Hu *et al. Biochar* (2025) 7:84
https://doi.org/10.1007/s42773-025-00473-z

Biochar

ORIGINAL RESEARCH Open Access

Improving the stability of black soil microbial communities through long-term application of biochar to optimize the characteristics of DOM components

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Abstract

Biochar has demonstrated to have ability to improve soil properties and boost plant productivity. However, the underlying mechanisms by which dissolved organic matter (DOM) fluorescent components and microbial communities in black soil regions contribute to plant productivity remain uncertain. To address this gap, a long-term field experiment was conducted in Northeastern China's black soil region, investigating how varying biochar application rates (0, 15.75, 31.50, and 47.25 t ha^{-1}) influence DOM fluorescence properties and the composition of soil microbial communities. Employing fluorescence excitation–emission matrix-parallel factor analysis (EEM-PARAFAC) and high-throughput sequencing, the research systematically analyzed how biochar amendments influence DOM composition, fluorescence properties, microbial diversity, and their interrelations. The findings demonstrated that biochar significantly modified DOM composition, increasing the proportions of protein-like and humic substances while enhancing its aromaticity and stability. A medium application rate (31.5 t ha^{-1}) notably improved alpha- and beta-diversity within the soil microbial community, optimized a co-occurrence network dominated by Proteobacteria and Acidobacteria, and facilitated key DOM transformations and nutrient cycling. In contrast, a high biochar application rate (47.25 t ha^{-1}) negatively impacted the stability of microbial communities. Structural equation modeling (SEM) revealed that biochar indirectly boosted crop yields by modulating DOM fluorescence and microbial community dynamics. The insights gained from this study provide practical guidance for optimizing biochar application rates, maximizing its benefits, and mitigating potential ecological risks in black soil systems.

Highlights

- A 6-year one-time application experiment of biochar was conducted in black soil farmland.
- The main network modules were correlated with the transformation of key DOM components.
- The biochar application amount of 31.50 t ha^{-1} could effectively promote DOM humification and bacterial diversity.

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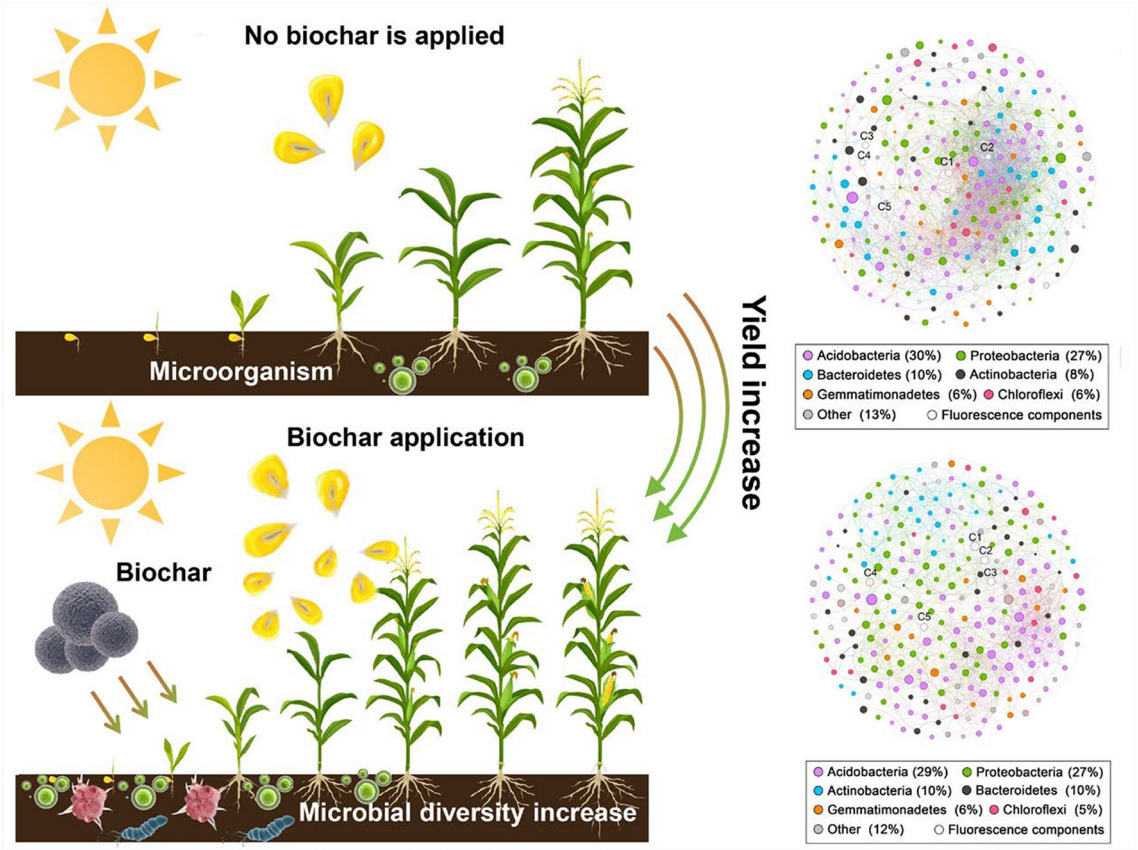
- High dose application of biochar (47.25 t ha^{-1}) had a negative impact on soil bacterial communities.
- Phylum Proteobacteria and Acidobacteria have obvious differences in DOM degradation and carbon pool stability.

Keywords Biochar, Black soil, DOM fluorescent components, Sampling period, Microplastics, Network analysis

Graphical Abstract



1 Introduction

Northeastern China's black soil region is considered one of the world's most productive agricultural areas, renowned for its abundant organic matter, well-structured soil, and remarkable ability to retain water (Wang et al. 2024a, b). However, extensive intensive farming, unsound land management, and climate change have caused significant soil degradation in this region, as marked by organic matter depletion, severe erosion, acidification, and compaction issues (Li et al. 2024a, b). These degradation processes not only weaken soil fertility and productivity but also pose threats to regional ecological stability and food security. Traditional soil improvement measures, including chemical fertilizer application, organic matter addition, and conservation tillage, indeed have some benefits. However, while chemical fertilizers can improve soil quality in the short term, they often exacerbate environmental issues such as water eutrophication and soil acidification (Nisar et al. 2021). Organic amendments such as manure and compost can improve soil structure and nutrient supply, but the high cost limited their utilization and raised the potential risk of pathogens (Ansari et al. 2019). Conservation tillage helps mitigate soil erosion but is less effective under conditions of severe nutrient depletion and organic matter loss (Lv et al. 2023).

Biochar, a carbon-dense substance produced through the pyrolysis of biomass, is distinguished by its

extensively developed porous structure. This network offers stable attachment points and protective barriers for soil microorganisms, simultaneously improving soil aeration and moisture retention (Wu et al. 2024). The surface properties of biochar exhibit a certain degree of hydrophobicity, which helps reduce water evaporation (Qi et al. 2025). During pyrolysis, the biomass undergoes high carbonization, resulting in exceptional thermal stability and resistance to decomposition. The tightly bonded carbon atoms in biochar molecules effectively limit microbial access to carbon, playing a vital role in soil carbon sequestration and structural improvement (Nan et al. 2020). DOM is a critical indicator of soil microenvironmental performance, significantly influencing microbial communities during crop growth, consequently impacting crop yield (Chen et al. 2024; Xia et al. 2024). DOM contains substances such as fulvic acid, humic substances, and proteins, and is highly effective in enhancing microbial biomass carbon (MBC), and enzymatic activity (Dong et al. 2022). The use of biochar can enhance the content of DOM, mitigate the risk of DOM loss, and promote its long-term stability within the soil. Furthermore, the surface of biochar is abundant in functional groups, including carboxyl and phenolic hydroxyl groups, which have the ability to bind with and exchange organic molecules in soil DOM. This interaction alters the molecular structure and chemical characteristics of DOM (Liu et al. 2025), improving its aromaticity and stability, and thus reinforcing its capacity for long-term sequestration in the soil. While biochar has demonstrated promising effects in enhancing soil fertility, facilitating carbon sequestration, and optimizing the quality of soil organic matter, there remains a gap in comprehensive research on the interactions between soil microbial communities and the evolution of DOM molecular structures in the black soil region of Northeast China.

