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The molecular composition of soil organic matter is regulated by bacterial community under biochar application

Zongkun Yang [a], Wenbo Liu [a,b,*], Xiaoge Fan [a], Han Gao [c], Xiangrui Xu [d], Cheng Liu [a], Yanjun Chai [a], Min Zhang [a], Marios Drosos [e], Shengdao Shan [a]

[a] Key Laboratory of Recycling and Eco-treatment of Waste Biomass of Zhejiang Province, School of Environmental and Natural Resources, Zhejiang University of Science and Technology, Hangzhou, China

[b] College of Resources and Environmental Sciences, Nanjing Agricultural University, Nanjing, China

[c] State Environmental Protection Key Laboratory of Soil Environmental Management and Pollution Control, Nanjing Institute of Environmental Sciences, Ministry of Ecology and Environment of China, Nanjing, China

[d] Research Institute for Urban Planning and Development, Hangzhou City University, Hangzhou, China

[e] Department of Agricultural, Forest, Food, and Environmental Sciences, University of Basilicata, Viale dell'Ateneo Lucano n. 10, Potenza, Italy

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ABSTRACT

Soil organic matter (SOM) consists of diverse carbon compounds, which are influenced by microorganisms that affect its turnover and stability. However, changes in SOM molecular composition following biochar application and their interactions with the soil bacterial communities remain poorly understood. We aimed to evaluate SOM molecular composition, soil bacterial communities, and carbon cycle functional genes of bacteria in soils treated with biochar using pyrolysis–gas chromatography-mass spectrometry (py-GC/MS) and amplicon sequencing. The py-GC/MS results indicated that biochar increased the molecular diversity and significantly altered the molecular composition of SOM. In biochar-treated soils, the abundance of lignin-derived products increases, while lipids levels decrease. Biochar application shifted the soil bacterial life-history strategy towards copiotrophy, characterised by a higher copiotroph/oligotroph ratio and ribosomal RNA operon copy number. Procrustes analysis revealed that SOM molecular composition was strongly correlated with both the bacterial community and carbon cycle functional genes. Specifically, the SOM composition was closely associated with Gammaproteobacteria, Acidobacteria, and Chloroflexi. Additionally, SOM network analysis indicated that biochar enhanced SOM molecular complexity (i.e., node count, edge count, and average degree) primarily due to the accumulation of lignin-derived products. These findings highlight the potential of biochar to reshape the molecular composition of SOM via microbially mediated processes.

1. Introduction

Soil organic matter (SOM), which is primarily composed of carbon, plays a vital role in regulating soil ecosystems, including biodiversity and agricultural productivity (Cotrufo and Lavallee, 2022). Currently, intensive agricultural practices are the primary contributors to soil carbon loss, with an average annual loss of soil organic carbon (SOC) estimated at approximately 0.3–1.0 Pg C (Tian et al., 2024). SOC loss causes a decline in soil fertility, reduced nutrient uptake by crops, and an increased risk of climate change. Owing to biochar porous structure, alkaline nature, abundant surface functional groups, and nutrient content, biochar has been recognised as a valuable soil amendment (Chi et al., 2024). As an environmentally friendly soil amendment, biochar not only provides a carbon source to the soil but also alters microbial communities, thereby influencing SOC turnover (Y. Chen et al., 2024a). Consequently, biochar is frequently employed as an effective amendment in agronomic practices to enhance SOM content and promote soil carbon sequestration in agricultural production.

SOM primarily originates from plant residues, soil microbial necromass, and metabolic by-products (Whalen et al., 2022). It consists of organic compounds of varying molecular sizes and carbon structures, including aromatic compounds, lignin-derived products, lipids, nitrogen compounds, polycyclic aromatic hydrocarbons, and polysaccharides (Jones et al., 2023; San-Emeterio et al., 2023). This molecular-level

* Corresponding author at: Key Laboratory of Recycling and Eco-treatment of Waste Biomass of Zhejiang Province, School of Environmental and Natural Resources, Zhejiang University of Science and Technology, Hangzhou, China.

E-mail address: wbliu@zust.edu.cn (W. Liu).

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approach provides insight into the origin and transformation of SOM components. SOM molecular composition is inevitably influenced by the ecosystem type and soil horizon (Davenport et al., 2023), as well as agronomic practices. A recent study indicated that increasing biochar application enhanced soil concentration of polycyclic aromatic hydrocarbons (PAHs) (Zhang et al., 2024). Recently, new perspectives for the microscale observation and characterisation of SOM components have been provided by modern analytical techniques such as solid-state ^{13}C nuclear magnetic resonance spectroscopy (Bahadori et al., 2021), nanoscale secondary ion mass spectrometry (Vidal et al., 2021), and pyrolysis–gas chromatography-mass spectrometry (py-GC/MS) (Jones et al., 2023). Py-GC/MS enables the identification of SOM components through the analysis of characteristic fragment ions generated during high-temperature pyrolysis without the need for soil sample pretreatment (Akoueson et al., 2021; Lyu et al., 2024). This method is highly regarded for its precise and detailed molecular analysis capabilities (Cui et al., 2024; Jensen et al., 2024). Therefore, employing py-GC/MS technology has significantly enhanced our understanding of SOM molecular characteristics. These methods also provide valuable insights into the effects of biochar application on SOM composition and transformation at the molecular level.

In addition to being regulated by exogenous carbon, SOM molecular components are shaped by the characteristics of soil microbial communities. Soil bacterial communities influence SOM formation and decomposition (Sokol et al., 2022). During SOM formation, soil bacterial cells in biofilms combine with polysaccharides, proteins, lipids, and other biopolymers to form extracellular polymeric substances (Op De Beeck et al., 2021) that bind to mineral particles to create SOM (Whalen et al., 2022). However, the rate and extent of microbial utilisation of organic substrates determine the SOM decomposition rate (Angst et al., 2021). A previous report indicated that copiotrophic bacteria preferentially mineralise easily decomposable organic matter molecules, whereas oligotrophic bacteria drive the decomposition of recalcitrant organic matter (Su et al., 2023). Therefore, the interactions and succession within the microbial communities, as well as different life history strategies, collectively determine the turnover rate of soil carbon. Research has shown that microorganisms drive significant changes in the complexity and diversity of SOM molecular composition (Davenport et al., 2023). For instance, changes in the microbial community composition facilitate lignin decomposition (Man et al., 2022), whereas mineral fertiliser application accelerates the decomposition of lipid compounds (Zou et al., 2023). However, how the molecular composition of organic matter is regulated by soil bacterial communities following biochar application, particularly when different microbial life strategies are considered, remains unclear.

Thus, we aimed to investigate changes in SOM molecular composition and soil bacterial communities in rice paddy soils subjected to different carbon treatments (no biochar application (NPK), perishable waste biochar (PWB), rice straw biochar (RSB), pig manure biochar (PMB)). We hypothesised that (i) biochar could increase SOM diversity and complexity and alter its composition, and (ii) biochar-related changes in SOM molecular composition are associated with bacterial communities, particularly those with varying life strategies. Disentangling the changes in SOM molecular composition and their relationship with soil bacterial communities can significantly advance our knowledge of the strategic management of agricultural soil carbon sequestration with biochar application.

