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Getting down to the mechanism of biochar effects on the functioning of plant-soil systems

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Getting down to the mechanism of biochar effects on the functioning of plant-soil systems

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Table of contents

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Biochar Amendment and Nitrogen Fertilizer Contribute to the Changes in Soil Properties and Microbial Communities in a Paddy Field

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Biochar amendment can influence the abundance, activity, and community structure of soil microbes. However, scare information is present about the effect of the combined application of biochar with synthetic nitrogen (N) fertilizer under paddy field condition. We aimed to resolve this research gap in rice field conditions through different biochar in combination with N fertilizers on soil nutrients, soil microbial communities, and rice grain yield. The present study involves eight treatments in the form of biochar (0, 10, 20, and 30 t ha^{-1}) and N (135 and 180 kg ha^{-1}) fertilizer amendments. The soil microbial communities were characterized using high-throughput sequencing of 16S and Internal transcribed spacer (ITS) ribosomal RNA gene amplicons. Experiential findings showed that the treatments had biochar amendments along with N fertilizer significantly advanced soil pH, soil organic carbon (SOC), total nitrogen (TN), soil microbial carbon (SMBC), soil microbial nitrogen (SMBN), and rice grain yield in comparison to sole N application. Furthermore, in comparison with control in the first year (2019), biochar amendment mixed with N fertilizer had more desirable relative abundance of microorganism, phyla Acidobacteria, Actinobacteria, Proteobacteria, and Verrucomicrobia with better relative abundance ranging from 8.49, 4.60, 46.30, and 1.51% in T7, respectively. Similarly, during 2020, bacteria phyla Acidobacteria, Actinobacteria, Bacteroidetes, Gemmatimonadetes, Planctomycetes, and Verrucomicrobia were resulted in higher and ranging from 8.69, 5.18, 3.5, 1.9, 4.0, and 1.6%, in biochar applied treatments, respectively, as compared to control (T1). Among the treatments, *Sphingopyxis* and *Thiobacillus* bacterial genus were in higher proportion in T7 and T3, respectively, as compared to other treatments and *Bacillus* was higher in T6. Interestingly, biochar addition significantly decreased the soil fungi phyla Ascomycota, Basidiomycota, Chytridiomycota, and Rozellomycota, in 2020 as compared to 2019. Whereas biochar addition to soil decreased *Echria*, *Kohlmeyeriopsis*, and *Westerdykella* fungal genus as compared to non-biochar treatments. The redundancy analysis showed that soil biochemical traits were positively correlated

with soil bacteria. In addition, correlation analysis showed that soil bacteria including Acidobacteria, Actinobacteria, Bacteroidetes, Planctomycetes, and Proteobacteria strongly correlated with rice grain yield. This study demonstrated that soil nutrients and bacteria contribute to an increase in rice yield in combined biochar amendment with lower N treatments.

Keywords: soil properties, biochar, soil fungi and bacteria, rice rhizosphere, grain yield

INTRODUCTION

Soil microorganisms play an important role in the soil ecosystem, which are important for soil quality and agricultural productivity. They have an impact on several critical and fundamental ecosystem processes, such as organic matter decomposition, nutrient mineralization, soil functionality, and plant nutrient uptake and growth (Bending et al., 2004; Khan et al., 2021). Soil management practices directly affect the abundance and structure of soil microbes (Li et al., 2012). Furthermore, bacteria are the most diverse and abundant group of soil microorganisms, which play a significant role in the decomposition and mineralization of organic matter and nutrients and the development of soil aggregates (Lian et al., 2019; Ali et al., 2020a; Bu et al., 2020), consequently, influencing soil fertility and plant growth (Tardy et al., 2015). Microorganisms have a distinct role in the decomposition and degradation of organic matter through extracellular enzyme and degrade the macromolecule to monomers to be utilized by the plant (Bending et al., 2004; Harris, 2009). Furthermore, the degradation of plant residues by microorganisms also leads to soil nutrient turnover and circulation, such as carbon (C) and nitrogen (N) cycling and soil aggregate formation (Brennan and Acosta-Martinez, 2017). Several studies reported that the soil pH, organic matter, and other soil properties influenced the soil community composition (Eldridge et al., 2015; Creamer et al., 2016; Pan et al., 2020). Previously, it is well reported that biochar application in combination with N fertilizer improves soil physiochemical properties and crop production (Ali et al., 2020a,b, 2021; Ullah et al., 2021a,b). However, the information on biochar in combination with synthetic N fertilizer on soil microbial and fungal community structure and composition under paddy field condition is not well reported.

Biochar is a carbon-rich, stable product that is produced by the burning of organic material (biomass) of agricultural and forestry wastes, animal bones, algae, and animal manures *via* a controlled process called pyrolysis (Lehmann and Joseph, 2015). Biochar has aromatic and heterocyclic C compounds in its chemical structure, making it resistant to microbial degradation (Ameloot et al., 2013; Anyika et al., 2015). As a soil amendment application, biochar has unique physical and chemical features. Biochar can increase crop growth, yield, and quality by improving soil chemical properties, boosting soil microbial biomass, and enhancing microbial growth and reproduction (Agegnehu et al., 2017; Yu et al., 2019). The addition of biochar to soil decreased soil compactness and affected soil water holding capacity and microbial growth (Liu et al., 2017; Li et al., 2018). Soil microbial populations, community structure, and physiological activity can be affected by biochar application to soil due to their sensitivity of soil microbes (Dempster et al., 2012; Dai et al., 2016). In addition, previous studies reported that application of biochar either increased or decreased the activities of the enzymes, which is related to the transformation of N, C, and P in soil (Bailey et al., 2011; Gomez et al., 2014). Furthermore, it is observed that increasing biochar rate proportionally increased soil microbial abundance (Gomez et al., 2014), whereas, Ameloot et al. (2014) reported the contrast results that 49 t biochar per hectare introverted microbial activity and condensed both extractable phospholipid fatty acid (PLFA) concentration and fungal abundance.

Biochar combined with N fertilizers not only significantly mitigated the problems of excessive fertilizer use such as environmental pollution but also improves soil microorganism abundance and soil enzymes (Ali et al., 2020a). This research is based on continuous long-term research in paddy fields under dual cropping systems, with the following research objectives: (i) characterize the influence of biochar and N application on rice grain yield, soil biochemical properties, and microbial community composition and function; (ii) examine the effect of fertilization on the relationships between microbial community and soil environmental factors; and (iii) measure the contributions of different fertilization regimes, soil biochemical traits, and microorganisms to enhance in rice production. The primary goal of this research is to provide a theoretical framework for sustainable agriculture practices to improve rice production with the use of biochar under lower chemical fertilizers.

MATERIALS AND METHODS

Site Description

The study was conducted at the research farm of Guangxi University, China (22°49′12″ N, 108°19′11″ E; 75 m), in 2019–2020. The climate is classified as subtropical monsoon, and the mean temperature and mean precipitation values of both years are shown in **Table 1** (local weather station). The soil (0–20 cm) is graded as Ultisols and is slightly acidic (pH 5.94), soil organic carbon (SOC) 15.10 g kg^{-1}, soil organic matter 25.8 g kg^{-1}, total N (TN) 1.35 g kg^{-1}, available N (AN) 134.7 mg kg^{-1}, available phosphorous (23.1 mg kg^{-1}), and available potassium (AK 233.6 mg kg^{-1}, with 1.36 g cm^{-3} soil bulk density (BD).

Biochar Production

Cassava straw was used in kilns with the temperature ranging 300–500°C by following the method previously documented by

TABLE 1 | Mean temperature and mean precipitation during both year.

Year	2019		2020	
Months	Mean Temp (°C)	Mean Precipitation (mm)	Mean Temp (°C)	Mean Precipitation (mm)
Jan	17	98	16	80
Feb	20	102	19	94
Mar	21	72	22	73
Apr	26	92	25	75
May	30	176	29	160
Jun	31	211	30	210
Jul	32	231	34	215
Aug	30	151	31	128
Sep	29	115	28	85
Oct	28	98	28	75
Nov	24	110	23	81
Dec	19	107	17	91

Mia et al. (2015). The properties of biochar were C (674.00 g kg^{-1}), H (3.81 g kg^{-1}), P (46.33 g kg^{-1}), N (5.43 g kg^{-1}), K (48.33 g kg^{-1}), S (2.39 g kg^{-1}), specific area (2.46 m^2 g^{-1}), and pore diameter (3.37 nm) with C:N ratio (124.12.) and are presented in our previous study (Ali et al., 2020a).

Experimental Design

The field experiment was conducted in a randomized complete block (RCB) design having three replications and a plot size of 3.9 by 6 m (23 m^{-2}) during 2019 and 2020. The experiment consisted of four biochar rates (0, 10, 20, and 30 ton ha^{-1}) and two N levels (135 and 180 kg ha^{-1}). Biochar amendment was applied once in 2019, whereas N application was applied in both years. The treatments combinations were as follows: T1 = 0 t B + N135 kg ha^{-1}, T2 = 0 t B + N180 kg ha^{-1}, T3 = 10 t B + N135 kg ha^{-1}, T4 = 20 t B + N135 kg ha^{-1}, T5 = 30 t B + N135 kg ha^{-1}, T6 = 10 t B + N180 kg ha^{-1}, T7 = 20 t B + N180 kg ha^{-1}, and T8 = 30 t B + N180 kg ha^{-1}. The cultivar "Zhenguiai" of noodle rice was utilized as a test crop. Plastic trays were used for the nursery and uniform seedlings were transplanted as two seedlings per hill and 13 rows per plot after 25 days. The locally recommended doses of phosphorus and potassium were applied at the rate of 75 and 150 kg ha^{-1}, respectively. The biochar was introduced to the field 25 days before the transplantation of seedlings. At the panicle initiation stage, when plant growth is at its highest, soil samples were collected near the rhizosphere. The soil samples were transported to the lab in an icebox and stored at −80°C for later use.

Soil Chemical Traits and Microbial Biomass

Soil samples were taken by a core sampler at depth 0–20 cm after the late-season rice harvest in 2019–2020. Soil sampling was done at different locations within each plot and combined to make a composite sample. The composite samples were divided into two parts, with one part frozen at −80°C for later DNA extraction and microbial biomass C and N measurement, and the second half was air-dried and utilized to determine soil chemical characteristics.

SOC was measured by the $K_2Cr_2O_7$-H_2SO_4 oxidation process followed by titration (Wang et al., 2014). To determine soil TN, a subsample of 200 mg was treated using the salicylic acid–sulfuric acid–hydrogen peroxide method previously described by Ohyama et al. (1991), and TN was determined using the micro-Kjeldahl technique according to Jackson (1956). In addition, soil pH and available N, P, and K were assessed by the methods of Lu et al. (2015). The fumigation extraction method was used to measure microbial biomass carbon (MBC) as defined by Brookes et al. (1985) and microbial biomass nitrogen (MBN) according to the method of Vance et al. (1987).

DNA Extraction and Sequencing

DNA samples were extracted using the Fast DNA^{TM} spin kit for soil (MP Biomedicals, US) following the manufacturer's instructions. The DNA concentration was measured using NanoDrop 2000 (Thermo Fisher Scientific, Wilmington, DE, United States), and the quality of PCR products was detected by 2% agarose gel electrophoresis. The V3–V4 region of the 16S rRNA gene was amplified with primer pairs 515F (GTGCCAGCMGCCGCGG) and 907R (CCGTCAATTCMTTTRAGTTT). The primer pair Internal transcribed spacer (ITS) 1F (CTTGGTCA-TTTAGAGGAAGTAA) and ITS 2R (GCTGCGTTC-TTCATCGATGC) was used to amplify the ITS 1 region of fungi. The PCR and sequencing processes were performed by Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China) using the Illumina MiSeq PE300 platform. The data were analyzed on the free Majorbio Cloud Platform.[1]

Processing of Illumina Sequencing Data

The paired reads were spliced using FLASH (version 1.2.3) software to merge the sequences before assembling a gene segment (Magoc and Salzberg, 2011). Chimeric sequences were identified and removed with a *de novo* method using USEARCH (version 8.1.1861) (Edgar, 2010). After the removal of the chimera, high-quality bacterial sequences were collected for subsequent analysis.

Effective bacterial sequences were separately subsampled for each sample for the subsequent statistical analysis. After subsampling, the data were processed using a modified SOP pipeline on the basis of USEARCH and the software package QIIME (Quantitative Insights Into Microbial Ecology v1.8.0) (Tian et al., 2015). Briefly, the selected sequences were clustered to operational taxonomic units (OTU) using a two-stage clustering algorithm with USEARCH (version 8.1.1861) at 97% sequence identity (Edgar, 2010). Representative sequences in each OTU were aligned to the SILVA reference alignment (Yilmaz et al., 2014). Taxonomy was assigned to each representative sequence using RDP with a minimum confidence of 85%.

[1]www.majorbio.com

Alpha and Beta Diversity Analysis

An OTU-based analysis method was used to evaluate the bacterial diversities in each sample from each plant (alpha diversity). To estimate the diversity index and species richness (alpha diversity) among the genotypes for each sample, OTU richness and Chao1, Simpson, and Shannon indices were calculated using QIIME software (v1.8.0), concerning a sequencing depth of 3%. Statistical analysis was performed using ANOVA with *p*-values to determine the significant differences in the diversity indices or species richness among the plant rhizosphere soil samples. The rarefaction curve and rank abundance curves were calculated at a 97% level of similarity of the OTUs.

Beta diversity analysis was used among all the samples for the similarity index determination of the community structure. At the OTU level of genotypes, beta diversity was calculated using weighted UniFrac distances and was visualized through PCoA (principal coordinate analysis). The weighted UniFrac distance matrices were clustered and evaluated by QIIME software (v1.8.0) and showed phylogenetic relationships among various communities and their abundance in the respective samples.

Rice Grain Yield

At maturity, the rice plants were harvested from the whole plot and rice grain yields was weighed. The dry weight of the rice grain was determined assuming adjusted 14% moisture content in rice grains.

Statistical Analysis

Statistics 8.1 analytical software was used to determine the analysis of variance among the treatments for each variable. Alpha diversity of bacteria and fungi including Simpson, Shannon, Chao1, and ACE indices was calculated using QIIME software (v1.8.0). Rarefaction curves of the species richness were plotted against the number of sequences, and the analysis of the dominant phyla was done using the Microbiome Analyst (Dhariwal et al., 2017). Redundancy analysis (RDA) was performed using the software package CANOCO5 (Microcomputer Power, Ithaca, United States) to measure soil properties and soil microbial diversity relationship. R (3.2) software was used to conduct correlation analysis among treatments for soil microbial abundance, soil properties, and grain yield. SmartPls3 software was used to analyze consistence multi-group analysis (MGAc) among the treatments for all attributes.

RESULTS

Soil Chemical Traits and Microbial Biomass

Co-application of biochar and mineral N significantly improved the soil pH, SOC, TN, MBC, and MBN in 0–20 cm soil depth, compared with sole chemical N fertilizer application (**Table 2**). In all measured traits, the effect was highest under high biochar amendment input with no significant differences between 20 and 30 t ha^{-1} of biochar application. Across the years, the treatments exhibited the same behavior. Compared to sole N-treated plots (T1 and T2), higher biochar applied treatments (T4 and T8) improved soil pH, TN, SOC, SMBN, and SMBC by 15, 38, 26.17, 94, and 129% respectively, during 2019. Similarly, in 2020, soil pH, TN, SOC, SMBN, and SMBC were increased by 16, 44, and 32% in T4, respectively, as compared to T1. Whereas soil BD was decreased by 7.5 and 9% in non-biochar treatments (T1 and T2) as compared to higher biochar applied treatment (T4). SMBN was averagely increased in T4, T7, and T8 by 50, 80, and 95%, respectively, during both years as compared to T1. Similarly, SMBC was averagely enhanced by 134, 126, and 128% in T4, T7, and T8, respectively, as compared to T1 during both years.

Sequencing Quality Control and Summary

After screening, pre-clustering, and chimera removal, a total of 1,318,797 reads of high-quality bacterial 16S rRNA from the V3–V4 region were obtained, with an average of 54,949 reads per sample, having 32 phyla, 75 classes, 107 orders, 168 families, and 250 genera during 2019. Whereas, during 2020, a total of 763,986 reads were amplified with an average reads of 31,832 per sample, having 25 phyla, 66 classes, 100 orders, 170 families, and 253 genera during 2020. The unique numbers of OTUs were 76, 66, 64, 65, 76, 99, 175, and 58 in T1, T2, T3, T4, T5, T6, T7, and T8, respectively (**Figure 1A**). In 2020, the unique numbers of OTUs were 1, 1, 1, 1 and 10 in T1, T5, T6, T7, and T8, respective (**Figure 1B**).

A total of 756,790 reads were amplified for the fungal population, with an average reads per sample 31,532, having 10 phyla, 22 classes, 50 orders, 74 families, 94 genera, and 102 species, during 2019. The unique numbers of OTUs were 1, 1, 2, 2, 1, 1, 0, and 3 in T1, T2, T3, T4, T5, T6, T7, and T8, respectively (**Figure 1C**). Similarly, during 2020, a total of 404,210 reads were amplified with an average reads per sample of 16,842, having 10 phyla, 20 classes, 47 orders, 69 families, 84 genera, and 94 species. The unique numbers of OTUs were 44, 24, 10, 19, 23, 23, 32, and 10 in T1, T2, T3, T4, T5, T6, T7, and T8, respectively (**Figure 1D**). The results showed that the fungal OTU numbers were significantly decreased in biochar applied treatments as compared to other treatments (**Supplementary Files-Presentation 1**- and **Table 1**).

Composition and Community Structure of the Rice Rhizosphere Microbiomes Under Different Biochar and Nitrogen Application

The bacterial relative abundance of major bacterial phylum in each treatment is shown in **Figure 2**. The dominant bacterial phylum across all the treatments was Proteobacteria, Acidobacteria, Actinobacteria, Bacteroidetes, Gemmatimonadetes, Planctomycetes, Verrucomicrobia, and Chloroflexi among the others. The results showed that Proteobacteria were more (> 5%) abundant in all the treatments. Higher relative abundance of Proteobacteria was found in T7 and T3 during 2019 and 2020, respectively, compared to other treatments. The second most abundant bacteria

TABLE 2 | Response of soil properties to different biochar and nitrogen fertilizer treatments.

Years	Treatments	pH (water)	TN (g kg^{-1})	SOC (g kg^{-1})	BD (g kg^{-1})	SMBN (mg kg^{-1})	SMBC (mg kg^{-1})
2019	T1	6.12c	1.40c	13.23c	1.35a	22.33d	174.9c
	T2	6.41b	1.50bc	12.95c	1.35a	26.66c	294.1b
	T3	6.49b	1.57b	15.14b	1.30b	30.66c	300.7b
	T4	7.08a	1.60b	16.06ab	1.27bc	33.66c	384a
	T5	6.23c	1.61b	16.55ab	1.26c	41b	410a
	T6	6.46b	1.87a	16.46ab	1.28bc	29.33c	314.0b
	T7	7.09a	1.95a	16.77ab	1.26c	40.33a	396.6a
	T8	7.12a	1.96a	17.11a	1.24d	43.66a	400.3a
	Lsd	0.32	0.11	1.64	0.05	8.11	42.4
2020	T1	6.00c	1.37c	12.54c	1.33a	24.66d	167c
	T2	6.37bc	1.47c	11.73c	1.30b	29c	187.6c
	T3	6.44ab	1.60b	14.99b	1.28bc	33.66b	281.6b
	T4	7.09a	1.63b	16.25ab	1.25bc	36.66b	357a
	T5	6.08b	1.64b	16.36ab	1.23bc	44.33a	367a
	T6	6.36a	1.89a	16.33ab	1.24bc	32.33b	259b
	T7	6.99ab	1.98a	16.64a	1.22c	43a	340.6a
	T8	7.02a	1.99a	16.65a	1.22c	47.33a	341.6a
	Lsd	0.26	0.12	1.52	0.07	7.49	38.59

TN, total nitrogen; SOC, soil organic carbon; BD, bulk density; MBC, microbial biomass carbon; and MBN, microbial biomass nitrogen. Within a column, values preceded by the same letters are not significantly different at $p \leq 0.05$. Note: T1 = 0 t B + N135 kg ha^{1}, T2 = 0 t B + N180 kg ha^{1}, T3 = 10 t B + N135 kg ha^{-1}, T4 = 20 t B + N135 kg ha^{-1}, T5 = 30 t B + N135 kg ha^{1}, T6 = 10 t B + N180 kg ha^{1}, T7 = 20 t B + N180 kg ha^{-1}, and T8 = 30 t B + N180 kg ha^{-1}.

were Chloroflexi and were not significantly influenced by biochar and N fertilizer. Among the treatments, Actinobacteria, Proteobacteria, and Verrucomicrobia were higher in relative abundance ranging from 4.3, 47.98, and 1.3% in T7 as compared to control treatment (T1) in 2019. Similarly, compared to control (T1), Acidobacteria and unclassified bacteria were observed higher in relative abundance ranging from 17.56 and 10.23% in T6 and T4, respectively, during 2019 as compared to the control treatments. Furthermore, the relative abundance of Bacteroidetes, Gemmatimonadetes, and Planctomycetes were higher ranging from 20.64, 7.85, and 6.96%, respectively, in T5 over control treatment (T1) in 2019. Chloroflexi and Firmicutes resulted in higher relative abundance by 9.85 and 9.81%, respectively, in T8 and then control treatment (T1) during 2019. Whereas, during 2020, Acidobacteria, Actinobacteria, Bacteroidetes, Gemmatimonadetes, Planctomycetes, and Verrucomicrobia resulted higher in biochar plots by 12.4, 7.15, 99.41, 62.7, 74.92, and 20.6%, respectively, as compared to control (T1) (**Figure 2**). Chloroflexi, Firmicutes, Proteobacteria, and unclassified bacteria were not significantly influenced by biochar and N application during 2020, where between the years, Chloroflexi, Firmicutes, and Proteobacteria were decreased in 2020 as compared to 2019.

The relative abundance of fungal species at phylum level among all the treatments during 2019 and 2020 is shown in **Figure 3**. The results showed that the relative abundance of major fungal phylum was decreased with the increase in biochar application in the second year. During both years, the Ascomycota fungi were the most abundant fungi followed by Rozellomycota, Basidiomycota, Mortierellomycota, Chytridiomycota, Zoopagomycota, and Glomeromycota across the treatments. The overall results showed that the relative abundance of phylum Ascomycota, Rozellomycota, and Basidiomycota fungi were significantly decreased in 2020 as compared to 2019. Among the treatments, the results showed that the lowest 5 and 17% relative abundance of Ascomycota was recorded in T3 (20 t B ha^{-1} under + 135 kg N ha^{-1}) and T4 (30 t B ha^{-1} under + 135 kg N ha^{-1}), respectively, as compared to control and other treatments during 2019. Similarly, a Chytridiomycota fungus was recorded less of relative abundance ranging from 0.34 and 1.91% in T3 and T4, respectively, as compared the rest treatments during 2019. Whereas during 2020, unclassified fungi were higher in T3, T4, T5, and T8 with the abundance of 50.08, 50.46, 50.44, and 51.46%, respectively. The lowest values of 48, 49.92, and 48.56% were recorded in T1, T2, and T6, respectively, for unclassified fungal abundance. Furthermore, the higher fungal species at phylum level followed the order Ascomycota > unclassified fungi > Rozellomycota > Basidiomycota > Mortierellomycota > Chytridiomycot Zoopagomycot > Glomeromycota > Aphelidiomycota across the samples.

The bacterial diversity at genus level influenced by different biochar and N fertilizer during 2019 is represented in **Supplementary File 1. Presentation 1** and **Figure 1**. A total of 250 classified species were found among all soil samples. *Thiobacter* was the most abundant bacteria at genus level after unclassified bacteria, followed by *Sphingopyxis*, *GP6*, *Geobacter Povalibacter*, *Sphingosinicella*, *Gemmatimonas*, *Aminicenantes genera incertae sedis*, *Gp18*, *Bellilinea*, and *Kofleria*. Among the treatments, T7 resulted in higher percent of *Sphingopyxis*, whereas T3 resulted in maximum percent of *Thiobacter* bacteria. Whereas, during 2020, a total number of classified

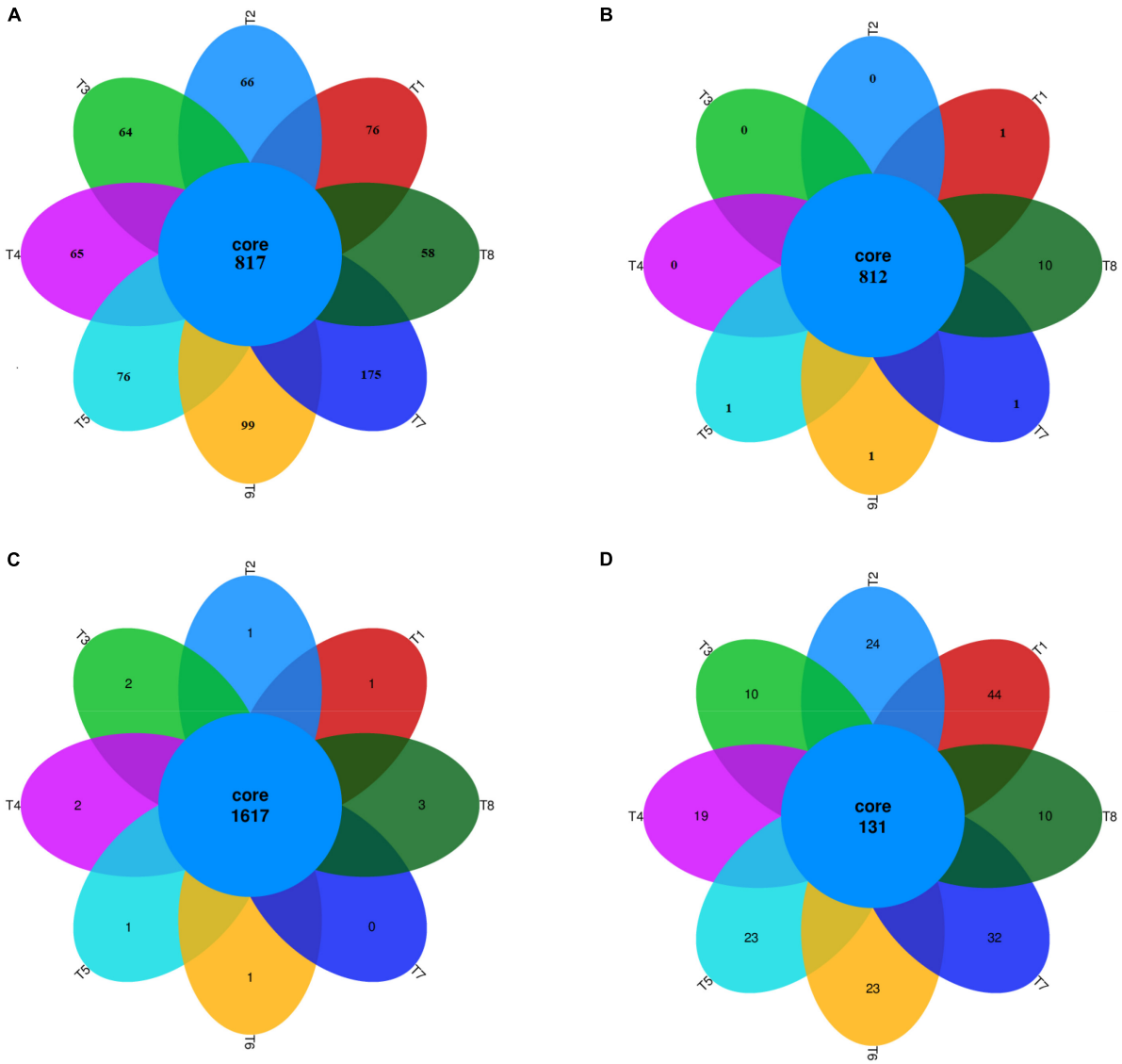

FIGURE 1 | Venn diagram. A Venn diagram representing unique and shred OTU's of bacteria and fungi during 2019 **(A,C)** and 2020 **(B,D)**, respectively, among the different treatments. T1 = 0 t B + N135 kg ha^{1}, T2 = 0 t B + N180 kg ha^{1}, T3 = 10 t B + N135 kg ha^{-1}, T4 = 20 t B + N135 kg ha^{-1}, T5 = 30 t B + N135 kg ha^{1}, T6 = 10 t B + N180 kg ha^{1}, T7 = 20 t B + N180 kg ha^{-1}, and T8 = 30 t B + N180 kg ha^{-1}.

bacteria species at genus level were 179, and the most abundant bacteria after unclassified were *Novosphingobium* followed by *Fictibacillus*, *Bacillus*, *Sphingomonas*, *Gp6*, *Clostridiumsensu stricto*, *Gemmatimonas*, *Aminicenantes genera incertae sedis*, *Thiobacillus*, *Spartobacteria genera incertae sedis*, *Gp16*, *Reyranella*, *Geobacter*, *Gp17*, *Streptomyces*, *Novosphingobium*, *Fictibacillus*, *Methanothrix*, and *Lysinibacillus* (**Supplementary File 1. Presentation 1** and **Figure 2**). Among the treatments, T7 and T5 resulted in higher percent of *Bacillus* bacteria. Furthermore, *Sphingomonas* was higher in T3 as compared to other treatments. The overall results showed that the classified fungi were decreased in 2020 as compared to 2019. Similarly, for fungal genus abundance, the top 10 fungi at genus level were *Gaeumannomyces*, *Myrothecium*, *Zopfiella*, unclassified *Pleosporales*, *Mortierella*, *Pyrenochaetopsis*, *Aspergillus*, unclassified *Mortierellales*, *Fusarium*, and *Cladosporium* among 49 species during 2019 (**Supplementary File 1. Presentation 1** and **Figure 3**). Whereas in 2020, the top 10 most abundant fungal species at genus level were *Echria*, *Panaeolus*, *Westerdykella*, *Tomentellopsis*, *Calocybella*, *Kohlmeyeriopsis*, *Mortierella*, *Zopfiella*, *Gaeumannomyces*, and *Schizothecium* among 37 species (**Supplementary File 1. Presentation 1** and **Figure 4**).

Alpha Diversity

To assess the diversity among the treatments, alpha diversity indices were calculated for each samples (**Supplementary File 1. Presentation 1**, **Table 2** and **Figures 5–6**). The rarefaction curve illustrated enough richness of observed OUTs

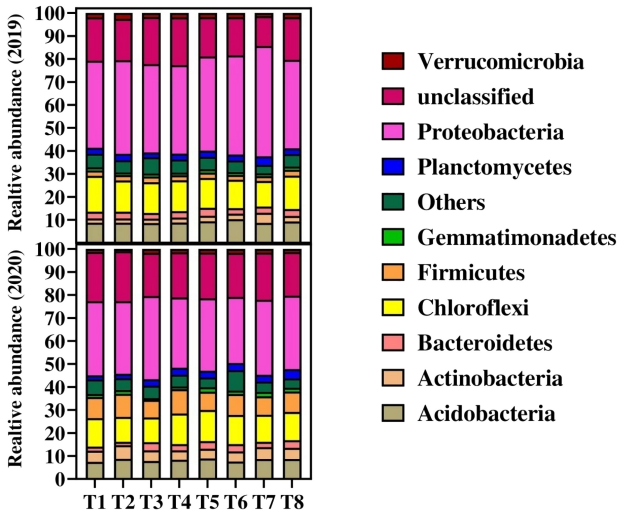

FIGURE 2 | Changes in relative abundance on bacterial species on phylum level in response to different treatments. Distinct colored columns represent different species, and the length of the columns represents the species' proportion. For treatment combination details (see **Table 1**).

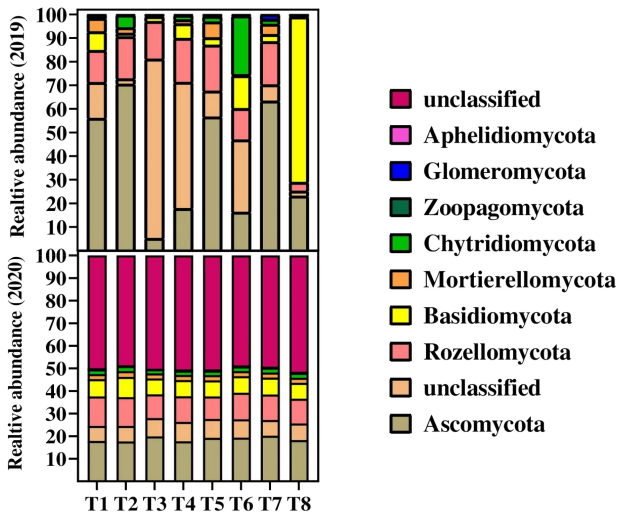

FIGURE 3 | Changes in relative abundance on fungal species on phylum level in to different treatments. Distinct colored columns represent different species, and the length of the columns represents the species' proportion. For treatment combination details (see **Table 1**).

and sequencing depth to examine microbial alpha diversity (**Supplementary File 1. Presentation 1** and **Figures 7–10**). According to the results Chao1, ACE, Shannon, and Simpson indices were decreased in 2020 as compared to 2019. In 2019, the control treatment showed slightly higher OTU richness (Chao1 = 15,459.03) as compared other treatments. The lowest OTU richness (Chao1 = 13,678.0) observed in T7 as compared to the rest of treatments (**Supplementary File 1. Presentation 1-Table 2** and **Figures 6A–D**). Whereas during 2020, the OTU richness was higher in T7 (Chao1 = 1,467.1), and the lowest OTU richness (Chao1 = 1,425.67) in T2. The higher ACE index of 2,299.07 and 1,459.30 was recorded in T2 in 2019 and 2020, respectively. The lowest ACE index of 2,205.90 and 1,417.06 was recorded in T6 and T7 during 2019 and 2020, respectively. Shannon and Simpson indices were recorded lowest in T6 (6.88) and T7 (0.995) as compared to the rest of treatments in 2019, whereas, in 2020, the Shannon and Simpson indices increased with biochar application as compared to control treatments.

In addition, the results of alpha diversity for fungi showed the opposite trend as compared to bacterial. The diversity indices among fungal samples were higher in 2019 and lower in 2020. Among the treatments, T4 resulted in a higher OTU richness (Choa1 = 190.36) in 2019, whereas T5 resulted in higher OTU richness (Chao1 = 956.51) in 2020. The lowest OTU richness for fungal taxonomic feature level was recorded in T6 (Chao1 = 151.05) and T2 (Chao1 = 920.3) during 2019 and 2020, respectively. Shannon and Simpson indices were not significantly affected by biochar and N treatments as compared to control treatments. However, variation do exist among the samples, for example, the higher Shannon index was recorded in T5 during 2019 and T4 during 2020.

Beta Diversity

PCoA was used to assess the similarity and dissimilarity for bacterial beta diversity among the treatments. According to the results, most of the samples of corresponding treatments tend to group, indicating that there is a similarity between the treatments as they clustered near to each other except T7 that tends to differ from other treatments in terms of rhizosphere microbial community. Moreover, 51% variation among the treatments was explained by PCoA1, whereas PCoA2 explains 13% of the total variation among the treatments during both years (**Figure 6**).

Beta diversity analysis (PCoA) of fungal community showed that the treatments tend to the group, presenting that there is a connection among the treatments as they clustered near to each other except T8 that tends to differ from other treatments in terms of rhizosphere during 2019. The results showed that treatments in 2019 and 2020 clustered in different quadrate that indicates that the difference among the treatments of in both years. T3, T6, and T2 were observed together in two different groups, which represents that these treatments were dissimilar for the fungal community in 2019. The variation among the treatments by PCoA1 and PCoA2 was explained by 35 and 18%, respectively, for the fungal community in both years.

Grain Yield

The biochar and N fertilizer combined application considerably affect the rice yield in both years (**Figure 5**). Biochar applied treatment including T8, T7, and T5 improved rice yield by 32.4, 31.8, and 31.7% as compared to non-biochar applied treatments (T1) in 2019. Similarly, in 2020, the grain yield of rice was higher in T8, T7, and T5 by 27.2, 23.5, and 27.6%, respectively, as compared to T1. Compared to T2 (no biochar + 180 kg ha^{-1}), the treatments T8, T7, and T5 significantly increased grain yield by 10.46, 10.56, and 10.87%, respectively, during both years. The lowest grain yield of rice during both years was recorded in non-biochar–treated plots (T1 and T2).

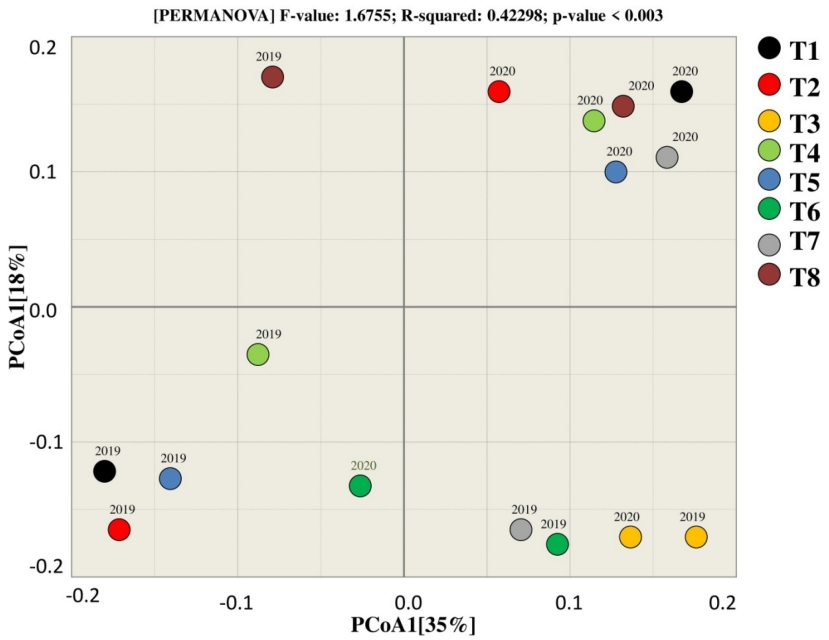

FIGURE 4 | Beta diversity analysis for estimating similarity and dissimilarity among the treatments for the fungal community.

Relationship Between Bacterial Community Composition and Soil Properties

RDA was performed to determine the strength of the association between the soil pH, SOC, TN, BD, SMBN, and SMBC contents and the diversity of the soil bacterial composition. **Figure 7** shows the relationship between bacterial communities (at phylum level) and the soil properties for the different treatments. Soil pH, TN, SOC, SMBN, SMBC, and grain yield occurred in same quadrant, which indicates that biochar has significant effect soil physiochemical properties and grain yield. The eight treatments took place in four different quadrants, showing that the fertilization treatments had a substantial effect on the composition of soil bacterial composition. Biochar applied at higher rate (T8) showed significant correlation with soil properties.

Figure 8 shows the Pearson correlation heatmap among the most abundant bacteria's, soil properties, and grain yield. Soil properties including soil pH (R = 0.82), TN (0.72), SOC (0.71), SMBC (0.64), and SMBN (0.76) were strongly positively correlated with grain yield. However, the relationship between soil BD (−0.75) and grain yield was strongly negatively correlated. Furthermore, the abundance of soil bacteria including *Acidobacteria* (0.37), Actinobacteria (0.24), Bacteroidetes (0.39), Planctomycetes (0.71), and Proteobacteria (0.32) strongly positively correlated with the grain yield of paddy rice, whereas, the abundance of Chloroflexi, Firmicutes, Gemmatimonadetes, and Verrucomicrobia showed no significant relationship with grain yield of rice. The relationship between the soil biochemical properties and soil bacteria abundance were also positively correlated, except soil BD.

For the measured indicators of all soil bacterial abundance, soil properties, and grain yield, a network plot (MGAc) among the treatments was created to understand the relationship among the treatments using SmartPls3 software (**Figure 9**). The results showed that, for all measured traits, the treatments without biochar applications (T1 and T2) were significantly dissimilar from the treatments with biochar application, whereas the treatments had biochar rate from 20 to 30 ton ha^{-1} under both N fertilizers (T4, T5, T6, and T8) were resulted in the same outputs for all traits across the years.

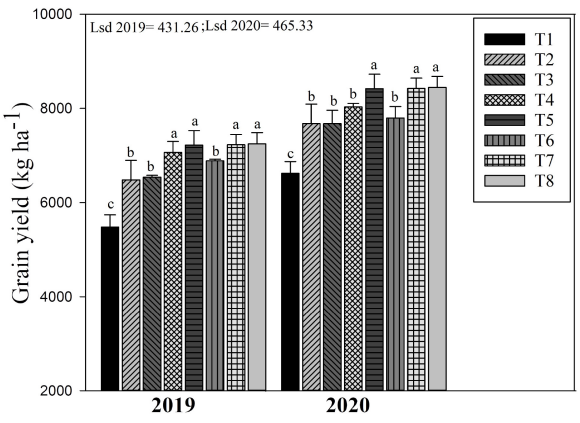

FIGURE 5 | Changes in grain yield of rice as influenced by different biochar rates in combination with different N fertilizers. The mean comparison was made using the least significant difference (LSD) test for treatments means based on the LSD test at 5%. Different letters on bars are not significantly different at $p < 0.05$. For treatment combination details (see **Table 1**).

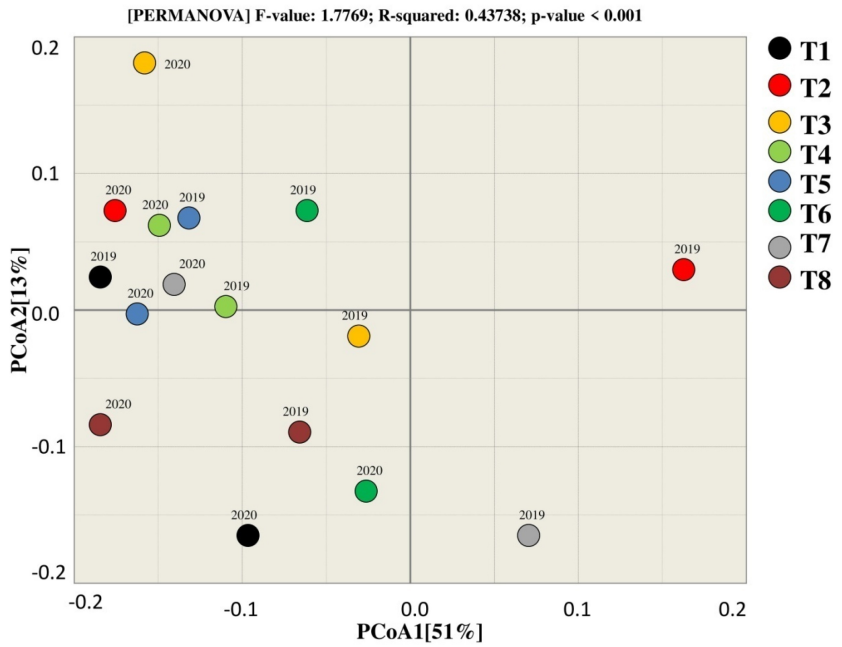

FIGURE 6 | Beta diversity analysis for estimating similarity and dissimilarity among the treatments for bacterial.

DISCUSSION

Biochar application and the use of N fertilizer are important agricultural management practices in sustainable development. Several reports have concluded that the use of biochar considerably improves soil health and crops yield. However, the effect of long-term biochar application in combination with chemical N fertilizer on paddy field soil properties and microbial community composition during 2 years is still unclear. This research explored the effect of biochar in combination with N fertilizer on paddy field soil properties, microbial functions, and bacterial community composition. The soil bacterial community is the essential component of soil ecology, responsible for enhancing soil health, and plants production (Luo et al., 2020).

Soil Properties

In the present study, biochar amendment in combination with N fertilizer significantly improved soil biochemical indicators (i.e., MBC, MBN, pH, SOC, and TN) compared with sole N fertilization (**Table 2**), whereas soil DB was decreased in biochar-treated soil as compared to sole N-applied treatments. The possible explanation for this increment might due to the biochar's higher porosity, higher surface area, and its large number of microspores (Ali et al., 2020a,b, 2021). Our results were in line with our previous study (Ali et al., 2021), showing that the biochar addition in combination with urea considerably improved the pH by 14%. Numerous studies have shown that soil pH can increase when biochar is applied, particularly in acidic soils, which can ameliorate the nutrient supply to plants (Ali et al., 2020a,b; Ullah et al., 2021a,b). Similarly, SMBN and SMBC were increased might due to the alkaline nature of biochar. Biochar amendment addition to acidic soils can improved the microbial activities and increases the microbe populations as documented in our previous study (Ali et al., 2021). The other possible explanation might be due to the inhibition of denitrification inhibitors, which are the major regulators of nitrification. Our findings are supported by the previous results of Zhou et al. (2017), who documented that SMBC and SMBN can be increased by 26 and 21% in biochar-treated soil as compared to control. Liu et al. (2016) also reported that biochar application improved soil MBN and MBC as compared to non-biochar applied treatments. However, in contrast to our results, Castaldi et al. (2011), Zavalloni et al. (2011), and Dempster et al. (2012) have documented that biochar has no significant effects on soil MBC.

Impact of Biochar Amendment in Combination With Nitrogen Fertilizer on the Abundances of Soil Bacteria and Fungi

The microbial population's diversity and richness are regarded critical for soil integrity, functionality, and sustainability, yet they are commonly diminished by current farming practices (Zhao et al., 2014; Khan et al., 2021). In the present study, the different biochar rates in combination with N fertilizer significantly affected the soil bacterial and fungal abundance (**Table 2**). Compared to control (T1), biochar application at the rate of 20–30 t ha^{-1} significantly increased soil bacterial abundance. The possible reason for these increments due to the increase in soil pH in biochar applied treatments. Several studies reported that soil physical and chemical properties indirectly affect soil microbial abundance (Lehmann et al., 2011; Cole et al., 2019).

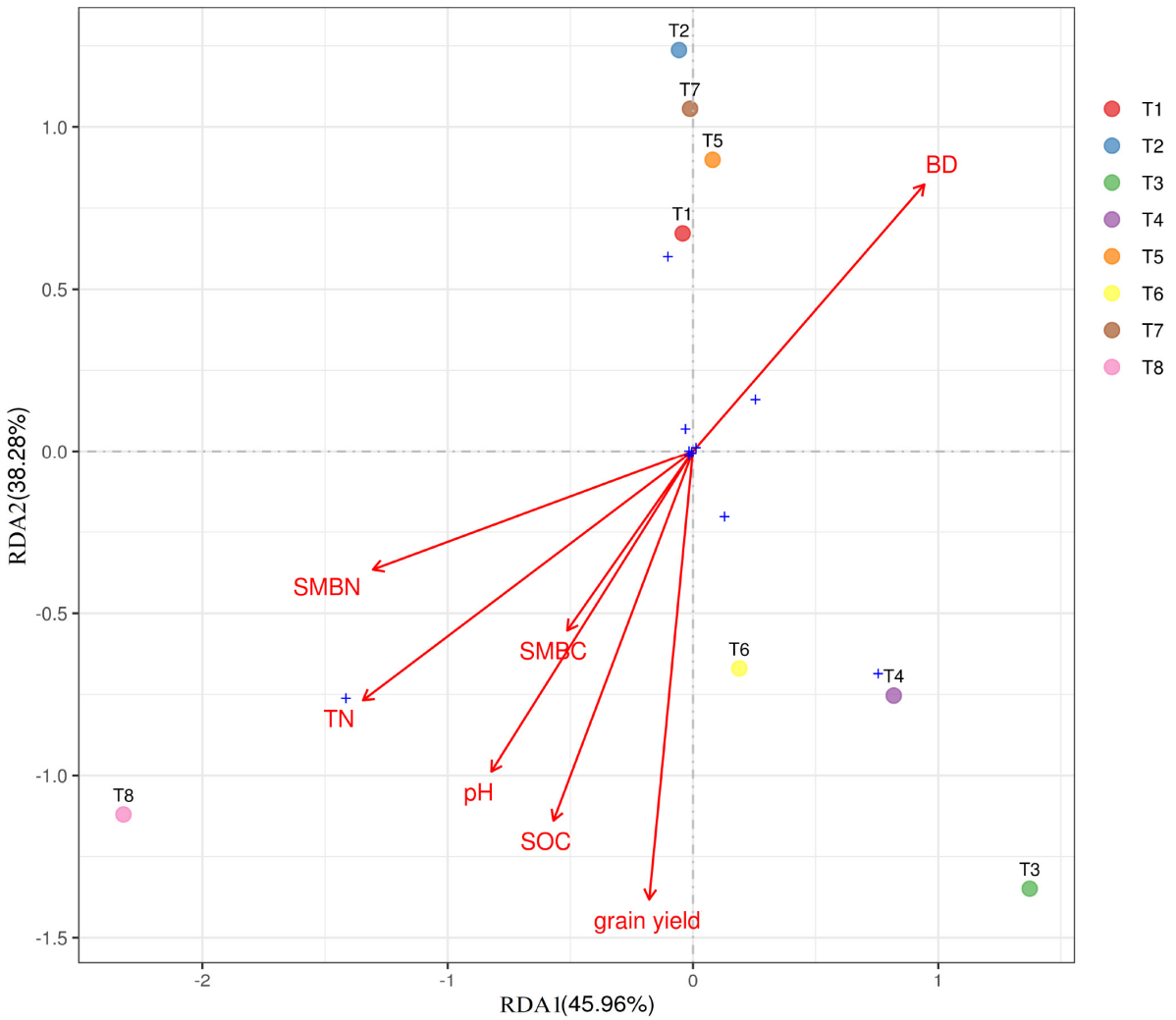

FIGURE 7 | Ordination plot of results from redundancy analysis to identify relationship between soil properties, grain yield (GY), and dominant bacterial phylum among the treatments. SMBN, soil microbial nitrogen; SMC, soil microbial carbon; TN, total nitrogen; SOC, soil organic carbon. For treatment combination details (see **Table 1**).

For example, soil pH is the most important factor for the change in bacterial abundance of soil (Yao et al., 2017; Liu et al., 2019; Zheng et al., 2019; Sun et al., 2020). Thus, biochar in combination with N fertilizer improved soil pH in our experiment, which consequently improved soil bacterial abundance. A similar study was reported by Yao et al. (2017) in that the addition of a higher rate of biochar increases bacterial abundance. Likewise, Chen et al. (2015) also reported that 40 tons of biochar ha^{-1} significantly increased bacterial 16S rRNA gene copy numbers by 35–62%. However, Luo et al. (2017) and Gao et al. (2021) reported that that biochar fertilizer in alkaline soil did not affect soil pH or bacterial abundance. Thus, our results suggested that an appropriate rate of biochar application in combination with N fertilizer improved soil bacterial abundance in the paddy field.

In this study, fungal abundance was decreased in the biochar-treated soil as compared to control. This increase might be due to an increase in soil pH as compared to control in biochar-treated soil. Yao et al. (2017) reported a similar finding that changes in soil fungus might be caused by changes in soil pH and nutrient content due to biochar addition. Because biochar has a higher pH, its effect would be exacerbated in acidic soil, where the changes in soil pH after applying biochar amendment would be more significant (Paz-Ferreiro et al., 2015; Xu et al., 2018). In contrast, Steinbeiss et al. (2009), Jones et al. (2012), Luo et al. (2017), and Gao et al. (2021), reported that biochar amendment could promote fungal growth as compared with the control soil.

Community Compositions of Soil Bacteria and Fungi Influences by Biochar and Nitrogen Application

Many studies have shown that biochar has a short- or long-term impact on bacterial and fungal community compositions (Yao et al., 2017; Li et al., 2020). However, the effects

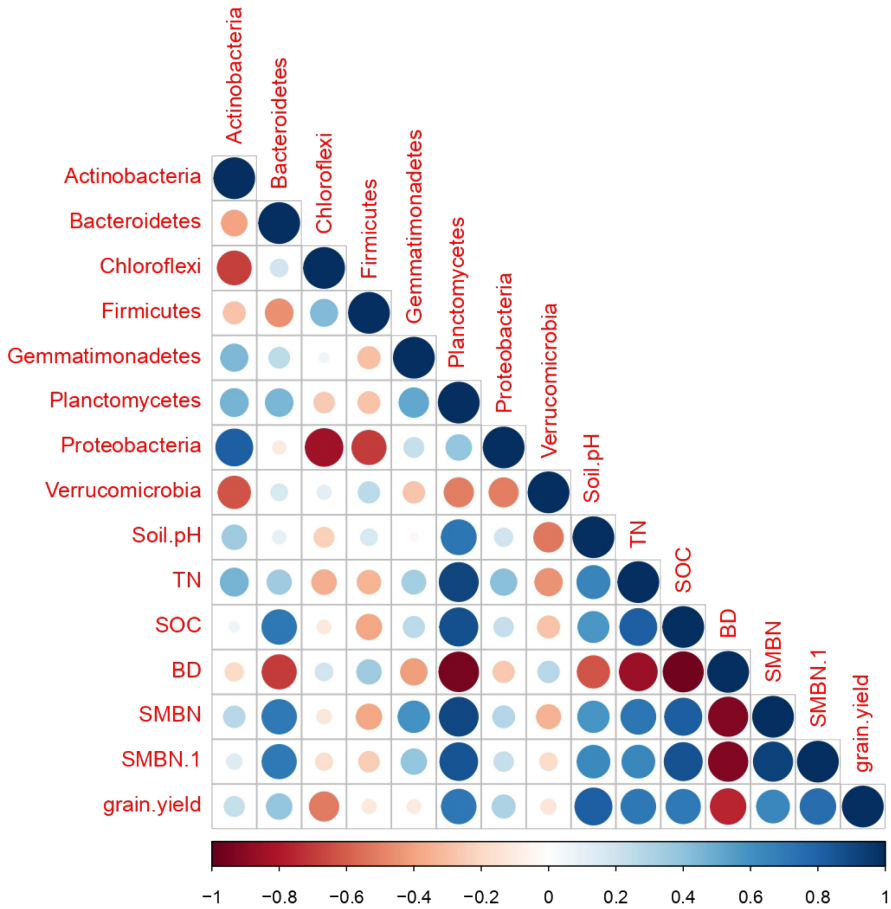

FIGURE 8 | Correlation analysis among treatments for soil microbial abundance, soil properties, and grain yield.

of biochar on community composition remain unclear. In the present study, the abundance of Proteobacteria, Actinobacteria, and Verrucomicrobia were observed higher in biochar-treated soil as compared to control. In terms of community composition and relative abundance, Proteobacteria accounted for the highest fraction in the soil, which is consistent with the findings (Ji et al., 2016; Yin et al., 2021). For a possible explanation, Proteobacteria are a eutrophic bacterium (Fierer et al., 2007), and, previously, it is well documented that biochar amendment improves soil properties (Ali et al., 2020a,b, 2021), leading to an increase in the Proteobacteria population.

The relative abundance of Acidobacteria, Chloroflexi, Actinobacteria, Bacteroidetes, Gemmatimonadetes, Planctomycetes, Verrucomicrobia, and Chloroflexi was higher in the first year of the experiment. However, in 2020, the frequency of these microbes has reduced. Most Acidobacteria are acidophilic bacteria, and their relative abundance is negatively correlated with soil pH. In our study, biochar increased soil pH as compared to control, which consequently decreased Acidobacteria. Similar reducing results of Acidobacteria abundance in higher soil pH (8.50 and 7.87) compared to low soil pH (5.35) were reported by Yin et al. (2021).

The relative abundance of Actinobacteria was increased in biochar-treated soil as compared to control. As gram-positive bacteria, Actinobacteria play a significant role in organic matter turnover, including cellulose and chitin decomposition (Ali et al., 2019). Bacteroidetes abundance was improved in the second year as compared to first year and was higher in biochar applied treatments as compared to control. The application of biochar to the soil as a carbon source promotes an increase in the relative abundance of Bacteroidetes in the soil, because Bacteroidetes bacteria are strongly correlated with the conversion of organic materials such as DNA, proteins, and lipids (Cottrell and Kirchman, 2000).

Biochar in combination with N fertilizer significantly affected the abundance of fungal community structure (**Figure 3**). In the present study, the dominant fungal phyla across the treatments were Ascomycota, Rozellomycota, Basidiomycota, Mortierellomycota, Chytridiomycota, Zoopagomycota, and Glomeromycota during both years. Compared to control, biochar application in combination with N fertilizer significantly decreases the relative abundance of Ascomycota, Rozellomycota, and Basidiomycota phyla, whereas, between the years, these phyla were more decreased in 2020 over 2019. The possible explanation for these condense in fungal phyla might due

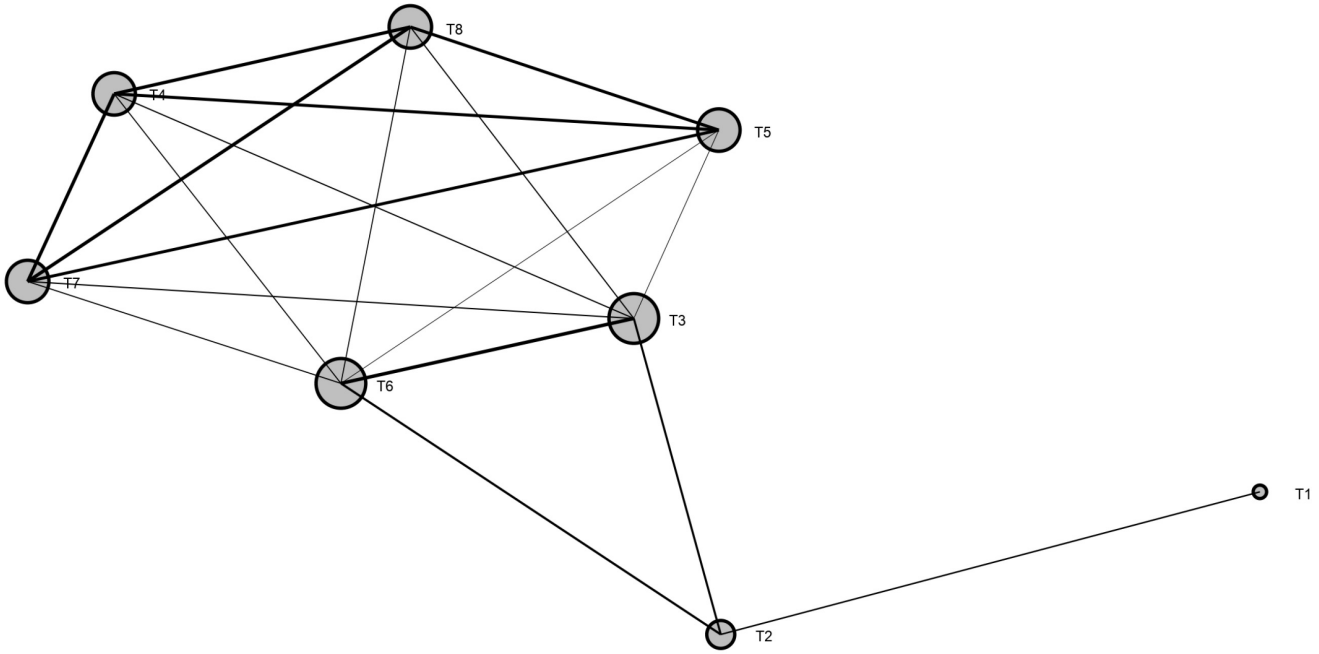

FIGURE 9 | Network plot (MGAc analysis) among the treatments for soil microbial abundance, soil properties, and grain yield. Size of the ball represents the variation among the treatments, and lines represent the interaction among the treatments. Note: T1 = 0 t B + N135 kg ha^{1}, T2 = 0 t B + N180 kg ha^{1}, T3 = 10 t B + N135 kg ha^{-1}, T4 = 20 t B + N135 kg ha^{-1}, T5 = 30 t B + N135 kg ha^{1}, T6 = 10 t B + N180 kg ha^{1}, T7 = 20 t B + N180 kg ha^{-1}, and T8 = 30 t B + N180 kg ha^{-1}.

to changes in soil chemical and physicochemical properties, especially soil pH. Furthermore, Heitkötter and Marschner (2015), Ullah et al. (2020) and Ali et al. (2020a,b), reported that biochar changes in soil physicochemical properties alter soil enzyme activities, which consequently resulted in the abundance and composition of soil fungi abundance and composition. Compared to 2019, reduction in Ascomycota during 2020 might be attributed to that biochar as a slow-release fertilizer can frequently take up to a year to see results. Further explanation for these changes might be that biochar as a microbial C source; the Dissolved organic carbon (DOC) probably promotes saprotroph growth and enhances their competitive capacity, leading to an overall decrease in diversity and a decline in fungal pathogens (Dai et al., 2018). Similar results to our finding were reported by Yin et al. (2021) that biochar decreased the relative abundance of Ascomycota, Basidiomycota, and Mortierellomycota. Another study reported that these fungi decompose organic matter, symbiotic fungi, parasitic or pathogenic fungi, and even other fungi (Hang et al., 2020; Liu et al., 2020). Furthermore, Chen et al. (2013) and Hale et al. (2014) documented that because of biochar porous nature protects soil from a variety of biological rivals. In addition, our study also reported that Mortierellomycota phyla were decreased in biochar-treated soil during both years. The possible reason for this change due to biochar provides carbon supply to the soil, and it is hypothesized that dominant fungal genera in soil are competing for carbon source, leading to a decrease in Mortierellomycota. Same results to our findings were observed by Yin et al. (2021) that Mortierella abundance was observed lesser in biochar-treated soil as compared to control.

Grain Yield

Co-incorporation of biochar and synthetic N fertilizer significantly increased rice grain yield compared to non-biochar applied plots (**Figure 5**). The enhancements in rice yield could be attributed to enhancement in soil pH, TN, SOC, MBC, and MBN (**Table 2**), which ultimately enhanced rice growth and biomass accretion by providing enough nutrients during the growing period. The results of the Pearson correlation heatmap confirmed that that the variations in soil nutrients were the factors that elucidated the greatest proportion of the difference in rice grain yield (**Figure 8**). Moreover, Akhtar et al. (2018), Iqbal et al. (2019) and Iqbal et al. (2020) reported that changes in crop yields are strongly allied with soil biogeochemical properties and microbial biomass production.

Relationships Between Bacterial Communities and Soil Biochemical Traits

Biochar application in combination with N fertilizer can cause physiochemical changes in soil, which can lead to changes in the composition of the bacterial community (Li et al., 2019). In the present study, we observed that biochar amendment considerably influenced soil quality traits as shown in **Table 2**. Furthermore, it is also reported that soil quality traits were positively correlated with the structure and composition of the bacterial community (Wu et al., 2020). **Figure 8** showed the relationship of bacterial community at phylum level and soil traits including soil pH, TN, SOC, MBN, and MBC for different biochar and N rates. In our findings, RDA showed that biochar

amendments in combination with N fertilizer had substantial effects on soil bacterial community and soil quality indicators compared with control (**Figure 7**). The dominant bacteria at the phylum level, i.e., Proteobacteria, Chloroflexi, and Acidobacteria, were positively correlated with soil properties, but the Proteobacteria were strongly associated with pH, SOC, and TN. It can be said that bacterial growth is strongly associated to the kind of fertilizer, and regulating the type and proportion of biochar amendment is an operative strategy for increasing bacterial growth. From what has been debated above, the application of biochar amendment in conjunction with reduced synthetic fertilizer may provide a faster growth environment for bacteria, thereby improving the bacterial community structure and soil fertility.

CONCLUSION

The results showed that biochar amendment in combination with N fertilizer increased soil physio-biochemical properties, improved rice grain yield, increased soil bacteria, and altered fungi abundance and community structure. The bacterial Chao1, ACE, and Shannon indices were increased and fungal Chao1, ACE, and Shannon indices were decreased in biochar applied treatments as compared to sole N-applied treatments. Biochar along with N fertilizer increased number of unique OTUs of bacteria and decreased number of unique OTUs of fungi in 2020. The relationship among soil properties and soil bacteria in the combined application of biochar and N were stronger than sole N-treated soil. Furthermore, the variation in soil bacteria and fungi was closely associated with soil properties (pH, SOC, BD, TN, MBC, and MBN) and rice grain yield, which suggested that the effects of biochar in combination with N on soil bacteria and fungi community were ultimately driven by the changes in soil chemical and physical properties. These results aimed to provide a reference and basic understanding for paddy soil improvement by combined application of biochar and N with good application prospects.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in the NCBI under accession number PRJNA797522.

AUTHOR CONTRIBUTIONS

IA and LJ designed the study and wrote the manuscript. PY, IA, AI, Imran, HL, AK, SU, QZ, and MG performed the data analysis and revised the manuscript. HZ, SW, XW, AK, and QZ performed the data curation. All authors approved the submission.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2022.834751/full#supplementary-material

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Iron-Modified Biochar Strengthens Simazine Adsorption and Decreases Simazine Decomposition in the Soil

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Currently, modified biochar has been successfully used in the remediation of soil polluted with heavy metals. However, the effects of the modified biochar on pesticides (such as simazine) are still unclear. Herein, the environmental fate of simazine, such as decomposition, leaching, and adsorption in unamended soil, in the soil amended with unmodified and modified biochar (biochar + $FeCl_3$, biochar + FeOS, biochar + Fe) were evaluated. In addition, an incubation experiment was also performed to observe the influence of modified biochar on the microbial community and diversity in the soil. The results showed that modified biochar significantly decreased the decomposition of simazine in the soil compared to its counterpart. Modified biochar also reduced the concentration of simazine in the leachate. Compared with the control, soil microbial biomass in the soil amended with unmodified biochar, biochar + $FeCl_3$, biochar + Fe, and biochar + FeOS was decreased by 5.3%, 18.8%, 8.7%, and 18.1%, respectively. Furthermore, modified biochar changed the structure of the microbial community. This shows that modified biochar could increase the soil adsorption capacity for simazine and change the amount and microbial community that regulates the fate of simazine in the soil. This study concludes that iron-modified biochar has positive and negative effects on the soil. Therefore, its advantages and side effects should be considered before applying it to the soil.

Keywords: iron-modified biochar, simazine, decomposition, adsorption, microbial community

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INTRODUCTION

As the second most detectable pesticide in surface and groundwater in the United States, Europe, and Australia, simazine can be found in water at hundreds of micrograms per liter (Cox et al., 2000; Troiano et al., 2001). This has raised many environmental issues, including human health, aquatic, and terrestrial ecosystems (Regitano et al., 2006; Rico et al., 2012). In order to prevent ground and surface waters from being contaminated with simazine, strategies were implemented to cut

down on the persistence of simazine in the environment. For example, organic matter content as a modifier was applied to the soil to regulate herbicide behavior (Cox et al., 2001; Odukkathil and Vasudevan, 2013). Multiple technologies that can be used to control pesticides in the soil, including physiochemical technologies and biological methods, have shortcomings such as low efficiency, long time, high cost, and even the introduction of new pollution, which result in several problems in the remediation of pesticide pollution (Fan and Song, 2014; Meriam Suhaimy et al., 2020) that have not been solved. In recent years, biochar application as an amendment for remediation of soil and water pesticide pollution has attracted increasing attention worldwide. Several studies indicated that biochar could effectively increase the adsorption of simazine in the soil, thus decreasing its risk of leaching into the environment and also reducing its risk of being directly being up-taken by plants (Jones et al., 2011a; Eibisch et al., 2015; Cheng et al., 2021). Simultaneously, some studies have reported that biochar inhibits simazine biodegradation (Cheng et al., 2017a) and reduces simazine efficacy for controlling weeds or killing pests. The studies mentioned above also show that the application of biochar can regulate the transport and decomposition of pesticides in the soil, thereby avoiding soil and water pollution.

Due to its high surface area and stronger cation exchange capacity, biochar can be used as a modifier to remediate soil contaminated by simazine (Zheng et al., 2010; He et al., 2019). When applied to the soil, the increased surface area and cation exchange capacity increase the soil adsorption capacity for simazine (Cheng et al., 2017a; Liu et al., 2018), resulting in lower pesticide concentrations in leachate (Larsbo et al., 2013; Tatarková et al., 2013) and crop residues (Yang et al., 2010). Additionally, when more simazine is adsorbed by the soil, the probability of soil microorganisms coming into contact with simazine is reduced, which reduces the rate of simazine decomposition (Cheng et al., 2017a). This also increases the risk of accumulating simazine in the surface soil. Therefore, biochar addition is a risk-benefit approach for simazine pollution control in agricultural soils. To take things a step further in enhancing the improvement effect, much-modified biochar is applied to agricultural soils to improve the soil adsorption capacity. However, this method can reduce the contact chance of simazine with soil microorganisms and may also reduce the number of microorganisms in the soil, thereby inhibiting the decomposition of simazine.

In recent years, biochar modified with iron materials has been widely used to control heavy metal (especially arsenic and cadmium) pollution in the agricultural soil (Wu et al., 2018, 2019; Pan et al., 2019). The addition of iron-based materials enhanced the specific surface area of biochar and improved its reactivity (Asmel et al., 2017; Peng et al., 2021). Simultaneously, iron can increase the number of specific adsorption sites and change the chemical activity of the adsorbent by changing the pH value (at the point of zero charges), ultimately promoting electrostatic interactions between heavy metals and the adsorbent surface (Vieira et al., 2017). This greatly improved the adsorption performance of biochar for heavy metals (Cui et al., 2021; Yuan et al., 2021). Inevitably, the above process also increases the adsorption of simazine by biochar and may even affect the composition and quantity of the soil microbial community, thereby changing the turnover of simazine in the soil. Although the remediation of iron-modified biochar on heavy metals has received significant attention, studies on their effects on simazine fate are rare, especially the influence of microbes regulated by iron-modified biochar on simazine decomposition. Therefore, this study was aimed at (1) comparing simazine fate, including decomposition, adsorption, and leaching in the soil amended with iron-modified biochar. It was also aimed at (2) investigating the possible mechanisms for simazine fate by iron-modified biochar and (3) evaluating the influence of iron-modified biochar application on the fate of simazine in soil.

MATERIALS AND METHODS

Biochar Preparation, Soil, and ^{14}C-Labeled Simazine

Unmodified biochar was produced from wheat (Triticum aestivum L.) straw collected from the Henfaes Research Centre Wales, North Wales, United Kingdom (53° 140N; 4° 100W). The preparation process was introduced by Cheng et al. (2021). The iron modification process was mainly completed according to the method developed by Wu et al. (2018). Briefly, biochar was first roasted and then impregnated with a mixture solution (100 ml, 0.75 mol/L) of $FeSO_4{\cdot}7H_2O$ and $FeCl_3$ solution (100 ml, 0.75 mol/L), in which the n ($FeSO_4{\cdot}7H_2O$): n (H_2O_2) ratio was set at 1:0.5. After that, the solution was stirred and filtered, followed by drying the unfiltered materials at room temperature. These materials were collected for subsequent use and named biochar-FeOS and biochar-$FeCl_3$. In addition, in this study, biochar was thoroughly mixed with zero-valent iron under a continuous flow of nitrogen gas (N_2), called biochar-Fe.

Soil was collected from the field in the Henfaes Research Centre, which is used for grassland and arable production. Soil (Eutric Cambsiol soil type) in the Ah horizon (0–15 cm, sandy loam) was collected in bags and transported to the laboratory. It was naturally dried and sieved through a 2 mm mesh sieve to remove plant residues and stones. The major properties of the soil are shown in **Table 1**, with additional properties displayed by Farrar et al. (2012) and Jones et al. (2012).

Simazine (6-chloro-N, N'-diethyl-1,3,5-triazine-2,4-diamine) was purchased from Sigma Chemical Co (St. Louis, MO, United States). Before the experiment, simazine was labeled with a simazine ^{14}C stock solution.

Experiment Design

In this study, five treatments were installed: (1) Soil without biochar (CK); (2) a mixture of unmodified biochar and soil at a ratio of 1:25 (BC); (3) a mixture of modified biochar (biochar-FeOS) and soil at a ratio of 1:25 (BC-FeOS); (4) a mixture modified biochar (biochar- $FeCl_3$) and soil at a ratio of 1:25 (BC-$FeCl_3$); (5) a mixture of modified biochar (biochar-Fe) and soil at a ratio of 1:25 (BC-Fe).

TABLE 1 | The physical and chemical characterization of soil.

pH	Ec (uScm^{-1})	TC (%)	TN (%)	TOC (mg/kg)	NO3^{-} (mg/kg)	NH4^{+} (mg/kg)
5.20 ± 0.02	87.73 ± 4.18	3.10 ± 0.05	0.34 ± 0.01	411.96 ± 25.57	4.13 ± 0.23	12.8 ± 0.20

Decomposition Experiment

Approximately 300 g of the prepared soil was packed into a PV box (L × W × H = 11 × 8 × 10.5 cm), and the humidity was adjusted to 70% of the water holding content (Jones et al., 2011b). These samples were stored at 20°C for 14 days for microbial recovery. Then 5.0g of soil was transferred into a 50ml centrifuge tube. Then, 0.5 ml of ^{14}C-labeled simazine (0.60 mg L^{-1}, 0.54 kBq mL^{-1}) was added to each tube. A 1-ml NaOH trap (1 M) was placed above the sample to capture CO_2 released from the sample. The NaOH traps were replaced on the 1st, 3rd, 5th, 7th, 10th, 14th, 17th, and 21st days. Finally, the tubes were sealed and incubated in the dark at 20°C for 21 days. The $^{14}CO_2$ content in the NaOH traps was determined by liquid scintillation counting using Optiphase 3 scintillation fluid (PerkinElmer Corp., Waltham, MA) and a Wallac 1404 liquid scintillation counter (PerkinElmer Corp). In addition, 10.0 g of other soil was collected and analyzed using phospholipid fatty acids (PLFA).

Adsorption Experiment

A series of batch experiments were performed to obtain the sorption isotherms of simazine by soil amended with or without biochar and iron-modified biochar. First, 2.0 g previously prepared soil sample was weighed into a 50 ml centrifuge tube. Before adding liquid, these loaded soil tubes were put into the oven at 80°C for 30 min to minimize microbial degradation (Kuzyakov and Jones, 2006). Then a 20ml solution containing ^{14}C-labeled simazine (0.05 Kbq ml^{-1}) was added to each tube. To balance the salt ionic, $CaCl_2$ was added to the tube at a concentration of 0.01 mol L^{-1} to balance the salt ionic. The concentration of simazine in the solution ranged from 0, 6.25, 12.5, 25.0, 50.0, and 100.0 μg/L. After that, the samples were shaken at 200 rpm for 24 h at 20°C. Then 1 ml of the supernatant was extracted from the soil solution and subjected to centrifugation at 4000 rpm for 10 min to determine ^{14}C activity. The ^{14}C activity measurement was the same as in the above introduction.

Leaching Experiment

The details of the leaching experiment were introduced by Jones et al. (2011a) and Cheng et al. (2017b). Briefly, 5 g of the previous soil sample was weighed into a 25 ml inverted syringe (2 cm in diameter). A 1 mm polypropylene mesh was placed at the bottom, and the other was placed on the soil's surface to prevent soil loss and solution shock. Then 1 ml of ^{14}C-labeled simazine (2.50 mg L^{-1}, 0.05 kBq ml^{-1}) was added to the soil surface. After that, a syringe pump was used to add distilled water at 0.2 ml/min after waiting for 1 h of equilibration period. The resulting leachate was sequentially collected (equivalent to 1, 2, 3, 4, 5, and 6 soil pore volumes), and its ^{14}C activity was determined as described above.

Analysis of Microbial Communities

PLFA analysis was used to provide a general profile of the microbial community and quantify total microbial biomass because PLFA is the main component of all microbes' cell membranes (Kim et al., 2018; Zang et al., 2020). Therefore, soil samples collected and stored at −80°C before adding simazine were undergone for PLFA analysis of microbial communities. PLFA was performed according to the method of Buyer and Sasser (2012) with different taxonomic groups classified as described in the study of Frostegård et al. (1993) with acknowledgment of the caveats raised by Frostegård et al. (2011). The soil was suspended in a solution of a methanol-chloroform-phosphate buffer. After filtration, the chloroform phase was separated, and the phospholipids were separated from glycolipids and neutral lipids by solid-phase extraction. The phospholipids were saponified and methylated to fatty acid methyl esters using an Agilent 6890 gas chromatograph equipped with a flame ionization detector and an Ultra-2 column (Kim et al., 2018).

Physicochemical Properties Analysis

All analyses for collected samples were repeated four times. Two solutions were prepared, in which one solution was prepared by mixing a soil dry sample with deionized water (1:2.5, w:v). Another solution was prepared by mixing biochar and deionized water suspension with standard electrodes (1:2.5, w:v). Available NO_3^- and NH_4^+ were measured using a colorimetric method (Mulvaney, 1996; Miranda et al., 2001) based on soil extractions (0.5 M K_2SO_4 extracted from biochar and soil). Soil organic carbon was measured using the $K_2Cr_2O_7$ oxidation method. Ash content of the biochar was measured according to the weight loss of biochar when combusted at 575°C for 16 h. Elemental C, N, H, and S abundances were determined using a Vario MACRO cube analyzer. O content was calculated based on the assumption that biochar is composed of C, N, H, and O only after deducting the ash content. The biochar's Cation exchange capacity (CEC) was determined according to the modified ammonium acetate method (Gaskin et al., 2008). WHC of the biochar was determined according to EBC (2012). The biochar's specific surface area (SSA) was measured using an Autosorb iQ/monosorb surface area analyzer (Quantachrome Instruments, Boynton Beach, FL, United States). Functional groups were determined using a Fourier transform infrared spectrometer (FTIR). The zeta potential was determined using a Malvern Zeta meter (Nano ZSE + MPT2, Malvern Panalytical Instruments Ltd., United Kingdom). The surface morphology of the biochar was observed using scanning electron microscopy (SEM) JSM-6460 LV Scanning Microscope (JEOL, Tokyo, Japan).

