C) indicate significant differences among the four durations ($p < 0.05$). M0, M6, M10, and M14 represent complete maize straw mulching durations of 0, 6, 10 and 14 years, respectively.

Table 2

Co-occurrence network topological properties at four maize straw mulching durations.

Treatment	Edges	Degree	Average path length	Betweenness centralization	Modularity	Diameter	Density	Positive edges (P)	Negative edges (N)	N/P
M0	40.00 ± 11.53b	0.71 ± 0.13b	1.67 ± 0.30b	0.00 ± 0.00b	0.88 ± 0.02a	3.67 ± 1.20b	0.006 ± 0.00b	33.33 ± 10.33b	6.67 ± 1.20b	0.22 ± 0.03b
M6	82.33 ± 0.33a	1.13 ± 0.01a	3.14 ± 0.13a	0.01 ± 0.00a	0.82 ± 0.00b	9.67 ± 0.67a	0.008 ± 0.00ab	60.33 ± 1.86a	22.00 ± 2.08a	0.37 ± 0.04a
M10	88.33 ± 4.26a	1.18 ± 0.03a	3.07 ± 0.09a	0.01 ± 0.00a	0.82 ± 0.01b	8.67 ± 0.33a	0.008 ± 0.00ab	66.67 ± 3.53a	21.67 ± 0.88a	0.33 ± 0.01ab
M14	74.00 ± 2.08a	1.15 ± 0.02a	3.09 ± 0.11a	0.02 ± 0.00a	0.78 ± 0.01b	8.67 ± 0.33a	0.009 ± 0.00a	52.33 ± 1.45ab	21.67 ± 0.88a	0.41 ± 0.01a

Data were presented as mean ± standard errors, and the different lowercase letters in the same column indicate that mean values are significantly different ($p < 0.05$). M0, M6, M10, and M14 represent complete maize straw mulching durations of 0, 6, 10 and 14 years, respectively.

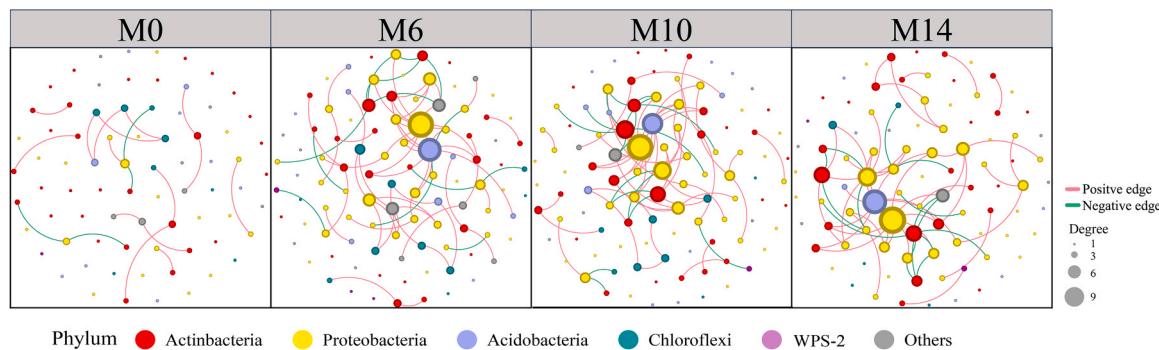


Fig. 3. Visualization of the soil bacterial co-occurrence network. Node colors indicate bacterial classifications at the phylum level and node size represent degree. Red and green links denote positive and negative connections between nodes, respectively. M0, M6, M10, and M14 represent complete maize straw mulching durations of 0, 6, 10 and 14 years, respectively.