2. Materials and methods

2.1. Experimental design and soil sampling

The field experiments were established in 2021 in Tongxiang City, Zhejiang Province, China (120.618°E, 30.637°N), a typical rice cultivation region with a subtropical monsoon climate (average annual temperature 20 °C, precipitation 1070 mm). The soil is classified as Vertisols, typical of paddy soils influenced by long-term rice cultivation, with the physicochemical properties: pH 7.07, total C 8.93 g kg^{-1}, total N 0.98 g kg^{-1}, total P 0.73 g kg^{-1}, and total K 23.66 g kg^{-1} (Yang et al., 2024). The field experiment had a randomised block design with four treatments (three replicates), as follows: (1) only chemical fertiliser and no biochar application (NPK); (2) chemical fertiliser and perishable waste biochar (PWB); (3) chemical fertiliser and rice straw biochar (RSB); (4) chemical fertiliser and pig manure biochar (PMB). The field rotation system is rice-fallow, with rice planted from June to October each year and the rest of the time being fallow. Biochar was produced by pyrolysis at 500 °C under anaerobic conditions. Biochar was applied once at the beginning of June 2021, at a rate corresponding to the total amount for six years, with no additional applications over the following six years. Fertiliser and biochar application rates are provided in Table S1, while the physicochemical properties and molecular compositions of the biochars are detailed in Tables S2 and S3.

Soil samples were collected following the rice harvest in the second cropping cycle. Considering the depth of the tillage layer in this region, soil at a depth of 0–15 cm was sampled from each plot. Each fresh soil sample was initially passed through a 2 mm sieve to remove small stones, root fragments, and coarse biochar particles. The sieved soil was then divided into two subsamples: one was stored at –80 °C for DNA extraction, and the other was air-dried for subsequent molecular analysis. After air-drying, residual biochar particles were manually removed. The dried soil was thoroughly ground and, as completely as possible, passed through a 0.15 mm sieve to ensure uniform particle size. This standardised preparation was critical for minimising size-related variability and ensuring comparability in SOM molecular composition analysis.

2.2. Measurement and classification of SOM molecules

The SOM molecular compositions of all samples were determined using py-GC/MS at Nanjing University. The py-GC/MS analysis was conducted using a multi-shot pyrolyzer (EGA/PY-3030D, Frontier Lab, Japan) paired with an ISQ single quadrupole GC–MS system (Thermo Fisher Scientific, USA). A DB-5MS capillary column (0.25 mm × 30 m, 0.25 μm, J&K Scientific, USA) connected to a quadrupole mass spectrometer was fitted to the GC system. Helium was used as the carrier gas at a constant flow rate of 1.0 mL/min in splitless mode. The GC temperature program was set to begin at 40 °C (held for 3 min), followed by an increase to 270 °C at 10 °C/min, and then maintained for an additional 5 min. Samples were pyrolyzed at 450 °C for 12 s before the signal acquisition. The temperatures of the py-GC/MS interface, injector, transfer line, and source were set to 250 °C, 250 °C, 270 °C, and 280 °C, respectively. Mass spectra were scanned within a range of 35–600 m/z at a rate of five scans per second, with the electron energy configured to 70 eV.

Thermo Scientific Xcalibur (version 4.1) software and the National Institute of Standards and Technology (NIST) compound library were used for peak analysis and compound identification (Gao et al., 2022; Jones et al., 2023). Compounds were categorized based on their sources, including aromatic compounds, lignin-derived products, lipids, nitrogen compounds, polycyclic aromatic hydrocarbons, and polysaccharides, and expressed as a percentage of the total sample peak area. Specifically, for compound identification, the compound with the highest probability at each retention time from the three replicates of each treatment was selected as the representative for that retention time, with its peak area recorded. For compound classification, classification data were first compiled from relevant studies (Almendros et al., 2018; Gao et al., 2022; Girona-García et al., 2019; González-Pérez et al., 2007; Grandy et al., 2009; Jones et al., 2023). Xcalibur software was then used to identify the molecular structure of each compound, and compounds were classified based on their functional groups. This approach ensures a robust and consistent method for compound identification and classification. Compounds of multiple or unknown origins were classified as "unknown origin.” Compounds with an abundance of less than 0.01 % across all samples were excluded from the analysis.

2.3. Amplicon sequencing and high-throughput quantitative PCR

DNA was isolated from soil samples with a FastDNA Spin Kit (MP Biomedicals), and its quality was assessed via a NanoDrop spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA).

To assess the bacterial communities, the V4–V5 hypervariable regions of the bacterial 16S rRNA gene were amplified using the primers 515F (5′-GTGCCAGCMGCCGCGG-3′) and 907R (5′-CCGTCAATTCMTTTRAGTTT-3′). Sequencing was performed by Genesky Biotechnologies, Inc. (Shanghai, China) using an Illumina NovaSeq 6000 platform. Raw reads were processed using the QIIME2 Analysis Platform (https://qiime2.org). The Cutadapt plugin was used to remove the adaptor and primer sequences. Quality control and amplicon sequence variant (ASV) identification were conducted using the DADA2 plugin with parameters set to p-max-ee = 2.0, p-trunc-q = 2, and p-chimera-method = consensus (Callahan et al., 2016). Taxonomic classification of representative ASV sequences was performed using a naïve Bayes classifier trained on the Ribosomal Database Project (RDP, version 11.5). To account for sequencing variations across samples, all datasets were rarefied into uniform 17,298 sequences for downstream analyses. All raw data have been submitted to the NCBI Sequence Read Archive (SRA) under BioProject ID PRJNA1153308.

High-throughput qPCR was performed on a Wafergen SmartChip Real-Time qPCR platform to analyse 34C-cycling functional genes following the previous method (Wang et al., 2014). Each DNA sample was amplified in triplicate with the detection limit set at 31 threshold cycles. If multiple primer sets were targeted at the same gene, it was considered a single functional gene.

2.4. rRNA operon copy number

The rRNA operon copy number at the community level was estimated using the rrnDB database (https://rrndb.umms.med.umich.edu/). Starting from the lowest taxonomic rank (genus), rrn copy numbers were matched. For operational taxonomic units (OTU) without rrn data, the average copy number of the parent taxa was used. The abundance-weighted average rrn copy number was calculated for each OTU. Finally, the abundance-weighted average run for each sample was determined by taking the product of the estimated run copy number and the relative abundance for each OTU and summing this value across all OTU in the sample (Wang et al., 2024).

2.5. Construction of SOM molecular co-occurrence network

The SOM molecular co-occurrence network was constructed using a method based on random matrix theory (Zhang et al., 2018). First, data filtering was performed to ensure reliability and eliminate potential false associations among OTUs. Subsequently, the adjacency matrices for all the samples were imported into R and converted into a network data format. Finally, subnetworks were selected and partitioned from the “global network” based on specified node or edge sets. The subnetworks for each sample were extracted, and their topological properties were calculated, including the number of nodes and edges, average degree, average path length, graph density, eigenvector centrality, and clustering coefficient. All networks were constructed using the Molecular Ecological Network Analysis pipeline and visualised using the interactive Gephi platform (https://gephi.org).