Statistical Analysis

As displayed in equation (1), the distribution coefficient (Kd) was calculated from the difference between the total amount added and the amount that stayed in the solution.

$$Kd = (C0 - Ce) \times V/W/Ce \quad (1)$$

where W is the weight of the soil sample, V is the volume of $CaCl_2$ including the simazine solution, C_0 is the concentration in the starting solution, and Ce is the concentration of simazine in the solution after adsorption.

The adsorbed results were fitted by the Langmuir and Freundlich models. The Langmuir model is expressed as Equation (2), and the Freundlich isotherm model is described as Equation (3).

$$\frac{C_e}{q_e} = \frac{1}{q_{max}K_L} + \frac{C_e}{q_{max}} \quad (2)$$

$$\ln q_e = \ln K_F + \frac{1}{n}\ln C_e \quad (3)$$

where q_e and q_{max} are the equilibrium and maximum adsorption capacities (mg/g), respectively, KL is the Langmuir constant related to the affinity of the binding sites (L/mg), and Ce is the equilibrium concentration of adsorbate in an aqueous phase (mg/L). K_f is a constant that represents Freundlich adsorption capacity, and n is a constant that represents the adsorption intensity.

The variables, including biochar and soil properties and ^{14}C activity in adsorption, decomposition, and leaching experiments among treatments, were first tested for normality and homogeneity of variance. Variables with normal distributions and equality of variance were analyzed using a one-way ANOVA with Fisher's least significant difference (LSD). Variables with non-normal distributions or unequal variance (decomposition and leaching) were studied non-parametrically using a Wilcoxon paired signed-rank test. All differences were considered significant at the $p < 0.05$ level. Linear regression was undertaken in Origin 2019b.0 (OriginLab Corp, Northampton, MA).

RESULTS AND DISCUSSION

Biochar and Iron-Modified Biochar Properties

The chemical and physical properties of the iron-modified biochar are listed in **Figure 1** and **Table 1**. Compared to BC (9.70), except for BC-Fe (9.44), the pH value was significantly lower in BC-$FeCl_3$ (1.95) and BC-FeOS (2.33) after iron modification. C, H, N, and O content of biochar or iron-modified biochar are shown in **Figure 1**. The C content in BC was 59.59%, 56.34% in BC + Fe, and 54.57% in BC + $FeCl_3$, whereas it was decreased to 17.35% in BC + FeOS. The O content of BC (11.33%) was significantly lower than BC + Fe (13.26%), BC + FeOS (39.53%) and BC + $FeCl_3$ (20.82%). Generally, biochar prepared above 250 degrees will be alkaline (Cheng et al., 2017a), probably because of oxygen-containing functional groups and carbonate substances in biochar (Yuan et al., 2011). The biochar prepared at 550°C showed strong alkalinity (9.70). This is consistent with the previous reports (Mandal et al., 2018; Tomczyk et al., 2020). However, after modification with $FeCl_3$ and FeOS, the pH of the modified biochar decreased significantly (**Figure 1B** and **Table 1**). Infrared spectroscopy results showed that the modified biochar's oxygen-containing functional groups changed significantly compared to pristine biochar (**Figure 1A**). At the same time, the composition of carbon, oxygen, hydrogen, nitrogen, and other elements has undergone obvious changes during the modification process (**Figure 2A**). This suggests that the carbonate substances in the pristine biochar have undergone significant chemical reactions. Although the change of oxygen-containing functional groups and carbonate substances during the modification process led to a decrease in pH value for the modified biochar, the main reason was that the hydrolysis reaction of FeOS and $FeCl_3$ occurred in (4) during the modification process, releasing a large amount of H ions that resulted in the strong acidity of BC-FeOS and BC-$FeCl_3$ (Wu et al., 2018; Zhang et al., 2020).

$$Fe^{3+} + H_2O = Fe(OH)_3 + H^+ \quad (4)$$

The atomic ratios of C, N, H, and O elements [H/C, O/C, and (N + O)/C] represent the aromaticity, hydrophilicity, and polarity of biochar (Wu et al., 2018). As evinced in **Figure 2A**, The carbon content of the pristine biochar was significantly reduced after the modification of FeOS, while the content of H, N, and O increased accordingly. The main reason is that the organic carbon and inorganic carbon undergo strong oxidation and neutralization reactions in the modification process of the pristine biochar, and CO_2 is generated and released into the atmosphere, resulting in a significant decrease in the carbon content of the modified biochar, which coincides as the relative content of H, N, and O elements rise. In addition, the atomic ratios of H/C, O/C, and (N + O)/C were significantly higher in BC-FeOS than in pristine biochar or other iron-modified biochar, indicating that BC-FeOS has high aromaticity, hydrophilicity, and polarity (**Figure 2B**).

The Influence of Iron Modified Biochar on Simazine Adsorption

As shown in **Figure 3**, iron significantly changed the adsorption of simazine on biochar. Kd values decreased from 57.7 L kg^{-1} in pristine biochar to 11.3L kg^{-1} in BC + FeOS. Nonetheless, the addition of iron-modified biochar significantly increased the adsorption of simazine from the soil compared to the adsorption of simazine by the control soil (Kd 7.2 kg^{-1}). In addition, according to the R^2 value of the Langmuir and Freundlich models ($R^2 > 0.97$, **Table 2**), the Freundlich models can better fit the adsorption of simazine on iron-modified biochar. The findings showed that adsorption capacity constants K_f were 0.014 in control, 0.136 in BC, 0.070 in BC-$FeCl_3$, 0.087 in BC-Fe, and 0.033 in BC-FeOS, respectively. Evidently, those adsorption capacity constants indicated that the iron modification significantly decreased the adsorption capacity compared with the pristine biochar. This view is supported by the

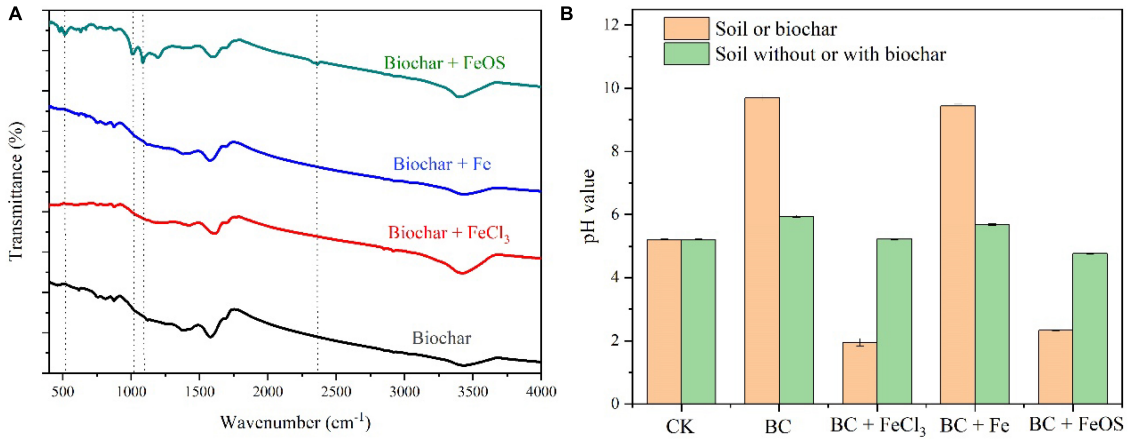

FIGURE 1 | FTIR spectra for the pristine biochar and iron-modified biochar **(A)** and pH value of soil before and after biochar amendment **(B)**.

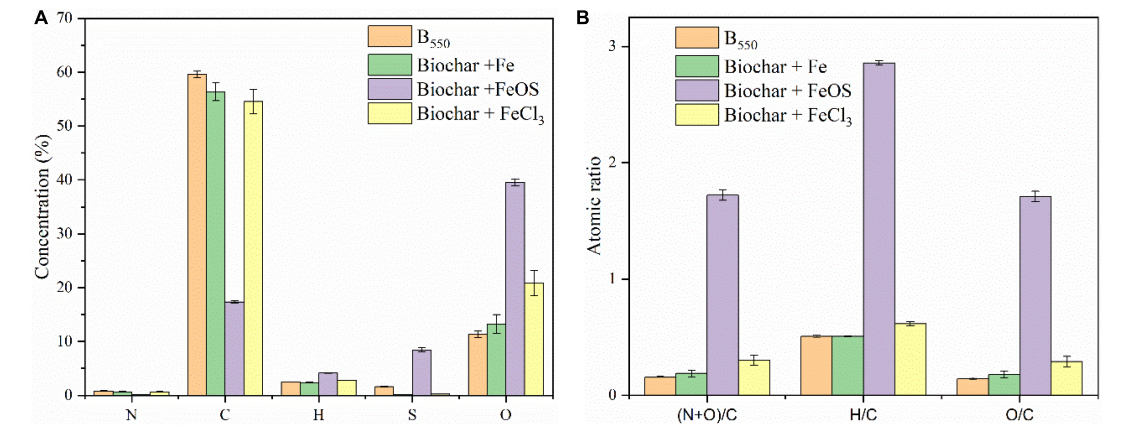

FIGURE 2 | The content of elements **(A)** and the molar ratio **(B)** of biochar or iron modified biochar.

TABLE 2 | The properties of biochar with and without iron modification.

	Biochar	Biochar-$FeCl_3$	Biochar- FeOS	Biochar-Fe
pH	9.70 ± 0.10	1.95 ± 0.12	2.33 ± 0.01	9.44 ± 0.07
Ec (uScm^{-1})	4.56 ± 0.29	12.27±0.92	5.92±0.28	4.27 ± 0.92
CEC (cmol kg^{-1})	2.07 ± 0.16	2.14 ± 1.10	18.03 ± 0.65	2.03 ± 0.18
WHC (%)	659.77 ± 9.14	411.41 ± 5.09	97.20 ± 5.71	222.40 ± 5.67
Zeta potential (mv)	−31.87 ± 1.91	−24.76 ± 0.73	−17.42 ± 1.91	−33.24 ± 1.98

partitioning results of simazine between solid and liquid phases at adsorption equilibrium (**Figure 3**).

Previous studies have shown that the organic carbon content in biochar has an essential effect on the adsorption of simazine (Yang et al., 2016; Feng et al., 2021). In this study, a series of complex oxidation reactions occurred during iron modification (Wu et al., 2018), resulting in lots of organic carbon in the pristine biochar, which was oxidized and escaped. As a result, the adsorption capacity of iron-modified biochar to simazine was reduced. The element content in biochar (**Figure 2A**) also showed low carbon content in the iron-modified biochar, especially in BC-$FeCl_3$ and BC-FeOS. In addition, the content of element C in biochar was inversely proportional to the adsorption capacity of biochar to simazine. The above phenomenon indicated that the carbon content in biochar greatly influences the adsorption capacity of biochar to simazine. However, iron modification reduced the carbon content in the modification process, which decreased the adsorption of biochar to simazine.

The Influence of Iron Modified Biochar on Simazine Leaching

The concentration of simazine in the leachate directly results from the performance of biochar in the adsorption process, which is associated with the adsorption capacity of biochar

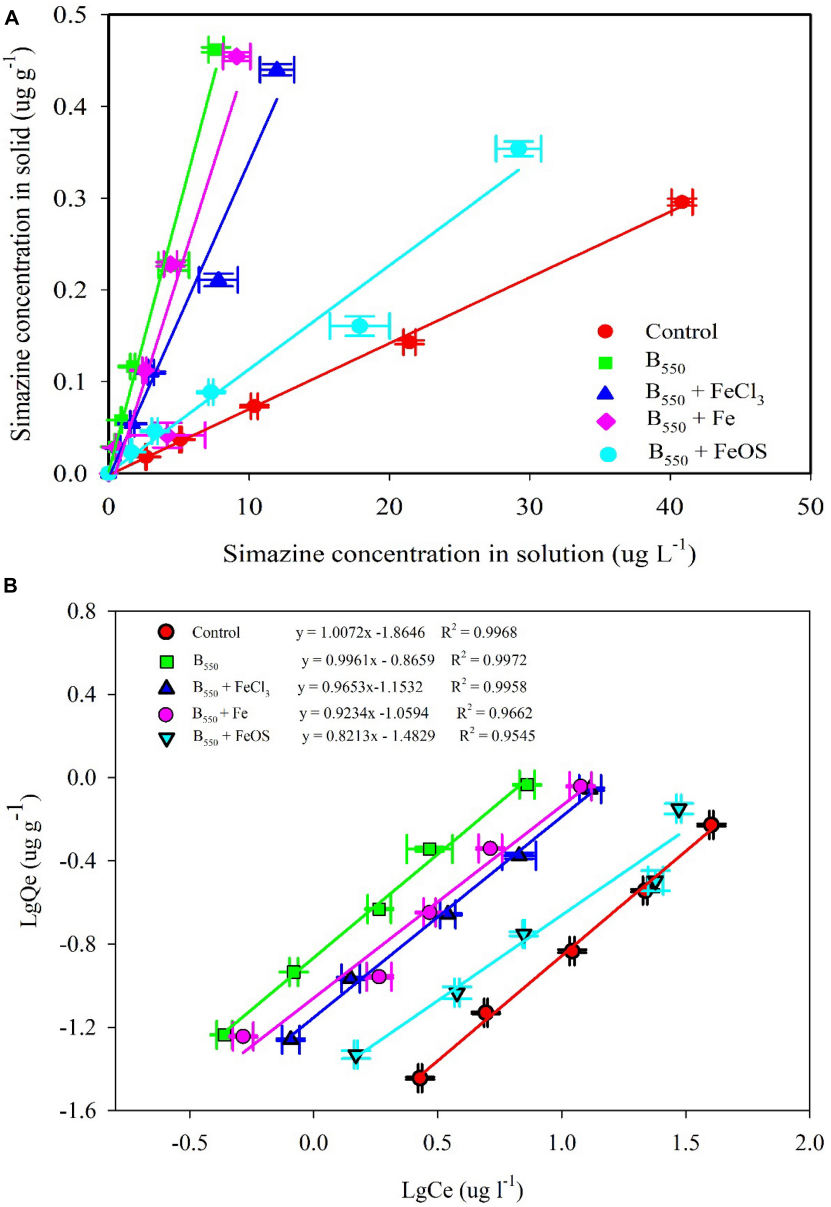

FIGURE 3 | The adsorption of iron modified biochar or pristine biochar on simazine. Distribution of simazine in solid and liquid phases **(A)** and Freundlich isotherms **(B)**.

(Liu et al., 2018). In this study, 63.36% of simazine was leached out in the control leachate, which has the highest content among all treatments. However, the exudation of simazine was 18.43% in BC, 32.95% in BC + FeOS, 16.86% in BC + Fe and 20.92% in BC + $FeCl_3$ (**Figure 4**). Apparently, the addition of biochar significantly reduced the concentration of simazine in the leachate. Moreover, the iron modification processes weakened the retention capacity of biochar for simazine, increasing groundwater pollution compared with pristine biochar. The desorption process is the reverse process of adsorption onto the adsorbent (Cabrera et al., 2014). The stronger the adsorption capacity of the adsorbent to the adsorbed substance, the less the adsorbed substance can be desorbed. The concentration of simazine in the leachate (**Figure 3**) and the adsorption of iron-biochar to simazine (**Figure 2A**) indicated that the content of simazine in the leachate was regulated by the adsorption capacity of biochar to simazine. Therefore, iron modification leads to a decrease in biochar adsorption capacity for simazine, which increases the potential risk of simazine migration into watercourses.

Regulation of the Microbial Community and Simazine Decomposition

The application of biochar can improve the physical and chemical properties of the soil (Keith et al., 2016). It can also increase the

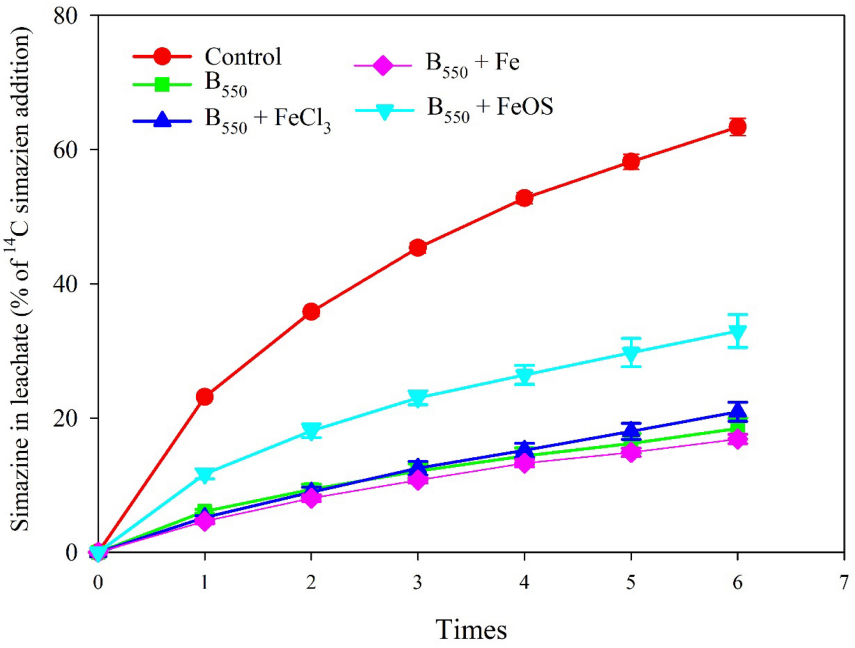

FIGURE 4 | The concentration of simazine in the leachate.

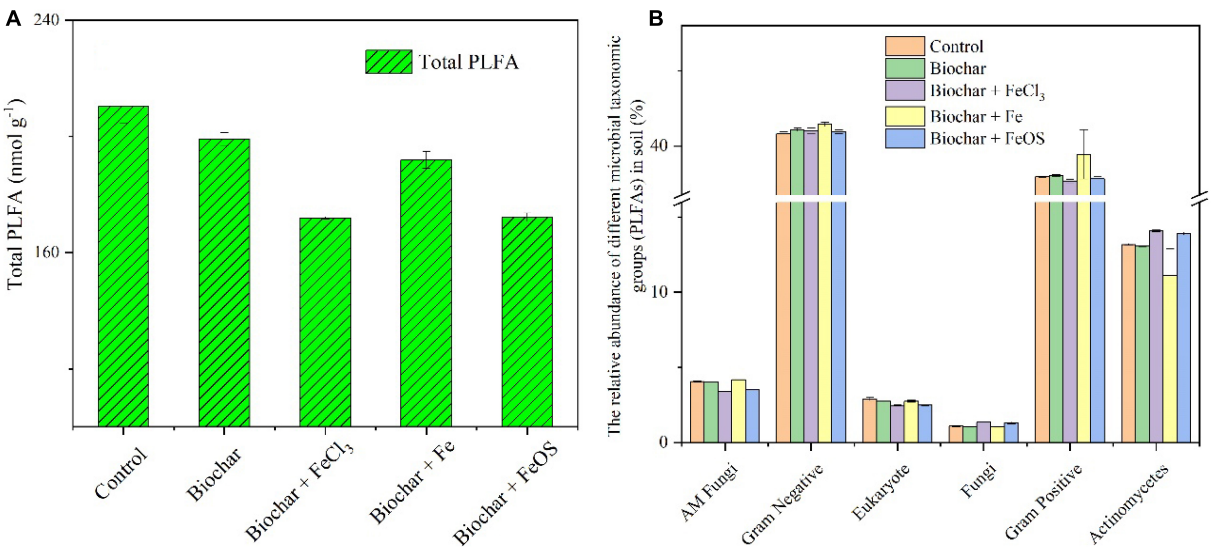

FIGURE 5 | The influence of iron modified biochar on the total biomass **(A)** and the relative abundance of different microbial taxonomic groups **(B)** (PLFAs) in soil.

ability of soil to retain water and fertilizer (Hariz et al., 2015). At the same time, it can directly provide nutrients, further affect the composition and activity of microorganisms in the soil (Yang et al., 2021), and regulate the amount of soil organic matter or exogenous organic matter decomposition (Cheng et al., 2017a). In addition, the addition of biochar can reduce the contact probability between exogenous organic matter and microbial decomposers, thereby reducing the decomposition of exogenous organic matter (Cheng et al., 2017b). The total microbial biomass and microbial community were also studied as shown in **Figures 5A,B**. After biochar application in the soil, the total microbial biomass decreased (**Figure 5A**). Compared to the control, the total microbial biomass in the soil with the addition of unmodified biochar decreased by 5%. The most significant effect was that the total microbial biomass in soils treated with BC + $FeCl_3$ and BC + FeOS additions decreased by 18%. The possible reason was the change in soil's physical and chemical properties due to biochar addition (Yang et al., 2021).

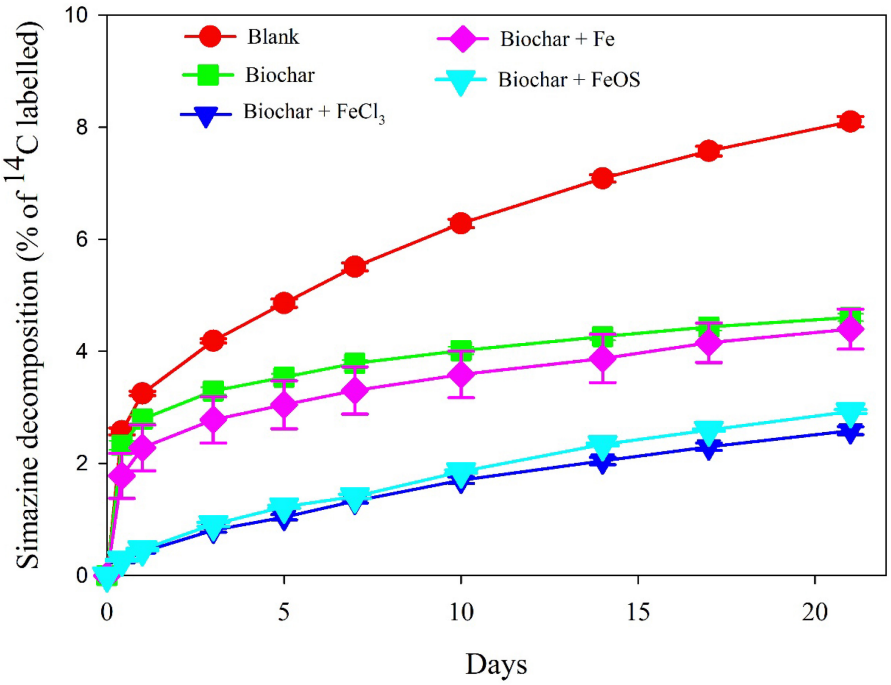

FIGURE 6 | The simazine decomposition in the soil amended with biochar or iron modified biochar.

According to the soil pH (**Figure 2B**), it is evidenced that soil acidification inhibits the growth of soil microorganisms (**Figure 5A**). In addition, the results in the composition of the community structure showed that the composition of *AM fungi* and *Eukaryote*s in the soil amended with BC + $FeCl_3$ or BC + FeOS was significantly lower than that of the control treatment (**Figure 5B**).

As shown in **Figure 6**, the decomposition of simazine in the soil was observed after iron-modified biochar was applied. As previously reported, biochar addition inhibited the decomposition of simazine in soil (Cheng et al., 2017b). Compared with the control, the decomposition ratio of simazine in the pristine biochar amended soil decreased by 43.13%, and the decomposition ratio of simazine in the BC + $FeCl_3$ and BC + FeOS addition treatments decreased by 68.09% and 63.88%, respectively. Obviously, the iron-modified biochar showed a more substantial inhibitory effect on the decomposition of simazine. Previous studies considered that the addition of biochar to the soil enhanced the adsorption of pesticides, thereby reducing the probability of contact between pesticides and soil microbial decomposers and reducing the decomposition rate of pesticides in the soil. This shows that the adsorption of pesticides by soil after biochar addition could regulate the decomposition rate of pesticides (Liu et al., 2018). However, the adsorption of iron-modified biochar to simazine and the decomposition of simazine in iron-modified amended soil showed that the adsorption of simazine in soil and the inhibition of simazine decomposition by biochar did not completely correspond. On the contrary, the effect of biochar on the total microbial biomass and the change of community composition compared to the decomposition of simazine indicates that the impact of biochar on the composition and structure of the microbial community played a prominent role in the decomposition of simazine in soil.

CONCLUSION

In summary, adding biochar modified with iron in the soil evinced a significant effect on simazine because it increased simazine adsorption and regulated microbial community content. Therefore, the adsorption of simazine in soil amended with iron-modified biochar was significantly higher. The decomposition of simazine in soil amended with iron-modified biochar was substantially lower. Compared to the control, the adsorption of simazine was increased in the treatment with biochar addition. However, compared to pristine biochar, iron modification decreased the adsorption of simazine. At the same time, iron-modified biochar addition to soil significantly reduced simazine decomposition. The comprehensive analysis has revealed the increased adsorption of simazine due to biochar addition, which decreased the probability of simazine exposure to microorganisms. The influence of iron-modified biochar on microbial biomass and the community was the main reason for simazine decomposition.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

HC: conceptualization and writing and experiment. DX and SL: review and editing. ZD: data curation. XW and WN: experiment. PH, DC, and DJ: conceptualization and English improvement. All authors contributed to the article and approved the submitted version.

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Biochar rebuilds the network complexity of rare and abundant microbial taxa in reclaimed soil of mining areas to cooperatively avert cadmium stress

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Understanding the interactions between the soil microbial communities and species is critical in the remediation of heavy metal-contaminated soil. Biochar has been widely applied as a stabilizer in the *in situ* remediation of cadmium (Cd)-contaminated soils in mining areas. However, the rebuilding of the microbial taxa of rare and abundant species by biochar and their cooperative resistance to Cd stress remains elusive. In this pursuit, the present study envisaged the effects of two types of biochars *viz*., poplar bark biochar (PB) and thiourea-modified poplar bark biochar (TP) on the rare and abundant bacterial and fungal taxa by using pot experiments. The results demonstrated that the PB and TP treatments significantly reduced the leached Cd content, by 35.13 and 68.05%, respectively, compared with the control group (CK), in the reclaimed soil of the mining area. The application of biochar significantly improved the physicochemical properties like pH and Soil Organic Matter (SOM) of the soil. It was observed that TP treatment was superior to the PB and CK groups in increasing the diversity of the soil abundant and rare species of microbial taxa. Compared with the CK group, the application of PB and TP enhanced and elevated the complexity of the microbial networks of rare and abundant taxa, increased the number and types of network core microorganisms, reshaped the network core microorganisms and hubs, and boosted the microbial resistance to Cd stress. Our results indicate the response of rare and abundant microbial taxa to biochar application and the mechanism of their synergistic remediation of Cd-contaminated soil, thereby providing technical feasibility for *in situ* remediation of Cd-contaminated soil in mining areas.

KEYWORDS

biochar, mine reclamation, Cd-contaminated soil, rare and abundant microbial taxa, microbial network

Introduction

Mining and industrial activities increase the risk of soil heavy metal contamination, especially by cadmium (Cd) (Huang et al., 2018; Kan et al., 2021). Cd is a highly toxic non-essential metal that is listed as the most hazardous heavy metal element by the United Nations (Zeng et al., 2019). Its long residence time in soil and high exposure risk to humans make it one of the priority pollutants (Xue et al., 2022). Mining activities often lead to serious surface damage and environmental pollution (Ma et al., 2021). Besides, the conventional mine reclamation adopts coal gangue and fly ash filling technology, which easily causes the leaching of harmful heavy metals, further aggravating the heavy metal pollution of the reclaimed mine (Dong et al., 2016). Once these lands are converted to agricultural land, they pose a great threat to food security, ecosystems, and human health (Lu et al., 2021; Zhu et al., 2022a). Therefore, there is an urgent need of an *in situ*, low-cost, and efficient remediation technology to solve the problem of Cd pollution in the reclaimed mines.

Compared with the physical and bioremediation strategies such as electrokinetic remediation, phytoremediation, and microbial remediation, the *in situ* stabilizer remediation has been widely applied in Cd-contaminated soil remediation, owing to the high efficiency and cost-effectiveness of the stabilizers in reducing the toxicity and bioavailability of Cd (Chen et al., 2018; Ma et al., 2020a,b). Since the stabilizers vary widely in performance, efficiency, and potential stabilization mechanisms, the selection and application method of the stabilizers plays a key role in the *in situ* remediation (Wang et al., 2021). Among the various Cd stabilizers, biochar has attracted great attention from scholars, owing to its multiple effects on remediation of Cd-contaminated soil, including reducing the bioavailability of Cd, alleviating soil acidification, and improving soil ecological functions (Ahmad et al., 2014; Chen et al., 2020). For a better stabilization effect, some studies have introduced exogenous elements in biochar (Gondek et al., 2018; Rajendran et al., 2019). These elements may combine with Cd in the soil to form more stable compounds which could be immobilized in the soil, achieving an efficient remediation.

Along with the efficient stabilization of Cd, extensive studies have focused and highlighted the ecological impact of biochar on the microbial taxa (Xu et al., 2022; Zhu et al., 2022c). In the natural environment, the abundance and distribution of species in microbial communities are non-uniform, with a few abundant species and a lot of rare species (Jiao and Lu, 2020). Different microbes play key roles in maintaining the ecosystem functions, including nutrient cycling, organic matter decomposition, soil health, and crop productivity (Jiao et al., 2018). However, various degrees of interference of Cd in soil may directly damage the normal physiological metabolism of the microorganisms, which may pose adverse effects on the microbial diversity along with the ecosystems (Wang et al., 2019). During soil remediation, abundant microbial taxa have drawn attention for their remarkable contributions to increase in the biomass and improvement of nutrient cycling (Qi et al., 2022). In the recent years, there is an increasing research on the importance of rare taxa in maintaining the ecosystem stability. Rare taxa have a high diversity and functional redundancy, and thus play an important role in ensuring the functions of the microbial communities (Hannula et al., 2017; Shu et al., 2021). Studies have revealed interactions of the intra-/inter-species between the rare and abundant species in resisting the disturbance due to the pollutants (Dong et al., 2021). They formed a complex ecological network and maintained the stability of the microbial network. Notably, some species, regardless of their amounts, occupied key positions in the ecological networks and were considered as key species for maintaining the stability of the community structure (Li et al., 2022). However, it is still unclear that whether these key species were abundant or rare, and their responses to environmental perturbations were not consistent all the time (Jiao and Lu, 2020; Zhao et al., 2022). For example, because of their poor resistance to heavy metals, almost all rare taxa in pristine soils were eliminated by heavy metals, resulting in a sharp decline in bacterial diversity. Studies have also shown that the diversity and community composition of rare taxa was more stable under the influence of climate change and other disturbances such as copper stress, freeze-thaw, and mechanical disturbance (Shade et al., 2014; Liang et al., 2020). During the *in situ* remediation of Cd-contaminated soil in mining areas, biochar changed the soil environmental conditions, including heavy metal concentrations, metal forms, soil pH, and available nutrients. These changes affected the aggregation and distribution of rare and abundant microbial taxa as well as their functions. Therefore, there is a need to identify the rare and abundant species and their interactions in the process of biochar remediation of Cd-contaminated soil.

In the present study, two high-efficiency Cd-stabilizing biochars *viz*., the poplar bark biochar (PB) and thiourea-modified poplar bark biochar (TP) were selected to conduct pot experiments for exploring the effects of biochar remediation in Cd-contaminated soil on the rare and abundant microbial taxa in mining areas and revealing their interactions and the mechanism of their synergistic control of Cd pollution with biochar. The specific goals of this study were as follows: (1) to elucidate the response of the rare and abundant taxa of bacteria and fungi to PB and TP application; (2) to explore the co-occurrence relationship of the rare and abundant taxa of bacteria and fungi under PB and TP application; and (3) to reveal the synergistic mechanism of biochar along with the rare and abundant microbial taxa against Cd stress. Our study can assist the prediction of the response of soil bacteria and fungi to biochar reclamation of Cd-contaminated soil in mining areas, and provide a technical support for further engineering

application of biochar in the *in situ* remediation of heavy metal pollution in mining areas.

Materials and methods

Biochar preparation and soil sampling

In this study, the bark of Italian poplar was selected as the biochar material. The bark was fully washed with deionized water and dried in an oven at 60°C for 24 h. After dried, it was fully compacted and placed in a tube furnace which was under argon and with temperature raised to 600°C at 5°C/min and then kept at this temperature for 2 h. Therefore, PB was obtained. TP was prepared following the same procedure using bark and thiourea at a mass ratio of 1:1. The prepared biochar was ground, passed through a 60-mesh sieve and stored. The biochar produced by this method exhibited an optimal Cd adsorption efficiency, according to our earlier findings (Zhu et al., 2020a).

Soil samples (0–20 cm deep) were collected from the Liuxin Coal Mine Reclamation Area, Xuzhou City, Jiangsu Province, China. The area had a temperate continental monsoon climate, with an annual precipitation of 800–930 mm and an annual average temperature of 14.2°C. The type of soil was cinnamon soil, containing 29.19 ± 1.33% sand, 32.69 ± 1.02% clay, and 38.12 ± 1.58% silt. The cropping system was wheat-rice rotation. The Cd content in the soil was 9.97 ± 0.01 $mg{\cdot}kg^{-1}$. More details of the biochar and soil are shown in **Supplementary Table 1**.

Pot experiment and soil physicochemical analysis

Pot experiments were performed in a temperature and humidity-controlled glass greenhouse. About 2.5 kg of soil was thoroughly mixed with biochar at a ratio of 2% (w/w) and filled into a polyethylene flowerpot with a height of 12 cm and a diameter of 13 cm. Our previous study confirmed that the best Cd stabilization effect was obtained at this biochar ratio (Zhu et al., 2020b). The treatment group without biochar was the control (CK) group, and the treatment group with poplar bark biochar and thiourea-modified biochar were the PB and TP treatment groups, respectively. A total of 10 replicas were set up for each treatment group. Cabbage seeds were sterilized with 2% hydrogen peroxide, washed with deionized water, and then sown at 2 cm depth for germination. Subsequently, the seeds were incubated at 25°C with 60% relative humidity for 30 days. Finally, 0–10 cm topsoil samples from three sampling points were collected from each pot and mixed together as the soil sample.

The soil pH and electrical conductivity (EC) was obtained by measuring a 1:2.5 (w/v) soil-water suspension using a pH meter (Fan et al., 2020). The soil organic matter (SOM) was determined by potassium dichromate oxidation-outer heating method (Luo Z. et al., 2020). NH_4^+-N was measured by spectrophotometry after extraction with potassium chloride, and NO_3^--N was determined by spectrophotometry after extraction with calcium chloride (Li et al., 2020). The available phosphate (AP) was determined by molybdenum blue method after extraction with ammonium bicarbonate (Zhu et al., 2022b). The enzymatic activities of β-glucosidase (BG), alkaline phosphatase (Pho), and urease (Ure) were determined by nitrophenol colorimetry, disodium phenyl phosphate colorimetry, and sodium phenoxide-sodium hypochlorite colorimetry, respectively (Zhu et al., 2022b). After the soil was digested with HNO_3^--HF, the total Cd content in the soil was analyzed by inductively coupled plasma mass spectrometer (ICP-MS) (Zhao et al., 2019). The four-step sequential extraction method proposed by the European Community Bureau of Reference (BCR) was adopted to determine the forms of Cd in soil samples, including the weak acid-soluble state, reducible state, oxidizable state, and residue state (Xu et al., 2020). The content of Cd in the leaching solution was determined according to the toxicity characteristic leaching procedure (TCLP) (Luo M. et al., 2020). The relative standard deviation (RSD) among replicate samples was smaller than 10%.

DNA extraction, PCR and high-throughput sequencing

DNA was extracted from soil samples using the FastDNA™ SPIN Kit for soil (MP Biomedicals, California, CA, United States) according to manufacturer's instructions. The V4-V5 region of 16S rRNA was amplified using primers 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′). The PCR program was as follows: 95°C for 3 min, followed by 27 cycles of (95°C for 30 s, 55°C for 30 s, and 72°C for 30 s), and 72°C for 10 min and finally cooled to 10°C. The PCR products of the various samples were detected by 2% agarose gel electrophoresis. A DNA Gel Extraction Kit (Axygen, United States) was used to recover the target PCR fragments. According to the above preliminarily quantitatively determined DNA amount from electrophoresis results, a Quant-iT™ PicoGreen™ dsDNA Assay Kit with fluorescent reagents was used to conduct fluorescence quantification on PCR amplification products using a microplate reader (BioTek, FLx800, United States). The samples were mixed in equal proportions. The TruSeq Nano DNA LT Library Prep Kit developed by Illumina (United States) was used to construct the DNA sequencing library. The constructed library was quantified by Qubit and qPCR. The qualified library was sent to the Shanghai Meiji Biotechnology

Co., Ltd., for sequencing with HiSeq 2500 PE2500 (Illumina, United States) (Li et al., 2020; Luo Z. et al., 2020).

A program called Trimmomatic (Version 0.33[1]) was used to filter the poor quality paired-end (PE) raw reads. FLASH (Version 1.2.11[2]) was used to merge the PE rends into one sequence. According to the barcode and primer information at the two ends of each sequence, Mothur (Version 1.35.1[3]) was used to assign the sequence to the corresponding sample, and finally an effective assembled sequence was obtained. USEARCH was adopted to cluster the assembled sequences (Version 10[4]), and the sequences were clustered into amplicon sequence variants (ASVs) with a sequence similarity of 97%. USEARCH was also run to remove chimera and singleton sequences. Then Silva[5] was carried out to annotate the representative sequences (Ma et al., 2021).

Identification of rare and abundant microbial taxa

To assess the response of the rare and abundant microbial taxa to the biochar treatments, the ASVs were classified into the following six categories based on the criteria used in Chen's study (Chen et al., 2019): (i) always abundant taxa (AAT), ASV with a relatively abundance of ≥1% in all samples; (ii) conditionally abundant taxa (CAT), ASV with a relatively abundance of >1% in some samples but never <0.01%; (iii) always rare taxa (ART), ASV with a relatively abundance of <0.01% in all samples; (iv) conditionally rare taxa (CRT), ASV with a relative abundance of <1% in all samples and <0.01% in some samples; (v) moderate taxa (MT), ASV with a relative abundance between 0.01 and 1% in all samples; (vi) conditionally rare and abundant taxa (CRAT), ASV with a relative abundance ranging from <0.01 to ≥1%. According to previous studies, AAT, CAT, and CRAT were combined as abundant taxa, while ART and CRT were combined as rare taxa.

Data process and analysis

The α-diversity indices of the rare and abundant microorganisms were obtained by the vegan package in R (Version 4.1.3). One-way analysis of variance (ANOVA) was adopted to test the significance of differences between the treatments, and the results were tested by honestly significant difference (HSD) test. Based on the Bray-Curtis dissimilarity matrix, the β-diversity of microorganisms was further determined. Subsequently, the differences in the different microbial communities were visualized by principal coordinate analysis (PCoA) with ggplot2 package. Permutational multivariate analysis of variance (PERMANOVA) was performed using the vegan package to elucidate the significant differences in the microbial subcommunity structure between the different groups. Using the pairwise Spearman rank-correlation matrix, co-occurrence networks were constructed with the help of the psych package. Robots (soft threshold $r > 0.60$) and statistically significant ($p < 0.05$) correlations were included in the network analysis. The visualization of co-occurrence networks was carried out in Gephi 0.9.2. Network topological characteristics including average degree, clustering coefficient and betweenness centrality were obtained by the igraph package. The network keystone ASVs were determined according to within-module connectivity (Zi) and among-module connectivity (Pi), and nodes with Zi ≥ 2.5 or Pi ≥ 0.62 were considered as the keystone ASVs. Mantel correlation was used to explore the relationship between the Bray-Curtis dissimilarity and Cd content of the rare and abundant microbial communities. Finally, the canonical correspondence analysis (CCA) was conducted using the vegan package to evaluate the impact of soil variables on the different microbial sub-communities.

Results

Changes in the reclaimed soil and plant properties after biochar application

The results showed that the effects of PB and TP on the physicochemical properties, activities of enzymes and Cd content of the soil in mining areas were significantly different ($p < 0.05$). Compared with the CK group, the leached Cd content in the PB and TP treatment groups was decreased by 35.13 and 68.05%, respectively, the acid-soluble Cd content decreased by 43.60 and 52.02%, respectively, and the residual Cd content was increased by 18.39 and 51.31%, respectively (**Figure 1**). Compared with the CK group, the PB treatment significantly increased the SOM and AP, while the TP treatment significantly increased pH, EC, and AP. The application of biochar showed nearly no effect on the soil ammonium nitrogen content, but they significantly dropped the soil nitrate nitrogen content (**Supplementary Table 2**). Compared with CK, PB, and TP significantly promoted crop growth ($p < 0.05$), plant height increased from 10.1 to 12.1 cm, and 13.4 cm, respectively, while leaf length increased from 5.59 to 6.09 cm, and 6.85 cm, respectively. Meanwhile, biomass of roots, stems, and leaves of PB treatment increased 23.91, 30.58, and 9.97%, as well as the TP treatment increasing 83.88, 97.15, and 21.80%, respectively (**Supplementary Figure 1**). Compared with the CK

1 http://www.usadellab.org/
2 https://ccb.jhu.edu/software/FLASH/
3 http://www.mothur.org
4 http://www.drive5.com/usearch/
5 https://www.arb-silva.de/

group, the PB, and TP treatments had no significant effects on the soil alkaline phosphatase activity, but they significantly increased the contents of urease. Compared with the CK group, the PB treatment significantly decreased the enzymatic activity of β-glucosidase, whereas the TP treatment increased it (**Supplementary Table 2**).

Distribution and diversity of the rare and abundant taxa in response to biochar application

There were 1,620,057 and 1,642,124 high-quality sequences of bacteria and fungi, respectively, in all samples after the quality control step, resulting 5,393 bacterial and 1,344 fungal ASVs, with a 97% sequence similarity. In case of bacteria, there were 956 rare taxa and 61 abundant taxa, while in case of fungi, 247 ASVs exhibited the rare taxa, and only 78 ASVs were classified as abundant taxa. The relative abundance of rare taxa was higher than that of abundant taxa. The soil samples were identified and classified into 39 bacterial phyla (**Supplementary Figure 2**). The top nine phyla with higher abundance accounted for more than 95% of the relative abundance. *Proteobacteria* is the most dominant phylum in all treatments, and the dominant phylum of abundant and rare bacteria is *Proteobacteria*, when its relative abundances of PB and TP treatments increased 30.47 and 93.46%, respectively, compared to CK group. The abundant *Sordariomycrtes* is dominant phyla of abundant fungi, while plylum *Dothideomycetes* belongs to the rare fungi.

The application of PB and TP did not change the Simpson index of abundant bacterial and fungal taxa, but both significantly increased the Simpson index of rare taxa ($p < 0.05$). The application of biochar significantly increased the richness of the rare bacterial and fungal taxa ($p < 0.05$), and no significant differences were seen in the richness of the abundant fungal taxa between the treatment groups. However, the PB and TP treatments significantly increased the richness of the abundant fungal taxa ($p < 0.05$; **Figure 2**). The results of PCoA analysis at the level of ASV exhibited a close distance between the PB and the CK group, indicating similar soil abundant and rare bacterial community structures between these two groups. The TP treatment resulted in very different abundant and rare bacterial community composition in the soil from those of the CK and PB treatments. The TP treatment showed a significant effect on the community composition of the rare and abundant bacteria ($p = 0.001$) (**Figure 3**). The similarity of rare and abundant fungal communities was less affected by biochar than that of the bacteria. Both the rare bacterial and fungal communities were less similar than their corresponding abundant communities, suggesting that the β-diversity of rare taxa was more susceptible to biochar application.

Co-occurrence networks of the rare and abundant microbial taxa in response to biochar application

To further explore the response of interactions between the microbial taxa with various abundances in biochar application, co-occurrence networks of rare and abundant microbial communities were constructed at the ASV level based on Spearman correlation. All the co-occurrence networks exhibited scale-free characteristics ($R^2 > 0.835$) (**Supplementary Table 3**), thereby indicating the non-random structures of the networks. In this study, the dominant modules in the networks were mostly composed of rare taxa. The rare nodes appeared at the edges in most of the abundant nodes.

In the bacterial networks of the CK, PB, and TP groups, the rare and abundant ASVs accounted for 79.02 and 20.98%, 90.00 and 10.00%, and 85.82 and 14.18% of the total nodes in the corresponding network, respectively (**Figure 4**), and 2 (both rare), 8 (all rare), and 6 (2 abundant and 4 rare) keystone ASVs were identified, respectively (**Supplementary Figure 3**). In the fungal networks of the CK, PB, and TP groups, the rare and abundant ASVs accounted for respective 53.12 and 46.88%, 52.63 and 47.37%, and 64.06 and 35.94% of the total nodes in the corresponding network, and 3 (2 abundant and 1 rare), 5 (4 abundant and 1 rare), and 5 (2 abundant and 3 rare) keystone ASVs were identified, respectively. The rare bacterial and fungal nodes in the CK, PB, and TP groups accounted for 54.59 and 16.43%, 62.27 and 14.44%, and 67.62 and 15.93% of the total nodes in the corresponding network, respectively. In the bacterial-fungal co-occurrence networks of the CK, PB, and TP groups, 13, 10, and 16 keystone ASVs were identified, with the abundant fungi accounting for 8/13, 7/10, and 1/2, respectively. The rare microbial taxa in the sub-network were the main body of the network, indicating that the taxonomic disappearance of these taxa may lead to the disintegration of the network and module. Thus, the rare taxa could be more important than the abundant taxa in maintaining the complexity of the microbial networks.

Relationships between biochar, microbial communities, microbial networks, and cadmium stress

As the Cd concentration decreased, the bacterial and fungal co-occurrence networks became more complex, with an increased number of nodes and connections. Especially in the TP treatment group, the number of nodes of the rare bacterial and fungal taxa was higher than that compared with those of the CK and PB groups (**Supplementary Table 3**). The three important node-level topological characteristics of the subcommunities, degree, betweenness, and clustering coefficient

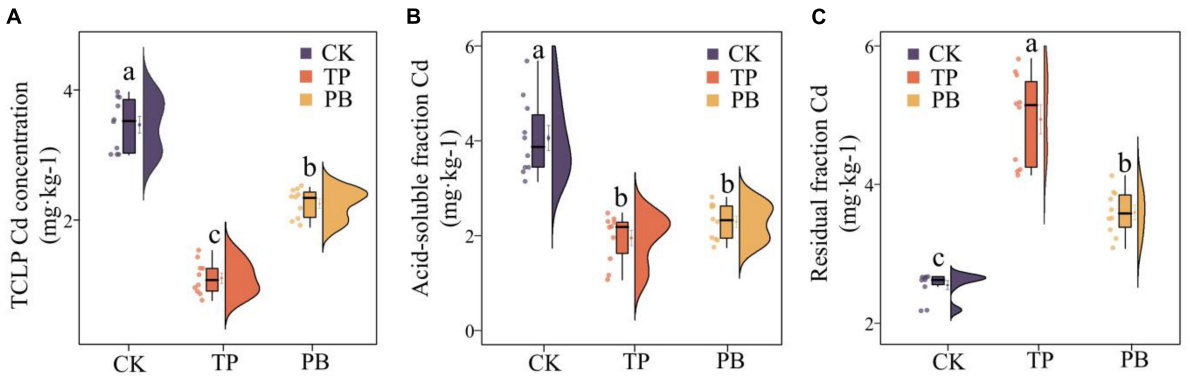

FIGURE 1
Leached cadmium (Cd) content **(A)** and distribution forms of Cd **(B,C)** in the control group (CK), poplar bark biochar (PB), and thiourea-modified poplar bark biochar (TP) soil treatment groups. Different letters indicate the values that differ significantly among CK, PB, and TP treatments at $p < 0.05$ [honestly significant difference (HSD) test].

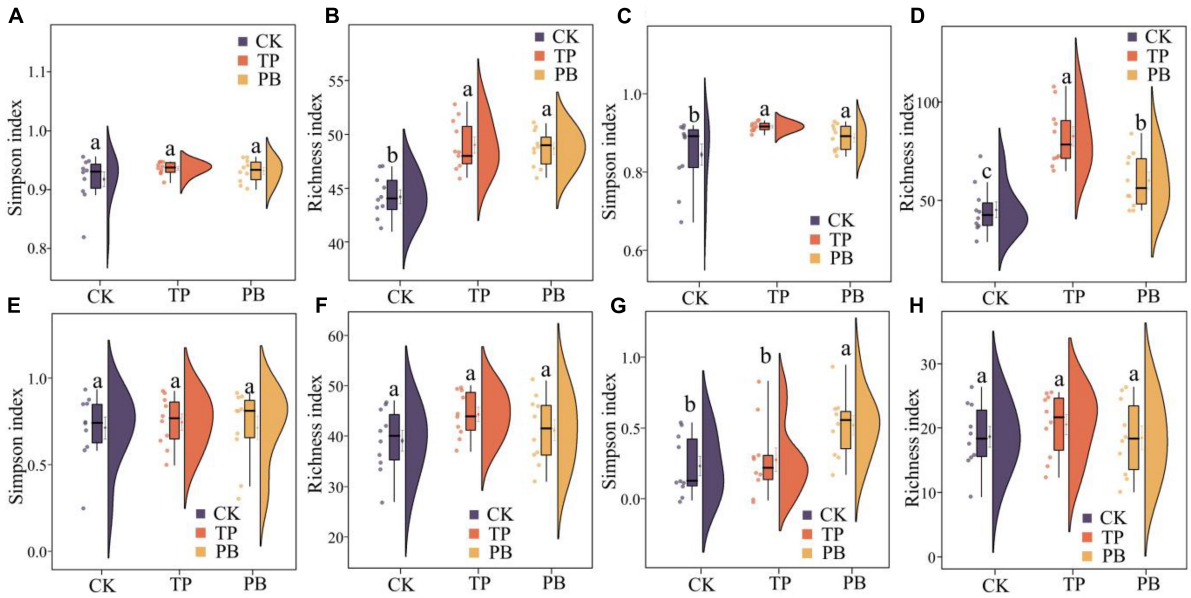

FIGURE 2
α-Diversity indices of abundant **(A,B)** and rare **(C,D)** bacterial and abundant **(E,F)** and rare **(G,H)** fungal communities in the control group (CK), poplar bark biochar (PB), and thiourea-modified poplar bark biochar (TP) treatment groups. Different letters indicate the values that differ significantly among CK, PB, and TP treatments at $p < 0.05$ [honestly significant difference (HSD) test].

showed that the betweenness of the TP group was significantly higher than that of CK and PB. A node exhibiting a high betweenness has a stronger control over the network. Therefore, the rare bacterial and fungal taxa may convey more information. The clustering coefficient of the TP group was significantly higher than that of the CK and PB, indicating that the interconnections between the adjacent points of microbial taxa in the TP group were higher. The results suggested that biochar affected the connections between the microbial communities, thereby increasing the complexity of the soil microbial community networks.

Spearman's correlation analysis showed that the community similarity between the rare and abundant bacterial taxa was significantly positively correlated with the leached Cd, acid-soluble Cd, oxidized Cd, reduced Cd, and residual Cd in the soil ($p < 0.001$, Figure 5). Soil Cd positively correlated with the community similarity of abundant fungal taxa, whereas the community similarity of the rare fungal taxa did not exhibit a significant correlation with Cd. Although the R^2 of the correlation analysis was small, the Mantel test demonstrated that the compositions of the rare bacterial and abundant fungal communities presented higher correlations with various Cd

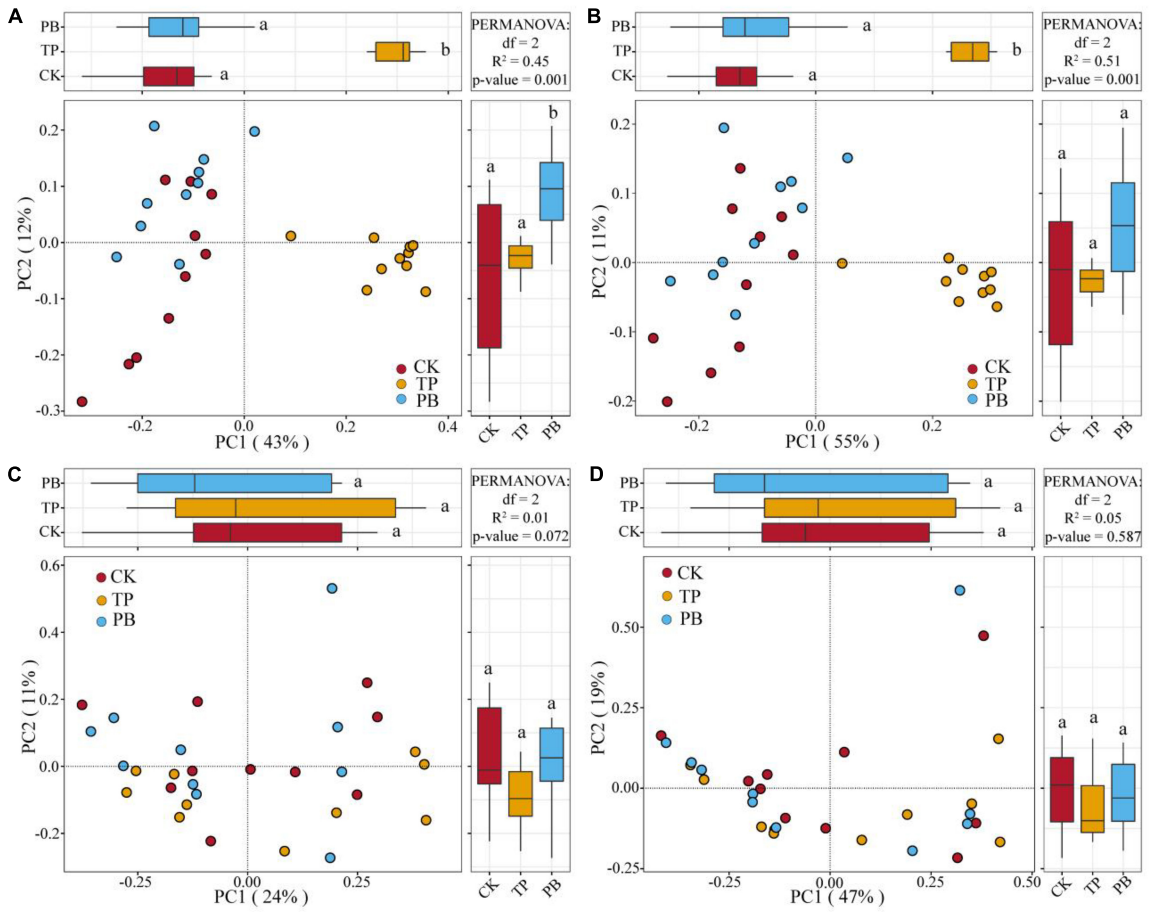

FIGURE 3
Principal coordinate analysis (PCoA) of the abundant and rare bacterial and fungal communities using Bray-Curtis distances. Different letters indicate the distribution of different treatments of samples along the PC1 and PC2 axes at $p < 0.05$. Abundant bacteria **(A)**, Rare bacteria **(B)**, Abundant fungi **(C)**, and rare fungi **(D)**.

forms in the soil than the compositions of the abundant bacterial and rare fungal communities. These results suggest that the changes in the rare and abundant microbial taxa may modulate the soil Cd levels after biochar application.

The Bray-Curtis distance-based CCA results showed that the rare and abundant bacterial taxa formed distinct and well-separated clusters in different treatment groups (**Figure 6**). However, the difference between the rare and abundant fungal taxa was not clear, thereby indicating that the changes in the soil properties after biochar treatment had a big difference in the impact of the rare and abundant taxa between the bacterial and fungal sub-communities. The impact on bacterial taxa was greater than that on fungi. The rare and abundant bacterial communities were mainly driven by pH, EC, SOM, AP, available nitrogen (AN), leached Cd content, and Cd forms. For fungi, BG, leached Cd content, and various forms of Cd were significantly associated with the changes in the abundant taxa. In the figure, the length of the arrow shows that the factor that showed the greatest influence on the number of soil microbial ASVs was the residual Cd. In addition, the sample points of the PB and TP treatments formed obtuse angles with the leached Cd, but acute angles with the residual Cd, indicating that the biochar treatment could help to reduce the leached Cd content and increase the residual Cd content.

Discussion

The application of biochar could decrease the soil Cd availability, which might lead to changes of the external environmental stress (Zhu et al., 2022b). In this study, OH^- ions released from the surface of the biochar particles significantly increased soil pH ($p < 0.05$), from 7.87 to 7.92 and 7.97, respectively, with the PB and TP treatments (**Supplementary Table 2**). Compared with the CK group, the application of biochar increased the buffering capacity of the soil system. The fixation of soil Cd, including the adsorption, complexation, and precipitation of Cd ions increased in an alkaline environment,

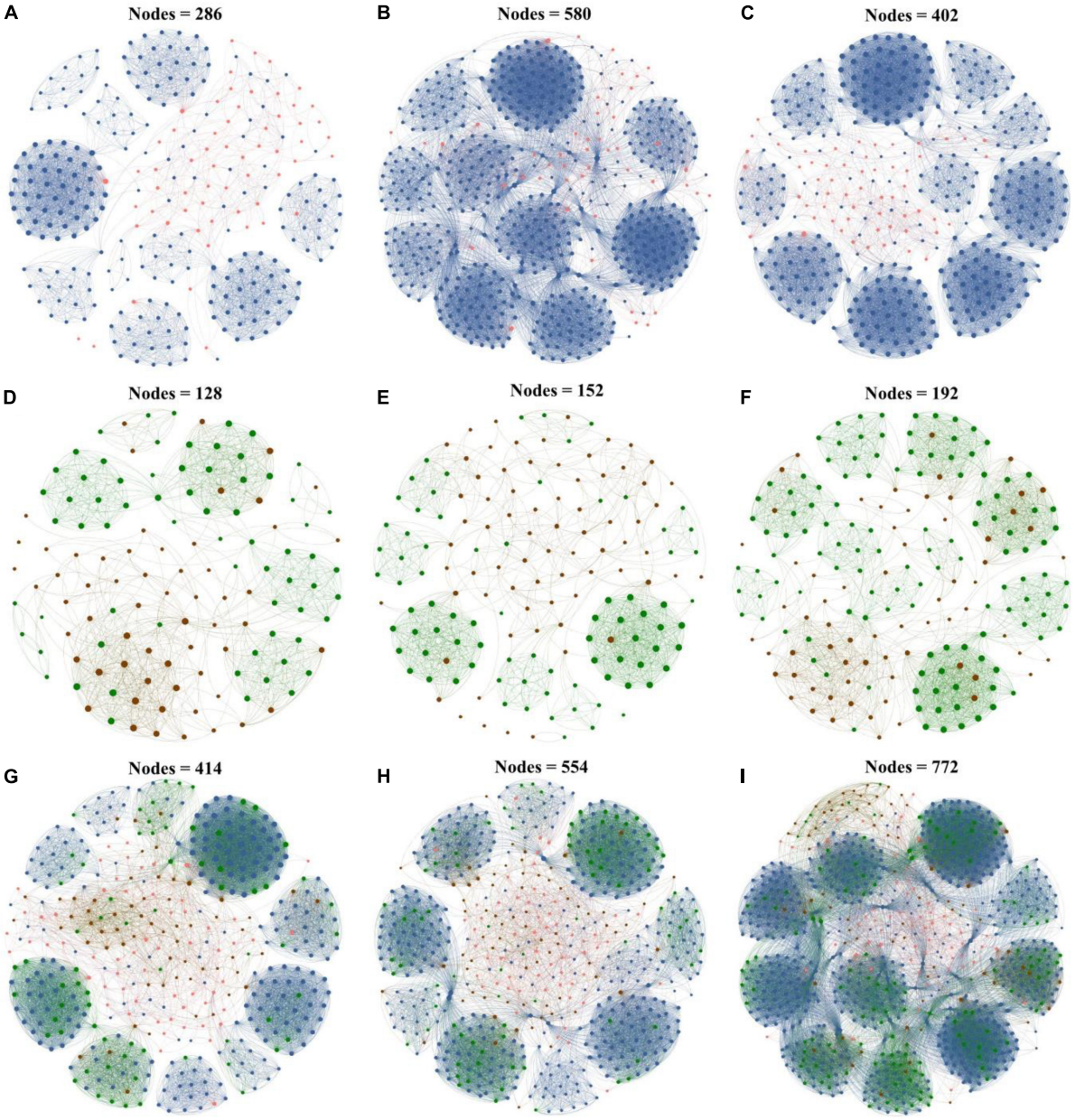

FIGURE 4
Microbial networks of the control group (CK), poplar bark biochar (PB), and thiourea-modified poplar bark biochar (TP) groups. **(A–C)** Rare and abundant bacterial networks; **(D–F)** rare and abundant fungal networks; and **(G–I)** rare and abundant bacterial and fungal networks. The size of a node is proportional to the degree of connectivity. Blue nodes: rare bacteria; pink nodes: abundant bacteria; green nodes: rare fungi; and brown nodes: abundant fungi.

and this has been confirmed in previous studies (Ehsan et al., 2014; Hou et al., 2019; Li et al., 2019). The elevation in pH did not only increase the negative charge on the soil components to boost Cd fixation (Tang et al., 2020), but also initiated and enhanced the ability of other adsorption factors to adsorb Cd (Yang X. et al., 2016). For example, soil pH was the primary factor affecting the phosphorus fixation in soil, because it ensured sufficient adsorption sites for phosphate ions, when physical deposition occurs on the biochar surface (Jin et al., 2019). Besides, earlier studies have revealed that biochar improves the soil agglomeration structure, which reduces the transfer of water into the soil surface and enhanced the soil aeration (El-Naggar et al., 2018; Bashir et al., 2020). This promotes the growth of plant roots and microbes, thereby increasing the Cd adsorption by plant root exudates and microorganisms (Mazhar et al., 2019; Harindintwali et al., 2020).

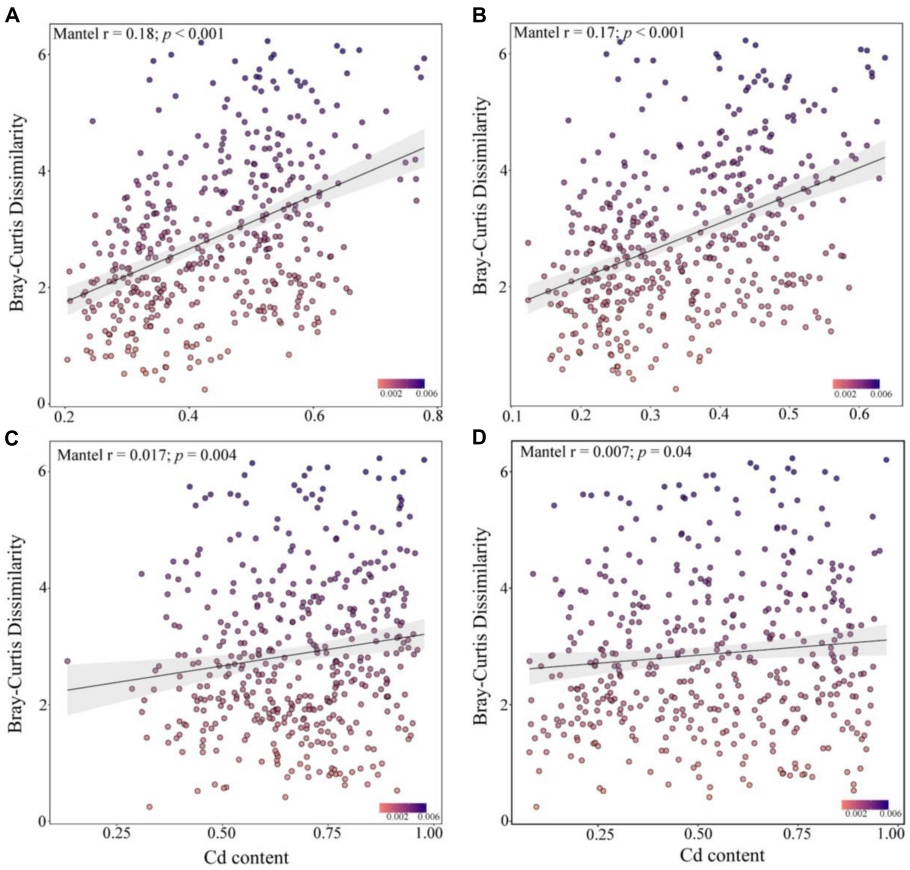

FIGURE 5
Mantel correlation between the dissimilarity of abundant bacteria **(A)**, rare bacteria **(B)**, abundant fungi **(C)**, and rare fungi **(D)** communities and the soil cadmium (Cd) levels.

A large number of alkaline cations get adsorbed on the surface of the biochar and improve the conductivity of soil particles. While OH^- gets neutralized by the H^+ ions absorbed by the clay minerals, the adsorption of Cd^{2+} occurs in the form of soil colloids (Ehsan et al., 2014). In addition, the sulfur element introduced by TP promotes the formation of cadmium sulfate, which cannot be easily oxidatively hydrolyzed and then greatly reduces the bioavailability of Cd (Gholami et al., 2019; Chen et al., 2020). It is generally found that adding biochar would decrease soil enzyme activity related to soil carbon mineralization (Zhu et al., 2022b). Bailey et al. (2011) have shown that the addition of biochar to soil has increased a series of enzymatic activities, and these enzymes were related with nitrogen utilization. The addition of biochar will inevitably change the soil carbon, nitrogen, and phosphorus nutrient cycle (Yang J. et al., 2016), and β-glucosidase, urease and alkaline phosphatase can be used as evaluation indicators. Elzobair et al. (2016) have proved that biochar had the ability to adsorb a variety of organic and inorganic molecules, as well as could inhibit several enzyme activities or enzyme substrates through adsorbing or blocking reaction sites. Moreover, the positive effects of biochar on soil enzymes (Ali et al., 2020) might have a relationship with the improvement of soil physicochemical properties. It is also worth noting that different additives showed the big divergence of effects on soil enzyme activity, which might be caused by the different constituent raw materials of these additives (Elzobair et al., 2016).

The adsorption of heavy metal ions on the biocarbon surface in the soil might change the soil C/N ratio, therefore, the addition of PB and TP have significantly increased the biomass of cabbage (**Supplementary Figure 1**). O'connor et al. (2018) have shown that high concentrations of Cd could pose potential negative effects on plant growth, whereas the addition of biochar could alleviate the fluidity and availability of heavy metals in soil. The macroscopic nutrients, for example, nitrogen, phosphorus, and alkaline cations (e.g., Ca^{2+} and Mg^{2+}), could directly or indirectly increase the plant productivity through providing some nutrients or improving the soil structure (Chen et al., 2018). The cumulative absorption of Cd in plant tissues is always distributed in roots and shoots. However, most of these absorbed metals prefer to retain in the roots, while a limited portion would transfer to the aboveground parts. Plant iron

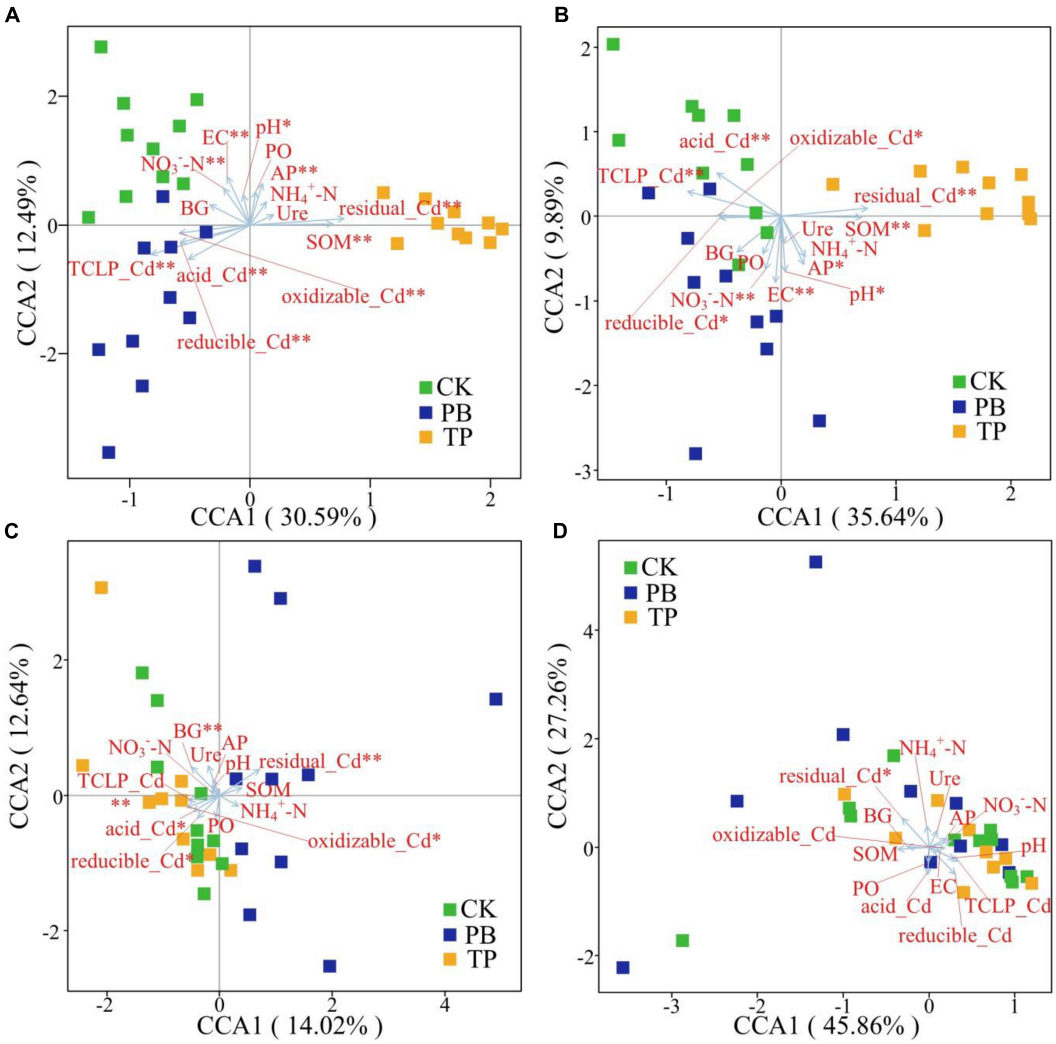

FIGURE 6
Identification of driving factors of β-diversity of the rare and abundant microbial communities in soil by Bray-Curtis distance-based canonical correspondence analysis (CCA). EC, electrical conductivity; SOM, soil organic matter; AP, available phosphorus; NO_3^--N, nitrate nitrogen; NH_4^+-N, ammonical nitrogen; BG, β-glucosidase; Ure, urease; PO, alkaline phosphatase. *Indicates statistically significant. *$p < 0.05$; **$p < 0.01$. Abundant bacteria **(A)**, Rare bacteria **(B)**, Abundant fungi **(C)**, and rare fungi **(D)**.

carriers released from plant roots are important factors affecting the availability of trace elements in the rhizosphere, and this mechanism may protect plants from heavy metal poisoning and reduce metal transfer to plant tissues (Lu et al., 2019). Iron chelates are synthesized and secreted by grasses. Biochar is an important donor of iron oxides. These non-protein amino acids can dissolve a small amount of soluble iron compounds in the rhizosphere.

There were clear differences in the diversity and distribution of the rare and abundant taxa of bacterial and fungal communities caused by biochar. In terms of diversity, compared with the CK group without biochar application, the biochar treatments exhibited little effect on the Simpson indices of the abundant soil bacterial and fungal taxa, but significantly increased the Simpson index of the rare bacterial taxa ($p < 0.05$), thereby proving a superior effect of TP than that of PB (Figure 2). Interestingly, the diversity of the rare microbes was higher than that of the abundant microbes in all treatments. Because of the high diversity of the rare microbes, the rare microbial taxa may have increased the functional redundancy of the community, thereby providing a wider ecological buffer space to cope with Cd stress (Zhao et al., 2022). In terms of distribution, the rare and abundant bacterial taxa were clearly separated given biochar treatments, and the separation in the TP group was more distinct (Figure 3), thus indicating an intensified reconstructing of the bacterial taxa by the TP treatment. The distributions of the rare and abundant fungal communities in the treatment groups were quite different, and

no clear clustering appeared. Biochar treatments may have little effect on the distribution and reconstructing the abundant and rare fungal taxa in the soil. Studies have shown that the rare microbial taxa can act as a microbial seed bank to safeguard the entire community (Dong et al., 2021; Xu et al., 2021). Our results demonstrated that the rare bacterial taxa (such as *cyanobacteria*) get activated by biochar, and thus maintained the bacterial community stability under pollution stress, which was not the case in the group with no biochar application. From the results of CCA analysis (**Figure 6**), it can be seen that the soil Cd was the primary factor affecting the distribution of the abundant and rare microbial communities in soil, especially the leached Cd content and residual Cd content in the soil. In addition, pH and SOM significantly affected the distribution of rare and abundant bacterial taxa. The applications of PB and TP increased the pH and buffering capacity of the soil, which further promoted the passivation of Cd and reduced its stress on microorganisms. The organic matter in biochar provided a nutrient-rich soil environment for microorganisms, especially TP, which greatly improved the soil nutrient status, thereby providing a good living environment for the abundant and rare microorganisms. After modification by biochar, the complexity of the microbial network increased, and the number and the types of core microorganisms was also elevated. The functional microorganisms such as *Proteobacteria* with pollutant degradation function (Banerjee et al., 2019) and *Actinomycetes* (Xu et al., 2017) with high metal resistance occupied the core positions of the network, maintaining the community stability under Cd stress.

Co-occurrence networks are an important means to explore the abundance patterns and internal relationships of the complex microbial communities (Kaya et al., 2020; Li et al., 2021). They display changes in the topology and characteristics of the rare and abundant microbial networks and serve to be a remarkable tool for the in-depth understanding of the stability of complex ecosystems. In the present study, the co-occurrence networks of microorganisms in all groups exhibited a power-law distribution, indicating that the networks were non-random and scale-free (**Figure 4**). Compared with the CK group, under the action of biochar in the PB and TP treatments, the abundant and rare taxa networks of bacteria and fungi were more complex, indicating that the contribution and tightness of the microbial networks were higher under the action of biochar. The frequent and diversified coupling relationship between the microorganisms provided a better buffer to the microorganisms to cope the Cd stress (Zhu et al., 2022b). Topological characteristics such as the degree of the nodes, betweenness, and closeness centrality suggested that the TP-modified microbial networks indicated a more complicated coupling between the rare and abundant taxa of bacteria and fungi. The reduction of TP in the leached Cd content and Cd acid-soluble state was higher than that in the PB and CK groups. The soil pH and available nutrients caused by TP created a good living environment to the soil microorganisms. Therefore, after the application TP, the complexity of the networks of the rare and abundant taxa increased, and the network structure was more organized. Furthermore, the application of biochar increased the number of core microorganisms in the network. In the bacterial networks, rare species of microorganisms accounted for a higher proportion of core microorganisms, such as *Proteobacteria, Actinobacteriota, Gemmatimonadota, Myxococcota, Chloroflexi, Patescibacteria, Bacteroidota*, and *Bdellovibrionota* (**Supplementary Figure 1**). Some of them were related to plant growth promotion and tolerance enhancement, such as *Gemmatimonadota* (Qiu et al., 2021), while some were associated with the metal-sensitivity, such as *Bacteroidota*. In the fungal networks, except for the TP treatment, most of the core microorganisms were abundant such as *Ascomycota* and *Basidiomycota* species. The phylum of *Ascomycota* was able to preferentially grow on the carbon-rich refractory materials and decompose cellulose, lignocellulose, and chitin in the soil. The main function of *Proteobacteria* is to promote the fixation of organic nitrogen and improve the adaptability to complex environments, which plays an important role in maintaining soil ecosystem functions. *Proteobacteria* is an abundant aerobic bacteria, which could degrade various pollutants and promote oxidative enzymes. Banerjee et al. (2019) have shown *Proteobacteria* have strong metabolic characteristics and environmental adaptability, and play an important role in immobilizing heavy metals and maintaining ecosystem functions. *Actinomycetes* have thicker peptidoglycan layers, which could provide high metal resistance (Xu et al., 2017). The phylum *Glomeromycota* has been reported that they could form a symbiotic relationship with plant roots, which bring about the increasing tolerance of plants to heavy metals (Lee et al., 2018). These microorganisms might be furtherly used to promote the stabilization of heavy metals in the soil, and enhance the fertility of the reclaimed soil, as well as speed up the agrochemical process of the soil in the mining area. It is worthy to mention that in the interaction networks between the bacteria and fungi, we found that most positions of the core microorganisms were occupied by fungi, while bacteria only occupied a small part. This indicated that under biochar modification, the fungal community can play a bigger role than bacteria in maintaining the stability of the microbial network, especially the abundant fungal community. The application of biochar can thus be beneficial to the formation of a more developed and healthy soil system, and it may serve as an important technical means to alleviate and solve the Cd stress in mining areas.