The formation of humic substances within DOM is intricately linked to soil microbial activity. As a result, biochar application not only fosters beneficial shifts in microbial communities but also facilitates the mineralization of unstable organic matter fractions (Li et al. 2024a, b). Due to its porous nature, biochar provides an extensive surface area for the attachment of various chemical functional groups, improving its ability to physically and chemically adsorb soil nutrients, thus increasing their availability to microorganisms. These characteristics contribute to the expansion and structural complexity of soil microbial networks while also strengthening microbial community resilience against environmental disturbances (Deshoux et al. 2023; Gui et al. 2025). However, it should be noted that the effect of biochar on soil microbial community is not fixed, but regulated by many factors. These factors include application rate, mixing method, soil pH and nutrient supply (Sandhu et al. 2019). At present, most studies tend to sample at a single growth stage of crops, ignoring the dynamic shifts of microbial communities during crop growth cycles and their interaction with biochar. The one-time sampling strategy may lead to the lack of key information, such as how the microbial community regulates the dynamic balance of soil nutrients in the later stage of crop growth, and how soil microorganisms respond to the persistence effect of biochar after harvest (Bello et al. 2021; Du et al. 2022). Focusing on specific time is conducive to exploring a series of regular phenomena in crop growth, but it also limits our systematic understanding of biochar regulation mechanisms. Therefore, it is very important to comprehensively study the effects of biochar application on soil microbial communities, and samples should be collected at crop planting and harvest stages for analysis. This approach will help elucidate its short- and long-term effects within agroecosystems, ultimately offering a scientific foundation for refining biochar application strategies.

In the soybean–maize rotation system in the black soil region of Northeast China, the effects of biochar addition on soil bacterial communities, especially their structural composition and network interactions, have not been fully studied. To investigate how varying biochar application rates influence the fluorescent composition of soil DOM, the structure of bacterial networks, and their corresponding interactions and regulatory mechanisms, a six-year field experiment was conducted in a typical black soil area. This study utilized EEM-PARAFAC combined with high-throughput sequencing techniques. The primary goal was to explore how different biochar application levels affect the fluorescence characteristics of DOM, as well as bacterial diversity, community composition, and network dynamics. The specific research aims were: (1) to assess the effects of biochar application on soil microbial communities, (2) to explore the connections between microbial communities, their co-occurrence networks, and the fluorescence properties of DOM, and (3) to evaluate how biochar application influences crop yields and uncover the underlying mechanisms driving these effects.

2 Materials and methods

2.1 Experimental site description

A long-term field experiment on biochar was launched in 2013 at the Modern Agricultural Demonstration Zone in Minzhu Town, Harbin, Heilongjiang Province (45°50′N, 126°51′E). This area is located in the black soil area of Northeast China, which is prone to soil degradation and is an ideal place to study the long-term effects of biochar application.

The area has a temperate continental monsoon climate, with an average annual rainfall of 486.4–543.6 mm. The average annual temperature is about 3.5 °C, exhibiting substantial seasonal fluctuations. The site has a black soil type with a silty loam texture. Prior to the commencement of the field experiment, an analysis of the initial soil properties was conducted (as presented in Table S1). These properties included a bulk density of 1.31 g kg^{-1}, an initial pH of 6.74, and an organic matter content of 29.87 g kg^{-1}. The cropping system used was soybean–maize rotation, mechanical sowing in May and harvesting in September. The soybean variety used is Heinong 43 (Mid-early maturity high protein soybean varieties, plant type convergence, lodging resistance, growth period of 118–125 days, protein content of 42.5–44.2%. It is suitable for planting in the first and second accumulated temperature zones of Heilongjiang), while the maize variety is Longgao L2(The plant type is semi-convergent, the culm is strong without lodging, the living culm is mature, and has good drought resistance and stress resistance. The growth period is about 122 days, which is suitable for planting in the first and second accumulated temperature zones of Heilongjiang Province).

2.2 Experimental design

The biochar used in this study was provided by Liaoning Provincial Biochar Engineering Technology Center. It was derived from the pyrolysis of maize stalks and exhibited a pH of 8.69. The particle size distribution of the biochar was as follows: 15.0% had a size smaller than 0.1 mm, 60.2% ranged between 0.1 mm and 2 mm, and 24.8% exceeded 2 mm. In addition, the biochar contained 3 g of Ca kg^{-1}, 2 g of Mg kg^{-1}, and 17 g of K kg^{-1}; additional properties are provided in Table S2. The biochar was applied at the following rates: 0 t ha^{-1} (CK), 15.75 t ha^{-1} (BC1), 31.5 t ha^{-1} (BC2), and 47.25 t ha^{-1} (BC3). Prior to the commencement of the experiment, the biochar was thoroughly mixed into the upper soil layers using rotary tillage. The experimental plots, each sized at 3.9 m × 10 m, were organized using a randomized block design, incorporating three replications. A uniform basal fertilizer application was applied to all plots, with the following nutrient rates: 358.7 kg ha^{-1} of N, 130.4 kg ha^{-1} of P_2O_5, and 125 kg ha^{-1} of K_2O. These nutrients were supplied in the form of urea, superphosphate, and potassium sulfate. No herbicides or pesticides were applied to any of the treatments.

2.3 Sample collection and determination

Soil samples were collected at both the planting and harvesting stages. Five soil cores (0–20 cm depth) were taken from randomly selected locations within each plot. The collected samples were transferred into sterile plastic bags, and any foreign materials, including leaves, stones, and roots, were carefully removed. Afterward, the samples were split into two portions: one was preserved at − 80 °C for microbial diversity analysis, while the other was left to air-dry in preparation for physicochemical property assessments.

Soil moisture content (MC), bulk density (BD), total nitrogen (TN), total phosphorus (TP), alkali-hydrolyzed nitrogen (AN), available phosphorus (AP), soil organic matter (SOM), and microbial biomass carbon (MBC) were determined using the methods outlined by Bao (2000). Dissolved organic carbon (DOC) was quantified using a Multi N/C 2100 S TOC analyzer (AnalytikJena, Jena, Germany). Particulate organic carbon (POC) was determined using the methods outlined by Liao et al. (2023). Once the maize crop had matured, it was harvested from the experimental plots, and the yield was calculated based on a standard grain moisture content of 14%.

2.4 Acquisition of EEMs soil DOM extraction and analysis

DOM was isolated from soil using a water-soil oscillation technique, employing a 1:5 (w/v) ratio of soil to deionized water. The sample was then adjusted to achieve a DOC concentration of 5 ± 0.10 mg L^{-1} and a pH of 7.42 ± 0.05. Measurements were conducted at 24 °C with a fluorescence spectrophotometer (F-7000, Hitachi, Japan). EEM spectroscopy was performed in three dimensions, with scanning excitation wavelengths between 200 and 550 nm and emission wavelengths between 250 and 550 nm.

2.5 Spectral index calculation and PARAFAC analysis

The DOMFlour toolbox, developed in MATLAB 2012a, was employed to remove Rayleigh and Raman scattering peaks. The methods used to calculate the freshness index (β/α), humification index (HIX), biological index (BIX), and fluorescence index (FI) in this study are detailed in Shi et al. (2024).

The underlying principles and formulas for PARAFAC analysis are based on those provided by Wang et al. (2024a, b) and are outlined as follows:

$$X_{ijk} = \sum_{n=1}^{N} a_{in} b_{jn} c_{kn} + e_{ijk}$$

$$i = 1,2,\cdots,I; j = 1,2,\cdots,J; k = 1,2,\cdots,K$$

The fluorescence intensity of sample i at emission wavelength j and excitation wavelength k is directly proportional to the concentration of fluorophore component n in the same sample, and the number of individual fluorophore components is denoted as N (Wu et al. 2022).

2.6 DNA extraction, PCR and Miseq sequencing

The extraction of total DNA was carried out using the E.Z.N.A.® Soil DNA Kit (Omega Bio-tek, Norcross, GA, USA). DNA quality and concentration were assessed through agarose gel electrophoresis and spectrophotometric analysis. The bacterial 16S rDNA gene's hypervariable V3-V4 region was amplified using the primer pair 338F/806R, as described by Dai et al. (2022). Amplification was conducted with an ABI GeneSol® 9700 PCR thermal cycler (ABI, CA, USA), employing a reaction mixture with standard components and cycling protocols. The resulting PCR products underwent high-throughput sequencing on the Illumina NovaSeq platform, performed by Majorbio Bio-Pharm Technology Co., Ltd.