2.6. Statistical analysis

To analyse the α-diversity of SOM molecular composition, bacterial community, and C cycling functional genes, we calculated the richness and Shannon indices using the ‘vegan’ package in R software (v4.3.2) (Hill et al., 2003). Analysis of variance (ANOVA) tests were then conducted to compare significant differences in richness and Shannon indices, copiotroph/oligotroph ratios, rrn copy numbers, C cycling gene abundance, and subnetwork topological properties across different treatments. To assess the β-diversity of SOM molecular composition and bacterial communities, Bray–Curtis distances were calculated using the vegdist () function from the ‘vegan’ package in R. Dissimilarities in distances were visualised using principal coordinate analysis (PCoA) plots, and non-parametric analysis of variance (Adonis) was used to examine differences between treatments. Procrustes and Mantel analyses were used to assess the correlations between SOM molecular composition and microbial communities as well as C cycling genes. Subnetworks were selected and partitioned from the global network using the subgraph () function from the ‘igraph’ package, and their topological properties were calculated (Ma et al., 2016). In addition, Pearson’s correlation analyses were conducted to evaluate the relationships between the topological structures of the subnetworks and environmental factors. All figures were generated using R software (‘ggplot2′ package, v4.3.2).

3. Results

3.1. Molecular composition of SOM in response to different types of biochar

The α-diversity of SOM molecular composition was assessed using the richness and Shannon indices (Fig. 1a and 1b). Compared with the NPK treatment, biochar application significantly increased both the SOM molecular richness and Shannon indices. In soils treated with the three types of biochar, molecular richness was the highest under RSB, followed by PWB and PMB, with no significant differences in the Shannon index. Additionally, the PCoA plot differentiated the SOM molecular composition across the different treatments (ADONIS, $R^2 = 0.77$, $p < 0.001$; Fig. 1c), highlighting the profound impact of biochar on the molecular signature of SOM. The differences in the relative abundances of the compounds were compared to further explore the changes in SOM molecular composition caused by biochar application (Fig. 1d and Table S3). The soil with biochar addition contains the following relative abundances of molecular components: lipids (26.1 ± 1.8 %), aromatic compounds (19.5 ± 0.8 %), lignin-derived products (10.4 ± 1.3 %), nitrogen compounds (12.8 ± 2.8 %), and polysaccharides (12.2 ± 0.8 %). (Table S4). Compared with the NPK treatment, the addition of biochar significantly increased the relative abundance of lignin-derived products and polycyclic aromatic hydrocarbons and decreased the abundance of aromatic compounds and lipids. However, PWB alone significantly increased the abundance of nitrogen compounds and polysaccharides. The richness of lignin-derived products was elevated in the RSB treatment, whereas that of nitrogen compounds was higher in the PMB treatment than in the NPK treatment (Table S5).

3.2. Effects of different types of biochar on soil bacterial communities and C-related cycling functional genes

The structural characteristics of the bacterial community under biochar application were evaluated by analysing community composition and microbial life strategy (copiotroph/oligotroph) (Fig. 2a and 2b). PCoA demonstrated that biochar application induced distinct shifts in the soil bacterial community composition (Fig. S1). Proteobacteria (33.52–36.38 %) was the dominant phylum in the different treatments, followed by Chloroflexi (20.81–24.78 %) and Acidobacteria (16.65–18.58 %). The application of biochar enhanced the relative abundances of Bacteroidetes and Proteobacteria, whereas it reduced the relative abundance of Chloroflexi. Furthermore, compared with PWB and PMB, applying RSB significantly increases the relative abundance of Nitrospirae and Verrucomicrobia (Table S6). In addition, biochar increased the relative abundance of copiotrophs (Fig. 2b) and the

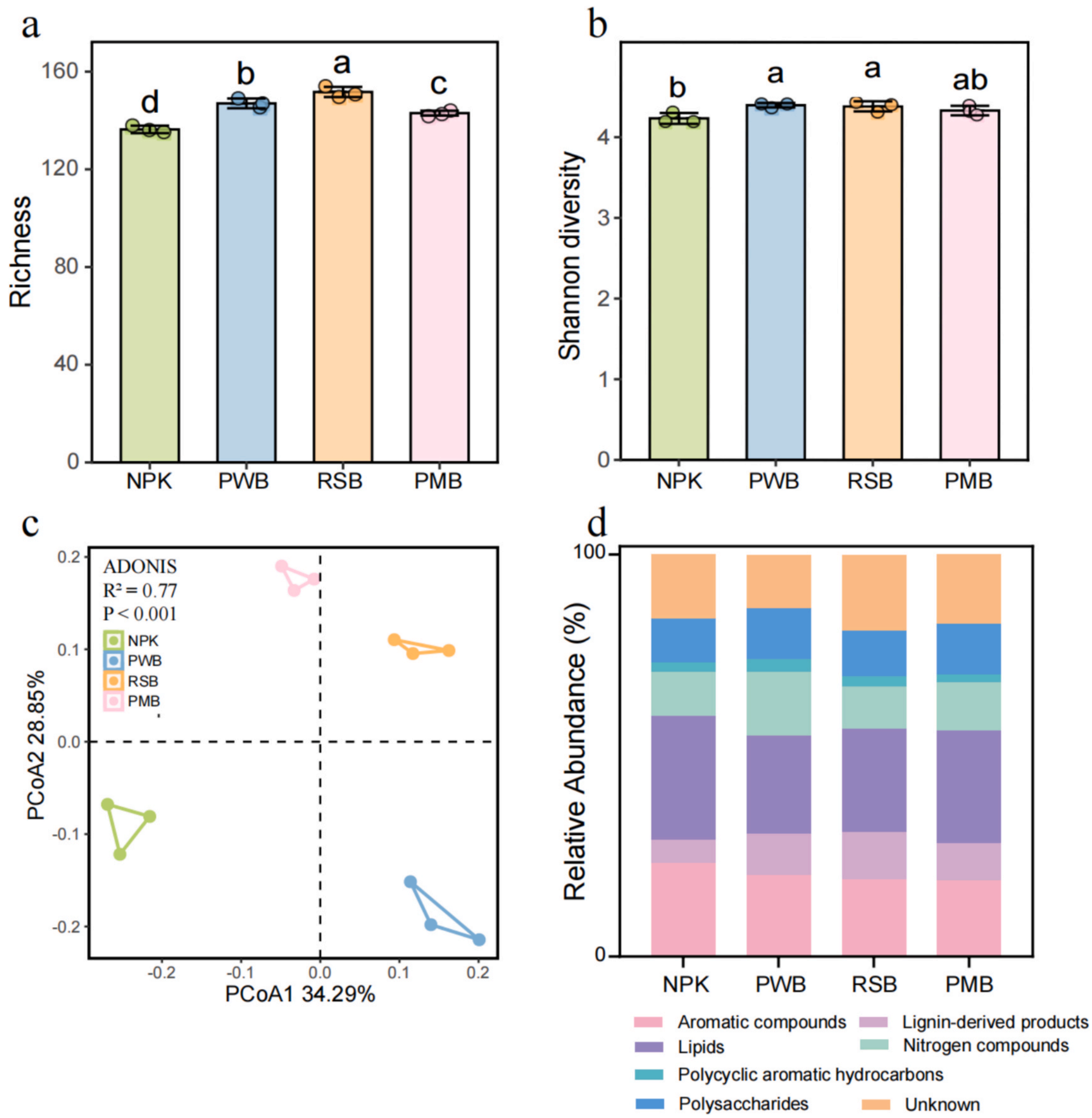

Fig. 1. The diversity indices of SOM molecular composition: richness index (a) and Shannon index (b). Different lowercase letters indicate significant differences between treatments (n = 3, Duncan test, $p < 0.05$). PCoA of SOM molecular composition (c), with ADONIS test showing inter-group differences based on the SOM molecular composition distance matrix. Stacked bar chart of the relative abundance of SOM molecular composition (d). NPK: chemical fertilizer and no biochar; PWB: chemical fertilizer and perishable waste biochar; RSB: chemical fertilizer and rice straw biochar; PMB: chemical fertilizer and pig manure biochar.