Conclusion

Understanding the interactions between microbial taxa is very important for the reclamation of heavy metal polluted

soils with biochar in mining areas. In the present study, the applications of PB and TP improved the physicochemical properties and enzymatic activities of the reclaimed soil in the mining area, and effectively reduced the soil Cd availability. Both PB and TP rebuilt the abundant and rare microbial communities in the Cd-contaminated soils. TP performed better in improving the diversity, structure and distribution patterns of the soil abundant and rare bacterial and fungal communities than PB. The network topology characteristics showed that the co-occurrence networks of bacteria and fungi modified by biochar exhibited a higher complexity and stability than that of CK, as well as increased the number and types of core microorganisms. The taxa that accounted for the majority of the core microorganisms in the bacterial and fungal networks, such as *Proteobacteria, Actinobacteria, Gemmatimonadota, Bacteroidota*, and *Basidiomycota* occupied the core hubs of the network and improved the resistance of the microbial communities to Cd stress. Our study can provide a technical support for the engineering application of biochar in the *in-situ* remediation of heavy metal pollution in mining areas.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/**Supplementary material**.

Author contributions

FC, YZ, and JM collected the samples. YZ, YY, and YC performed the experiments. YZ performed the data analyses and wrote the manuscript. FC, JM, XG, and LW helped to perform the analysis with constructive discussions. FC performed the supervision, project administration, and funding acquisition. All authors contributed to the revisions during the editing process and read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2022.972300/full#supplementary-material

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Responses of microbial necromass carbon and microbial community structure to straw- and straw-derived biochar in brown earth soil of Northeast China

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Soil microbial organisms are conducive to SOC sequestration. However, little attention has been given to the contributions of living MBC and microbial necromass carbon to the SOC pool under biochar and straw amendments. The aims of the study were to explore (1) the effects of maize straw and biochar on MBC, POC, MAOC, DOC and microbial necromass carbon; (2) the contribution of MBC and microbial necromass carbon to the SOC pool; and (3) the relationships among the soil microbial community structure, microbial necromass carbon and other SOC fractions under maize straw and biochar application for nine consecutive years. Three treatments were studied: CK (applied chemical fertilizer only), BC (biochar applied annually at a rate of 2.625 t ha^{-1} combined with chemical fertilizer), and SR (straw applied annually at a rate of 7.5 t ha^{-1}). Both biochar and straw increased the SOC contents after nine successive maize plant seasons; the DOC and MAOC contents were also increased by biochar and straw amendments. Biochar had advantages in increasing POC contents compared to straw. Biochar and straw increased MBC contents by 48.54% and 60.83% compared to CK, respectively. Straw significantly increased the Galn, GluN, MurA, ManN and total amino contents ($P < 0.05$); however, biochar significantly increased the Galn and GluN contents ($P < 0.05$) but had no impact on the MurA contents and decreased the ManN contents. Biochar mainly increased the fungal-derived necromass carbon contents but had no effect on the bacterial-derived necromass carbon, and straw increased both the bacterial- and fungal-derived necromass carbon contents. Straw had no influence on the ratios of microbial necromass carbon accounting for SOC and MAOC, but biochar decreased the ratios in the

current study. Similarly, biochar mainly increased the fungal PLFA and total PLFA contents compared to CK, but straw increased bacterial PLFAs, fungal PLFAs and Actinomycetes PLFAs. Maize yield were increased by 7.44 and 9.16% by biochar and straw application, respectively. These results indicate that biochar stimulates fungal activities and turnover to contribute to the stable soil carbon pool and that biochar also improves POC contents to improve the soil organic carbon sink.

KEYWORDS

biochar, straw, microbial necromass carbon, phospholipid fatty acids, carbon sequestration

Introduction

The soil organic carbon (SOC) pool is the largest terrestrial carbon pool compared to the atmospheric and vegetation carbon pools (Stockmann et al., 2013). SOC concentration and storage are vital to soil fertility, climate change mitigation and food security (Lal, 2006, 2016). SOC can be separated from stable and active carbon pools by its nature. However, the mechanisms of the stable carbon pool still need to be elucidated (Blankinship et al., 2018).

The SOC storage mechanisms could be identified *via* two main methods for a long time due to physical protection by aggregates from microbial organisms and chemical protection by mineral materials through organo-mineral complexes (Han et al., 2016). Soil aggregates could successfully use barriers to keep SOC from being accessible to microorganisms (Six et al., 2004). Soil aggregates can be divided into macroaggregates (>250 μm) and microaggregates (53–250 μm) by their sizes, formation mechanism and properties (Elliott, 1986). Mineral-associated organic carbon (MAOC) is formed by mineral components and SOC molecules *via* ligand exchange or van der Waals forces (Bai et al., 2018). Although previous studies have researched aggregates and MAOC, no obvious ground for their formation or composition has been found (Blankinship et al., 2018).

SOC can be divided into microbial-derived and plant-derived SOC by its origin (Angst et al., 2021). In general, plant residue is considered the main source of SOC formation, and soil microbes act as decomposers but do not contribute (Kogel-Knabner, 2017). The plant-derived SOC has been decomposed by soil microbial organisms from the plant residues. Soil microbial organisms can also utilize plant residues by biosynthesis for growth. Ultimately, the carbon contained in the microbial necromass enters the soil carbon pool by the entombing effect (Liang et al., 2017; Liang, 2020). The current consensus is that microbial-derived materials play a vital role in stabilizing the SOC pool (Kallenbach et al., 2016; Cotrufo et al., 2019). Although microbial biomass turnover is fast in soils, the proportion of microbial biomass carbon constitutes only a small fraction of SOC. Microbial necromass carbon is seen as a fraction of the stable carbon pool (Liang et al., 2017). Amino sugars are typical biomarkers that contain substances in the cell walls of microbial necromass. Amino sugar measurements are important detection methods for researching the presence of soil microbial necromass (Amelung et al., 2008). Amino sugars are almost negligible in plant residues. Amino sugars in soil are very complicated; the most important categories of amino sugars comprise glucosamine (GluN), muramic acid (MurA), mannosamine (ManN) and galactosamine (GalN) (Zhang and Amelung, 1996). Specifically, MurA is a typical marker of bacterial necromass, as it only occurs in bacterial cell walls; chitin in fungal cell walls is the major component of GluN; and GluN is also found in bacterial necromass (Wang et al., 2021). The origin of GalN is still unclear, so GalN is frequently considered nonspecific. ManN also originates from bacteria and fungi, but distinguishing its origin from bacteria or fungi is still difficult (Liang et al., 2007). In recent years, researchers have recognized the importance of the contributions of microbial necromass to the stable SOC pool, which might be more than 50% in croplands (Wang et al., 2021).

SOC contents are indicators of soil productivity and sustainability because SOC acts as the carbon source for microbes and is critical for retaining soil fertility and productivity (Lal, 2016). Maintaining SOC contents at a relatively high level is essential for maintaining soil fertility and productivity. Soil microbial necromass carbon could contribute to half of the SOC contents in the global cropland (Wang et al., 2021). Therefore, microbial necromass carbon also plays an important role in remaining soil fertility and productivity. Consecutive maize straw mulching has been shown to increase both maize yield and soil microbial necromass carbon contents

Abbreviations: SOC, soil organic carbon; MBC, microbial biomass carbon; POC, particulate organic carbon; DOC, dissolved organic carbon; MAOC, mineral-associated organic carbon; Galn, galactosamin; GluN, glucosamine; MurA, muramic acid; ManN, manosamine; PLFAs, phospholipid fatty acids; MWD, mean weight diameter; GMD, geometric mean diameter.

in a previous study (Liu et al., 2020). To date, no obvious evidence has shown the linkage between microbial necromass carbon and maize growth. The accumulation of microbial necromass carbon could increase the soil stable carbon pool as a result of the entombing effect. This would be beneficial for SOC storage, and higher SOC would be better for soil quality and productivity. Northeast China is situated at one of the golden maize belts and is a main grain-producing area. Intensive cultivation and a growing demand for food due to increased population have led to soil degradation and the decline in SOC (Lal, 2009). Suitable practices are needed to improve SOC and maintain soil fertility. Straw returning is an effective way to enhance soil fertility and SOC contents (Zhao et al., 2018; Tian et al., 2020). However, straw return would lead to more greenhouse gas emissions and adverse carbon neutralization. Therefore, turning straw resources into biochar is an effective method for carbon storage. Biochar is a carbon-rich solid product produced by biomass *via* pyrolysis and oxygen-limited conditions (Chen et al., 2019). Biochar usually contains a large amount of carbon, and the carbon in biochar is mainly aromatic carbon (Chen et al., 2019). This extremely stable carbon has stayed in soils from millennial to centennial timescales. At the very beginning of the study, biochar was used as a soil amendment to enhance soil organic carbon sequestration due to its large carbon sequestration potential (Bolan et al., 2021). However, biochar has multifunctional values beyond carbon storage in actual production, such as porous materials for mitigating greenhouse gas emissions, catalysts in industry, nanomaterials in industry, feed supplements in animals to improve animal health, and even immobilizing agents in organic contaminants and heavy metals in soil and water (Kumar et al., 2020; Bolan et al., 2021; Lin et al., 2022). Several studies have researched the effect of biochar on microbial necromass. A 34-month incubation study investigated the metabolic traits of microbial communities in aged biochar, and the results indicated that biochar has the potential to protect SOC by mediating bacterial metabolic capacities (Sun et al., 2016). Soil microbial activity can also be enhanced by the application rate of biochar doses, and the stability of microbial necromass is also well-maintained by biochar amendments (Zhang et al., 2021a,b). Previous studies have shown that biochar can stimulate soil microbial activity and improve MBC (Yang et al., 2017b; Fang et al., 2018; Dai et al., 2021). However, little attention has been given to the effect of biochar on living microbial organisms and dead necromass and the contribution of and relationship among MBC, microbial necromass carbon and SOC. The objectives of this study were to investigate (1) the effects of maize straw and biochar on soil MBC, POC, MAOC, DOC and microbial necromass carbon, (2) the contribution of MBC and microbial necromass carbon to the SOC pool and (3) the relationships among soil microbial community structure, microbial necromass carbon and other SOC fractions under maize straw and biochar application for nine consecutive years.

Materials and methods

Field experimental site and experimental design

The field experiment was conducted at the Shenyang Agricultural University field experiment station (41°49′N, 123°33′E) starting in May 2013. This station is situated in Northeast China, one of the three gold maize belts of the word. The climate is a warm continental monsoon climate. The frost-free period is ∼ 150 days. The entire growth season is ∼130–150 days. The annual average precipitation is approximately 705 mm, and the mean temperature is approximately 7.9°C. The soil type at this site is classified as Haplic Luvisols by WRB classification. The basic soil properties at the beginning of the experiment are shown in Yang et al. (2017a). The 9-year field experiment was conducted from May 2013 to October 2018. The field experiments included three treatments: CK (mineral NPK fertilizer applied only), BC (biochar applied annually at a rate of 2.625 t ha^{-1} together with mineral NPK fertilizer), and SR (maize stover returned at a rate of 7.5 t ha^{-1} together with mineral NPK fertilizer). The biochar application rate was according to the 35% inversion rate of maize stover biomass of 7.5 t ha^{-1} charred during pyrolysis. The mineral NPK fertilizer was applied at rates of urea (120 kg N ha^{-1}), calcium superphosphate (60 kg P_2O_5 ha^{-1}) and potassium sulfate (60 kg K_2O ha^{-1}). All fertilizers were applied once before sowing. The cropping pattern was continuous maize cropping. The area of each plot was 3.6 × 10 m^2. Three replicates of each treatment were arranged in a randomized block design.

Maize stover and biochar

The biochar applied in the study was produced by Jinhefu Agriculture Development Company, Liaoning, China. The pyrolysis conditions were ∼450°C, and the pyrolysis duration lasted 90 min. Maize stover was collected from the field and then broken down into pieces of 5–7 cm. Both biochar and maize stover were applied by hand on the soil surface. Subsequently, a rotary cultivator was used to uniformly mix the amendments with the soil. The initial properties of biochar and maize stover were detailed in Yang et al. (2017a).

Sampling and analysis

Topsoil (0–20 cm) was collected in early October 2021 after nine growing seasons. Undisturbed soil samples were collected in each treatment for soil aggregate separation. Undisturbed soil samples were collected by the profile method (dig a profile, cut the undisturbed soil to a vertical depth of 20 cm, and

then hold the samples in aluminum boxes) and from five randomly selected locations in all plots. Then, all samples from the same plot were mixed together and transported to the laboratory. Bumping was avoided to protect the undisturbed soil samples during transportation. During the air-drying process, the undisturbed soil samples were sieved through an 8 mm mesh. Then, the samples were stored for aggregate analysis. The wet-sieving method was used to measure water stable aggregate fractions (Elliott, 1986). Different aggregate fractions were separated by a series of sieves (2,000 μm, 250 μm and 53 μm). The four aggregate fractions were (1) >2,000 μm (large macroaggregates), (2) 250–2,000 μm (small macroaggregates), (3) 53–250 μm (microaggregate), and (4) <53 μm (silt+clay fraction). The detailed procedure is shown in Sun et al. (2021).

Bulk soil samples were also collected from each plot at the same time. To achieve representative samples, five random sampling points were chosen in each plot. Topsoil (0–20 cm) was abundantly mixed together adequately and then placed in sealable plastic bags. These bulk samples are transported to the laboratory and divided into two parts. One part of the samples was stored fresh to analyze soil MBC and PLFAs. The other samples were air-dried and stored for the detection of amino sugars and other chemical properties.

Determination of amino sugars

The amino sugar contents in soils were detected by the method described by Zhang and Amelung (1996). First, the air-dried soil samples were sieved through a 0.25 mm mesh. The sieved samples containing 0.3 mg N were hydrolyzed under N_2 conditions with 6 M HCl (10 min) at 105°C for 8 h. The hydrolysate was filtered and dried with an evaporator. The samples were dissolved in deionized water, and the pH was adjusted to 6.6–6.8 by KOH (1 M) and HCl (0.01 M) solutions. Next, the supernatant was collected for freeze-drying, and the precipitate was removed by centrifugation (10,000 g, 10 min). Using methanol to wash the residues to recover the amino sugars, these amino sugars were transformed into aldononitrile derivatives that were extracted by 1.5 ml dichloromethane solution. The amino sugar derivatives were dissolved in 300 μL hexane and ethyl acetate solvent (v:v = 1:1) for final analysis until the removal of dichloromethane by N_2. These amino sugar derivatives were separated on a Thermo ICS5000 ion chromatograph (ICS5000, Thermo Fisher Scientific, USA) equipped with a Dionex™ CarboPac™ PA20 column (150*3.0 mm, 10 μm). Soil total amino sugars were calculated by the sum of the MurA, GluN, ManN and Galn contents. MurA and GluN were used to calculate bacterial residue carbon and fungal residue carbon, respectively.

Analysis of phospholipid fatty acids

The soil PLFA method was used to analyze the composition of the soil microbial community (Frostegard and Baath, 1996). In brief, fresh soil samples were freeze-dried by a vacuum freeze dryer (Labconco* FreeZone). Freeze-dried soil (4 g) was extracted twice by a single-phase chloroform-methanol-citrate buffer (v:v:v = 1:2:0.8). All the supernatant was collected and mixed together as one sample. Then, chloroform and citric acid buffer were added, and the chloroform layer was separated after incubation overnight in the dark and dried with N_2 at 30°C. Phospholipids were separated into neutral lipids, glycolipids and phospholipids by standard solid phase extraction (SPE) tubes (6 mL, 500 mg, Supelco Inc., Pennsylvania, USA). Then, the phospholipids were methylated by 1:1 methanol-toluene and 0.2 M KOH solution to transform into their respective fatty acid methyl esters. Methyl non-adecanoate fatty acid (19:0) was set up as an internal standard to quantify the concentrations of phospholipids before quantitative analysis of phospholipid fatty acids. The fatty acid methyl esters were identified by gas chromatography (Agilent 7890A, USA) equipped with MIDI peak identification software (Version 4.5; MIDI Inc., USA). The microbial community composition was classified according to phospholipid fatty acid markers, phospholipid fatty acids 16:1ω7c, 17:0 cyclo ω7c, 18:1ω7c, 19:0 cyclo ω7c, 15:0 iso, 15:0 anteiso, 16:0 iso, 17:1 iso w9c, 17:0 anteiso were used as biomarkers for bacteria; 18:2ω6c, 18:1ω9c, 16:1ω5c for fungi; and the sum of PLFA content was used to represent the total microbial PLFAs (Klamer, 2004; Bach et al., 2010; Landesman and Dighton, 2010; Xu et al., 2022).

Determination of soil chemical properties and maize yield

One part of the fresh soil samples was used to determine the soil microbial biomass carbon. The determination of soil MBC was determined by the chloroform fumigation method (Vance et al., 1987). Briefly, fresh soil samples (equivalent to 10 g of oven-dried soil) were weighed in glass beakers. Then, the samples were fumigated and non-fumigated for 24 h at 25°C in the dark. After the fumigated and non-fumigated processes, all samples were extracted by K_2SO_4 solutions (0.5 M) immediately. After shaking and centrifuging all the extracted samples, the supernatant was filtered through a 0.22 μm filter and detected by a TOC analyzer (Multi C/N 3100, Analytik Jena, Germany). Soil organic carbon and aggregate-associated organic carbon were detected by an Elementar Vario max Analyzer (Elementer Macro Cube, Germany) after sieving through a 0.15 mm mesh. The soil organic carbon fraction was isolated by density fractionation as described by Fang et al. (2018). Soil DOC was extracted by deionized water as described by Dong et al.

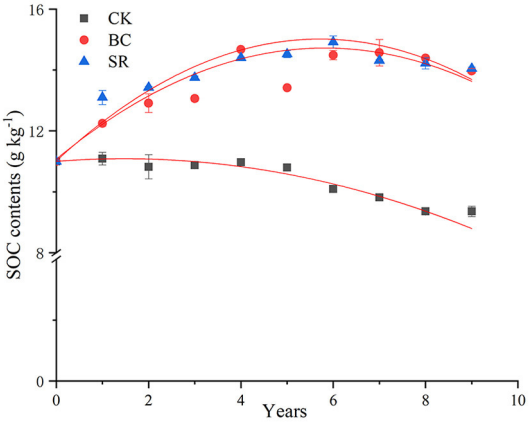

FIGURE 1
Dynamics of SOC contents during straw and biochar application for 9 consecutive years. The data of the first 3 years was cited from Yang et al. (2017a). Bars represent standard deviations ($n = 3$).

(2019). Briefly, 10 g air-dried samples were weighed in flasks, and 50 ml deionized water was added to all flasks. All flasks containing soil samples were placed on a shaker and shaken at 230 rpm for 30 min. Then, all flasks were centrifuged at 4,000 × g for 40 min. The supernatant was filtered through a 0.45 μm filter and analyzed. All filtered supernatants were detected by a TOC analyzer (Multi C/N 3100, Analytik Jena, Germany). POC and MAOC were separated by a 1.8 g cm^{-3} sodium iodide solution. Briefly, 10 g of air-dried soil samples (sieved through 1 mm mesh) were weighed in one plastic centrifuge tube, and then 50 mL of 1.8 g cm^{-3} sodium iodide solution was added to the centrifuge tube. After shaking on a reciprocating shaker and centrifuging in a low-speed centrifuge, all the supernatant with floating particles was collected and filtered by a glass-fiber filter. The NaI solution was collected for reuse. This process was repeated twice as shown before. The floating samples that were filtered were washed with deionized water three times, and this fraction was POC. The residues in the centrifuge tube were also washed with deionized water three times to remove the residue NaI. The residue fraction in the centrifuge tube was MAOC. All POC and MAOC samples were oven-dried at 60°C until constant weight. All samples were weighed and stored for analysis. The POC and MAOC were also measured by an Elementar Vario max Analyzer (Elementer Macro Cube, Germany). At harvest, all ears of maize plants in the middle two rows in each plot were collected to measure the maize yield.

Calculations and statistical analysis

Soil aggregate stability is traditionally expressed by the mean weight diameter (MWD), geometric mean diameter (GMD) and macroaggregates ($R_{>250\mu m}$) (Mazurak, 1950; van Bavel, 1950). The calculation equation is displayed as follows:

$$MWD = \frac{\sum_{i=1}^{n} xiWi}{\sum_{i=1}^{n} Wi} \quad (1)$$

$$GMD = EXP\left[\frac{\sum_{i=1}^{n} milnxi}{\sum_{i=1}^{n} mi}\right] \quad (2)$$

where xi is the average diameter of every aggregate fraction, Wi is the weight percentage of every aggregate fraction, and mi is the weight of different aggregate fractions.

Soil MBC was calculated by the following equation:

$$MBC\ (mg\ kg^{-1}) = \frac{\text{Extracted } C_{fumigated\ soil} - Extracted\ C_{non-fumigated\ soil}}{K} \quad (3)$$

where K is a correction factor of 0.45 (Vance et al., 1987).

$$fungal\ necromass\ carbon\ (mg\ g^{-1}) = \left(GluN/179.17 - 2 \times mmol\ MurA/251.23\right) \times 179.17 \times 9 \quad (4)$$

$$bacteria\ necromass\ carbon\ \left(mg\ g^{-1}\right) = MurA(mg\ g^{-1}) \times 45 \quad (5)$$

where 179.2 is the molecular weight of GluN, 251.23 is the molecular weight of MurA, 9 is the conversion coefficient of fungal GluN to fungal necromass carbon, and 45 is the conversion coefficient from MurA to bacterial necromass carbon in equation 5 (Appuhn et al., 2006).

All data were processed by Office Excel 2016 and are expressed as the mean ± standard deviation. One-way analysis of variance (ANOVA) was used to test the differences among treatments. Multiple comparisons were performed by the least significant difference (LSD) method using IBM SPSS 22.0 software (New York, USA). All figures were generated by Origin 2022 software (Origin Lab Inc., Northampton, USA).

Results

Effects of maize straw and straw biochar on SOC contents

As shown in Figure 1, from 2013 to 2018, the SOC contents in the topsoil layer (0–20 cm) showed different dynamics. BC and SR enhanced the SOC content with annually applied organic materials, but the SOC contents decreased in the CK treatment compared to the initial level during the field experiment. The SOC level dynamics exhibited two separate stages in BC and SR, with a rapid accumulation stage (2013–2016) and a slow fluctuation stage (2017–2021).

TABLE 1 Effect of straw and straw biochar on SOC fractions.

Treatments	DOC ($mg\ kg^{-1}$)	MBC ($mg\ kg^{-1}$)	POC ($g\ kg^{-1}$)	MAOC ($g\ kg^{-1}$)
CK	34.16 ± 2.99b	114.86 ± 14.71b	2.65 ± 0.01c	6.55 ± 0.16c
BC	73.99 ± 14.09a	170.62 ± 9.89a	5.36 ± 0.10a	8.42 ± 0.19b
SR	89.74 ± 4.96a	184.73 ± 13.39a	4.57 ± 0.10b	9.22 ± 0.10a

Different lowercase letters indicate significant differences (P<0.05) among different treatments. Data are means ± standard deviations, n = 3. SOC, soil organic carbon; MBC, microbial biomass carbon; POC, particulate organic carbon; DOC, dissolved organic carbon; MAOC, mineral-associated organic carbon.

TABLE 2 The proportions of different SOC fractions accounting for SOC contents.

Treatments	DOC/SOC	MBC/SOC	POC/SOC	MAOC/SOC
		%		
CK	0.36 ± 0.03b	1.23 ± 0.14a	28.36 ± 0.51c	70.01 ± 0.51a
BC	0.53 ± 0.10a	1.22 ± 0.07a	38.40 ± 0.93a	60.28 ± 0.99b
SR	0.64 ± 0.03a	1.32 ± 0.10a	32.51 ± 0.20b	62.61 ± 0.29b

Different lowercase letters indicate significant differences (P < 0.05) among different treatments. Data are means ± standard deviations, n = 3.

TABLE 3 Effect of straw and straw biochar on soil amino sugars.

Treatments	GalN ($mg\ kg^{-1}$)	ManN ($mg\ kg^{-1}$)	GluN ($mg\ kg^{-1}$)	MurA ($mg\ kg^{-1}$)	Total amino sugars ($mg\ kg^{-1}$)
CK	400.43 ± 0.51c	123.02 ± 0.50b	432.23 ± 0.12c	27.67 ± 0.50b	983.36 ± 1.04c
BC	481.68 ± 0.69b	114.88 ± 0.18c	538.31 ± 0.92b	27.24 ± 0.48b	1,162.11 ± 1.83b
SR	567.12 ± 0.31a	134.36 ± 0.70a	669.56 ± 0.33a	32.31 ± 1.20a	1,403.35 ± 0.71a

Different lowercase letters indicate significant differences (P < 0.05) among different treatments. Data are means ± standard deviations, n = 3. Galn, galactosamin; GluN, glucosamine; MurA, muramic acid; ManN, manosamine.

Effects of maize straw and straw biochar on SOC fractions

In this study, biochar and straw amendments caused significant changes in different SOC fractions after 9-year field experiments (Table 1). BC and SR enhanced the DOC contents by 116.59 and 162.70%, respectively. The proportions of DOC accounting for SOC also increased in the BC and SR groups compared to the CK group (Table 2), and the ratio of DOC/SOC followed the trend SR = BC>CK, which indicated that the ratio of DOC/SOC in the BC and SR groups was significantly higher than that in the CK group.

Compared to that in the CK group, the MBC contents increased by 48.54% and 60.83% in the BC and SR treatments, respectively. The contents of MBC in the BC and SR groups were non-significantly different after the 9-year field experiment ($P > 0.05$). The ratio of MBC/SOC was non-significantly different among the three treatments ($P > 0.05$).

BC and SR significantly enhanced soil POC (Table 1). The soil POC contents increased by 102.09 and 72.19% in the BC and SR groups, respectively. The proportions of POC accounting for SOC followed the trend BC>SR>BC, which indicated that the ratio of POC/SOC was higher in the BC group than in the SR and CK groups.

The MAOC contents were significantly enhanced by the BC and SR treatments after the 9-year field study (Table 1). The MAOC contents followed the trend SR>BC>CK. The BC and SR treatments increased the MAOC contents by 28.49 and 40.70%, respectively. The MAOC/SOC ratio followed the trend of CK>SR=BC, which indicated that the MAOC proportion of SOC was higher in the CK group than in the BC and SR groups (Table 2).

Effects of maize straw and straw biochar on soil amino sugars and microbial necromass carbon

After the 9-year field experiment, soil total amino sugars and different amino sugars showed different trends (Table 3). The GalN content was the highest in the SR group among the three treatments, followed by the BC treatment and the CK group. Compared to that in the CK group, the content

TABLE 4 Effect of straw and straw biochar on soil microbial necromass carbon contents.

Treatments	Bacterial-derived carbon g kg^{-1}	Fungal-derived carbon g kg^{-1}	Microbial necromass carbon g kg^{-1}
CK	1.24 ± 0.10b	3.53 ± 0.02c	4.78 ± 0.15c
BC	1.23 ± 0.10b	4.50 ± 0.03b	5.72 ± 0.20b
SR	1.45 ± 0.01a	5.61 ± 0.10a	7.07 ± 0.12a

Different lowercase letters indicate significant differences ($P < 0.05$) among different treatments.

TABLE 5 The ratio of microbial necromass carbon accounting for SOC and MAOC.

Treatments	Microbial necromass carbon/SOC %	Microbial necromass carbon/MAOC%
CK	51.08 ± 0.92a	72.98 ± 1.84a
BC	40.96 ± 0.25b	67.97 ± 1.51b
SR	50.28 ± 0.38a	76.64 ± 0.89a

Different lowercase letters indicate significant differences ($P < 0.05$) among different treatments.

of GalN increased by 20.29 and 41.63% in the BC and SR groups, respectively. The ManN content followed the trend SR>CK>BC. The ManN content was highest in the SR group, followed by the CK group and the BC group. The GluN content had the same trend as the GalN content, which followed the trend SR>BC>CK; the BC and SR treatments enhanced the GluN content by 24.54 and 54.91%, respectively. BC treatment had no effect on the MurA content after the 9-year field experiment, SR treatment significantly enhanced the MurA content in the current study, the MurA content was significantly higher in the SR group than in the BC and CK groups ($P < 0.05$), and no significant difference was observed in the MurA content between the CK and BC groups ($P > 0.05$). The soil total amino sugar content was calculated by the contents of the above amino sugars; the total amino sugar content was highest in the SR group, followed by the BC group and the CK group. Compared to that in the CK group, the total amino sugar content increased by 18.18 and 42.71% in the BC and SR groups, respectively.

Bacterial-derived carbon and fungal-derived carbon are shown in Table 4. After the 9-year field experiment, no significant differences in bacterial-derived carbon content were observed between the BC and CK groups ($P > 0.05$), the bacterial-derived carbon content in the SR group was significantly higher than that in the BC and CK groups ($P<0.05$), and the bacterial-derived carbon content in the SR group increased by 16.80 and 18.64% compared to that in the CK and BC groups, respectively. Both BC and SR treatment enhanced the fungal-derived carbon content compared to the CK group ($P < 0.05$). Compared to that in the CK group, the fungal-derived carbon content increased by 27.16% in the BC group and 58.74% in the SR group. Soil microbial necromass carbon was calculated by the sum of bacterial-derived carbon and fungal-derived carbon. The soil microbial necromass carbon followed the trend of SR>BC>CK. Compared to the CK treatment, the BC and SR treatments enhanced the microbial necromass carbon by 19.68 and 47.81%, respectively.

The ratio of microbial necromass carbon accounting for SOC and MAOC is shown in Table 5. We found that the ratio of microbial necromass carbon accounting for SOC and MAOC in the BC group was significantly lower than that in the CK and SR groups ($P < 0.05$). The difference in the ratio of microbial necromass carbon accounting for SOC and MAOC between the CK and SR groups was not significant ($P > 0.05$).

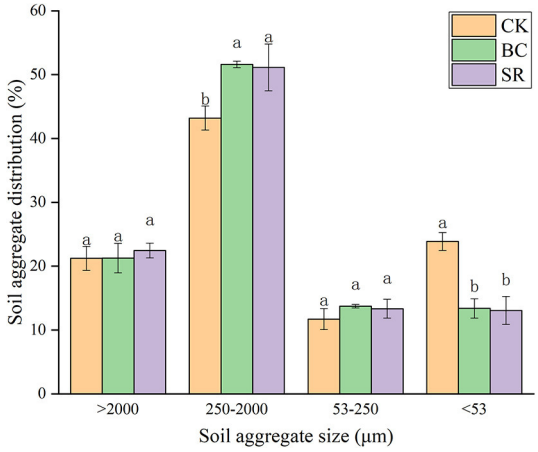

FIGURE 2
Effect of maize straw and straw-derived biochar on soil aggregate distribution in Year 2021. The error bars represent the standard deviations of the mean values ($n = 3$). Different lowercase letters indicate significant differences between the different treatments.

Effects of maize straw and straw biochar on soil aggregates

As shown in Figure 2, small macroaggregates (250–2,000 μm) dominated in brown earth, and the proportions of these small macroaggregates ranged from 43.20∼51.61%. The microaggregate proportions were the lowest of all the aggregate fractions, ranging from 11.71∼13.73%. No differences were observed in large macroaggregates and microaggregates among treatments ($P < 0.05$). Compared to that in the CK group, the proportion of small macroaggregates increased by 19.46 and 18.37% in the BC and SR groups, respectively. No differences were observed in the small macroaggregate fraction between the

TABLE 6 Effect of maize straw and straw-derived biochar on soil aggregate stability in 2021.

Treatments	MWD mm	GMD mm	Macroaggregates%
CK	1.57 ± 0.10a	0.50 ± 0.05b	64.42 ± 3.04b
BC	1.67 ± 0.11a	0.71 ± 0.07a	72.89 ± 1.78a
SR	1.72 ± 0.03a	0.74 ± 0.06a	73.60 ± 2.79a

The error bars represent the standard deviations of the mean values (n = 3). Different lowercase letters indicate significant differences between the different treatments. MWD, mean weight diameter; GMD, geometric mean diameter.

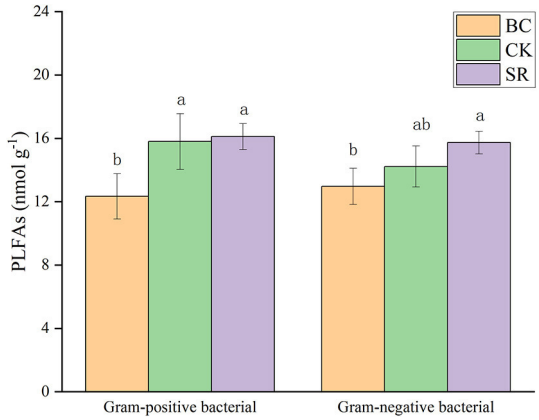

FIGURE 3
Effect of maize straw and straw-derived biochar on gram-positive and gram-negative microbial PLFAs. The error bars represent the standard deviations of the mean values (n = 3). Different lowercase letters indicate significant differences between the different treatments.

BC and SR groups ($P < 0.05$). Both the BC and SR treatments decreased the silt+clay fraction. Compared to that in the CK group, the silt+clay fraction decreased by 43.90 and 45.27% in the BC and SR groups, respectively.

The aggregate MWD and GMD in the BC and SR groups were higher than those in the CK group (Table 6). However, the difference in MWD among treatments was not significant ($P > 0.05$), the difference in GMD in the BC and SR groups was significantly higher than that in the CK groups ($P < 0.05$), and the BC and SR treatments increased the GMD by 42.24 and 47.58%, respectively. The macroaggregate content was also enhanced significantly by amendments ($P < 0.05$). Compared to that in the CK group, the macroaggregate content increased by 13.13 and 14.24% in the BC and SR groups, respectively.

Effects of maize straw and straw biochar on soil phospholipid fatty acids

As shown in Figure 3, soil total microbial PLFAs were significantly increased by biochar and straw application ($P <$ 0.05), but no difference was observed between the BC and SR groups ($P > 0.05$). Soil microbial PLFAs dominated in all treatments over fungal PLFAs and actinomycetes PLFAs (average of 29.26 vs. 7.34 and 9.01 nmol g^{-1}). Both BC and SR enhanced soil bacterial PLFAs, but only SR significantly enhanced microbial PLFAs ($P < 0.05$). Both BC and SR enhanced the soil fungal PLFA content, which followed the trend of SR>BC>CK. The soil actinomycete PLFA content showed the same trend as the bacterial PLFA content.

As shown in Figure 3, gram-positive bacterial and gram-negative bacterial PLFAs were significantly affected by biochar and straw application ($P < 0.05$). The BC and SR treatments significantly enhanced the gram-positive bacterial PLFAs ($P <$ 0.05), and only the SR treatment significantly enhanced the gram-negative PLFA content. No differences were observed between the BC and SR treatments for either gram-positive or gram-negative PLFA contents ($P > 0.05$).

The BC and SR treatments both significantly increased the fungal/bacterial PLFA ratio in the current study ($P < 0.05$)

TABLE 7 The ratios of total amino sugars to total phospholipid fatty acids (PLFAs) for different treatments.

Treatments	Fungi/Bacterial PLFA	Total amino sugars/total PLFAs
CK	0.22 ± 0.01b	25.49 ± 2.80a
BC	0.26 ± 0.01a	24.99 ± 2.66a
SR	0.27 ± 0.00a	27.82 ± 1.33a

The error bars represent the standard deviations of the mean values (n = 3). Different lowercase letters indicate significant differences between the different treatments.

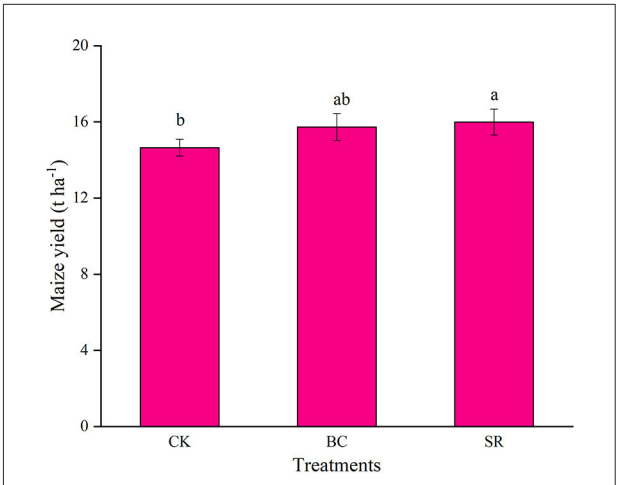

FIGURE 4
Effect of maize straw and straw-derived biochar on maize yield. The error bars represent the standard deviations of the mean values (n = 3). Different lowercase letters indicate significant differences between the different treatments.

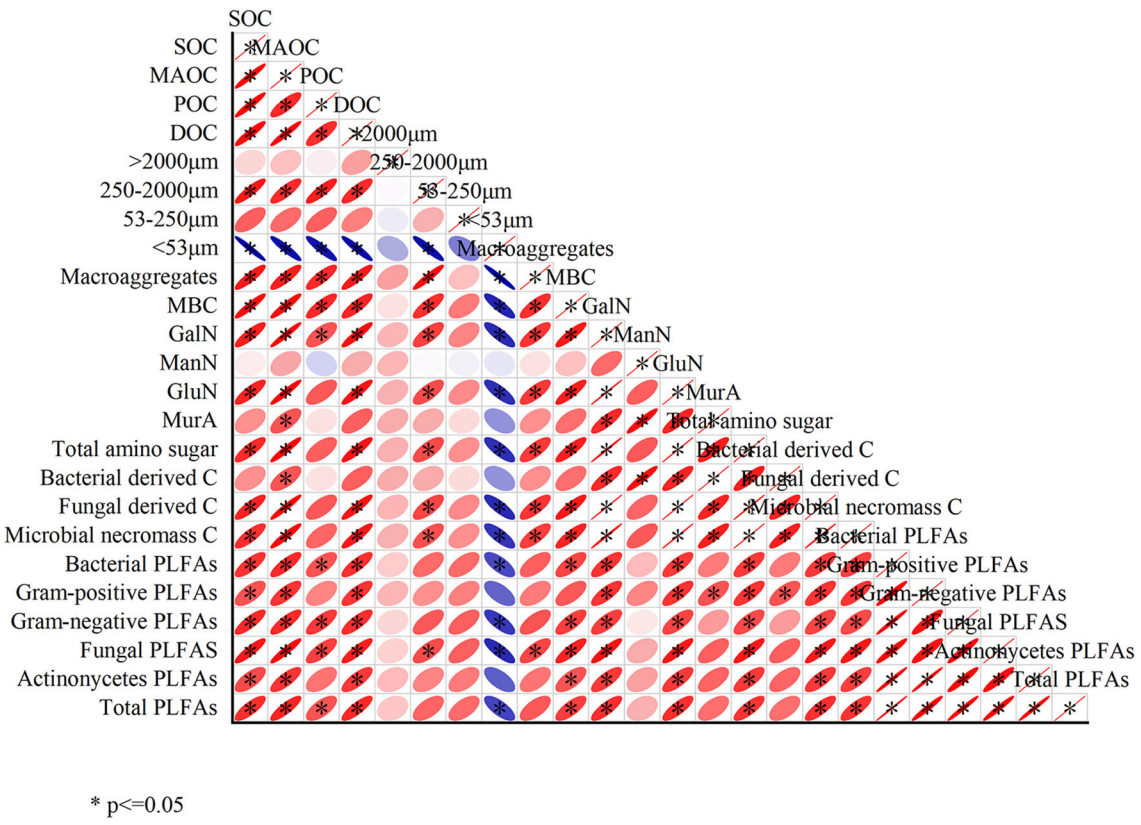

FIGURE 5
Relationships among different variables. *indicate significance at $p < 0.05$.

(Table 7). Compared to that in the CK group, the ratio of fungal/bacterial PLFAs was increased by 14.96 and 20.95% in the BC and SR treatments, respectively. No significant differences in the ratio of total amino sugar/total PLFA contents were found among treatments ($P > 0.05$) (Table 7).

Effects of maize straw and straw biochar on maize yields

As shown in Figure 4, maize yield were significantly increased by biochar and straw application ($P < 0.05$), but no difference was observed between the BC and SR groups ($P > 0.05$). Compared to CK, maize yield were increased by 7.44 and 9.16% in the BC and SR treatments, respectively. So biochar and straw could increase maize yield after nine-year field experiment.

Relationships among soil properties

The correlations among different variables are shown in Figure 5. Almost all the variables showed a positive correlation except the silt+clay fractions (<53 μm). SOC and MAOC were significantly positively correlated with GalN, GluN, total amino sugars, bacterial PLFAs, fungal PLFAs, actinomycetes PLFAs and total PLFAs ($P < 0.05$). The silt+clay fraction was significantly negatively correlated with SOC, MAOC, POC, DOC, macroaggregates, MBC, GalN, GluN, total amino sugars, fungal-derived C, microbial necromass carbon, bacterial PLFAs, fungal PLFAs, and total PLFAs.

The RDA results showed that the soil amino sugar content, microbial necromass carbon content and PLFA content were significantly related to the SOC and aggregate fractions (Figure 6). The environmental variables could explain 89.71% of the total variance.

Discussion

Long-term effects of maize straw and straw biochar on SOC dynamics and SOC fractions

Different straw management practices (such as straw return and biochar amendment) were useful ways to improve SOC

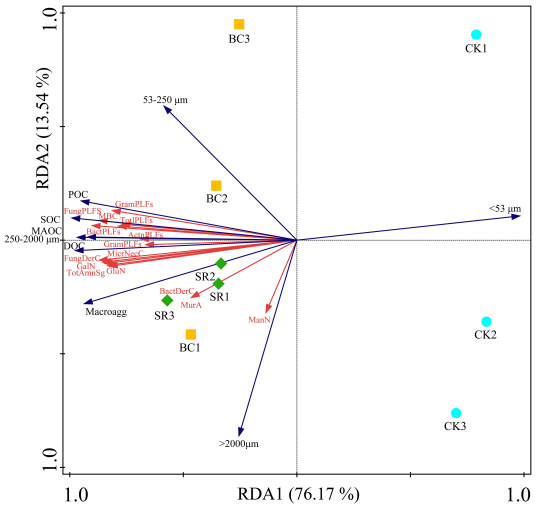

FIGURE 6
Redundancy analysis (RDA) showing the effect of different environmental variables on the microbial community.

TABLE 8 The fitting equations in different treatments.

Treatments	Fitting equation	R^2
CK	$y = 11.0+0.1188x-0.0404x^2$	0.98
BC	$y = 11.0+1.4036x-0.1229x^2$	0.96
SR	$y = 11.0+1.2557x-0.1080x^2$	0.98

contents in the plow layer. The SOC content decreased during the 9-year field experiment in the CK group, indicating that continuous planting would deplete SOC. SOC dynamics are vital for global food and nutritional security due to their nutrient supplementation (Lal, 2016). In the current study, both biochar and straw enhanced SOC contents during the 9-year field experiment. In the first 4 years (2013–2016), the SOC content increased rapidly in the BC and SR treatments, followed by a slow fluctuation period (Table 8). The SOC content is regulated by carbon input and the mineralization process (Cotrufo et al., 2015). The amount of carbon input was different in the BC and SR treatments. In theory, the amount of carbon input in the SR and BC groups was 3.22 t annually and 1.73 t annually, respectively. The SOC content was not significantly different between the BC and SR groups after the 9-year field study. This result indicated that biochar has more advantages in improving SOC in farmland because of the high stability of biochar carbon (Dong et al., 2016).

In a recent framework, the SOC pool was divided into two carbon pools (POC and MAOC) by physical properties, which could be better for SOC accrual and persistence (Castellano et al., 2015; Cotrufo et al., 2015). MAOC refers to the organic molecules that combine with minerals or aggregate within highly stable fine microaggregates (Leuthold et al., 2022). The possible mechanisms of the formation of MAOC include hydrogen bonding, cation bridging, anion exchange, ligand exchange, coulombic attraction, and van der Waals forces (Bai et al., 2018). In the current study, both biochar and straw significantly enhanced the MAOC content, and the MAOC content in the SR group was significantly higher than that in the BC group (Table 1). The results indicated that biochar will inevitably participate in the biogeochemical process in the soil. In previous studies, biochar particles could be sufficiently associated with minerals based on their superficial functional groups (Chia et al., 2012; Burgeon et al., 2021). Straw decomposed by soil microorganisms can release polysaccharides and organic acids (Jastrow, 1996). These polysaccharides and organic acids play a positive role in the formation of MAOC (Choudhury et al., 2014). Our results also indicated that straw return plays a positive role in the formation of MAOC and soil aggregate stability. In general, soil POC usually refers to the primary SOC fraction composed of structural materials derived from plants or microorganisms that have undergone decomposition and fragmentation but little to no depolymerization (Leuthold et al., 2022). However, biochar as a soil amendment could also add biochar particles to soil conditions. Biochar particles (pyrolysis organic carbon) can be transformed from plants by thermal or combustion processes, and these biochar particles contain highly condensed aromatic rings (Lehmann and Joseph, 2015). Strictly speaking, the POC fraction in the biochar treatment was not the same as the traditional POC fraction. However, by using the physical separation method, POC and MAOC could be separated and accurately studied as different fractions according to their properties. We could sufficiently study the distribution of different SOC fractions in distinct physical fractions. In this study, the BC treatment had the highest POC content and proportions accounting for SOC compared to the CK and SR treatments. This result could be explained by the highly condensed aromatic properties of biochar carbon. The proportion of MAOC accounting for SOC in the CK group was the highest of all three treatments, and the proportion of POC accounting for SOC in the CK group was the lowest of all three treatments. This result was similar to that of previous studies, in which it was found that most organic carbon was stored in the MAOC fraction, especially in soils with low organic carbon contents (Cotrufo et al., 2019). Our results suggest that biochar and straw amendments could improve both the POC and MAOC contents. In previous studies, the SOC stock showed no significant increase in response to long-term continuous organic amendment inputs; this phenomenon is defined by carbon saturation (Six et al., 2002; Feng et al., 2014). Carbon saturation is mainly reflected by MAOC (Cotrufo et al., 2019). Thus, biochar has more potential than straw in carbon sequestration as it increases both MAOC and POC contents.

Soil DOC has been shown to have an extremely fast turnover rate and easy degradability, so DOC is crucial to SOC turnover

and CO_2 emissions (Vila-Costa et al., 2020). Studies of the effect of biochar amendment on soil DOC have revealed distinct results. Dong et al. (2019) suggested that biochar applied once has little effect on DOC contents and composition after a 5-year field experiment. Yang et al. (2017b) reported that biochar application decreases the DOC content compared to the CK treatment. Biochar could also enhance the DOC content in both acidic and neutral soils (Smebye et al., 2016). Straw return has a positive effect on improving the DOC content (Ye and Horwath, 2017; Gmach et al., 2020). The effect of biochar on the DOC content still requires further research. In this study, biochar amendment played a similar role as straw in improving the DOC content, as both biochar and straw increased the DOC content compared to that in the CK group. No significant difference in DOC content was found between the BC and SR groups. Straw decomposed by soil microorganisms could release small organic molecules to improve the DOC content; biochar also contains dissolved organic carbon, which could enhance the DOC content. The soil DOC content was determined by the input and output of soil organic C under various biogeochemical processes, such as decomposition, sorption and leaching (Bolan et al., 2011). Biochar had different effects on the DOC content, and these distinct results were attributed to the differences in biochar type, soil type, climate and cultivation management. In this study, the increase in DOC content by biochar could be explained by biochar enhancing soil microorganism activity and the MBC content, so more DOC could be released from SOC by soil microbial decomposition.

Effect of maize straw and straw biochar on soil aggregates

Soil aggregates are the basic units in soil and impact many soil functions because they determine nutritional element contents and spatial distribution, the interactions between the solid and liquid interphase, and heat flow and capacity (Yudina and Kuzyakov, 2019). Biochar applied as a soil amendment has led to inconsistent results in previous studies. Different results were usually attributed to the different biochar feedstocks, distinct soil types, experimental durations and environments. Straw return usually plays a positive role in soil aggregation. Because straw resources are easily decomposed by soil microbes, additional binding agents and biological binding agents are released during the decomposition process (Dai et al., 2019; Lian et al., 2022). Our study also showed the same trend as that shown in previous studies. Biochar also played a positive role in the soil aggregation process. Biochar as a soil amendment still contains large amounts of non-pyrolyzed organic residue, which can stimulate soil microbial activity (Wang et al., 2017). Biochar can also absorb labile organic carbon as the substrate of soil microbial organisms (Liang et al., 2010). Soil MBC is a kind of biological binding agent associated with the soil aggregation process (Guo et al., 2018). Biochar and straw both increased the MBC content in the current study. Increasing the MBC content *via* biochar application might be a way to increase the soil macroaggregate content and aggregate stability.

Effect of maize stover and straw biochar on soil necromass carbon and PLFAs

In this study, organic amendments increased amino sugars after consecutive crop seasons, except for the ManN content (Table 3). Both biochar and straw could provide substrates for soil microbes and improve microbial activity Yang et al. (2017b). The organic amendments increased the MBC content in this study. Both biochar and straw increased the GalN and GluN contents in the order of SR>BC>CK. The DBX and GluN contents had high proportions of the total amino sugar content, which indicated that fungi were more impacted than bacteria by the organic amendments. GalN was once thought to be closely related to bacterial-derived carbon (Joergensen et al., 2010); however, fungi could contribute more GalN to the total amino sugar content than bacteria under some conditions (Engelking et al., 2007). Further research is needed to quantify the origin of GalN in the future. GluN mainly exists in chitin, and the decomposition of chitin was much slower than that of MurA (Ding et al., 2013), so the fungi-derived carbon content was higher than the bacterial-derived C content. Biochar had no significant effect on the MurA content compared to the CK treatment, but straw amendment significantly increased the MurA content. This result could be explained by the difference in the metabolisms of microbial communities or decomposition rates of MurA in the BC and SR treatments. Microbial necromass carbon is an important component contributing to the stable SOC pool (Liang et al., 2017). In the current study, biochar and straw both enhanced fungi-derived carbon and total microbial necromass carbon. Biochar had no effect on the bacterial-derived carbon contents compared to CK, and straw still increased the bacterial-derived carbon compared to the CK and biochar amendments (Table 4). Biochar amendment decreased the proportion of microbial necromass carbon accounting in SOC and MAOC, but no differences were observed between the straw amendment and CK treatment (Table 5). The proportions of microbial necromass carbon accounting for the SOC pool varied from 40.96 to 51.08%, and these results showed that microbial necromass carbon accounted for almost 50% in the current study. Although biochar had the potential to increase the microbial necromass carbon concentration, biochar amendments promoted carbon sequestration by POC and microbial necromass carbon in this study.

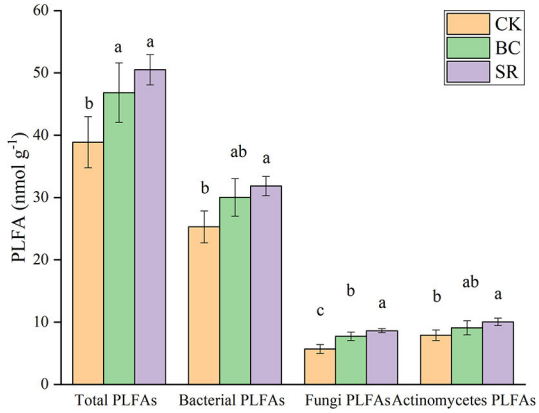

FIGURE 7
Effect of maize straw and straw-derived biochar on soil PLFA. The error bars represent the standard deviations of the mean values ($n = 3$). Different lowercase letters indicate significant differences between the different treatments. PLFA, phospholipid fatty acid.

Both biochar and straw as soil amendments increased the total PLFAs (Figure 7). These results indicated that biochar and straw could improve soil microbial activity and were significantly changed by organic amendments. Bacterial PLFAs were more abundant than other PLFAs (fungi, actinomycetes). This result was inconsistent with the microbial necromass carbon. Fungi-derived carbon was dominant in the soil microbial necromass carbon, but the bacterial PLFA content was dominant in the soil PLFAs. This result could be because the fungi-derived carbon was hard to decompose, but the bacterial-derived carbon was easy to decompose (Ding et al., 2013), as the cell walls of fungi were more recalcitrant than bacterial cell walls (Baldock and Skjemstad, 2000). Our results are similar to those of previous studies (Li et al., 2015).

Conclusion

This study demonstrated that both biochar and straw had a positive effect on SOC dynamics and different SOC fractions after a 9-year field experiment. Biochar had the advantage of improving the POC content, but straw had the advantage of improving the MAOC. This result is due to the stable properties of biochar. Biochar and straw both increased the DOC and MBC contents, but no differences were observed between biochar and straw in DOC and MBC. This result indicates that biochar and straw could increase both the labile organic carbon fractions and microbial activity. Both biochar and straw increased the fungi-derived necromass carbon, total necromass carbon, fungi PLFAs and total microbial PLFAs. Biochar had no significant effect on the bacterial-derived necromass carbon and bacterial PLFAs. Maize yield increased by 7.44% and 9.16 after biochar and straw application for 9 years. Compared to straw, biochar could improve SOC mainly by fungal-derived necromass carbon and POC in the field.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

QS: conceptualization, data curation, formal analysis, investigation, methodology, resources, software, and writing-original draft. XY: conceptualization, data curation, formal analysis, and resources. ZB: formal analysis and investigation. JG: data curation and software. JM: supervision, writing–review and editing, project administration, and funding acquisition. XH, ZL, WC, and YL: supervision and writing-review and editing. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Contrasting effects of maize litter and litter-derived biochar on the temperature sensitivity of paddy soil organic matter decomposition

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Organic matter input regulates the rate and temperature sensitivity (expressed as Q_{10}) of soil organic matter (SOM) decomposition by changing microbial composition and activities. It remains unclear how the incorporation of litter-made biochar instead of litter affects the Q_{10} of SOM decomposition. Using a unique combination of two-and three-source partitioning methods (isotopic discrimination between C3/C4 pathways and ^{14}C labeling), we investigated: (1) how maize litter versus litter-made biochar (of C4 origin) addition influenced the Q_{10} of SOM (C3 origin) under 10°C warming, and (2) how the litter or biochar amendments affected the Q_{10} of ^{14}C-labeled fresh organic matter (FOM) after long-term incubation. Compared with biochar addition, litter increased the rates and Q_{10} of mass-specific respiration, SOM and FOM decomposition, as well as the contents of SOM-derived dissolved organic C (DOC) and total phospholipid fatty acids (PLFA). Litter-amended soils have much higher activities (V_{max}) of β-glucosidase, N-acetyl-β-glucosaminidase, and leucine aminopeptidase, suggesting larger enzyme pools than in soils with biochar. The Q_{10} of enzyme V_{max} (1.6–2.0) and K_m (1.2–1.4) were similar between litter- and biochar-amended soils, and remained stable with warming. However, warming reduced microbial biomass (PLFA) and enzyme activity (V_{max}), suggesting decreased enzyme production associated with smaller microbial biomass or faster enzyme turnover at higher temperatures. Reductions in PLFA content and enzyme V_{max} due to warming were larger in litter-amended soils (by 31%) than in the control and biochar-amended soils (by 4–11%), implying the active litter-feeding microorganisms have a smaller degree of heat tolerance than the inactive microorganisms under biochar amendments. The reduction in enzyme activity (V_{max}) by warming was lower in soils with biochar than in the control soil. Our modeling suggested that the higher Q_{10} in litter-amended soils was mainly caused by faster C loss under warming, linked to

reductions in microbial biomass and growth efficiency, rather than the slightly increased SOM-originated substrate availability (DOC). Overall, using straw-made biochar instead of straw *per se* as a soil amendment lowers the Q_{10} of SOM and FOM by making microbial communities and enzyme pools more temperature-tolerant, and consequently reduces SOM losses under warming.

KEYWORDS

priming effects, warming, three-source partitioning, enzyme Michaelis–Menten kinetics, phospholipid fatty acid, biochar

Introduction

Climate warming, concomitant with rising atmospheric CO_2 concentration, is projected to elevate the Earth's temperature by 1.5–3.5°C by 2100 (IPCC, 2022). It is highly uncertain whether warming will accelerate the transfer of the enormous global soil C stock to the atmosphere, which implies a positive feedback between climate and the terrestrial C cycle (Davidson and Janssens, 2006). Estimation of the temperature sensitivity of soil organic matter (SOM) decomposition (usually expressed as Q_{10}, the factor by which the decomposition rate increases with a 10°C temperature rise) is therefore critical to future climate projections (Jones et al., 2005; Todd-Brown et al., 2013). The Q_{10} of SOM decomposition is partly determined by substrate availability (Gershenson et al., 2009; Pang et al., 2015), which in turn is controlled by soil C stabilization mechanisms (Conant et al., 2011). Further, Q_{10} is tightly linked to microbial decomposer characteristics, such as C use efficiency (CUE) and extracellular enzyme kinetics (Allison et al., 2010; Bradford, 2013).

A major microbial regulation over SOM decomposition is through catalysis by extracellular enzymes, a rate-limiting step of decomposition generally modeled as temperature-dependent Michaelis–Menten kinetics (Davidson et al., 2006, 2011):

$$V = \frac{V_{\max} \times [S]}{K_m + [S]}$$

where V is the decomposition rate of the substrate, $[S]$ is the substrate concentration in the soil solution or solid phase, V_{max} is the maximum rate of the enzyme-catalyzed reaction, and K_m is the half-saturation constant (the substrate concentration at which V equals half V_{max}) which is indicative of substrate-enzyme affinity. V_{max} and K_m are both intrinsically temperature-sensitive, and their relative changes with temperature determine the apparent temperature sensitivity of reaction rates, which is particularly important at low $[S]$ (Razavi et al., 2015). V_{max}, K_m and their Q_{10} are crucial parameters in new-generation soil biogeochemical models that link microbial physiology to C processes (Allison, 2012; Wieder et al., 2014). Decreases in V_{max} or increases in K_m with warming may contribute to microbial thermal acclimation by warming (Allison et al., 2018).

Fresh C supply stimulates microorganisms to secrete enzymes and thereby promote SOM decomposition, which is termed the "priming effect" (Kuzyakov, 2010). Moreover, the temperature sensitivity of soil C mineralization (either of SOM or fresh C input) could be increased by new substrate inputs (Zhu and Cheng, 2011), whereas substrate shortage tends to have the opposite effect (Moinet et al., 2018; Su et al., 2022). This was attributed to the positive correlation between Q_{10} and the item $[S]$ (i.e., the substrate concentration) in the Michaelis–Menten equation, because the effects of increasing V_{max} with temperature are more strongly counterbalanced by increasing K_m at lower $[S]$ (Davidson et al., 2006). However, the possible microbiological mechanisms underlying the changed Q_{10} under exogenous substrate inputs, such as the temperature responses of soil enzyme kinetics (V_{max} and K_m) and microbial physiology (e.g., CUE and microbial turnover), have rarely been considered. In addition, few studies have disentangled the temperature sensitivity of SOM and newly added fresh substrates (Zhu and Cheng, 2011; Wei et al., 2021), which should behave differently under climate warming given their distinct decomposability (Davidson and Janssens, 2006).

Converting plant biomass (tree, grass, or crop residues) into biochar by pyrolysis, and applying biochar to the soil, is a measure of abating climate change by C sequestration (Lehmann, 2007; Woolf et al., 2010). This is primarily based on the chemical inertness of biochar and its very long residence time in the soil (hundreds to thousands of years; Kuzyakov et al., 2014), especially when compared with the rapid decomposition of plant litter. It should be noted that plant biomass pyrolysis to biochar deprives soil organisms of a substantial amount of labile C, which would normally return to the soil under natural conditions, thereby profoundly affecting ecosystem processes. Many studies have compared the effects of litter and litter-derived biochar on greenhouse gas emissions, N cycling, enzyme activities, and microbial C utilization (Wu et al., 2013; Shen et al., 2014; Liu et al., 2020). However, it has not been considered that converting litter to biochar, which decreases labile C inputs to the soil, may lower the temperature sensitivity of SOM decomposition. This is because a lack of utilizable C reduces the growth of microbial biomass and

extracellular enzyme production, lowering the depolymerization of SOM (and hence SOM-derived substrates, [*S*]). In addition, microbial communities with greater growing biomass are more temperature-sensitive (Larionova et al., 2007). This should result in a higher Q_{10} of SOM decomposition under litter than under biochar amendment (Thiessen et al., 2013). On the other hand, biochar may reduce the temperature responses of SOM mineralization by lowering microbial activities (e.g., the metabolic quotient; Zhang et al., 2022). To date, however, the effects of litter and litter-derived biochar on the temperature sensitivity of soil C decomposition have not been assessed.

The goals of this study were (1) to compare the effects of litter and litter-derived biochar on the Q_{10} of the decomposition of SOM and freshly added organic substances, and (2) to investigate the underlying mechanisms from the perspective of enzyme kinetics, microbial physiology, and substrate availability. Soils amended with maize litter or litter-derived biochar were subjected to 10°C warming at the early and late stages of a long-term incubation. The Q_{10} of SOM decomposition can be distinguished from that of biochar or litter based on the distinct isotopic signatures of C4 (maize) and C3 (SOM) materials. After long-term incubation, we applied a secondary addition of ^{14}C-labeled wheat litter to all soils to assess how prior amendments affected the Q_{10} of fresh C inputs. The temperature responses of Michaelis–Menten kinetics of soil enzymes, microbial phospholipid fatty acid (PLFA) profiles, and soil substrate availability (dissolved organic matter) for microorganisms were analyzed to elucidate the mechanisms responsible for the Q_{10} of organic matter decomposition. The unique combination of isotopic approaches, with analyses of PLFA biomarkers and enzyme kinetics, provides useful information about how the lability of amendments influences soil C feedbacks to warmer climates.

Materials and methods

Soil collection and biochar production

Soil was collected from the plow horizon (Ap horizon, 0–10 cm) of an old paddy rice field located in northern Jiangsu Province, China. The region is characterized by a typical subtropical climate, with an annual precipitation of 1,000 mm and an average temperature of 14°C. The soil had a silty texture (silt: 88%; clay: 3.5%) and could be tentatively classified as Anthrosol (WRB, 2015). Soils from ten points in the field were homogenized by passing through a 2-mm sieve, and handpicked to remove plant residues and stones prior to incubation. The basic soil properties are listed in Table 1.

Biochar was prepared from maize litter (leaves) at 400°C and 650°C. Finely ball-milled maize litter was passed through a 2-mm sieve and tightly filled into a ceramic crucible (iØ/oØ = 46/50 mm × 40 mm high) prior to pyrolysis in a muffle furnace. The temperature of the muffle furnace was slowly raised from room temperature to 400 or 650°C at a rate of 4.2°C min^{-1} and kept at the set temperature for 4 h. The charring process yielded biochar with a mass equivalent to 30 and 15% of the initial litter mass at 400°C and 650°C, respectively. The biochar was milled and 0.5 mm-sieved before being added to the soil. The basic properties of biochar are listed in Table 1.

TABLE 1 Basic properties of the soil and amendments of maize litter and biochar.

	Soil	Maize litter	Biochar (400°C)	Biochar (650°C)
Total C (%)	1.95 ± 0.03	45.4 ± 0.05	49.1 ± 0.09	58.1 ± 0.17
Total N (%)	0.19 ± 0.001	1.41 ± 0.01	2.54 ± 0.02	1.9 ± 0.03
C:N ratio	10.2 ± 0.01	32.3 ± 0.22	19.3 ± 0.10	30.6 ± 0.53
pH	6.82 ± 0.10	ND	8.69 ± 0.01	9.30 ± 0.01
DOC (mg/g)	0.05 ± 0.004	ND	1.31 ± 0.25	1.53 ± 0.24
δ^{13}C (‰)	−27.3 ± 0.21	−12.3 ± 0.33	−12.07 ± 0.13	−12.23 ± 0.04

Values are means ± standard errors (n = 3).

Experimental layout and soil incubation procedures

Long-term soil incubation was conducted with four treatments, i.e., soils with no amendment (Control), soils amended with maize litter (Litter), biochar produced at 400°C (BC400), and biochar produced at 650°C (BC650). The maize litter was added at a rate of 30 mg g^{-1} soil (o.d. basis), while the rates of biochar addition were 30 and 15% (the yield rate of biochar) of litter addition rate for BC400 (9.2 mg g^{-1} soil) and BC650 (5.6 mg g^{-1} soil), respectively. Biochar was amended at such rates so that the litter mass required to produce the added biochar was equivalent to the added maize litter in the litter treatment. All soils were adjusted to 50% water-holding capacity and incubated at 20°C in 150 ml glass flasks, which were loosely capped, and distilled water was added periodically to maintain constant soil moisture.

On day 21 of the incubation, soils of 18 g dry weight were transferred into small plastic vials and placed into airtight 1.2-L jars, together with another vial containing 15 ml 1 M NaOH solution. Thereafter, three replicates per treatment were maintained at 20°C, while the other three were incubated at 30°C for 44 days to mimic a short-term soil warming event. Such magnitude of temperature rise was larger than those estimated in various climate change projections (IPCC, 2022), but was generally adopted in incubation studies to maximize temperature effects over short time scales (e.g., Fang et al., 2014; Xu et al., 2022). Soil CO_2 emitted over the study period, as well as its isotopic composition, was determined after 44 days of warming (Figure 1).

Short-term warming was also performed after 417 days of long-term incubation, but additional treatments were set up with the secondary addition of dried ^{14}C-labeled rice leaves. Rice litter instead of maize litter was used because crop rotations commonly occur in paddy fields of the study area. A portion of soils (7 g on a dry weight basis) keeping the original treatments (Control, Litter,

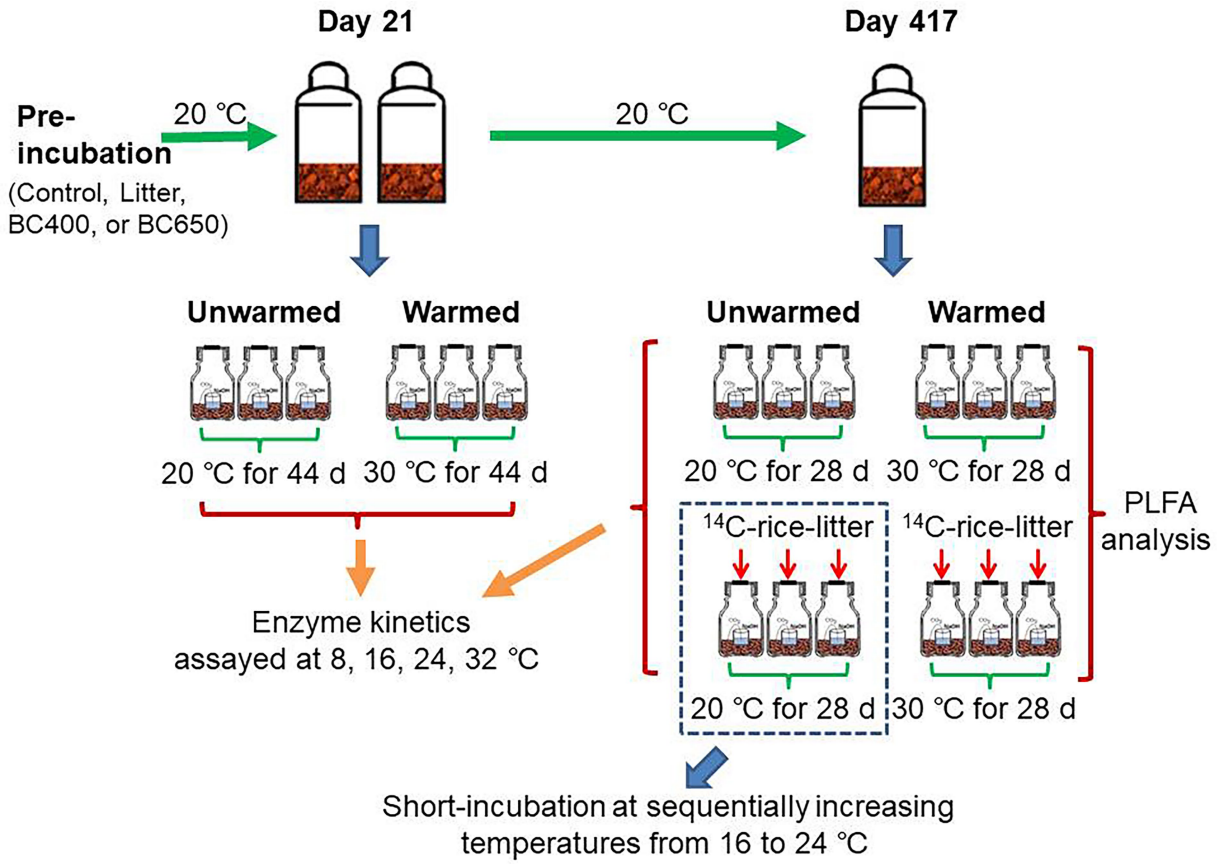

FIGURE 1
Layout of long-term incubation and warming experiments. Briefly, soils without/with litter or biochar addition were incubated at 20°C for 21 and 417 days before being incubated in 1.2L glass jars with NaOH traps for a short period at 20°C (unwarmed) and 30°C (warmed). Part of soils at day 417 received secondary amendments of ^{14}C-labeled rice litter prior to the warming. Additional incubation at sequentially increasing temperatures was conducted for soils that had received secondary ^{14}C-labeled litter addition at day 417 and had been subsequently incubated in glass jars at 20°C for 48 days.

BC400, or BC650) were treated as above, that is, incubated at 20°C and 30°C with NaOH vials. The remaining soils received a secondary incorporation of ^{14}C-labeled rice leaves at a rate of 30 mg g^{-1}, in addition to their original amendments, and were then placed at 20°C and 30°C for short-term warming. The ^{14}C-labeling procedures for rice have been described by Ge et al. (2012). Briefly, rice seedlings of roughly 0.1 g dry weight were transplanted and grown in an air-tight chamber for continuous labeling with $^{14}CO_2$ generated from $Na_2{}^{14}CO_3$ for >2 months, after which their leaves were harvested. The warming lasted 28 days, following which soil CO_2 emissions, its ^{13}Csignatures, and ^{14}C activities were analyzed.

To further investigate Q_{10} of the freshly added organic matter, an incubation regime using sequentially changing temperatures was adopted for soils receiving the secondary litter amendment after short-term warming was completed. Briefly, the incubation temperature was slowly decreased from 20°C to 16°C at a rate of 1°C every 6 h, and then increased from 16, 18, 20, 22, to 24°C at a step of 2°C every 3–9 days. The duration at each temperature depends on the specific CO_2 emission rate. The NaOH solution used for CO_2 trapping was collected and replenished at the end of the incubation at each temperature. The trapped CO_2 and its ^{14}C signal were analyzed later.

Chemical and isotopic analysis

For soils undergoing short-term warming on days 21 and 417, the amount and ^{13}C or ^{14}C signatures of NaOH-trapped CO_2 were determined using the following procedure. First, if ^{14}C activity was analyzed, a 5 ml aliquot was removed from the collected 15 ml NaOH solution and stored until ^{14}C measurement on a scintillation counter (LS-6500, Beckman, Germany). The remaining NaOH solution was precipitated with excess 1 M $SrCl_2$ and titrated with 0.5 M HCl to quantify the trapped CO_2. The precipitate ($SrCO_3$) was washed with 50 ml deionized water by centrifuging at 9,000 rpm and discarding the supernatants, which was repeated three times. Finally, the $SrCO_3$ precipitate was dried at 50°C for ^{13}C analysis using a MAT 253 isotope ratio mass spectrometer (IRMS) equipped with a Kiel IV Carbonate Device (Thermo Scientific, United States; precision: ±0.04‰).

Soil pH was measured in deionized water extracts at a soil:water ratio of 1:5 using a pH electrode (Mettler Toledo FE28, Switzerland), whereas the pH of the biochar was measured at a biochar:water ratio of 1:15. To determine the dissolved organic C (DOC) content, soils or biochar were extracted with 0.05 M K_2SO_4 at a soil:K_2SO_4 ratio of 1:4 or biochar:K_2SO_4 ratio of 1:10. The total organic C of the K_2SO_4 extracts was analyzed using a TOC analyzer (Multi N/C 2100, Analytik Jena, Germany). The total C and N, as well as the ^{13}C composition, of the soil/biochar solids were determined using an elemental analyzer (Vario Macro Cube, Elementar, Germany) coupled to an IRMS (MAT 253, Thermo Finnigan, United States; precision: ±0.10‰).

Enzyme assays

Unwarmed and warmed soils at both early and late incubation stages were analyzed for enzyme kinetics to reflect how different amendments affected the temperature sensitivities of enzyme V_{max} and K_m (Figure 1). The kinetics of three enzymes targeting soil C-and N-containing substrates, β-glucosidase (BG; EC: 3.2.1.21), N-acetyl-β-glucosaminidase (NAG; EC: 3.2.1.14), and leucine aminopeptidase (LAP; EC: 3.4.11.1), were analyzed at different temperatures. Enzyme assays were performed following the method described by Allison et al. (2018). Briefly, homogenous soil slurries were prepared by dispersing 3 g of moist soil in 120 ml buffer. The buffer contained 14 g L^{-1} citric acid, 6.3 g L^{-1} boric acid, 12.1 g L^{-1} Trizma base, 11.6 g L^{-1} maleic acid, and 19.5 g L^{-1} NaOH, and were adjusted to the same pH (6.8) with soil. Thereafter, 300 µl soil homogenate was combined with75 µl of substrates in each well of a 96-well microplate, which was incubated at 8, 16, 24, and 32°C for 4 h (but 2 h for BG and LAP on day 21). All enzyme activities were assayed for Michaelis–Menten kinetics, with seven substrate concentrations spanning the range of 10–600 µM.

Phospholipid fatty acid analysis

The extraction and analysis of PLFAs followed the procedures described by Ge et al. (2017). Fatty acids were extracted from 3 g of freeze-dried samples in 15.2 ml of chloroform:methanol:citrate (1:2:0.8) buffer. Phospholipids in the extracts were separated from neutral lipids and glycolipids by using a silica-bonded phase column (SPE-Si, Supelco, Poole, UK). Subsequently, the phospholipids were methylated to fatty acid methyl esters (FAMEs), which were quantified using a gas chromatograph (N6890, Agilent, USA) and identified using the MIDI Sherlock Microbial Identification System 4.5 (Newark, DE, USA). The 19:0 methyl ester was used as an internal standard. PLFA analysis was only conducted for warmed and unwarmed soils at the late incubation stage, to examine how different microbial groups responded to the 10°C warming and whether this was changed by secondary litter addition (Figure 1).

PLFA markers for various microbial groups are listed in Supplementary Table S1, with monounsaturated fatty acids used as indicators for gram-negative bacteria, iso-and anteiso-branched fatty acids for gram-positive bacteria, 10-methyl fatty acids for actinomycetes, and 18:2ω6c and 18:1ω9c for fungi (Zhang et al., 2017). Two calculated indicators, the ratio of two cyclopropyl fatty acids (cy17:0 and cy19:0) to their precursors (Cy/Pre), and the degree of PLFA unsaturation, were used to reflect microbial responses to temperature stress. The PLFA unsaturation was calculated as follows:

$$\text{Unsaturation} = \sum \frac{[PLFA_{\text{unsat}}] \times N_{db}}{\text{total PLFA}} \times 100\% \quad (1)$$

where $[PLFA_{\text{unsat}}]$ refers to the concentration of a specific unsaturated PLFA in the sample, and N_{db} is the number of double bonds in the PLFA.

Statistical analyses

The stable C isotopic composition of samples is expressed as $\delta^{13}C$ values defined by:

$$\delta^{13}C\,(‰) = \left[\left(R_{\text{sample}} / R_{\text{V-PDB}}\right) - 1\right] \times 1{,}000 \quad (2)$$

where R_{sample} and $R_{\text{v-PDB}}$ are the $^{13}C/^{12}C$ ratios in the samples and Vienna Pee Dee Belemnite (V-PDB) standard, respectively. When no ^{14}C-labeled rice litter was involved, the contributions of maize-originating litter/biochar and native SOM to soil CO_2 emission was calculated using a simple two-source mixing model:

$$C_L = C_t \times \frac{\delta^{13}C_t - \delta^{13}C_{\text{SOM}}}{\delta^{13}C_L - \delta^{13}C_{\text{SOM}}} \quad (3)$$

$$C_{\text{SOM}} = C_t - C_L \quad (4)$$

where C_L, C_{SOM}, and C_t are the C from maize litter, native SOM, and bulk soil (mg kg^{-1}), respectively; $\delta^{13}C_L$, $\delta^{13}C_{\text{SOM}}$, and $\delta^{13}C_t$ refer to the $\delta^{13}C$ values (‰) of maize litter (−12.32 ± 0.33‰), SOM (−27.33 ± 0.21‰), and total soil C, respectively.