2.7 Statistical analysis

Statistical analyses were conducted utilizing the R program (v4.0.5). Bacterial amplicon sequence variant (ASV) richness was assessed using DADA2 software (Callahan et al. 2016). Principal coordinates analysis (PCoA) based on the Bray–Curtis distance matrix examined microbial community composition differences among samples. PERMANOVA test evaluated the statistical significance of structural variations between sample groups. Additionally, LEfSe analysis (LDA > 3.6, $P < 0.05$) identified bacterial taxa with significant changes in relative abundance over time, from the phylum to genus level.

To explore the associations between DOM composition and bacterial communities, networks were constructed by linking ASVs to the five DOM fluorescence components. Only ASVs with a relative abundance exceeding 1.5% and present in more than 80% of the soil samples were included in the network, ensuring focus on the most abundant ASVs. Spearman correlation coefficients were used to assess ASV relationships. Pairwise Spearman rank correlations (r) were calculated to assess the connections between ASV abundance and the proportions of individual DOM fluorescence components, with only those correlations where $r > 0.4$ (or $r < -0.4$) and P-values < 0.05 being retained. For correlations between ASVs, only those with $r > 0.7$ were considered. Network analysis was conducted using the igraph, vegan, and psych packages in R software, while network visualization and module detection were carried out using the Gephi (v0.9.2) interactive platform (Web Atlas, Paris, France).

Variation partitioning analysis (VPA) assessed the contributions of biochar rates, soil properties, and bacterial communities to DOM fluorescence across sampling periods. Distance-based redundancy analysis (RDA) examined how soil physicochemical factors and fluorescence components influenced microbial structure. Structural equation modeling (SEM) was conducted using the lavaan package. Histogram and box diagram were plotted using Origin 2020 (Origin Lab Inc., MA, USA).

3 Results

3.1 Composition and fluorescence intensity of soil DOM

During the planting period, Component C1 (Fig. A1) in soil DOM was identified as a protein-like substance (Kulkarni et al. 2017). Component C2 (Fig. A2) was identified as fulvic acid-like material (Dai et al. 2022). Component C3 (Fig. A3) was identified as a humic-like substance (Liu et al. 2019). Component C4 (Fig. A4) was identified as a humic acid-like substance (Gao et al. 2017). Component C5 (Fig. A5) was identified as fulvic acid-like material (Osburn et al. 2011). During the harvesting period, component C1 (Fig. B1) in soil DOM was identified as a humic acid substance (Dai et al. 2022). Component C2 (Fig. B2) was identified as a fulvic acid-like substance, featuring two excitation peaks (Vines et al. 2020). Component C3 (Fig. B3) represented humic-like substance (Lin et al. 2020). Component C4 (Fig. B4) was identified as a fulvic acid-like substance (Osburn et al. 2011). Component C5 (Fig. B5) was linked to tyrosine-like substance (Kida et al. 2019). The Fmax results showed that the content and distribution of these fluorescence components varied with treatments and sampling periods (Fig. 1. A6, A7, B6, B7). Compared with the soil at the planting period, the soil DOM after harvest had a higher Fmax value. During the planting period, C1 had the highest proportion, accounting for 29.30 – 40.43% of total Fmax, and gradually increased with biochar application. Compared with CK, the proportion of protein-like substance in BC3 treatment significantly increased by 11.13%; the proportion of C3 in BC2 and BC3 treatments was significantly decreased by 5.18% and 11.73% ($P < 0.05$), respectively; the proportion of C4 in BC1, BC2, and BC3 treatments was significantly reduced by 1.89%, 2.07%, and 4.41% ($P < 0.05$), respectively.

During the harvesting period, C1 had the highest proportion, accounting for 33.10 – 35.16% of the total Fmax. The proportion of C2 in BC2 treatment was the highest, which was significantly higher than CK (1.36%, $P < 0.05$). Different from the soil at the planting period, the soil after harvest had no tryptophan-like substance in the protein component and was replaced by C3. The Fmax of BC1 was significantly higher than CK ($P < 0.05$), whereas medium and high-dose biochar treatments showed no significant difference from CK.

3.2 Spectral index of soil DOM

FI, BIX, HIX, and β/α were used to evaluate changes in DOM characteristics under different biochar treatments and sampling periods (Fig. 2). These spectral indices provide crucial insights into the source, local

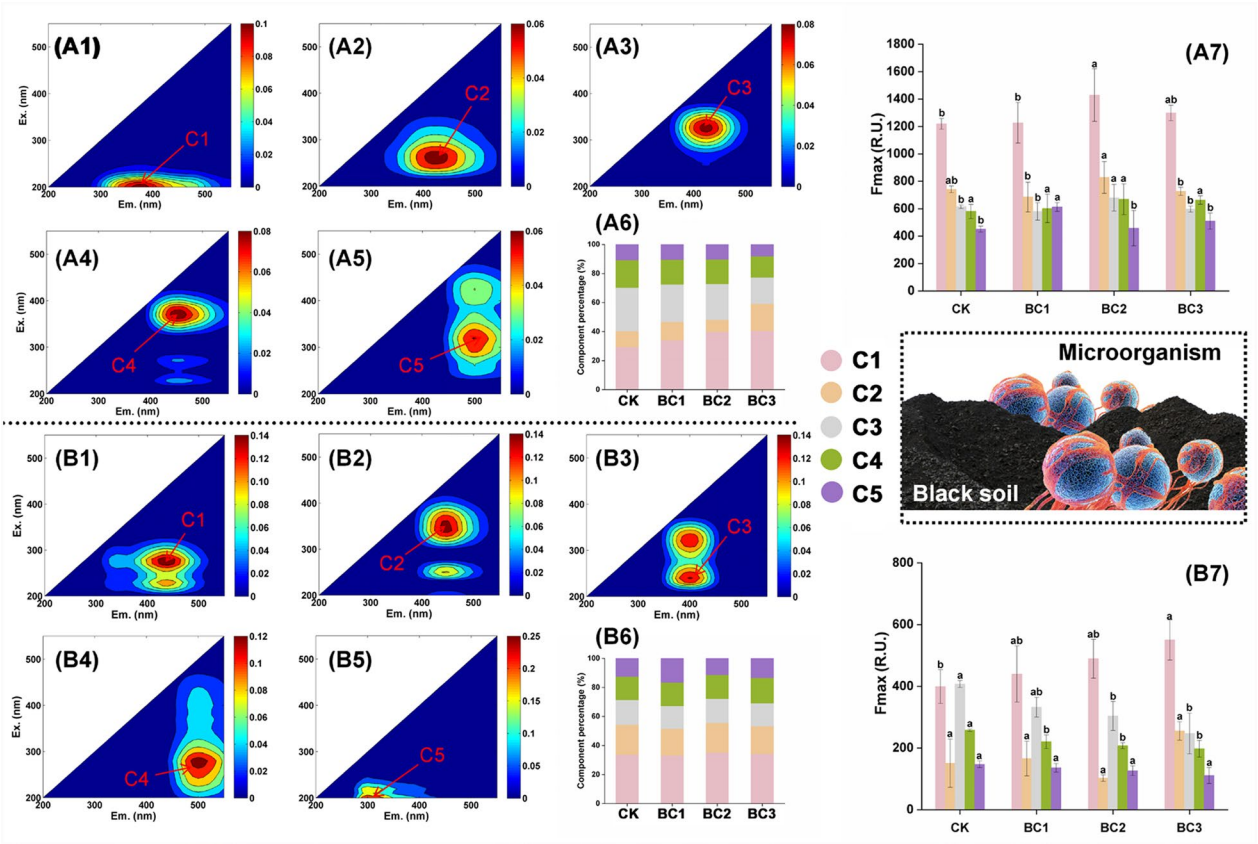


Fig.1 The PARAFAC model was used to identify the five fluorescent components (**A1**, **A2**, **A3**, **A4**, **A5**), component percentage (**A6**), and Fmax (**A7**) of the soil during the planting period. The PARAFAC model was used to identify the five fluorescent components (**B1**, **B2**, **B3**, **B4**, **B5**), component percentage (**B6**), and Fmax (**B7**) of the soil during the harvesting period. Different letters indicate significant differences among treatments ($P < 0.05$)