copiotroph/oligotroph ratio (Fig. 2c). Compared with the NPK treatment, the addition of PWB, RSB, and PMB resulted in a 5.2 %, 3.42 %, and 4.96 % increase in the relative abundance of copiotrophic bacteria, respectively. The copy number of ribosomal RNA gene operons (rrn) in bacterial genomes is a conserved phylogenetic trait at the genus levels, serving as an effective predictor of growth rate and nutrient utilization efficiency in individual organisms. The results indicated that the addition of biochar significantly increased the rrn copy number (Fig. 2d), with no significant differences among the three types of biochar treatments. Compared with the PMB treatment, the relative abundance of C-fixation genes was higher under PWB and RSB treatments, while the opposite trend was observed for C degradation genes (Fig. 3). However, the application of biochar did not significantly modify the overall relative abundance of C-fixation genes or C degradation genes (Fig. 3a and b). The relative abundance of genes involved in cellulose and pectin degradation was significantly reduced in the PMB treatment compared to the NPK treatment (Fig. 3c). There were no significant differences in the α-diversity indices (richness and Shannon) of C-cycling functional genes among the treatments (Fig. S2c and S2d).

3.3. Relationship between SOM molecular composition, bacterial communities, and C-related cycling functional genes

Procrustes analysis based on Bray–Curtis distances revealed a good fit and a significant correlation between SOM molecular composition and the bacterial community ($M^2 = 0.1357$, $p = 0.001$) and C-cycling functional genes ($M^2 = 0.1346$, $p = 0.001$). These results suggest that changes in SOM molecular composition may be influenced by variations in the bacterial community and C-cycling functional genes (Fig. 4a and 4b). The Mantel test results demonstrated that both bacterial life strategy and C-cycling functional genes affected the molecular composition of the SOM (Fig. 5). Specifically, the genes involved in C-related degradation and fixation were significantly correlated with polycyclic aromatic hydrocarbons ($P < 0.05$; Fig. 5a). The heatmap revealed a negative correlation between polycyclic aromatic hydrocarbons and several carbon degradation-related genes such as lignin (*glx* and *mnp*), chitin (*chiA*), and hemicellulose (*xylA* and *manB*) degradation genes (Fig. 5b). Lignin-derived products were significantly negatively correlated with the pectin-degrading gene *pgu*, the hemicellulose-degrading

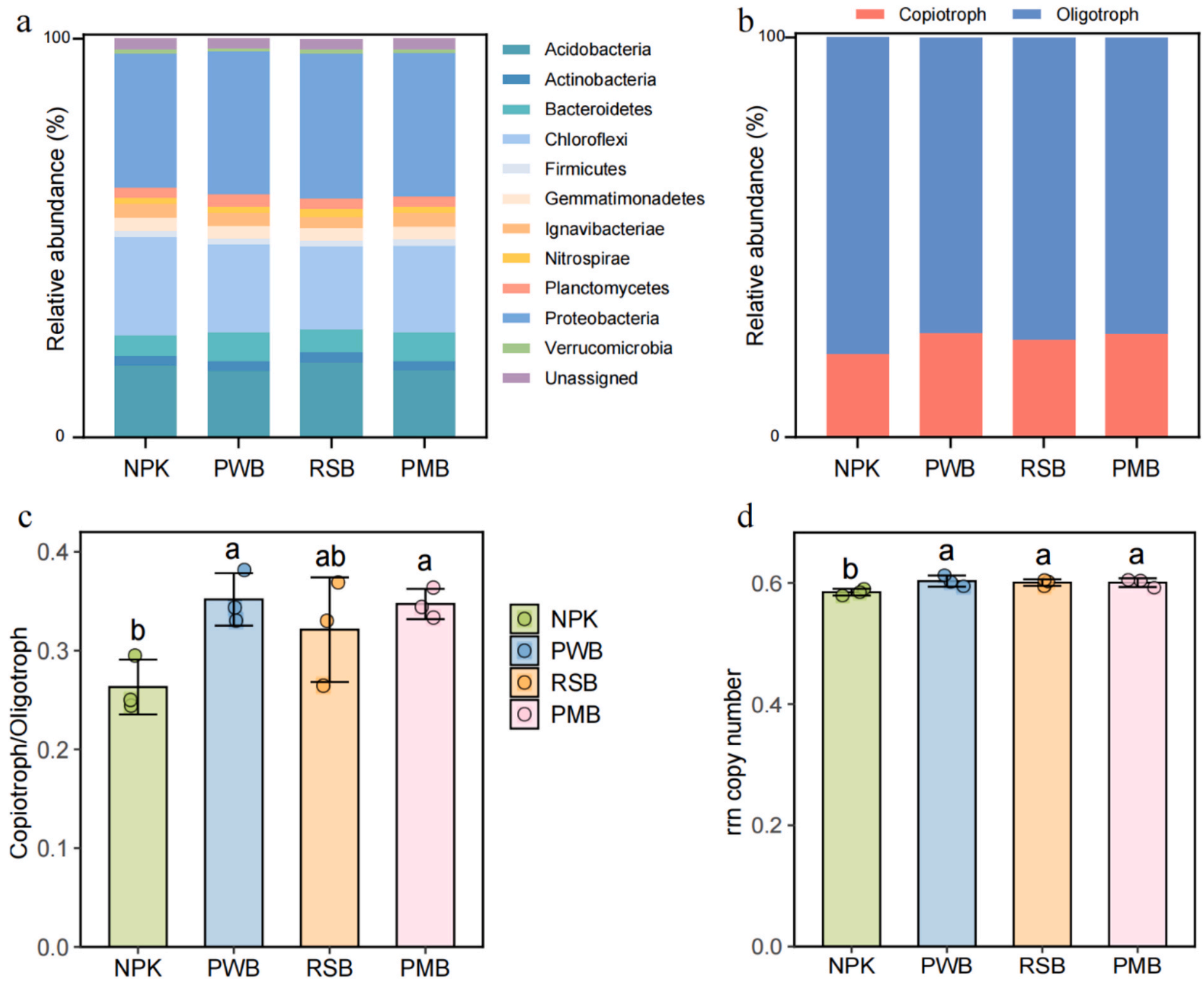

Fig. 2. Biochar effects on bacterial composition and on the copiotroph to oligotroph ratio. Changes in the relative abundance of bacterial phyla (a), relative abundance of copiotrophic and oligotrophs bacteria (b), ratio of copiotroph to oligotroph bacteria (c), and average rrn copy number at the community level (d). Based on the soil bacterial life history strategy, the bacterial community was categorized into copiotrophic bacteria (e.g., Bacteroidetes, β-Proteobacteria, γ-Proteobacteria, and Firmicutes) and oligotrophs bacteria (e.g., Acidobacteria, Actinobacteria, Chloroflexi, α-Proteobacteria, δ-Proteobacteria, Gemmatimonadetes, Nitrospirae, Planctomycetes, and Verrucomicrobia). Different lowercase letters indicate significant differences between treatments (n = 3, Duncan test, $p < 0.05$).