When both maize-originating litter/biochar and ^{14}C-labeled rice litter were present in the soil, the total CO_2 released from the soil was partitioned into three C sources, i.e., the maize-derived litter or biochar, the rice litter, and native SOM, using the approach of Blagodatskaya et al. (2011). In the first step, the contributions of ^{14}C-labeled rice litter and other non-rice C sources were calculated based on their specific ^{14}C activity.

$$C_{\text{rice}} = \frac{\left(DPM_s - DPM_{bl}\right) \times V_{\text{NaOH}}}{{}^{14}C_{\text{RL}} / [\text{C}]_{\text{RL}}} \quad (5)$$

$$C_{\text{non-rice}} = C_{\text{bulk}} - C_{\text{rice}} \quad (6)$$

where C_{rice} (mg C), $C_{non\text{-}rice}$ (mg C), and C_{bulk} (mg C) are C derived from rice, non-rice sources (maize and native SOM), and bulk soil, respectively; DPM_s and DPM_{bl} are the ^{14}C activity (decay per minute, DPM ml^{-1}) of the NaOH solution for samples and the blank, respectively; $^{14}C_{RL}$ (DPM g^{-1}) and $[C]_{RL}$ (mg C g^{-1}) are the specific ^{14}C activity and C content of the rice litter, respectively; and V_{NaOH} is the volume (ml) of NaOH for CO_2 trapping. In the second step, C from non-rice sources was partitioned into native SOM and maize-originating C (litter or biochar), according to the following equations:

$$\delta^{13}C_{\text{non-rice}} = \frac{C_t \times \delta^{13}C_t - C_{\text{rice}} \times \delta^{13}C_{\text{rice}}}{C_{\text{non-rice}}} \quad (7)$$

$$C_{\text{maize}} = C_{\text{non-rice}} \times \frac{\delta^{13}C_{\text{non-rice}} - \delta^{13}C_{\text{SOM}}}{\delta^{13}C_{\text{maize}} - \delta^{13}C_{\text{SOM}}} \quad (8)$$

$$C_{\text{SOM}} = C_{\text{non-rice}} - C_{\text{maize}} \quad (9)$$

where C_{rice} ($\delta^{13}C_{rice}$), $C_{non\text{-}rice}$ ($\delta^{13}C_{non\text{-}rice}$), C_{maize} ($\delta^{13}C_{maize}$), C_{SOM} ($\delta^{13}C_{SOM}$), and C_t ($\delta^{13}C_t$) refer to C (δ^{13}Cvalues) from rice ($\delta^{13}C_{rice}$: -25.75 ± 0.22‰), non-rice sources, maize (litter or biochar), native SOM, and bulk soil, respectively. The Q_{10} for the decomposition of total soil C and specific C pools was calculated as the ratio of their mineralization rates at 30°C to those at 20°C.

The kinetic parameters for enzymes, half-saturation constant (K_m), and maximal velocity (V_{max}) at each assay temperature were derived by fitting soil enzyme activities at increasing substrate concentrations to the Michaelis–Menten equation. Fitting was performed using the nonlinear least squares (NLS) function in R 4.1.0. Q_{10} for V_{max} and K_m was calculated using the following equation:

$$\text{Q}_{10} = \left(\frac{V_{\text{max}32}}{V_{\text{max}8}}\right)^{\frac{10}{24}} \text{ or } \left(\frac{K_{\text{m}32}}{K_{\text{m}8}}\right)^{\frac{10}{24}} \quad (10)$$

where V_{max32} (K_{m32}) and V_{max8} (K_{m8}) are the fitted V_{max} (K_m) values at the assay temperatures of 32 and 8°C, respectively. The relationships between V_{max} or K_m and assay temperature were exponential (except for K_m of LAP on day 417), and V_{max} and K_m were log-transformed when plotting them versus assay temperature. Mass-specific respiration was expressed as CO_2 emitted per unit PLFA content over a certain incubation period.

One-way analysis of variance (ANOVA) was used to test the effects of amendments on soil C, Q_{10} and PLFA contents, followed by Duncan's post-hoc test. For the changing-temperature incubation, an one-way repeated-measures ANOVA was conducted to test the between-treatment differences in the decomposition rates of ^{14}C-labeled rice litter. Principal component analysis (PCA) was applied for ordination of the PLFA composition of the soil samples using PC-ORD 5 (MjM Software, United States).

Modeling analysis of variables determining Q_{10} of SOM mineralization

We constructed a simple modeling analysis of the roles of enzyme kinetics (V_{max} and K_m), soil substrate availability (using DOC as a proxy), and microbial physiological variables (microbial turnover) in determining Q_{10} of SOM mineralization over a short period of warming after 417 days of incubation. Following previous studies (Allison et al., 2010), we assumed SOM mineralization to be a Michaelis–Menten process affected by microbial CUE:

$$R(T) = \frac{V_{\text{max-T0}} \times Q_{10\text{-vmax}}^{(T-T0)/10} \times [S]}{K_{\text{m-T0}} \times Q_{10\text{-km}}^{(T-T0)/10} + [S]} \times (1 - \text{CUE}) \quad (11)$$

where $R(T)$ is the temperature-dependent soil respiration rate originating from SOM at a given time point, $V_{max\text{-}T0}$ ($K_{m\text{-}T0}$) is V_{max} (K_m) at a reference temperature T0, $Q_{10\text{-}vmax}$ ($Q_{10\text{-}km}$) is the intrinsic Q_{10} for enzyme V_{max} (K_m), and [S] is the SOM-derived substrate content at the incubation temperature T. Based on soil incubation data at 20°C and 30°C, the instantaneous Q_{10i} of SOM mineralization at any time point can be derived as

$$Q_{10\text{i}} = \frac{R(30)}{R(20)} = \frac{D_f \times Q_{10\text{-vmax}} \times \left(1 + [S]_{20} / K_{\text{m}20}\right)}{Q_{10\text{-}K\text{m}} / Q_{10[\text{S}]} + [S]_{20} / K_{\text{m}20}} \times Q_{10\text{-R\%}} \quad (12)$$

$$D_f = \frac{V_{\text{max}}(30)}{V_{\text{max}}(20)} \quad (13)$$

$$\text{Q}_{10\text{-R\%}} = \frac{1 - CUE_{30}}{1 - CUE_{20}} = \frac{1 - D_{\text{f}} \times CUE_{20}}{1 - CUE_{20}} \quad (14)$$

where $[S]_{20}/K_{m20}$ is the ratio of substrate content ([S]) to K_m at 20°C, $Q_{10[S]}$ is the ratio of SOM-derived substrate content at 30°C to that at 20°C, $CUE_{20(30)}$ is CUE at 20 (30) °C, D_f is the decay factor by which enzyme pools (indicated by V_{max} and linked to microbial biomass) were decreased due to soil warming, and

$Q_{10\text{-R\%}}$ is the temperature sensitivity of the proportion of microbial assimilated C loss *via* cell respiration (i.e., C that is not ultimately used for biomass construction) as a function of CUE at different temperatures. We only considered Q_{10i} at the late incubation stage in the absence of secondarily added rice litter, because in this case, soil respiration before warming should have reached a near-equilibrium state, and thus Q_{10i} could be easily linked to the temperature sensitivity of cumulative SOM mineralization (Q_{10t}):

$$Q_{10t} = \sum_{i=1}^{t} \frac{Q_{10i} \times R_{20}}{t \times R_{20}} = \sum_{i=1}^{t} Q_{10i} / t \quad (15)$$

where Q_{10i} corresponds to a short time interval (an hour) of the warming period t and R_{20} is the SOM mineralization at 20°C, which is assumed to be invariant with time.

The ratio of DOC from soils incubated at 30°C to that incubated at 20°C was used to approximate $Q_{10[S]}$ (Tables 2, 3). $Q_{10\text{-vmax}}$ and $Q_{10\text{-Km}}$ in Equations (11, 12) were parameterized with measured values for BG at 20°C (Table 3), considering that BG enzymes catalyze the hydrolysis of cellobiose and other organic substrates, and their kinetics should be highly correlated with that for overall SOM mineralization. However, $Q_{10\text{-R\%}}$ had to be estimated based on previously reported CUE values in the literature. $[S]_{20}$ was estimated by fitting the measured BG activities at varying substrate concentrations to a modified Michaelis–Menten equation (Larionova et al., 2007):

$$V = \frac{V_{max} \times \left([C] + [S]_{20}\right)}{K_m + [C] + [S]_{20}} \quad (16)$$

where V is BG activity at the exogenous substrate concentration of [C] at 20°C, V_{max} is the maximum reaction velocity of BG, K_m is the half-saturation constant, and $[S]_{20}$ is the concentration of substrates derived from native SOM at 20°C.

Sensitivity analysis was employed to find the variables that exerted the largest influence on Q_{10i}. We then investigated the effects of changing $[S]_{20}/K_{m20}$ on Q_{10i}, with and without considering $Q_{10\text{-R\%}}$ by setting it to 1 or the estimated value. Finally, the evolution of Q_{10t} for cumulative SOM mineralization was simulated at a 1 h time step over 720 h of incubation according to Equation (13), with the assumption that D_f (which indicates the effects of warming on microbes) decreases linearly with time over 240 h, due to the gradual reduction of microbial biomass or enzyme pools.

Results

Mineralization of soil organic matter pools and their Q_{10}

At the initial stage of soil incubation, decomposition of maize litter dominated CO_2 efflux under litter addition, where SOM mineralization was even lower than that in the unamended control soil (Figure 2A). In contrast, biochar amendments accelerated SOM mineralization by 30% relative to that of the control at 20°C. Raising the incubation temperature to 30°C resulted in a much higher Q_{10} (3.5) of SOM in the litter-amended soils than in the control (Q_{10} close to 1) and biochar-amended soils (Q_{10} = 1.5, Figure 2B).

After 417 days of incubation, litter decomposition greatly declined, as 58% of amended maize litter was already decomposed (data not shown), and SOM decomposition contributed 92% to total soil CO_2 emission (Figure 2C). SOM mineralization in the litter-amended soils became higher than that in the control, particularly at 30°C. The Q_{10} of total C and SOM mineralization was higher under

TABLE 2 Dissolved organic C (DOC), total phospholipid fatty acids (PLFA), and mass-specific respiration (R_{mass}) at the late incubate stage.

Treatments	DOC (mg C kg^{-1})[a]			Total PLFA (nmol g^{-1})			R_{mass} (μg C nmol^{-1} PLFA d^{-1})[b]		
	20°C	30°C	$R_{30/20}$	20°C	30°C	$R_{30/20}$	20°C	30°C	$R_{30/20}$
− ^{14}C rice litter									
Control	56 ± 2.7b	53 ± 6.18b	0.94 ± 0.03a	38.0 ± 1.8a	31.1 ± 2a	0.82 ± 0.05a	0.42 ± 0.05a	0.42 ± 0.03b	0.99 ± 0.08b
Litter	77 ± 35a	91 ± 21.99a	1.1 ± 0.1a	57.3 ± 4a	34.0 ± 4.2a	0.59 ± 0.07b	0.34 ± 0.02a	0.78 ± 0.08a	2.29 ± 0.24a
BC400	55 ± 8.6b	54 ± 2.28b	0.99 ± 0.01a	34.3 ± 3.1a	31.2 ± 1.3a	0.91 ± 0.04a	0.45 ± 0.11a	0.48 ± 0.06b	1.07 ± 0.13b
BC650	54 ± 5b	53 ± 17.08b	0.99 ± 0.18a	40.3 ± 11a	32.8 ± 2a	0.94 ± 0.06a	0.53 ± 0.08a	0.40 ± 0.03b	0.74 ± 0.06b
+ ^{14}C rice litter									
Control	85 ± 49a	85 ± 37a	0.87 ± 0.15a	71.0 ± 0.2a	44.7 ± 2.3a	0.63 ± 0.03a	1.34 ± 0.02b	2.22 ± 0.04bc	1.66 ± 0.03d
Litter	86 ± 27a	110 ± 2.1a	1.27 ± 0.01a	56.4 ± 3.5a	33.2 ± 1.7a	0.59 ± 0.03a	1.54 ± 0.04a	3.49 ± 0.07a	2.27 ± 0.05b
BC400	70 ± 16a	76 ± 58a	1.18 ± 0.2a	77.2 ± 10a	43.0 ± 1.8a	0.56 ± 0.02a	1.04 ± 0.02c	2.2 ± 0.03c	2.12 ± 0.03c
BC650	93 ± 27a	90 ± 5.9a	1.14 ± 0.02a	87.0 ± 3.8a	38.3 ± 7.1a	0.44 ± 0.08a	0.85 ± 0.01d	2.35 ± 0.02b	2.75 ± 0.02a

$R_{30/20}$ is the ratio of DOC or PLFA at 30°C–20°C. For each temperature with or without secondary ^{14}C litter addition, lowercase letters in a column indicate significant differences between treatments. Values are presented as mean ± standard error (n = 3).

[a]DOC in litter-and biochar-amended soils without ^{14}C-litter addition almost entirely originated from SOM as indicated by ^{13}C signatures (not shown for clarity) of DOC.

[b]Respiration was averaged over 28 days of incubation to calculated R_{mass}.

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TABLE 3 Parameters used to simulate temporal changes in the instantaneous (Q_{10i}) and cumulative (Q_{10t}) temperature sensitivity of SOM mineralization depending on soil amendments.

Parameter	Units	Control	Litter	BC400 &BC650
D_f	1	0.84	0.73	0.92
$Q_{10\text{-vmax}}$	1	1.86	1.86	1.86
$Q_{10\text{-Km}}$	1	1.40	1.40	1.40
$Q_{10[Soils]}$	1	0.94	1.10	0.99
$[Soils]_{20}$	μm	12	24	12
K_{m20}	μm	57	70	60
$[Soils]_{20}/K_{m20}$	1	0.21	0.34	0.20
CUE_{20}	1	0.20	0.50	0.20
CUE_{30}	1	0.17	0.36	0.18
$Q_{10\text{-R\%}}$	1	1.04	1.27	1.02

litter amendment (Q_{10} = 1.4 for total C and SOM) than in the control or biochar-amended soils (Figure 2D). The mass-specific respiration (R_{mass}, for total CO_2 emission) was similar between soils with different amendments at 20°C, but was significantly higher in litter-amended soils at 30°C (Table 2). In addition, the temperature response of R_{mass}, expressed as the ratio of R_{mass} at 20°C to 30°C ($R_{30/20}$), was significantly larger under the litter amendment.

The secondary addition of ^{14}C-labeled rice litter on day 417 greatly increased total C and SOM mineralization, as well as R_{mass}, in all soils, with or without prior amendments (Table 2; Figure 2E). For soils that received fresh litter, R_{mass} was highest in soils with prior maize litter addition at 20°C and 30°C (Table 2). Q_{10} of SOM was increased by the secondary litter addition from 0.6–1.3 to 0.9–1.6, with the highest Q_{10} (1.6) in the original maize-litter-amended soils (Figure 2F). The Q_{10} for the newly added rice litter

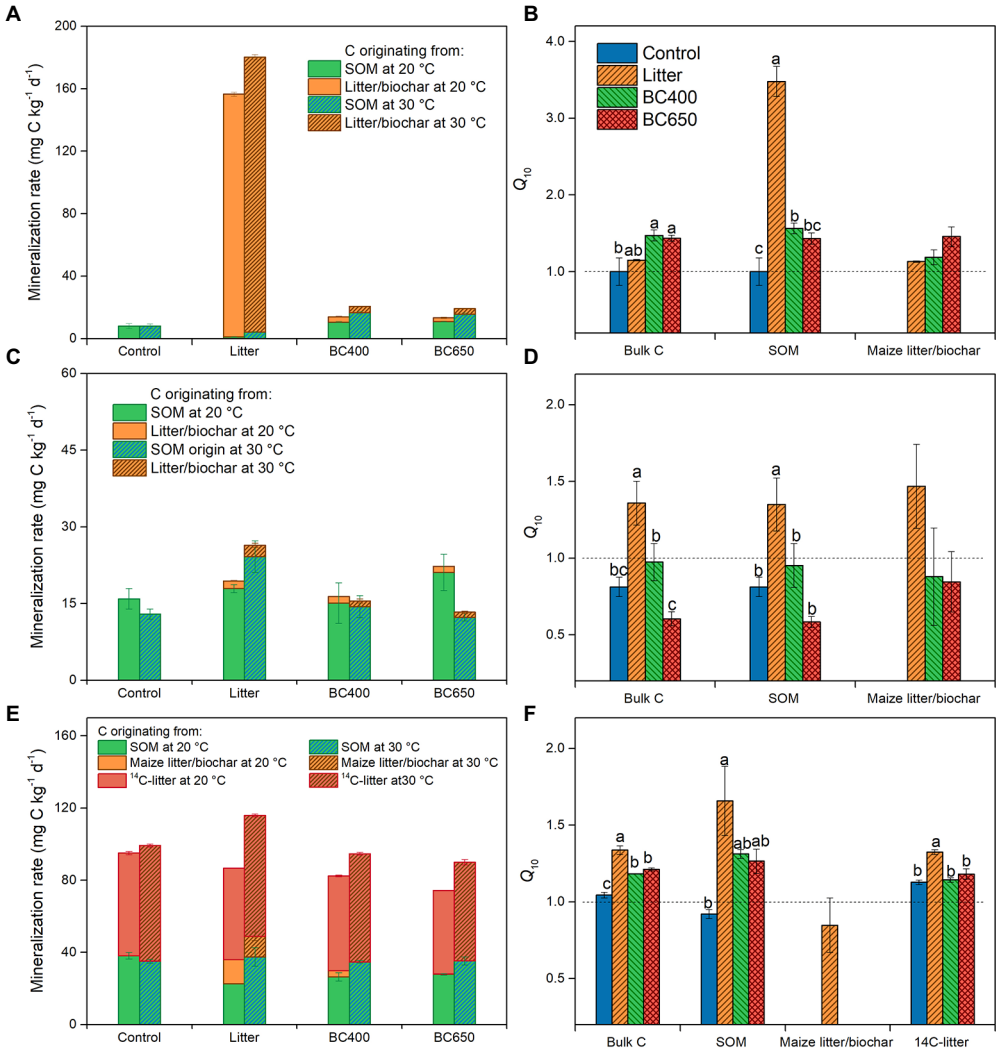

FIGURE 2
Mineralization of organic C pools in soil **(A–C)** and their Q_{10} **(D–F)** at the early incubation stage **(A,D)**, and at the late incubation stage without **(B,E)** and with **(C,F)** secondary litter amendment. Error bars represent standard errors (n=3). Lowercase letters above bars in **(D–F)** indicate significant differences between the treatments (p<0.05). There were initially four treatments (Control, Litter, BC400, and BC650). At the late incubation stage, part of the soils kept their earlier treatments, whereas the remaining all received secondary fresh litter (^{14}C-labeled) in addition to their prior amendments. Both the early- and late-stage soils were warmed over short periods by incubating soils at 20°C (unwarmed) and 30°C (warmed).

per se was also higher in soils with prior maize litter addition (1.3) than that in the original control or biochar-amended soils (1.1). The temperature response of R_{mass} was higher in soils with secondary litter addition (1.3) than in soils without the secondary addition (2.2).

The results of the sequentially increasing temperature incubation revealed an exponential relationship between the newly added ^{14}C-labeled rice litter decomposition (microbial-biomass-specific) and temperature in the original maize-litter-amended soil (Figure 3), resulting in a relatively high Q_{10} of 1.6. In comparison, mass-specific ^{14}C-litter decomposition rates and the corresponding Q_{10} (1.3) were lower in the original control and biochar-amended soils, particularly within the temperature range of 20–24°C. The ^{14}C-litter decomposition was similar in the prior control and biochar-amended soils.

Temperature dependence of enzyme kinetics and activities

All enzyme activities, and their kinetic parameters V_{max} (Supplementary Figure S1) and K_m (Supplementary Figure S2; except for LAP at the late incubation stage), showed exponential relationships with assay temperature (8–32°C). Q_{10} for V_{max} had a mean value of 1.8, which was larger than that for K_m (1.4). At the early and late incubation stages without secondary litter addition, V_{max} was generally highest under maize-litter addition ($p < 0.05$). Overall, K_m was not affected by these amendments. The Q_{10} of V_{max} and K_m of all three enzymes across assay temperatures were similar between amendments at either the early or late incubation stage, with or without secondary litter addition.

In most cases, warming from 20 to 30°C decreased V_{max} (data not shown) and enzyme activities (Figure 4). The magnitude of the decreases in enzyme activities (expressed as $R_{30/20}$, the ratio of activities at 30 to 20°C) was greater in the maize-litter-amended soils than in biochar-amended soils. $R_{30/20}$ ranged between 0.5 and1. Notably, $R_{30/20}$ was mostly close to 1 under the two biochar amendments (BC400 in particular), i.e., declines in enzyme activities were minimal, but $R_{30/20}$ could be as low as 0.6–0.7 in maize-litter-amended soils. Overall, secondary litter addition decreased $R_{30/20}$ (particularly for NAG, with $R_{30/20}$ decreasing to approximately 0.5) for soil with or without prior amendments. For all the enzymes, K_m showed no consistent response to warming (Supplementary Figure S2). The Q_{10} of V_{max} and K_m (Supplementary Figures S1, S2) were similar between the warmed and unwarmed soils (both $p > 0.05$).

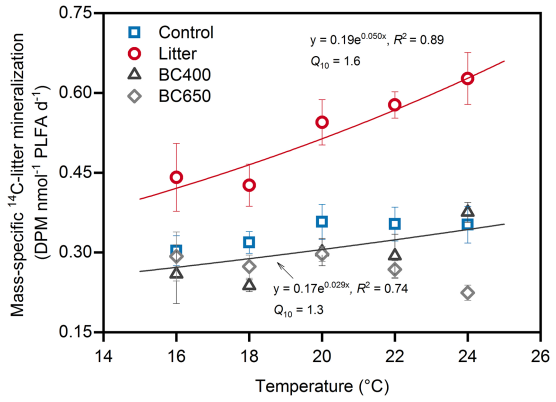

FIGURE 3
Decomposition of the secondarily added ^{14}C-labeled rice litter at sequentially changing incubation temperatures. The decomposition was expressed as microbial-mass-specific rate. Error bars identify standard errors (n=3). The sequential-warming experiment was conducted for soils (with original treatments of Control, Litter, BC400 or BC650) that had been incubated at 20°C for 417 days, after which fresh ^{14}C-labeled rice litter was added before incubation at 20°C for another 48 days. The two fitted exponential equations describe the relationship between decomposition rate and temperature. The decomposition rates of ^{14}C-litter were similar between the control and soils with prior amendments of BC400 and BC650; thus only one equation was given for these treatments.

PLFA composition and temperature stress indicators

The PLFA data indicated substantial differences in the microbial composition between the soils with maize litter and biochar. At the initial incubation stage, maize litter addition caused a 300% increase in total PLFA and a 13-fold increase in fungal PLFAs (Supplementary Table S2). In contrast, the total PLFA and PLFA composition were similar between the control and soils amended with BC400 or BC650. After 417 days, the PCA results demonstrated remarkable differentiation in microbial composition in response to warming and secondary litter addition (Figure 5). Raising the incubation temperature reduced the total PLFA content (Table 2) and PLFA markers of nearly all microbial groups (Figures 6A,B), regardless of the presence of secondary litter input. Without secondary litter addition, the magnitudes of such reductions, reflected in the ratio of PLFA at 30–20°C ($R_{30/20}$), were largest in soils receiving maize litter. Amending with fresh litter lowered $R_{30/20}$ in all soils, indicating that the total PLFA became more sensitive to warming.

Two calculated PLFA indicators of temperature stress, the Cy/Pre ratio and PLFA unsaturation, responded significantly to warming and secondary litter addition (Figures 6C,D). Cy/Pre increased with warming, with the magnitude of increase (the ratio at 30–20°C) being larger following secondary litter addition (Figure 6E). The PLFA unsaturation was greatly increased by secondary litter addition, but dropped after warming (Figure 6F).

Modeling analysis of factors influencing Q_{10} of SOM

The default parameter values used in the modeling analysis are listed in Table 3. Most of these variables (D_f, $Q_{10\text{-vmax}}$, $Q_{10\text{-Km}}$,

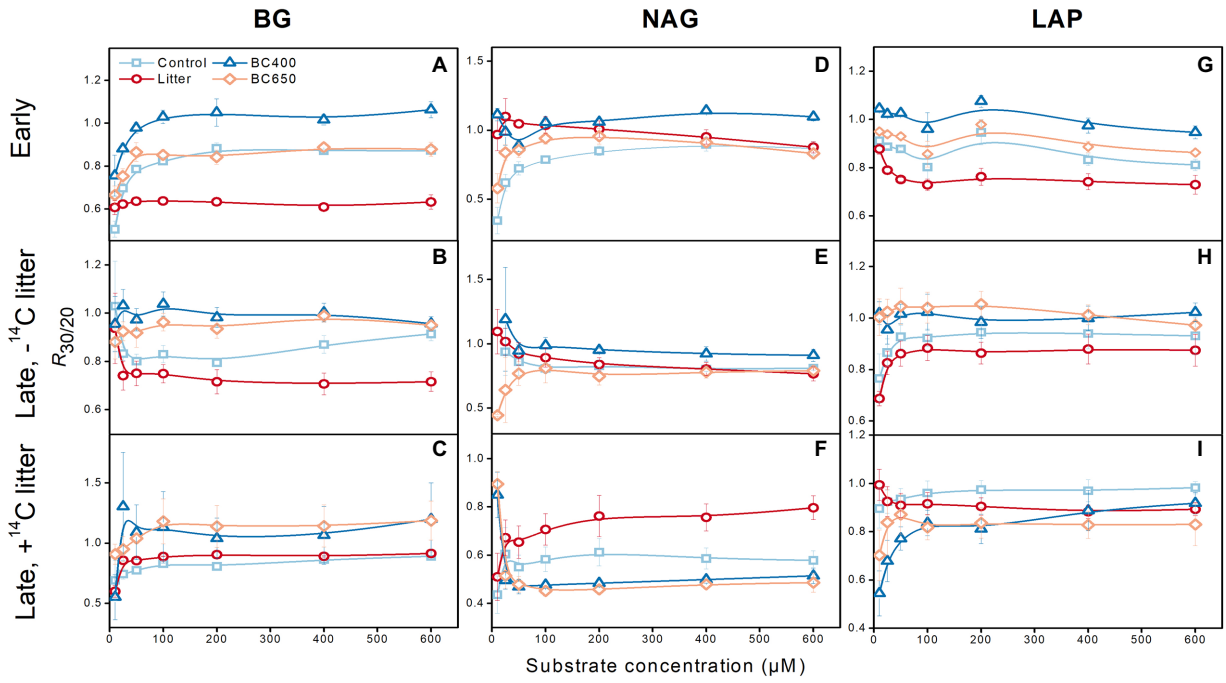

FIGURE 4
Effects of warming on enzyme activities for β-glucosidase (BG, **A–C**), N-acetyl-β-glucosaminidase (NAG, **D–F**) and leucine aminopeptidase (LAP, **G–I**). The warming effect was expressed as the ratio of enzyme activities (averaged over assay temperatures) in warmed (incubated at 30°C) versus unwarmed soils (incubated at 20°C; $R_{30/20}$). Error bars represent standard errors (n=3). At the early incubation stage there were four treatments (Control, Litter, BC400, and BC650). At the late incubation stage, part soils kept their earlier treatments, whereas the remaining received secondary fresh litter (^{14}C-labeled), in addition to their prior treatments. Both the early-and late-stage soils were subject to warming.

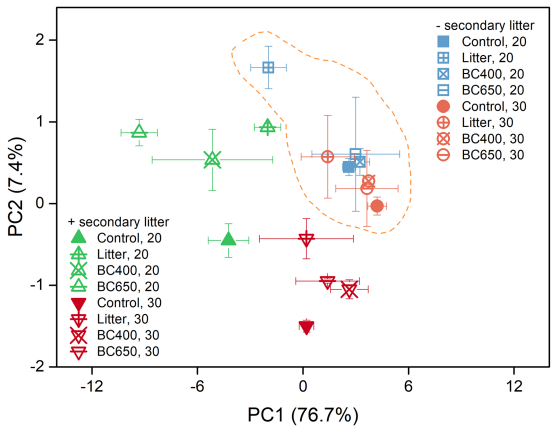

FIGURE 5
Ordination graph from the principal component analysis (PCA) of soil phospholipid fatty acid (PLFA) profiles at the late incubation stage, with or without secondary litter amendment. The percentage of variation explained by each principal component is given in the brackets beside each axis. Error bars indicate standard errors (n=3). Soil under all four treatments (Control, Litter, BC400, and BC650) were secondarily amended with fresh rice litter or kept their original treatments at the late incubation stage. All soils were then placed at 20°C (unwarmed) and 30°C (warmed) to mimic a short-term warming event. Samples surrounded by the dashed line did not receive secondary litter addition.

and $Q_{10[S]}$) were well constrained by our enzyme data (Supplementary Figures S1, S2), and DOC in unwarmed and warmed soils (Table 2). The substrate availability ($[S]_{20}$) in the control soil was approximated by fitting BG activity to Equation 16. The $[S]_{20}$ of the litter-amended soil was assumed to be double that of the control, to simulate the priming of litter on SOM depolymerization, although the measured DOC (mainly derived from SOM) was only 50% higher under litter addition. $Q_{10\text{-}R\%}$, which measured the increasing proportion of microbial assimilated C loss *via* cell respiration with warming, was a function of D_f and CUE at 20°C (Equation 14). However, the CUE was not measured and had to be estimated. Reported CUE values for litter could be as high as >0.6 (Lashermes et al., 2016; Joergensen and Wichern, 2018; Sauvadet et al., 2018), but we adopted a value of 0.5 for the litter-amended soils, because CUE might have declined with time. For the control and biochar amendments, we used a much lower CUE value (0.2), as reported by Spohn et al. (2016) for a C-poor subsoil, which resembled C-depleted soils after 417 days of incubation. It is reasonable to use a higher CUE for microbes living on energy-rich litter than for those using only SOM (Joergensen and Wichern, 2018). This resulted in a higher $Q_{10\text{-}R\%}$ under litter amendment compared to those under the control and biochar amendments. This between-amendment pattern of $Q_{10\text{-}R\%}$ was mainly dictated by the lower D_f for litter amendments, and was robust to the estimated CUE values for specific amendments across a wide range (Figure 7).

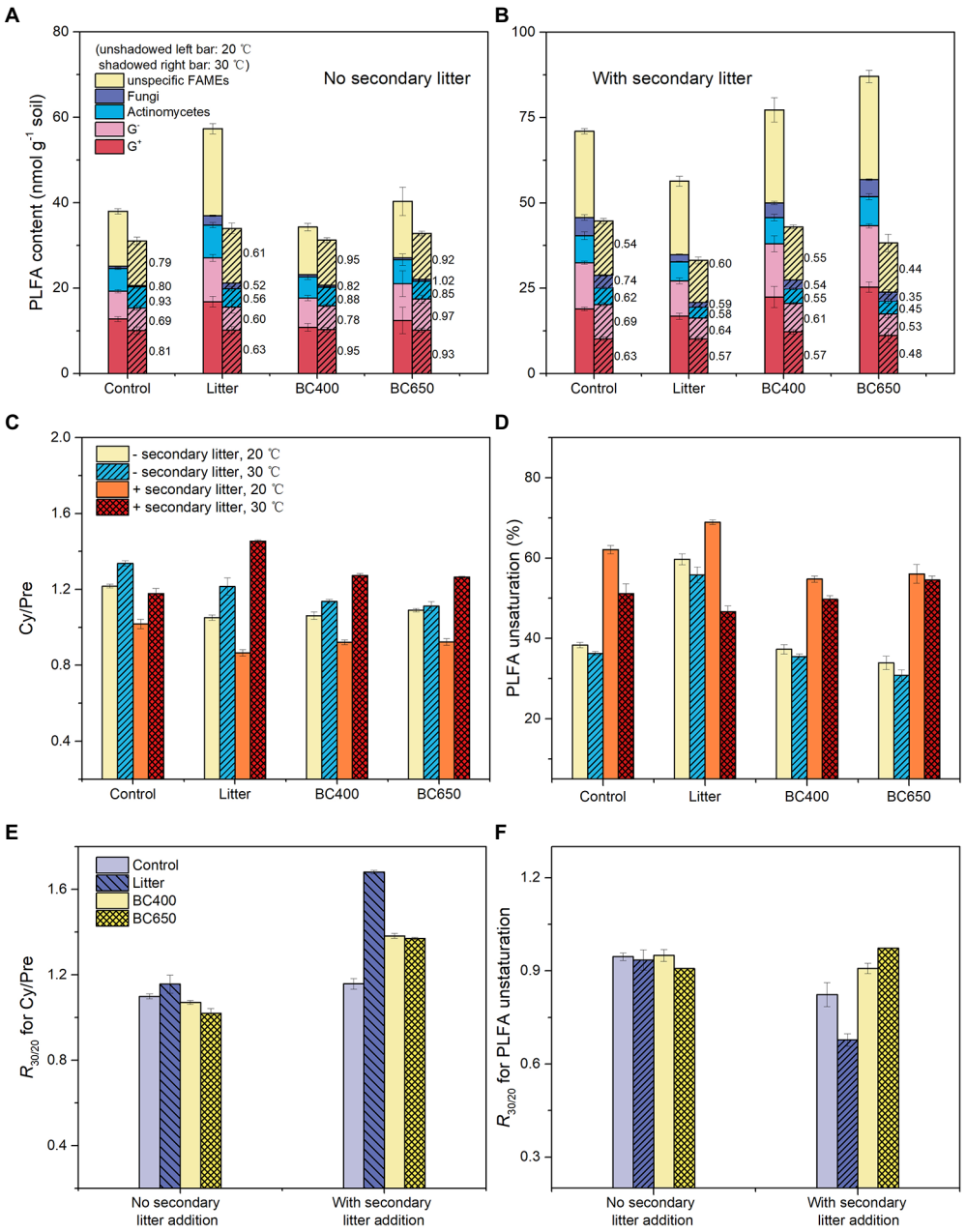

FIGURE 6
Phospholipid fatty acid (PLFA) content and temperature stress indicators calculated from PLFA data at the lateincubation stage: **(A,B)** PLFAs belonging to various microbial groups, with their sensitivities to warming (the ratio of levels at 30°C–20°C) given by the number alongside the bars; **(C,D)** ratio of cyclopropyl fatty acids (cy17:0 and cy19:0) to their precursors (Cy/Pre) and PLFA unsaturation; **(E,F)** sensitivities of Cy/Pre and PLFA unsaturation to warming (ratio of levels at 30°C–20°C, $R_{30/20}$). Soil under four prior treatments (Control, Litter, BC400, and BC650) with or without secondary addition of fresh rice litter were placed at 20°C (unwarmed) and 30°C (warmed) to mimic a short-term warming event. Error bars indicate standard errors (n=3). Note that the contents of all PLFAs were lower at 30°C than 20°C.

We mainly focused on the influence of substrate availability (indicated by $[S]_{20}/K_{m20}$) and microbial physiological characteristics (mainly the temperature-dependence of CUE, which determined $Q_{10\text{-}R\%}$) on the temperature sensitivity of SOM decomposition. First, we investigated the effects of substrate availability by varying $[S]_{20}/K_{m20}$ without considering $Q_{10\text{-}R\%}$ (with $Q_{10\text{-}R\%}$ set as 1), and found that the instantaneous Q_{10i} for SOM increased with $[S]_{20}/K_{m20}$ (Figure 8A). However, Q_{10i} was insensitive to $[S]_{20}/K_{m20}$ (Table 4). Even if we assumed a two-fold substrate content under litter amendment relative to that of the control (Table 3), this only slightly affected Q_{10i} (1.11 and 1.13 for control and litter-amended soils, respectively). The corresponding simulated temperature sensitivities for SOM (instantaneous and cumulative) were lowest in litter-amended soils (Figures 8B,C),

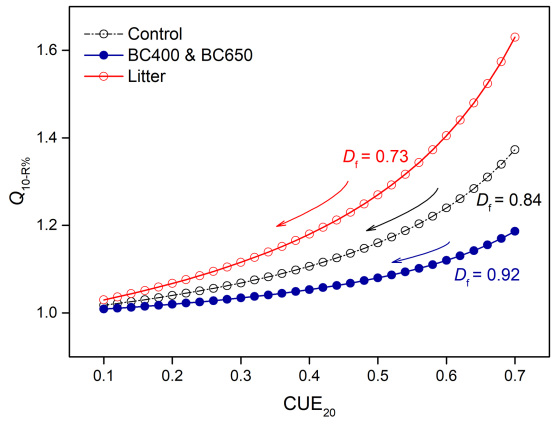

FIGURE 7
Simulated $Q_{10\text{-}R\%}$ as a function of microbial carbon use efficiency at 20°C (CUE_{20}), depending on soil amendments (litter or litter-made biochar). $Q_{10\text{-}R\%}$ reflects the loss of assimilated C due to decreasing CUE under warming, and was calculated according to Equation (14). D_f indicates the magnitude of CUE declines as temperature increases from 20 to 30°C. Lower D_f is used for litter-amended soils, where microbial biomass and enzyme pools decrease more under warming. Note that $Q_{10\text{-}R\%}$ was consistently higher in soils with lower D_f, and at a fixed D_f the loss of assimilated C becomes less temperature-sensitive (i.e., $Q_{10\text{-}R\%}$ decreases) as CUE decreases.

which was contradictory to our experimental observation. Therefore, the higher substrate availability in litter-amended soils alone could not account for the greater temperature sensitivity of SOM mineralization.

However, when we considered a higher $Q_{10\text{-}R\%}$ (i.e., larger warming-induced declines in microbial CUE) in litter-amended soils, the temperature sensitivities of SOM exceeded those in the control and biochar-amended soils (Figures 8D–F). The resultant pattern of Q_{10t} for SOM across amendments (i.e., Control ~ BC400 ~ BC650 < Litter) agreed with our experimental observations (Figure 2D). This could be because that Q_{10i} of SOM was much more sensitive to $Q_{10\text{-}R\%}$ than to $[S]_{20}/K_{m20}$ (sensitivity: 1 versus 0.04 for $Q_{10\text{-}R\%}$ and $[S]_{20}/K_{m20}$, respectively; Table 4). Based on these results, $Q_{10\text{-}R\%}$ was a more important determinant of the temperature sensitivity of SOM decomposition than substrate availability ($[S]_{20}/K_{m20}$).

Discussion

Less temperature-tolerant microbial communities In soil with litter amendments

Soil enzymes may adapt to warmer environments with rigid structures to enable better substrate affinity (Bradford et al., 2008; German et al., 2012), which tends to increase the Q_{10} of V_{max} but decreases that of K_m, as hypothesized by Allison et al. (2018). The Q_{10} for V_{max} and K_m, however, remained stable under short-term warming (Supplementary Figures S1, S2), suggesting little thermal adaptation of the enzyme structure and function. It is plausible that the new sets of isoenzymes produced in warmed soils maintain a relatively constant Q_{10} (Razavi et al., 2016). This may also account for the insignificant warming effects on K_m (Supplementary Figure S2).

Nevertheless, warming decreased V_{max} and enzyme activities (Figure 4), which could be associated with reductions in microbial biomass (Figures 6A,B) rather than enzyme thermal adaptation. V_{max} in soil usually reflects the enzyme pool size (Wallenstein et al., 2010), which, in turn, is linked to microbial biomass (Allison et al., 2010). Indeed, for soils at the late incubation stage, warming decreased the total PLFA content by approximately 24%, at a magnitude comparable to that for enzyme activities (15%) and V_{max} (18%). There were also positive correlations between warming-induced declines in PLFA and enzyme activities, particularly for NAG and LAP (Supplementary Figure S3). Notably, warming reduced microbial biomass more in the soil with litter (Figures 6A,B; Table 2), where microorganisms grew actively. These results suggest that microbial communities activated by litter were less tolerant to high temperatures than inactive microbes under biochar amendments.

Warming-induced enzyme denaturation should not be the major mechanism of V_{max} decline, because the loss of extracellular enzyme activities occurs slowly in soil owing to the stabilization of enzyme molecules on mineral surfaces (Allison, 2006; Schimel et al., 2017). For instance, it is very unlikely that warming-induced denaturation decreased the activities of enzymes such as NAG by nearly 50% following secondary litter input (Figure 4F). In addition, denaturation reduces the binding sites of enzymes, weakens substrate-enzyme affinity, and decreases K_m. However, we detected a relatively similar K_m between warmed and unwarmed soils, with or without litter addition. Decreased microbial biomass and enzyme production together with accelerated enzyme turnover (Conant et al., 2011) are more important controlling factors than denaturation for the declining V_{max} and apparent enzyme activities with soil warming.

Decreased microbial CUE or growth efficiency at higher temperatures, due to higher maintenance energy costs or waste metabolism (Bradford, 2013), accounts for the warming-induced reduction in microbial biomass (Tucker et al., 2013). This was supported by the overall higher mass-specific respiration at 30°C than at 20°C at the late incubation stage (Table 2). Moreover, warming increased the microbial synthesis of cyclopropyl and saturated fatty acids (Figures 6C,D), which can counter membrane fluidity at high temperatures (Suutari and Laakso, 1994; Wixon and Balser, 2013), but is energetically expensive (Zogg et al., 1997). Because of the trade-off between microbial growth and stress tolerance (Malik et al., 2020), this unavoidably reduces resource allocation to microbial growth, thereby decreasing microbial growth and CUE. Higher temperatures may also increase microbial death rates (Joergensen et al., 1990).

The mechanisms underlying the lower heat tolerance of actively growing microbial communities under litter input are still

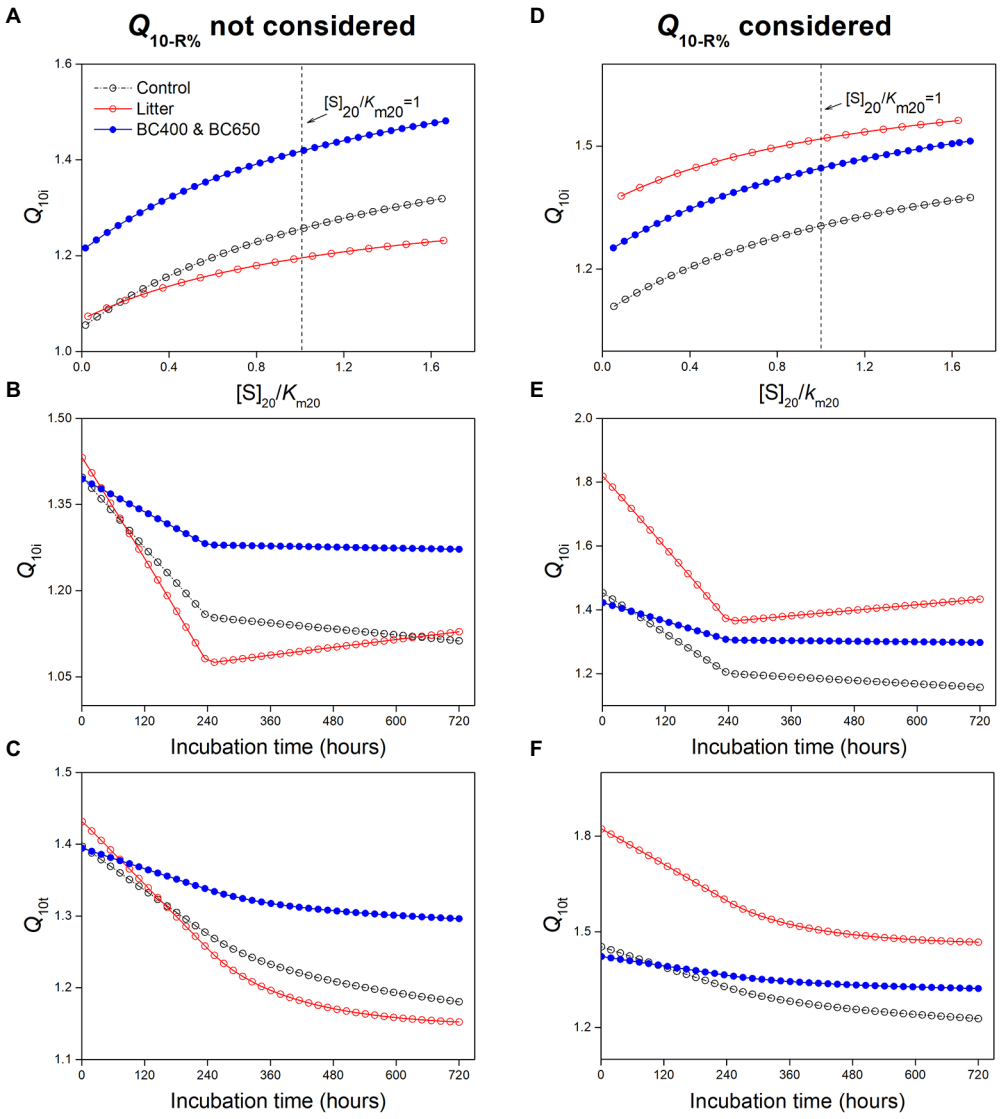

FIGURE 8
Results of the modeling analysis: **(A–C)** effects of $[S]_{20}/K_{m20}$ (ratio of SOM-derived substrate concentration to K_m at 20°C) on instantaneous temperature sensitivity (Q_{10i}) of SOM **(A)**, the modeled Q_{10i} of SOM using parameter values in Table 3 **(B)**, and cumulative temperature sensitivity (Q_{10t}) of SOM mineralization with time based on modeled Q_{10i} **(C)** without considering $Q_{10\text{-}R\%}$ in Equation (12); **(D–F)** relationship between Q_{10i} and $[S]_{20}/K_{m20}$ **(D)**, and the modeled Q_{10i} **(E)** and Q_{10t} **(F)** of SOM mineralization with $Q_{10\text{-}R\%}$ set to values in Table 3.

not clear. We hypothesize that the growing r-strategists stimulated by labile litter (Loeppmann et al., 2016) are less stress-tolerant than inactive and starving microbes (oligotrophs or K-strategists; Lipson, 2015) under biochar amendments. Starvation makes microbial species (mainly K-strategists) more resistant to stress (e.g., heat, UV; Nyström et al., 1992; Hartke et al., 1998). Specific proteins and lipids induced by starvation are produced to cope with environmental stress (Hartke et al., 1998). On the other hand, the r-strategists under C-abundant conditions may mainly invest energy into growth rather than stress resistance. Indeed, microorganisms growing on litter demonstrated lower cyclopropyl and saturated fatty acid levels (mainly at 20°C, Figures 6C,D), suggesting that they synthesized fewer heat-resistant compounds than K-strategists (Wixon and Balser, 2013). Overall, the distinct microbial life strategies, and corresponding energy allocation tradeoff between growth and stress resistance, might underlie microbial heat tolerance under litter and biochar amendments.

Increased resistance of enzymes and microbes to warming under biochar amendments

Biochar addition preserved enzyme activities at high temperatures, as evidenced by the lower warming-induced decline in enzyme activities in biochar-amended soils relative to the control or litter-amended soils (Figure 4). Biochar stimulated the

TABLE 4 Sensitivity analysis of Q_{10i} values (instantaneous temperature sensitivity, Equation 12) to key parameters depending on biochar and maize litter addition.

Parameter	Input range		Sensitivity[a]		
	Lower	Upper	Control	Litter	BC400 & BC650
D_f	0.4	1	1.00	1.00	1.00
Q_{10_vmax}	0.5	5	1.00	1.00	1.00
Q_{10_Km}	0.5	5	0.87	0.79	0.87
$Q_{10[S]}$	0.5	2	0.86	0.80	0.87
$[S]_{20}/K_{m20}$	0.01	2	0.05	0.03	0.04
$Q_{10\text{-}R\%}$	1	3	1.00	1.00	1.00

The input ranges of these parameters cover all possible values. Where lower and upper input refer to the lower and upper bounds of the input values of each parameter, and lower and upper outputs are the corresponding Q_{10i}, respectively.
[a]Sensitivity of Q_{10i} to an input parameter was assessed by:

$$\text{Sensitivity} = \frac{\log_{10}(\text{upper output}) - \log_{10}(\text{lower output})}{\log_{10}(\text{upper input}) - \log_{10}(\text{lower input})}$$ following Allison et al. (2010).

biosynthesis of saturated fatty acids, which is beneficial to microbial temperature resistance (Wixon and Balser, 2013), as seen from the lower PLFA unsaturation in unwarmed biochar-amended soils receiving secondary litter addition (Figure 6D).

In addition, biochar may create biologically favorable soil space (microbial niches) near its surfaces, i.e., the "charosphere" formed by the adsorption of water, nutrients and biomolecules (Luo et al., 2013; Quilliam et al., 2013). The charosphere may have contributed to the persistence of enzyme activity in the biochar-amended soils under warming conditions.

Lower Q_{10} of soil organic matter decomposition under biochar than under litter

Q_{10} of SOM decomposition is often increased by the addition of labile substrate (Gershenson et al., 2009; Liu et al., 2021). However, the effects of labile C addition on the temperature sensitivity of SOM versus FOM remain poorly understood (Wei et al., 2021). Herein, Q_{10} of both SOM and FOM increased with litter input, which was not observed with biochar addition (Figures 2, 3). Therefore, using litter-made biochar as a soil amendment instead of litter may lower the responses of both SOM and FOM decomposition to warming.

Substrate availability (i.e., item [*S*] in the Michaelis–Menten equation) increases the Q_{10} of soil CO_2 emission, which has frequently been emphasized previously (Pang et al., 2015; Liu et al., 2021). The generally low [*S*] in soils is a major constraint on the temperature sensitivity of many C cycling processes (Davidson et al., 2006). However, only a few studies have focused on the relationship between substrate availability and Q_{10} of SOM under fresh C inputs (Wei et al., 2021). Zhu and Cheng (2011) ascribed the increased Q_{10} of SOM with plant rhizodeposits to increased SOM-derived substrate availability, as stimulated by enzyme production. Despite more SOM-originating substrates (DOC, Table 2) in the litter-amended soils, we consider this to be a minor contributor to the Q_{10} of SOM. This is because Q_{10} is directly related to the substrate-K_m ratio ($[S]/K_m$) rather than the substrate content *per se* ([*S*]; Equation 12). The Q_{10}, however, was insensitive to $[S]/K_m$ values in our modeling (Table 4; Figure 8A). Furthermore, $[S]/K_m$ for SOM generally had small values (approximately 0.2 here), as SOM-derived [*S*] was commonly low relative to K_m (Larionova et al., 2007; Allison et al., 2010), and was unlikely to be substantially improved given the low energy availability of SOM (Gunina and Kuzyakov, 2022).

Our modeling suggested that Q_{10} of SOM was much more sensitive to warming-induced declines in microbial CUE (and hence loss of assimilated C with temperature, $Q_{10\text{-}R\%}$) than to substrate availability (Figure 8). This is partly because in our modeling (Equation 12), Q_{10} had a linear relationship with $Q_{10\text{-}R\%}$, but a saturating (Michaelis–Menten-like) relationship with substrate availability ($[S]/K_m$). In addition, if only substrate availability was considered, the modeled Q_{10} for SOM would be lowest under the litter amendment (Figures 8A–C), which was opposite to our experimental observations (Figure 2).This was because microbial biomass and enzyme activities declined the most with warming in litter-amended soils, which had strong negative impacts on Q_{10} that could not be counteracted by the slightly higher $[S]/K_m$ than under biochar amendments. The litter-amended soils had the highest simulated Q_{10} only when assigned the highest $Q_{10\text{-}R\%}$ (Figure 8F). This was reasonable because their mass-specific respiration and microbial biomass was much more sensitive to temperature elevation (Table 2; Figure 6), supporting that warming decreased microbial C utilization for growth (i.e., CUE; Li et al., 2019) to greater extents in litter-amended soils. However, the importance of microbial physiology to Q_{10} of SOM decomposition has often been neglected in previous studies (Zhu and Cheng, 2011; Su et al., 2022). Recently, Xu et al. (2022) found that vegetable field soils in warmer regions tended to have lower CUE and higher Q_{10} of SOM decomposition, highlighting the possible control over Q_{10} by CUE. Therefore, Q_{10} may be modeled as a function of microbial physiological parameters such as CUE.

The regulation of temperature sensitivity by microbial CUE may also account for the high Q_{10} of FOM inputs in soils with earlier litter additions. Following secondary litter addition, substrate availability was similar between all soils (DOC, Table 2). However, the Q_{10} of mass-specific decomposition of FOM was evidently higher in soils with earlier maize-litter amendments (Figure 3), suggesting that CUE in litter-amended soils might decline more with warming than in biochar-amended soils (Liu et al., 2020). Presumably, microbial community composition in prior litter-amended soils was more dominated by the temperature-intolerant r-strategists, making microbial CUE more temperature-sensitive even after FOM inputs. On the other hand, CUE of biochar-amended soils might be higher under warming as it could be facilitated by the beneficial effects of the "charosphere" on microbial cells and enzyme molecules (Luo et al., 2013; Quilliam et al., 2013).

Overall, through modeling analysis, we tentatively postulate that under exogenous C inputs, microbial physiology may outweigh substrate availability in controlling Q_{10} of the SOM (Figure 9). Added organic C should have a limited influence on substrate release from SOM, a mixture of substances with low energy availability that is consequently unfavorable to microbes (Gunina and Kuzyakov, 2022). In comparison, substantial changes in microbial life strategies (e.g., r versus K), as well as their heat tolerance, may occur under labile C inputs, greatly modifying the final Q_{10} for SOM decomposition. Future modeling of the temperature sensitivity of SOM decomposition should place greater emphasis on the microbial physiological changes (particularly decreasing CUE) in response to both warming and substrate availability.

Conclusion

Compared with biochar amendment, litter increased the rates and Q_{10} of both SOM and FOM decomposition. Litter addition stimulated microbial growth and activities to yield more extracellular enzymes, but the actively growing microbes were less resistant to warming than inactive microbes. Biochar had almost no effect on microbial growth, but made enzyme activities more resistant to high temperatures. This was possibly linked to the existence of the "charosphere," i.e., the biologically favorable space in the vicinity of biochar surfaces, due to the adsorption of water, nutrients, enzymes and substrates. Theoretically, greater warming-induced losses of microbial biomass and enzyme pools in litter-amended soils should lower Q_{10}. However, the litter-amended soils still had higher Q_{10} of SOM and FOM than biochar-amended soils, because warming accelerated microbial C loss (as reflected in the mass-specific respiration) to greater extents under litter inputs due to dominance of temperature-intolerant r-strategists. The acceleration of assimilated C loss also explains why litter-amended soils showed greater magnitudes of microbial biomass decline in response to warming. Despite the increased SOM-derived substrate availability by priming under litter

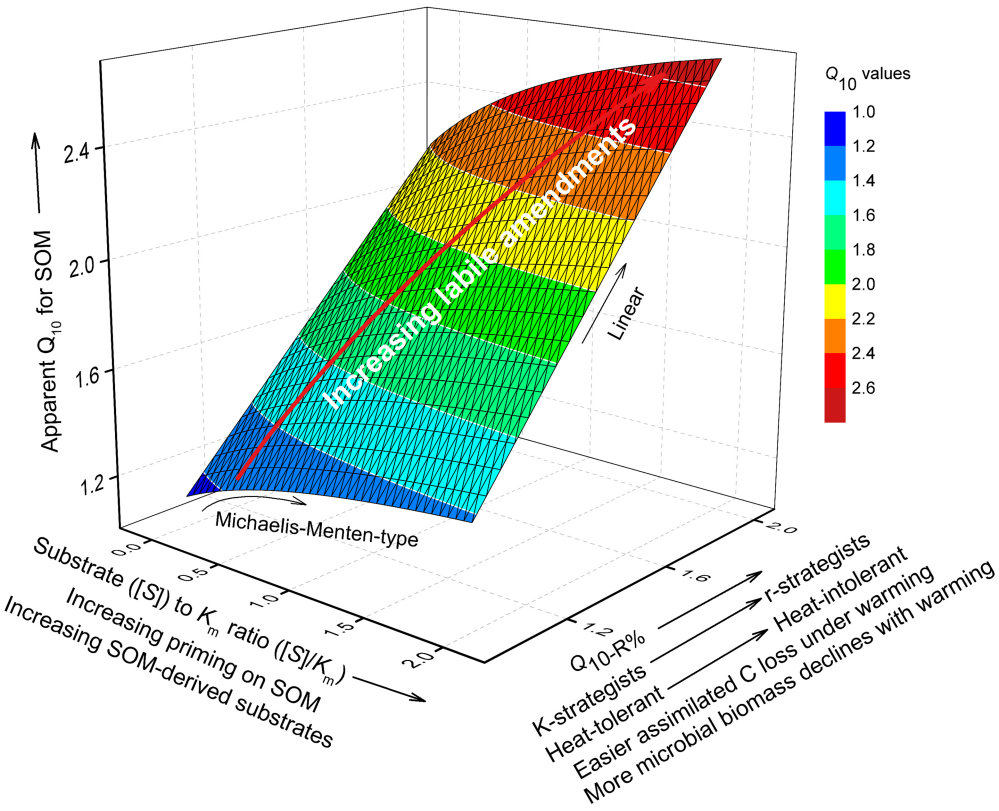

FIGURE 9
Conceptual figure showing the contribution of substrate availability and microbial physiological parameters on apparent Q_{10} of SOM decomposition, with the priming effect considered. The influence of substrate availability ([S]) is manifested in the item $[S]/K_m$ (Equation 12), i.e., the ratio of substrate availability to half saturation constant (K_m) in Michaelis–Menten kinetics. $[S]/K_m$ may increase due to the priming of SOM depolymerization by labile C inputs. The influence of microbial physiology on Q_{10} is mainly through $Q_{10\text{-}R\%}$, i.e., warming-accelerated loss of assimilated C from microbial cells. $Q_{10\text{-}R\%}$ increases with the proliferation of r-strategists under labile C inputs, which leads to higher microbial biomass that is less resistant to warming, and hence more easily loses assimilated C at high temperatures. According to the 3D shape, the Q_{10} of SOM is much more sensitive to increasing $Q_{10\text{-}R\%}$ than to increasing $[S]/K_m$. The white lines are the contours of Q_{10}. The red arrow indicates the trajectory of Q_{10} changes with $[S]/K_m$ and $Q_{10\text{-}R\%}$ owing to increasing labile amendments to soil.

inputs, our modeling results suggest this as a lesser contributor to the higher Q_{10} of litter-amended soils than the changing microbial physiology under warming (i.e., microbes more easily lose C by respiration at higher temperatures). Overall, we highlighted soil microbial physiological characteristics (e.g., microbial biomass, enzyme pools, mass-specific respiration, CUE, and their temperature dependence) as critical determinants of the temperature sensitivity of SOM decomposition, in addition to previously emphasized substrate availability. Whether the greater microbial vulnerability to warming under labile litter inputs is associated with the stress intolerance of r-strategists merits further investigation. Overall, we propose that rather than returning pure straw to the soil (a common agricultural practice), incorporation of straw-made biochar, or a combined application of biochar and straw, may be a better option to slow down SOM decomposition in agroecosystems in a warming climate.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

JC, CF, and ZD conceived and designed the research. JC, TG, and MN conducted the experiments and soil analysis. JC wrote the manuscript. JC, YK, and SA analyzed the data and edited the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2022.1008744/full#supplementary-material

SUPPLEMENTARY FIGURE S1
Relationship between log(V_{max}) and assay temperature of enzyme kinetics in unwarmed (at 20°C) and warmed soils (30°C). Error bars indicate standard errors (n=3).

SUPPLEMENTARY FIGURE S2
Relationship between log(K_m) and assay temperature of enzyme kinetics in unwarmed (at 20°C) and warmed soils (30°C). Error bars indicate standard errors (n=3).

SUPPLEMENTARY FIGURE S3
Relationship of warming-induced declines in soil enzyme activities (measured by $R_{30/20}$, the ratio of levels at 30–20°C) to that in total PLFA at the late incubate stage. Soils were amended with or without secondary rice litter inputs. The $R_{30/20}$ for enzyme activities was averaged over substrate concentrations of 200–600 μm (Figure 4). Two exponential equations were fitted for LAP and NAG enzymes. The correlation for BG was insignificant due to two outliers (within the dashed area).

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Biochar-mediated changes in the microbial communities of rhizosphere soil alter the architecture of maize roots

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Aeolian sandy soil is a key resource for supporting food production on a global scale; however, the growth of crops in Aeolian sandy soil is often impaired due to its poor physical properties and lack of nutrients and organic matter. Biochar can be used to enhance the properties of Aeolian sandy soil and create an environment more suitable for crop growth, but the long-term effects of biochar on Aeolian sandy soil and microbial communities need to be clarified. Here, a field experiment was conducted in which biochar was applied to a maize (*Zea mays* L.) field in a single application at different rates: CK, 0 Mg ha^{-1}; C1, 15.75 Mg ha^{-1}; C2, 31.50 Mg ha^{-1}; C3, 63.00 Mg ha^{-1}; and C4, 126.00 Mg ha^{-1}. After 7years of continuous maize cropping, verify the relationship between root architecture and soil microbial communities under biochar application using a root scanner and 16S/ITS rRNA gene sequencing. The application of biochar promoted the growth of maize. Specifically, total root length, total root surface area, total root volume, and root biomass were 13.99–17.85, 2.52–4.69, 23.61–44.41, and 50.61–77.80% higher in treatments in which biochar was applied (C2, C3, and C4 treatments) compared with the control treatment, respectively. Biochar application increased the diversity of bacterial communities, the ACE index, and Chao 1 index of C1, C2, C3, and C4 treatments increased by 5.83–8.96 and 5.52–8.53%, respectively, compared with the control treatment, and significantly changed the structure of the of bacterial communities in rhizosphere soil. However, there was no significant change in the fungal community. The growth of maize roots was more influenced by rhizosphere bacteria and less by fungal community. A microbial co-occurrence network revealed strong associations among rhizosphere microorganisms. The core taxa (Module hubs taxa) of the bulk soil microbial co-occurrence network were closely related to the total length and total surface area of maize roots, and the core taxa (Connectors taxa) of the rhizosphere soil were closely related to total root length. Overall, our findings indicate that the application of biochar promotes the growth of maize roots in aeolian sandy soil through its effects on bacterial communities in rhizosphere soil.

KEYWORDS
biochar, microbial community, maize, root architecture, rhizosphere

Introduction

Aeolian sand soil is one of the important reserve resources of cultivated in the world (Ge et al., 2015). This soil type is mainly present in areas with low precipitation, large diurnal temperature fluctuations, and sandstorms, such as deserts, grasslands, and semi-desert grasslands (Driessen et al., 2001). Approximately 18% of China's land area (1.74×10^6 hm^2) has aeolian sandy soil, and the area with aeolian sandy soil continues to grow (Kari et al., 2021). However, there are major challenges to growing crops in aeolian sandy soil because of its low content of organic matter and nutrients, as well as its poor water and fertilizer retention properties (Han et al., 2021). There is thus a need for more studies to explore the efficacy of using different approaches to enhance the properties of aeolian sandy soil.

Biochar is one potentially effective approach for enhancing the properties of aeolian sandy soil. Biochar is a solid, carbon (C)-rich product that is highly stable in soil, and it is produced *via* the high-temperature pyrolysis of biomass materials, including crop straw, rice husk, and livestock manure, under anoxic conditions (Sohi et al., 2010). The amount of straw produced on a global scale is substantial; straw is rich in nutrients, as it contains nearly half of the nutrients absorbed by crops (Lal, 2005). However, straw is often discarded and burned rather than used in crop production, and this practice results in an unnecessary waste of resources, as well as environmental pollution (Langmann et al., 2009). The reuse of straw to make biochar can reduce the environmental pollution associated with straw burning and enhance the properties of soil when biochar is applied to the soil (Zhang et al., 2021). Biochar has a loose and porous structure, and the physical properties of soil change following its application to soil (Soinne et al., 2014). For example, biochar can enhance the aeration and water-holding properties of soil (Glaser et al., 2002), increase the specific surface area and porosity of soil, and reduce soil bulk density (Novak et al., 2009; Busschei et al., 2010). Biochar is also rich in C and nutrients; thus, the application of biochar to soil can substantially increase the C content of soil and promote the conversion of soil C, nitrogen (N), and phosphorus (P; Lehmann et al., 2006). Biochar can also make the soil environment more suitable for the growth of soil microorganisms, promote the metabolic activities of soil microbes (Zhu et al., 2017), and increase the abundance and diversity of microbial communities (Siedt et al., 2021).

Several studies have shown that biochar application can have a substantial effect on soil microbial communities (Anderson et al., 2011; Luo et al., 2017; Hu et al., 2020; Akhil et al., 2021). Soil microbial populations were significantly increased in the long-term effect of biochar (Wardle et al., 2008; Kolb et al., 2009), but high application rates of biochar reduced soil microbial populations (Dempster et al., 2011). However, few studies have characterized the effects of biochar application on the microbial communities in rhizosphere soil (i.e., the root–soil interface). Biochar application can increase the biomass of pine roots and maize roots by 300% (Wardle et al., 1998) and from 88 to 92% (Yamato et al., 2006), respectively. Biochar can also have direct and indirect effects on the structure and diversity of soil microbial communities in the rhizosphere (Kolton et al., 2011; Yu et al., 2018). For example, biochar application was shown to increase the relative abundance of *Pseudomonas*, *Bacillus*, and *Trichoderma* in rhizosphere soil in a 6-week pot experiment (Jaiswal et al., 2018a). Biochar application was also shown to lead to significant increases in the diversity and evenness of rhizosphere bacterial communities in a 3-month experiment (Graber et al., 2010). The application of biochar over 4 consecutive years had a substantial effect on the structure of the soil fungal community; however, biochar application had no noticeable effect on fungal diversity (Yin et al., 2021). Overall, biochar application changes the soil physical (Lu et al., 2014; Nelissen et al., 2015) and chemical properties (Kimetu and Lehmann, 2010). The improvement of soil nutrient content can directly promote plant root growth (Abiven et al., 2015). In addition, biochar application can also change the soil microbial community by altering soil properties (Ding et al., 2016). In turn, soil microorganisms can act on crop roots (Bourceret et al., 2022). However, few studies have examined the long-term effects of biochar, determined the most appropriate application rate of biochar, as well as the relationship between root architecture and soil microbial communities under biochar application.

Here, we aimed to (1) identify the most suitable biochar application rate for fertilizing aeolian sandy soil; (2) characterize the long-term effects of biochar addition on the properties of aeolian sandy soil, the architecture of crop roots, and the diversity and structure of microbial communities in bulk and rhizosphere soil; and (3) clarify the relationships among soil, crop root architecture, and microbial communities under biochar addition. To address these aims, we conducted a field experiment in which biochar was applied to a maize field with aeolian sandy soil. We then characterized changes in the properties of aeolian sandy soil, the architecture of maize roots, and microbial communities following 7 years of continuous cropping and a single biochar application.

Materials and methods

Overview of the study area

Our study was conducted at the Battery Soil Improvement Experimental Station, 121 Regiment, Agricultural 8th Division, Shihezi Reclamation Area, Xinjiang Uygur Autonomous Region, China (43°26′–45°20′N, 84°58′–86°24′E) (Figure 1). The study area features an arid semi-desert climate with an average annual temperature of 7.5°C, 2,525 h of annual sunshine, a frost-free period of 169 days, 225 mm of annual rainfall, and 1,250 mm of annual evaporation. The aeolian sandy soil comprised 53.2% sand, 27.2% powder, and 19.6% clay grains (Ma, 2021).

Experimental design

Our experiment was conducted on a mobile dune that was bulldozed in 2014. The experiment was conducted in a randomized group design with five treatments varying in the rate of biochar application: CK, 0 Mg ha^{-1}; C1, 15.75 Mg ha^{-1}; C2, 31.50 Mg ha^{-1}; C3, 63.00 Mg ha^{-1}; and C4, 126.00 Mg ha^{-1}. There were three plots (4.6 m × 7 m) per treatment (Figure 1). Biochar was applied to each plot in separate applications, and it was mixed well with the soil at a depth of 0–30 cm (only applied only one time in 2014 layout experiment). Wheat straw was the source of the biochar used in the experiment. The biochar was carbonized at 450°C for 5 h, crushed, and filtered through a 2-mm mesh sieve. The properties of the biochar were as follows: pH, 8.21; organic C (OC), 1.38 g/kg;

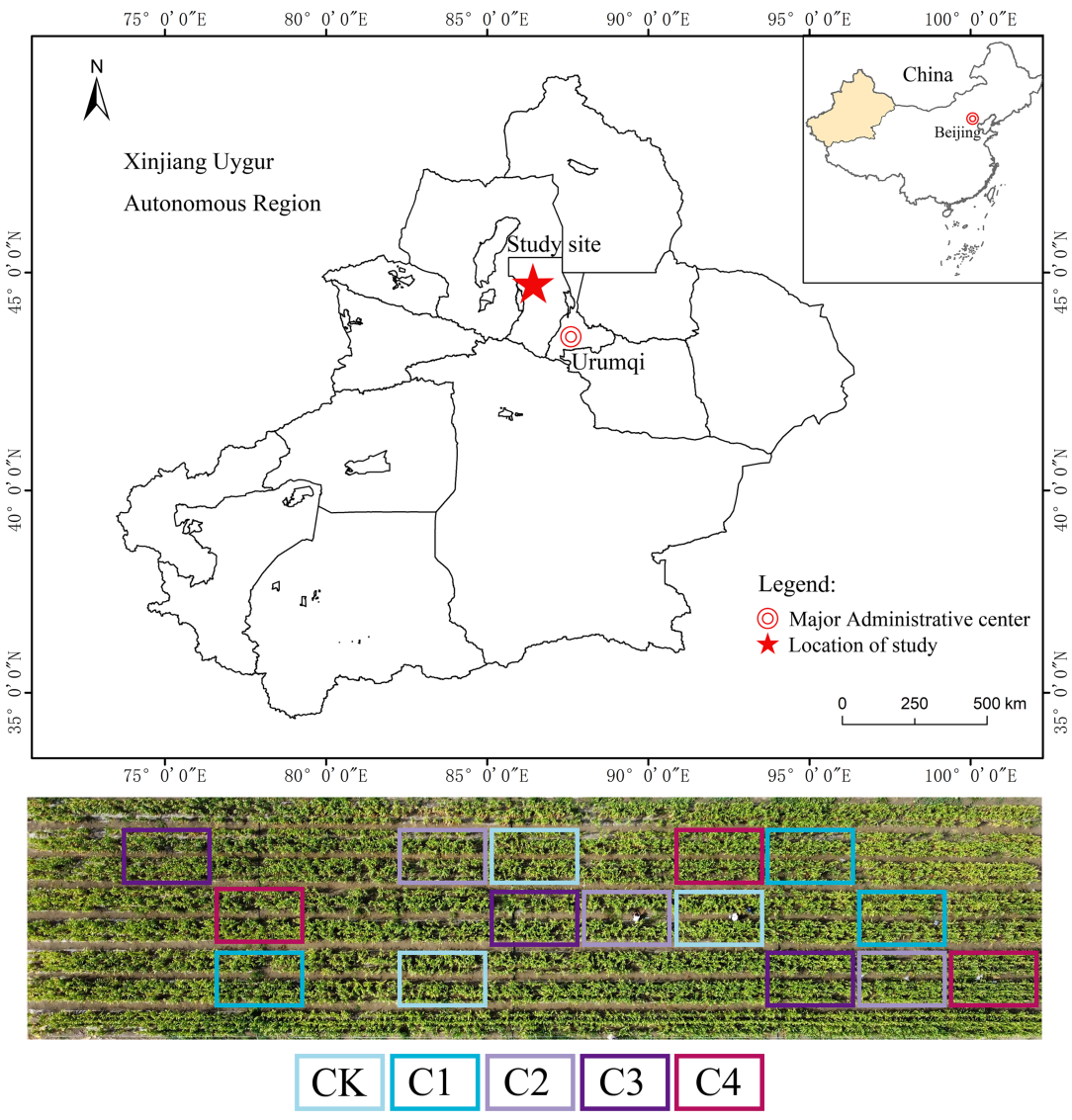

FIGURE 1
Study area within China.

available nitrogen (AN), 7.40 mg/kg; available phosphorus (AP), 4.60 mg/kg; and available potassium (AK), 97.00 mg/kg. Maize (Xin Yu 53) was sown in May and harvested in September each year from 2014 and 2021. Only one crop was planted in our experimental plots per year; plants were irrigated *via* under-membrane drip irrigation; and fertilizer application and other management practices were based on those used by local farmers. The total amount of irrigation per year was 4800.0 m^3 ha^{-1}, and the total amount of fertilizer applied was 258 kg ha^{-1} N, 123 kg ha^{-1} P_2O_5, and 81 kg ha^{-1} K_2O.

Sample collection and processing

Many scholars found increased microbial diversity and a more uniform distribution of bacterial communities at the rhizosphere level during maturation (Schmidt and Eickhorst, 2014; Kwak et al., 2018; Schmidt et al., 2018). Therefore, we chose to collect soil samples from maize stage R6 (black layer) in 2021. Firstly, maize roots were collected. To avoid interactions between plots, two maize plants were randomly selected in the center of each plot (3.6 m × 5.0 m; ensure that the sampling interval of each plot is two meters apart), and then rectangular soil blocks (30 cm × 30 cm × 30 cm) were cut vertically downward around the maize roots. After slapping away large chunks of soil, and carefully separate the roots from the soil (Nazih et al., 2001). The collected maize roots were brought back to the laboratory. Secondly, when collecting maize roots, rhizosphere soil is collected by shaking it off from the roots in the air (Wang et al., 2009). One of these samples was placed in a self-sealing bag, all other plant residues in the soil samples were removed, air dried, and sieved (1 and 0.25 mm) for subsequent determination of basic rhizosphere soil chemical properties. The other sample was immediately placed in a sterile centrifuge tube (2 ml) and stored in a liquid nitrogen tank at −80°C for subsequent characterization of the soil microbial community. There were six replicates for each treatment.

Thirdly, bulk soil samples were collected. Also due to avoid interactions between plots, we selected the center of each plot (3.6 m × 5.0 m) as the collection area and collected two mixed soil samples using the five-point sampling method at a depth of 0–30 cm. One of the samples was placed in self-sealing bag, all other plant residues in the soil samples were removed, air dried, and sieved (1 and 0.25 mm) for subsequent determination of basic bulk soil chemical properties. Another sample was placed in sterile centrifuge tube (2 ml) in liquid N tanks at −80°C for subsequent characterization of soil microbial communities. Finally, we also collected two ring knife samples from each plot to determine the physical properties of the soil. Six replicate samples were taken for each treatment.

Basic physicochemical properties of soil and maize root architecture

The collected plant roots were brought back to the laboratory, and the soil particles on the root surface were carefully washed with water. Images of maize roots were digitized using an Epson Perfection V850 Pro scanner, and WinRHIZO software was used to measure total root length, total root surface area, and total root volume. Root biomass is the weight recorded after drying at 105°C until reaching constant weight. A pH meter (Mettler Toledo FE28-Standard, Switzerland) was used to measure soil pH at water: soil ratio of 2.5:1. The H_2SO_4–$K_2Cr_2O_7$ external heating method (Hu et al., 2022), alkali diffusion method, spectrophotometry (Shimadzu UV-1780, Japan; Shao et al., 2019), flame photometry (Shanghaiyuefeng FP6400, China; Chen et al., 2021), the drying method, H_2SO_4-$HClO_4$ digestion—spectrophotometry (Shimadzu UV-1780, Japan), H_2SO_4-$HClO_4$ digestion—flame photometry (Shanghaiyuefeng FP6400, China; Mei et al., 2021), and an elemental analyzer (Euro EA3000, Italy) were used to measure the content of SOC, AN, AP, AK, soil moisture content, total P (TP), total K (TK), and total N (TN), respectively.

DNA extraction and sequencing

Total DNA was extracted using the Power Soil DNA Isolation Kit Power DNA Extraction Kit, and DNA integrity and purity were examined. The V3-V4 region of the bacterial 16S rRNA gene was amplified using the primers 338F (5′-ACTCCTAGGGAGGAGCA-3′) and 806R (5′-GGACTCH VGGGTWTTAT-3′) and combined with adapter sequences and barcode sequences (Quast et al., 2013). The fungal ITS1 gene was amplified using the primers ITS1 (5′-CTGT CATTAGGGAGAGAGA-3′) and ITS2 (5′-GCTGCGTTCTT CA TCGATGA-3′) and combined with adapter and barcode sequences (Kõljalg et al., 2013). PCR reactions were conducted in 50-μl systems with 100 ng of template DNA, 1.5 μl of primer (10 μmol/L), 25 μl of 2× PCR buffer for KOD FX Neo (Toyobo, Japan), 1.0 μl of KOD FX Neo DNA polymerase (1.0 U/μl; Toyobo, Japan), and 10 μl of dNTP (2 mmol/L). The thermal cycling conditions were as follows: pre-denaturation at 95°C for 5 min; 25 cycles of denaturation at 95°C for 40 s, annealing at 55°C for 40 s, and extension at 72°C for 40 s; and a final extension at 72°C for 7 min. A 1.8% agarose gel electrophoresis was used to detect the PCR products; an Illumina HiSeq 2500 platform was then used to sequence the quality-checked libraries.