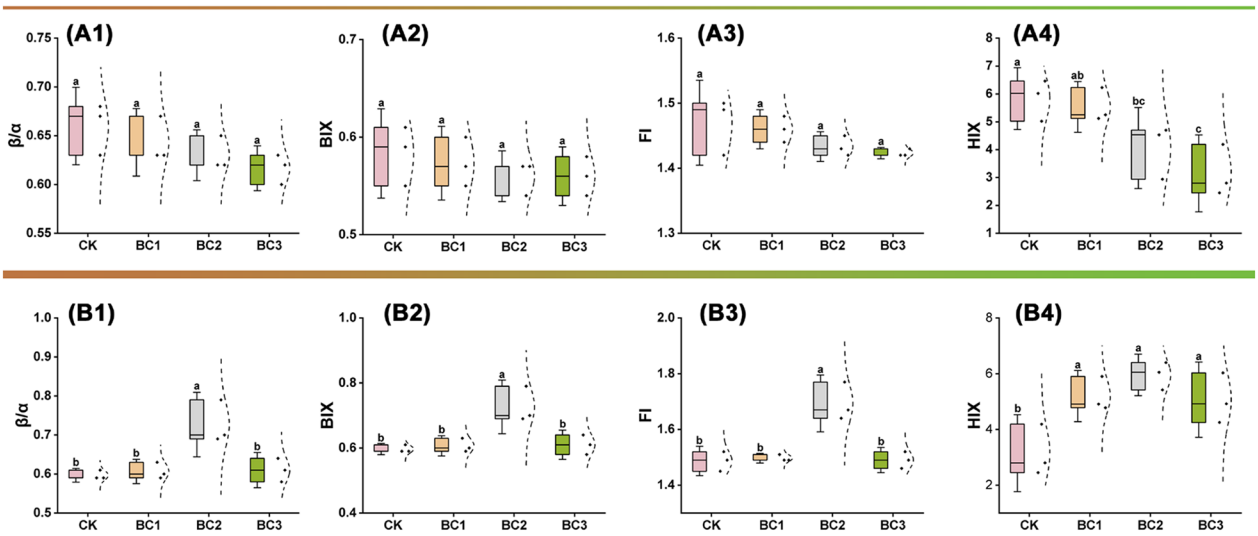


Fig. 2 The freshness index, biological index, fluorescence index and fluorescence index of soil DOM during the planting period (**A1**, **A2**, **A3**, **A4**) and harvesting period (**B1**, **B2**, **B3**, **B4**) under long-term biochar application. Different letters indicate significant differences among treatments ($P < 0.05$)

production, and humification level of DOM. The FI values ranged from 1.4 to 1.7, indicating the dual origin of DOM as both endogenous and exogenous in soil aggregates. During planting-period, the FI values decreased along with increasing biochar application rate (Fig. 2A3, B3). Besides, the FI values of each treatment in the harvesting period were higher than those in the planting period. Two-way ANOVA indicated that FI value was not influenced by biochar application rate and sampling period, but was significantly affected by their interaction. During the harvesting period, the FI value in the BC2 treatment was significantly higher than in other treatments. During the whole sampling period, HIX showed different trends with the amount of biochar application. During the planting period, compared with CK, all biochar application treatments significantly reduced HIX and decreased with the increase of biochar application. During the harvesting period, compared with CK, all biochar application treatments significantly enhanced HIX, and increased first and then decreased with the increase of biochar application. Likewise, the changes in β/α followed a pattern similar to HIX. The BC2 treatment exhibited notably higher values of HIX and β/α during the harvest period in comparison to the other treatments, indicating that the application of biochar at a rate of 31.50 t ha^{-1} considerably improved soil biological activity.

3.3 Soil microbial richness and diversity

A total of 23,273 ASVs were identified through MiSeq sequencing. These ASVs were predominantly distributed among the following phyla: Proteobacteria (35.68%), Actinobacteria (6.38%), Bacteroidetes (8.85%), Chloroflexi (6.73%), Firmicutes (2.95%), Planctomycetes (3.22%), and Basidiomycota (8.06%). While the treatments had no significant effect on soil bacterial richness, it was significantly influenced by sampling time and their interaction. During the harvest period, the BC2 treatment exhibited the highest bacterial richness, significantly higher than that of the CK and BC3 treatments (Fig. 3A). Principal coordinate analysis (PCoA) based on Bray–Curtis distance revealed the effects of biochar application rate and planting period on bacterial community structure. During the growing season, PCoA analysis revealed that PCo1 and PCo2 explained 36.74% and 15.40% of the variation in soil bacterial community composition, respectively. After harvesting, PCo1 and PCo2 explained 30.78% and 16.09% of soil bacterial community variation, respectively (Fig. 3B2). As shown in PCoA ordination, all treatments clearly separated from each other.

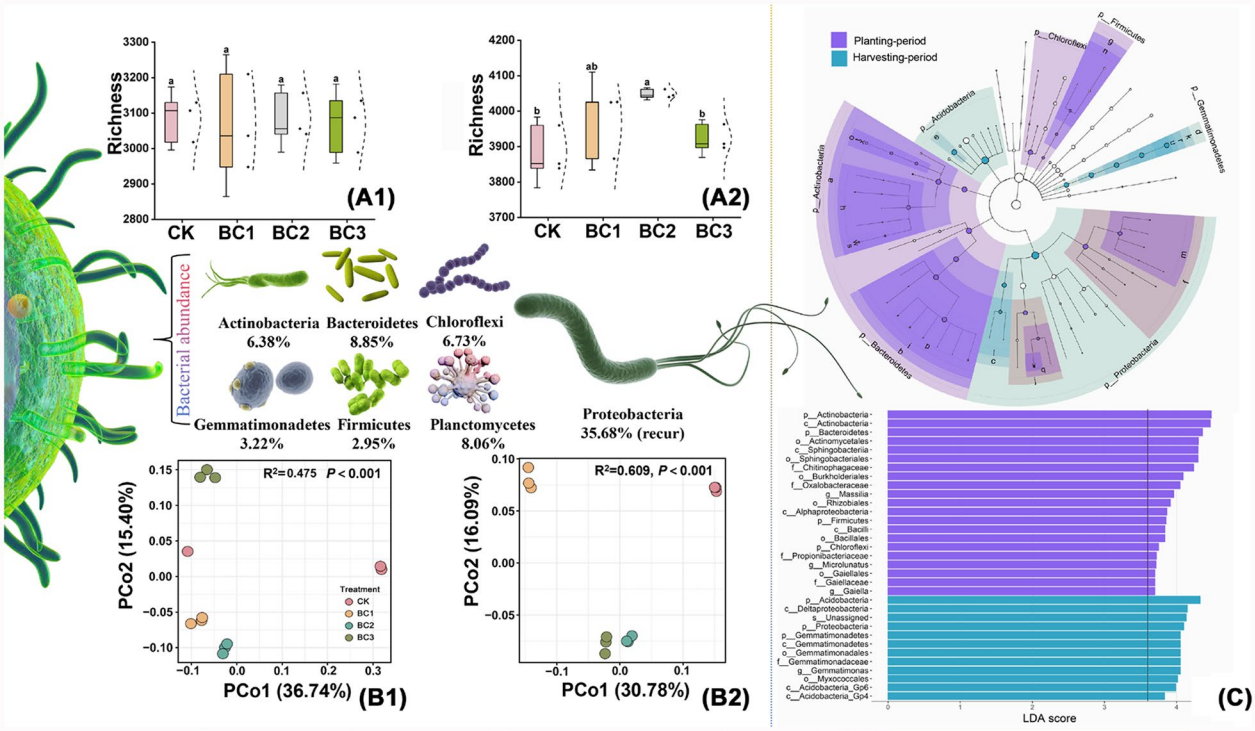


Fig. 3 The soil bacterial community richness index (**A1**) and β diversity (**B1**) among planting period; the soil bacterial community richness index (**A2**) and β diversity (**B2**) among harvesting-period. The LEfSe analysis under different sampling periods (**C**). Different letters indicate significant differences among treatments ($P < 0.05$)

This pattern was further confirmed by PERMANOVA analysis, which revealed that bacterial community composition was significantly influenced by biochar addition (Fig. 3B1, B2). All biochar application treatments exhibited substantial differences in soil bacterial communities compared to CK, with BC3 showing the largest difference from CK. Additionally, the CK treatment in the planting period was more dispersed than other treatments, while the treatments in the harvesting period were more concentrated. These interpretations were supported by PERMDISP analysis ($F=9.16$, $P=0.005$).

LEfSe analysis highlighted the differences between sampling periods in the soil bacterial community comosition under biochar application treatments, identifying significantly enriched taxa that reflected shifts in relative abundance (Fig. 3C). A total of 33 biomarkers were detected (planting period, 21; harvesting period, 12). Representative soil bacteria during the crop planting period mainly belonged to the phyla Actinobacteria, Bacteroidetes, Chloroflexi, and Firmicutes, while were primarily from the phyla Acidobacteria, Proteobacteria, Gemmatimonadetes, and Nitrospirae during the harvesting period.