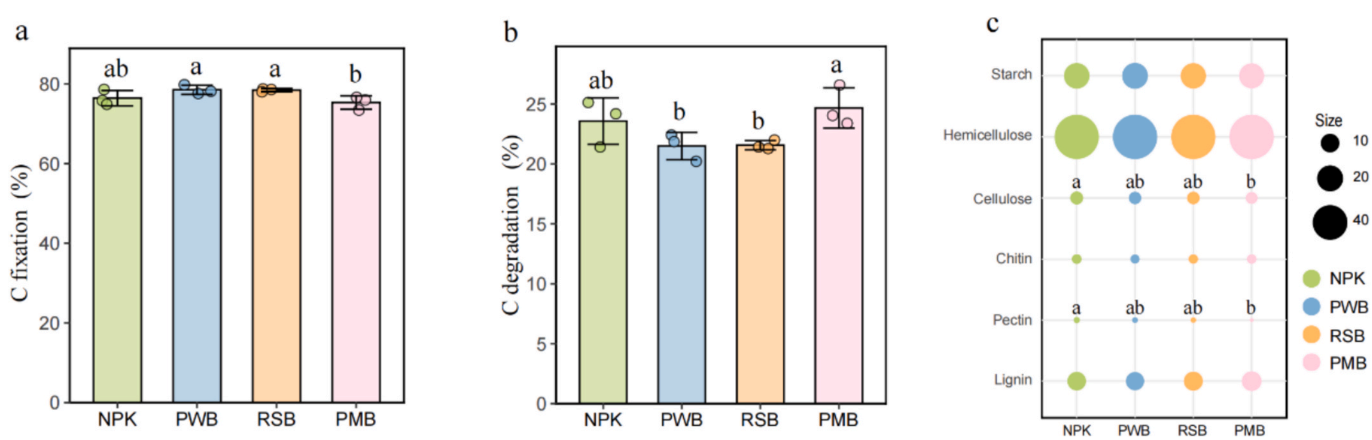

Fig. 3. Effects of biochar on the relative abundance of C-fixation genes (a) and C-degradation genes (b). Bubble chart of C-degradation gene subcategories (c). Different lowercase letters indicate significant differences between treatments (n = 3, Duncan test, $p < 0.05$). Subcategories of C-degradation functional genes: Lignin (*glx*, *lig*, *mnp* and *pox*), Pectin (*pgu*), Chitin (*chiA*), Cellulose (*cdh*, *cex* and *naglu*), Hemicellulose (*xylA*, *manB* and *abfA*), Starch (*amyA*, *amyX*, *apu*, *sga* and *iso_plu*).

gene *abfA*, and the C fixation-related genes (*pccA* and *accA*). Furthermore, lignin-derived products showed a negative correlation with the oligotrophic Chloroflexi (Fig. 5c). Aromatic compounds showed a positive correlation with Chloroflexi but a negative correlation with Gammaproteobacteria and the copiotroph/ocligotroph ratio.

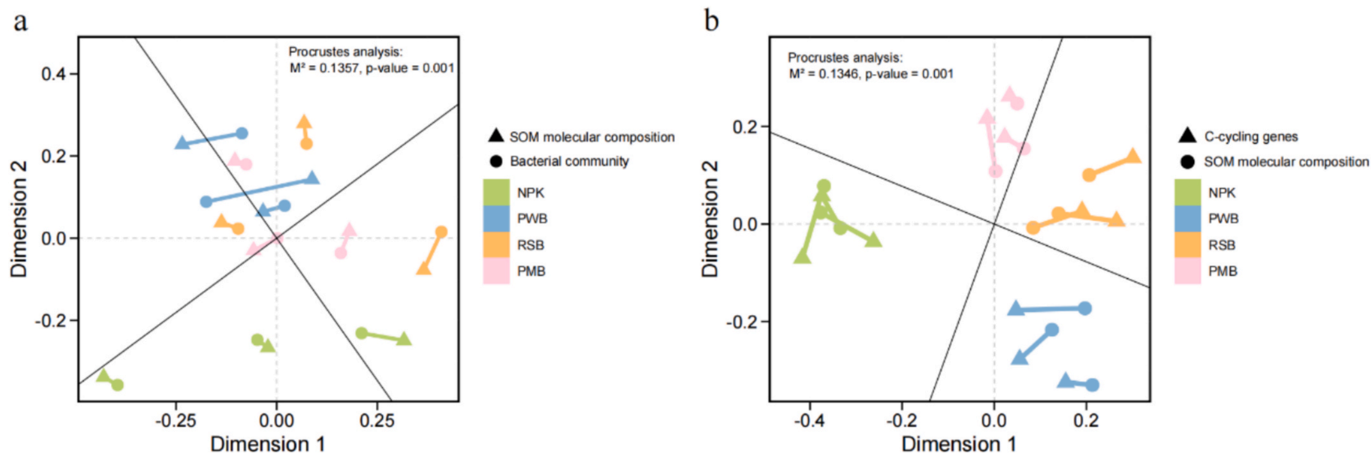

Fig. 4. Procrustes analysis linking SOM molecular composition with bacterial communities (a) and carbon cycling functional genes (b).