Bioinformatics analysis

The reads for each sample were spliced into tags according to the overlap among reads using FLASH software (version 1.2.11, http://ccb.jhu.edu/software/FLASH/); these raw tags were then filtered using Trimmomatic software (version 0.33) to obtain high-quality tags. The final data were obtained after chimeric sequences were removed using UCHIME (version 8.1). USEARCH software (version 10.0) was used to cluster the tags at the 97% similarity level. Operational taxonomic units (OTUs) were annotated using

the Silva taxonomic database (Release 132, http://www.arb-silva.de) for bacterial OTUs and the Unite taxonomic database (Release 8.0, https://unite.ut.ee/) for fungal OTUs. Taxonomic ranks were assigned using the RDP Classifier (version 2.2, http://sourceforge.net/projects/rdpclassifier/) with a minimum confidence estimate of 80%. Mothur (version v.1.30, http://www.mothur.org/) was used to analyze the diversity of microbial communities. Linear discriminant analysis (LDA) effect size (LefSe) analysis[1] was used to determine the effect of biochar addition on the abundance of each component of the microbial communities. The logarithmic LDA score indicating significant differences was 3.0 (Zhou et al., 2019).

Data analysis

R (version 4.0.2) was used to analyze the data, and the significance of differences among treatments ($p < 0.05$) was determined using a least significant difference test in the Agricolae package. The abundances of microbial communities were added to the histograms using Origin software. Microbial taxa with abundance greater than 0.1% were selected, and microbial co-occurrence networks and Zi (intra-network module connectivity)-Pi (inter-network module connectivity) plots were made using the igraph package in R. Here, Network hubs ($Zi > 2.5$; $Pi > 0.62$), Module hubs ($Zi > 2.5$; $Pi \leq 0.62$), connectors ($Zi \leq 2.5$; $Pi > 0.62$), and peripherals ($Zi \leq 2.5$; $Pi \leq 0.62$) were defined according to their Zi and Pi threshold value (Poudel et al., 2016). Network hubs, Module hubs, and connectors mean the nodes were highly connected within or between modules, and he can act as a core taxa (Guimerà and Amaral, 2005; Toju et al., 2018). Correlations of soil microbial communities with soil physicochemical properties and maize root architecture were determined using the corrplot software package.

Results

Soil properties and maize roots affected by biochar application

The application of biochar had a significant effect on the soil moisture content and soil bulk density (Supplementary Table S1). The soil moisture content was 3.09% lower in the C4 treatment than in the CK treatment. Soil bulk density was 0.34, 1.45, 2.04, and 2.34% lower in the C1, C2, C3, and C4 treatments than in the CK treatment, respectively.

In the bulk soil, biochar application altered the content of AN, AP, total phosphorus (TP), AK, and total potassium (TK; Supplementary Table S2). The soil AN content was 36.72, 65.91, 138.79, and 143.88% higher in the C1, C2, C3, and C4 treatments than in the CK treatment, respectively, and these differences were significant. The content of AP was 12.60 and 42.00% higher in the C3 and C4 treatments than in the CK treatment, respectively, and these differences were significant. The content of TP was 7.14 and 14.29% higher in the C3 and C4 treatments than in the CK treatment, respectively, and these differences were significant. The AK content was 15.84, 17.83, and 28.96% higher in the C2, C3, and C4 treatments than in the CK treatment, respectively. The TK content was 20.88% higher in the C4 treatment than in the CK treatment, and this difference was significant.

In the rhizosphere soil, the SOC content was 17.00 and 23.85% higher in the C3 and C4 treatments than in the CK treatment, respectively, and these differences were significant; however, there were no significant differences in the pH and TN among treatments (Supplementary Table S2). The AN, AP, AK, TP, and TK content were higher in the C1, C2, C3, and C4 treatments than in the CK treatment, and these differences were significant. Specifically, AN was 10.53, 46.22, 55.92, and 106.58% higher; AP was 8.10, 19.25, 27.34, and 35.94% higher, and AK was 13.14, 18.58, 18.24, and 53.31% higher in the C1, C2, C3, and C4 treatments than in the CK treatment, respectively.

The application of biochar had a significant effect on the architecture of maize roots (Figure 2). The total root length and total root surface area of maize were significantly higher in the C2, C3, and C4 treatments than in the CK treatment. Specifically, the total root length was 13.99, 17.85, and 15.78% higher and the total root surface area was 2.52, 4.69, and 3.99% higher in the C2, C3, and C4 treatments than in the CK treatment, respectively. The total root volume was 11.21, 23.61, 36.92, and 44.41% higher in the C1, C2, C3, and C4 treatments than in the CK treatment, respectively, the root biomass was 50.61, 74.78, and 77.80% higher in the C2, C3, and C4 treatments than in the CK treatment, respectively, and these differences were significant.

Microbial community diversity affected by biochar application

The addition of biochar had no significant effect on the diversity of bacterial and fungal communities in bulk soil; however, biochar addition had a significant effect on the diversity of bacterial and fungal communities in rhizosphere soil (Figure 3). The ACE index was 7.05, 5.83, 6.79, and 8.96% and the Chao 1 index was 6.98, 5.52, 6.44, and 8.53% higher in the C1, C2, C3, and C4 treatments than in the CK treatment for bacterial communities in rhizosphere soil, respectively (Figure 3B). No significant differences in the ACE, Chao 1, Simpson, and Shannon indexes of the fungal communities were observed among biochar treatments and the CK treatment (Figure 3D). However, the Chao 1 index was 24.14% lower in the C4 treatment than in the C1 treatment; the Simpson index was 1.99% lower in the C2 treatment than in the C1 treatment; and the Shannon index was 9.75 and 10.04% lower in the C2 and C4 treatments than in the C1 treatment, respectively.

1 http://huttenhower.sph.harvard.edu/lefse/

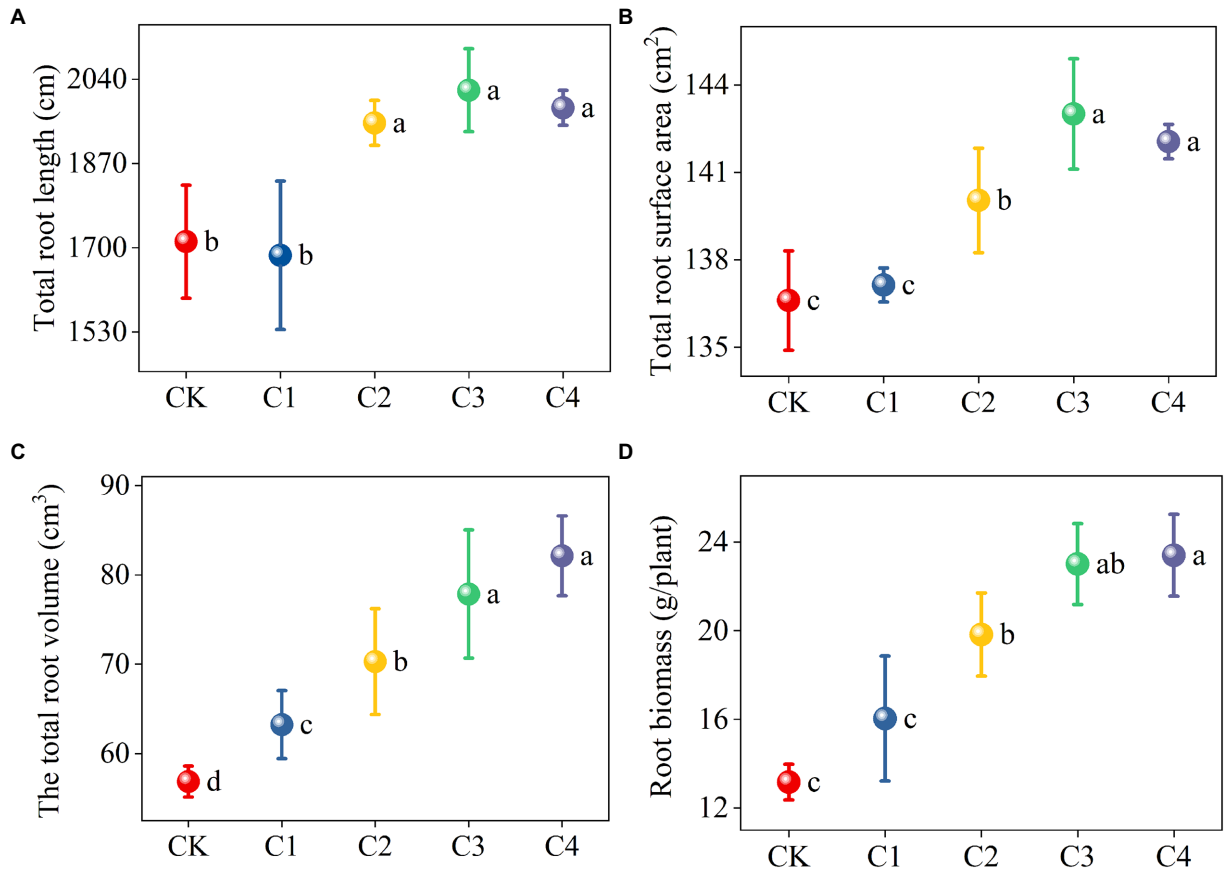

FIGURE 2
Effect of biochar addition on the architecture of maize roots. Different lowercase letters indicate significant differences under different levels of biochar application ($p<0.05$). **(A)** Effect of biochar addition on the total root length of maize. **(B)** Effect of biochar addition on the total root surface area of maize. **(C)** Effect of biochar addition on the total root volume of maize. **(D)** Effect of biochar addition on the root biomass of maize.

Microbial community structure affected by biochar application

Changes in the top 10 bacterial phyla in terms of relative abundance (Figure 4) were characterized in the soil samples from the different treatments. Variation in the structure of the bacterial community was low in bulk soil (Figure 4A). Firmicutes was the most abundant phylum (average abundance of 42.73%), followed by Proteobacteria (average abundance of 25.07%). The structure of the bacterial community was more variable in rhizosphere soil (Figure 4B). The relative abundance of Firmicutes was 20.53, 31.16, 42.09, 37.17, and 33.51% in the CK, C1, C2, C3, and C4 treatments, respectively. The relative abundance of Proteobacteria was 36.06, 30.41, 23.98, 28.71, and 29.86% in the CK, C1, C2, C3, and C4 treatments, respectively. The structure of fungal communities was less variable in bulk and rhizosphere soil (Figures 4C,D). Ascomycota was the most abundant phylum (average abundance of 70.70%), followed by Basidiomycota (average abundance of 16.78%).

In this study, the top 50 genus in terms of relative abundance were selected to demonstrate the changes in bacterial and fungal community composition in bulk and rhizosphere soil (Supplementary Figures S1, S2). In bulk soil, *Lactobacillus* was the most abundant bacterial genus (average abundance of 15.09%). C4 treatment significantly altered bulk soil bacterial genus, such as *Akkermansia*, *Mycoplasma*, *Ensifer*, etc. (Supplementary Figure S1A). The structure of the bacterial community was more variable in rhizosphere soil. After biochar application, *Lactobacillus* abundance was significantly increased by 3.66, 7.75, 6.72, and 5.21% in C1, C2, C3, and C4 treatments, respectively. *Sphingomonas* abundance was significantly reduced by 1.81, 4.73, 1.91, and 1.63% in C1, C2, C3, and C4 treatments, respectively (Supplementary Figure S1B). An unclassified genus was the most abundant fungal genus (average abundance of 37.60%) in bulk soil (Supplementary Figure S2A). In rhizosphere soil, the average abundance of this genus was 32.55% (Supplementary Figure S2B).

Linear discriminant analysis (LDA) effect size analysis revealed significant differences among treatments in 14 bacterial taxa (Figure 5C; Supplementary Figure S1A) and 15 fungal taxa (Figure 5A; Supplementary Figure S2A) in bulk soil. In the rhizosphere soil, significant differences among treatments were

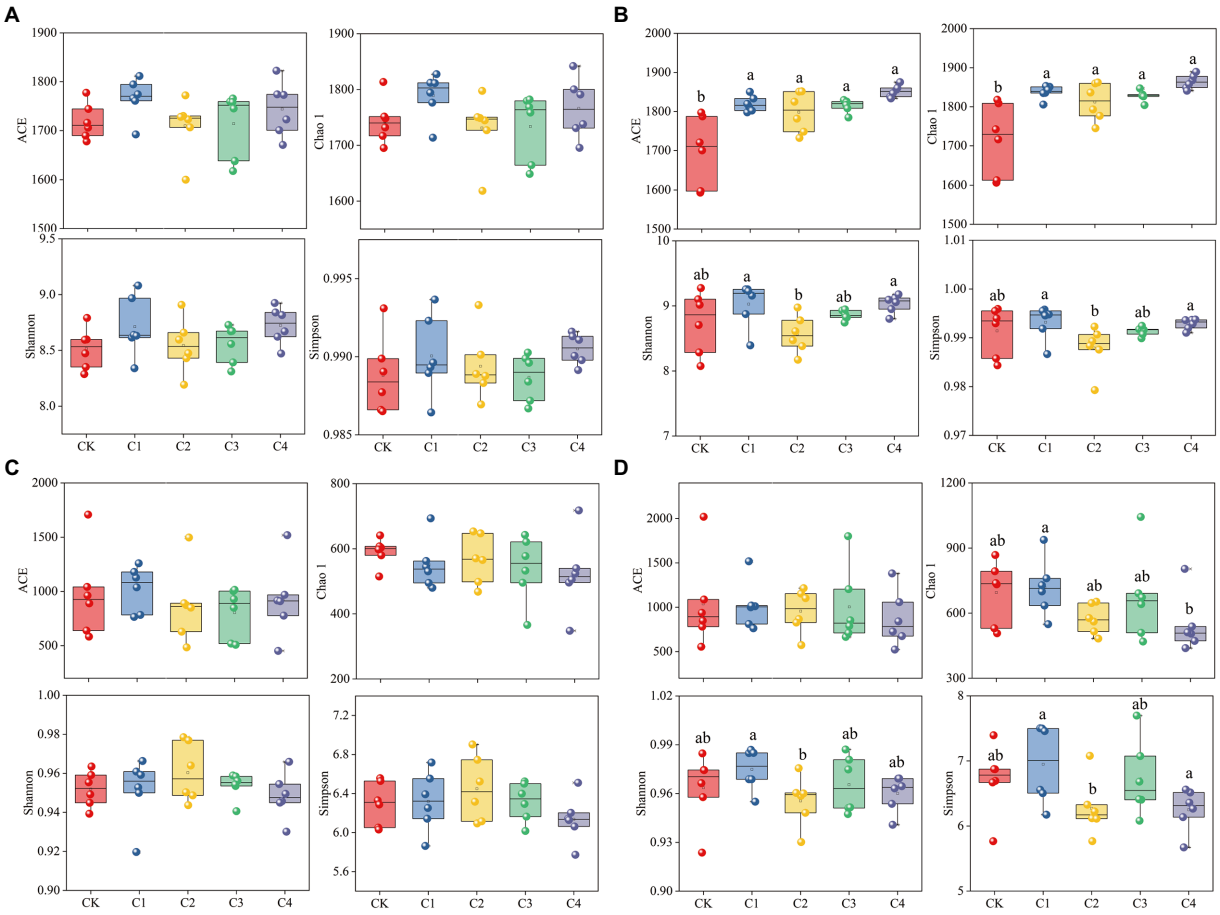

FIGURE 3
Effect of biochar addition on the α-diversity of soil microbial communities. **(A)** α-diversity of the bacterial communities in bulk soil. **(B)** α-diversity of the bacterial communities in rhizosphere soil. **(C)** α-diversity of the fungal communities in bulk soil. **(D)** α-diversity of the fungal communities in rhizosphere soil. Different lowercase letters indicate significant differences among biochar application treatments ($p<0.05$).

observed in 14 fungal taxa (Figures 5B,D and Supplementary Figure S2B) and 114 bacterial taxa (Figure 5D and Supplementary Figure S1B). The largest difference observed between treatments was in Firmicutes, which was 21.56% more abundant in the C2 treatment than in the CK treatment.

Correlation analysis of soil, microbial communities, and root architecture

Soil physicochemical properties and maize root architecture were highly correlated with microbial communities in rhizosphere soil at the phylum level; by contrast, correlations of soil physicochemical properties and maize root architecture with the microbial communities in bulk soil microbes at the phylum level were weak (Figure 6). In rhizosphere soil (Figure 6B), SOC, AN, AP, AK, TP, TK, total root volume, and root biomass were highly significantly and positively correlated with Fibrobacteres and negatively correlated with Olpidiomycota; AN, AP, and total root length were significantly and negatively correlated with Planctomycetes; and AN, AP, total root length, total surface area, total volume, and root biomass were significantly and negatively correlated with RsaHF231.

We constructed a microbial co-occurrence network (Figure 7A) to clarify interrelationships among microorganisms. Bulk soil microorganisms could be divided into three modules, which were referred to as Module 1, Module 2, and Module 3. Rhizosphere soil microorganisms could be divided into two modules, which were referred to as Module 1 and Module 2 (Figure 7B). In the bulk soil group, the bacterial–fungal mutualistic network contained 328 nodes and 10,036 edges, and the average path length was 61.195; in the rhizosphere soil group, the bacterial–fungal mutualistic network contained 328 nodes and 14,472 edges, and the average path length was 88.244 (Table 1).

Zi-Pi plots were constructed to identify the core OTUs in the microbial networks (Figures 7C,D). We identified a total of 189 connection points and three module centroids in bulk soil microbial network (Figure 7C). There were 98 connection points and four module centroids in the rhizosphere soil microbial network (Figure 7D). Ascomycota was the most

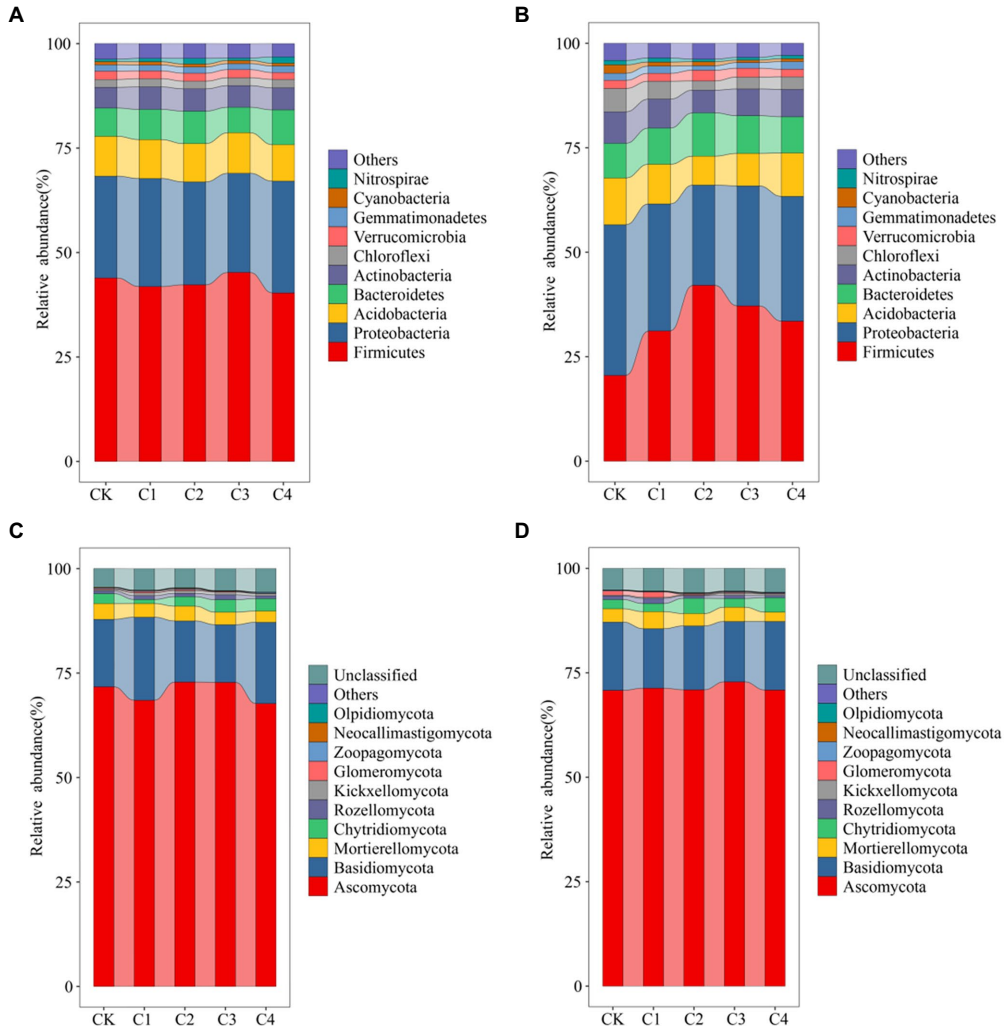

FIGURE 4
Effect of biochar addition on the structure of soil bacterial communities. **(A)** Bacterial communities in bulk soil at the phylum level (TPO10). **(B)** Bacterial communities in rhizosphere soil at the phylum level (TPO10). **(C)** Fungal communities in bulk soil at the phylum level (TPO10). **(D)** Fungal communities in rhizosphere soil at the phylum level (TPO10).

abundant phylum (average abundance of 69.10%) among connector's taxa in the bulk soil co-occurrence network, followed by Basidiomycota (average abundance of 12.98%). Ascomycota was the most abundant phylum among hubs taxa in the bulk soil co-occurrence network, and there was extensive variation in the abundance of Ascomycota among treatments; the relative abundance of Ascomycota was 62.64, 96.31, 70.92, 87.76, and 64.62% in the CK, C1, C2, C3, and C4 treatments, respectively. This was followed by Chytridiomycota, and its relative abundance was 37.36, 3.69, 29.08, 12.24, and 35.38% in the CK, C1, C2, C3, and C4 treatments, respectively. Ascomycota was the most abundant phylum (average abundance of 62.43%) among connectors taxa in the rhizosphere soil co-occurrence network, followed by Basidiomycota (average abundance of 9.53%). Basidiomycota was the most abundant phylum among hubs taxa in the rhizosphere soil co-occurrence network, and there was extensive variation in the abundance of Basidiomycota among treatments; the relative abundance of Basidiomycota was 65.93, 50.60, 57.58, 45.97, and 4.82% in the CK, C1, C2, C3, and C4 treatments, respectively. This was followed by Mortierellomycota, which had relative abundances of 34.07, 49.40, 42.42, 54.03, and 95.18% in the CK, C1, C2, C3, and C4 treatments, respectively.

Connectors taxa were not significantly correlated ($p > 0.05$) with total root length, total root surface area, and total root volume (Figure 8); hubs taxa were significantly correlated ($p < 0.05$) with total root length and total root surface area in bulk soil (Figure 8A). In rhizosphere soil, connectors taxa were significantly correlated with total root length ($p < 0.05$); hubs taxa were not significantly correlated with total root length, total root surface area, and total root volume ($p > 0.05$; Figure 8B).

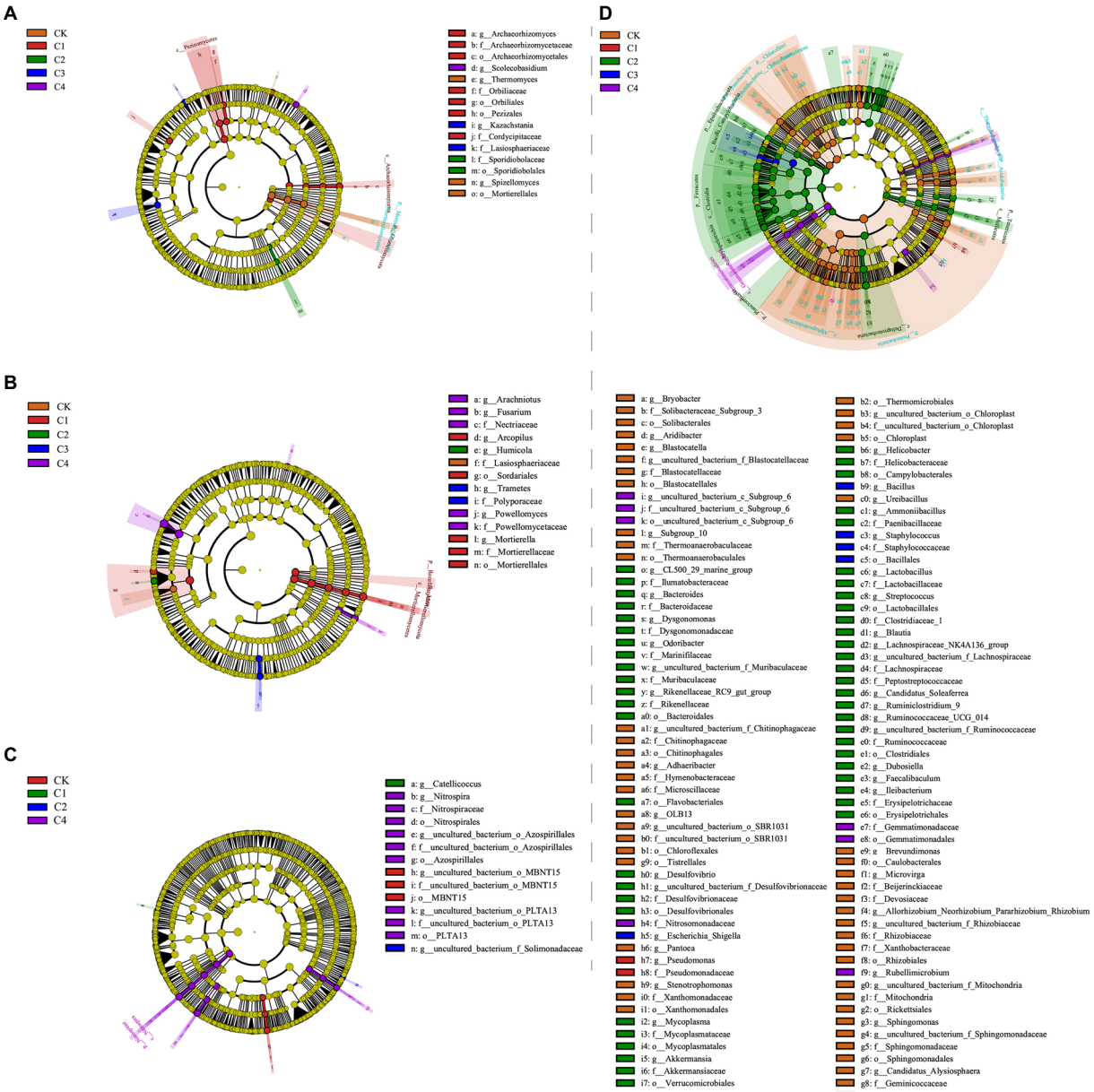

FIGURE 5
Analysis of the effect of biochar addition on soil microbial communities at the phylum and genus level using Linear discriminant analysis (LDA) effect size (LEfSe). **(A)** Fungal communities in bulk soil. **(B)** Fungal communities in rhizosphere soil. **(C)** Bacterial communities in bulk soil. **(D)** Bacterial communities in rhizosphere soil.

Discussion

Soil microorganisms have a substantial effect on the flow of energy and material cycling of ecosystems. Studies of soil microbes have been a major focus in soil and environmental investigations (Yao et al., 2006; Todd-Brown et al., 2012). The plant–root environment has a major effect on microbial communities, and microbial communities can affect the growth of plant roots (Vejan et al., 2016; Trivedi et al., 2020). The application of biochar can make the soil environment more suitable for soil microorganisms and thus affect microbial activity (Li et al., 2020a). However, the relationship between crop root architecture and microbial communities under biochar addition has not been extensively studied. Here, we analyzed the long-term effects of biochar addition on the architecture of maize roots and microbial communities using 16S/ITS rRNA gene sequencing.

Effect of biochar application on the architecture of maize roots

The results of our experiment showed that even a single application of biochar can have a significant effect on the growth

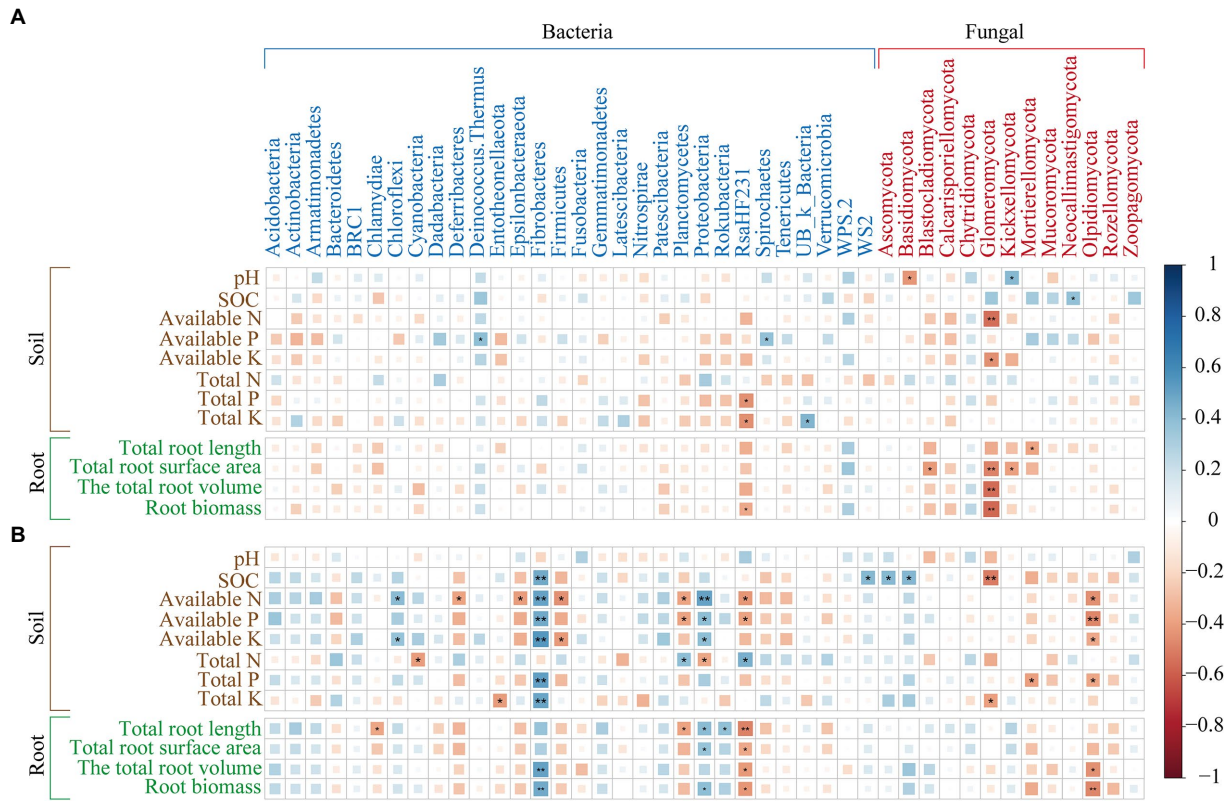

FIGURE 6
Relationships among soil microbial communities, soil physicochemical properties, and maize root architecture. **(A)** Communities in bulk soil. **(B)** Communities in rhizosphere soil. *p<0.05; **p<0.01.

of maize roots after 7 years. This is consistent with the results of several studies showing that the application of biochar enhances soil properties, which promotes the growth and development of roots (Abiven et al., 2015). However, the results of recent studies examining the effects of biochar application on the growth and development of plant roots are variable. In some studies, biochar has been shown to have a positive effect on the root growth of crops (Crane-Droesch et al., 2013). In other studies, biochar has been shown to have a negative effect on root growth, including toxic effects that force plants to grow more roots to meet their water and nutrient needs (Hodge, 2004; Peng et al., 2010). Our findings demonstrate that biochar has a positive effect on root growth (Figure 2), and the enhancement of the physicochemical properties of soil by biochar is one of the driving forces of this positive effect (Olmo et al., 2016). Correlation analysis between soil properties and root growth (Supplementary Figure S5) revealed a positive correlation between AP and TP in the rhizosphere soil and root growth. Previous studies have indicated that P reacts with various chemical and biological components in the soil and that increases in P alter maize root secretions and root symbionts, which in turn increases the growth of the lateral roots of maize (Lynch, 2011; Bourceret et al., 2022). This conclusion was confirmed by the changes in soil chemistry, where the application of biochar significantly increased the nutrient content, such as AP and TP in the rhizosphere soil (Supplementary Table S2), which provided the plant with nutrients required for root growth (Ding et al., 2016). However, soil bulk density was negatively correlated with root growth (Supplementary Figure S5). It showed that the total root length, total root surface area, and total root volume tended to increase as the soil bulk density decreased. This is in agreement with previous studies that a lower bulk density increases soil porosity leading to increased aeration, which affects root distribution and growth (Bengough and Young, 1993; Goodman and Ennos, 1999). Notably, we found that the application of biochar significantly reduced the soil moisture content of C4 treatment (Supplementary Table S1), which is inconsistent with previous studies, which found that the application of biochar improved the water retention and effective moisture of the soil (Baccile et al., 2009; Laird et al., 2010; Glab et al., 2018). Our study found that opposite results in administering doses of higher biochar (126.00 Mg ha^{-1}). First of all, previous studies found that charcoal applications greater than 80.00 Mg ha^{-1} instead reduced soil water-holding properties, which may be due to the increase of soil aeration pore space and the decrease of capillary pore space, resulting in the decrease of soil water holding capacity (Gao et al., 2011; Carvalho et al., 2016). Secondly, maize is a deep-rooted crop with a root system that can grow up to 1 m deep. Excessive application of biochar in shallow Soils can affect

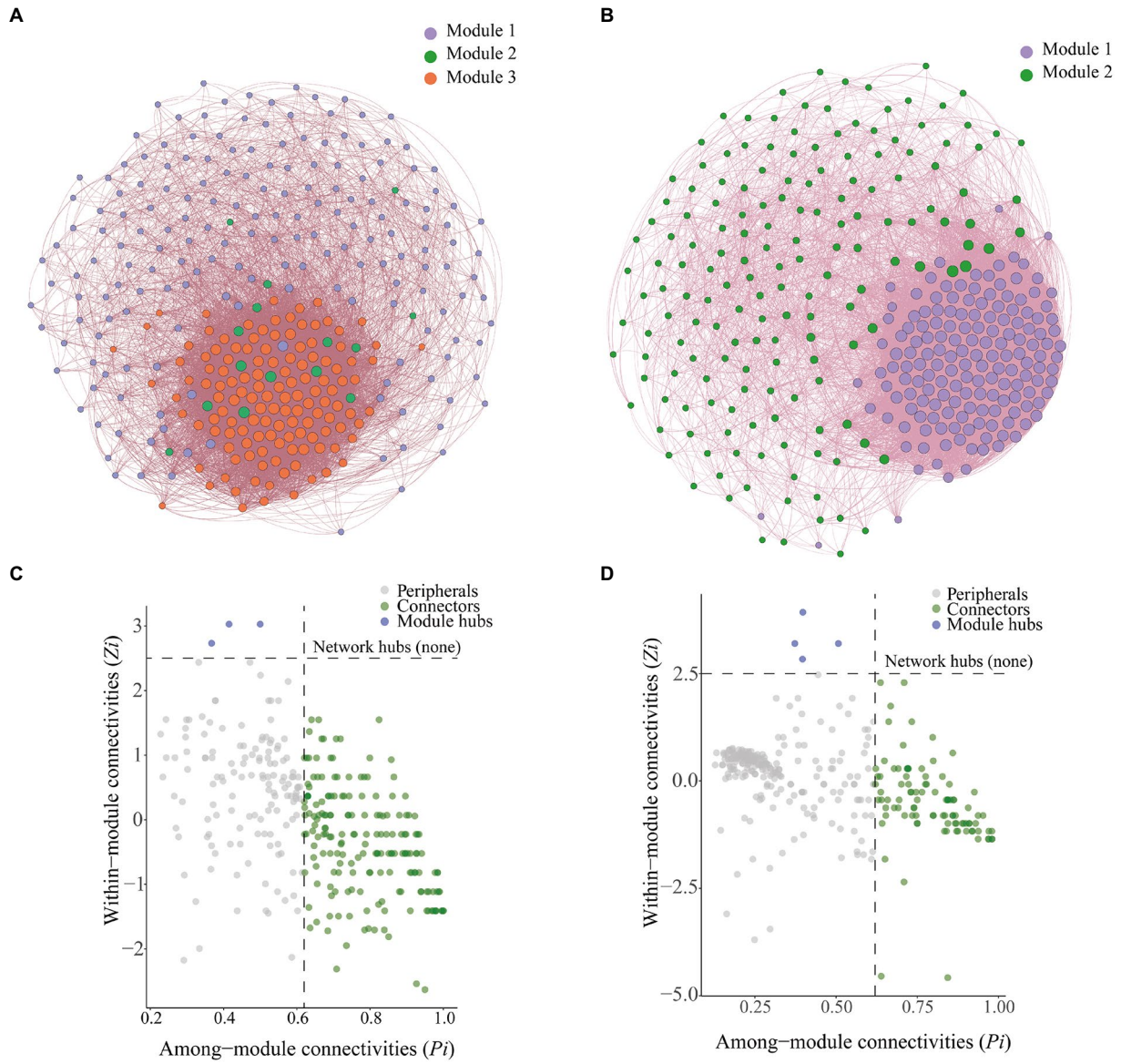

FIGURE 7
Co-occurrence networks and analysis of soil microbial communities. **(A)** Co-occurrence network of bacteria and fungi with relative abundance greater than 0.1% in bulk soil. **(B)** Co-occurrence network of bacteria and fungi with relative abundance greater than 0.1% in rhizosphere soil. **(C)** Zi-Pi plots of bacteria and fungi with relative abundance greater than 0.1% in bulk soil. **(D)** Zi-Pi plots of bacteria and fungi with relative abundance greater than 0.1% in rhizosphere soil.

TABLE 1 Co-occurrence network analysis coefficients.

Treatment	The number of nodes	The number of edges	Average degree	Weighted average	Mean clustering coefficient	Mean path length
Bulk	328	10,036	61.195	87.762	0.466	2.023
Rhizosphere	328	14,472	88.244	155.682	0.568	1.912

the water holding capacity of the soil around the maize root system (Feng et al., 2021). Thirdly, the previous study was conducted after the fourth month and after the 39th day of biochar application, while our study was conducted after 7 years of biochar application (Laird et al., 2010; Glab et al., 2018) and aging biochar may affect soil moisture content. Finally, we also found that the biomass of maize roots was greatest when 126.00 Mg ha^{-1} biochar was applied (Figure 2), maize roots may have absorbed more

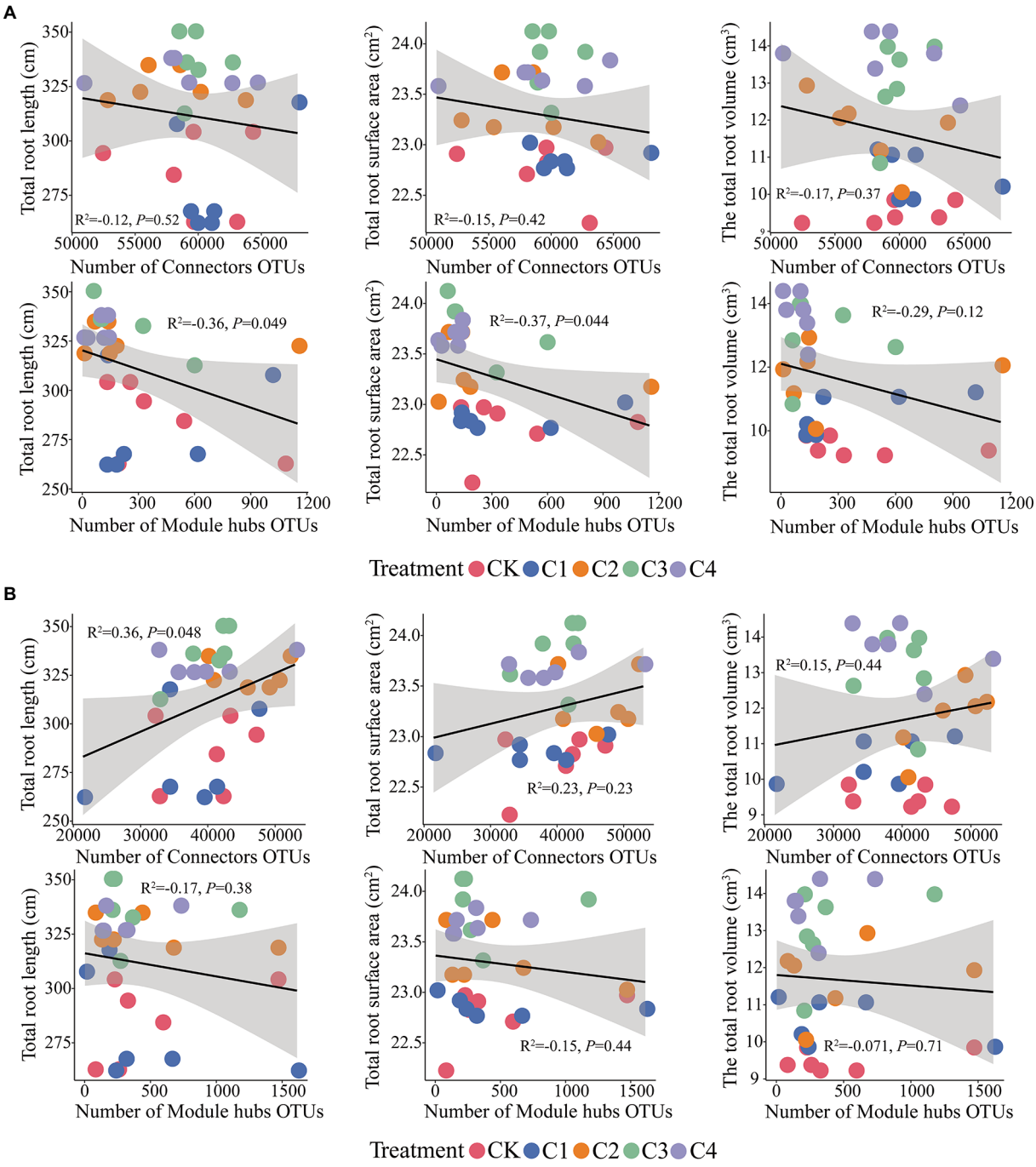

FIGURE 8
Effect of core microbial taxa on the architecture of maize roots. **(A)** Effect of microbial taxa in bulk soil on the architecture of maize roots. **(B)** Effect of microbial taxa in rhizosphere soil on the architecture of maize roots.

water, resulting in the reduction of soil water content (Dardanelli et al., 2004).

Changes in the roots of plants might also be affected by the interaction between biochar and rhizosphere soil microbial communities (Glaser et al., 2002; Liang et al., 2006; Li et al., 2020a). The application of biochar increases the production of exudates by roots and provides nutrients and energy for microbial metabolism and growth, which alters the relationship between rhizosphere microorganisms and plants (Ma et al., 2019). These findings are consistent with the results of our study; rhizosphere soil microorganisms had a closer relationship with maize root growth than bulk soil microorganisms under biochar application (Figure 6). In addition, rhizosphere microorganisms accumulate around the root system and enhance the bioavailability of

insoluble minerals, which increases the uptake of minerals by the roots and provides nutrients to the plant thus changing the maize root structure (Trivedi et al., 2020).

Differences in rhizosphere soil and bulk soil bacterial and fungal communities

We found that biochar application had a more pronounced effect on rhizosphere soil bacterial communities than on fungal communities. Previous studies have shown that environmental factors have stronger effects on bacterial communities than on fungal communities (de Vries et al., 2018; Yang and Wu, 2020). This might stem from the fact that bacteria can be more readily adsorbed by biomass charcoal than fungi (Pietikäinen et al., 2000); bacteria can also more rapidly adapt to changes in soil nutrients associated with biomass charcoal compared with fungi (Lehmann et al., 2011). Several non-mutually exclusive explanations might explain these observations. Previous studies have suggested that biochar has an indirect effect on the growth of bacteria and a direct effect on the growth of fungi. Specifically, biochar species have direct effects on the abundance of fungi, whereas bacteria are primarily affected by changes in soil properties associated with biochar application (Yang and Wu, 2020). This is consistent with our finding that the application of biochar significantly enhanced soil physicochemical properties (Supplementary Tables S1, S2). The contents of SOC, AN, AP, and AK in rhizosphere soil increased after biochar application. Researchers have found similar results with Tobacco, biochar addition increased the richness and diversity of the bacterial community in the tobacco rhizosphere, which was related to the soil physical and chemical properties (Zhang et al., 2017). In addition, fungi degrade the recalcitrant C in biochar more readily than bacteria, can grow in the pores of biochar, and use additional resources (Lehmann et al., 2011). In this study, the length of time (7 years) since biochar application, the low content of recalcitrant C in biochar, and the destruction of the pores over time might explain the weak effect of biochar application on fungi in our study. Therefore, bacterial communities were more affected by biochar treatment than fungal communities.

We found that Firmicutes was the dominant bacterial phylum in the soils at our study site, and members of this phylum are known to be well adapted to survive extreme conditions (Hayward et al., 2010). The soils at our study site are sandy, poor in nutrients, and low in organic matter (Han et al., 2021). Our study site is located in Central Asia, which experiences an arid, semi-desert climate, and this type of climate is highly suitable for the growth of thick-walled fungi. Soil potassium (K) is the main factor affecting the distribution of Firmicutes (Vollú et al., 2014). Our correlation analysis confirmed this expectation, as K was significantly correlated with the abundance of Firmicutes in rhizosphere soil (Figure 6).

There were significant differences in the structure of the soil bacteria communities between rhizosphere soil and bulk soil under biochar application. Previous studies have shown that Firmicutes comprises a large portion of the bacterial community in rhizosphere soil (Teixeira et al., 2010; Ramos et al., 2019). However, we found that the abundance of Firmicutes was higher in bulk soil (40.34–45.25%) than in rhizosphere soil (20.53–42.09%; Figure 4). Firmicutes are known to generate desiccation-resistant endospores (Heulin et al., 2012; Ramos et al., 2019). Thus, we suspect that the high relative abundance of Firmicutes in bulk soil might stem from the production of large amounts of bacilli by members of the genus *Bacillus*, which have been shown to comprise approximately 21.41% of all bulk soil bacteria under unfavorable conditions (Song et al., 2013). Alternatively, the difference in the structure of the bacterial communities between rhizosphere soil and bulk soil might be explained by root exudates (Harel et al., 2012; Kolton et al., 2016; Coskun et al., 2017; Jaiswal et al., 2018b). Root exudates are a key source of nutrients for rhizosphere bacteria and have a substantial effect on the structure of soil microbial communities (Gu et al., 2020). We suggest that the application of biochar might alter the structure of the microbial communities of rhizosphere soil by increasing root-produced secretions and providing nutrients and energy that aid microbial metabolism and growth (Ma et al., 2019); the application of biochar could also contribute to differences in the structure of the bacteria communities in rhizosphere soil and bulk soil.

Effect of biochar application on soil microbial communities

Our findings revealed that the application of biochar resulted in a significant increase in the α-diversity of bacterial communities in rhizosphere soil (Figure 3B). These findings are consistent with the results of previous studies showing that the application of biochar can lead to significant increases in the diversity of rhizosphere soil bacteria (Graber et al., 2010). The application of biochar has also been shown to significantly increase the α-diversity of bacterial communities in the rhizosphere soil of apple trees (Cao et al., 2021). Increases in soil nutrients might alter the structure and diversity of soil microbial communities (Hamer et al., 2004; Lehmann et al., 2011). We found that the application of biochar significantly increased the content of nutrients in rhizosphere soil (Supplementary Table S2), and this likely affects the diversity of the bacterial communities in rhizosphere soil (Toyama et al., 2011).In rhizosphere soil bacterial communities, the relative abundance of Firmicutes was significantly increased, and the abundance of Actinobacteria and Acidobacteria were decreased when biochar was applied at rates of 15.75–126.00 Mg ha^{-1}. They were identified as biochar decomposers (Khodadad et al., 2011; Pezzolla et al., 2015). Firmicutes are fast-growing copiotrophs, and biochar provides nutrients (Fierer et al., 2007) and growth sites (Li et al., 2022), enhancing their competitiveness in the bacterial colonies. The relative abundance of soil acidobacteria was negatively correlated with soil pH, and the abundance of Acidobacteria decreased with the increase of pH (Mao et al., 2012; Männistö et al., 2007; Lauber et al., 2008; Chu et al., 2010; Griffiths et al.,

2011). However, there was no significant correlation between the relative abundance of Acidobacteria and soil pH values in this study (Figure 6). This may be influenced by other environmental factors in the soil (Navarrete et al., 2013). The abundance of Acidobacteria decreased may be caused by different Acidobacteria subgroups, or even different Acidobacteria bacteria in the same subgroup, which have different responses to soil environmental factors (Jones et al., 2009; Zhang et al., 2014). The application of willow branch biochar (17.00–68.00 Mg ha^{-1}) has been shown to increase the relative abundance of Actinobacteria (Prayogo et al., 2014); however, we found that biochar application resulted in a decrease in the abundance of Actinobacteria. This might stem from differences in the type of biochar applied (Wheat straw was the source of the biochar used in this experiment). The application of biochar does not appear to affect the growth of Actinobacteria in soil over short periods; however, the abundance of Actinobacteria tends to increase in the long term (Xu et al., 2020). This finding indicates that the effect of biochar application on microorganisms varies depending on the length of time since biochar application. In addition, the higher amount of available C in the biochar used in the present study, may explain the decrease in Actinobacteria in biochar, whose abundance was supposed to be associated with the degradation of recalcitrant carbon compounds (Bai et al., 2020).

The structure of microbial communities has also been shown to vary with habitat type and crop type (Kolton et al., 2011), suggesting that habitat type and crop type can have substantial effects on microbial communities (Kolton et al., 2016). We found that the application of biochar (15.75–31.50 Mg ha^{-1}) resulted in significant decreases in the abundance of Proteobacteria and Alphaproteobacteria in the rhizosphere soil. As previous studies have shown that the abundance of Proteobacteria is higher in soils with high C availability (Fierer et al., 2007), it not consistent with our results, we found that application of biochar reduced the decline in relative abundance of proteobacteria. Firstly, our study found that there was no significant correlation between the abundance of Proteobacteria and SOC, but was positively correlated with AN, AP, and AK in the rhizosphere soil (Figure 6), it consistent with previous studies (Dai et al., 2018). It may be that the changes in other soil nutrients mask the role of SOC. Secondly, some scholars found that the abundance change of Firmicutes was completely opposite to that of Proteobacteria (Li et al., 2020b). Although biochar application improved soil nutrients to some extent, other microorganisms showed more competitiveness in this process (e.g., Firmicutes in this study; Gregory et al., 2015; Herrmann et al., 2019). Finally, different from other studies, our study was carried out in the seventh year after biochar application, so it may have different effects on Proteobacteria.

The role of core soil microorganisms in maize root growth

We identified the core microbial taxa in the soil by constructing soil microbial co-occurrence networks (Figure 7). We found that there were stronger interactions among rhizosphere soil microorganisms than among bulk soil microorganisms. This might stem from the fact that rhizosphere soil is richer in nutrients than bulk soil (Supplementary Table S2); consequently, competitively superior taxa become dominant, and over time this can lead to the establishment of an equilibrium among dominant taxa (Nielsen et al., 2014; Li et al., 2021). We also found that core microbes were closely related to the growth of maize roots (Figure 8). This might stem from the role of core microbiota in promoting nutrient uptake by maize. Given that the content of nutrients accessible to maize in aeolian sandy soil is low, core microorganisms facilitate the uptake of nutrients by maize roots, which promote root growth (Yeoh et al., 2016). Analysis of the composition of core microorganisms revealed that the fungal phyla Basidiomycota and Ascomycota and the bacterial phylum Firmicutes play key roles in the growth of maize roots (Supplementary Figure S3). Previous studies have shown that the abundance of Ascomycota is affected by soil properties, as ascomycete fungi decompose organic matter around plant roots and promote their growth (Bastida et al., 2013). Basidiomycete fungi can decompose complex organic compounds in the soil; they thus play a key role in the formation of humus in the soil, and their activity promotes the growth of plant roots (Kjøller and Rosendahl, 2014). Previous studies of lemon rhizosphere soil have shown that *Bacillus cereus*, *Bacillus simplex*, and *Bacillus* sp. (all of which are thick-walled bacteria) promote the growth of primary roots and lateral roots, and this effect was achieved through the release of volatile organic compounds that altered the architecture of the root system (Egidi et al., 2019). These findings are consistent with the results of our study.

In summary, analyzed in relation to root growth and soil physicochemical properties, the application of 126.00 Mg hm^{-2} biochar had the best promotion effect on maize root growth after 7 years of biochar application. Biochar application changed maize root architecture by affecting soil physical properties, chemical properties, and soil microbial communities. Biochar application significantly altered soil moisture content, bulk density, and nutrient content, and can directly promote plant root growth (Abiven et al., 2015). Application of biochar affected the growth, development, and metabolism of soil bacteria by altering soil physicochemical properties (Zhu et al., 2017; Siedt et al., 2021). It increases the diversity of the rhizosphere soil bacterial community and changes the microbial structure (Ding et al., 2016), which in turn can maintain plant root growth (Bourceret et al., 2022). At the same time, core microorganisms play a key role in promoting nutrient uptake and root growth in the maize root system (Yeoh et al., 2016). Therefore, future research needs to pay more attention to the long-term effects of multiple factors on the architecture of maize roots.

Conclusion

Our study showed that 7 years after application of biochar significantly promoted the growth of maize roots, with the best

effect when biochar was applied at 126.00 Mg ha^{-1} and biochar application had a major effect on the bacterial communities in rhizosphere soil. The microbial communities of rhizosphere soil and bulk soil significantly differed. The bacterial communities in rhizosphere soil and core microorganisms play key roles in shaping the architecture of the maize root system. These findings enhance our understanding of the relationships between the architecture of maize roots and microorganisms in aeolian sandy soils. Additional studies are needed to characterize changes in root architecture and the soil microbial community during the entire growth period of maize through long-term field experiments.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found at: https://www.ncbi.nlm.nih.gov/, PRJNA855115.

Author contributions

HY, YH, MC, and XS conceived and designed the study and wrote the manuscript. HY, YH, MC, CQ, ZY, and HJ were responsible for performing the field and laboratory work. HY, YH, XS, and HJ analyzed the data. ZY, XZ, WX, and GT helped to perform the analysis with constructive discussions. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2022.1023444/full#supplementary-material

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More microbial manipulation and plant defense than soil fertility for biochar in food production: A field experiment of replanted ginseng with different biochars

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The role of biochar–microbe interaction in plant rhizosphere mediating soil-borne disease suppression has been poorly understood for plant health in field conditions. Chinese ginseng (*Panax ginseng* C. A. Meyer) is widely cultivated in Alfisols across Northeast China, being often stressed severely by pathogenic diseases. In this study, the topsoil of a continuously cropped ginseng farm was amended at 20 t ha^{-1}, respectively, with manure biochar (PB), wood biochar (WB), and maize residue biochar (MB) in comparison to conventional manure compost (MC). Post-amendment changes in edaphic properties of bulk topsoil and the rhizosphere, in root growth and quality, and disease incidence were examined with field observations and physicochemical, molecular, and biochemical assays. In the 3 years following the amendment, the increases over MC in root biomass were parallel to the overall fertility improvement, being greater with MB and WB than with PB. Differently, the survival rate of ginseng plants increased insignificantly with PB but significantly with WB (14%) and MB (21%), while ginseng root quality was unchanged with WB but improved with PB (32%) and MB (56%). For the rhizosphere at harvest following 3 years of growing, the total content of phenolic acids from root exudate decreased by 56, 35, and 45% with PB, WB, and MB, respectively, over MC. For the rhizosphere microbiome, total fungal and bacterial abundance both was unchanged under WB but significantly increased under MB (by 200 and 38%), respectively, over MC. At the phyla level, abundances of *arbuscular mycorrhizal* and *Bryobacter* as potentially beneficial microbes were elevated

while those of *Fusarium* and *Ilyonectria* as potentially pathogenic microbes were reduced, with WB and MB over MC. Moreover, rhizosphere fungal network complexity was enhanced insignificantly under PB but significantly under WB moderately and MB greatly, over MC. Overall, maize biochar exerted a great impact rather on rhizosphere microbial community composition and networking of functional groups, particularly fungi, and thus plant defense than on soil fertility and root growth.

KEYWORDS

soil amendment, manure compost, medicine plant, allelochemicals, beneficial microbes, microbial networking, organic molecules, plant defense

Introduction

Globally, soil degradation is one of the major threats to food security and sustainable agriculture, with organic carbon loss, acidification, destabilization of aggregates, and structure, as well as loss of biodiversity of soils observed extensively in China, European, and Lain American countries (Bindraban et al., 2012; Smith et al., 2016). Consequently, soil health, defined as its capacity to perform continued provisioning of ecosystem services including supporting plant growth and quality (Lehmann et al., 2020; Janzen et al., 2021), has been at risk across the globe. The decline of soil health impacts not only food production but also food quality, through the deciphering of the complex relationships between soil, food, and human health (Oliver and Gregory, 2015). This has been a serious concern in the consensus of One Health (Banerjee and van der Heijden, 2022) and has been taken into intergovernmental actions of the EU and UN.[1]

Among the drivers of soil degradation, continuous cropping or replanting plants in a field has been concerned as a major challenge for sustainable agriculture. This is regarded mainly with the loss of biocontrol of soil-borne plant diseases (Pankhurst and Lynch, 2005), which occur often with the accumulation of phenolics (Li et al., 2010), either autotoxins or allelopathy, in rhizosphere *via* increased root exudation. Such problems of phytotoxic phenolic compounds in root exudate are frequently observed in replanted vegetables (Yu et al., 2003) and medicine crops (Wu et al., 2008), and often in association with fungal pathogenic diseases (Ye et al., 2006). In practice, organic amendments have been widely applied to alleviate soil degradation, without confronting crop production (Pepper et al., 2019; Ali et al., 2022), although globally advocated for climate-friendly agriculture (Paustian et al., 2016; Rumpel et al., 2018).

As a root tuber medicine crop, Panax ginseng Meyer (P. ginseng) is widely produced in northeastern Asia, where the soil is mostly mollic Alfisols rich in organic matter. Since the 2010s, ginseng has been increasingly cultivated in farmlands, often continuously replanting, across Northeast China and Korea (Li et al., 2020). In the area around the forest of Changbai Mountain in Northeast China, ginseng is harvested normally after 4–5 years of growing in an orchard (Li et al., 2014). However, the production of replanted ginseng often fails in the same field due to soil-borne diseases resulting from continuous cropping (Ying et al., 2012). This has been observed with a significant decline in soil quality and in plant growth along with a disordered soil microbiome and food web system affected by the high contents of allelochemicals in root exudates in the rhizosphere (Li et al., 2011). As reported by Shan (2009), replanting ginseng leads to organic matter loss and the associated soil compaction and acidification. As an existing practice, compost of manure available extensively from livestock production in the area has been increasingly used as an organic amendment to ginseng fields (Eo and Park, 2013). Even so, ginseng yield and quality are much limited under continuous cropping, and farmers' incomes are severely stressed (Punja et al., 2008).

The problem of auto-allelopathy of root-derived phenolics and soil-borne diseases with P. ginseng under continuous cropping (Wu et al., 2008) has been later linked to the activities of root cell wall degrading enzymes (CWDEs) impacted strongly by pathogens in the root–soil interface (Kubicek et al., 2014; Jaiswal et al., 2018). Pathogens are known to produce an array of CWDEs including pectinases, cellulases, xylanases, phosphatases, and cutinases, enforcing the break-down of root cell walls made of cellulose, pectins, hemicelluloses, and structural proteins (Annis and Goodwin, 1997) and of soil organic matter in competition for nutrients (Agrios, 2005). In response to continuous cropping stress of Rehmannia glutinosa, cucumber, and tobacco (Zhou and Wu, 2012b; Wu et al., 2015), root exudates enriched mainly of phenolic compounds cannot only be autotoxins for plants but also tend to promote the growth of soil-borne pathogens, while

1 https://www.un.org/food-systems-summit

inhibiting beneficial microbes (Kong et al., 2008; Pollock et al., 2011). As a result, the soil microbial community is shifted with the short-term accumulation of these allelopathic compounds in root exudates as readily accessible carbon substrates, reshaping the soil–root–microbe interaction and in turn plant growth (Zhou et al., 2012, 2014). How the amount and composition of the phenolic allelochemicals derived from root exudate could change with soil fertility and plant health improvement and impact pathogens, enzyme activities, and microbial community in the rhizosphere has been poorly understood for ginseng growth under continuous cropping.

The organic amendment generally promotes microbial growth but may induce some saprophytic pathogenic fungi and potentially enhance soil-borne fungal pathogens (Bonanomi et al., 2006), particularly when given manure as a food source (Bending et al., 2004; Fang et al., 2011). For example, the application of MC increases the incidence of root rot and root loss of Panax ginseng (Eo and Park, 2013). Moreover, livestock manure is concerned with the pollution risk of potentially toxic heavy metals, pathogens, residual antibiotics, and antibiotic resistance genes (Bloem et al., 2017). Alternatively, biochar produced *via* pyrolysis of waste biomass of crop residues, manure, and even sewage sludge (Pan et al., 2017) has been proven as a multi-functional organic amendment for clean and safe food production beyond carbon sequestration (Lin et al., 2020; Bolan et al., 2022). Particularly, biochar could act as a strong absorbent of organic compounds (Graber and Kookana, 2015) for its relatively high surface area and micro-porosity (Ahmad et al., 2014; Ma et al., 2018). For instance, Jaiswal et al. (2018) reported an absorption capacity of up to 300 g kg^{-1} for pathogenic enzymes by pinewood biochar. Activated charcoal could efficiently absorb a variety of root exudate alleles, including lactic acid, benzoic acid, vanillin acid, and succinic acid, as well as markedly increase the yield of taro (Colocasia esculenta Schott) under continuous cropping (Asao et al., 2003). There has been increasing evidence for the immobilization of allelochemicals from root exudates (Gu et al., 2017), for the suppression of soil-borne pathogens (Jaiswal et al., 2017), and for improvement of microbial diversity and metabolic activity in amended soil (Kolton et al., 2016; Li et al., 2019), by biochar following amendment to soils under fruits or vegetables. Indeed, biochar effects on crop productivity (Liu et al., 2013) and quality (Ke et al., 2022), on microbial community (Xu et al., 2021), and on nutrient availability (Tesfaye et al., 2021) could vary with feedstocks, pyrolysis condition, and soil condition. Using wood and maize biochar compared to manure amendment, the microbiome in the soil–root interface is markedly regenerated, and ginseng production is very significantly recovered in an orchard under continuous cropping (Liu et al., 2022). It remains unclear how changes in soil quality, amount, and composition of root exudates, enzyme activities, and microbial community are inter-linked to impact the root growth and quality in biochar-amended soil compared to compost-amended soil.

In this study, we hypothesize that biochar may promote plant growth and disease defense of ginseng in replanted soil mediated jointly by soil physical, biochemical, and biological improvements. We further hypothesize that these promoting effects may differ among biochar types varying in physical and chemical properties. In a continuously cropped Alfisol ginseng field from Northeast China, a 3-year field experiment was conducted with soil amendment of biochars, respectively, from the wood residue, crop residue, and livestock manure, in comparison to conventional MC. Soil edaphic change was analyzed with a biophysical assay of soil aggregates and a chemical assay of nutrients while microbial community composition and root exudates and enzyme activity in ginseng rhizosphere were portrayed, respectively, by molecular biology assay and biochemical assay. The objectivity of this study is to understand how the growth of replanted ginseng is promoted with biochar amendment, through a concurrent improvement of soil fertility and rhizosphere manipulation for enhanced biocontrol. This study aims to provide new insight into the potential of using biochar to improve soil and plant defense for safe and healthy food production seriously stressed by the soil-borne disease under continuous cropping.

Materials and methods

Experimental site and soil

The field experiment was carried out in Xiaoshan village (41°23′N, 127°32′E), Fusong County, Baishan Municipality of Jilin Province of China (**Figure 1**). The local climate is cold temperate and humid with a mean annual temperature of 4°C and precipitation of 800 mm as well as a sunshine time of 2352.5 h over the period of 2015–2019. Being formed on weathered basalt under pine forest, the clayey-loam textured soil was classified as an aquic-mollic Alfisol as per Soil Taxonomy (USAD ARS 2009). The local area had been a typical ginseng production area in Northeast China since 150 years ago but the cultivation of ginseng in managed farms had been prevailing since the ban on deforestation in 2009. Resultantly, the amendment of livestock MC and/or direct replacement of topsoil had been conventionally practiced for sustaining soil fertility. A ginseng field under continuous cropping was selected for an experiment on a household farm, and the topsoil (0–15 cm) was newly replaced before ginseng sowing for the experiment. The soil properties of topsoil were as follows: a pH (H_2O) of 5.07, organic carbon (SOC) of 10.05 g kg^{-1}, total nitrogen of 1.09 g kg^{-1}, available P of 26.47 mg kg^{-1}, and available K of 224.24 mg kg^{-1}.

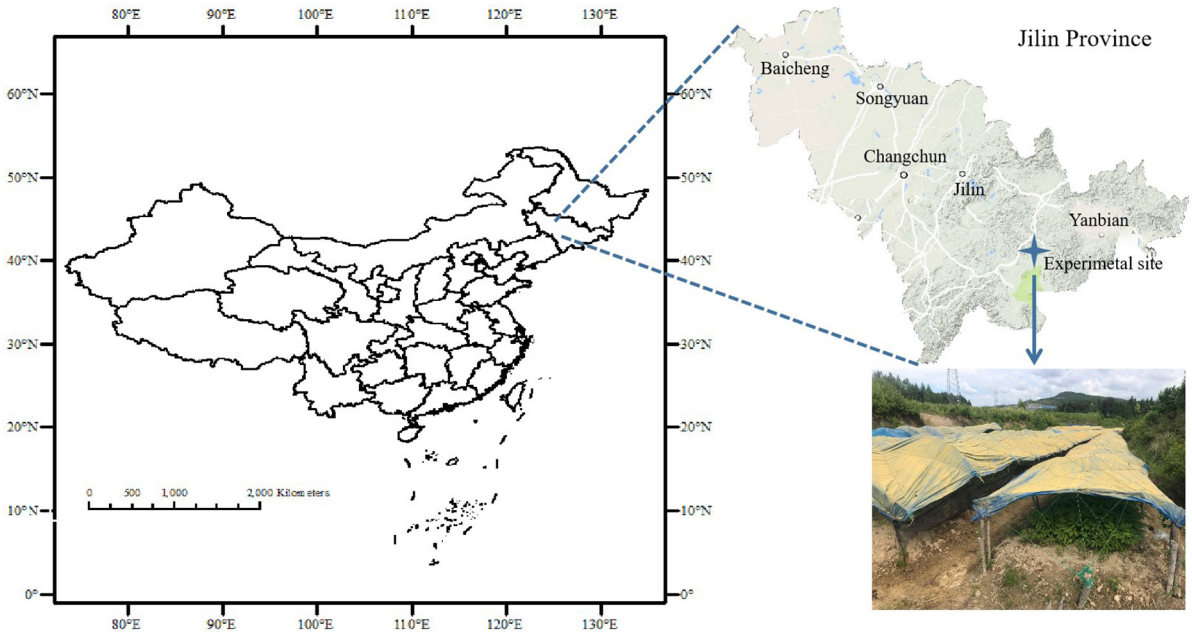

FIGURE 1
Site location of the experiment in Fusong County, Jilin Province, Northeast China.

Manure compost, biochar, and biochar compound fertilizer

In this study, commercially available biochar, respectively, from manure, wood waste, and maize residue was used to amend the ginseng field in comparison to MC. Both pig manure biochar (PB) and wood waste biochar (WB) were provided by Zhejiang Jinguo Environment Protection Co., Ltd., Zhejiang, China, while maize residue biochar (MB) was provided by Shanxi Gongxiao Company Ltd., Shanxi, China. Commercial manure compost (MC), with pig manure fermented under ambient conditions, was purchased from Qingdao Diendi Biological Technology Co., Ltd., China. The PB was produced *via* pyrolysis of air-dried swine manure at 700°C under an anoxic condition in a pyrolysis kiln. Maize biochar (MB) was pyrolyzed of maize residue at a temperature in a range of 350–550°C in a partially oxic vertical kiln. However, wood biochar (WB) was a by-product of steam generation using anoxic pyrolysis of wood chips at a temperature of 600–650°C in a gasifier. Along with the biochar for soil amendment, biochar compound fertilizer (BCF) was used as a nutrient supplier, which was manufactured of mineral nutrients blended with biochar and commercially provided by Beijing Sanju Green Technology Co., Ltd., Beijing, China. Before applying to the field, all the biochar materials were ground and passed through a 2-mm sieve. The properties of all these materials used are listed in **Table 1**, while additional properties of the biochars are organized in **Supplementary Table 1**.

Experimental design

In April 2018, a field experiment was initiated for ginseng grown in newly replaced topsoil. The topsoil was treated with organic soil amendment at a dry mass dosage of 20 t ha^{-1}, respectively, of MC as control, PB, WB, and MB. A treatment plot was 8.2 m^2 (5.1 m × 1.6 m) in an area separated with a 0.2-m width strip in between (**Supplementary Figure 1**). Before ginseng seeds sowing, the required amount of an amendment material was hand-spread onto the soil surface of a plot and subsequently incorporated evenly to a depth approximately of 15 cm with a wooden ranker following a tilling operation. Biochar was amended at one time,while the amendment of MC was split in two halfs; half was amended before seeds sowing and another half was amended in April of the subsequent year, by hand spreading and incorporating into the topsoil. For nutrient supply, a mineral compound fertilizer (MCF) was supplied at 900 kg ha^{-1} for the control of MC, while BCF was applied at 600 kg ha^{-1} for the biochar treatments. One week following the amendment, ginseng seeds were sown in each plot at a rate of 30 g m^{-2}.

A treatment was replicated in triplicates, and all the treatment plots were arranged in a complete randomized block design. Throughout the ginseng growing period of 2018–2020, 3 years following sowing, all the farm management activities followed the conventional practices by the local ginseng farmers, including plant protection with pesticides and weed control, and kept consistent across all the treatment plots.

Plant sampling and analysis

Observation of plant traits was performed, respectively, on September 2018 and June 2020. In a plot, five plants were randomly selected to measure the size and gross fresh biomass. Plant leaf SPAD value was measured with a portable chlorophyll meter (SPAD 502, Konica Minolta Sensing, Japan) while leaf area was measured with a leaf area meter but leaf weight was measured with an electronic balance.

Ginseng root observation and sampling were conducted in the field while harvested in June 2020. Root tubers were separated, and the diameter and length were measured with a vernier caliper. The root tubers from selected ginseng plants were sampled and pooled for a treatment plot, sealed in a plastic bag, and shipped to the laboratory in an ice-box within 24 h following field sampling.

At the laboratory, a root sample was crashed/chopped and homogenized. A major portion of the sample was oven-dried at 75°C to constant weight (recorded as root biomass) and then ground to pass through a 0.25-mm sieve. Following Kim et al. (2012), the contents of ginsenoside monomers were determined with liquid chromatography (LC-1100 system, Agilent, Beijing, China), with the protocol given in **Supplementary Information** available online.

Soil sampling and analysis

A ginseng rhizosphere sample for microbiome analysis was collected at ginseng harvest, as per the protocols described by Butler et al. (2003). In detail, all ginseng roots in a plot were carefully picked-up and gently hand-shaken to remove the soil material attached, then collected, pooled, and homogenized. Following the ginseng rhizosphere sampling, a composite bulk topsoil (0–15 cm) sample was obtained with 5 individual subsamples randomly collected using a stainless steel shovel in a treatment plot. Immediately after collecting, samples of both rhizosphere and bulk topsoil were sealed in steel stainless cans, placed in an ice box, and shipped to the laboratory within 24 h following sampling in the field.

At arrival, a rhizosphere sample was immediately stored at −80°C prior to microbial deoxyribonucleic acid (DNA) extraction. A fresh bulk topsoil sample was hand crashed, removed of gravel and plant debris, sieved to pass through a 2-mm sieve, and homogenized. Of this sample, one portion was air-dried and ground to pass a 0.25 and a 0.15-mm sieve, respectively, before physicochemical analyses following the protocols described by Lu (2000). Another portion was stored at 4°C for soil water-stable aggregates separation, detailed in **Supplementary Information**, as per Smith et al. (2014), for the measurement of microbial biomass carbon and nitrogen following Vance et al. (1987).

Extraction, identification, and quantification of phenolic acids

Phenolic acids from ginseng root exudates were determined with the methods described by Zhou and Wu (2012a). A portion of a fresh rhizosphere sample was sieved to pass through a 2-mm sieve and homogenized. Subsequently, 5.xx g of such a sample was weighed and added to 25 ml of 1 M NaOH and agitated for 24 h on a reciprocal shaker at 30°C. The contents were then spun in a vortex generator for 30 min at the maximum speed, and the suspension was centrifuged at 10,000 rpm for 10 min to obtain liquid supernatant. Following an adjustment to pH 2.5 using 9 M HCl, the solution was extracted with ethyl acetate five times. The resultant extracts were pooled and dried with a rotatory evaporation drier (ZLS-1, Herexi, China) at 35°C. The residue was again dissolved in 5 ml methanol in an ultrasonic tank for 5 min and subsequently injected into the column of an Agilent HPLC-mass spectrometry (Vanquish, Thermo, USA). With the Waters HPLC system (C18 column: Inertsil ODS-SP, 4.6 × 250 mm, 5 μm), the mobile phase A was methanol, and the mobile phase B was 2% glacial acetic acid. The flow rate was kept constant at 0.7 ml/min. While detection was performed at 280 nm, the injection volume was 20 μl and the column temperature was maintained at 30°C. Mass spectral quantification was allowed with a 6,460 Triple Quad LC/MS, operated in the ESI mode with a negative polarity, and scanned by normal mass range from 50 to 240 m/z. Identification and quantification of phenolic compounds were guaranteed by comparing retention times and areas with the internal standards.

TABLE 1 Basic properties of amendment materials and fertilizer used in the experiment.

Material	Ph (H_2O)	Org. C (g kg^{-1})	Total N (g kg^{-1})	Total P (g kg^{-1})	Total K (g kg^{-1})
MC	7.73	302.30	9.30	13.40	15.60
PB	9.67	486.23	11.85	16.99	12.88
WB	10.15	463.63	2.97	5.55	7.22
MB	9.95	601.92	6.78	3.08	25.71
MCF	7.05	n. d.	160.00	65.32	132.77
BCF	5.00	150.01	137.30	61.24	118.80

MC, manure compost; PB, pig manure biochar; WB, wood biochar; MB, maize biochar; MCF, mineral compound fertilizer; BCF, biochar compound fertilizer. n.m., not measured.

Deoxyribonucleic acid extraction, real-time qPCR analysis, and Illumina HiSeq sequencing

A rhizosphere soil sample was extracted for total microbial DNA using a Power Soil^{TM} DNA Isolation Kit (MoBio, CA, USA). The qPCRs were performed in a 25-μl volume containing 10 ng DNA, 0.2 μM of each primer, 0.2 mg ml^{-1} BSA, and 12.5 μl of SYBR premix EX Taq^{TM} (Takara Shuzo, Shiga, Japan). Standard curves were generated using triplicate 10-fold dilutions of plasmid DNA harboring cloned target genes, respectively, for bacteria and fungi. Melting curve analysis was done to confirm that specific amplification was not due to primer dimers or other artifacts following each assay. The qPCR amplification efficiencies were 103% for the bacterial 16S rRNA gene and 98% for the fungal ITS gene, all with R^2-values > 0.99.

Bacterial and fungal community compositions were portrayed with sequencing target amplicons using the Illumina HiSeq 2500 platform. The V3–V4 region of the bacterial 16S rRNA gene was targeted with the primer pair 341F/806R, while the fungal ITS region was with the primer pair ITS1F/ITS2R. The 2% (w/v) agarose gel electrophoresis was used to examine PCR products. The bands were purified with the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, USA) and quantified using the QuantiFluor^{TM}-ST (Promega, USA). Purified amplicons were concentrated on Illumina HiSeq in an iso-molar concentration and sequenced with the standard protocol.

The obtained raw sequences were trimmed using QIIME and UPARSE pipelines (Edgar, 2013). Briefly, the remaining sequences were translated into amino acids using the Fun Gene Pipeline when the sequences were screened for quality to remove the barcodes, primers, and low-quality sequences. Chimeric sequences and singletons were removed using the UCHIME algorithm. The remaining high-quality sequences were clustered into operational taxonomic units (OTUs) at a 97% similarity cutoff. BLAST algorithm was used to retrieve the NCBI GenBank database, and the representative sequences of each OTU were classified and identified. OTUs were implemented based on QIIME software to calculate the rarefaction curves and community diversity indices. Herein, bacterial community functions were predicted using PICRUSt (Langille et al., 2013) and plotted in the KEGG Orthology classification scheme while fungal functions using FUNGuild (Nguyen et al., 2016) with OTU data.