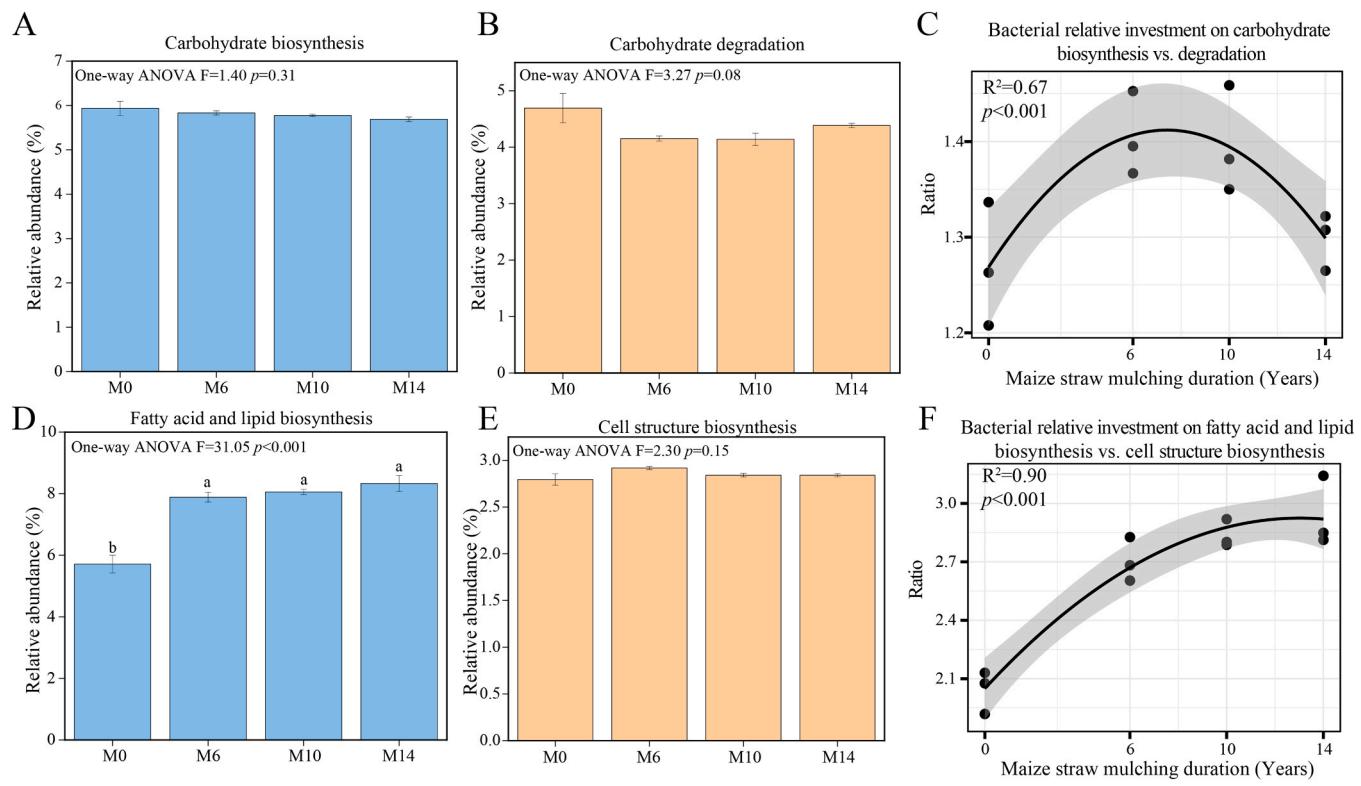


Fig. 4. Predicted bacterial metabolic pathways. Panels (A) to (C) display the relative abundances of carbohydrate biosynthesis, degradation, and their ratio, respectively. Panels (D) to (F) show the relative abundances of fatty acid and lipid biosynthesis, cell structure biosynthesis, and their ratio, respectively. For panels (C) and (F), the significant relationships between the duration of maize straw mulching and each variable are indicated by R-squares and p-values in the plots. Different lowercase letters indicate significant differences between durations ($p < 0.05$). M0, M6, M10, and M14 represent complete maize straw mulching durations of 0, 6, 10 and 14 years, respectively.

capable of efficiently utilizing the input recalcitrant components over the long term (14 years). Our further observation of a steadily rising Copio/Oligo ratio, though with a slowing rate, indicated that straw mulching alleviated copiotrophic bacterial C starvation at the early stage. As substrate complexity increased in the later stages, diverse ecological niches favored the proliferation of both oligotrophic and copiotrophic bacteria, each adapting to their preferred substrates.