The RDA analysis results demonstrated the relationships between sampling periods, treatments, fluorescence components (C1–C5), soil physicochemical properties, and dominant microbial phyla (Fig. 4A). During the crop planting period, CK treatment was positively correlated

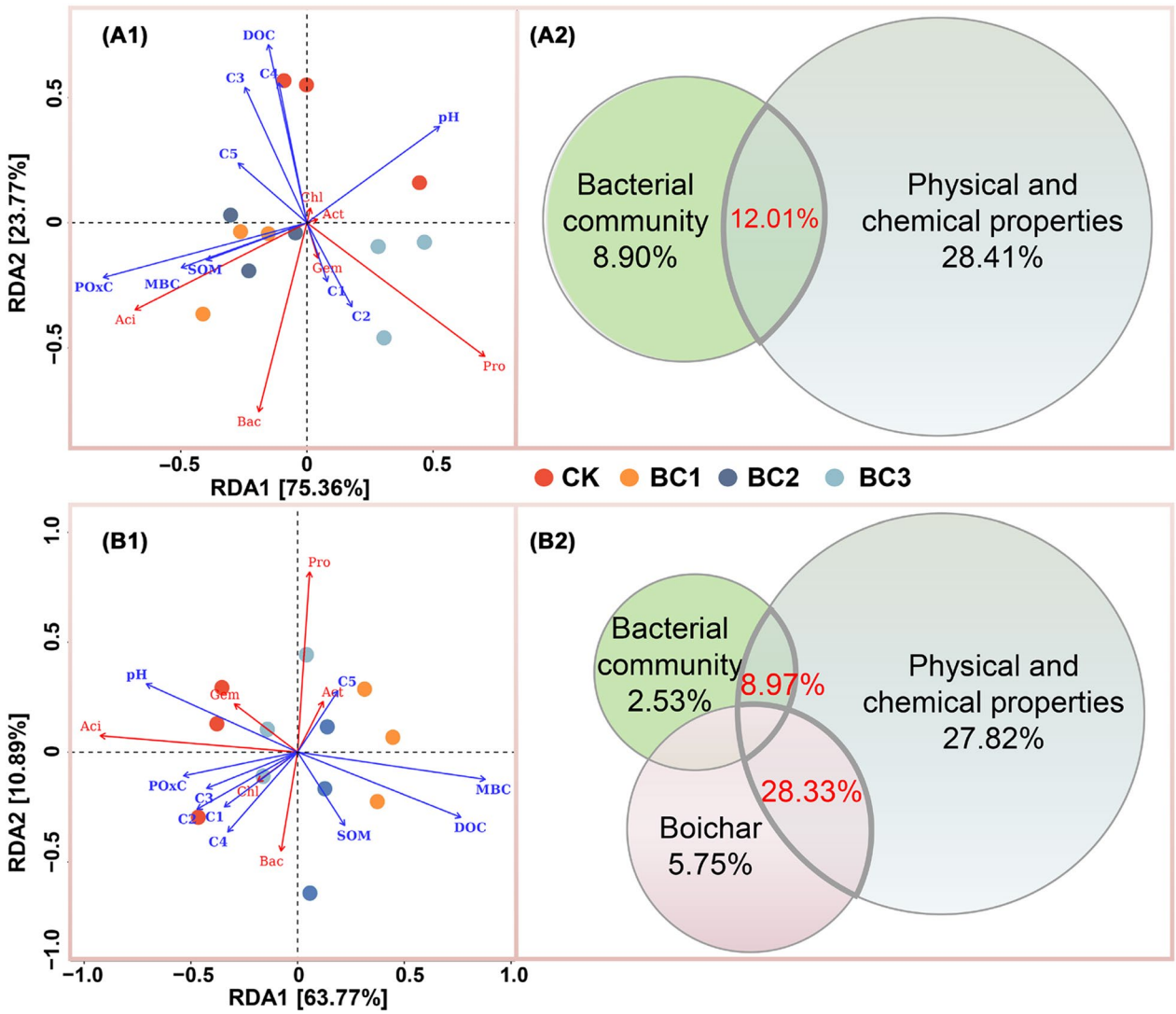


Fig. 4 RDA analysis of environmental factors and microbial community composition in crop planting period (**A1**) and harvesting period (**A2**) under biochar application. VPA analysis showed the effects of biochar application rate, soil properties and microbial community on fluorescence components crop planting period (**B1**) and harvesting period (**B2**) under long-term biochar application

with pH and DOC, while biochar treatments were positively correlated with MBC, SOM, and POC. The distribution characteristics of microbial phyla showed that Actinobacteriota and Bacteroidota were mainly positively correlated with CK treatment, whereas Proteobacteria and Acidobacteriota were positively correlated with BC2 and BC3 treatments (Fig. 4A1). During the crop harvesting period, CK treatment was significantly positively correlated with pH and POC, while biochar treatments were positively correlated with DOC, SOM, MBC, and MC. The distribution of microbial phyla indicated that Proteobacteria were positively correlated with BC2 and BC3 treatments, while Actinobacteriota and Bacteroidota were more closely associated with CK treatment (Fig. 4B1).

VPA analysis revealed the contributions of biochar application rates, soil physicochemical properties, and bacterial communities to soil DOM fluorescence components (Fig. 4B). Throughout the planting period, variations in bacterial communities and soil physicochemical characteristics collectively accounted for 49.32% of the fluorescence composition. Specifically, bacterial communities contributed 20.91%, while soil physicochemical properties explained 40.42% (Fig. 4B1). In contrast, during the harvesting period, biochar application rates, bacterial communities, and soil physicochemical parameters together explained 73.74% of the fluorescence composition, with contributions of 14.72%, 30.86%, and 56.15%, respectively (Fig. 4B2).

3.4 Analysis of soil microbial co-occurrence network

Spearman correlation analysis at the genus level (Fig. S1) revealed strong associations between DOM fluorescent components and dominant bacterial genera in both soils before planting period and harvesting period. During the crop planting period, more than half of the dominant genera were associated with C1 and C2, and nearly all fluorescent components, except C4, were linked to the dominant genera.

To gain deeper insights into the association between microbial communities and DOM components, co-occurrence networks were employed to quantitatively evaluate the Spearman correlations between ASVs and the five fluorescent components present in soils during both the planting and harvesting periods of the crop cycle (Fig. 5A, B, D, E). The soil microbial network was