Co-occurrence networks analysis

OTUs with a relative abundance of less than 0.01% were removed to reduce rare OTUs in the data set. Then, the psych and igraph packages in the R software (Version 3.5.1) were used to analyze the preprocessed data and calculate the Spearman correlation and the network properties. Only the results with a cut-off at an absolute $r > 0.6$ and a $p < 0.05$ after adjusting by Benjamini–Hochberg's false discovery rate were retained for further network visualization using the "gephi" software (Version 0.9.2)[2] (Benjamini and Hochberg, 1995).

Data processing and statistical analysis

The Sloan neutral community model (NCM) was used to evaluate the potential importance of stochastic processes to soil microbial community assembly (Sloan et al., 2006). To explore the relative effects of stochastic and deterministic processes on microbial communities, Levins' niche breadth (B) index was calculated both for bacteria and fungi (Mo et al., 2021).

All data were expressed as means plus/minus standard deviation and processed with Microsoft Excel version 2020. All statistical analyses were performed with ANOVA using SPSS software (Version 20.0). Treatment means were compared using Duncan's test, while the significance of a correlation was assessed with Pearson's test. Statistical significance was set at $p < 0.05$.

Results

Soil properties, ginseng growth, and root production

The edaphic properties of topsoil sampled at ginseng harvest are represented in **Table 2**. Obviously, soil fertility at 3 years following amendment was overall improved with biochar treatments compared to conventional MC. In detail, soil pH was elevated insignificantly with PB and MB but significantly (by 0.4 units) with WB though soil EC more or less increased under biochar amendments. The increase over MC of SOC and available P was small with PB but great (>50%) with WB and MB. Available K, whereas, was enriched by 1–2 folds with all the biochars. As for physical changes, the mean weight diameter of water-stable aggregates was unchanged with WB while greatly (by over 50%) increased with PB and MB in comparison to MC. Soil bulk density, whereas, was reduced insignificantly with PB but significantly with WB and MB. Such a trend was followed by soil porosity along with a significant increase in moisture, with all the biochar treatments. Finally, soil MBC was significantly increased by ca 30% with all the biochar, with microbial C/N ratio insignificantly changed. In addition, the microbial quotient was unchanged with MB, slightly reduced with PB, and moderately elevated with WB compared to MC. However, soil C/N ratio was unchanged with PB, while lifted moderately with MB and greatly with WB.

2 https://gephi.org/

TABLE 2 Basic properties of topsoil (0–15 cm) sampled at ginseng harvest 3-year following organic amendment at 20 t ha^{-1}.

Treat-ment	pH (H_2O)	B.D. (g cm^{-3})	SOC (g kg^{-1})	Total N (g kg^{-1})	Available P (mg kg^{-1})	Available K (mg kg^{-1})	Aggr-MWD (μ m)
MC	4.59 ± 0.16b	1.06 ± 0.05a	11.34 ± 0.9b	0.98 ± 0.01a	24.79 ± 0.20c	162.23 ± 14.16c	273.23 ± 22.80b
PB	4.72 ± 0.03b	1.02 ± 0.06ab	15.72 ± 0.44a	1.08 ± 0.18a	26.72 ± 3.59c	298.67 ± 24.87b	404.51 ± 33.56a
WB	4.98 ± 0.01a	0.97 ± 0.03bc	15.61 ± 0.79a	1.09 ± 0.08a	39.70 ± 1.45b	438.26 ± 18.00a	292.21 ± 16.45b
MB	4.64 ± 0.07b	0.90 ± 0.01bc	15.56 ± 2.63a	1.13 ± 0.20a	51.45 ± 2.79a	454.40 ± 27.09a	443.54 ± 20.17a
	Porosity (%)	**CEC (cmol kg^{-1})**	**Moisture (%)**	**EC (μs cm^{-1})**	**MBC (mg kg^{-1})**	**MBN (mg kg^{-1})**	**MBC/MBN**
MC	60.18 ± 1.81c	25.15 ± 0.95a	14.89 ± 0.93c	15.62 ± 3.06c	99.79 ± 7.75b	7.52 ± 2.67c	14.74 ± 6.50a
PB	61.54 ± 2.39bc	26.71 ± 1.35a	17.44 ± 0.64b	35.37 ± 6.20ab	128.56 ± 31.17a	11.41 ± 0.43ab	9.58 ± 2.94a
WB	63.47 ± 0.99ab	27.62 ± 2.02a	19.56 ± 0.47a	27.13 ± 1.86b	136.25 ± 12.12a	9.88 ± 1.47bc	13.88 ± 0.87a
MB	65.91 ± 0.37a	28.14 ± 2.09a	16.97 ± 0.82b	37.73 ± 6.54a	134.14 ± 26.88a	14.44 ± 2.25a	9.37 ± 1.75a

MC, manure compost; PB, WB, and MB, biochar, respectively, of swine manure, wood residue, and maize residue. Different letters indicate significant differences ($p < 0.05$) between treatments.

Data on survival rate and root biomass of ginseng plants sampled for, respectively, the 1st and 3rd growing seasons are organized in Figure 2, while the relevant changes in growth traits of the ginseng plants are listed in Supplementary Table 2. With all the biochar treatments compared to MC, ginseng root biomass was increased by about onefold for the 1st season and by 20–40% for the 3rd season following amendment. Across the treatments, the survival rate was between 80–90 and 65–80%, respectively, for the 1st and 3rd growing seasons. The survival rate was not significantly different among the treatments for the 1st growing season but was higher significantly with WB and MB though unchanged with PB for the 3rd growing season. In contrast, plant traits showed a divergent change either with different parameters or across the treatments. For the 1st growing season, plant height, leaf weight, and root diameter were all increased at varying extents, while leaf chlorophyll (SPAD) and root length were unchanged, with the biochar treatments over MC. Comparatively, plant height, root length, and diameter, as well as leaf chlorophyll were all increased in the 3rd growing season following amendment.

Changes in root quality in terms of the total content of ginsenosides are shown in Figure 2C, while the data of ginsenosides monomer saponins of ginseng roots harvested are provided in Supplementary Table 3. The total ginsenosides content of the harvested ginseng roots was unchanged with WB but significantly increased with PB by 32% and MB by 56%, over MC. To note, for the treatments of biochar amendment, root contents both of total ginsenosides and the key monomers of Rf, Rc, Rb2, Rb3, Rd, and Rh2 were higher with MB than with PB and WB.

Root phenolic acid metabolites

The contents of phenolic acids detected in the rhizosphere soil sampled at harvest, after three growing seasons following amendment, are shown in Table 3. Of the total 17 molecules of phenolic acids, 8 molecules including vanillic, benzoic, p-Hydroxycinnamic, vanillin, 4-Hydroxybenzoic, trans-Ferulic, syringic, and 3,4-Dihydroxybenzoic acid were detected at an abundance over 100 μg kg^{-1} soil and contributed by over 94% to the total. As plotted in Figure 3, the total abundance of these dominant phenolic acids was all significantly decreased, in line with a significant reduction in the total content of the phenolic acids, with the biochar treatments over MC. In detail, the decrease was by over 55% with PB, by 35% with WB, and by 45% with MB.

Microbial abundance and community structure of rhizosphere microbiome

Data of total gene abundance and community structure at genus level of bacteria and fungi of rhizosphere soil sampled at harvest after the 3rd growing season following amendment are organized in Figure 4. Similarly, the change in the composition of the top 10 phyla of bacterial and fungal is graphed in Supplementary Figure 3. Gene abundance of bacteria was unchanged with WB while significantly increased by 28% with PB and by 38% with MB, respectively, over MC; Differently, fungal gene abundance was unchanged with PB and WB but increased by twofold with MB. The sequences obtained across the treatments were classified into 26 phyla for bacteria and 10 phyla for fungi. For bacteria, the top 10 phyla were dominated by *Proteobacteria* (29–39%), *Acidobacteria* (23–36%), and *Actinobacteria* (6–18%), followed by *Verrucomicrobia* (1–19%), *Chloroflexi* (3–11%), and *Gemmatimonadetes* (3–5%) with the other 4 phyla in small abundance (<2%). Thereby, the proportion of *Proteobacteria* was decreased with biochar amendments over MC. However, the proportion of both *Verrucomicrobia* and *Chloroflexi* was unchanged with PB but increased significantly with WB and MB, while the reverse

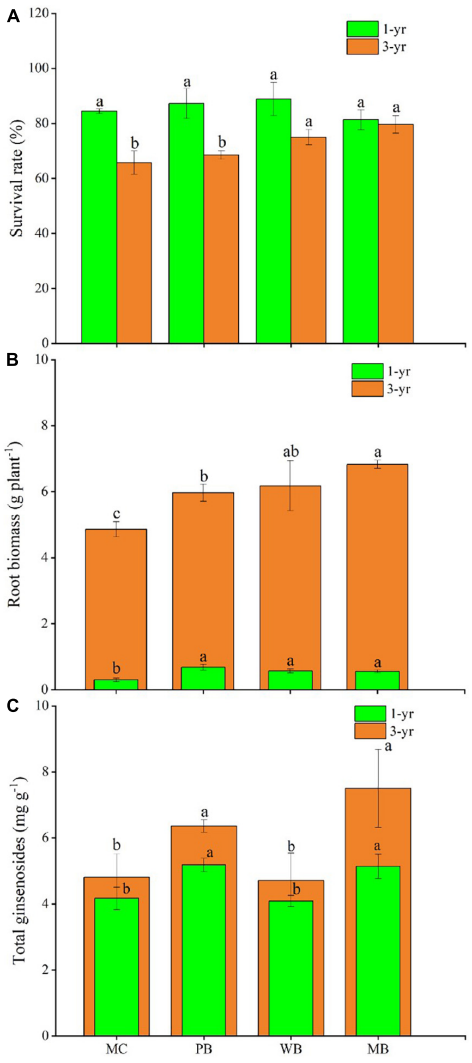

FIGURE 2
Survival rate of ginseng seedlings **(A)**, root biomass **(B)**, and content of ginsenosides (Li Yang **C**) of ginseng on the 1 and 3 year following soil amendment at 20 t ha^{-1}. MC, amendment of manure compost at 20 t ha^{-1}; PB, WB, and MB, amendment of pig manure, wood, and maize biochar at 20 t ha^{-1}. Different letters above the bars indicated a significant difference among treatments at $p < 0.05$.

was true for that of *Actinobacteria*. Meanwhile, the top 10 fungal phyla were dominated by *Ascomycota* (52–67%), followed by *Basidiomycota* (17–21%), *Mortierellomycota* (5–13%), and *Glomeromycota* (3–6%) with others in a proportion below 1%. Hereby, the proportion of *Ascomycota* markedly increased (by 20–30%) with the biochar treatments over MC. Like the changes in bacterial phyla, the proportions of *Mucoromycota*, *Chytridiomycotawas,* and *Mortierellomycota* decreased, while *Glomeromycota* increased, significantly with WB and MB.

As shown in **Figures 4A,B** total of 258 bacterial genera were shared by all four treatments, while one specific genus of *Buchnera* appeared only in the control MC. Based on the top 20 bacterial genera, the abundance of *Bryobacter*, *Candidatus Solibacter,* and *Candidatus Udaeobacter* was increased, while *Sphingomonas*, *Granulicella*, *Phenylobacterium*, *Arthrobacter*, *Sphingomonas,* and *Rhodanobacter* were decreased significantly with the biochar amendments compared to MC. Unlike bacteria, there were 154 shared fungal genera among the four treatments, while 1, 2, 29, and 18 distinct genera appeared in MC, PB, WB, and MB, respectively. Moreover, the abundance of *Aspergillus*, *Paraglomus*, *Chaetomium,* and *Leohumicola* was increased, while *Mortierella*, *Fusarium*, *Solicoccozyma*, *Ilyonectria*, *Saitozyma*, *Athelopsis,* and *Clavulinopsis* decreased significantly with WB and MB though insignificantly with PB, respectively, in comparison to MC.

Richness (Chao1) and Shannon diversity of both bacterial and fungal communities, calculated based on the rarefied sequences, are given in **Supplementary Figure 4**. Generally, the richness (Chao1) of the bacterial community was unchanged under PB while increased under WB and MB, and the Shannon index was unchanged with the biochar amendments over MC. For fungi, richness (Chao1) and Shannon index were unchanged under PB while both increased under WB and MB, respectively. For the β-diversity of both rhizosphere bacteria and fungi community, the treatments of WB and MB were clearly separated from treatment PB and the control of MC with the principal coordinate analysis (PCoA) (ANOSIM, $p < 0.001$) (**Figure 5**). Also, the Bray–Curtis dissimilarity of bacterial ($R = 0.769$, $p = 0.001$) and fungal community ($R = 0.796$, $p = 0.001$) was significant among the treatments.

Abundance of the dominant genus of the rhizosphere microbiome

The abundances of dominant genera (a relative proportion > 0.5%), 25 for bacteria and 7 for fungi, are shown in **Supplementary Table 4**. Compared to MC, the abundance of the fungal genus of *Archaeorhizomyces* was increased by 27% under WB while decreased by 42 and 39% under PB and MB, respectively. Comparatively, the abundance of *Mortierella* was greatly decreased with all the biochar amendments, at an extent of 51–156%. The abundance of *Paraglomus* was unchanged under WB while increased by 2–4 folds under PB and MB. Again, the abundance of *Solicoccozyma* was unchanged under WB, while decreased by folds under PB and MB.

For bacterial genus, the abundances of *Acidothermuss*, *Burkholderia- Caballeronia- Paraburkholderia*, *Gemmatimonas*, *Phenylobacterium*, *Pseudolabrys*, *Rhodanobacter,* and *Sphingomonas* were decreased by 0.6–6 folds, while those of *Bryobacter* and *Candidatus-Udaeobacter* increased by 0.4–26 folds under PB. The abundances of *Acidothermuss*, *Bryobacter*, *Candidatus_Solibacter,* and *Gemmatimonas* increased by 0.3–2 folds, while those of

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TABLE 3 Phenolic acids (μg kg^{-1}) in root exudates collected in ginseng rhizosphere 3-year following organic amendment at 20 t ha^{-1}.

Treat-ment	Vanillic acid	Benzoic acid	Vanillin	p-Hydroxy-cinnamic acid	4-Hydroxybenzoic acid	Trans-Ferulic acid	Syringic acid	3,4-Dihydroxy-benzoic acid
MC	1496.7 ± 167.8a	1285.9 ± 162.8a	902.3 ± 111.8a	1276.1 ± 219.5a	870.4 ± 109.1a	448.7 ± 82.55a	246.3 ± 24.89a	156.2 ± 15.67a
PB	632.9 ± 88.40c	910.2 ± 51.17a	512.7 ± 57.44b	426.8 ± 134.2b	399.7 ± 136.1b	395.2 ± 89.65a	145.6 ± 38.62b	100.8 ± 11.27b
WB	1050.9 ± 171.6b	1011.0 ± 70.42a	675.6 ± 55.41ab	380.1 ± 56.35b	670.4 ± 104.0ab	252.5 ± 62.50a	126.9 ± 22.53b	122.7 ± 23.34b
MB	742.6 ± 127.0c	603.6 ± 136.6b	422.5 ± 119.1b	872.3 ± 193.9ab	406.2 ± 117.6b	341.1 ± 144.9a	133.0 ± 13.17b	107.7 ± 8.69b
Treat-ment	**Trans-cinnamic acid**	**Syring-aldehyde**	**Salicylic acid**	**Protocatechu-aldehyde**	**Hydro-cinnamic acid**	**Gallic acid**	**Caffeic acid**	**HDC acid**	**Phe**
MC	75.58 ± 14.26a	65.59 ± 14.84a	65.63 ± 4.07a	60.26 ± 2.93a	56.88 ± 14.33a	13.04 ± 3.28a	9.85 ± 1.73ab	1.83 ± 0.51a	1.1 ± 0.25a
PB	34.92 ± 16.49b	51.64 ± 14.59ab	25.9 ± 7.10ab	34.94 ± 13.62b	18.07 ± 2.44c	10.46 ± 1.98a	14.14 ± 1.53a	1.95 ± 0.62a	0.87 ± 0.13a
WB	84.69 ± 16.40a	39.1 ± 16.64b	40.44 ± 4.92ab	39.78 ± 8.91b	57.65 ± 8.64a	10.62 ± 2.12a	11.54 ± 2.21ab	1.13 ± 0.31a	1.08 ± 0.07a
MB	70.05 ± 12.34a	38.35 ± 4.87b	39.04 ± 10.81b	37.86 ± 9.52b	31.31 ± 13.34b	13.09 ± 5.26a	7.71 ± 2.01b	1.68 ± 0.85a	0.79 ± 0.13a

MC, manure compost; PB, WB, and MB, biochar, respectively, of swine manure, wood residue, and maize residue. HDC acid, 4-Hydroxy-3,5-dimethoxy-cinnamic acid; Phe, L-Phenylalanine. MC, manure compost; PB, WB, and MB, biochar, respectively, of swine manure, wood residue, and maize residue. Different letters indicate significant differences ($p < 0.05$) between treatments.

FIGURE 3
Changes in total phenolics content of the rhizosphere soil sampled on the 3-year following soil amendment at 20 t ha^{-1}. MC, amendment of manure compost at 20 t ha^{-1}; PB, WB, and MB, amendment of pig manure, wood, and maize biochar at 20 t ha^{-1}. Different letters above the bars indicated a significant difference among treatments at $p < 0.05$.

Burkholderia-Caballeronia-Paraburkholderia, *Granulicella*, *Rhodanobacter*, and *Sphingomonas* were decreased by 0.3–3 folds under WB. Under MB compared to MC, however, the abundances of *Acidothermuss*, *Burkholderia- Caballeronia-Paraburkholderia*, *Candidatus_Solibacter*, *Gemmatimonas*, *Granulicella*, *Phenylo-bacterium*, *Rhodanobacter*, and *Sphingomonas* were decreased by 14%∼3 folds while that of *Candidatus_Udaeobacter* increased by 28-folds.

Co-occurrence network of rhizosphere microbial communities

Co-occurrence networks of rhizosphere microbial communities were constructed to visualize the relationships among bacterial and fungal OTUs under the treatments (Figure 6 and Table 4). Clearly, the networks of bacterial and fungal taxa were significantly different among the treatments. In general, the bacterial networks had more numbers of nodes and edges under PB, WB, and MB over MC, with the average degree being higher under WB and MB than under PB. Similar to the bacterial community, the number of nodes and edges, the average degree, the average clustering coefficient, and the modularity of fungi networks were unchanged under PB while higher under WB and MB, compared to under MC.

Bacterial and fungal community assembly processes and functions

The differences among the fields for functional traits of the bacterial community are depicted with the popular KEGG pathway (KO tier 2) classification scheme as shown

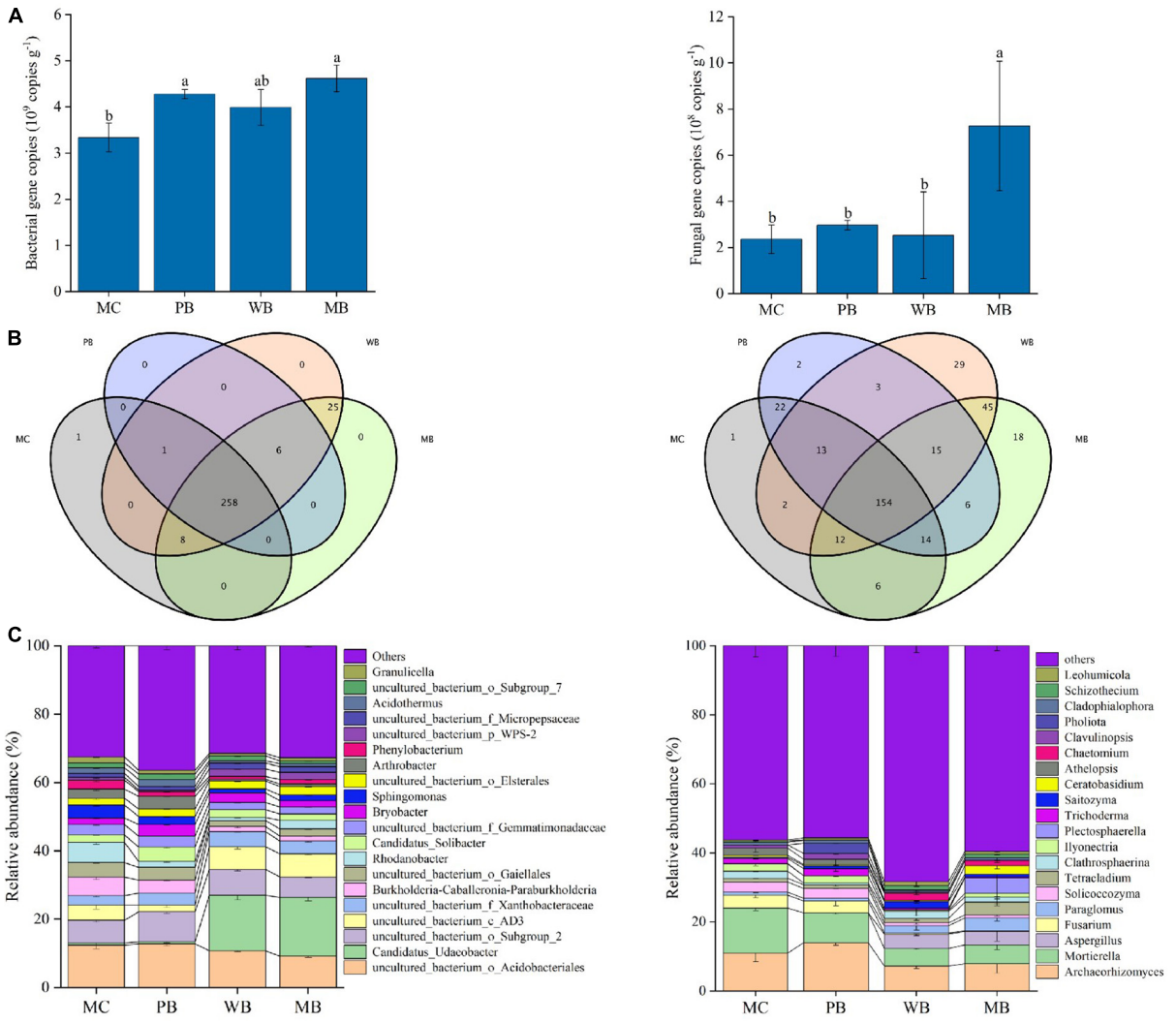

FIGURE 4
Gene abundance **(A)**, exclusive and shared of OTUs at the genus level **(B)**, and top 20 genera composition **(C)** of bacterial (left) and fungal (right) of the rhizosphere soil sampled on the 3-year following soil amendment at 20 t ha^{-1}. MC, amendment of manure compost at 20 t ha^{-1}; PB, WB, and MB, amendment of pig manure, wood, and maize biochar at 20 t ha^{-1}. Different letters above the bars indicated a significant difference among treatments at $p < 0.05$.

in Figure 7A. Among second-tier functional categories, the significantly lower relative abundance of functions with "cell motility," "amino acid metabolism," "metabolism of other amino acids," "lipid metabolism," "signal transduction," and "membrane transport" was observed under the biochar amendments over MC. Moreover, the abundance of those functional traits with "metabolism of cofactors and vitamins," "folding, sorting and degradation," "energy metabolism," "energy metabolism," "translation," "replication and repair," "nucleotide metabolism," and "cancers: specific types" were significantly higher under WB and MB than under MC.

The change in the relative abundance of fungi sequences assigned to functional guilds with ecological significance was observed with the different treatments in this study. When compared in trophic modes, the proportion of the "Plant pathogen" group to the total fungal community was significantly decreased with all biochar amendments of PB, WB, and MB compared to MC. Also, the proportion of "Arbuscular mycorrhizal" and "Dung saprotroph" was very significantly increased while that of "Soil Saprotroph" was decreased under WB and MB, respectively, compared to MC. In addition, the proportion of "Endophyte" and "Soil Saprotroph" was significantly increased, while "Ectomycorrhizal" decreased under PB over MC.

The average niche breadth across the treatments was significantly higher for the bacterial community than for the fungal community (Figure 7B). The biochar treatments significantly increased bacterial niche breadth over MC.

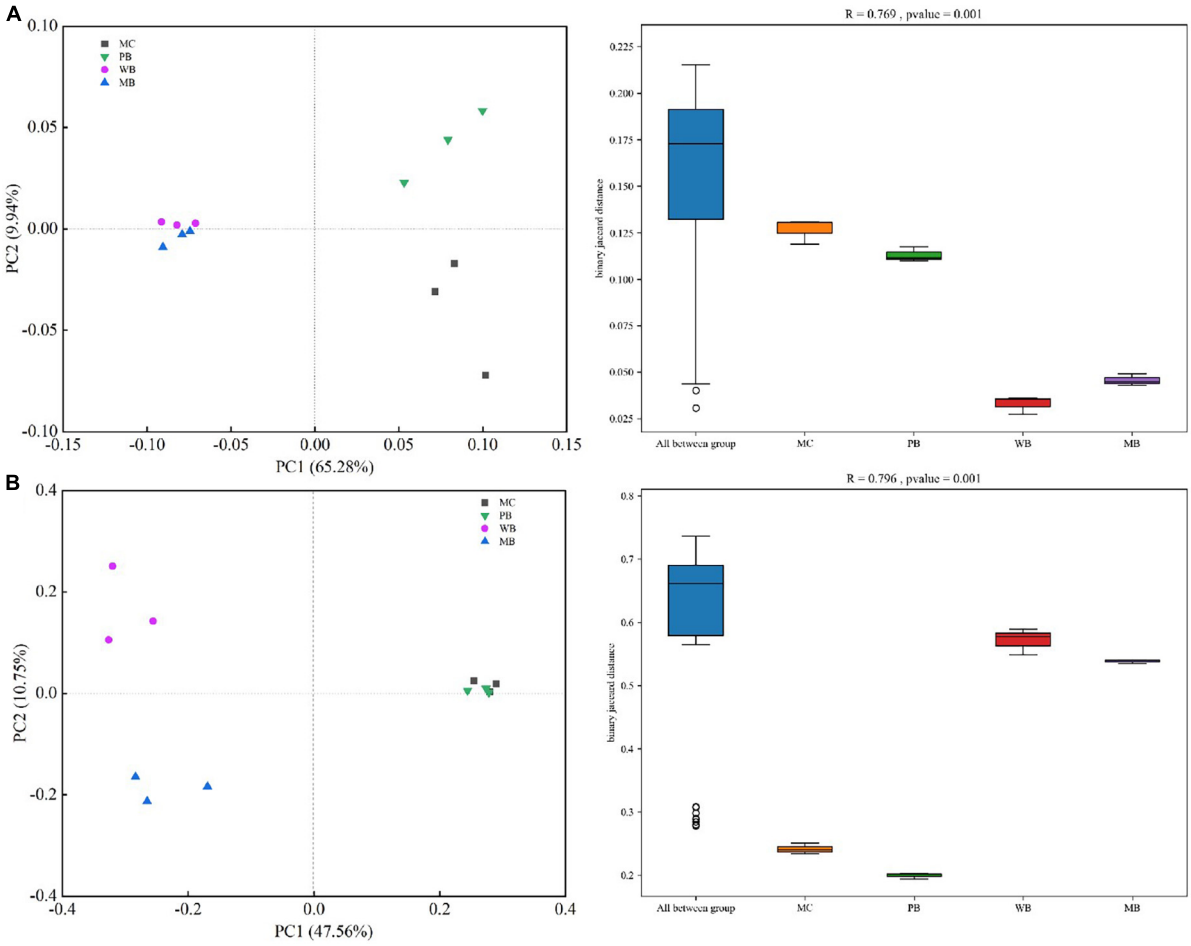

FIGURE 5
Principal coordinate analysis (PCoA) ordinations based on unweighted UniFrac distance metric and analysis of similarity (ANOSIM) based on binary Jaccard distance of bacterial **(A)** and fungal **(B)** community composition of the rhizosphere soil sampled on the 3-year following soil amendment at 20 t ha^{-1}. MC, amendment of manure compost at 20 t ha^{-1}; PB, WB, and MB, amendment of pig manure, wood, and maize biochar at 20 t ha^{-1}.

Differently, the fungal niche breadth was significantly increased under WB while decreased under PB though unchanged under MB, compared to MC. The contribution of stochastic processes on bacterial and fungal community assembly was investigated by the Sloan NCM (**Figure 7C**). Thereby, the explained fraction of variation was higher for the fungal community ($R^2 = 0.306$) than for the bacteria community ($R^2 = 0.184$).

Discussion

Divergent biochar impacts: Soil fertility, plant growth, and root quality

With continuous cropping of ginseng, the topsoil derived from acid Alfisols under the forest was already low in organic carbon and in the available pool of major nutrients such as N and P, being a basic and primary factor for soil borne-disease and root yield loss (Wu et al., 2020b). Being conducted on a farm under continuous cropping with renewed topsoil, this study clearly showed an overall improvement of soil fertility, although divergent across the soil fertility attributes (**Table 2**), following amendment with biochar over MC. Despite the alkaline nature of the biochars used (**Table 1**), soil pH was not elevated significantly except with WB with a pH up to 10. This again reflected the soil acidification stress under continuous ginseng growing (Yang et al., 2004; Wu et al., 2008). Compared to MC, the amendments of biochar, regardless of biochar types, significantly but strongly increased the organic carbon, available P and K, and soil MBC. Such a marked increase in the available nutrient pool of P and K with biochar amendment had been widely observed (Tesfaye et al., 2021). Hereby, the increase in SOC across the treatments was not parallel to the C input from the organic amendments (**Table 1**)

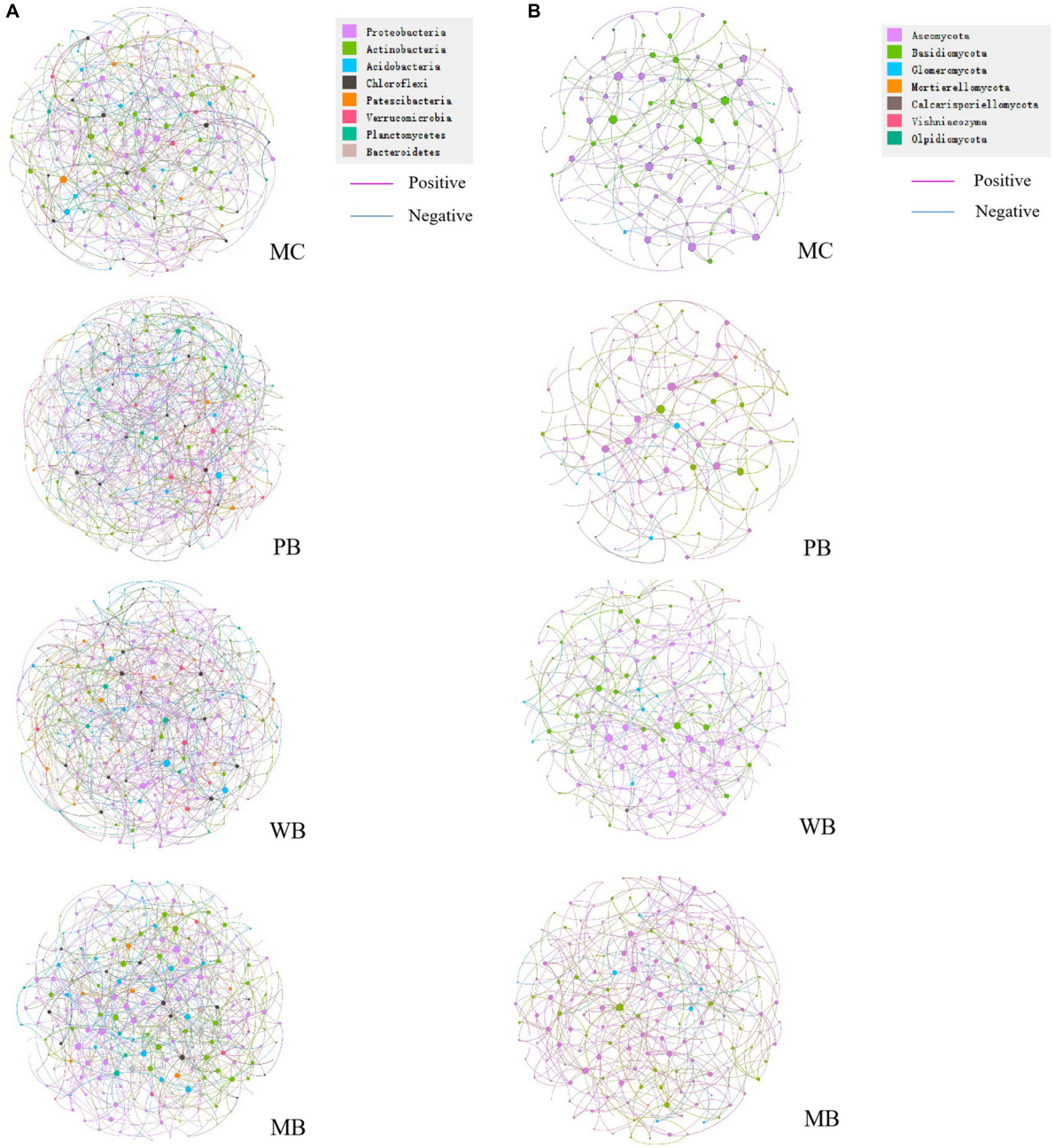

FIGURE 6
Bacteria> **(A)** and fungal **(B)** co-occurrence networks colored by their phyla, based on Spearman's correlations, of rhizosphere soil sampled on 3-year following amendment at 20 t ha^{-1}. The size of each node is proportional to the number of connections (degree), and the thickness of each edge is proportional to the value of Spearman's correlation coefficients. The red edges denote negative interactions while blue edges positive interactions, between two nodes. MC, amendment of manure compost at 20 t ha^{-1}; PB, WB, and MB, amendment of wood, maize, and pig manure biochar at 20 t ha^{-1}.

as a small portion of carbon was retained under MC while over half of C input persisted in soil under the biochar treatments. Manure C is susceptible to microbial decomposition (Lal, 2015; Pepper et al., 2019), while recalcitrant carbon in biochar is generally high in stability against decomposition (Fang et al., 2014; Lehmann et al., 2015b). Although the original soil fertility was lower than that in our previous study (Liu et al., 2022), soil fertility overall was improved markedly with WB and MB (by 13–14%) and slightly with PB (by 6%) over MC. Our recent studies confirmed the better performance of biochar than their unpyrolyzed biowastes including swine manure, in enhancing soil organic matter and improving soil quality (Lin et al., 2020;

Feng et al., 2021). Albeit, these changes could not be directly linked to the nutrient contents (**Table 1**) and other physical and biochemical properties (**Supplementary Table 1**) of the organic amendments. This indicates topsoil fertility resulted from a complexed soil–plant–microbe interaction over the 3 growing seasons of ginseng following the amendment treatment.

In this study, root biomass and quality (ginsenosides content hereby) and survival rate of the replanted ginseng plants harvested were modified at varying extents by the organic amendment (**Figure 2**). The greater change among the treatments in survival rate observed in the 3rd growing season than the 1st one represented obviously the pathogenic disease impact over the 2 years of continued growing of ginseng, which was often attributed to autotoxins by root exudates (Wu et al., 2008; Xiao et al., 2019). It is worth noting that the treatment effect was divergent among the root biomass, root quality (concerned with ginsenosides contents), and plant survival. Unlike the change in root biomass related to soil fertility change, the changes in survival rate under WB (14%) and MB (21%) and in ginsenosides content under PB (32%) and MB (56%) appeared divergent among the treatments, showing independent of overall soil fertility change. This suggested a wider variation but a greater role of biochar treatments on plant biocontrol and plant quality (function of biosynthesis of ginsenosides in this study). On the one hand, the change in the biochar amendments in ginsenosides content was in line with the content of total N, P, and K of the biochar amended (**Table 1**). High-quality ginseng (high contents of ginsenosides) was normally found in undisturbed habitats in forest soil rich in SOC and nutrients of N, P, and K (Lu et al., 2014; Fang et al., 2022). On the other hand, the change in survival rate did not follow the trend of total phenolics from the root exudates (**Figure 3**), previously concerned with the auto-toxicity to ginseng root growth (Wu et al., 2008; Wu et al., 2020b). In the studies by Asao et al. (2003) and Wu et al. (2020a), the great yield increase in plant roots following the application of activated carbon was concerned with the immobilization of a variety of alleles accumulated with the plant roots under continuous cropping. Instead, the survival rate in this study was very significantly correlated with soil porosity and soil available P content though generally related to soil fertility conditions (**Table 1**) under the organic amendments. Unlike PB and MB, the treatment of the nutrient-poor WB (**Table 1**) without a significant change in soil aggregation and microbial N and C/N ratio (**Table 2**) caused no change in total ginsenosides content despite a significant positive change in survival rate. With the focus on either soil quality (Bünemann et al., 2018) or soil health (Lehmann et al., 2020), soil fertility, plant growth, and microbial activity may have a very complexed interaction of soil biotic and abiotic factors and biophysical, biochemical, and biological processes in a given ecosystem (Lu et al., 2020; Liu et al., 2022). The role of soil biophysical improvement in biological soil health and plant growth has been recently highlighted (Lehmann et al., 2020). The role of soil–biochar–plant root–microbes in the rhizosphere (Lehmann et al., 2015a) should be explored for understanding the system-acquainted resistance potentially induced by biochar amendments (Jaiswal et al., 2017). The change in survival rate could be further linked to the improvement of soil biological health based on enhanced allelochemical degradation and microbiome manipulation.

Microbiological impact by biochar: Abundance vs. functional traits

In this study, with the positive change in MBC and MBN, the microbial quotient (0.82–0.88%) was hardly modified with the biochar amendments over MC (**Table 2**). Relative to MC, the positive change in MBN was lower under WB (31.4%) than PB (51.7%) and MB (92.0%) despite a 28–36% change in MBC across the biochar amendments. The wide variation of MBC/MBN ratio within and among the treatments reflected a potential shift of microbiome in the rhizosphere following the amendment of different biochars. In this study, biochar amendment caused a generally greater impact on microbial biomass (**Table 2**) and bacterial and fungal gene abundance (**Figure 4**). Crop productivity improvement was averaged at 11% (Liu et al., 2013), and a mean increase in microbial growth and metabolic activity was up to 17% (Zhou et al., 2017). Following Lehmann et al. (2011), microbial growth was promoted, and community composition was greatly altered with biochar amendments, as seen with the distant separation by PCA analysis in **Figure 4**. For the microbial genera, Chao 1 index was increased significantly (by 16 and 18%) for bacteria and for fungi (by 30 and 33%), with WB and MB though unchanged with PB. Shannon diversity index was unchanged among all treatments for bacteria while significantly increased (by 10 and 18%) with WB and MB though unchanged with PB. The changes in microbial community composition and diversity may be related to the pore volume of biochar (**Supplementary Table 1**), and the soil properties changes following biochar amendment (**Supplementary Figures 5, 6**). The pore volume of manure biochar is 0.21 $cm^3\ g^{-1}$, which is lower than WB (0.30 $cm^3\ g^{-1}$) and maize biochar (0.29 $cm^3\ g^{-1}$) so that wood and maize biochar amendment provides more habitat for soil microbes. This result was reported by Lehmann et al. (2011). Similarly, the co-occurrence parameters were all higher under PB, WB, and MB for bacterial but under WB and MB for fungi, compared to MC. The extents by which these were changed over MC were more or less parallel to the trend of microbial diversity changes with the treatments.

In addition, biochar amendment led to wider niche breadths than MC for both bacteria and fungi (**Figure 7B**). Soil microorganisms in biochar-amended soil could adapt to a wide range of micro-niches. In a work on straw biochar's effect on the network of rhizosphere fungi by Wang et al.

TABLE 4 Topological properties of co-occurrence network of rhizosphere soil following organic amendment at 20 t ha^{-1}.

	Treatment	Nodes	Edges	Average degree	Average path length	Average clustering coefficient	Modularity
Bacteria	MC	205	512	4.995	3.675	0.114	10
	PB	220	567	4.148	3.611	0.112	11
	WB	245	672	5.486	3.567	0.089	9
	MB	250	734	5.872	3.424	0.117	10
Fungi	MC	113	177	3.133	4.478	0.062	10
	PB	111	170	3.063	4.550	0.113	10
	WB	172	339	3.942	4.110	0.108	11
	MB	177	380	4.294	4.294	0.083	12

MC, manure compost; PB, WB, and MB, biochar, respectively, of swine manure, wood residue, and maize residue.

(2019), the nodes, edges, average path length, and fungal network modularity increased following biochar in addition to ryegrass soil. High-complexity networks normally tended better stability against environmental stresses with buffering through networking (Landi et al., 2018).

Moreover, biochar could affect the metabolic processes of pathogenic microorganisms, which inhibits mycelial growth and abates the virulence reported by Gu et al. (2017) and Wu et al. (2020a). With the biochar amendments (PB, WB, and MB) over MC (**Table 3** and **Figure 3**), the contents of the total and dominant phenolic acids (over 100 $\mu g\ kg^{-1}$ in concentration) were markedly declined (by 35–55%), though the composition structure unchanged. Over the 3 growing seasons, the level of total phenolic acids under MC amounted to 7.03 mg kg^{-1}, being folds higher than that in newly planted soil (Wu et al., 2016). Indeed, forest soils with wild ginseng were high in bacterial and fungal abundance, and no allergy-chemical obstacle was observed (Fang et al., 2022). While gene abundance of a dominant genus (abundance $>$ 0.5%) either of bacteria or of fungi (**Figure 8**) was significantly correlated with the total or monomer content of phenolic acids, the biochar amendment induced reduction evidenced improved biodefense, or system-acquainted resistance (Jaiswal et al., 2017), against allelopathic compounds impacts on soil microbes. The relative abundance of plant pathogens (**Figure 7A**), especially *Fusarium* spp. and *Ilyonectria* spp. (**Figure 4C**), was reduced under biochar amendments. *Fusarium* spp. and *Ilyonectria* spp. were reported as the main pathogens causing ginseng root rot (Punja et al., 2008; Shao et al., 2021). As observed in the studies by Kong et al. (2008), Pollock et al. (2011), and Dong et al. (2018), these pathogens tended to degrade the phenolic acids for their energy. Often, antagonist microbes potentially capable of biocontrol suppressed and soil pathogens accumulated ultimately leading to growth obstacles with the replanted *Panax notoginseng* (Miao et al., 2016; Dong et al., 2018; Luo et al., 2019). Therefore, the relative abundance of pathogens confirmed the improved biocontrol for root pathogenic diseases, as clearly shown in the previous study with continuous cropping (Liu et al., 2022). Such reduction was more or less in line with the ginseng survival and root biomass harvested with the application of biochar (**Figure 2A**). Hence, higher survival rate and root production were ensured with lower disease incidence probably through the suppression of pathogens including *Fusarium* spp. and *Ilyonectria* spp. together decrease in root release of phenolic acids under biochar amendment (**Supplementary Figure 2**). This added to the finding by Jaiswal et al. (2018), who reported immobilization and deactivation of pathogenic enzymes and toxic metabolites with biochar from eucalyptus wood chips and greenhouse pepper waste.

For the biochars amended in the experiment, WB and MB had a higher content of fixed carbon and pore volume, thus providing a higher capacity to absorb the phenolic acids, than PB. While Asao et al. (2003) reported that the use of activated charcoal effectively decreased phenolic acids by root exudates, the ginseng survival rate was linked to soil pH elevation, and physical properties improved in a previous experiment in a continuously cropped ginseng farm (Liu et al., 2022). Biochar application significantly improves soil proteobacteria relative abundance, and most of the ammonia-oxidizing bacteria including nitrogen-fixing bacteria, ammonia-oxidizing bacteria, cellulose-decomposing bacteria, nitrifying bacteria, and denitrifying bacteria belong to proteobacteria, which plays a significant role in nitrogen recycling that is beneficial for the plant roots (Zhang et al., 2022). Generally, maintenance of soil structure and nutrient conditions was the key driver to enhance plant stress resistance under biochar soil amendment (Jaiswal et al., 2017). The present study again supported that biochar from maize residue and wood waste was better than manure biochar in deactivating the phenolic root exudates. Thus, the great reduction of phenolic acid concentration in the rhizosphere under biochar treatments could be ascribed to either retarded root exudation of these compounds or enhanced immobilization of these compounds in biochar-amended soil. Unfortunately, the respective contribution remained unclear.

Following Oliver and Gregory (2015) and Banerjee and van der Heijden (2022), the overall improvement of one health (soil–root–microbes) for ginseng production using organic amendments was tentatively assessed concerning the

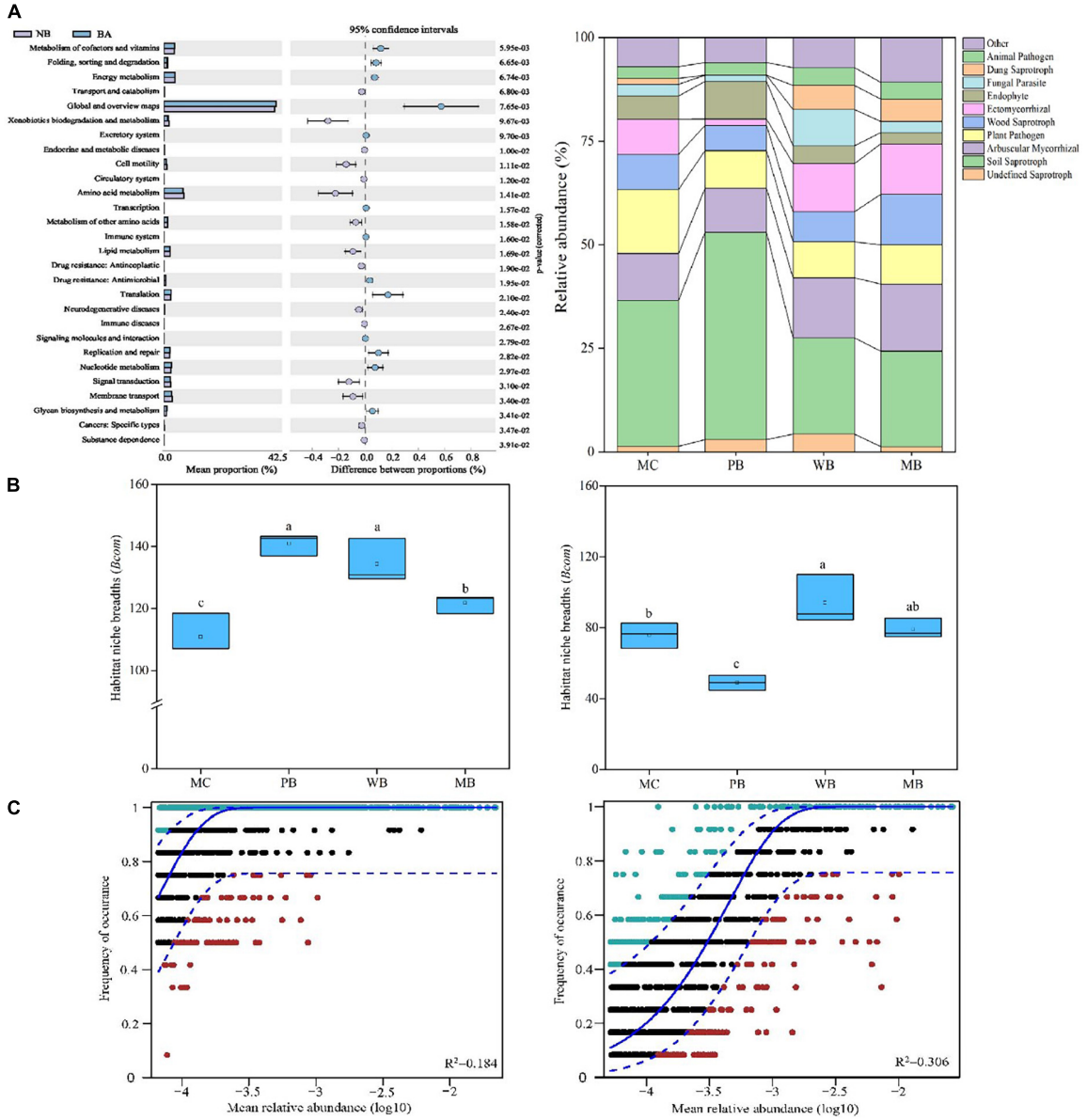

FIGURE 7
Functional traits **(A)**, mean habitat niche breadth **(B)**, and fit of the Sloan neutral community model **(C)** of bacterial (left) and fungal (right) community of rhizosphere soil sampled on 3-year following amendment at 20 t ha^{-1}. Functional traits of the bacterial community on KEGG pathway (KO tier 2) at DNA level of topsoil (0–15 cm) following amendment at 20 t ha^{-1} with biochar (BA, average of WB and MB) relative to manure (no biochar, NB). (Left), a relative abundance of different functions of both fields; (Right), size of the proportion differences in the BA soils from the NB soil at 95% confidence interval, with the significance at $p < 0.05$. Fungal functions using FUNGuild with OTU data. For Sloan neutral community model, solid blue line represents the best fit to the Sloan neutral community model, and dashed blue line represents 95% confidence intervals around the neutral community model prediction. OTUs that occur more or less frequently than predicted by the neutral community model are shown in green and red, respectively. R^2 indicates the fit to this model.

synergism between the key ecological services provided by soil. These key services were concerned with plant production, carbon sequestration, nutrient conservation, plant defense, and microbial biomass and diversity (**Supplementary Figure 7**). Maize residue-derived biochar rich in micro-pores (Ma et al., 2018) and organic molecules (Bian et al., 2022) synergistically promoted soil, plant, and microbes' system health. Beyond soil C sequestration, maize biochar ensured ginseng root production and quality while profoundly shifting microbial community composition and networking, and relevantly plant defense. As recently argued by Bolan et al. (2022), such multifaceted functionality could be a shifting paradigm for biochar application in the agricultural system toward carbon neutrality. Amendment of crop residue biochar, maize biochar,

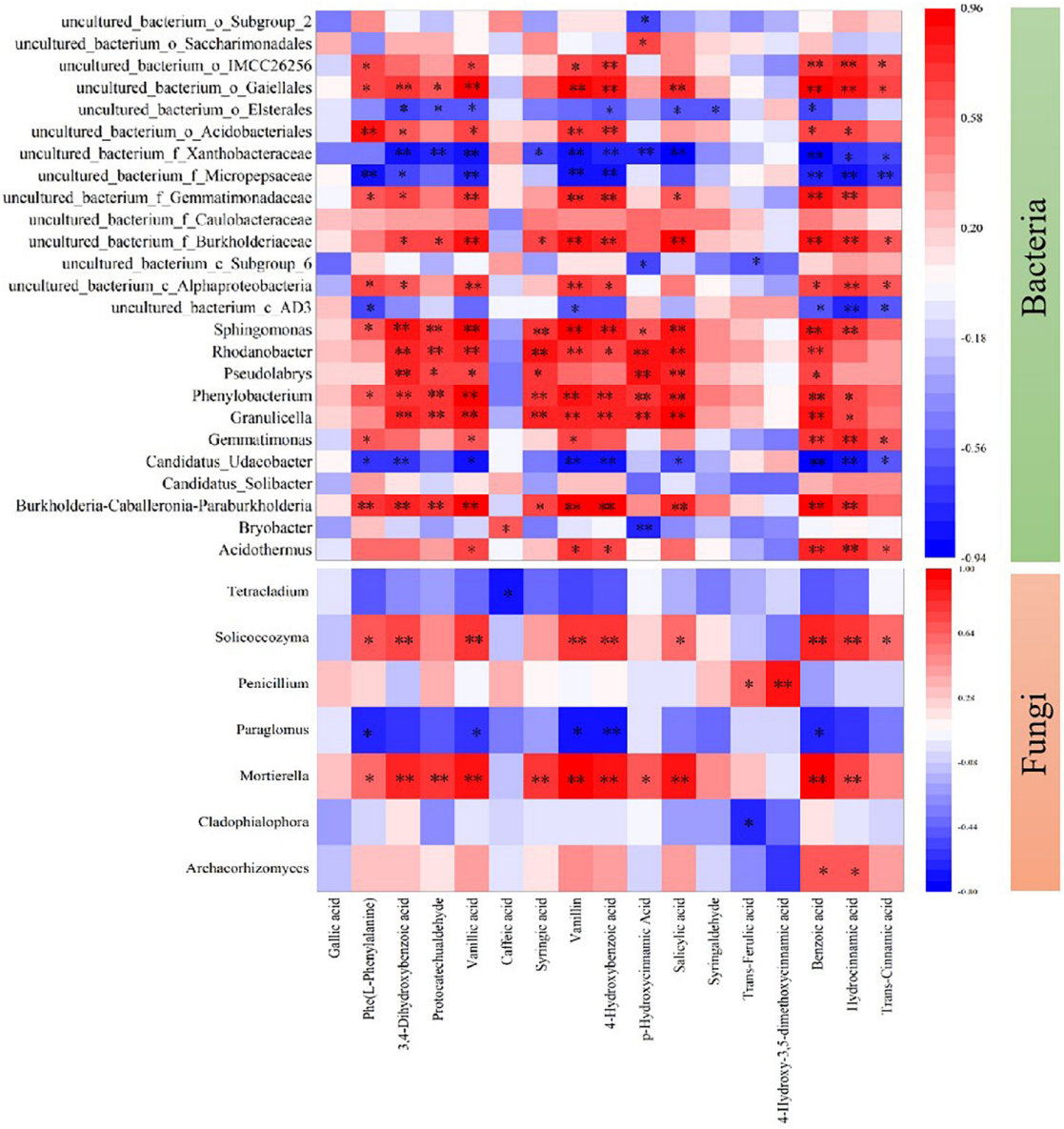

FIGURE 8
Spearman's rank-order correlation between the content of phenolic acids and the relative abundance of dominant bacteria and fungi at the genus level. Only statistically significant correlations are present, and the key from green to red represents the negative correlation to the positive correlation. MC, amendment of manure compost at 20 t ha^{-1}; PB, WB, and MB, amendment of pig manure, wood, and maize biochar at 20 t ha^{-1}. *$p < 0.05$; **$p < 0.01$.

in particular, could be a practical approach as the nature-based solution (United Nations Environment Programme [UNEP], 2022). The interlinks between biochar, soil, rhizosphere microbes, and plant growth/metabolism deserve further studies.

Conclusion

In this study, a profound effect of biochar was portrayed on reducing root-derived allelopathy phenolics and in turn soil born pathogenic fungi with enhancing microbial diversity and networking, besides soil fertility improvement, in replanted P. ginseng field. Our work demonstrated more biodefense against plant pathogenic disease than plant productivity with biochar amendments over MC. Among the biochars used, maize biochar enabled a synergistic promotion of soil, plant, and microbes' system health, thus contributing to ginseng quality improvement. Therefore, the MB in this study could be taken as a strategic solution to sustain soil health and quality production of functional root crops in continuously cropped soils.

Data availability statement

The data presented in this study are deposited in the NCBI repository, accession number: PRJNA786724.

Author contributions

CL: experiment, sample analysis, data processing, and manuscript drafting. RX: experiment performance and sample analysis. MT: field experiment, sampling, and data collection. XL, RB, LY, JZ, KC, and XZ: experiment and data inspection. MD, LL, SS, and SJ: supervision and data interpretation. GP: experiment design, data inspection and analysis, and manuscript editing. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2022.1065313/full#supplementary-material

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Impacts of organic materials amendment on the soil antibiotic resistome in subtropical paddy fields

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The organic material amendment has been proven to change the soil antibiotic resistance genes (ARGs) profile, which may threaten human health through the food chain, but the effects and mechanisms of different organic materials on ARGs in paddy soils are less explored. In this study, a field experiment was set up with the treatments of conventional chemical fertilization (NPK) and common organic material amendment [rice straw (RS), swine manure (SM), and biochar (BC)] to explore the effects and mechanisms. In total, 84 unique ARGs were found across the soil samples with different organic material amendments, and they conferred resistance to the major antibiotic classes. Compared with NPK, SM significantly increased the detected number and relative abundance of ARGs. A higher detected number of ARGs than NPK was observed in BC, whereas BC had a lower relative abundance of ARGs than NPK. Compared with NPK, a detected number decrease was observed in RS, although abundance showed no significant differences. Compared with other treatments, a higher detected number and relative abundance of mobile genetic elements (MGEs) were observed in BC, indicating a higher potential for horizontal gene transfer. There were significantly positive relationships between the relative abundances of total ARGs and MGEs and the bacterial abundance. The network analysis suggested the important role of MGEs and bacterial communities in shaping the ARGs profile. Mantel test and redundancy analysis (RDA) suggested that soil carbon, nitrogen, and C/N were the major chemical drivers of the ARGs profile. The risk of ARGs spreading to the food chain should be considered when applying SM and biochar, which shifted the ARGs and MGEs profiles, respectively. Pre-treatment measures need to be studied to reduce the dissemination of ARGs in paddy fields.

KEYWORDS

organic materials, biochar, antibiotic resistance genes, bacterial community, paddy soil

1. Introduction

The emergence and prevalence of antimicrobial resistance (ARGs) pose a major threat to public health (WHO, 2014); it has gained numerous concerns. The environmental microbes that carried ARGs had similar gene sequences to clinical pathogens, suggesting the potential transmission of ARGs from the environment to the pathogens of human beings or *vice versa*. Most importantly, more and more evidence proved that ARGs in manured soil can be transferred to the phyllosphere of vegetables (Zhang et al., 2019). Thus, the bloom and dissemination of ARGs in agroecosystems have potential risks to agricultural production and food security (He et al., 2020). Furthermore, native soil microbes could acquire ARGs *via* horizontal gene transfer mediated by mobile genetic elements (MGEs) from exogenous microorganisms (Chen et al., 2017). Thus, it is important to explore the antibiotic resistome in the agricultural ecosystem for assessing the potential risk.

Antibiotic resistance is an ancient and natural phenomenon (D'Costa et al., 2011; Shen et al., 2019), and human activities such as livestock production, composting, and manure fertilization have put selective pressure on antimicrobial resistance in various environments (Zhu et al., 2013; Forsberg et al., 2014). Soil is probably the largest habitat for microbes and one of the largest reservoirs of ARGs (Forsberg et al., 2012; Nesme et al., 2014), especially agricultural soil, which suffered from human activities. ARGs were widely detected in paddy fields (Zhao et al., 2020), vegetable farmlands (Xu et al., 2021), and even in the phyllosphere of vegetables (Chen et al., 2018; Zhou et al., 2019). In recent years, many studies have documented that livestock manure, reclaimed water, sewage sludge, and heavy metals play pivotal roles in profiling the patterns of ARGs in the soil environment (He et al., 2020). Agricultural soils play a critical role in sustaining crops and the food supply. To promote food production and sustainable development, chemical fertilizers and organic materials (for instance, livestock manure, straw, and biochar) were widely applied in croplands (Tiedje et al., 2019). Livestock manure application could introduce the ARGs that they carried into the soil and place selective pressure on soil indigenous stocktickerARG-bearing microbes *via* residual antibiotics or (and) heavy metals (Chen et al., 2017, 2019; Han et al., 2018). Fertilization not only influences soil physicochemical properties such as pH, available nitrogen, and soil organic matter contents but also affects microbial diversity, abundance, and community structure (Xie W. et al., 2018; Sun et al., 2019; Chen P. et al., 2021). A great number of studies have been conducted to assess the impacts of fertilizer application on the soil microbiome and function guilds (Dai et al., 2018; Jia et al., 2020). Organic materials, such as crop straw, straw-derived biochar, and swine manure (SM), are usually applied with chemical fertilizers to croplands for soil fertility improvement. Nevertheless, the impacts of different organic materials input on the antibiotic resistome in paddy fields are still less explored.

Different fertilizers make distinct contributions to the structure and function of microbial communities in agricultural soils. Long-term overuse of chemical fertilizers decreased soil pH and then shifted the structure of the soil bacterial community (Dai et al., 2018), even inhibiting the activity of bacteria (Lin et al., 2016; Jia et al., 2020). Additionally, the influences of chemical fertilizers on the relative abundance of ARGs in different tillage systems were inconsistent in previous reports (Wang F. et al., 2018; Han et al., 2021). Organic fertilizers, such as livestock manure, composting, and sludge, were widely amended into paddy soil. Consequently, the microbes carrying ARGs from animals or humans were introduced into the soil and shifted the structure of native microbial communities in the soil (Macedo et al., 2021). Generally, manure application promotes the propagation of ARGs, for instance, genes conferring resistance to sulfonamide (Tang et al., 2015; Lin et al., 2016; Xu et al., 2021). Recently, biochar was applied to improve soil fertility and reduce soil pollutants, including antibiotics and heavy metals (Ye et al., 2016; Wang S. et al., 2021). Biochar derived from various organic materials such as rice straw (RS), wheat straw, and maize straw can increase soil fertility and supply a unique habitat for microbes, then directly or indirectly change the abundance and diversity of microbes (Zhang G. et al., 2021). Previous studies showed that biochar addition reduced the relative abundance of ARGs in arable soils (Ye et al., 2016; Duan et al., 2017), while some found that biochar containing heavy metals could increase the relative abundance of ARGs (Ding et al., 2019). RS incorporation is another common agricultural practice, which significantly influences bacterial community composition and abundance (Zhang S. et al., 2021). To some extent, soil bacterial abundance and communities shifting accounts for the ARGs profiles feature. Organic materials input influences the microbial composition and abundance, which in turn impacts the shape of ARGs. It is essential to evaluate the effects of different organic materials input on the ARGs profile in the soils for further risk assessment. In this study, a field experiment was conducted with the aims (1) to determine the effect of different organic materials amendment on antibiotic resistome and bacterial communities in paddy soils and (2) to explore the underlying mechanisms of the effects of organic materials on the paddy soil resistome.

2. Materials and methods

2.1. Sampling and DNA extraction

The field experiment was conducted at the field experiment station (113° 19′ 52″ E, 28° 33′ 04″ N) of the Institute of Subtropical Agriculture, Chinese Academy of Sciences, which is located in Changsha County, Hunan Province in southern China. The study site had a subtropical humid monsoon climate, with an annual mean precipitation of 1,330 mm and an annual air temperature of 17.5°C (Shen et al., 2014).

The treatments of the field experiment with the double rice cropping included CK (without nitrogen fertilizer), NPK (NPK chemical fertilizers only), RS (chemical NPK fertilizers combined with RS at a rate of 6 t dry matter ha^{-1} in each rice season), SM (chemical NPK fertilizers combined with SM at a nitrogen supply ratio of 1:1), and BC (chemical NPK fertilizer combined with straw-derived biochar at a rate of 24 t dry matter ha^{-1} applied only once). Except for the CK treatment, all the treatments had the same N fertilizer application rate in each rice season. The experimental plots for each treatment were set up in triplicate. The chemical NPK fertilizers were composed of urea (120 kg and 150 kg N ha^{-1} in early and later rice seasons, respectively, with the application rate for basal, tillering, and panicle fertilizers at a ratio of 5:3:2), superphosphate (75 kg P_2O_5 ha^{-1} used as basal fertilizer), and potassium chloride (100 kg K_2O ha^{-1} used as basal fertilizer). The field experiment started in 2012.

Topsoil samples (0–20 cm) for all the plots of the treatments were collected with a sterilized shovel after harvest in the late rice season of 2019. One part of the soil samples for each plot was put in liquid nitrogen immediately for transport to the lab and stored at −80°C until use, and another part of the soil samples was put on ice for transport to the lab and stored at 4°C until use. Chemical properties of soil samples, including total nitrogen (TN), total phosphorus (TP), total organic carbon (TOC), available phosphorus (AP), nitrate (NO_3^--N), and ammonium (NH_4^+-N) were measured as described previously (Wang C. et al., 2021). The total microbial DNA of the soil was extracted using a DNeasy PowerSoil kit from 0.5 g fresh soil according to the manufacturer's instructions (Qiagen, Inc.). The concentration of extracted DNA was detected using NanoDrop One, and DNA was stored at −80°C until use.

2.2. Quantitative PCR analysis

A high-throughput qPCR (HT-qPCR) was used to quantify the ARGs and MGEs in soil samples. The array included 384 primers targeting ARGs (319) and MGEs (57); additionally, the taxonomic marker genes were included in the array (Stedtfeld et al., 2018; Kanger et al., 2020; Pu et al., 2020). All reactions were performed in the Takara SmartChip real-time PCR system, as described previously. Three technical replicates were performed for each sample, and a non-template negative control was included in each HT-qPCR run (Kanger et al., 2020). A threshold cycle (Ct) of 31 was used as the detection limit, and only all three replicates with Ct lower than 31 were regarded as genes detected in that sample. The relative abundance of detected ARGs was calculated using a previously reported formula (Han et al., 2021).

Real-time quantitative PCR was performed to quantify total bacterial 16S rRNA gene copies using a primer set as previously described (Pu et al., 2020). The 25-μL reaction mixture contained 12.5 μL of premixture (Takara, Japan), 0.5 μL of each primer (10 μM), 1 μL of DNA (~10 ng μL^{-1}), and 10.5 μL of RNase-free water. The bacterial 16S rRNA gene primer set and amplification condition were the same as HT-qPCR (Pu et al., 2020).

2.3. Bacterial 16S rRNA gene amplicon sequencing and taxonomic analyses

The bacterial composition in different fertilization practices was surveyed by prokaryotic 16S rRNA gene amplicon sequencing with primer pair 338F and 806R, which target the V3–V4 variable region of the 16S rRNA gene (Xu et al., 2016). The amplicon sequencing was performed on the Illumina MiSeq System (PE300) by Shanghai Majorbio Bio-Pharm Technology Co., Ltd.

Microbiome bioinformatics was performed with qiime2 2019.7 (Bolyen et al., 2019). The raw sequencing reads were demultiplexed and quality filtered using the q2-demux plugin, followed by denoising with DADA2 (Callahan et al., 2016) (*via* q2-dada2). Taxonomy was assigned to ASVs using the q2-feature classifier (Bokulich et al., 2018) against the SILVA taxonomy database (release 132) based on a 97% sequence similarity threshold (McDonald et al., 2012). The raw sequences have been deposited in the NODE.[1]

1 https://www.biosino.org/node/project/detail/OEP002404

2.4. Statistical analysis and data visualization

One-way analysis of variance (ANOVA) followed by the Student-Newman–Keuls test was carried out in SPSS 22.0 to compare the difference in diversity and the relative abundances of ARGs and MGEs across different treatments. $P < 0.05$ was considered to be statistically significant. The relative abundance of *aac(3)-Via* is one to three orders of magnitude higher than that of other detected genes, which will seriously affect the analysis results, so *aac(3)-Via* was discarded during analysis. A Venn diagram was generated to visualize the number of shared ARGs between different treatments using the Evenn (Chen T. et al., 2021). The difference in the relative abundance of ARGs and the community compositions of bacteria among different fertilization approaches was visualized by principal coordinates analysis (PCoA) based on the Bray–Curtis dissimilarity distances using the "vegan" package in R (Oksanen et al., 2020). A Mantel test was conducted to assess the correlations between soil properties, MGEs, bacterial abundance, bacterial diversity, and ARGs based on Bray–Curtis dissimilarity matrices with 999 permutations using the "linkET" package in R. Furthermore, transformation-based redundancy analysis (RDA) was carried out to explore the relationship between the composition of ARGs and soil chemical and biological parameters using the "vegan" package in R (Oksanen et al., 2020).

Networks were illustrated to explore the co-occurrence pattern between MGEs, bacterial taxa, and ARGs based on the Spearman correlation coefficients. The Spearman correlation coefficient (ρ) > 0.6 and $P < 0.01$ were regarded as statistically robust correlations (Li et al., 2015). The correlation coefficient matrices were imported into Gephi 0.92 for visualization (Bastian et al., 2009), and the network topology was explored by the Fruchterman-Reingold algorithm.

3. Results

3.1. Diversity and abundance of ARGs under different organic materials input

A total of 84 ARGs and 14 MGEs were detected across all samples (**Figure 1A**). These detected ARGs conferred resistance to the major eight antibiotics commonly used in the clinic or husbandry: aminoglycosides, beta-lactams, fluoroquinolones, MLSB (macrolides-lincosamides-streptogramins B), sulfonamides, tetracyclines, vancomycin, and others. Compared to CK, nitrogen and organic materials input significantly increased the number of detected ARGs in paddy soil regardless of the organic material types (**Figure 1B**). Under the treatments with the same nitrogen application rate, SM detected the highest number of ARGs ($P < 0.05$), followed by BC, NPK, and RS, in descending order. The three most frequently detected ARG classes, conferring resistance to aminoglycoside, multidrug, and MLSB, accounted for 28.6, 16.7, and 15.5% of the total number of detected ARGs, respectively. Interestingly, the plasmid-mediated colistin resistance determinant *mcr-1* gene was detected in all samples, which was first detected in animal guts and conferred resistance to the "last resort," polymyxin. No genes conferring resistance to sulfonamide were detected in CK, and only one was detected in other treatments. In addition, the

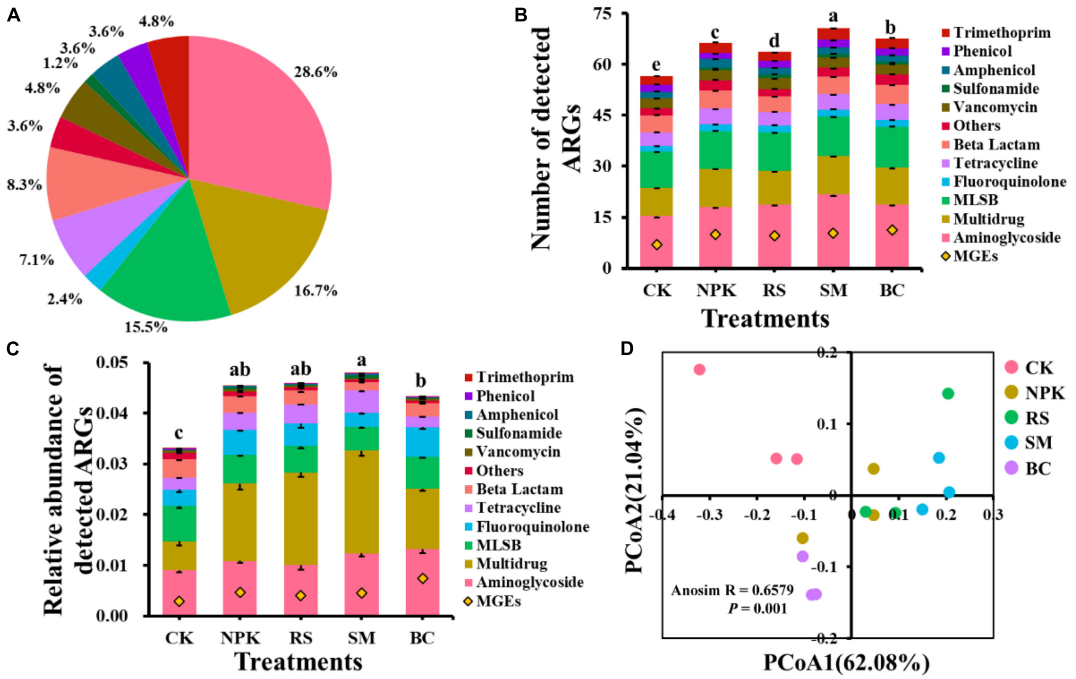

FIGURE 1
Classification of ARGs based on the antibiotic to which they confer resistance **(A)**. Detection number **(B)** and relative abundance **(C)** of ARGs categorized by antibiotic type detected in different management practices. The PCoA analysis is based on the relative abundance of ARGs using Bray–Curtis distances **(D)**. MLSB, macrolide-Lincosamide-Streptogramin B. Different letters above the bars indicate a significant difference ($P < 0.05$) across different treatments.

number of shared ARGs among different treatments was shown in the Venn diagram (Figure 2A). Nitrogen and organic material applications changed the ARGs profiles in soils. Compared to CK, the number of unique ARGs detected in different organic materials ranged from 1 to 8 (Figure 2). There were 57 ARGs found in all the treatments. In terms of MGEs, the detected number for the BC treatment was significantly higher than that for other organic materials treatments ($P < 0.05$), while no significant differences were observed in the detected number of MGEs among NPK, RS, and SM (Figure 3A). The HT-qPCR array detected a wide type of MGEs, including two insertional sequences, one integrase, two plasmids, and one transposase (Figure 3C).

For the relative abundance of ARGs, the genes conferring resistance to aminoglycoside, multidrug, and MLSB shared the highest relative abundance among all the treatments (Figure 1C), with the relative abundance in the range of 63.3–66.8, 7.78–7.5, and 4.08–9.52%), respectively, which, in total, accounted for 80.6–86.9% of the total abundance of ARGs. Compared to CK, the treatments with nitrogen fertilizer application significantly increased the total relative abundance of ARGs ($P < 0.05$). Under the same rate of nitrogen input, the total relative abundance of ARGs was the highest in the SM treatment and the lowest in the BC treatment ($P < 0.05$), and no significant differences were observed among RS, NPK, and BC ($P > 0.05$). There was no significant variance observed in the relative abundance of ARGs conferring resistance to amphenicol, trimethoprim, and vancomycin across all treatments, whereas variance was observed in other classes of ARGs among all treatments (Supplementary Table 2). For example, a higher total relative abundance of genes refer resistance to multidrug was observed in SM and RS than in other treatments under the same nitrogen input rate ($P < 0.05$), and BC was lower than NPK ($P < 0.05$). Regarding the relative abundance of total MGEs, an obvious increment was observed after nitrogen input and organic materials input (Figure 3B). Under the same nitrogen rate application, BC owned the highest detected number and relative abundance of total MGEs than other treatments ($P < 0.05$), while no significant variances were observed among other organic materials treatments. Divergent compositions of MGEs were observed among different treatments with different relative abundances (log-transformed, Figure 3C).

Differences in the comprehensive composition of ARGs among different treatments were assessed further by PCoA analysis based on the Bray–Curtis distance of the relative abundance of ARGs. The results showed that soil samples from CK were distinctly separated from others amended with organic materials (Figure 1D). Approximately 83.1% of the total variation was explained by the first and second axes of the ARGs structure.

3.2. Bacterial abundance, diversity, and community structure

The absolute abundance of the 16S rRNA gene in CK was 4.48×10^9 copies per gram of dried soil, which was significantly lower than those of the treatments with nitrogen fertilizer application. There was no significant difference in the absolute abundances of the 16S rRNA gene among the treatments with the same rate of nitrogen input (Supplementary Figure 1). For the bacterial Shannon-Winner index, there was no significant difference across all the treatments (Supplementary Figure 1).

Across all soil samples, the dominant bacterial phyla were *Proteobacteria* (33.3%), *Chloroflexi* (17.0%), *Acidobacteria* (10.8%),

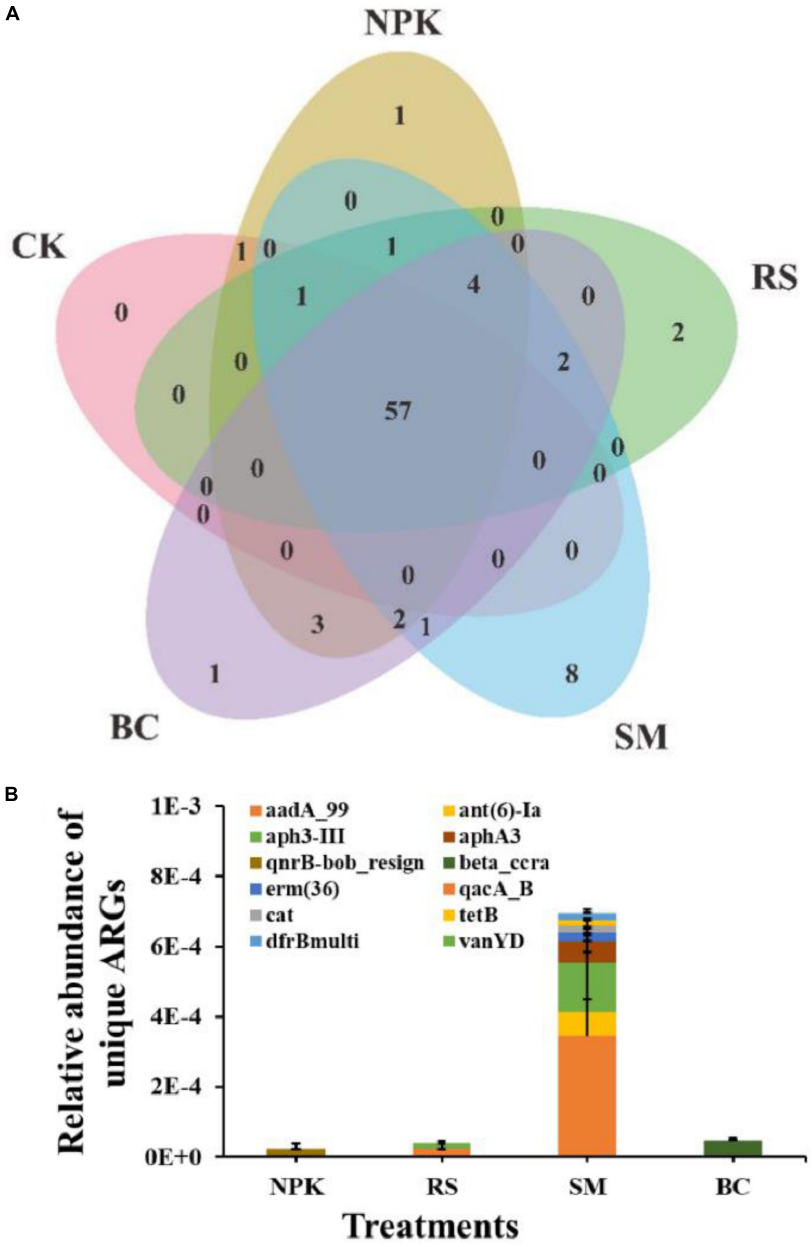

FIGURE 2
A Venn diagram showing the ARG numbers shared by different treatments **(A)**, and the relative abundance of unique genes detected in different treatments **(B)**.

and *Nitrospirae* (9.2%), accounting for more than 70% of the total bacterial sequences (Supplementary Figure 1). The ANOVA analysis was performed to find the variation in the relative abundance of bacterial phyla across all treatments. Only *Nitrospirae* and *Bacteroidetes* showed statistical differences across all five treatments, while the left phyla showed no significant differences across the treatments. Briefly, the relative abundance of *Nitrospirae* was the highest ($P < 0.05$) for the BC treatment as compared with other treatments with nitrogen fertilizer application, and there were no significant differences among the treatments of NPK, RS, and SM. Under the same nitrogen input rate, the relative abundances of *Bacteroidetes* for the treatments with organic materials input (RS, SM, and BC) were lower than that of NPK ($P < 0.05$). In addition, the PCoA based on the Bray–Curtis distance metrics showed no significant differences in bacterial community composition among all fertilization practices (Supplementary Figure 1).