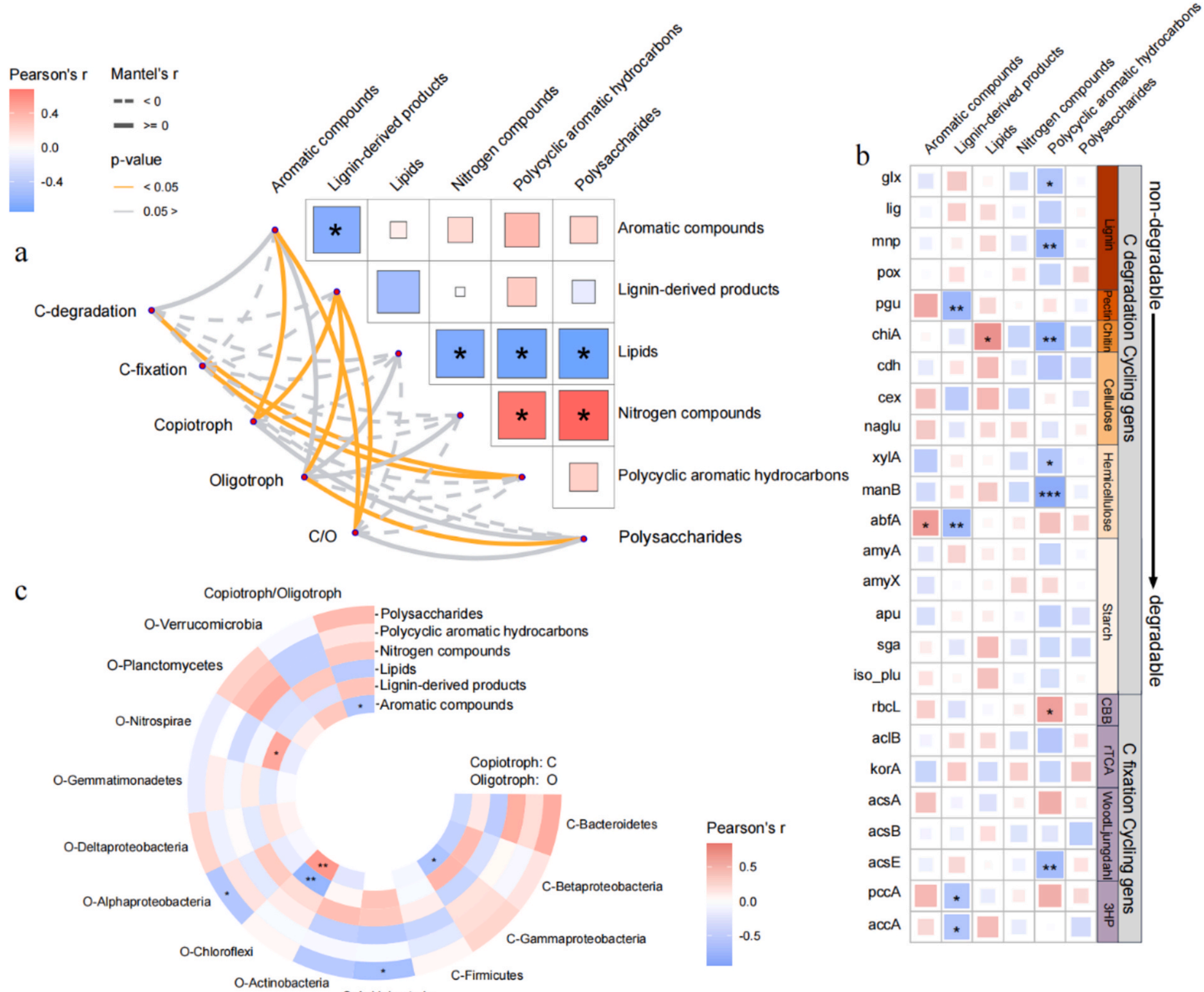

Fig. 5. Relationships between SOM molecular composition, C-degradation, C-fixation functional genes, copiotroph and oligotroph bacteria based on Pearson's correlation and Mantel test analysis (a). Correlation heatmap showing potential relationships between the abundance of SOM molecular composition and the relative abundance of carbon cycling functional genes (b) copiotroph and oligotroph bacteria (c). Blue and red colors represent negative and positive correlations between two variables, respectively. The deeper the color, the stronger the relationship. For each panel, color intensity is proportional to the Pearson's correlation coefficient. (***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.4. Co-occurrence network in SOM molecular level

To assess the different co-occurrence patterns of SOM molecular composition after biochar application, a co-occurrence network of molecular compounds was constructed (Fig. 6). Overall, 365 molecular compounds participated in interactions within the co-occurrence

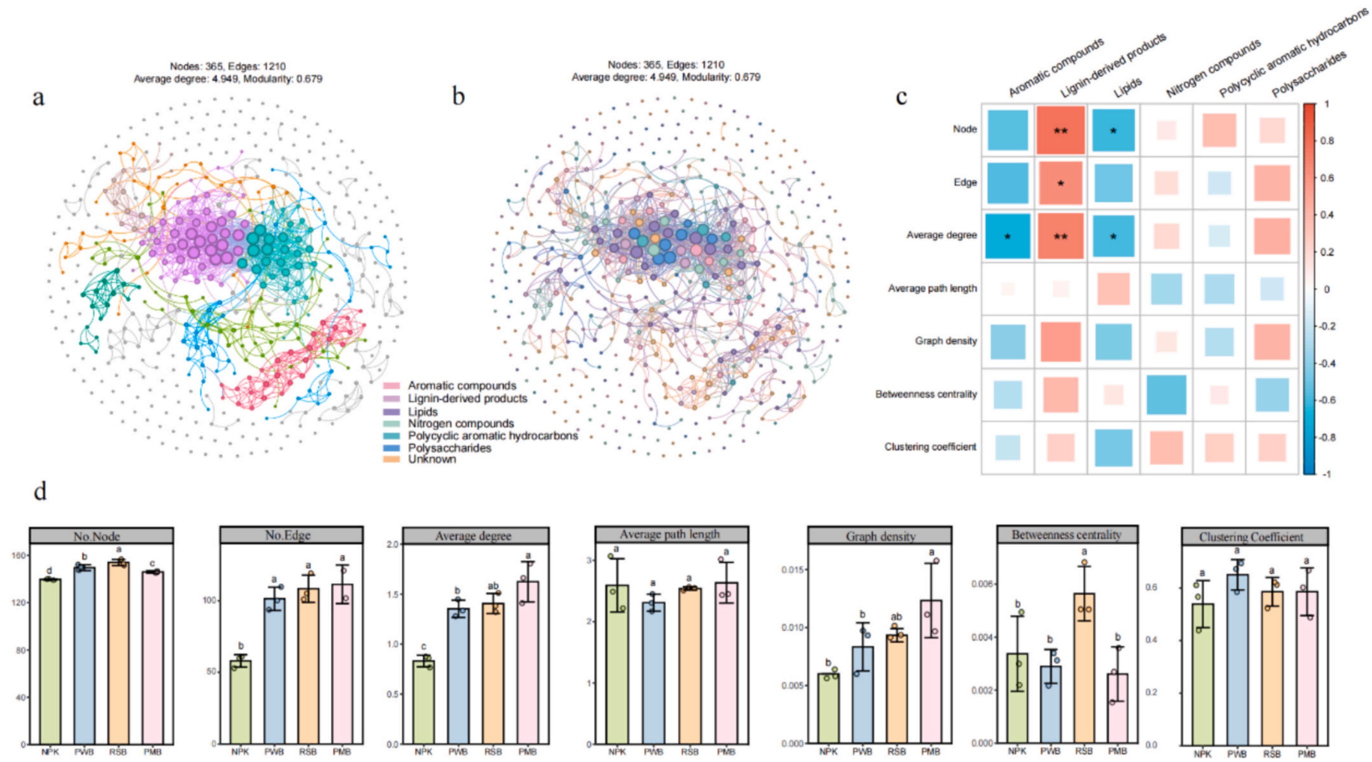

Fig. 6. Module classification of SOM molecular composition (a) and co-occurrence network of compound classifications (b). Pearson's correlation between SOM molecular compounds and properties of the co-occurrence network (c). Comparison of network topological structures under different treatments (d). Different colors represent negative and positive correlations between two variables, respectively. The deeper the color, the stronger the relationship. For each panel, color intensity is proportional to the Pearson's correlation coefficient (deep blue, r = − 1; deep red, r = 1). (*** indicates $p < 0.001$; ** indicates $p < 0.01$; * indicates $p < 0.05$). Different lowercase letters indicate significant differences between treatments (n = 3, Duncan test, $p < 0.05$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

network, resulting in 1,210 interaction relationships. The weighted degree proportions of the main components among the nodes and edges were as follows: lipids (24.1 %), aromatic compounds (14.8 %), nitrogen compounds (13.4 %), lignin-derived products (12.1 %), polysaccharides (11.9 %), and polycyclic aromatic hydrocarbons (6.7 %). With the application of the three types of biochar (PWB, RSB, and PMB), the nodes, edges, average degree, and graph density of the SOM molecular network differed significantly among all four treatments. Specifically, the nodes showed significant differences across all treatments, highlighting the distinct impacts of each biochar type on the SOM molecular network structure. This suggests that biochar enhances the complexity of the co-occurrence networks of organic matter molecular components. Spearman's correlation analysis revealed that lignin-derived products were positively correlated with the nodes, edges, and average degree, whereas aromatic compounds and lipids were negatively correlated with the average degree (Fig. 6c). These results suggest that lignin-derived products play a supporting role in the co-occurrence network between the organic matter molecules.