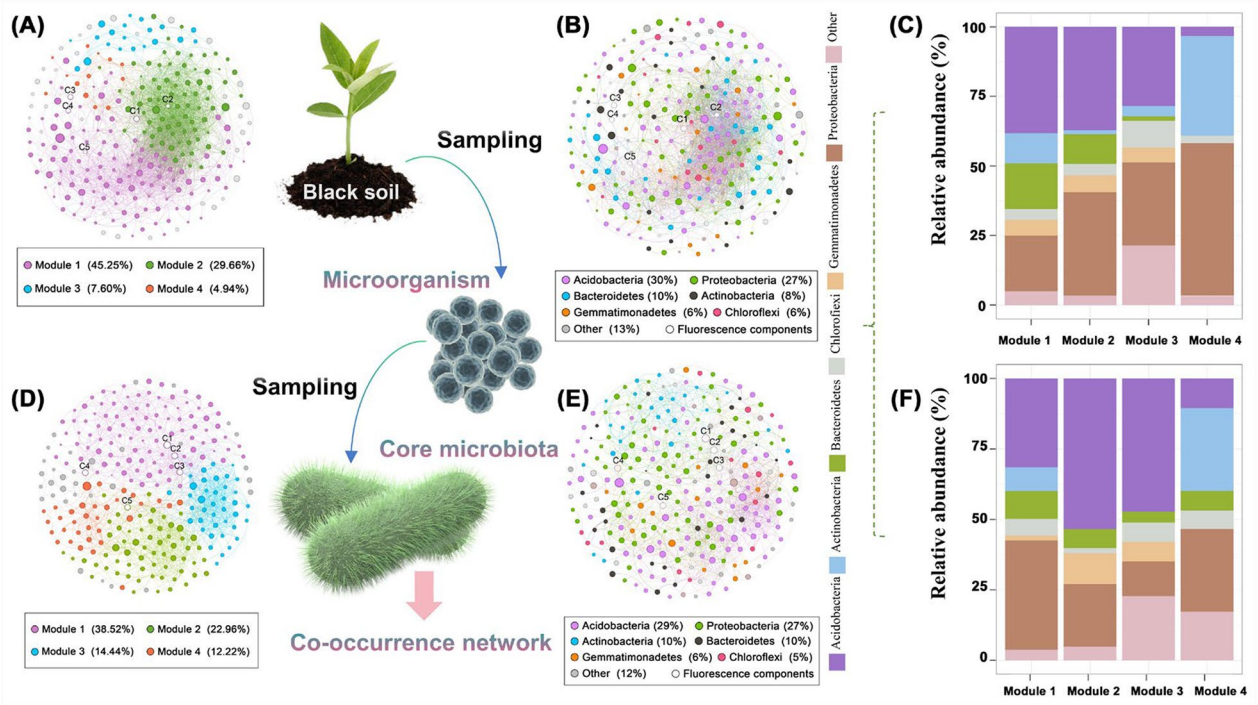


Fig. 5 The soil bacterial co-occurrence network in planting period and harvesting period. The nodes of planting period (**A**) and harvesting period (**D**) are colored according to different types of modularity classes. The nodes of the planting period (**B**) and the harvesting period (**E**) are colored according to the phylum. The relative abundance of major bacterial phylum in different modularity classes of planting period (**C**) and harvesting period (**F**). C1-C5 represents the contribution percentage of each fluorescent component. The size of the node was proportional to the relative abundance of the species, and the connection line between ASV and fluorescence component can only be performed when the absolute value of r exceeded 0.4 (or less than −0.4) and the *P* value was less than 0.05. For the correlation between ASVs, only the case where the absolute r value was greater than 0.7 was considered

primarily composed of phyla Proteobacteria, Acidobacteria, Bacteroidetes, Actinobacteria, Gemmatimonadetes, and Chloroflexi (Fig. 5B, E). The co-occurrence network of soil during planting period consisted of 263 nodes and 2428 edges, while it comprised 270 nodes and 1287 edges during the harvesting period, indicating more complex bacterial interactions during the crop planting period than harvesting period.

Module analysis of soil bacterial networks during the planting and harvest periods divided the nodes into four modules (Module 1–4). In the soil during the planting period, Modules 1 and 2 were dominated by Acidobacteria and Proteobacteria. Module 1 included C3, C4, and C5, while C1 and C2 were in Module 2 (Fig. 5A, C). In the soil during the harvesting period, Modules 1 and 2 were also dominated by Acidobacteria and Proteobacteria. Module 1 contained C1, C2, and C3, while C4 and C5 were in Module 2 (Fig. 5D, E). Notably, the relative abundance of Actinobacteria in Module 2 was much lower compared to other modules.

Further, heatmap visualization was employed to highlight the relationships between the abundance of fluorescent components and bacterial genera. During the planting period (Fig. S2A), component C1 and C2 had significant positive correlations ($P < 0.01$) with multiple genera, particularly genera within the phyla Actinobacteria and Bacteroidetes. During the harvesting period (Fig. S2B), components C1, C2 and C3 were closely associated with genera within the phyla Proteobacteria and Acidobacteriota. During the crop growth period, the fluorescent components of soil DOM exhibited a stronger correlation with bacterial genera belonging to the phylum Actinobacteria. In contrast, during the harvesting stage, these components were more closely linked to genera classified within the phylum Proteobacteria.

Furthermore, we constructed soil bacterial co-occurrence networks under different biochar application rates to determine the effect of biochar on soil bacterial networks (Fig. 6). The average degree in CK treatment was lower than in other treatments, indicating that

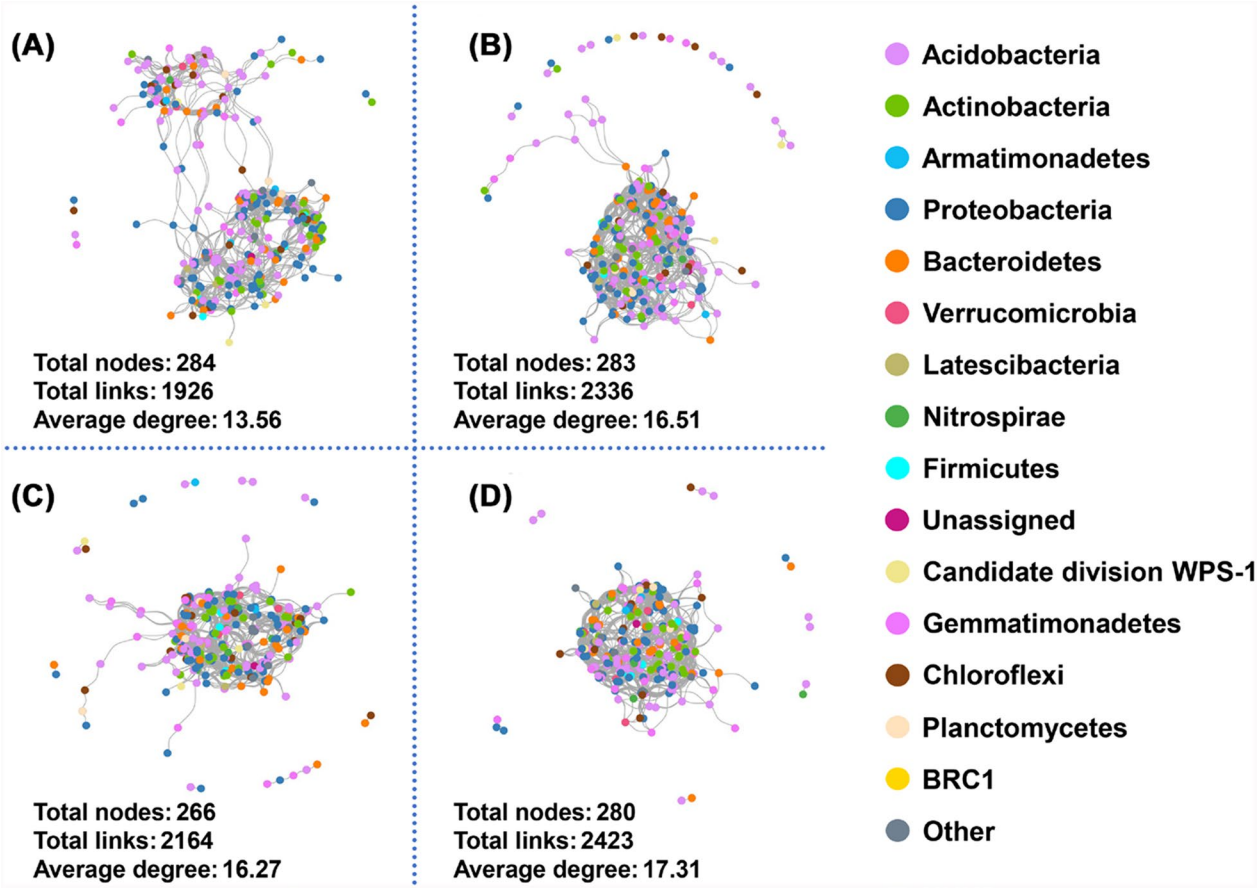


Fig.6 Soil bacterial co-occurrence networks across treatments. CK (**A**), BC1(**B**), BC2 (**C**) and BC3 (**D**). Note colors denote ASVs classified at the phylum level

biochar application increased the bacterial network complexity.

3.5 Effect of biochar application rate on crop yield

This study used crop yield as a key indicator of agricultural productivity in the black soil region. Compared with CK treatment, crop yields in BC1 and BC2 treatments were significantly increased ($P<0.05$) by biochar application (5.17% and 7.11%, respectively) (Fig. 7B). SEM analysis revealed that long-term biochar application indirectly influenced crop yield by affecting the change of DOM and microbial communities, and 70% variation of yield could be explained (Fig. 7A). The rate of biochar application exhibited a direct effect on MBC ($\lambda=0.65$, $P<0.001$) and FI ($\lambda=0.29$, $P<0.05$). Additionally, crop yield was directly influenced by microbial richness ($\lambda=0.33$, $P<0.01$), PCoA2 ($\lambda=-0.63$, $P<0.05$), and MBC ($\lambda=0.21$, $P<0.001$) (Fig. 7B).