3.3. Correlations between ARGs and MGEs

The network was composed of 57 nodes (50 ARGs and 7 MGEs) and 151 edges (Figure 4A). A total of 19 ARGs co-occurred with MGEs, and some of them were highly detected frequencies and relative abundances, such as *ermS*, *tetA(P)*, and *mdtG*. The clusters of nodes (modules) were found in the network, and there were eight modules. The nodes that connected intensively with each other were regarded as the "hubs" and used as the indicators of co-occurring ARGs in the same module. In the largest three modules (I, II,

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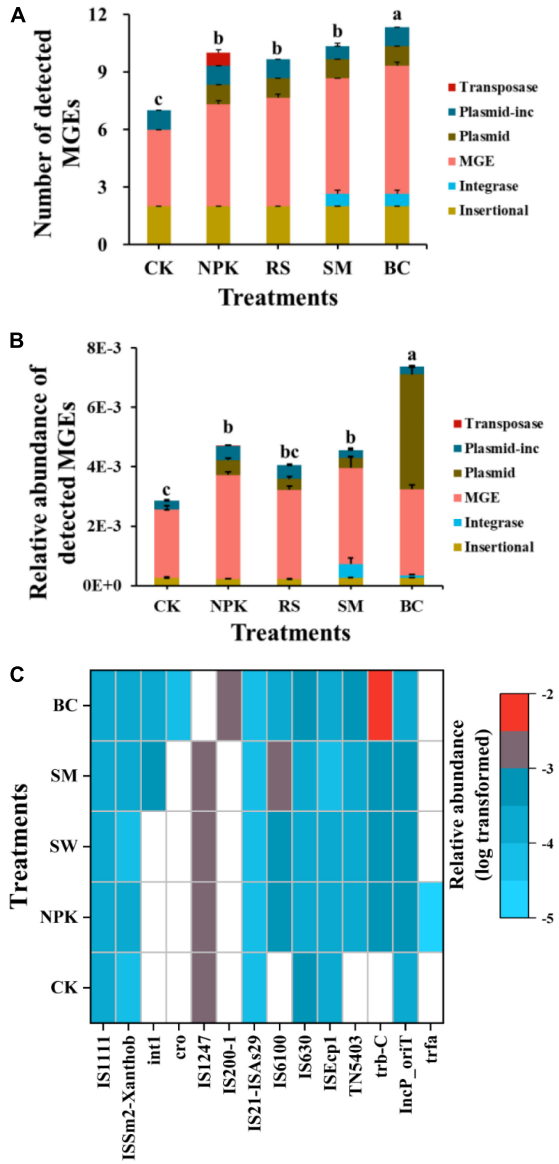

FIGURE 3
Detection number **(A)** and relative abundance **(B)** of MGEs categorized by MGE type detected in different management practices and the relative abundance of individual MGEs detected in soil samples **(C)**.

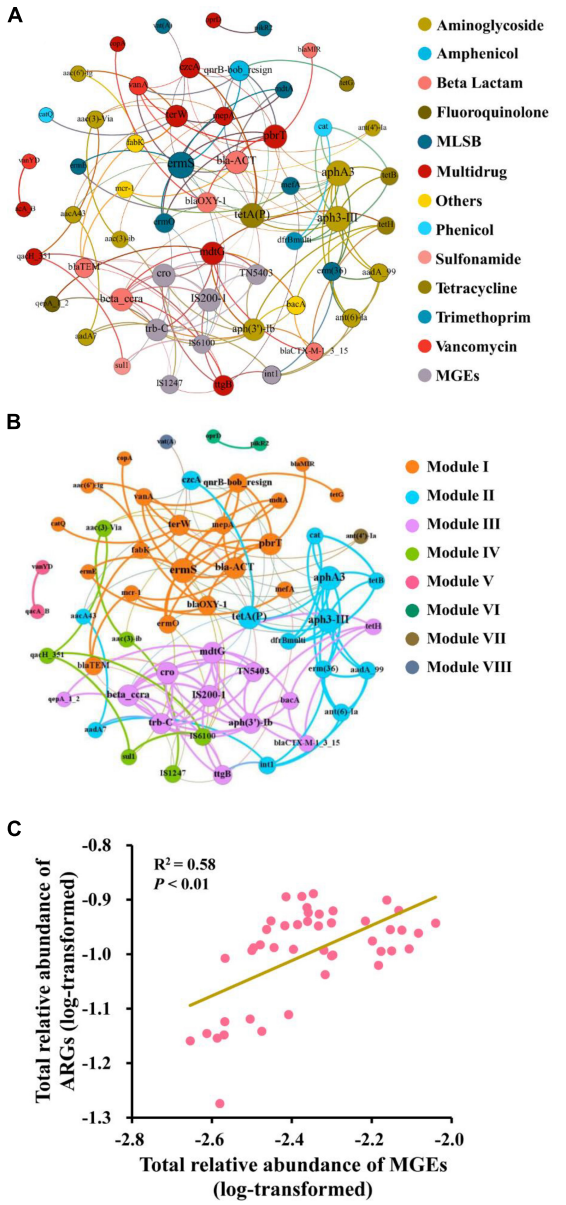

FIGURE 4
Correlation between ARGs and MGEs. Network illustrating the co-occurrence patterns among the detected ARGs and MGEs across all the soil samples. The nodes with different colors represent different categories of ARGs and MGEs **(A)** or different modules **(B)**. Correlation between the relative abundance of total ARGs and MGEs **(C)**.

and III), the gene *ermS*, *tetA(P)*, and *mdtG* were the hubs of these major modules, respectively (Figure 4B). Mostly, four MGEs (*IS200-1*, *TN5403*, *trbC*, and *cro*) are located in Module III. Furthermore, Pearson correlation analysis indicated that the relative abundance of total ARGs had a significantly positive relationship with the relative abundance of total MGEs ($r^2 = 0.58$, $P < 0.01$) (Figure 4C).

3.4. Relationships between bacterial communities, soil properties, and ARGs

The co-occurrence patterns between ARGs and bacterial taxa (class level) were studied using the network analysis based on the Spearman correlation relationships ($\rho > 0.6$, $P < 0.01$). The non-random co-occurrence patterns between bacterial taxa and ARGs could provide indirect evidence of potential host information for ARGs. This network was composed of 168 nodes (73 ARGs, 14 MGEs, and 81 bacterial taxa) and 231 edges. As shown in Figure 5A, *Thermodesulfovibrionia* (*Nitrospirae*) connected with 10 ARGs and three MGEs, which was the largest bacterial node, followed by *Subgroup_6* (*Acidobacteria*) and *Deinococci* (*Deinococcus-Thermus*). *For ARGs, ttgB connected with 10 bacterial taxa, which was the largest node of ARGs, followed by qach_351. In terms of MGEs, IncP_oriT was the largest node of MGEs, followed by IS6100 and int1*. Furthermore, bacterial 16S rRNA gene copies showed a significantly positive correlation with the relative abundance of total ARGs ($r^2 = 0.74$, $P < 0.001$) (Figure 5B), and the Mantel test showed that bacterial 16S rRNA gene copies had a significant positive correlation with the composition of ARGs ($r = 0.25$, $P < 0.05$) (Figure 6A). In

addition, MGEs' abundance, soil TN, TOC, C/N (C/N ratio), and pH also showed significant correlation with ARGs composition by mantel test ($P < 0.01$, Figure 6A). RDA analysis was performed to further verify and identify the main drivers of ARGs composition. For all treatments, the RDA analysis explained 87.1% of the total variability in the ARGs structure, and the first two axes account for 64.54% (Figure 6C). ARGs in the CK were separated from those in the nitrogen input treatments along the first axis. The contribution of bacterial abundance (16S rRNA gene copies) and abundance of total MGEs to the variation of ARGs accounted for 12.43 and 10.25%, respectively ($P < 0.05$). TN, TOC, and C/N were the top three soil properties that contributed to the abundance of ARGs, accounting for 11.38, 11.31, and 11.91%, respectively ($P < 0.05$). TN also made a great contribution to the detected number of ARGs (11.26%, Figure 6B). Furthermore, TOC was the main influencing factor of MGEs detected number (15.75%, Figure 6D) and abundance (29.37%, Figure 6E). The abundance of bacterial and MGEs were the main biotic factors shaping the ARG patterns, while TN, TOC, and C/N were the main abiotic drivers. The bacterial abundance played the most important biotic role in shaping the abundance of ARGs, while the MGEs' abundance played the most important role in profiling the number of ARGs.

4. Discussion

4.1. Effects of nitrogen and organic materials on antibiotic resistome in paddy soil

In this study, nitrogen fertilizers induced dramatic shifts in soil properties and microbial communities and also played a crucial role in shaping soil antibiotic resistome. In the previous studies, the increase and/or decrease in ARGs abundance induced by chemical fertilizer use were observed, even with a limited effect. For example, the *tetA* gene abundance in grassland was enhanced by nitrogen input (Nõlvak et al., 2016), while depletion of most *tet* genes was observed in the paddy-upland rotation system (Lin et al., 2016) with chemical fertilizer application as compared with a control with no fertilizer applied. In this study, the diversity and abundance of ARGs were higher in NPK than in CK, and the compositions of ARGs were obviously different between the two treatments. That implied an important role for nitrogen application in shaping ARGs pattern. The unique gene detected in NPK (*qnrB-bob_resign*) and the gene shared by CK and NPK (*terW*) could be considered as chemical fertilizers input-induced. Compared to CK, although only a slight increment in soil TN was observed in NPK (Supplementary Table 1), this would lead to an increase in bacterial abundance, especially nitrogen-favored organisms, and then affect resistome composition (Forsberg et al., 2014).

Straw incorporation in croplands is an effective approach to straw utilization, which provides various kinds of carbon for the microorganisms, and bacterial abundance or structure change would induce the ARGs shifting. In this study, compared to NPK, straw incorporation increased soil organic carbon content. Although straw incorporation did not significantly increase the abundance of bacteria and total ARGs, it changed the abundance of different types of ARGs (Supplementary Table 2). These results indicated that straw incorporation might provide carbon for the growth of soil bacteria,

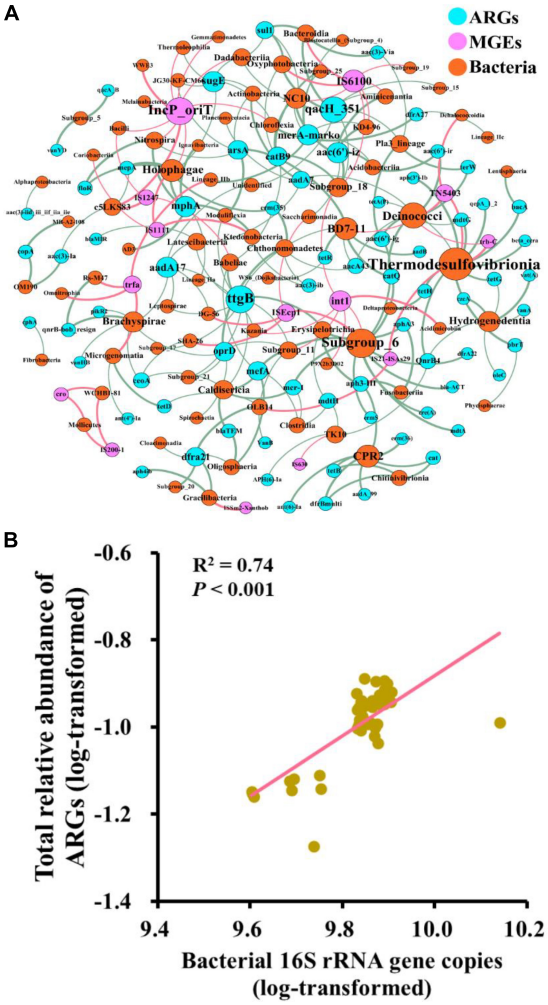

FIGURE 5
The network of co-occurrence patterns among ARGs, MGEs, and bacterial taxa at the class level **(A)** and the correlation between 16S rRNA gene copies and the relative abundance of total ARGs **(B)**.

especially that carried ARGs (*qacA_B* and *vanYD*) only detected in RS. Straw incorporation was reported to promote the simultaneous elimination of antibiotics and related ARGs in the paddy soil by changing the dissolvable organic carbon and bacterial structure (Zhang et al., 2022). This study's results differed from the observed results on microcosmic scales. Therefore, the influence on ARGs should be further explored when straw is returned to the paddy field.

Animal manure is applied into croplands as organic fertilizer to improve soil quality, and the manure carrying antibiotic-resistant bacteria (ARB) and ARGs could simultaneously be transported into agricultural soils, which could increase the abundance and diversity of ARGs in soils (Tiedje et al., 2019; He et al., 2020). In the present observation, compared to NPK, SM significantly increased the diversity, slightly increased the abundance of ARGs in the soil, and gained the highest ARGs detected number across all treatments. These results were similar to the previous studies in which manure amendment dramatically shifted the ARGs patterns in agricultural soils (Zhu et al., 2013; Chen et al., 2017, 2019). The increasing diversity of ARGs in swine-manured soil may be due to several reasons. First, SM is an important host habitat for ARGs carried microbes, which can be directly imported into the soil by fertilization

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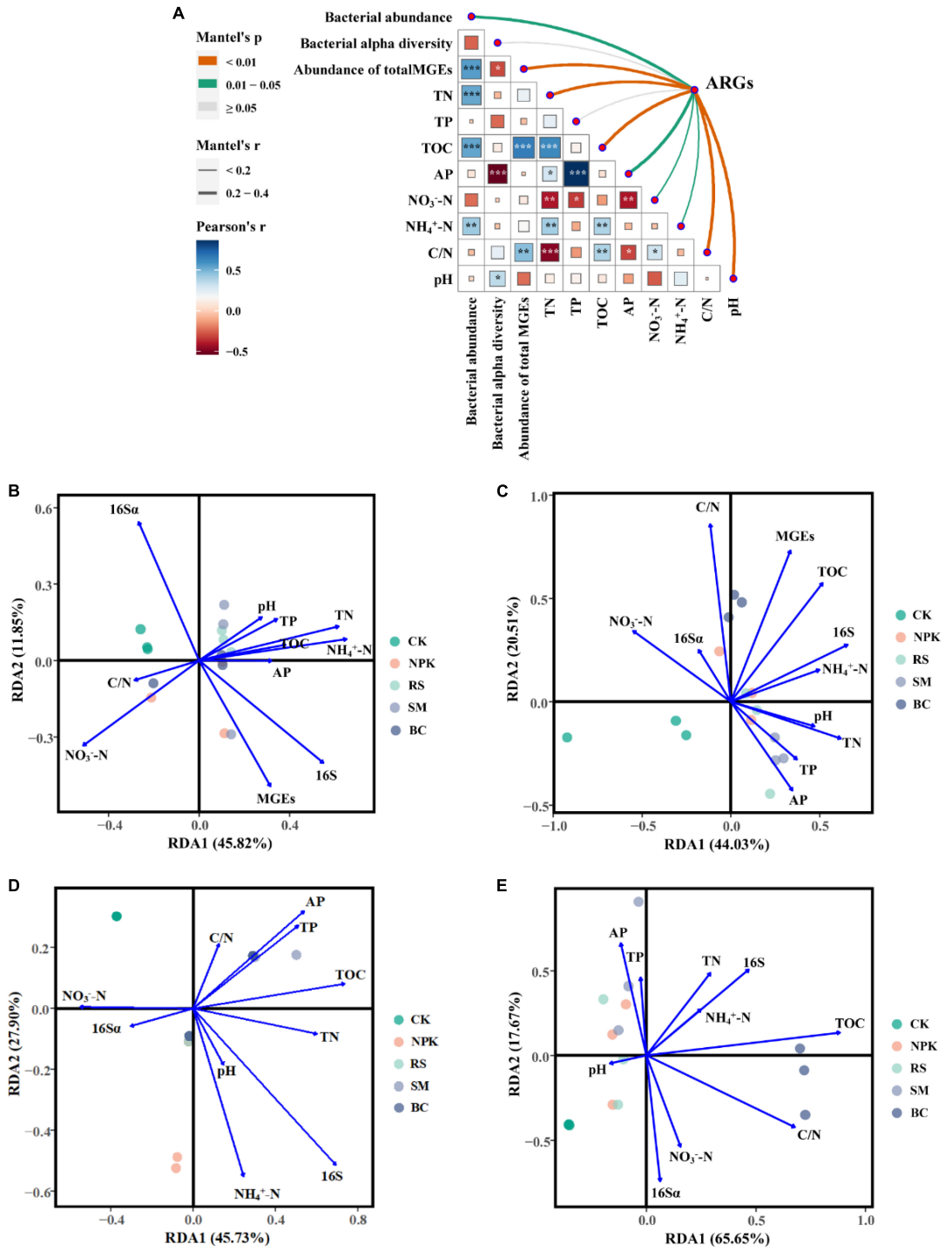

FIGURE 6
Pairwise comparisons of environmental factors (soil properties, bacteria, and MGEs) are shown, with a color gradient denoting Pearson's correlation coefficient **(A)**. The composition of ARGs was related to each environmental factor by Mantel tests. Edge width corresponds to the Mantel's r statistic for the corresponding distance correlations, and edge color denotes the statistical significance based on 999 permutations. Redundancy analysis (RDA) of the effects of environmental factors on detected number **(B)** and ARGs abundance **(C)** across all treatments, and the effects of environmental factors on MGEs detected number **(D)** and abundance **(E)** across all treatments. In all figures, C/N means the ratio of soil total carbon and total nitrogen, TOC means soil total carbon, TN means soil total nitrogen, TP means soil total phosphorus, AP means soil available phosphorus, NO_3^--N means soil nitrate nitrogen content, and NH_4^+-N means soil ammonium nitrogen content. **(B–E)** The 16S means the bacterial abundance, and the 16Sα means the bacterial alpha diversity (Shannon-Winner index).

(Zhu et al., 2013). Although not all manure-derived ARGs can persist for a long duration in soil, some of them can still be observed. In this study, eight unique ARGs were only detected in manured soil samples, indicating that manure-borne ARGs were introduced into the agroecosystems (Xie W.Y. et al., 2018). Second, antibiotic resistance bacteria in manure cannot survive in the soil for the long term due to niche shifting, but the native soil bacteria can acquire ARGs through horizontal gene transfer by MGEs, such as

transposons, integrons, and broad-host-range plasmids (Jain et al., 1999; Domingues and Nielsen, 2019), which plays an important role in the dissemination of ARGs in various environments. The *int1* gene had the highest relative abundance in the SM-amended soils in this study. These results mean that manure not only introduced novel ARGs to soils but also might introduce or boost MGEs in soil, resulting in the spread of ARGs.

Biochar derived from the pyrolysis of carbon-rich biomass has been widely amended into the soil to increase soil fertility, enhance carbon sequestration, and increase soil water-holding capacity (Xu et al., 2016; Ding et al., 2019; Chen P. et al., 2021). Thus, the changes in soil properties due to biochar amendment might induce the shift of ARGs composition. Due to its high porosity and large surface area, biochar is also applied as an effective adsorbent for the control of soil organic pollutants, such as pesticides and phenols. Biochar can reduce the oxytetracycline and sulfonamide concentrations as well as the corresponding resistance genes (Ye et al., 2016; Duan et al., 2017). Soil biochar amendment was reported to enhance the retention of the ARGs in a former study (He et al., 2021). Furthermore, biochar application has been reported to significantly increase the abundance of bacteria in plastic shed soil (Wang F. et al., 2021), which might change the abundance of ARGs. In this study, compared to NPK, biochar application increased the number of detected ARGs but did not significantly change the relative abundance of ARGs. It might be due to that the special structure of straw-derived biochar provided a special niche and enhanced the soil nutrient (carbon, nitrogen, etc.) contents for the growth of ARGs carried bacteria, which has grown to exceed the detection limits. The improvement of soil organic carbon with biochar amendment was observed from the same field in previous studies (Wang C. et al., 2018; Liu et al., 2021; Wang S. et al., 2021), indicating the biochar might shift the ARGs through increased TOC in paddy soil. Moreover, higher diversity and abundant MGEs were detected in biochar-applied paddy soil than in other treatments; these MGEs contained integrons (*intI1*), insertion sequences (*IS1111*), plasmids (*IncP-oriT*), etc., which mediated the horizontal transfer of ARGs as demonstrated widely in previous research (Post and Hall, 2009; Di Cesare et al., 2016; Kishida et al., 2016). This result indicated a higher frequency of horizontal gene transfer in biochar-applied paddy soil. Therefore, more research needs to be conducted to assess the effects of biochar on the fate of ARGs at the field scale.

4.2. Co-occurrence among ARGs, MGEs, and bacterial taxa

Network analysis indicated the co-occurrence patterns among ARGs, MGEs, and bacteria. In this study, the "hub" genes *ermS*, *tetA(P)*, and *mdtG* might be used as the marker genes in the fertilization soils. Furthermore, Module III was composed of several ARGs and MGEs, indicating that these antibiotic-resistance genes might make horizontal gene transfer *via* the assistance of linked MGEs (Zhu et al., 2013).

Soil is an important reservoir of microbes and ARGs. Soil bacteria are the major producers of bioactive substances, such as antibiotics, and at the same time, they are the hosts of numerous ARGs. The network for the relationship between ARGs and bacterial taxa could provide the potential host information of co-occurring ARGs. For instance, *ttgB* and *qacH_351* genes were multidrug resistance genes that were detected in many opportunistic human pathogens (*Pseudomonas aeruginosa*, *Escherichia coli*, and *Acinetobacter baumannii*), and most of these pathogens belong to *Proteobacteria*, but the network showed that *ttgB* and *qacH_351* had many other potential hosts not belong to *Proteobacteria* (The National Center for Biotechnology Information, 2019). Pathogens can acquire various ARGs from pathogens and non-pathogens through horizontal gene transfer *via* MGEs (Forsberg et al., 2012; Wright, 2019), such as integrons, transposons, plasmids, insertion sequences, and phages. The pathogens carrying various ARGs might be developing into "superbugs," which would further pose a risk to human health *via* direct infection and the food chain (He et al., 2020). Although *Proteobacteria* is one of the dominant phyla in our study, it is weakly correlated with ARGs, indicating that the main host of ARGs in this study may be non-dominant bacteria, and this result was also reported in a previous study (Hu et al., 2017).

4.3. Correlations among soil properties, bacteria, MGEs, and ARGs

Soil chemical properties and bacterial characteristics play a pivotal role in shaping ARGs patterns in different croplands (Tiedje et al., 2019; He et al., 2020). In this study, compared with NPK, organic materials input significantly changed the soil properties, especially the content of carbon and nitrogen (**Supplementary Table 1**), and shifted the profile of ARGs in paddy soils, but showed minor effects on bacterial communities, indicating the resilience of soil indigenous bacteria to the application of the organic material (Macedo et al., 2021). On the one hand, organic materials supply sufficient nutrients for bacterial growth and reproduction, then shift the bacterial abundance and diversity, and finally impact the composition of ARGs. On the other hand, organic matters such as SM might put select pressure on the native soil bacteria due to the heavy metals and/or antibiotics existing, which would then induce the change of ARGs profile. In this study, nitrogen and organic materials input significantly increased the bacterial abundance and abundance of ARGs, and a positive correlation was found between them. This indicated the potential contribution to the increased abundance of some ARGs up to and above the detection limits. This result was inconsistent with a previous study, in which bacterial community structure played a crucial role in shaping the ARGs profile of paddy soil (Xiao et al., 2016). That means the diversity and abundance of ARGs in paddy soils were determined by distinct factors, which need to be further explored.

Organic materials application increased the nutrient content in the soil, which made a contribution to bacterial shifting and then influence the ARGs. Mental test and RDA confirmed that bacterial abundance, TN, and TOC were the major drivers in shaping the profiles of the antibiotic resistome in presently observed soils. That result was consistent with a previous study in farmlands (Cheng et al., 2016), in which positive correlations were observed between the total abundance of ARGs and TN, TP, and TOC. In this study, TN and TOC were significantly correlated with bacterial abundance (**Figure 6A**), indicating the increment of bacterial abundance with the application of organic materials due to the TN and TOC increment.

In addition, the indigenous bacteria in the soil competed with the exogenous, long-term application of fertilizers, especially livestock manure, stimulated the horizontal gene transfer, and induced the retention and dissemination of ARGs (Udikovic-Kolic et al., 2014).

In most previous studies, the critical role of MGEs in the distribution of ARGs had been proved widely. In this study, abundant and diverse MGEs had been detected, and a significant correlation with ARGs was demonstrated by correlation analysis, a co-occurrence network, and the mantel test. This indicated the important potential role of MGEs in shaping the ARGs profile, which may occur *via* horizontal gene transfer (conjugation, transduction, and transformation) as found in a previous study (Hu et al., 2017).

Our study showed that not all organic materials combined with chemical fertilizer increased the diversity and abundance of ARGs significantly, and straw incorporation even reduced the number of resistance genes detected. SM application introduced many exogenous ARGs into paddy soils. In addition, organic materials application, especially biochar addition changed the abundance of the MGEs, which had a positive correlation with ARGs abundance. This indicated the potential horizontal gene transfer playing a crucial role in the spread of ARGs in paddy soils. Thus, it is necessary to consider the risk of ARGs when amending organic materials in paddy fields, for balancing the risks (dissemination of ARGs) and benefits (improvement of soil fertility).

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/**Supplementary material**.

Author contributions

ZL: investigation, methodology, and writing—original draft. JPS and FW: writing—original draft. MW: investigation and methodology. JLS: conceptualization, funding acquisition, and writing—review and editing. YL, QZ, and JW: methodology and writing—review and editing. All authors gave the final approval and agreed to be accountable for all aspects of the work.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2022.1075234/full#supplementary-material

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Effects of biochar in combination with varied N inputs on grain yield, N uptake, NH_3 volatilization, and N_2O emission in paddy soil

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Biochar application can improve crop yield, reduce ammonia (NH_3) volatilization and nitrous oxide (N_2O) emission from farmland. We here conducted a pot experiment to compare the effects of biochar application on rice yield, nitrogen (N) uptake, NH_3 and N_2O losses in paddy soil with low, medium, and high N inputs at 160kg/ha, 200kg/ha and 240kg/ha, respectively. The results showed that: (1) Biochar significantly increased the rice grain yield at medium (200kg/ha) and high (240kg/ha) N inputs by 56.4 and 70.5%, respectively. The way to increase yield was to increase the rice N uptake, rice panicle number per pot and 1,000 grain weight by 78.5–96.5%, 6–16% and 4.4–6.1%, respectively; (2) Under low (160kg/ha) N input, adding biochar effectively reduced the NH_3 volatilization by 31.6% in rice season. The decreases of pH value and NH_4^+-N content in surface water, and the increases of the abundance of NH_4^+-N oxidizing archaea and bacteria (AOA and AOB) communities contributed to the reduction of NH_3 volatilization following the biochar application; (3) Under same N input levels, the total N_2O emission in rice season decreased by 43.3–73.9% after biochar addition. The decreases of *nir*K and *nir*S gene abundances but the increases of *nos*Z gene abundance are the main mechanisms for biochar application to reduce N_2O emissions. Based on the results of the current study, adding biochar at medium (200kg/ha) N level (N200+BC) is the best treatment to synchronically reduce NH_3 and N_2O losses, improve grain yield, and reduce fertilizer application in rice production system.

KEYWORDS

biochar, N fertilizer, rice yield, NH_3 volatilization, N_2O emission

1. Introduction

China is a large rice growing country, with about 3.10×10^7 ha of rice field cultivation area. China rice production accounts for 27% of global rice production and about 35% of China's total grain production (Ullah et al., 2023). To meet the global population in rice demand, farmers apply inorganic nitrogen (N) fertilizer to increase rice yield (Chen C.R. et al., 2013; Chen T.T. et al., 2013; Chen et al., 2014). However, farmers often apply excessive N fertilizer inputs (more than 300–350 kg/ha/year) in the pursuit of maximum rice yields (Ali et al., 2020). Previous studies have demonstrated that approximately 50% of applied inorganic fertilizer N is lost either through emissions or leaching, which have detrimental effects to the atmosphere and water

environment (Cao et al., 2013; Li et al., 2017). Of which, ammonia (NH_3) volatilization is one major N loss pathway and account for 10–60% of the total N fertilizer input in the rice season. Moreover, nitrous oxide (N_2O) emissions from rice fields can account for 7–11% of N_2O emissions from agricultural fields in China (Zou et al., 2007; Cai et al., 2017). The N_2O emission can deplete the stratosphere and accelerate climate change. Therefore, ensuring food security while reducing fertilizer N environmental losses by coupling other soil additives is a major ongoing concern in terms of agricultural production and ecological environment in China.

In previous literatures, several management practices have been recommended to reduce N losses in paddy fields such as the use of biochar. Biochar is a type of solid material produced by high-temperature pyrolysis carbonization of biomass under anaerobic conditions. It is characterized with well-developed pore structure, high carbon content and large specific surface area, which results to it having high stability and strong adsorption performance (Zou et al., 2007; Tian et al., 2020). Biochar itself carries micronutrients for crop growth (Pyoungchung et al., 2011) and can also increase soil carbon stocks, promote nutrient cycling and sequestration, and improve crop yields (Gaskin et al., 2008; Novak et al., 2009; Ali et al., 2020). For example, Mcconnell et al. (2007), demonstrated that biochar application with additional N (240 kg/ha) fertilizer increased rice yield by 49.7%, attributing to the increasing effective spike number, spike grain number and 1,000 grain weight. In addition, biochar application in agricultural fields can improve soil quality and reduce nutrient losses, thus increasing crop yields (Steiner et al., 2007). Apart from influencing yields, biochar application has been widely used to reduce pollutants in agricultural production processes (Sun et al., 2019). The amended biochar itself can adsorb NH_3 and achieve NH_3 volatilization reduction by enhancing soil N fixation capacity and increasing soil nitrification rate (Chen C.R. et al., 2013; Chen T.T. et al., 2013). However, the presence of high salt-based ions such as calcium and magnesium in biochar has been shown to have the potential to exchange with hydrogen and aluminum ions in the soil, etc., and this effect usually raises soil pH and leads to increased NH_3 volatilization (Wang et al., 2011; Clough et al., 2013). Meanwhile, the effect of biochar on N_2O emissions from rice fields has been inconsistently reported. Soil NH_4^+ content is a key factor affecting N_2O emission rate, and biochar addition can slow down the denitrification process and reduce soil NH_4^+ content, thus reducing the N_2O emission rate (Wang et al., 2011; Yang et al., 2020). However, some studies have also found no effect of biochar on soil N_2O emission, or even a promoting effect (Clough et al., 2010; Wang et al., 2011). Several studies have demonstrated that the effects of biochar addition on NH_3 volatilization and N_2O emissions is highly influenced by environmental factors, biochar application rate, soil type and cropping system (Sha et al., 2019). However, limited studies investigated the comprehensive effects of biochar on NH_3 volatilization and N_2O emission in a whole crop grow cycle. Therefore, there is a need to undertake studies that cover a major share of the gaseous N loss pathways in rice production.

The integrated evaluation of crop yield, soil NH_3 volatilization and N_2O emission in response to N fertilizer application and exogenous substance addition has been a key area of research in the evaluation of the effects of on-farm management practices. However, most of the published recent studies have evaluated the effect of biochar application to agricultural fields based on a single level of N application rate (Ma et al., 2013). However, there is no clear evidence on the application of biochar with different rates of N fertilizer. This could help to underpin the best combination of biochar and N fertilizer with less N losses and without reducing rice yields. Hence, this study evaluated the effects of biochar addition on rice yield, NH_3 volatilization and N_2O emission at different levels of N supply. Further, explain the mechanistic effect of biochar on N losses based on N uptake, field water pH and ammonium N (NH_4^+-N), soil NH_4^+-N, and nitrate (NO_3^--N) contents and functional microbial gene abundance by a pot experiment. The results and conclusions of the study can provide technical support and theoretical basis for the mutual interaction of N fertilizer and biochar to achieve N fertilizer reduction and biochar resource conservation, crop yield stabilization and environmental protection in rice production.

2. Materials and methods

2.1. Background information and soil column set-up

Test soil for this study was collected at depth of 0–20 cm from an approximately 30 ha paddy field in Zhoutie town of Yixing city (31° 28′ N, 119° 59′ E), Jiangsu Province, which is located at the Taihu Lake region of China. This region has a subtropical monsoon climate, with an annual mean air temperature and rainfall of 15.7°C and 1,177 mm, respectively. The soil was mixed, air-dried for approximately 10 days, ground and sieved through a 2-mm nylon sieve, and repacked layer-wise (0–10 and 10–20 cm) into soil columns (inner diameter 35 cm, height 28 cm) at similar bulk density (1.3 g/cm^3) as in the field. About 20 kg soil was filled into each soil column according to the volume of soil column and the soil bulk density. The selected properties of 0–20 cm topsoil was as follows: pH 6.38 (soil: water ratio 1: 5), soil organic matter 29.2 g/kg, total N 1.72 g/kg, available P 23.1 mg/kg, available K 159.3 mg/kg, and CEC 22.6 cmol/kg. In this experiment, biochar was produced using wheat straw that had been heated to 500°C. The reactor was heated by a stepwise procedure under oxygen-limited conditions. For pyrolysis, the temperature was raised to 500°C at a rate of 5°C/min and held constant for 8 h. The measured properties of the biochar were pH 9.80, total N 0.81%, total C 37.5%, and BET surface area 32.0 m^2/g. At the initiation of the experiment, at the same time of repacking soil into column/pot, biochar was mixed with the top layer (0–20 cm) soil.

2.2. Experimental treatments and rice management

This current experiment constituted seven treatments; urea only at low, medium, and higher rates of N with 160, 200, and 240 kg/ha, named NN160N200, andN240, respectively; N160, N200, and N240 plus biochar at 5 t/ha, named N160 + BC, N200 + BC, and N240 + BC, respectively; Meanwhile a and control treatment without urea and biochar was tested. The N fertilizer rates used on this study were based on deficiency, sufficiency, and over application. Each treatment was replicated three times, therefore there were totally 21 soil columns, Biochar was evenly incorporated into each soil column into the soil at a depth of 15 cm at the initiation of the experiment.

Rice (*Oryza sativa* L., var. Nangeng 46) seedlings (28 days old) were transplanted into the soil columns (three holes in each column and two plants per hole) on July 1, 2021. The total fertilizer N application was split into a basal dressing at transplanting, and two top-dressing during the season in the ratio of 40–30–30%, respectively. Application periods were July 1, July 16, and August 14 in 20,218. Calcium superphosphate and potassium chloride were applied to all treatments including the CK at the rates of 90 kg/ha (P_2O_5) and 150 kg/ha (K_2O), respectively, at the time of transplanting as basal fertilizer. The floodwater was drained at a mid-season drainage period from July 31 to August 7, 2021 to control invalid tillering and improve the rice root development. During other times, a 3–5 cm depth floodwater level was maintained with tap water. Weeds and pests were controlled according to the local farmers' traditional practices. The rice shoots (including straw and grain) were manually harvested on November 8, 2021.

2.3. Sampling and measurements

2.3.1. Crop

Rice plants were harvested at the physiological maturity stage to determine grain yield and its components. Before harvesting, the plant height was measured using a ruler, and the yield-related agronomic traits (number of panicles, number of grains per panicle, and 1,000 seed weight) were also recorded. Rice straw and grain were oven-dried at 105°C for 30 min, and then dried at 80°C until constant weight. The dried plant samples were ground into powder using a high-speed crusher (DS-YM-001), passed through a 0.2 mm nylon sieve. Ground plant samples were kept in sealed containers until digestion. Sub-sample (0.25 g) of ground plant samples were digested in a mixture of H_2SO_4 and H_2O_2 and used for determination of total N content using the Kjeldahl method detailed in Sun et al. (2015). Rice NUE was calculated using Equation 1 outlined by Dong et al. (2015):

$$\mathrm{NUE}(\%) = \frac{N_F - N_0}{N} \times 100\% \quad (1)$$

Where N_F and N_0 denote the N uptake as measured at harvest in the fertilizer applied and the control treatments (kg/ha), respectively, while N denotes the N fertilizer added rate (160, 200, and 240 kg/ha in the current work).

To reflect the leaf chlorophyll content, SPAD values of rice leaves were measured using the chlorophyll content meter (SPAD-502Plus, Japan) at the tillering, earing, and maturation stage, respectively. This was done by selecting three rice plants from each soil column and three leaves of each rice plant were measured. Therefore, the SPAD values presented in this study represented average SPAD value of the three plants in each replicate (Li et al., 2019).

2.3.2. NH_3 volatilization

The daily NH_3 volatilization rates were measured at three N fertilizer applications, using the sponge absorption method (Rochette et al., 2013). The gas-capturing device was made of polyvinylchloride (PVC) plastic tube with an inner diameter of 15 cm and a height of 15 cm. Two sponges with a thickness of 2 cm and a diameter of 16 cm were dipped in 15 mL of phosphoglycerol (50 mL of phosphoric acid plus 40 mL of glycerol and then diluted to 1,000 mL with deionized water) and placed in a plastic tube. The lower sponge was 5 cm from the bottom of the tube and the upper sponge was at the top of the tube.

During sampling, the lower sponge was taken out (8: 00 am), immediately sealed in a bag, and replaced in the gas-capturing device with a new sponge also beforehand dipped in phosphoglycerol. The upper sponge was replaced once every 2 days. The sampled sponge was placed into a 500 mL plastic bottle, submerged in 300 mL of 1 M KCl, and shook at 180 r per minute for 1 h. The NH_4^+-N concentration in the extract was determined by an autoanalyzer (SKALAR San^{++} System, Netherlands). The NH_3 volatilization was calculated using Equation 2 outlined below:

$$\omega = \frac{m \times V_m \times V_e}{V_s} \times 10^{-3} \quad (2)$$

where, ω: NH_3 content in a single collection device (mg); m: NH_4^+-N concentration (mg/L); V_m: the volume of solution used to measure absorbance after constant volume (mL); V_e: the KCl solution volume for extracting ammonium from sponge (mL); V_s: the volume of extracting solution used for measurement (mL). The NH_3 emission factor and yield-scale NH_3 volatilization were calculated according to that introduced in our previous work (Min et al., 2021).

2.3.3. N_2O emission

The gas samples for N_2O determination were collected using the modified closed chamber method as described in Min et al. (2021). The chamber was a transparent Plexiglas cylinder with 100 cm height and 36 cm inner diameter (adjusted for the height of rice plant and the pot size), and covered with Al foil to exclude light. It was fitted into a grove at the bottom (for sealing by tap water in the grove) and had a small fan at the top to properly mix gas before sampling.

Gas samples were collected using a plastic syringe at 15 min intervals. We took the gas samples on the 2nd, 4th, 6th, and 8th day after each N fertilization application and during water drainage period. Thereafter, sampling was done every 10 days until harvest. The sponge absorption device was temporarily moved out during the N_2O measurement, to avoid any disturbance that may occur. When calculated the NH_3 flux, we adjusted the cover time according to the fact. Gas sample collection was done between 6:00–8:00 a.m. Meanwhile, air temperature in each collection device was recorded at collection. Four gas samples were collected using a 50-mL medical syringe at 0, 15, 30, and 45 min after the collection device was sealed. The gas samples were then injected into pre-evacuated 50 ml vacuum bottles fitted with butyl rubber lids for laboratory analysis. The N_2O concentrations were determined using a gas chromatograph (Agilent 7890B, Agilent Technologies, United States) at 350°C equipped with an electron capture detector (ECD). Total N_2O emission was calculated from the individual fluxes and the interval times (Sun et al., 2022).

2.3.4. NH_4^+-N, NO_3^--N concentrations and pH in overlying water

Overlying water samples were collected using a syringe on the same day and time as NH_3 volatilization samples collection. Collected water samples were filtered through a 0.45 μm membrane, then analyzed for pH using a combined reference electrode (Φ255 pH/temp/mV meter, Coulter Bechman Co., United States). A sub-sample of 50 ml filtered water was stored in clean plastic bottles at −20°C for further analysis. The concentrations of NH_4^+-N and

NO_3^--N in overlying water were determined by an autoanalyzer (SKALAR San^{++} System, Netherlands).

2.3.5. Soil properties

At the end of the experimental period after rice harvest, three soil cores in each pot were randomly sampled at 0–20 cm depth using a soil drill (50 mm in diameter), top layer soil was sampled at selected points. Soil samples were composited, mixed manually, placed in self-sealing bags, and brought back to the laboratory in collar box with ice. Soil samples were divided into two parts: one was stored at −80°C for molecular analysis and another at −20°C for analysis of other properties. The soil pH was measured in the 1:2.5 (w/v) soil: water suspension using a combined reference electrode (Φ255 pH/temp/mV meter). Soil samples were extracted with 2.0 M KCl (1:5 soil: extractant, w/v) for NH_4^+-N and NO_3^--N determination. Soil extracts were filtered through a 0.45 μm membrane filter and the NH_4^+-N and NO_3^--N concentrations were determined using an autoanalyzer (SKALAR San^{++} System, Netherlands). The gene copy numbers of AOA and AOB *amo*A, *nir*K, *nir*S, and *nos*Z of soil samples were determined by Shanghai Majorbio Biomedical Co., Ltd. according to the procedures detailed in Chu et al. (2020) and Ye et al. (2021).

2.4. Data analysis

A statistical analysis was performed using SPSS 22.0, and one-way analysis of variance (ANOVA) was used to determine the significance of the difference between treatments. The level of significance was measured using Duncan's multiple-comparison test ($p < 0.05$).

3. Results

3.1. Rice yield and nitrogen uptake

3.1.1. Rice yield and its component factors

The results in Table 1 showed that N200 and N240 treatments significantly ($p < 0.05$) increased the straw biomass by 54.0 and 77.0% relative to the control treatment. Both N160 and N160 + BC had no difference in straw biomass compared to the control. The combinations of BC with N200 and N240 significantly ($p < 0.05$) increased rice straw biomass by 79.5 and 42.3% compared to N200 and N240 alone, respectively. However, there was no difference in straw biomass between N160 + BC and N160. Interestingly, the grain yield in N added treatments (70.53–145.50 g/pot) were significantly ($p < 0.05$) higher than the no N added control treatment (42 g/pot). Moreover, N200 + BC and N240 + BC treatments significantly ($p < 0.05$) promoted the rice grain yield by 31.9 and 70.6% relative to N200 and N240 treatments, respectively. Nevertheless, amendment of biochar induced no difference in rice grain yield at low N input level (160 kg/ha).

Moreover, the number of panicles and 1,000 grain weight of rice in N200 + BC treatment were significantly ($p < 0.05$) 16 panicles/pot and 6.1% higher than N200, respectively. Compared with N240 treatment, however, N240 + BC treatment significantly ($p < 0.05$) increased the number of panicles, grains per panicle and 1,000 grain weight of rice by 6 panicles/pot, 35.9, and 4.4%, respectively (Table 1).

3.1.2. Rice plant height and leaf SPAD

At the tillering stage, either N fertilizer reduction or biochar addition had no effect on rice plant height (Figure 1A), but the SPAD value of rice leaf in N240 + BC treatment was significantly increased by 8.6% ($p < 0.05$) compared with N240 treatment (Figure 1B). At earing and maturation stages, rice plant height of rice was significantly ($p < 0.05$) increased by 5.0–11.0% and 7.1–19.5%, and rice leaf SPAD value was significantly ($p < 0.05$) increased by 9.9–13.4% and 17.6–29.9% ($p < 0.05$) when biochar was applied at medium (200 kg/ha) and high N (240 kg/ha) application levels. Among the different treatments at both early and maturing stage, the N200 + BC treatment had the highest values in terms of rice plant height and leaf SPAD value (Figure 1).

3.1.3. N content and uptake of rice straw and seeds

No significant difference in N content and uptake by rice straw among the treatments with N fertilizer but without biochar (Table 2). However, the seed N content and uptake increased when N addition increased from 160 kg/ha to 200 kg/ha, but decreased when N addition further increased to 240 kg/ha The N200 treatment

TABLE 1 Effects of biochar application and N fertilizer reduction on rice straw biomass, grain yield and its component factors.

Treatment	Straw biomass (g/pot)	Grain yield (g/pot)	Yield component factors		
			Panicle number	Grain number per panicle	Thousand seed weight (g)
Control	96.03 ± 24.47 d	42.63 ± 4.64 d	16.00 ± 2.00 f	109.00 ± 14.00 b	24.73 ± 0.58 ab
N160	115.50 ± 6.71 cd	72.47 ± 1.75 c	29.00 ± 4.00 de	117.00 ± 11.00 ab	23.93 ± 0.42 b
N160 + BC	118.55 ± 6.77 cd	70.53 ± 1.96 c	27.00 ± 3.00 e	120.00 ± 10.00 ab	24.27 ± 0.50 b
N200	147.88 ± 13.11 bc	93.03 ± 19.6 b	32.00 ± 3.00 cd	119.00 ± 20.00 ab	24.00 ± 0.20 b
N200 + BC	265.44 ± 14.02 a	145.50 ± 3.00 a	48.00 ± 3.00 a	128.00 ± 20.00 ab	25.47 ± 0.23 a
N240	169.97 ± 14.93 b	82.20 ± 2.00 bc	35.00 ± 2.00 c	103.00 ± 11.00 b	24.27 ± 0.50 b
N240 + BC	241.87 ± 30.72 a	140.23 ± 7.48 a	41.00 ± 3.00 b	140.00 ± 7.00 a	25.33 ± 0.70 a

Data in the table are mean ± standard deviation ($n = 3$); different lowercase letters in the same column indicate significant differences between treatments ($p < 0.05$).

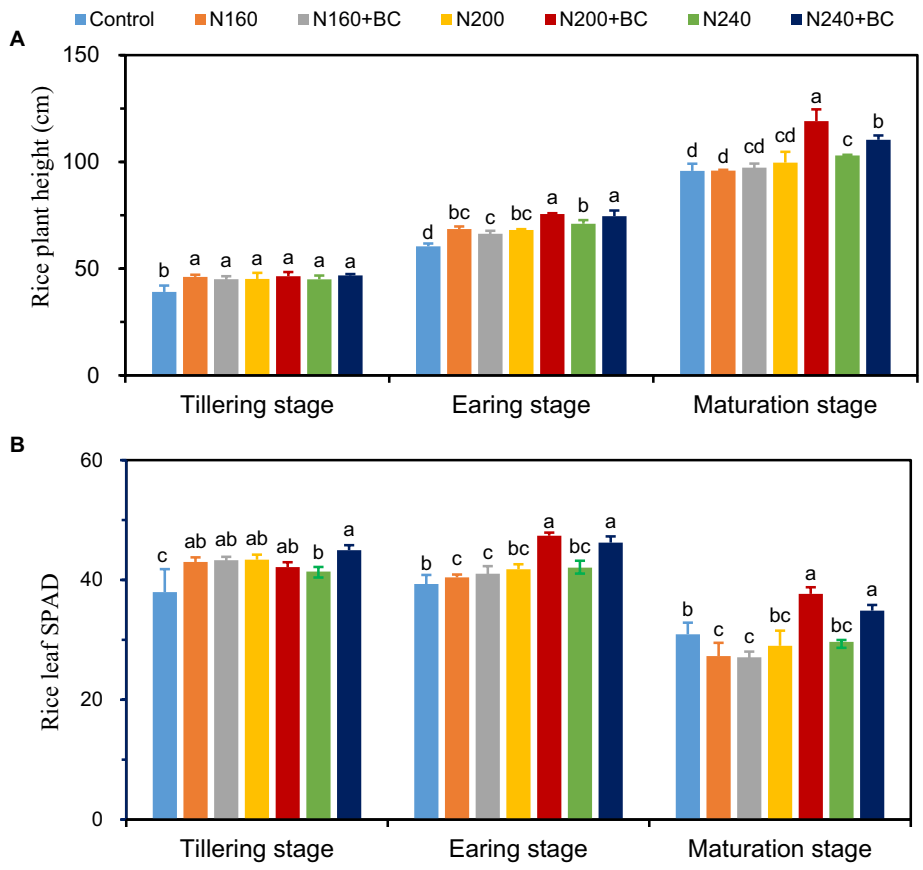

FIGURE 1
Effects of biochar application and N fertilizer reduction on rice plant height **(A)** and flag leaf SPAD **(B)** at different growth stages. Different lowercase letters above the columns indicate significant differences between treatments (p<0.05).

TABLE 2 Effect of biochar addition and N fertilizer reduction on total N content and uptake of rice straw and grain.

	Nitrogen content (g/kg)		Nitrogen uptake (g/pot)		
Treatment	Rice straw	Rice grain	Rice straw	Rice grain	Total
Control	4.39 ± 0.01 c	18.20 ± 0.10 cd	0.36 ± 0.03 b	0.73 ± 0.03 d	1.09 ± 0.06 d
N160	4.36 ± 0.18 c	18.58 ± 0.36 bcd	0.50 ± 0.05 b	1.35 ± 0.03 c	1.85 ± 0.02 c
N160 + BC	4.54 ± 0.27 c	18.08 ± 0.54 d	0.54 ± 0.03 b	1.27 ± 0.02 c	1.81 ± 0.02 c
N200	4.40 ± 0.09 c	20.39 ± 0.68 a	0.65 ± 0.07 b	1.90 ± 0.46 b	2.56 ± 0.53 b
N200 + BC	6.15 ± 1.69 b	20.19 ± 0.21 a	1.64 ± 0.46 a	2.94 ± 0.09 a	4.57 ± 0.37 a
N240	4.15 ± 0.09 c	19.34 ± 1.25 abc	0.71 ± 0.22 b	1.59 ± 0.13 bc	2.30 ± 0.08 bc
N240 + BC	7.29 ± 0.08 a	19.63 ± 0.48 ab	1.76 ± 0.27 a	2.75 ± 0.18 a	4.52 ± 0.38 a

Data in the table are mean ± standard deviation (n = 3); different lowercase letters in the same column indicate significant differences between treatments (p < 0.05).

showed a significant (p < 0.05) increase of 9.7 and 40.7% in seed N content and uptake, compared with N160 treatment, respectively. Biochar addition significantly (p < 0.05) increased rice straw N content by 39.8–75.7% and N uptake by 148–152% at medium and high N supply. Overall, biochar addition exerted no effect on seed N content of rice receiving same N fertilizer, while biochar addition significantly (p < 0.05) increased seed N uptake by 54.7–73.0% at middle (200 kg/hm^2) and high (240 kg/hm^2) N supply. Meanwhile, the total N uptake (straw + seeds) in N200 + BC and N240 + BC treatments was 1.8 and 2.0 times higher compared to N200 and N240 treatments, respectively (Table 2).

3.2. NH_3 volatilization, emission factor and yield-scale NH_3 volatilization

As shown in Supplementary Figure 1, during BF observation, the peak NH_3 volatilization rate (24.95–67.03 mg/pot/d) was observed on the third days after BF, while the peak NH_3 volatilization rate (29.48–110.63 mg/pot/d) was observed on the first day after SF2. Except for the N240 treatment, NH_3 volatilization could reach its peak (41.33–74.84 mg/pot/d) within the first 3 days after SF1. The NH_3 volatilization rate in all N fertilizer applied treatments dropped to a low level as that in the control treatment within 7 days after N each fertilizer application conducted.

The cumulative NH_3 volatilizations in the rice season under N applied treatments were 0.20–0.53 g/pot, accounting for 10.5–28.7% of the fertilizer N input into the rice paddy. The NH_3 losses after BF, SF1 and SF2 were 0.06–0.18 g/pot, 0.09–0.23 g/pot, and 0.05–0.20 g/pot, respectively (Table 3). N160 + BC treatment reduced the NH_3 volatilization at both SF1 (38.9%) and SF2 (37.2%) observations, compared with N160 treatment, and this effect resulted in an overall significant ($p < 0.05$) reduction in cumulative amount of NH_3 volatilization by 31.6%. Moreover, the application of biochar at N160 level significantly ($p < 0.05$) reduced the NH_3 emission factor by 37.2%. Nevertheless, the total NH_3 losses and emission factor in the rice season were not influenced by biochar at 200 kg/ha and 240 kg/ha applications. Interestingly, biochar addition significantly ($p < 0.05$) reduced yield-scale NH_3 volatilization at all three N application levels by 29.7, 52.7, and 46.3% relative to N160, N200, and N240 kg/ha supply, respectively (Table 3).

3.3. N_2O emission

Under no biochar additions, N_2O emissions in the rice season increased significantly ($p < 0.05$) with the increasing N application (Figure 2). Biochar addition significantly ($p < 0.05$) reduced the cumulative N_2O emissions in rice season by 54.1, 43.3, and 73.9% relative to N160, N200, and N240, respectively. Meanwhile, results in Figure 2 show that the cumulative N_2O emissions of N200 + BC and N240 + BC treatments in rice season can be reduced to the N160 level, and the N_2O emissions of N160 + BC treatment can be reduced to the control treatment level.

3.4. NH_4^+-N and NO_3^--N concentrations in overlying water

3.4.1. NH_4^+-N concentration in overlying water

The peak NH_4^+-N concentrations in the overlying water of each treatment during the BF occurred on the 2–5 days (16.1–29.0 mg/L) after N fertilizer application (Figure 3A). Except for the N240 treatment, on the first day after SF1 and SF2 applied, the NH_4^+-N concentrations of overlying water came to the peak with 27.8–77.5 mg/L and 71.7–172.4 mg/L, respectively (Figures 3B,C). After reaching the peak, the NH_4^+-N concentration in the overlying water of each treatment decreased rapidly to no significant difference among all treatments (Figure 3).

At the BF observation, compared with N200, the average NH_4^+-N concentration of overlying water in N200 + BC decreased by 8.5%. However, biochar addition into low (160 kg/ha) and high (240 kg/ha) N supplied treatments increased the average NH_4^+-N concentration of overlying water by 4.4 and 33.9%, respectively. After the SF1 and SF2 applied, the addition of biochar at low (160 kg/ha) N supply level increased the average NH_4^+-N concentration of overlying water by 10.0 and 11.8%, respectively. Nevertheless, biochar addition at medium (200 kg/ha) and high (240 kg/ha) N supply levels reduced the mean overlying water NH_4^+-N concentrations by 20.3–54.5% and 31.0–53.7%, respectively, during the same periods.

3.4.2. NO_3^--N concentration in overlying water

During the BF, the addition of biochar at all three levels of N supply increased the mean NO_3^--N concentration in the overlying water by 16.2, 3.8, and 11.0%, respectively (Figure 4A). During the SF1, the addition of biochar at the medium (200 kg/ha) N level increased the mean NO_3^--N concentration in the overlying water by 66.0%, but at the high (240 kg/ha) N level it decreased the mean NO_3^--N concentration in the overlying water by 41.3%. During the SF2, the average NO_3^--N concentration in the overlying water of N200 + BC and N240 + BC treatments was 3.5 and 1.2 times higher than that of N200 and N240 treatments, respectively (Figures 4B,C).

3.5. Soil properties

3.5.1. Topsoil NH_4^+-N and NO_3^--N content

During the BF, biochar had varied effects on soil NH_4^+-N content under low (160 kg/ha) and high (240 kg/ha) N fertilizer application conditions. The soil NH_4^+-N content in N160 + BC treatment was significantly ($p < 0.05$) higher than N160 treatment by 9.8%, while that in N240 + BC was significantly ($p < 0.05$) lower than N240 treatment by 15.0% (Figure 5A). The soil NH_4^+-N contents in the N240 + BC treatment were significantly ($p < 0.05$) 48.8 and 79.7% higher than N240 treatment during the SF1 and SF2, respectively.

The soil NO_3^--N content was not remarkably affected by N fertilizer application and biochar addition during the BF and SF1 (Figure 5B). During the SF2, biochar amendment at medium (200 kg/

TABLE 3 Effect of biochar application and N fertilizer reduction on the accumulation of NH_3 volatilization, emission factor and yield-scale NH_3 volatilization at different fertilization periods and the whole reproductive period of rice.

Treatment	NH_3 volatilizations (g/pot)				Emission factor	Yield-scale NH_3 volatilization
	BF	SF1	SF2	Accumulation	%	g/kg
Control	0.02 ± 0.00 d	0.01 ± 0.00 d	0.03 ± 0.01 f	0.06 ± 0.01 d	–	1.31 ± 0.09 d
N160	0.06 ± 0.01 c	0.18 ± 0.08 ab	0.14 ± 0.01 b	0.38 ± 0.09 b	28.7 ± 7.6 a	5.25 ± 1.22 b
N160 + BC	0.06 ± 0.02 c	0.11 ± 0.01 c	0.09 ± 0.01 c	0.26 ± 0.01 c	18.0 ± 1.0 b	3.69 ± 0.24 c
N200	0.07 ± 0.02 c	0.13 ± 0.04 bc	0.06 ± 0.02 de	0.27 ± 0.03 c	15.0 ± 2.1 bc	2.98 ± 0.81 c
N200 + BC	0.06 ± 0.02 c	0.09 ± 0.03 c	0.05 ± 0.01 ef	0.20 ± 0.04 c	10.5 ± 3.0 c	1.41 ± 0.30 d
N240	0.15 ± 0.02 b	0.18 ± 0.03 ab	0.20 ± 0.02 a	0.53 ± 0.02 a	28.1 ± 1.0 a	6.47 ± 0.81 a
N240 + BC	0.18 ± 0.00 a	0.23 ± 0.02 a	0.07 ± 0.2 cd	0.49 ± 0.02 a	25.3 ± 1.2 a	3.47 ± 0.08 c

BF, basal fertilizer; SF1, first supplementary; SF2, second supplementary fertilization of urea-N in rice season. Data are means ± SD ($n = 3$); different lowercase letters in the same column indicate significant differences between treatments ($p < 0.05$).

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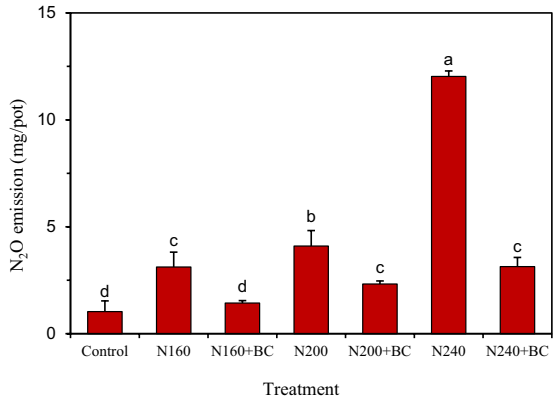

FIGURE 2
Effects of biochar application and N fertilizer reduction on cumulative N_2O emission from rice paddy. Different lowercase letters above the columns indicate significant differences between treatments (p<0.05).

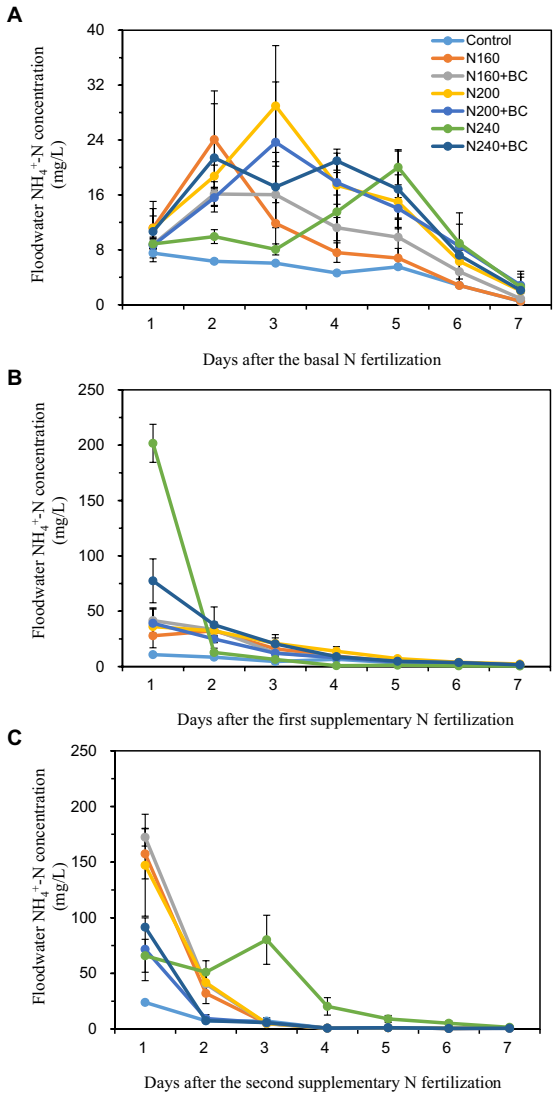

FIGURE 3
Effect of biochar application and N fertilizer reduction on ammonium nitrogen (NH_4^+-N) concentration in overlying water after applications of basal fertilizer (BF, **A**), first supplementary fertilizer (SF1, **B**) and second supplementary fertilizer (SF2, **C**).

ha) N levels significantly ($p < 0.05$) increased the soil NO_3^--N content, and the N200 + BC treatment had 3.3 times more soil NO_3^--N content than the N200 treatment (Figure 5B).

3.5.2. Community abundance of ammonia oxidizing and denitrifying bacteria

Table 4 shows that the number of functional genes of ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB) *amo*A in paddy soil ranged from 2.23–6.22 × 10^5 copies/g and 1.73–5.09 × 10^5 copies/g, respectively. And the number of functional genes of denitrifying bacteria *nir*S, *nir*K and *nos*Z ranged from 1.51–16.34 × 10^7, 2.76–14.03 × 10^6 copies/g and 0.33–3.83 × 10^6 copies/g. For all treatments, the *nirS* gene showed higher dominance role. The community abundance of AOB and denitrifying bacteria showed a pattern of decreasing with increasing N application levels (Table 4). However, in N160 + BC and N240 + BC treatments, the soil AOA abundance significantly ($p < 0.05$) increased by 37 and 146%, relative to their counterparts N160 and N240 treatments, respectively. However, soil AOB abundance under medium (200 kg/ha) N supply conditions was significantly ($p < 0.05$) increased by 1.5 times with the addition of biochar.

N160 + BC had significantly ($p < 0.05$) lower number of *nir*S, *nir*K, and *nos*Z functional genes by 78.0, 25.3, and 73.1%, respectively compared to N160. In contrast, the copies of *nir*S, *nir*K, and *nos*Z in N240 + BC soil were 10.8, 3.7, and 7.4 times significantly ($p < 0.05$) higher than N240 soil. No significant effect of biochar on the copies of all three denitrifying bacterial functional genes was found at medium (200 kg/ha) N supply level.

4. Discussion

4.1. Effect and mechanism of biochar on rice yield

Both exogenous material addition and N fertilizer application played a crucial role in the formation of rice yield (Timsina et al., 2001; Zhang et al., 2020). The results of this study showed that the combinations of N200 + BC and N240 + BC were effective in improving rice yield (Table 1). Similar results have been confirmed by Feng et al. (2021). They demonstrated that application of N fertilizer at 240 kg/ha with biochar increased the rice yield by 9.3% in a pot experiment. Results presented in the current study demonstrated that the addition of biochar at medium (200 kg/ha) and high (240 kg/ha) N levels promoted rice grain yield through increasing effective panicle number and 1,000 seed weight. However, there was no significant difference in panicle number, grain number per panicle and 1,000 seed weight between N160 + BC and N160 treatments, explaining the no yield-increasing effect of biochar on rice at 160 kg/ha N addition. Meanwhile, it has been shown that biochar can increase the growth rate of seedling rice and its mineral element uptake and dry matter accumulation (Ray et al., 2012). Further, combination of biochar and N at 240 kg/ha increased the rice plant height and leaf SAPD values (Figure 1). Therefore, this effect can also be inferred that promoting

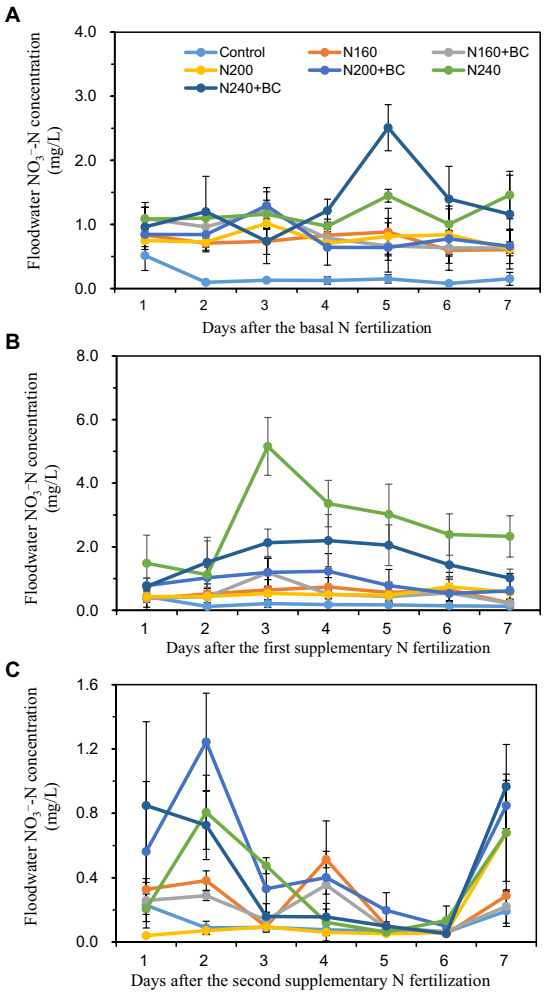

FIGURE 4
Effect of biochar application and N fertilizer reduction on nitrate N (NO_3^--N) concentration of overlying water after applications of basal fertilizer (BF, **A**), first supplementary fertilizer (SF1, **B**) and second supplementary fertilizer (SF2, **C**).

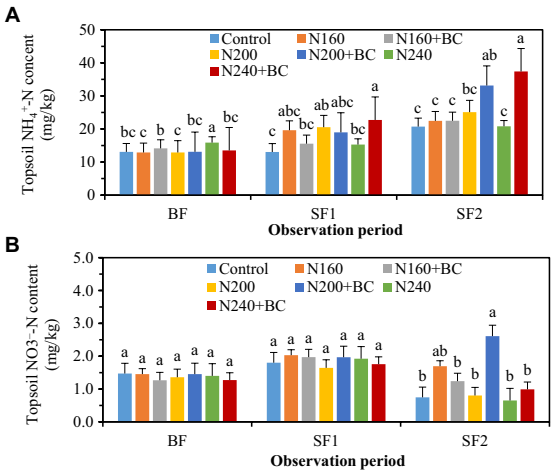

FIGURE 5
Effects of biochar addition and N reduction on the contents of NH_4^+-N **(A)** and NO_3^--N **(B)** in soil at different fertilizer stages. The BF, SF1, and SF2 refer to the basal, first and second supplementary fertilizations, respectively. Different lowercase letters above the columns indicate significant differences between treatments (p<0.05).

dry matter accumulation in rice and increasing leaf SPAD values are also one of the mechanisms of biochar to increase crop yield.

In terms of nutrient uptake, biochar addition has been well reported to improve crop N fertilizer utilization (Van Zwieten et al., 2010; Huang et al., 2018). Similarly, in this experiment we found that the addition of biochar at N200 and N240 treatments increased rice seed N and total plant N uptake capacities (Table 2). Therefore, promoting N uptake and utilization of rice is the second mechanism of biochar to improve rice yield under 200–240 kg/ha N supply conditions. The increase in N uptake under these treatments can be associated with the effect of biochar increasing the activity of soil microorganisms by providing them with an unstable carbon source. The presence of a C source can promote soil N mineralization thus improving soil N availability, and promotes plant N uptake (Saifullah et al., 2018). In addition, the addition of biochar can improve the stability of soil aggregates and crop root architecture (especially increase the number of fine roots) (Backer et al., 2017), as well as promote the uptake of other fast-acting nutrients by rice, thus ensuring the nutrient supply for the whole growth cycle of rice.

4.2. Effect of biochar on soil NH_3 volatilization under different N fertilizer levels

This study found that the effect of biochar on NH_3 volatilization in rice season was related to N input level, with a significant reduction of NH_3 volatilization only at low (160 kg/ha) N level (Table 3). Under different experimental conditions, different views on the impact of biochar addition on NH_3 volatilization in rice field ecosystem including promotion, reduction, and no impact, were reported (Meng et al., 2019; Shaukat et al., 2019). Liu et al. (2020) reported that appropriate N reduction form 180 kg/ha to 150 kg/ha with biochar helped to reduce NH_3 volatilization and these results are consistent with the findings of our present study. In addition to environmental factors such as wind speed and temperature, the main factors affecting NH_3 volatilization in rice fields include soil and overlying water pH and NH_4^+-N concentration, especially the changes in the corresponding indicators within 1 week after N fertilizer application (Soares et al., 2012; Mandal et al., 2018). The reduction effects of biochar on NH_3 volatilization under low (160 kg/ha) N condition in this experiment were mainly found the SF1 (39%) and SF2 (36%). As shown in the Supplementary Figure 2, the addition of biochar at low (160 kg/ha) N input at both the SF1and SF2 reduced the mean pH of overlying water by 0.05 units. In addition, the mean soil NH_4^+-N and pH decreased by 4.07 mg/kg and 0.07 units, respectively, during the SF1 (Figure 5A; Supplementary Figure 3). In this study, the reduction of pH value and NH_4^+-N content by the N160 + BC at the SF1 and SF2 is linked to the reduction of NH_4^+-N potential for conversion to NH_3, thus inhibiting the volatilization of NH_3 (Feng et al., 2022).

Further, the addition of biochar at low (160 kg/hm^2) N levels can increase the community abundance of AOB (Table 4). This is because previous study has found that the surface structural properties of

TABLE 4 Effect of biochar application and nitrogen fertilizer reduction on the abundance of soil ammonia oxidizing and denitrifying bacteria communities.

	AOA	AOB	*nir*K	*ni* S	*nos*Z
Treatment	10^5 copies/g	10^5 copies/g	10^6 copies/g	10^7 copies/g	10^6 copies/g
Control	2.4 ± 0.62 c	2.44 ± 4.49 c	8.73 ± 0.49 b	3.02 ± 0.09 d	0.59 ± 0.01 cd
N160	3.28 ± 0.64 c	3.74 ± 0.16 b	14.03 ± 1.88 a	12.21 ± 2.65 b	3.83 ± 0.85 a
N160 + BC	4.50 ± 9.37 b	3.84 ± 7.62 b	10.48 ± 0.88 b	2.69 ± 0.41 d	1.03 ± 0.05 cd
N200	2.23 ± 7.09 c	2.04 ± 4.99 d	3.16 ± 1.32 c	7.53 ± 1.39 c	1.37 ± 0.37 c
N200 + BC	3.32 ± 7.88 c	5.09 ± 0.42 a	5.02 ± 1.01 c	6.85 ± 2.11 c	1.26 ± 0.11 c
N240	2.53 ± 0.55 c	1.73 ± 5.54 d	2.76 ± 0.14 c	1.51 ± 0.15 d	0.33 ± 0.04 d
N240 + BC	6.22 ± 7.96 a	2.89 ± 3.37 cd	10.16 ± 2.69 b	16.34 ± 1.76 a	2.45 ± 0.80 b

Different lowercase letters in the same column indicate significant differences between treatments ($p < 0.05$).

biochar could provide habitat for AOB and increase the abundance and activity of AOB, which is consistent with the results of this work (Cheng et al., 2012). Increased abundance of AOB communities enhances soil nitrification, which can improve soil utilization of NH_4^+-N and reduce NH_4^+-N concentration in the soil liquid phase, thus reducing NH_3 volatilization from rice fields (He et al., 2019). The soil urease activity directly affects the urea hydrolysis process, and largely determines the soil NH_3 emission rate and amount (Yi et al., 2021). Previous studies have shown that biochar can adsorb urease molecules, and then protect the binding sites of enzymatic reaction, thus to prevent the enzymatic reaction and reduce NH_3 volatilization (Noyce et al., 2015). In this experiment, the addition of biochar at low (160 kg/ha) N level may inhibit soil urease activity and thus decreased NH_3 volatilization from rice fields, but the exact effect needs to be further investigated. In addition, according to the suggestion of Nannipieri et al. (2019), more frequent soil sampling should be conducted, after the N fertilization, to reveal the enzymatic mechanism of biochar effects on the NH_3 volatilization from paddy systems.

4.3. Effect of biochar on soil N_2O emission under different N fertilizer levels

The results of this experiment showed that biochar addition under different N application conditions was effective in reducing N_2O emissions in the rice season (Figure 2), and this finding is consistent with previous report (Fan et al., 2020). The effect of biochar on N_2O emissions is associated with the ability of biochar to alter the conversion of soil N nutrients (NO_3^--N and NH_4^+-N) (Harter et al., 2014; Liu et al., 2019). Nguyen et al. (2017) found that biochar addition reduced the effective source of N for soil nitrifying and denitrifying bacteria, which in turn contributed to the reduction of N_2O emissions. In this study, soil NH_4^+-N and NO_3^--N contents were reduced by 0.91 and 0.23 mg/kg, respectively, after biochar addition with N at 160 kg/ha (Figures 5A,B), which corresponded with nitrification and denitrification inhibited, thus reducing N_2O emissions. In addition, pH changes are also the main controlling factor for differences in soil N_2O emission between the treatments with or without biochar (Wang et al., 2018), especially when the nitrification processes dominate are reduced in soils with lower pH (Yoo et al., 2016). In this study, the average pH values of the soil following biochar addition at 200–240 kg/ha were reduced (Supplementary Figure 3), which also contributed to the reduction of soil N_2O emissions by biochar addition at medium and high N inputs. Our results further demonstrated that under different N application levels, the mechanism of biochar to reduce N_2O emission is varied.

Functional soil microbial communities represented by *nir*S, *nir*K, and *nos*Z genes play key roles in regulating N_2O emissions (Wang et al., 2013; Harter et al., 2016). In this study, biochar application at low (160 kg/ha) N levels decreased soil *nir*S and *nir*K gene copy numbers, while biochar application at high (240 kg/ha) N levels increased soil *nos*Z gene copy numbers (Table 4). According to previous study, the reduction of N_2O emissions as biochar was linked to the decreased *nir*S and *nir*K genes copies but the increased *nos*Z gene copies or a decrease in the ratio of (*nir*K + *nir*S)/*nos*Z in other word (Shi et al., 2019). Duan et al. (2018) found that biochar could increase the abundance of *nos*Z genes in soil, thus effectively reducing N_2O emissions, like the results of this study. The addition of biochar at low (160 kg/ha) N level in this study inhibited soil autotrophic nitrification and denitrification processes by reducing soil *nir*S and *nir*K gene abundance, and at medium (200 kg/ha) N level by reducing soil *nir*S gene abundance. In contrast, the addition of biochar at high (240 kg/ha) N levels is effective in reducing N_2O emissions by increasing the abundance of soil *nos*Z genes, increasing N_2O reductase activity, and promoting the catalytic process of N_2O reduction to N_2.

5. Conclusion

The combined effects of biochar addition and N reduction on rice yield and N uptake, NH_3 and N_2O losses in paddy soil and the underlying mechanisms were evaluated by a soil column experiment. The main conclusions were:

(1) The yield increasing effect of biochar can be realized at medium (200 kg/ha) to high (240 kg/ha) N inputs, attributing to the improving N uptake and increasing panicle number, grain number per panicle and 1,000 seed weight. In particular, the highest yield was achieved by combination of biochar and N fertilizer at medium (200 kg/ha) level.

(2) Biochar addition only effectively reduced the NH_3 volatilization in rice season with low (160 kg/ha) N supply condition. Interestingly, however, at equal N input level, yield-scale NH_3 volatilizations were reduced by biochar addition.