4.2. Temporal changes of soil bacterial co-occurrence network and metabolic potentials under continuous maize straw mulching

The co-occurrence network characteristics of the bacterial community further revealed the increasing resource competition among groups as maize straw return continued. The bacterial network evolved toward greater complexity and exhibited more interactions, particularly negative ones under all the maize straw mulching durations (Table 2), supporting our earlier inference that increased phylogenetic diversity among bacteria caused intensified interspecies competition for resources. Moreover, a significant reduction in the modularity of the bacterial network under maize straw mulching (Table 2) suggested that interactions among species have become more extensive and interconnected, ultimately enhancing resource acquisition across the bacterial community (Li et al., 2022). Under such intensified resource competition, we unexpectedly found two key nodes in Proteobacteria and Acidobacteria with a higher positive connections with other species (Fig. 3), suggesting that cross-feeding interactions could also emerge among certain species in response to complex organic matter input (Kong et al., 2020; Kost et al., 2023). Nonetheless, during 10–14 years, several new, highly connected nodes emerged within the phylum Actinobacteria, predominantly displaying negative interactions with other species (Fig. 3). Such intensification of resource competition between

oligotrophic bacteria and the others may limit the rapid thriving of bacterial community, which could explain the gradual decline in the growth rate of copiotrophic bacterial species.

Based on the above community succession and interactions, bacterial growth was promoted by straw return; however, its growth rate and C utilization efficiency were constrained by the duration of straw mulching, as evidenced by metabolic pathway predictions. Specifically, although straw mulching enhanced the potential for bacterial biomass synthesis, the relative increase in fatty acid and lipid biosynthesis pathways after 6 years was minimal (Fig. 4D). This confirmed that the growth rate of the bacterial community was constrained at the stage of 10–14 years of maize straw mulching. This aligns with the finding observed by Marschner et al. (2011) and Chen et al. (2015), where microbial growth rates gradually stabilized following long-term input of complex substrates. Furthermore, although bacterial carbohydrate biosynthesis efficiency improved after 6 years of straw mulching, as indicated by an increased biosynthesis versus degradation ratio (Fig. 4C), this trend reversed with continued mulching, suggesting that while bacteria became more capable of acquiring carbohydrates, their utilization efficiency declined with prolonged mulching duration. As bacterial growth and labile C utilization became increasingly constrained, it may shift to favor the synthesis of bacterial-derived stable C, as indicated by the non-increasing ratio of relative investment in cell membrane versus structure biosynthesis during the 10–14 years (Fig. 4F). This suggested that after 10 years of maize straw return, the conditions with a higher amount of recalcitrant plant residue retention favored the conversion of straw-derived C into more stable bacterial components, contributing to the observed increase in the C/N ratio (Table 1). In this study, we used PICRUSt2 to predict bacterial metabolic functions based on community structural traits. However, we acknowledge its limitations, such as genomic reference bias and insufficient