4 Discussion

4.1 Effect of long-term application of biochar on soil DOM spectrum

Soil DOM is a vital active component of soil organic matter, comprising humic acids, proteins, organic acids, and amino acids. It plays a central role in nutrient cycling and microbial metabolism and is crucial for soil carbon pool stability (Feng et al. 2024). This study demonstrated that biochar application significantly altered the quality and composition of soil DOM, with its effects were regulated by application rate and sampling period. EEM-PARAFAC analysis revealed that biochar application increased the content of component C1 in DOM of planting period, indicating significantly enhanced microbial decomposition activity. The accumulation of these microbial metabolic byproducts suggests that biochar promotes organic matter decomposition and enhances the bioavailability of DOM (Bolan et al. 2023; He et al. 2024). Meanwhile, the reduced proportions of component C2 and C3 substances reflect a propitious shift of DOM towards more degradable and biologically active forms.

During the harvesting period, C1 became the dominant DOM component, and the transformation of protein-like substances indicated that long-term biochar application induced DOM stabilization. This stabilization was mainly reflected in the significant increase in humification degree and aromaticity, suggesting that biochar, by altering the source and quality of DOM, not only enhances short-term nutrient supply but also improves the long-term stability of soil organic carbon (Gross et al. 2024).

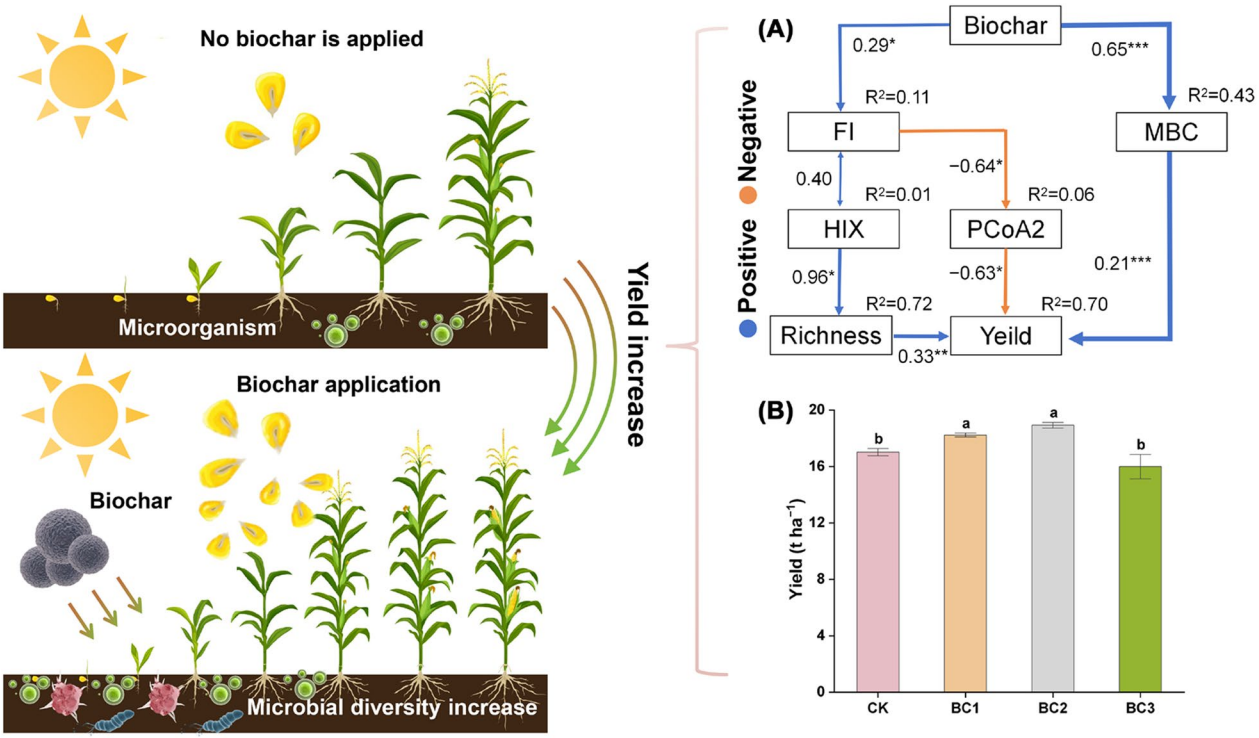


Fig. 7 SEM showed the pathway between biochar application rate, soil fluorescence characteristics, bacterial community structure and crop yield (**A**), and the yield changes of different treatments (**B**). (χ^2/df = 1.380, GFI = 0.912, CFI = 0.966, GFI = 0.959, $P=0.167$). The asterisks indicate the significance level:* $P<0.05$;** $P<0.01$;*** $P<0.001$. Different letters indicate significant differences among treatments ($P<0.05$)

In this research, the FI values for all treatments ranged from 1.4 to 1.7, while BIX values remained below 1. The observed variations in FI and BIX over time provided further insights into the dual regulatory role of biochar in influencing both the origin and composition of DOM. During the planting period, FI values decreased with increasing biochar application, indicating the dominance of exogenous DOM inputs. In contrast, during the harvesting-period, FI values increased significantly, suggesting enhanced microbial transformation and utilization of DOM. Medium-dose biochar treatment (BC2) significantly increased HIX values (HIX > 5), indicating the accumulation of humic components following organic matter decomposition, contributing to soil carbon pool stability (Das et al. 2021). Our study further confirmed that medium-dose biochar application (BC2) not only enhanced the humification degree of DOM but also achieved more efficient carbon and nutrient cycling in the soil–plant system. Long-term studies have also shown that biochar can promote the long-term stabilization of DOM and improve the carbon metabolism activity of soil microorganisms under different application rates. In the treatment of 30 t ha-1, the structure of DOM changed significantly to a more stable direction, which enhanced the stability of soil carbon pool (Cong et al. 2023; Han et al. 2023).

4.2 Effects of long-term biochar application on soil bacterial communities

The results of this study suggested that biochar significantly impacted the diversity and structure of soil microbial communities. Biochar has been previously reported to promote root development and increase exudate production, which supports the reorganization and optimization of microbial communities (Ferreira et al. 2024). This research revealed significant variations in the effects of different biochar application rates on the dominant microbial groups. In the BC2 treatment, the greatest bacterial richness was recorded. This treatment showed a significant increase in the relative abundance of Proteobacteria and Acidobacteria compared to other treatments. As r-strategists, Proteobacteria are capable of rapidly degrading recalcitrant components of dissolved organic matter (DOM), particularly polysaccharides, thus facilitating the release of nutrients that are readily available for crop growth. Acidobacteria, as k-strategists, prefer stable organic matter and play key roles in humic and fulvic acid transformations (Hale et al. 2019; Yang et al. 2023). This functional differentiation indicates that biochar regulates soil ecological functions by selectively promoting microbial groups.

The addition of biochar can affect nutrient cycling, decomposition and energy flow in soil by changing the abundance and community composition of soil microorganisms. This is because biochar application changes the physical and chemical properties of soil and provides a good habitat for soil bacteria, resulting in changes in bacterial community structure and diversity (Gao et al. 2021). Wang et al. (2024a, b) monitored the soil bacterial community for six consecutive years after biochar application, and the results showed that the relative abundance of Acidobacteria, Actinobacteria, Nitrospirae and Gemmatimonadetes increases with the application level of biochar in soil.

However, high-dose biochar application (BC3) may disrupt the balance of microbial communities. This study found that high-dose biochar excessively promoted the growth of specific microbial groups (e.g., Proteobacteria and Actinobacteria), leading to reduced diversity. High application rates of biochar can curtail microbial diversity by promoting the rapid growth of specific microorganisms, thereby changing soil microbial community structure. Additionally, certain studies indicate that high biochar application rates have led to a reduction in the biomass of both gram-positive and gram-negative bacteria, particularly in the initial months following application. Studies have also shown that this effect may result from soil pH changes and nutrient imbalances caused by excessive biochar (Li et al. 2020). Therefore, scientific and rational selection of biochar application rates, especially in long-term soil management, is crucial for improving soil quality and ensuring sustainable agriculture (Bolan et al. 2023; Qiu et al. 2023). In this study, we observed that the biochar-amended soil showed higher microbial network connectivity than the unamended soil, which enhanced the ecological resilience of the soil and provided stable microbial support for crop growth, especially in the treatment with biochar application rate of 31.50 t ha^{-1}. Long-term biochar application can promote nutrient cycling and organic matter decomposition, thus supporting the sustainability of soil ecosystems and agricultural productivity (Yuan et al. 2023).