4. Discussion

4.1. Biochar increased the molecular diversity of SOM and altered its composition and complexity

Based on the first hypothesis, biochar (PWB and RSB) addition enhanced the diversity and complexity of the SOM molecular composition, thereby altering the characteristics of its molecular components (Fig. 1). The molecular diversity of one plant-derived C is modulated by the 'microbial funnel effect', leading to convergence. It is well-established that microbes metabolise complex molecular constituents into CO_2 and microbial biomass, a process that results in SOM becoming increasingly aligned with microbial metabolic byproducts, including cellular waste, exudates, and secondary metabolites (Camenzind et al., 2023; Gleixner, 2013). This continual turnover and accumulation of microbial residues underpin the emergence of 'microbial funnels'. In this study, the observed increase in molecular diversity was not surprising, as previous research has confirmed similar phenomena under exogenous organic amendments such as organic fertilisers, crop residues, and biochar (Chen et al., 2021; Song et al., 2024). One possible reason for this is that biochar inherently contains a wide range of organic molecules, such as fatty acids, alcohols, phenols, and ester compounds (Chen et al., 2019), which directly influence the molecular composition of SOM through biochar-derived carbon, thereby potentially becoming a direct component of SOM and contributing to an increase in soil pH (S. Chen et al., 2024). Another possible reason is that biochar indirectly enhances molecular diversity by regulating the soil bacterial community composition, plant litter, and root exudates. For example, molecular diversity positively correlates with root exudates and bacterial diversity (Gao et al., 2024; Panchal et al., 2022). In addition, the increase in molecular α-diversity of SOM under biochar coincides with the increase of dissolved organic matter (DOM), as DOM is the primary substrate for soil microorganisms to participate in organic matter turnover (Li et al., 2023; Ling et al., 2022).

The three different biochar treatment (PWB, RSB, and PMB) exhibited distinct SOM molecular compositions (Fig. 1). Compared to rice straw and perishable waste biochar, pig manure biochar contains higher nutrient levels (e.g., TN, TP, and TK) (Table S2), which promote microbial growth and metabolism, accelerating the decomposition and transformation of organic matter, thereby altering the molecular composition of SOM (Luo et al., 2023). Additionally, due to its high content of various organic substances, pig manure biochar, after pyrolysis, contains more functional groups that strongly interact with cations, enhancing its cation exchange capacity (CEC) and stabilising SOM through adsorption, thereby reducing its decomposition and mineralization (Hossain et al., 2020). The addition of various biochars increased the relative abundance of lignin-derived products (Table S4). However, the relative abundance of nitrogen compounds showed no statistically significant differences between RSB and PMB treatments, indicating that the increase in nitrogen compounds was not consistent across all biochar types. Biochar could modulates the fate of lignin in soils through two interconnected processes: (i) Enhanced microbial decomposition: Biochar stimulates lignin depolymerization by promoting microbial activity (Ling et al., 2022; Rombolà et al., 2022), thereby increasing the release of lignin-derived oligomers; (ii) Physical-chemical stabilization: The porous architecture and reactive surface functionalities of biochar adsorb these oligomers, effectively shielding them from enzymatic attack and subsequent mineralization (Dai et al., 2021). Concurrently, biochar-mediated nutrient enrichment (e.g., N and P) enhances rice root exudation, which may contribute additional lignin-related metabolites to the rhizosphere (Jin et al., 2022). Crucially, the detection of lignin-derived compounds via py-GC/MS reflects their total pool in soils, which integrates both decomposition-derived fragments and potential direct inputs from biochar or plant exudates. In contrast, biochar treatment reduced the relative abundance of lipids (Table S4). The application of biochar may alter the environmental conditions of the soil, such as pH, temperature, and moisture content. These changes can accelerate the degradation of specific free lipids and short-chain lipid components, leading to a decrease in their abundance (Chen et al., 2021; Zou et al., 2023). Additionally, lipids, which are energy-rich organic compounds, are preferentially utilised by microbial communities (Gunina and Kuzyakov, 2022). The introduction of biochar accelerated the use of fatty acids from lipids for cellular biopolymer synthesis. Notably, PWB elevated the abundance of polycyclic aromatic hydrocarbons, which can persist in the soil and inhibit microbial activity, thereby posing risks to agricultural ecosystems and human health. Therefore, it is crucial to comprehensively assess the dual effects of different types of biochar on SOM molecular composition tailored to specific agricultural application scenarios.

Despite certain limitations of py-GC/MS, primarily arising from interactions with the apolar column during chromatography (Gregoris et al., 2023), these interactions tend to prolong the retention times of non-polar compounds (i.e., lipids and lignin-derived products), leading to their overrepresentation in the analysis. In contrast, polar compounds, such as polysaccharides and nitrogen-containing compounds, are typically underestimated due to weaker interactions with the apolar column, resulting in faster elution (Derenne and Quénéa, 2015). In future studies, using alternative column types, such as polar columns, will help mitigate the overestimation of non-polar compounds, thereby improving the accuracy of the analysis. Nevertheless, py-GC/MS remains a valuable tool for analysing the molecular composition of SOM. It is worth noting that soil samples for py-GC/MS analysis were sieved through a 0.15 mm sieve to remove coarse biochar particles, small stones, and other impurities, thereby ensuring compatibility with instrument sensitivity and analytical precision. Although this sieve size is finer than the 2 mm mesh used for microbial analysis, both sample types underwent thorough homogenization prior to analysis. For microbial assays, DNA was extracted following mechanical disruption with magnetic beads, which efficiently fragment soil aggregates and microbial cells into fine particles (Liu et al., 2021; Luo et al., 2017). Thus, despite the initial difference in sieving, both protocols yielded comparably disintegrated material suitable for respective analyses. Fine sieving minimizes particle-size-related variability and ensures consistent molecular characterization (Davenport et al., 2023; Jensen et al., 2024). Although slight variability may arise from different sieving protocols, the methods employed in this study maintained internal consistency. Trace amounts of biochar-derived compounds may remain post-sieving; however, their impact on the overall results is expected to be negligible. Future research will aim to further standardize sample preparation procedures to improve the accuracy and reproducibility of both molecular and microbial analyses.

The SOM molecular network is crucial for driving molecular diversity and revealing how C material inputs influence molecular co-occurrence network. Our results show that biochar increases the molecular network topological indices (i.e., node, edge, and average degree), thereby enhancing network complexity (Fig. 6). The increase in molecular network complexity is driven by microbial metabolism, diversity of external organic carbon inputs, and chemical coupling and interactions between molecular components (Lehmann and Kleber, 2015; Schmidt et al., 2011). Positive correlations between nitrogen compounds and polysaccharides indicate synergistic effects between molecular components, such as the mutual enhancement of transformation and metabolism (Zongtang Yang et al., 2024) (Fig. 5a). First, nitrogen compounds possess abundant functional groups that can participate in coordination reactions with hydroxyl groups on polysaccharide chains to form stable complexes (Keiluweit et al., 2015). Second, nitrogen compounds provide essential nutrients for microbial growth, thereby enhancing the release of amylase and β-glucosidase during polysaccharide decomposition. Increased enzymatic activity promotes the decomposition and transformation of nitrogen compounds. Negative correlations suggest competition for molecules, potentially due to limited resources or mutual inhibition, leading to changes in metabolic priority. For instance, competition for aromatic compounds and lignin-derived products may prompt microbes to adjust their metabolic pathways and prioritise easily accessible energy sources (Fig. 5a) (Machado et al., 2021). When one compound is abundant, its associated metabolic pathways may intensify, whereas degradation of the other compound is inhibited. During the decomposition of lipids and polysaccharides (Fig. 5a), they may compete for soil moisture, nutrients, and the composition and activity of microbial communities, thus affecting their decomposition rates and efficiencies. The positive correlation between lignin-derived products and network nodes, edges, and average degree was likely due to the direct increase in their abundance caused by biochar application (Fig. 6c). However, the negative correlation between lipids, nodes, and the average degree likely stems from biochar-induced soil changes that accelerate lipid decomposition and reduce lipid diversity and abundance, thereby lowering the microbial demand (Wang et al., 2020; Xiang et al., 2022). This lipid decline reduces microbial energy storage, intensifies nutrient competition, and decreases network diversity. Therefore, biochar indirectly affects network topology by influencing the microbial community, ultimately increasing the complexity of the SOM molecular composition network.