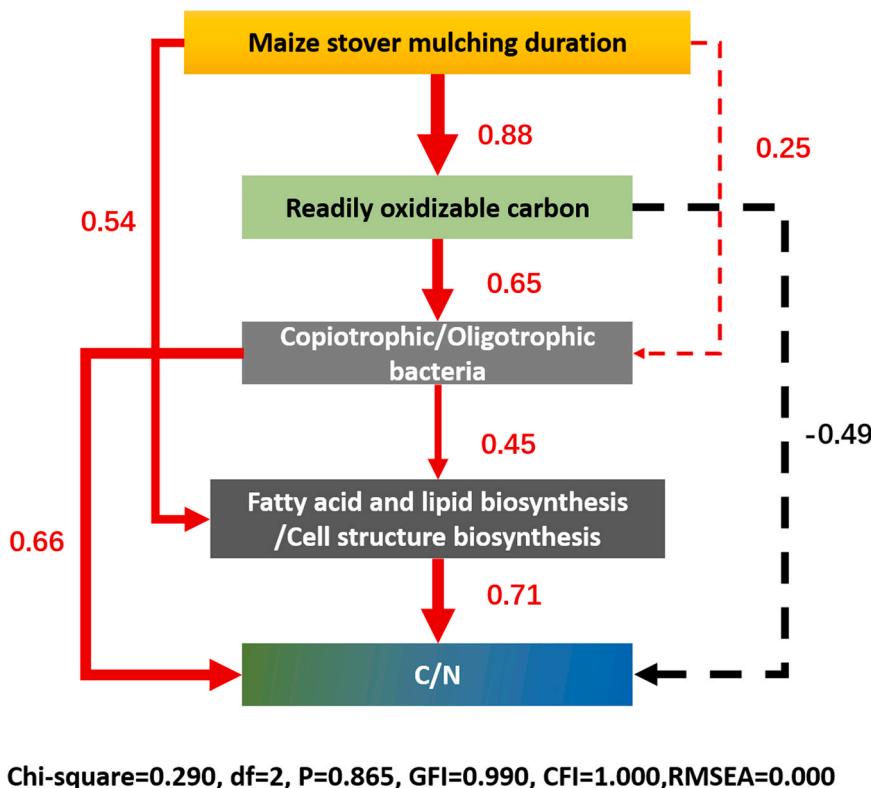


Fig. 5. Structural equation model that shows the causal linkages between soil bacterial traits and soil organic matter buildup. The solid red arrows indicate significant positive effects, while the dashed red and black arrows represent non-significant positive and negative relationships, respectively. The width of the arrows indicates the strength of the standardized path coefficients, which are the numbers adjacent to the arrows. Copiotrophic/Oligotrophic bacteria, the ratio of copiotrophic to oligotrophic bacterial relative abundance. Fatty acid and lipid biosynthesis/Cell structure biosynthesis, the ratio of relative abundance of metabolic pathways associated with fatty acid and lipid biosynthesis to those associated with cell structure biosynthesis. C/N, the ratio of soil organic carbon to total nitrogen.

strain-level resolution (Douglas et al., 2020), highlighting the need for future validation through complementary approaches.

4.3. The coupling relationship between soil bacterial community and soil organic matter pool buildup

Integrating soil bacterial traits with the dynamics of SOM pool, we found that bacterial communities responded rapidly, while SOM quantity and quality occurred changes gradually. As soil bacterial responses progressed through three phases, ranging from rapid growth to resource competition and finally to less efficient carbohydrate utilization, SOM pool buildup evolved from initial stability to accelerated accumulation, ultimately enhancing both its quantity and C availability. Supported by SEM analysis (Fig. 5), we confirmed both their coupled relationship and our hypothesis that the sensitive response of bacterial communities to the input of exogenous organic matter played a key role in driving the accumulation and turnover of SOM. Accordingly, we proposed the following potential bacterial mechanisms driving SOM buildup over time (Fig. 6).

Compared to soils without straw return, the short-term (6 years) application of straw alleviated bacterial community C starvation, triggering bacterial proliferation, faster growth, and stronger interactions among species. Although this process intensified negative interactions between bacteria, cross-feeding among dominant species and other taxa also increased, facilitating resource acquisition and promoting the rapid growth of the bacterial community. This was reflected in higher bacterial metabolic investments in carbohydrate biosynthesis and biomass yield. However, such rapid bacterial growth could inevitably accelerate the turnover of habitat resources (Kuzyakov and Blagodatskaya, 2015), which explained why the magnitude of SOM, as well as C availability did not significantly increased during this phase. Mid-term (10 years) and

long-term (14 years) applications of straw mulching further intensified resource competition between certain Actinobacteria and other species, which somewhat constrained the growth rate of the bacterial community and the increment of bacterial biomass biosynthesis. The enhanced resource acquisition capacity could facilitate the release of recalcitrant components derived from maize straw. Therefore, from the 10th year onward, the relative retention of plant-derived C is expected to increase (Li et al., 2023), favoring stable structural bacterial C biosynthesis (Fig. 4F), which further contributed to the faster accumulation of C than N in soil (Table 1). Between the 10th and 14th years, a key difference was the lower relative bacterial investment in carbohydrate biosynthesis versus degradation in the latter (Fig. 4C). Such reduced efficiency in carbohydrate utilization at M14 likely reflected an augmented N demand to support further bacterial metabolic improvements in high C/N habitats (Cui et al., 2020). Correspondingly, the soil at M14 exhibited not only a higher quantity of SOM, but also a greater C/N ratio, and increased C availability, as evidenced by elevated ROC concentrations (Table 1). Our study primarily focused on the bacteria that play a critical role in the internal cycling of SOM. However, we acknowledge that soil fungi also have a significant and often underappreciated role in transforming plant-derived C into soil (Koechli et al., 2019; Zhao et al., 2021b). Investigating their temporal dynamics and interactions with bacteria in future studies would help better integrate microbial traits with SOM accumulation and turnover in agroecosystem.