4.3 Synergistic patterns between soil bacterial communities and DOM under biochar application

Biochar application significantly enhanced the complexity and stability of soil ecological networks through the interaction between soil bacterial communities and DOM. This study demonstrated that biochar played a vital role in regulating soil carbon cycling by influencing DOM components and microbial network characteristics, thereby improving overall ecosystem functions. Microbial network analysis revealed the impact of biochar application on community symbiotic patterns.

The microbial network during the planting period had the highest number of nodes (263) and edges (2428),

indicating more active interactions among soil bacteria and higher functional diversity at this stage (Li et al. 2023). However, during the harvest period, the number of nodes increased to 270, but the number of edges decreased to 1287, suggesting a reduction in microbial collaboration intensity while optimizing ecological network stability which can lead to more efficient nutrient cycling and organic matter decomposition (Wang et al. 2021). RDA and VPA results further indicated that the effects of biochar application on DOM fluorescent components were indirectly mediated by bacterial community structure. The physical and chemical properties of soil, as well as the contribution of microbial communities to the formation and stabilization of DOM, vary across different planting periods (Deshoux et al. 2023). During the planting period, soil physicochemical properties contributed more to DOM (explaining 49.32%), while bacterial communities contributed relatively less (explaining 20.91%). However, during the harvesting period, the role of bacterial communities in DOM significantly increased (explaining 30.86%), highlighting the dominance of bacteria in the DOM stabilization process. Most studies on short-term application of biochar or year-by-year application of biochar showed that biochar application increased the pH of agricultural soil by 0.5–1.6 (Xu et al. 2022). This is inconsistent with our results, which may be attributed to the fact that this study conducted soil sampling after 6 years of one-time application of biochar, and biochar reduced soil pH through oxidation. Soil DOC has been shown to have an extremely fast turnover rate and easy degradability, Biochar could enhance the DOC content in both acidic and neutral soils (Sun et al. 2022). The increase of DOC content in soil organic carbon by biochar is due to the increase of soil microbial activity and MBC content by biochar, which changes the microbial community structure, so that soil microorganisms decompose and release more DOC.

Soil DOM shapes microbial communities, and microorganisms affect DOM transformation (Lucas et al. 2016). The network pattern suggests a possible interaction between key microbes and DOM components. The bacterial network was divided into five modules during the planting and harvesting periods. In addition, the decrease in the number of biochar application nodes, the increase in the number of connections, and the shortening of the average path length mean that the competition of microorganisms for substrates is enhanced (Figs. 5, 6). These results showed that the application of biochar could change the symbiotic network pattern of key bacteria and DOM components. The correlation between microorganisms and DOM components may be due to the good growth of microorganisms on the existing substrates, or it may be because the substrates exist but are not consumed by existing microorganisms and therefore persist. In this study, Proteobacteria were positively correlated with N-compounds, polysaccharides, plant-derived long-chain alkanes and some aromatic compounds (Ar25), suggesting a strong adaptability to muti-source substrates (Ling et al. 2022). Also, Acidobacteria have genes for degrading complex carbohydrate compounds, which allows them to degrade a wide range of compounds (e.g. cellulose, hemicellulose, amino acids, starch, and other metabolic intermediates) (Hale et al. 2019; Yang et al. 2023). Actinobacteriota and Gemmatimonadota thrived on a portion of the same DOM molecule. As slow-growing oligotrophs, Chloroflexi are more likely to survive in extreme environments, and their preferential use of SOM has been rarely reported (Sazykina et al 2022). Planctomycetota are slow to decompose plant-derived organic matter (Ivanova et al. 2018).

The relationship between key microbial groups and DOM chemical components is different at the "micro" and "macro" levels, indicating that there is a complex interaction between DOM and microbial communities. DOM components have a strong correlation with key soil microbial groups, that is, they belong to the same category, which has been confirmed by other studies (Li et al. 2018; Zhang and Shen 2022). However, biochar application significantly complicated the interaction between bacterial community and DOM fluorescent components, indicating that bacterial community played a stronger role in DOM production and consumption.

4.4 Changes in DOM fluorescence characteristics mediated by soil microorganisms impacted crop yield

Biochar significantly affected crop yield by regulating soil DOM fluorescence characteristics and microbial community structure. The current study, based on SEM, revealed the critical link between microbially mediated DOM dynamics and crop yield. The results showed that biochar application at different doses had dose-dependent effects on DOM fluorescent components, indirectly influencing crop yield through microbial mediation.

Medium-dose biochar treatment (BC2) significantly improved DOM bioavailability and stability while increasing crop yield by 7.11%. This effect was mainly attributed to biochar-regulated dynamic changes in DOM fluorescent components (e.g., tryptophan and humic substances), which provided abundant carbon sources and nutrients for soil microorganisms while enhancing DOM humification and aromaticity, further stabilizing the soil carbon pool. SEM path analysis revealed that changes in DOM fluorescence characteristics had a significant indirect contribution to crop yield ($R^2 = 0.62$), indicating that biochar application enhanced

crop growth through synergistic interactions between microbes and DOM.

Additionally, the study found that optimization of microbial networks was a key driver for biochar-induced crop yield improvements. The microbial co-occurrence network under medium-dose biochar treatment was more complex, with key taxa such as Proteobacteria and Acidobacteria playing central roles in DOM decomposition and transformation. These functional groups could breakdown protein and polysaccharide components in DOM, releasing key metabolites like amino acids that promote plant growth while stabilizing DOM components to enhance soil nutrient availability (Ye et al. 2020).

Our findings also revealed that even after six years of biochar application to the soil, biochar continued to promote maize growth and yield. Some studies have shown that biochar application significantly increased maize yield over an eight-year period, with increases ranging from 9.4% to 35.5% (Cong et al. 2023). This helps us to better understand the long-term effects of biochar on maize growth and yield. In contrast, high-dose biochar application (BC3) had a negative impact on yield, reducing it by 6.05%. SEM analysis indicated that excessive biochar may inhibit the activity of sensitive microbes and increase the proportion of recalcitrant DOM components, thereby altering soil pH and nutrient balance, consequently reducing nutrient conversion efficiency (Huang et al. 2023; Song et al. 2024). After years of application, biochar not only enhanced the stability of soil DOM, but also promoted the circulation of key nutrients by changing the structure of microbial communities. This long-term effect helps to improve soil fertility and crop yield stability, especially in areas with high drought or climate change, and the continuous application of biochar has an important positive effect on crop production (Kätterer et al. 2019).

Moderate biochar application significantly enhanced crop yield through microbially mediated changes in DOM fluorescent components. This finding provided scientific evidence for optimitum biochar application strategies and highlighted the crucial role of soil microbe-DOM interactions in agricultural ecosystem management. Future studies could further explore the interactions between biochar and other functional soil components to achieve more efficient agricultural production and ecosystem services.

5 Conclusions

This study systematically investigated the long-term effects of biochar application rates on DOM and microbial community structure in black soil region of Northeast China. The study revealed the interaction mechanisms between DOM and microbial communities mediated by biochar. The findings indicated that a single application of medium-dose biochar (31.50 t ha^{-1}) led to a significant enhancement in fluorescence indices, including FI, BIX, and HIX, as well as an increase in bacterial richness at the time of crop harvest. Biochar application also contributed to greater complexity and stability in the overall bacterial network. Notably, the network of bacteria exposed to the medium-dose biochar treatment (31.50 t ha^{-1}) exhibited higher complexity and a more tightly integrated structure compared to those subjected to other biochar application rates. The study found that Proteobacteria were associated with humic-like components, while Acidobacteria were linked to protein-like components, indicating their potential roles in DOM formation and transformation. At the same time, long-term application of biochar had potential help to improve soil fertility and crop yield stability. These findings provide scientific evidence and empirical references for optimizing biochar application strategies in agricultural production.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1007/s42773-025-00473-z.

Supplementary material 1.

Acknowledgements
Not applicable

Author contributions
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Funding
This work was sponsored by the National Key Research and Development Program of China (2023YFD1500500) and the Strategic Priority Research Program of the Chinese Academy of Sciences (XDA28130200), and Innovation Capacity Building Project of Beijing Academy of Agricultural and Forestry Sciences (KJCX20240506).

Data availability
All data gathered or analyzed in this study are included in the article.

Declarations

Competing interests
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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Received: 6 February 2025 Revised: 28 April 2025 Accepted: 9 May 2025
Published online: 23 June 2025

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