4.2. Biochar regulates bacterial community composition and carbon-related functional genes

In this study, biochar altered the bacterial community composition (Fig. 2 and S1), which is consistent with a previous report (H. Yang et al., 2023). Soil microbial communities are influenced by C input (Yu et al., 2021). Biochar promotes the growth of Bacteroidetes and Proteobacteria while suppressing Chloroflexi (Table S6), a phenomenon known as 'unfair competition'. Based on existing life-history strategy classifications, bacterial communities can be differentiated into copiotrophic and oligotrophs (Li et al., 2021). A significant increase in the abundance of copiotrophic bacteria was observed following biochar application (Fig. 2c). For example, the relative abundance of Chloroflexi (oligotrophs) decreased, while the abundance of Bacteroidetes and γ-Proteobacteria (copiotrophic) increased, similar to the results reported in previous studies (Campos et al., 2020; Sheng and Zhu, 2018). The addition of biochar can enhance soil nutrient content and alter the soil's acid-base environment (Hossain et al., 2020). These changes in nutrient availability and environmental conditions favour the growth and proliferation of copiotrophic bacteria. In addition, rrn copy number is an important indicator of bacterial community ecological strategies and is used to reflect copiotroph/oligotroph selection microbial characteristics at the community level (Sinsabaugh et al., 2009). It is generally believed that bacterial communities with a higher rrn copy number are copiotrophic bacteria, whereas those with lower rrn copy numbers are oligotrophs bacteria (Y. Yang et al., 2023). The addition of biochar led to the predominance of copiotrophic bacteria, which contributed to community reconstruction. In nutrient-rich soils, this process accelerates SOM decomposition and mineralisation, promoting bacterial community growth and leading to the formation of competitive copiotrophic bacteria populations. Overall, copiotrophic bacteria in nutrient-rich environments tend to increase carbon emissions (Jia et al., 2023), a phenomenon closely linked to their high metabolic activity and rapid growth characteristics. In contrast, oligotrophic bacteria tend to reduce carbon emissions, thereby promoting soil carbon stability and accumulation (Su et al., 2023). Therefore, biochar alters the bacterial community habitat, enabling copiotrophic bacteria to proliferate rapidly and consequently increasing the rrn copy number.

In this study, compared to the PMB treatment, both PWB and RSB treatments significantly reduced the abundance of C-degradation genes (Fig. 3), which contrasts with previous findings (Liao et al., 2023). Biochar may reduce the occurrence of C-degradation reactions by adsorbing C-degrading enzymes or inhibiting their activity, thereby directly decreasing the expression and functional activity of C-degrading genes (Feng et al., 2023). Thus, the addition of biochar, a stable and recalcitrant carbon source, may reduce the microbial demand for easily degradable carbon, potentially affecting the activity of enzymes involved in carbon degradation. However, the observed changes in microbial activity could be influenced by both biochar-derived and native SOM components. Notably, the abundance of C-degrading genes remained unchanged, which may suggest a potential stabilization of SOC by limiting decomposition processes, thereby enhancing carbon sequestration in agricultural systems.

4.3. Molecular composition of SOM is related to bacterial community composition and microbial life strategies

Based on our second hypothesis, a consistent correlation among SOM molecular composition, soil microbial communities, and C-cycling functional genes was observed (Fig. 4). Different microbial communities occupy distinct ecological niches within ecosystems, and their interactions with SOM can be conceptualised as a food web in which microbes decompose organic matter to provide energy and nutrients for other organisms (Cotrufo et al., 2013). The composition and function of microbial communities change in response to variations in SOM molecular composition, reflecting the adaptation and selection processes of microbes in specific environments, which in turn influence the stability of soil ecosystems and nutrient cycling capacity (Domeignoz-Horta et al., 2021). Consistent with the findings of a previous study (Chen and Sinsabaugh, 2021), variations in microbial abundance and C-cycling functional genes significantly affected the molecular composition of SOM. In paddy soil systems, differences in SOM composition are closely linked to microbial degradation processes, which play a critical role in shaping the molecular characteristics of SOM (Su et al., 2023). In this study, both copiotrophic and oligotrophic bacteria were closely associated with the molecular compound lignin-derived products (Fig. 5a). As hypothesised, lignin-derived products negatively affect the growth, proliferation, and metabolism of the oligotrophic bacterium Chloroflexi (Fig. 5c). Additionally, biochar may alter the supply of C sources and electron acceptors, affecting microbial energy metabolism and causing some microorganisms to shift their metabolic activities away from C degradation towards other functions, thereby reducing the abundance of C degradation genes (Azeem et al., 2023; Y. Chen et al., 2024b). C degradation genes promote SOM decomposition and enhance soil nutrient content. When soil nutrients are abundant, microorganisms can access them more readily, which, in turn, leads to an increase in the abundance of C-degradation genes. A recent study suggested that variations in genes encoding the degradation of different carbon sources may influence microbial metabolic activities by modulating the microbial carbon pump process (Huang et al., 2024). Thus, SOM molecular compounds can directly influence microbial communities and C-cycling functional genes, and these three components are interrelated and mutually influential. However, further in-depth research of SOM at the molecular level is needed to elucidate the impact and relationships between SOM molecular compounds, bacterial communities, and C-cycling functional genes.

5. Conclusions

Changes in SOM molecular composition were revealed under different biochar inputs from the perspective of bacterial community composition and C cycling functional gene interactions. In contrast to the application of mineral fertilisers, biochar to some extent increased the α-diversity of SOM molecular compounds and markedly reshaped their composition. Microbial communities associated with C degradation and fixation genes exhibited significant predictive and associative relationships with SOM molecular compounds, which are regulated by different C material inputs. The accumulation of lignin-derived products in biochar-treated soils enhanced the complexity of the SOM molecular co-occurrence network. Thus, the application of biochar may accelerate SOM molecular characteristics transformation, which could potentially improve ecosystem functions and services, and contribute to sustainable agricultural production.

CRediT authorship contribution statement

Zongkun Yang: Writing – original draft, Investigation, Data curation. **Wenbo Liu:** Writing – review & editing, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Xiaoge Fan:** Investigation. **Han Gao:** Writing – review & editing, Supervision. **Xiangrui Xu:** Writing – review & editing, Resources. **Cheng Liu:** Writing – review & editing, Resources. **Yanjun Chai:** Writing – review & editing, Resources. **Min Zhang:** Writing – review & editing, Supervision, Resources. **Marios Drosos:** Writing – review & editing, Resources. **Shengdao Shan:** Writing – review & editing, Supervision, Resources.

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.geoderma.2025.117308.

Data availability

Data will be made available on request.

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