5. Conclusions

This study provides temporal evidence that bacterial traits are critical in the buildup of SOM in no-till systems with maize straw mulching. Our results suggest that bacterial traits, such as phylogenetic diversity, composition, functional group succession (Copio/Oligo ratio), co-

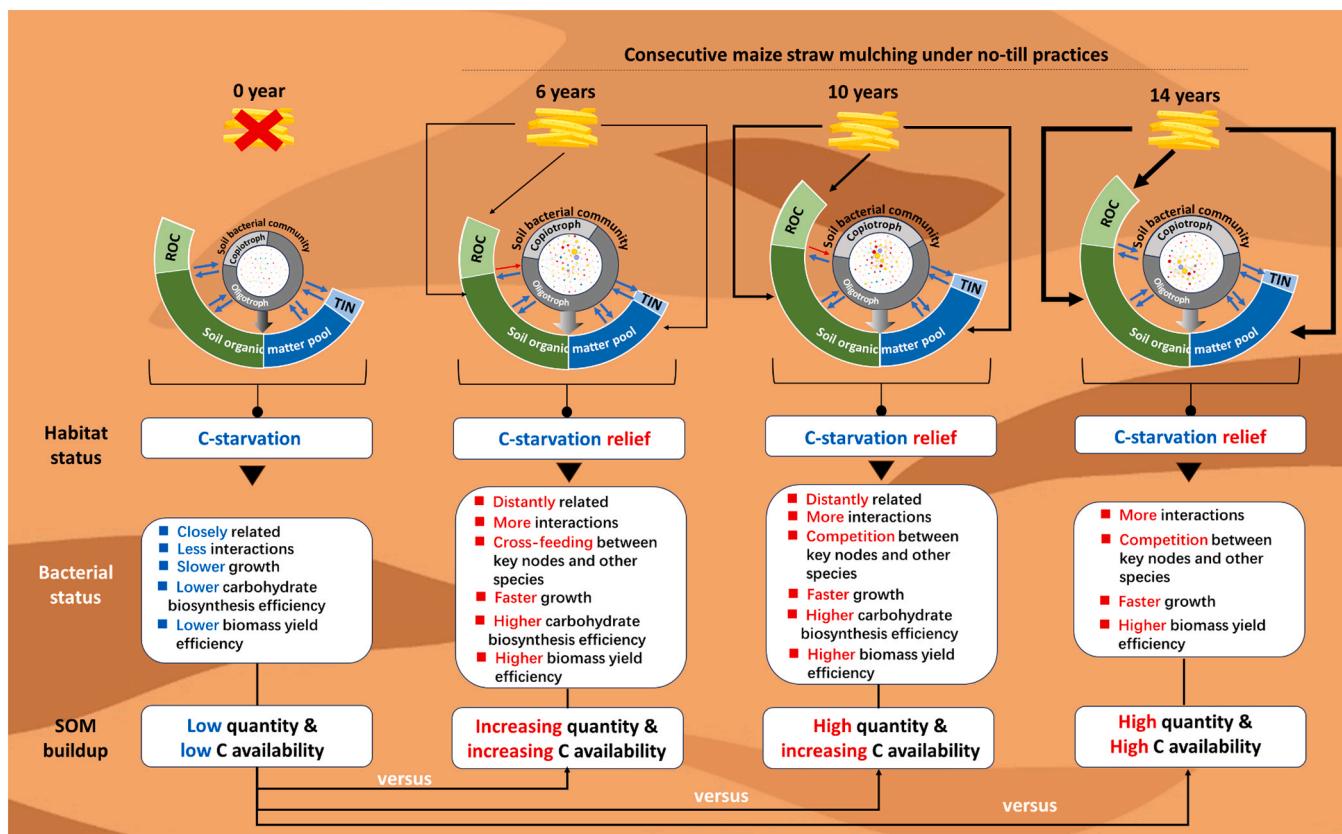


Fig. 6. Conceptual illustration depicting the potential mechanisms underlying the synergies between bacterial communities and the accumulation and quality of soil organic matter (SOM) in response to varying durations of maize straw mulching. The green portion of the circle represents the total soil organic carbon (C) content, while the blue portion indicates the total nitrogen content. TIN, total inorganic nitrogen, which is represented by the sum of ammonium nitrogen and nitrate nitrogen; ROC, readily oxidizable carbon. Bidirectional blue arrows illustrate the interactions between organic matter components and the soil bacterial community. Blue arrows pointing away from the bacterial community indicate its potential contribution to these components through biosynthesis, while blue arrows pointing towards the bacterial community represent bacterial degradation of those components. Starting from the top and moving counterclockwise, each pair of arrows corresponds to carbohydrate, aromatic compound, organic nitrogen, and inorganic nitrogen metabolism, respectively. Arrow thickness reflects the relative contribution of bacterial communities and their extent of decomposition for the same component. The red arrows indicate relatively more investment on carbohydrate biosynthesis compared with 0 year. Arrows from maize straw to the C and nitrogen circles indicate that, with increasing years of maize straw mulching, the exogenous organic C and nutrients input rises. For soil bacterial community, the inner circle illustrates the bacterial network, while the outer circle represents the relative proportions of copiotrophic and oligotrophic bacteria. The text boxes in the first row beneath the illustration describe the C status of bacterial habitats corresponding to each duration. The text in the second and third rows summarizes the bacterial state and SOM buildup status, respectively, for each duration compared to the 0-year control based on experimental results. To better highlight differences between treatments, we used blue and red font colors to emphasize the parts where variations were observed.

occurrence network properties, and metabolic potentials, responded more rapidly to maize straw return than SOM pool capacity enlargement. By integrating these bacterial structuring and metabolic traits, we observed a survival strategy shift in the bacterial community from rapid growth to resource competition, and ultimately to less efficient carbohydrate utilization, along a gradient of increasing maize straw mulching durations. This progression corresponded to SOM buildup, from no significant changes in SOM quantity and C availability to increased quantity but no changes in C availability, and eventually to both enhanced quantity and C availability. Causal relationship analysis, based on chemical and microbial properties, further demonstrated that maize straw mulching could promote SOM buildup by influencing key traits related to bacterial growth strategies, which are driven by labile C availability. Overall, we conclude that maize straw mulching serves as a substantial C source for bacterial growth, with bacterial survival strategies evolving over time to regulate SOM quantity and quality. These findings underscore the importance of evaluating multiple bacterial traits and incorporating temporal considerations to better understand SOM sustainability in agroecosystem. Our findings suggest that consecutive return of crop residues with high C to N ratios may diminish microbial efficiency in converting exogenous C into SOM. To enhance C

sequestration and soil fertility in arable ecosystems, strategic management adjustments that optimize resource stoichiometry, such as cover cropping, organic manure application, and legume incorporation, might effectively enhance microbial C use efficiency and support sustainable conservation agriculture.

CRediT authorship contribution statement

Zhangmi He: Writing – original draft, Visualization, Investigation, Formal analysis. **Xuefeng Zhu:** Writing – original draft, Visualization, Funding acquisition, Formal analysis, Conceptualization. **Feng Zhou:** Writing – review & editing, Investigation, Funding acquisition. **Mengtao Zhu:** Investigation. **Xuelian Bao:** Investigation. **Fangbo Deng:** Investigation. **Hongbo He:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Xudong Zhang:** Funding acquisition, Conceptualization.

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. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.agee.2025.109816](https://doi.org/10.1016/j.agee.2025.109816).

Data availability

Data will be made available on request.

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