(3) Biochar addition was effective in reducing the N_2O emissions from rice paddy receiving inorganic N fertilizer from low (160 kg/ha) to high (240 kg/ha) inputs. Varied changes in the functional genes, including *nir*S, *nir*K, and *nos*Z, explained the inhibiting effects of biochar on the N_2O emission at different N inputs.

(4) We recommend the addition of biochar at medium (200 kg/hm^2) N levels, which can archive the synergistic benefits of reducing inorganic N fertilizer, promoting crop yield, and decreasing N environmental losses.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

ZY and CY conducted the experiment, obtained the data, and drafted the manuscript. PJ drafted and revised the manuscript. HS designed the experiment, provided the fund support, and drafted and revised the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2023.1174805/full#supplementary-material

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Contrasting effects of organic materials versus their derived biochars on maize growth, soil properties and bacterial community in two type soils

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The objective of this study was to assess the benefit of applying biochar instead of its feedstock in enhancing soil quality. To accomplish this, we investigated the short-term effects of two organic materials and their derived biochars on maize growth, soil properties, and microbial community in fluvo-aquic and red soil with a pot experiment. Five treatments were applied to each soil, namely, the addition of straw, manure, straw-derived biochar, manure-derived biochar, and the control with no addition of any organic materials and biochar. Our results revealed that straw decreased the shoot biomass of maize in both soils, while straw-derived biochar, manure and manure-derived biochar increased it by 51.50, 35.47 and 74.95% in fluvo-aquic soil and by 36.38, 117.57 and 67.05% in red soil compared with the control, respectively. Regarding soil properties, although all treatments increased soil total organic carbon, straw and manure exhibited more pronounced effects on improving permanganate-oxidizable carbon, basal respiration, and enzyme activity compared with their derived biochars. Manure and its biochar had more significant effects on improving soil available phosphorus, whereas straw and its biochar exhibited more ameliorating effects on available potassium. Straw and manure consistently decreased bacterial alpha diversity (Chao1 and Shannon index) and altered bacterial community composition in the two soils by increasing the relative abundances of Proteobacteria, Firmicutes, and Bacteroidota and decreasing those of Actinobacteriota, Chloroflexi, and Acidobacteriota. More specifically, straw had a greater effect on Proteobacteria, whereas manure affected Firmicutes more. While straw-derived biochar had no effect on bacterial diversity and bacterial community composition in both soils, manure-derived biochar increased bacterial diversity in the fluvo-aquic soil and altered bacterial community composition in the red soil by increasing the relative abundances of Proteobacteria and Bacteroidota and decreasing that of Firmicutes. In summary, owing to the input of active organic carbon, straw and manure exhibited more pronounced short-term effects on soil enzyme activity and bacterial community compared with their derived biochar. Furthermore, straw-derived biochar was found to be a better option than straw in promoting maize growth and nutrient resorption, while the choice of manure and its biochar should be determined by the soil type.

KEYWORDS

organic material, biochar, soil properties, soil enzyme activity, soil bacterial community

1. Introduction

China is one of the largest agricultural countries in the world, with less than 9% of the world's cultivated land feeding nearly 20% of the world's population (Liu Z. J. et al., 2021). Such outstanding achievement is largely ascribable to the use of chemical fertilizers in China (Zhu and Jin, 2013). However, the unscientific use of chemical fertilizers and ignorance concerning organic and microbial inputs have resulted in soil fertility degradation, such as acidification, salinization, nutrient imbalance, and microecological disorders (Guo et al., 2010; Gupta et al., 2022). Concomitantly, the organic matter of cropland soils decreases with intensive agricultural management. Hence, a fundamental shift toward agricultural green development is required to ensure sustainable food security and protect the ecological environment (Davies and Shen, 2020).

The canonical practices used to reverse soil fertility, particularly organic matter, are straw returning and organic fertilization. The former has been demonstrated to reduce soil bulk density, increase porosity, enhance the available nutrient content, and promote soil organic carbon storage and stability, thereby improving crop productivity (Zhu et al., 2015). Furthermore, straw returning was found to improve soil microbial richness and diversity (Sun et al., 2015). These benefits have also been observed for organic fertilization (Ren et al., 2018; Rayne and Aula, 2020). Moreover, Sun et al. (2015) noted that organic fertilization exerted a more positive influence on soil microbial diversity than straw returning. In addition, straw returning has a few disadvantages. First, it decreases the soil water content and temperature and, as a result, declines the seedling emergence rate (Zhao J. L. et al., 2019). Second, straw incorporation can lead to the deficiency of the soil available nitrogen because of the high carbon-to-nitrogen ratio of straw, and aggravate the competition for nitrogen between crop and soil microbes and finally negatively affect crop growth and yield (Li et al., 2016). Third, though straw returning improves soil physicochemical properties, such as water-storage capacity and porosity, it also provides a more suitable living environment favoring various pathogens and insect eggs, which aggravates crop diseases and insect pests (Liu T. et al., 2016). In addition, straw returning increases the emission of greenhouse gases such as carbon dioxide (Liu et al., 2014). Compared with straw returning, manure is a type of traditional fertilizer with a lower carbon-to-nitrogen ratio and may exhibit better fertilizer efficiency, while it can contribute to greenhouse gas emissions in different treatment processes, such as manure storage, fermentation, and application to soil (Chadwick et al., 2011). Inadequately treated manure used as fertilizer is more likely to carry harmful substances such as pathogens and parasite eggs (Wan et al., 2020).

Biochar is a type of solid multifunctional material with rich carbon, developed pore structure, huge specific surface area, rich oxygen-containing functional groups, high aromatization, and stable properties. It is produced by high-temperature pyrolysis (usually <700°C) of biomass materials from agricultural, forestry, and animal husbandry wastes under anaerobic conditions such as straw, litter, livestock manure, and other biomass materials (Ahmad et al., 2014; Chen et al., 2019). Compared with direct straw returning, biochar can better reduce soil bulk density, increase soil permeability, improve soil buffering function, enhance soil water and nutrient retention capacity, adjust soil acid–base balance, promote crop root elongation and growth, and prevent issues such as a low seedling emergence rate and yield decline (Tan et al., 2017). Because of the high-temperature treatment of biochar, the parasite eggs and pathogens possibly carried by the biomass materials are completely killed, which avoids the risk of diseases and insect pests at the later stage of crop growth. Notably, previous studies have shown that the mean residence time of biochar in soil is approximately 2,000 years, while the half-life is approximately 1,400 years (Kuzyakov et al., 2009). As a beneficial soil amendment, biochar has recently gained increasing attention in modern agriculture (Tan et al., 2017; Chen et al., 2019; Palansooriya et al., 2019).

As the biogeochemical cycle and material metabolism of elements in the soil are driven by soil microbes, the composition of soil microbes is directly related to soil fertility and crop productivity. Furthermore, soil biological stability is largely affected by the soil microbial community structure, which is crucial to the stability of the terrestrial ecosystem (Griffiths and Philippot, 2013). As a strategy to enhance soil fertility, organic amendments strongly influence soil microbial community directly by their own and indirectly through changing soil physico-chemical properties, while different organic amendments have different mechanisms of action. Straw and manure are rich in dissolved organic carbon, which is the organic carbon source for microorganisms present in the soil. Thus, straw and manure exhibit rapid changes in the microbial community composition. However, the long-term effects are gradually weakened due to the consumption of dissolved organic carbon. Ros et al. (2006) noted that microbial communities did not show major changes after soil was amended with bio waste, green waste, manure, and sewage sludge after more than a decade. Biochar shows substantial short- and long-term effects on soil microbial communities by space for colonization and changing soil conditions (e.g., moisture and pH). It can persist in the soil for a long period as it contains a large amount of stable carbon that is resistant to decay. The porous structure of biochar can adsorb nutrients and water in soil and provide a good habitat for soil microbes (Palansooriya et al., 2019). This could explain why straw biochar exhibited more pronounced effect on the abundance and diversity of bacteria compared with straw in a field experiment carried out for four consecutive years (Li et al., 2020). Therefore, to comprehensively evaluate the various positive effects of biochar on the soil properties and microbes compared with its raw material, it is necessary to undertake a shorter-term study. This is especially important for crops with short growing period, such as summer maize.

Furthermore, it has been proved that biochar can enhance soil health and alter the composition and structure of the soil microbial community (Sun et al., 2016; Sheng and Zhu, 2018). However, different results have also been reported. Liao et al. (2021) found that biochar amendment had limited impacts on rhizosphere bacterial community composition in alkaline calcareous soils. Zhang et al. (2019) also demonstrated that biochar could alter the bacterial communities in acidic soil but not alkaline soil. The differences in soil types tested and feedstocks could be the prime reasons for these discrepancies (Li et al., 2020). It is equally necessary to assess the effects of biochar with different feedstocks on soil fertility and microbial diversity, particularly in different types of soil. In this study, alkaline fluvo-aquic soil and acid red soil were selected as the test soils to explore the short-term effects of two highly disparate and widely sourced biomass materials, namely, wheat straw and swine manure, and their derived biochars on maize growth, soil properties, and microbial diversity. We hypothesized that (1) straw and manure can more rapidly alter soil bacterial community and enzyme activities rather than their derived biochar in

short term and (2) the response of maize growth, soil chemical and biological characteristics to biochar input was positive, but the beneficial effect of biochar should be dependent on its feedstocks.

2. Materials and methods

2.1. Materials

Two type soils were selected for this experiment. The fluvo-aquic soil was collected from Xinxiang, Henan province (35.4°N, 114.4°E), the red soil was collected from Sanming, Fujian province (26.8°N, 116.8°E). Soil samples were collected from the surface (0–20 cm) and hand-picked to remove obvious plant debris, air-dried, ground, and sieved through a 2 mm sieve, and then reserved for the pot experiment. The fluvo-aquic soil properties were as follows: 9.93 g kg^{-1} total organic carbon, 75.25 mg kg^{-1} available N, 16.25 mg kg^{-1} available P, 186.30 mg kg^{-1} available K and a soil pH of 8.21. The red soil properties were as follows: 11.27 g kg^{-1} total organic carbon, 114.33 mg kg^{-1} available N, 15.91 mg kg^{-1} available P, 95.20 mg kg^{-1} available K and a soil pH of 4.55.

Two types of biochar were prepared from wheat straw and swine manure, which named as SBC and MBC, respectively. The preparation of biochar was as follows: first, the two biomass materials were oven dried and pulverized with a 1 mm sieve, and then the powder was compacted in ceramic crucible equipped with a cover and pyrolyzed for 4 h at 550°C in a muffle furnace under oxygen-limited condition, naturally cooled it to room temperature, and bag it for later use. The chemical properties of the biomass material and biochar listed in Supplementary Table S1.

2.2. Experimental design

A pot experiment was conducted in natural condition to study the effects of biomass materials and their derived biochar to maize growth, soil nutrients and microbial diversity. Each pot (10 cm height and 9 cm diameter) was filled with 200 g soil and was fertilized with the following amounts of macronutrients: N 150, P_2O_5 100, K_2O 100 mg kg^{-1} soil supplied with NH_4NO_3, KH_2PO_4, K_2SO_4, respectively. There were five treatments for each soil, including the application of wheat straw (S), swine manure (M), wheat straw-derived biochar (SBC), swine manure-derived biochar (MBC) and the control with no addition of any organic materials and biochar (CK), the addition amount of two types of biochar was 2 g per pot at a rate of 1% (w/w), the addition rate of wheat straw or swine manure was calculated according to biochar addition amount divided by each biochar production rate, with the addition amount of wheat straw (biochar production rate: 27%) being 7.41 g per pot and swine manure being (biochar production rate: 37%) 5.41 g per pot. The treatments in fluvo-aquic soil and red soil were referred to as F_CK, F_S, F_SBC, F_M, F_MBC, R_CK, R_S, R_SBC, R_M, R_MBC, respectively. Each treatment was performed in four repeats (pots).

The experiment was arranged on April, 2021 at Henan Institute of Science and Technology (35.3°N, 113.9°E), Xinxiang, China, in a rain-sheltered wire house under open-air conditions. After all fertilizers were evenly mixed with the soil, three maize seeds were sown per pot and thinned to one per pot after seedling emergence, all treatments were managed consistently. The experiment was finished 45 days after emergence of maize. At the end of the experiment, both soil and plant samples were collected. The plants were carefully taken out from soils, The plant samples were washed and separated into roots and shoots, dry weight of each part was weighed, and then crushed for nutrient element analysis. After removing the plants, the soil remaining in each pot was mixed well and split into three subsamples for subsequent analysis. One was stored at 4°C for soil respiration and enzymatic activity, one was air-dried and stored for soil physicochemical properties analysis, and one subsample was stored at −80°C for molecular ecological assays.

2.3. Analysis of soil physicochemical properties

Air-dried soil samples were triturated with a wooden roller, passed through a sieve of 1 mm for soil pH, available N, available P and available K analysis, and passed through a sieve of 0.15 mm for soil total organic carbon analysis. The experimental parameters were measured according to the soil physicochemical analysis handbook (Bao, 2008). Soil total organic carbon (TOC) was measured using the chromic and titration procedure. The soil pH was determined potentiometrically in 1:2.5 soil/distilled water suspensions after shaking. The soil available N (AN) was determined using alkaline hydrolysis diffusion, the soil available P (AP) was determined using Olsen's method, and the soil available K (AK) was extracted with 1 mol L^{-1} of ammonium acetate and determined using a flame photometer. Soil permanganate oxidizable carbon (POXC) was determined by the method as the description of Lefroy et al. (1993).

2.4. Analysis of soil enzymatic activities and soil basal respiration

The fresh soil samples stored in 4°C were used for analysis of soil enzymatic activities and soil basal respiration (SBR). Soil basal respiration (SBR) referenced our previous study described and slight changed (Zhang et al., 2015). Briefly, the 20 g fresh soil was incubated with 10 mL of 0.1 M NaOH for 24 h at 37°C to absorb the CO2, and then the residual alkali was titrated with standardized HCl. The activities of several soil enzymes, including urease (UA), sucrase (SU), catalase (CA) and β-glucosidase (GLU), were determined according to the textbook edited by Li et al. (2008). The mean soil enzyme (GMea) activity was calculated based on the geometric mean of all tested enzymes (Zhang et al., 2015), the formula is given as:

$$\mathrm{GMea} = \left(\mathrm{urease} \times \mathrm{sucrase} \times \mathrm{catalase} \times \beta - \mathrm{glucosidase}\right)^{1/4}.$$

2.5. Soil DNA extraction, high-throughput sequencing and bioinformatic analysis

Total microbial genomic DNA of each soils was extracted using the E.Z.N.A.® soil DNA Kit (Omega Bio-tek, Norcross, GA, United States) according to manufacturer's instructions. The 1.0% agarose gel electrophoresis and a NanoDrop® ND-2000

spectrophotometer (Thermo Scientific Inc., United States) were used to determine the concentration and quality of DNA. The hypervariable region V3-V4 of the bacterial 16S rRNA gene were amplified with primer pairs 338F (5'-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5'-GGACTACHVGGGTWTCTAAT-3′) by an ABI GeneAmp® 9,700 PCR thermocycler (ABI, CA, United States) (Liu C. et al., 2016). The PCR reaction mixture including 4 μL 5 × Fast Pfu buffer, 2 μL 2.5 mM dNTPs, 0.8 μL each primer (5 μM), 0.4 μL Fast Pfu polymerase, 10 ng of template DNA, and ddH_2O to a final volume of 20 μL. Amplification conditions for PCR were as follows: initial denaturation at 95°C for 3 min, followed by 27 cycles of denaturing at 95°C for 30 s, annealing at 55°C for 30 s and extension at 72°C for 45 s, and single extension at 72°C for 10 min, and end at 10°C. Triplicate amplifications were performed on all samples. The PCR product was extracted from 2% agarose gel and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) according to manufacturer's instructions and quantified using Quantus™ Fluorometer (Promega, USA).

Purified amplicons were pooled in equimolar amounts and paired-end sequenced on an Illumina MiSeq PE300 platform (Illumina, San Diego, United States) according to the standard protocols by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China). The raw sequencing reads were deposited into the NCBI Sequence Read Archive (SRA) database (Accession Number: PRJNA888047).

The biological analysis process was as follows: after the sample separation of the PE reads obtained by MiSeq sequencing, the quality control and filtering of the double-terminal Reads were carried out according to the sequencing quality, and the optimized data after the quality control splicing was obtained by splicing according to the overlap relationship between the two-terminal Reads. Then the sequence denoising method (DADA2) was used to process the optimized data to obtain the representative sequence and abundance information of ASV (Amplicon Sequence Variants). Based on the representative sequence and abundance information of ASV, a series of statistical or visual analysis could be carried out, such as species taxonomy analysis, community diversity analysis, species difference analysis, correlation analysis and phylogenetic analysis. The structural equation model (SEM) was constructed to assess how soil properties and basal respiration directly and indirectly affected soil bacterial diversity and enzyme activities in two soils. The model assumes was run by the AMOS 18.0 software (IBM, Chicago, IL, United States). Adequate model fits were indicated by the χ^2 test ($p > 0.05$), goodness-of-fit index (GFI), and a low root-mean-square error of approximation (RMSEA) (< 0.001) (Hooper et al., 2008).

2.6. Statistical analysis

The significant differences between treatments were determined using one-way ANOVA (Duncan's multiple comparisons at 95% confidence level). Mean values ± standard deviations were reported in this study. Bioinformatic analysis of the soil microbiota was carried out using the Majorbio Cloud platform.[1] Based on the ASVs information, alpha diversity indices including Chao1 richness and Shannon index were calculated with Mothur v1.30.1 (Schloss et al., 2009). The similarity among the microbial communities in different samples was determined by principal coordinate analysis (PCoA) based on Bray–curtis dissimilarity using Vegan v2.5–3 package. The PERMANOVA test was used to assess the percentage of variation explained by the treatment along with its statistical significance using Vegan v2.5–3 package. The distance-based redundancy analysis (db-RDA) was performed using Vegan v2.5–3 package to investigate effect of soil physicochemical properties on soil bacterial community structure. Forward selection was based on Monte Carlo permutation tests (permutations = 9,999). Values of the *x*- and *y*-axis and the length of the corresponding arrows represented the importance of each soil physicochemical properties in explaining the distribution of taxon across communities.

3. Results

3.1. Plant biomass and accumulation of nitrogen, phosphorus, and potassium

Plant biomass under different treatments were listed in Figure 1. In fluvo-aquic soil, the root biomass of maize in F_SBC and F_MBC signifcantly increased by 44.90 and 47.65% respectively, compared with F_CK. For the red soil, maize root biomass in R_SBC and R_M signifcantly increased by 22.42 and 11.98%, respectively, compared with R_CK. The shoot biomass of maize in SBC, M and MBC were higher by 51.50, 35.47 and 74.95% in fluvo-aquic soil and by 36.38, 117.57 and 67.05% in red soil compared with CK. Conversely, treatment with S significantly reduced the shoot biomass of maize in two soils. In addition, the root and shoot biomass treated by SBC were higher than S in two soils. For manure and its biochar, MBC showed superiority over M in fluvo-aquic soil, while the opposite situation was observed in red soil.

In terms of nutrient accumulation in maize plants, compared to CK, the shoot nitrogen accumulation was increased by F_SBC, F_M and F_MBC in fluvo-aquic soil, and increased by R_M and R_MBC in red soil (Figure 2A). The root and shoot phosphorous accumulation were increased by M and MBC in two soils compared with CK, while the effect of S and SBC treatment were not significant (Figure 2B). Moreover, the root potassium accumulation was increased by S and SBC, and the shoot potassium accumulation was increased by SBC, M, and MBC in two soils, compared with CK.

3.2. Soil physicochemical properties and enzyme activities

Soil physicochemical properties under different treatments were listed in Table 1. Soil pH was decreased by F_S and F_M compare to CK in fluvo-aquic soil, while it was increased by R_SBC, R_M and R_MBC in red soil. The TOC of the two soils were increased by all the treatments, while the POXC were increased by F_S, F_M, and F_MBC in the fluvo-aquic soil and increased by R_S and R_M in the red soil, compared with CK. Furthermore, F_S and F_M could increase the AN in the fluvo-aquic soils, while R_S, R_SBC, and R_MBC decreased in the red soil. M and MBC could increase the AP of the two soils, while S and SBC had stronger promotion on soil AK.

1 https://cloud.majorbio.com

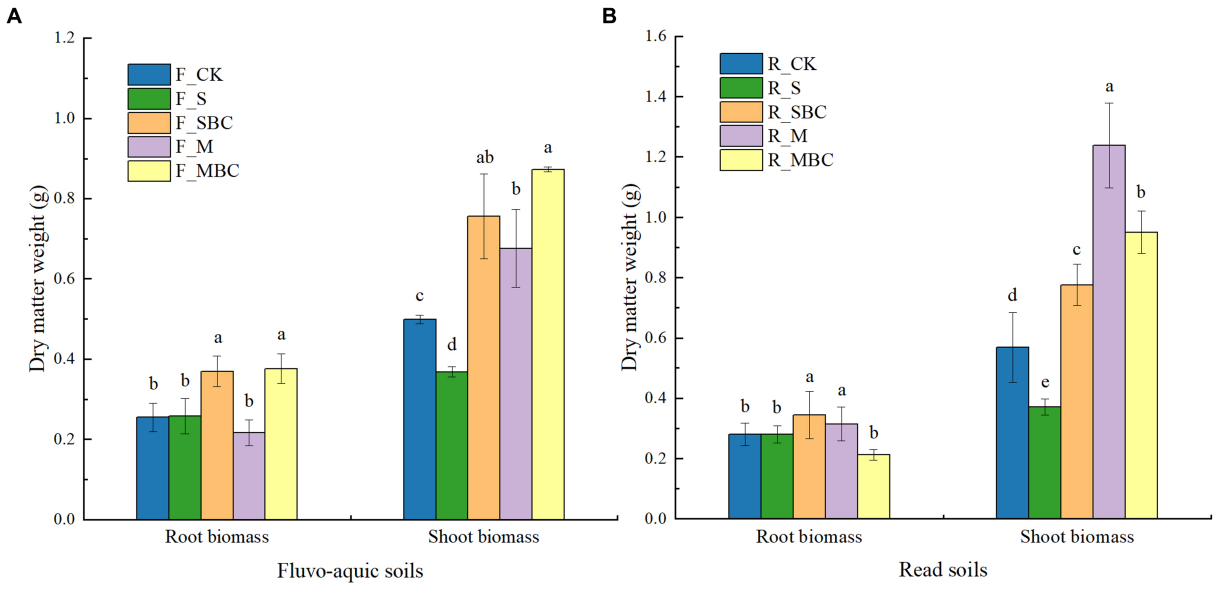

FIGURE 1
Short-term effects of organic materials and their derived biochars on maize root and shoot biomass in fluvo-aquic soil **(A)** and red soil **(B)**. Data in the figure are represented by mean±SD (n=4). Error bars indicate standard deviation. The different lowercase letters on error bars represent significant differences (p<0.05).

Soil basal respiration and enzyme activities under different treatments were listed in Table 2. In the two soils, the SBR of S, M and MBC was higher than that of the CK, and the greatest increase was observed at S treatment. Compared with CK, F_S and F_M in the fluvo-aquic soil and R_S, R_M, and R_MBC in the red soil could increase the UA activity, and F_S in the fluvo-aquic soil and all the treatments in the red soil significantly increased the SU activity. F_MBC in the fluvo-aquic soil and R_SBC in the red soil had no significant effect, the other treatments could significantly increase the soil CA activity versus CK. In addition, compared with CK, S and M could significantly increase while SBC significantly decreased the GLU activity in the two soils, and the effect of MBC on the GLU activity in the two soils was opposite, decreasing in the fluvo-aquic soil and increasing in the red soil (Table 2). Finally, the geometric mean of enzyme activities (GMea) of the assayed enzyme activities increased 26.4 and 21.0% by S and M, and decreased 4.8% by SBC in the fluvo-aquic soil, while increased 215.9, 30.0, 283.1, and 66.2% by S, SBC, M and MBC in the red soil (Table 2).

3.3. Alpha and beta diversities of bacteria

For the bacterial α diversity, both S and M significantly reduced the bacterial Chao1 index and Shannon index of the two soils, while SBC had no significant effect on the bacterial Chao1 index and Shannon index in the two soils. MBC had no significant effect in the red soil but significantly increased the bacterial Chao1 index and Shannon index in the fluvo-aquic soil (Figures 3A–D).

The PCoA analysis displayed that the application of different organic materials affected the different distributions of bacterial communities in the two soils. The first two principal components, PC1, and PC2 accounted for 41.9 and 16.76% in fluvo-aquic soil and 44.41 and 24.35% in the red soil, respectively. The ANOSIM analysis depicted that in the fluvo-aquic soil, F_SBC and F_MBC were closely clustered with F_CK, while F_S and F_M were separated from F_CK. In the red soil, R_SBC and R_CK were also closely clustered, R_MBC, R_S and R_M were separated from R_CK (Figures 3E,F).

3.4. Bacterial community members

Across all the tested soil samples, there were 12 bacterial phyla with average relative abundance exceeding 1%, and these taxa altogether accounted for more than 95% of the total bacterial recovered sequences. Among them, Proteobacteria, Actinobacteriota, and Firmicutes were the first three dominant communities, with an average relative abundance of 21.2–51.5%, 9.8–25.8%, and 5.2–35%, respectively. First, F_S significantly increased the abundance of Proteobacteria in the fluvo-aquic soil, while R_S, R_M, and R_MBC significantly increased in red soil. Second, F_S and F_M significantly decreased the abundance of Actinobacteriota in the fluvo-aquic soil, while R_M was significantly reduced in the red soil. Furthermore, S significantly increased the abundance of Firmicutes in the fluvo-aquic soil and reduced in the red soil; M significantly increased in the two soils, and R_MBC significantly decreased in the red soil (Figure 4A).

At the genus level, the bacteria genera with the top 50 abundances were clustered and presented in a heat map for ease of visualization (Figure 4B). The top 20 genera had average relative abundances in all treatment more than 1.0%, accounting for 39.33% in total. Among them, Bacillus (belonging to Firmicutes), Sphingomonas (belonging to Proteobacteria), Massilia (belonging to Proteobacteria), Frateuria (belonging to Proteobacteria) and Clostridium_sensu_stricto_1 (belonging to Actinobacteriota) were the five most dominant genera. Across the board, the relative abundance of Massilia and Frateuria were higher in red soil compared to fluvo-aquic soil, and were significantly increased by M and S treatment, respectively. The relative

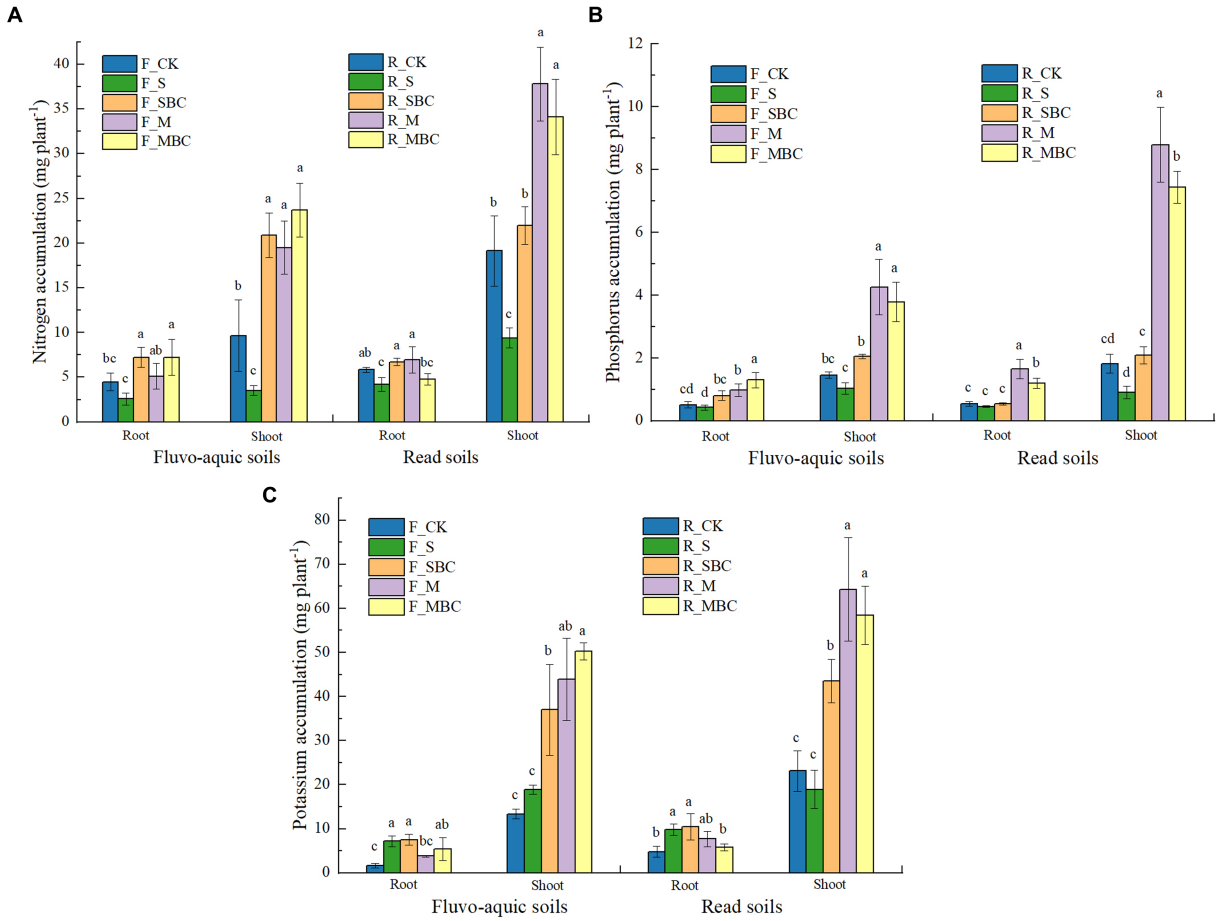

FIGURE 2
Short-term effects of organic materials and their derived biochars on maize root and shoot nitrogen **(A)**, phosphorus **(B)**, and potassium **(C)** accumulation. Data in the figure are represented by mean±SD (n=4). Error bars indicate standard deviation. The different lowercase letters on error bars represent significant differences (p<0.05).

TABLE 1 Soil physiochemical properties in different treatments.

Treatment	pH	TOC	POXC	AN	AP	AK
		gkg^{-1}	gkg^{-1}	mgkg^{-1}	mgkg^{-1}	mgkg^{-1}
F_CK	8.34 ± 0.10a	9.54 ± 0.57c	2.74 ± 0.09b	98.00 ± 2.86c	27.74 ± 3.24c	255.25 ± 5.38d
F_S	7.80 ± 0.05c	14.87 ± 1.13a	4.60 ± 0.42a	132.13 ± 5.98b	19.98 ± 1.78d	560.50 ± 8.19a
F_SBC	8.23 ± 0.14ab	16.38 ± 1.89a	2.65 ± 0.33b	98.00 ± 4.95c	28.02 ± 3.18c	536.00 ± 33.51a
F_M	8.18 ± 0.07b	12.50 ± 1.43b	4.56 ± 0.70a	141.75 ± 2.02a	110.50 ± 8.16a	455.25 ± 20.71b
F_MBC	8.34 ± 0.08a	11.84 ± 1.10b	3.80 ± 0.94a	97.13 ± 8.27c	59.11 ± 6.03b	375.50 ± 12.48c
R_CK	4.53 ± 0.05d	12.64 ± 0.62c	2.76 ± 0.07c	161.88 ± 8.75a	23.55 ± 2.41c	135.75 ± 13.07d
R_S	4.51 ± 0.19d	19.31 ± 1.81a	4.79 ± 0.76a	135.63 ± 8.75b	23.36 ± 1.42c	587.00 ± 10.49a
R_SBC	4.96 ± 0.08b	16.97 ± 0.40b	2.84 ± 0.12c	130.38 ± 7.76bc	21.61 ± 1.01c	505.25 ± 8.54b
R_M	4.75 ± 0.09c	16.65 ± 1.18b	3.52 ± 0.43b	167.13 ± 8.75a	61.28 ± 2.32a	279.00 ± 39.76c
R_MBC	5.72 ± 0.05a	16.26 ± 0.69b	2.81 ± 0.45c	117.25 ± 12.94c	49.95 ± 1.73b	280.50 ± 23.30c

TOC, total organic carbon; POXC, permanganate oxidizable carbon; AN, available nitrogen; AP, available phosphorus; AK, available potassium. Data in the table are represented by mean ± SD (n = 4). For each soil, within a column, the different lowercase letters represent significant differences (p < 0.05).

abundance of Clostridium_sensu_stricto_1 was increased by M treatment in two soils. The clustering indicates that, in the fluvo-aquic soil, F_SBC and F_MBC were clustered together with F_CK, which indicated that their categories were similar at genus level, but F_S and F_M were considerable different from them. Similar results were observed in the red soil, two types of biochar treatments and control

TABLE 2 Soil basal respiration and enzyme activities in different treatments.

Treatment	SBR	UA	SU	CA	GLU	GMea
	$mgkg^{-1}$ $24h^{-1}$	mgg^{-1} $24h^{-1}$	mgg^{-1} $24h^{-1}$	mlg^{-1}	ug g^{-1} h^{-1}	
F_CK	34.98 ± 5.38c	2.78 ± 0.16b	26.80 ± 0.90bc	4.46 ± 0.24c	93.45 ± 3.62d	13.26 ± 0.43c
F_S	84.05 ± 20.82a	3.86 ± 0.18a	28.50 ± 0.08a	5.31 ± 0.10a	135.73 ± 2.83a	16.77 ± 0.21a
F_SBC	30.50 ± 1.82c	2.71 ± 0.03b	25.35 ± 1.46c	4.91 ± 0.17b	75.61 ± 2.05e	12.63 ± 0.17d
F_M	59.30 ± 8.54b	3.87 ± 0.15a	28.26 ± 0.37ab	5.21 ± 0.10a	116.55 ± 2.95b	16.05 ± 0.14b
F_MBC	45.70 ± 11.70bc	2.73 ± 0.08b	26.40 ± 1.55c	4.53 ± 0.22c	104.31 ± 6.02c	13.57 ± 0.40c
R_CK	24.45 ± 3.38c	0.34 ± 0.03d	5.75 ± 0.38c	1.01 ± 0.17d	27.47 ± 2.43c	2.71 ± 0.15e
R_S	62.63 ± 5.90a	1.17 ± 0.19b	15.75 ± 3.29a	2.21 ± 0.05c	134.44 ± 4.03a	8.55 ± 0.70b
R_SBC	20.50 ± 4.40c	0.44 ± 0.06d	14.62 ± 0.61ab	1.06 ± 0.13d	22.78 ± 1.85d	3.52 ± 0.25d
R_M	35.25 ± 6.16b	2.44 ± 0.05a	12.98 ± 1.00b	4.33 ± 0.24a	84.73 ± 2.73b	10.37 ± 0.08a
R_MBC	33.40 ± 3.60b	0.60 ± 0.05c	12.16 ± 0.86b	2.61 ± 0.45b	21.76 ± 0.83d	4.50 ± 0.32c

SBR, soil basal respiration; UA, urease; SU, sucrose; CA, catalase; GLU, β-glucosidase; GMea, geometric mean of enzyme activities. Data in the table are represented by mean ± SD ($n = 4$). For each soil, within a column, the different lowercase letters represent significant differences ($p < 0.05$).

were generally clustered together, which was different from the two types of biomass material treatments (Figure 4B).

3.5. Relationship between soil microbial community structure, soil enzyme activities and soil properties

The RDA analysis revealed the relationship between the bacterial community and the soil physicochemical properties, which sufficiently explained the influence of the environmental factors on the change in the bacterial community structure at the genus level. The first axis and the second axis explained 38.19 and 23.75% of the total variance, respectively (Figure 5A). According to the Mantel test (Supplementary Table S2), pH, TOC, AN, AP, UA, SU, CA, and GLU significantly influenced the bacterial community structure in the two soils.

The correlation analysis demonstrated that *Proteobacteria*, the first dominant phylum, was positively correlated with almost all the environmental factors, among which SBR and GLU had the highest correlation, all of which reached an extremely significant level ($p < 0.001$); POXC and AK ($p < 0.01$); and TOC, UA, and CA ($p < 0.05$). *Actinobacteriota* had a high correlation with environmental factors pH, POXC, AN, and SBR, among which there was a significant positive correlation with pH ($p < 0.05$), an extremely significant negative correlation with POXC and AN ($p < 0.001$), and an extremely significant negative correlation with SBR ($p < 0.01$). In contrast to *Actinobacteriota*, *Firmicutes* was only negatively correlated with the pH ($p < 0.05$) and positively correlated with AN ($p < 0.001$), and both of the two bacterial phyla had the highest correlation with AN (Figure 5B).

Structural equation models (SEMs) provided good fits to the data, and explained 94 and 86% of the variance in GMea in fluvo-aquic soil and red soil, respectively (Figure 6). AN, AP and POXC had indirect effects on GMea by changing SBR in fluvo-aquic soil, among which, AN and POXC strongly and positively contributed to SBR, whereas AP had the opposite effect. However, in red soil, pH, AN, AP and POXC exhibited direct effects on GMea, AP and POXC positively contributed to GMea, while pH and AN showed negative contribution. Moreover, POXC strongly and positively contributed to SBR, and AP positively contributed to soil bacterial diversity in red soil.

4. Discussion

As is well known, organic amendments can improve soil fertility, promote soil organic carbon storage and stability, and enhance crop growth (Zhu et al., 2015). In this study, the shoot biomass of maize was increased by straw-derived biochar, manure, and manure-derived biochar (Figure 1). In parallel, the potassium accumulation in shoots were increased by straw-derived biochar, while shoot nitrogen, phosphorus, and potassium accumulation were all increased by manure and its biochar in both soils. Notably, our results showed that straw-derived biochar had more significant improving effect on maize shoot biomass and nutrient resorption compared with straw in the two soils due to the significant promotion on root growth (Figures 1, 2), because of root is important organs for plant fitness and are responsible for the absorption of water and nutrients. The promoting effect of straw biochar on root growth were consistent with the findings of previous studies (Tan et al., 2017; Liu X. Y. et al., 2021). Both manure and its biochar could increase the shoot biomass in two soils and exhibited superiority over straw and its biochar owing to the higher contents of mineral nitrogen and phosphorus and the enhanced soil nitrogen and phosphorus supply and plant nitrogen and phosphorus accumulation (Table 1; Figures 1, 2). Therefore, it is apparent that the effects of the organic amendments to the soil are derived directly from its nutrient content in the short term, especially the plant bioavailable nutrients. A systematic review also found that positive yield increases were generally associated with the nutrient contents of the biochar particles, and manure biochars with high nitrogen and phosphorus contents displayed excellent yield-increasing effect (Spokas et al., 2012). Predictably, for different feedstock type biochars, manure-derived biochar with higher nitrogen and phosphorus concentrations showed more pronounced promoting effect on maize growth during the early growing stage (Figure 1). An unexpected observation was that the shoot biomass of maize was decreased by straw compared with the control, although straw had

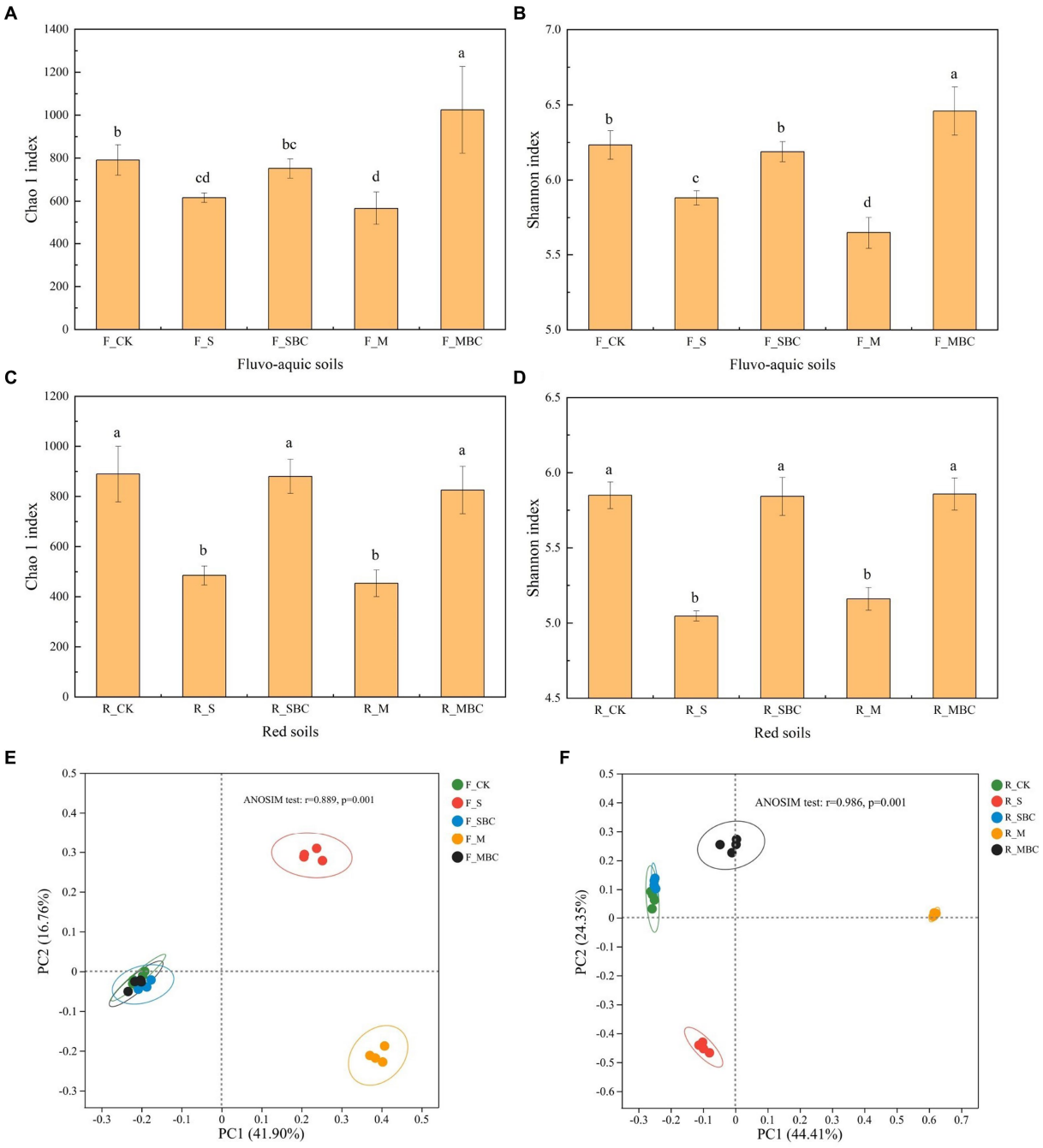

FIGURE 3
ASV-based diversity indices of bacterial community in fluvo-aquic soil **(A,B)** and red soil **(C,D)** and ASV-based PCoA analysis of bacterial community structure in fluvo-aquic soil **(E)** and red soil **(F)**. Data in the figure are represented by mean±SD (n=4). Error bars indicate standard deviation. The different lowercase letters on error bars represent significant differences (p<0.05).

a positive effect on improving the total organic carbon, permanganate-oxidizable carbon, available potassium, basal respiration and enzyme activities in two soils. We speculate that the large quantities straw input decreased the contact area between roots and soil in the early stage, in turn, reduced the water and nutrient uptake efficiency of the plants. Morris et al. (2009) also found that placing seed with or near to straw residue could cause a restriction in crop establishment. Apparently, further studies are warranted to explore such phenomenon.

The pH of soils has generally been found to increase following biochar application, particularly in acidic soils (Zhang et al., 2015; Wu et al., 2020). Our experiments revealed that the addition of the two biochars did not significantly alter the pH of the fluvo-aquic soil, which was related to the higher pH of the fluvo-aquic soil itself. Furthermore, manure-derived biochar increased soil pH more than straw-derived biochar in the red soil because biochar was alkaline after anaerobic pyrolysis, and manure contained more alkaline components than straw (Siedt et al.,

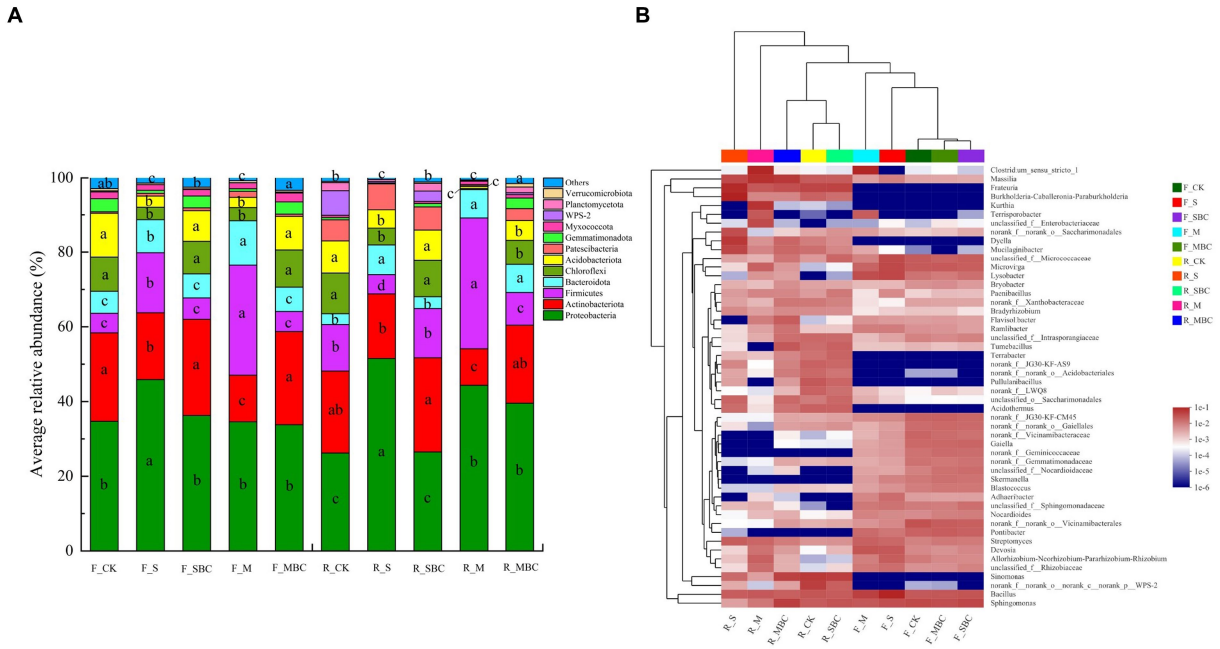

FIGURE 4
Relative abundance (>1%) of the major phyla of bacterial communities **(A)** and heatmap of differential relative abundances of microbial genera (top 50) **(B)** for different treatments. The different lowercase letters represent significant differences ($p<0.05$).

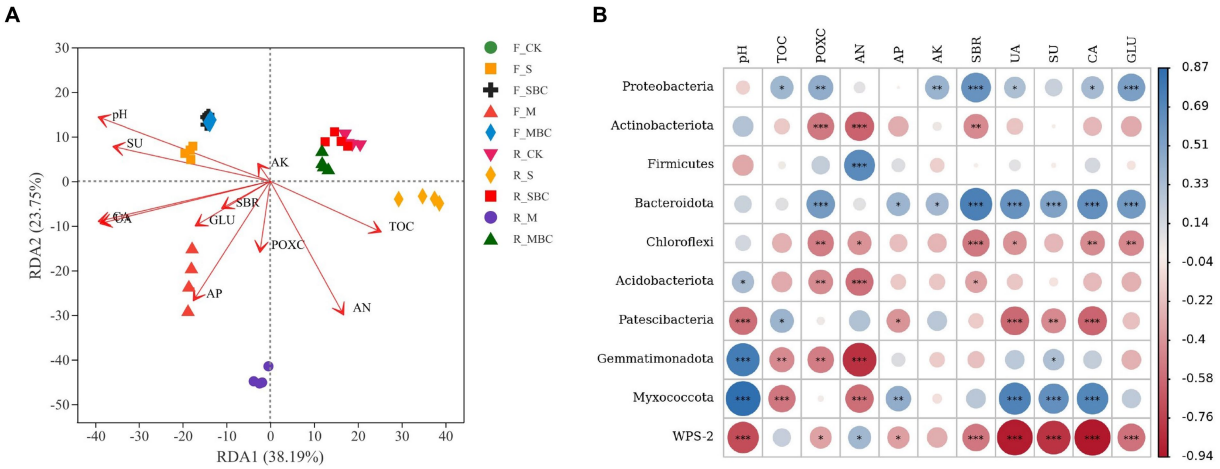

FIGURE 5
ASV-based db-RDA analysis of the relationship between bacterial communities at the genus level and soil environmental factors **(A)** and correlations between soil properties and dominant bacterial phyla **(B)**. TOC, total organic carbon; POXC, permanganate oxidizable carbon; AN, available nitrogen; AP, available phosphorus; AK, available potassium; SBR, soil basal respiration; UA, urease; SU, sucrose; CA, catalase; GLU, β-glucosidase. *$p<0.05$; **$p<0.01$; ***$p<0.001$.

2021). Multiple studies had shown that the effect of biochar on the nutrient availability in soils were depended on soil and biochar types (Brassard et al., 2016; Jin et al., 2016). In this study, we found that two types of biochar reduced the content of available nitrogen in red soil significantly, but had no significant effect in fluvo-aquic soil (Table 1). These findings are consistent with other previously published trials (Zhang et al., 2015; Wu et al., 2020). Nitrification is essential step in the soil nitrogen cycle, which has an optimal point at pH 8.0 and does not occur at a pH lower than 5.5 (Sahrawat, 2008), therefore the NH_4^+-N is generally the dominant nitrogen form in acid red soil. Based on the above analysis, the available nitrogen content in red soil decreased with biochar amendment, possibly because biochar with abundant oxygen-containing functional groups and high porous showed better adsorption capacities on NH_4^+-N compared to NO_3^--N. Additionally, direct nutrient input may be the main reason for biochar to affect soil phosphorus and potassium supply, we found manure and its biochar with higher phosphorus

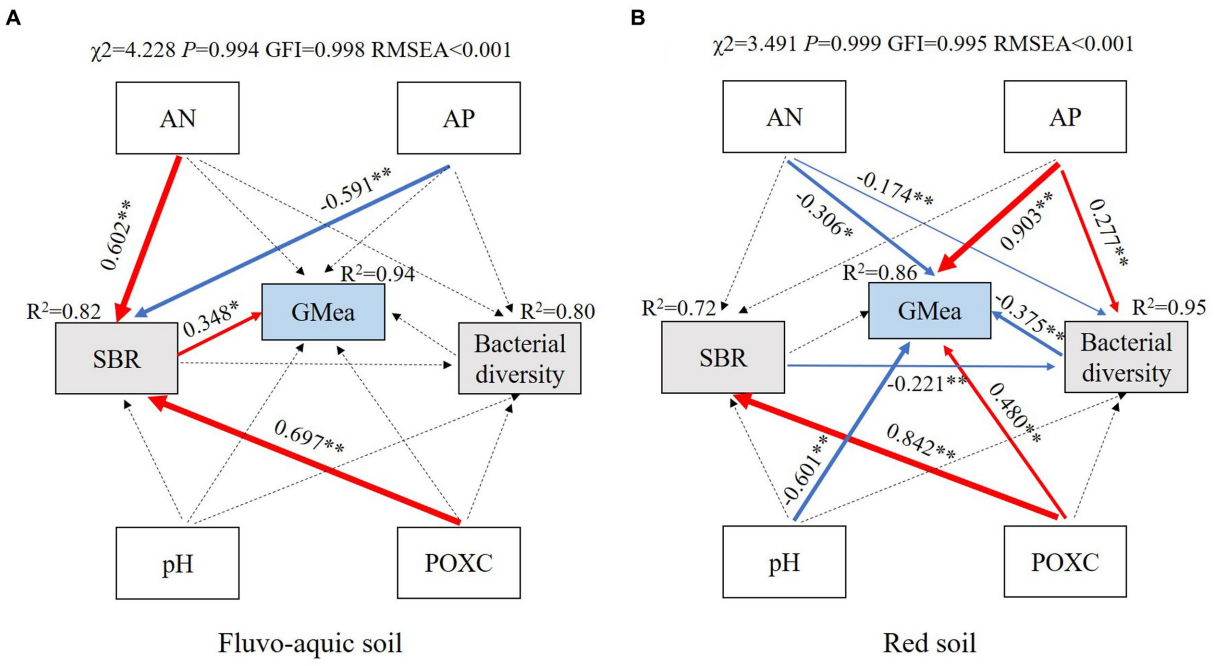

FIGURE 6
Structural equation models showing the direct and indirect effects of soil physiochemical properties, soil basal respiration, bacterial diversity and soil geometric mean of enzyme activities in fluvo-aquic soil **(A)** and red soil **(B)**. Solid and dashed arrows indicate significant and nonsignificant relationships, and red and blue arrows indicate positive and negative correlations, respectively. Numbers adjacent to the arrows are path coefficients, and the width of the arrows is proportional to the strength of the path coefficients. Significance levels are indicated: *p<0.05; **p<0.01. POXC, permanganate oxidizable carbon; AN, available nitrogen; AP, available phosphorus; SBR, soil basal respiration; Bacterial diversity, bacterial Shannon index; GMea, geometric mean of enzyme activities.

concentrations had a more ameliorating effect on soil available phosphorus, whereas straw and its biochar with higher potassium concentrations exhibited a better effect on increasing available potassium (Supplementary Table S1; Table 1). Furthermore, four organic materials significantly increased the total organic carbon of the two soils, while straw and manure significantly increased the permanganate-oxidizable carbon content and soil basal respiration in two soils rather than their biochars. It has been demonstrated that a positive relationship exists between soil basal respiration and soil permanganate-oxidizable carbon content (Zhang et al., 2021). This can be attributed to the fact that the easily oxidized organic carbon in straw and manure is utilized by the soil microorganism as the energy source and emitted carbon dioxide in the short term, while the input by biochar is predominantly stable carbon that is resistant to decay (Zhao et al., 2020). In short, straw and manure showed greater effect on improving POXC content and basal respiration compared with their derived biochar in both fluvo-aquic and red soil.

Soil enzymes, as bioactive indicators for evaluating soil quality, are often affected by soil physiochemical properties and microorganisms (Ghosh et al., 2020). In the present study, we found straw and manure showed more significant promoting effect on soil enzyme activities compared with their biochar in both fluvo-aquic soil and red soil (Table 2). This may be attributed to the input of easily oxidized organic carbon in straw and manure as demonstrated previously, which could quickly modulate the microbial community composition and change soil enzymes during a short-term experiment (Wei et al., 2019). To test this hypothesis, structural equation models were performed in two soils. The results showed that soil basal respiration, which showed strong positive connection with soil available nitrogen and easily oxidized organic carbon, was great predictor for soil enzyme activities in fluvo-aquic soil, while soil available phosphorus and easily oxidized organic carbon were positive regulator of soil enzyme activities in red soil (Figure 6). This may explain why the soil treated by manure derived biochar showed higher soil geometric mean of enzyme activities compared with straw derived biochar in both fluvo-aquic soil and red soil.

The results of alpha diversity indicated that straw and manure significantly reduced the Chao1 and Shannon index in both fluvo-aquic and red soil (Figure 3), which was different from previous studies (Zhao S. C. et al., 2019; Li et al., 2021). The results could be inconsistent because ours was a short-term experiment, soil microbes were sensitive to external additives, and straw and manure imported large amounts of labile organic carbons into the soil which created a relatively easy-to-use nutrient environment for the soil microbes. Thus, some bacteria with a high utilization efficiency of labile organic carbons grew faster, in turn, inhibiting the growth of the other bacteria in a short period. Our experiment on the main bacterial phylum also proved that straw and manure significantly increased the relative abundances of Proteobacteria, Firmicutes, and Bacteroidota in the two soils, and they occupied three of the top four dominant phyla. Therefore, the niche of the other phyla was severely compressed (Figure 4A). The same study also found straw and manure reduced the complexity of the soil bacterial co-occurrence

networks, which is a key component of bacterial biodiversity (Liu et al., 2020; Ge et al., 2021; Chen et al., 2022). In addition, manure-derived biochar significantly increased the Chao1 index and the Shannon index of bacteria rather than straw-derived biochar in the fluvo-aquic soil (Figures 3A,B). This can be attributed to the more reasonable carbon-to-nitrogen ratio and richer nutrients in manure-derived biochar (Supplementary Table S1), which could significantly increase the POXC content of the fluvo-aquic soil (Wu et al., 2021; Table 1). Additionally, soil available phosphorus was another key factor that affected the diversity of soil bacteria (Chen et al., 2017), and manure-derived biochar enhanced available phosphorus of the fluvo-aquic soil more considerably (Table 1). In summary, as expected, straw and manure showed more pronounced short-term effects on soil bacterial diversity and community structure compared with their derived biochars.

As is well known, soil environment variations are the principal driving forces of changes in microbial diversity and community compositions in soil. Members of different communities prefer different ecological niches. Organic materials and their derived biochars affect soil microbial community primarily by altering the soil physicochemical properties in the short term. The RDA results revealed that soil properties contributed to over 60% of the alterations in the composition of the bacterial community (Figure 5A), suggesting that these soil environmental factors played a dominant role in the construction of the microbial community structure. Furthermore, soil bacterial community composition exhibited different responses to straw and manure addition, with more marked effect of straw on the relative abundance of Proteobacteria and of manure on Firmicutes at the phylum level (Figure 4A). Zhang et al. (2021) also found the significantly increasing effect of straw retention on the relative abundance of Proteobacteria, which could be because many members of phylum Proteobacteria are important saprophytes capable of decomposing plant debris and more efficiently utilized cellulose as a carbon source, such as the genus Bradyrhizobium (Ge et al., 2021). We also found the abundance of Proteobacteria showed significant positive correlation with soil basal respiration and β-glucosidase, which were increased by straw addition significantly in two soils (Table 2; Figure 5B). Moreover, soil N availability favored the growth of Firmicutes, which is a dominant diazotrophic phyla (Wang et al., 2022). Therefore, manure increased the abundance of Firmicutes due to its best improving effect on soil available N. The positive correlation between Firmicutes abundance and soil available N could also demonstrate the above speculations (Table 2; Figure 5B). For two biochars, there was no significant difference between straw-derived biochar and control, while manure-derived biochar could altered bacterial community composition in the red soil, by increasing the relative abundance of Proteobacteria and Bacteroidota, and decreasing that of Firmicutes, the increased soil pH, basal respiration, enzyme activities and decreased soil available N are likely to be one of the main reasons, the results of correlation analysis can support these suggestions (Figure 5B). Some studies have displayed that Proteobacteria are significantly regulated by the soil nutrient indicators, and Firmicutes are generally positively correlated with the soil AN and negatively correlated with the soil pH (Lu et al., 2020; Muneer et al., 2022).

5. Conclusion

This study explored the short-term effects of two organic materials and their derived biochars on maize growth, soil properties, and microbial community structure in fluvo-aquic and red soil. Our research demonstrated that straw-derived biochar is more effective than straw in improving maize shoot biomass and nutrient resorption, because of its significant promotion on root growth. For manure and its biochar, although both exerted a positive effect on maize shoot biomass, manure-derived biochar amendments showed superiority over manure in the fluvo-aquic soil, the opposite situation was observed in the red soil. In addition, due to the input of the labile organic carbons, straw and manure showed more pronounced short-term effects on soil basal respiration, enzyme activity and bacterial community structure versus their derived biochar. In summary, in our opinion, straw-derived biochar had more obvious advantage than straw in promoting maize growth and nutrient resorption at seedling stage, while the choice of manure or its biochar should be determined by the soil type.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary material.

Author contributions

YZ and XY conceived the research, performed the experiments, and analyzed the data. YZ, XY, XL, FW, and CS wrote and edited the manuscript. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Effects of biochar amendment and organic fertilizer on microbial communities in the rhizosphere soil of wheat in Yellow River Delta saline-alkaline soil

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The biochar and organic fertilizer amendment have been used as an effective practice to increase soil fertility. Nevertheless, the mechanisms of microbial community response to organic fertilizer and biochar application on saline-alkali soil have not been clarified. This study investigated the effects at different concentrations of organic fertilizer and biochar on the microbial community of wheat rhizosphere soil under field experiment in the Yellow River Delta (China, YRD), using high-throughput sequencing technology. Biochar and organic fertilizer significantly influenced in most soil parameters ($p < 0.05$), apart from soil moisture content (M), pH, total nitrogen (TN) and soil total phosphorus (TP). Proteobacteria and Actinobacteriota were found in the rhizosphere soil as the main bacterial phyla, and the main fungal phyla were Ascomycota and Mortierellomycota. The soil bacterial and fungal communities under organic fertilizer were distinct from CK. Furthermore, redundancy analysis (RDA) directed that changes in bacterial communities were related to soil properties like pH, available phosphorus (AP), and total organic carbon (TOC), while pH, AP and TP, were crucial contributors in regulating fungal distribution. The correlation between soil parameters and bacteria or fungi varied with the application of biochar and organic fertilizers, and the interaction between the bacteria and fungi in organic fertilizer treatments formed more connections compared with biochar treatments. Our results indicated that biochar was superior to organic fertilizer under the contents set up in this study, and soil parameters increased with biochar and organic fertilizer application rate. The diversity and structure of soil bacteria and fungi differed with the application of biochar and organic fertilizer. The research provides a reference to rational application of organic fertilizer and biochar improvement in saline-alkali soil.

KEYWORDS

biochar, organic fertilizer, microbial communities, wheat, saline-alkali soil

1. Introduction

The Yellow River Delta (YRD), located in the warm-temperate zone, is gradually being degraded as one of the important deltaic agricultural economic zones in China (Fang et al., 2005; Luo et al., 2016; You et al., 2021). The saline-alkali land of the YRD is approximately 2,400 km^2, which is about half of the area. Restricted by salinity stress and nutrient deficiencies, the vegetation structure in this area is simple and biodiversity is low (Luo et al., 2016; Li et al., 2019). Soil microorganisms play an important controller for plant growth and stress tolerance and are often used as an indicator of soil quality within evaluations (Salas-González et al., 2020; Wang et al., 2021). Consequently, appropriate amendments need to be selected which can improve the soil physicochemical and biological properties in order to realize sustainable usage of saline-alkali soil in the YRD.

Organic fertilizers are a positive option for regulating soil properties and plant growth in degraded soil (Le et al., 2016). Application of organic fertilizers in saline-alkali soil offers a better option for increasing organic carbon content in soil and crop yields by providing essential plant nutrients and organic materials (Demelash et al., 2014). Many searches have indicated that the addition of organic fertilizers to soil enhances organic carbon content, soil structure, cation-exchange capacity, and nutrient quality (Le et al., 2016; Gu et al., 2023). Organic fertilizers are also known to stimulate soil microbial biomass (Zhao et al., 2020; Liu et al., 2021), enzyme activities (Plaza et al., 2004; Ge et al., 2009; Insam et al., 2015), and to promote changes in community structure and abundance (Zhao et al., 2016). Nevertheless, organic fertilizers application does not always enhance soil microbial diversity, their impact depends on the duration of application, the source and nature of the fertilizer, soil type, and tillage conditions (Xu et al., 2021). Thus, the impacts of different quantities of organic fertilizer on the soil microbial community should be investigated using organic fertilizers with the goal of reclaiming saline-alkali soils in the YRD.

Biochar has attracted tremendous attention for its soil ameliorating effects, and it enhances carbon storage, soil fertility and quality, and contaminant immobilization and transformation (Zhu et al., 2017). Biochar is an alkaline material, rich in recalcitrant carbon and surface functional groups (Lehmann et al., 2011; Zhou et al., 2019). Because of these reasons, biochar has also been considered as a popular and promising soil conditioner that can improve crop yields (Kang et al., 2018; Zhang et al., 2019). In most researches, biochar has been able to increase the biomass of microorganisms in the soil, with significant changes in microbial community composition possibly explain the biogeochemical impacts of biochar on element cycles, pathogenic bacteria, and crop growth (Lehmann et al., 2011). Several studies have demonstrated that biochar indeed affects soil microbial communities (Chen et al., 2013; Liu et al., 2017; Zhang et al., 2018), and have reported possible effects of biochar on microbial community abundance and structure (Sun et al., 2013). These conflicting results are mainly due to variations in soil type, biochar variety, biochar production conditions and duration, application rate, and time (Dangi et al., 2019). Thus, understanding the soil microbial community and its reactions to diverse biochar amendments will inform a new approach for alleviating poor soil physicochemical and biological properties, and thus provide a practical management approach for sustainable agricultural production in saline-alkali soils.

Wheat (*Triticum aestivum*) is a leading staple crop and is cultivated all over the world, with approximately 2.1 million km^2 under cultivation (Meng et al., 2019). Previous study observed that the use of organic fertilizer and biochar could improve soil quality and performance of wheat (Meng et al., 2019). Researches have also indicated that rhizosphere bacteria and fungi can contribute directly to plant growth (Compant et al., 2010; Lehmann et al., 2011). However, in saline-alkali soil, the reaction of the rhizosphere microbial community after organic fertilizer and biochar amendment applications has not been well investigated. Therefore, in this study, the YRD saline-alkali soil was used as the research target to determine the effects of different amounts of organic fertilizer and biochar additions on (i) the nutrient profile and physicochemical characteristics of the soil; and (ii) the rhizosphere bacterial and fungal communities of wheat in the target site. We hypothesized that moderate amounts of organic fertilizer and biochar amendment would enhance soil physicochemical properties and create soil bacterial and fungal communities which alleviate stress induced by saline-alkali soil in the YRD.

2. Materials and methods

2.1. Biochar and organic fertilizer

The biochar used in this study was sourced from Taiyu Bioengineering Co., Ltd. (Qixia, China). It was derived from apple shoots and processed at a temperature of 450°C for 1 day. The biochar had a pH value of 7.52 and an electrical conductivity (EC) of 0.35 ms/cm. And it contained 70.2% carbon, 0.35% nitrogen, 0.13% available phosphorus, and 1.53% available potassium. Organic fertilizer was provided by Yangfeng Agricultural Technology Co., Ltd. (Weifang, China), based on maize straw and mushroom residue as the main composting substrate. The pH value of organic fertilizer was 7.94, with an EC of 3.25 ms/cm. The organic fertilizer had a high organic matter content more than 60% and nitrogen, phosphorus, and potassium contents more than 6%.

2.2. Field experiments

Field experiments were made in the Yellow River Delta Institute of Modern Agriculture, Shandong Academy of Agricultural Sciences, Dongying, China (118.37°N, 37.17°E). The primary soil type of the experimental plot was a typical saline-alkali of YRD, the specific texture and type of which are shown in Gu et al. (2023). Winter wheat (*Triticum aestivum* L.) was sown in October 2017and harvested in June 2018.We established six treatments with three replications: no biochar or organic fertilizer (CK); low biochar (BL): 10.0 t/ha; medium biochar (BM): 20.0 t/ha; high biochar (BH): 30.0 t/ha; low organic fertilizer (ML): 7.5 t/ha; medium organic fertilizer (MM): 10.0 t/ha. Test plot was designed in random block design with three replicates, the plot area was 10 m × 15 m with 1 m gaps between plots. Organic fertilizer and biochar were spread on the soil surface and then evenly tilled 0–20 cm before planting the crop. Other field management

followed local management practice. At the end of the field trial, wheat rhizosphere soil was obtained from the tilled area (0–20 cm) and collected and processed according to Gu et al. (2022a). Then, the collected samples were split into two parts: one for high-throughput sequencing, the other for soil physicochemical properties after being air-dried to remove impurities.

2.3. Analysis of soil physicochemical properties

Soil moisture content (M) was measured by the weight method. pH was measured with a water quality analyzer (Shanghai Lechen LC-MP-41 T). Soil electrical conductivity (EC) was measured at 10 cm soil level with a conductivity meter (SANXIN SX836). Other nutrients were assayed according to our previous study (Gu et al., 2023). Alkaline nitrogen (AN) and total nitrogen (TN) contents of soil were analyzed using the Analytik Jena Nitrogen Elemental Analyzer Multi-N/C 2100/2100S, Germany. Available phosphorus (AP) was extracted by $NaHCO_3$ and measured on a continuous flow analyzer (AMS Alliance, Futura, France). Soil total phosphorus (TP) was measured by wet digestion with $HClO_4$-H_2SO_4 and quantified using inductively coupled plasma emission spectrometry (Agilent 5,800 ICP-OES, United States). Available potassium (AK) was with CH_3COONH_4 (pH 7.0) and measured by inductively coupled plasma emission spectrometer (Agilent 5,900 SVDV, USA). Organic matter in soil was quantified using a potassium dichromate oxidation and carbon analyzer (OI Analytical Aurora 1,030 TOC, USA).

2.4. Soil microbial community analysis

DNA was extracted from wheat rhizosphere soil samples by FastDNA® Spin Kit for Soil (MP Biomedicals, USA) and the quality of the extraction was checked by NanoDrop 2000 luminometer. The extracted DNA was amplified by polymerase chain reaction (PCR) technique. The 16S gene in the V3-V4 region of the rhizosphere soil samples was sequenced using primers 338F (5′-ACTCCT ACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGG GTATCTAAT-3′), while the internal transcribed spacer (ITS) were sequenced using primers ITS1F (5′-CTTGGTCATTTAGA GGAAGTAA-3′) and ITS2R (5′-GCTGCGTTCTTCATCGATGC-3′). All sequencing was performed in triplicate and the results obtained are stored in the NCBI SRA database (PRJNA987399).

2.5. Data analysis

All data were calculated using Excel 2023 for mean and standard deviation. Duncan multiple range test and Pearson correlation analysis were conducted with DPS Statistics 18.10. Data related to microorganisms are counted and processed on the Majorbio Cloud Platform[1] Figures were performed using Origin Pro 2022 software.

1 www.majorbio.com

3. Results

3.1. Soil physicochemical properties

The impact of organic fertilizer and biochar application on the soil properties and nutrient profile of saline-alkali soil is shown in Table 1. In general, biochar amendment and organic fertilizer caused significant changes in most soil parameters ($p < 0.05$), apart from M, pH, TN and TP. Compared to CK, all treatments significantly reduced the EC and increased AK ($p < 0.05$). The concentration of AN was significantly increased in BH as compared to CK and the BL treatment ($p < 0.05$), while being no significantly different from the other treatments. In comparison to CK, AP levels were significantly increased under BL, BM, and BH treatments ($p < 0.05$), however, the AP levels were also significantly decreased by the ML and MM treatments compared with CK ($p < 0.05$), but the increase was lower than the BL, BM, and BH treatments. The level of TOC was significantly higher in the BL, BM, and BH treatments than in CK ($p < 0.05$), while in the ML and MM treatments compared to CK no obvious changes were observed. The TC level was significantly higher in the BL, BM, BH and MM treatments as compared to CK and the ML treatment ($p < 0.05$).

3.2. Microbial α-diversity

After read-quality filtering, 1,378,473 high-quality bacterial sequences and 1,017,877 high-quality fungal sequences were obtained. Bacterial and fungal sequences in this study were clustered into 2,326 and 309 OTUs, respectively, when grouped at 97% sequence similarity. Bacterial and fungal OTUs sparsity curves inclined towards a saturation plateau, which demonstrated that the data were large enough to detect most of the bacterial and fungal taxa in the rhizosphere soil (Supplementary Figure S1). As shown in Table 2, the OTUs and the Shannon and Chao1 index were compared under various conditions in the bacterial and fungal colonies, respectively. The OTUs and the Shannon and Chao1 index of the bacterial community amongst the six conditions were: 2109.33–2150.67 (OTUs), 6.31–6.50 (Shannon), and 2231.69–2253.98 (Chao1). For the fungal community, the values were: 192.33–242.00 (OTUs), 2.58–3.49 (Shannon), and 216.42–254.68 (Chao1; Table 2). There were no significant differences in OTU richness or the Chao1 index of the bacterial communities among the six conditions ($p > 0.05$). Nevertheless, the Shannon index of the bacterial communities was significantly increased in the BM treatment versus with the CK and MM treatment ($p < 0.05$), and was not significantly affected compared with others. When compared to CK, the OTUs or Shannon and Chao1 indices of the fungal communities under biochar amendment and organic fertilizer treated were no significant differences ($p > 0.05$).

3.3. Microbial community composition and structure

After classification analysis, wheat rhizosphere soil was identified for 32 bacterial phyla and 11 fungal phyla. The most abundant bacterial phyla were Proteobacteria, Actinobacteriota, Acidobacteriota, Bacteroidota, and Chloroflexi across the soil samples,

TABLE 1 Effect of biochar and organic fertilizer on the physical and chemical properties of soil.

	CK	BL	BM	BH	ML	MM
M(%)	7.72 ± 1.00a	8.36 ± 1.71a	7.92 ± 1.82a	8.22 ± 0.94a	8.88 ± 2.51a	9.64 ± 3.17a
pH	8.04 ± 0.087a	8.05 ± 0.032a	8.07 ± 0.042a	8.06 ± 0.081a	7.99 ± 0.036a	7.98 ± 0.040a
EC(ms/cm)	370.89 ± 16.45a	309.33 ± 19.36d	266.76 ± 17.81e	332.78 ± 19.71b	319.77 ± 13.61c	333.33 ± 18.57b
AN(mg/kg)	100.93 ± 7.57b	97.60 ± 9.32b	107.48 ± 7.40ab	118.03 ± 3.22a	104.94 ± 4.16ab	103.43 ± 9.75ab
TN(g/kg)	1.49 ± 0.08a	1.53 ± 0.19a	1.62 ± 0.16a	1.57 ± 0.21a	1.44 ± 0.13a	1.58 ± 0.22a
AP(mg/kg)	59.17 ± 3.85d	80.01 ± 4.35c	87.82 ± 4.05b	95.48 ± 5.06a	41.26 ± 2.37f	51.18 ± 3.63e
TP(g/kg)	1.13 ± 0.06ab	1.27 ± 0.19a	1.29 ± 0.03a	1.30 ± 0.12a	1.06 ± 0.02b	1.08 ± 0.07b
AK(mg/kg)	195.29 ± 7.96d	211.21 ± 10.55c	235.37 ± 8.73b	273.10 ± 11.26a	205.40 ± 9.52c	241.18 ± 12.41b
TOC(g/kg)	9.48 ± 0.09d	13.83 ± 1.85c	18.53 ± 3.70b	21.92 ± 3.94a	9.90 ± 0.36d	10.85 ± 2.94cd
TC(g/kg)	16.33 ± 1.15e	23.84 ± 1.19c	31.94 ± 1.73b	37.80 ± 1.80a	17.07 ± 1.62e	18.71 ± 1.06d

Data represent mean ± standard deviation (SD) of three biological replicates. Different lowercase letters within a row indicate significant differences ($p < 0.05$).

TABLE 2 Operational taxonomic unit richness and diversity indices of different samples.

Treatment	Bacterial index			Fungal index		
	OTUs	Shannon	Chao 1	OTUs	Shannon	Chao 1
CK	2127.33 ± 38.81a	6.35 ± 0.10bc	2236.60 ± 34.49a	213.33 ± 12.50ab	3.23 ± 0.30a	238.34 ± 9.02a
BL	2129.33 ± 50.46a	6.46 ± 0.08ab	2235.80 ± 43.47a	229.00 ± 9.17ab	3.44 ± 0.50a	254.46 ± 14.35a
BM	2150.67 ± 48.34a	6.50 ± 0.09a	2249.28 ± 42.01a	227.33 ± 10.12ab	3.23 ± 0.36a	245.13 ± 8.27a
BH	2141.33 ± 36.23a	6.43 ± 0.08ab	2246.95 ± 24.44a	242.00 ± 10.82a	3.49 ± 0.30a	254.68 ± 13.61a
ML	2125.33 ± 45.76a	6.46 ± 0.06ab	2231.69 ± 31.98a	192.33 ± 33.50b	2.58 ± 0.83a	216.42 ± 44.35a
MM	2109.33 ± 36.53a	6.31 ± 0.02c	2253.98 ± 20.00a	208.67 ± 34.99ab	3.01 ± 0.69a	224.55 ± 32.68a

Data represent mean ± standard deviation (SD) of three biological replicates. Different lowercase letters within a row indicate significant differences ($p < 0.05$).

which represented more than 80% of the total bacterial population (Figure 1A). Of these, the highest relative abundance of Proteobacteria was found in all treated soils, which was followed by the Actinobacteria group. Ascomycota was the richest fungal community in the soil samples in terms of relative abundance, which ranged from 86 to 94% of the total communities tested (Figure 1B). Mortierellomycota ranked second for relative abundance in CK, BL, BM, BH, and MM, while Olpidiomycota ranked second for relative abundance in ML (Figure 1B).

The heatmap of the 30 genera of most abundant classified bacterial and fungal were showed in Figure 2. At the bacterial genus level, *Sphingomonas*, *norank_f__Geminicoccaceae*, *Skermanella* and *Arthrobacter* were the top 4 dominant genera, and their relative abundances together represented approximately 13.4% of all sequences (Figure 2A). The distribution of the genera differed significantly across the different samples and belonged to eight phyla, including Proteobacteria (13 genera), Actinobacteriota (7), Acidobacteriota (4), Bacteroidota (1), Chloroflexi (1), Firmicutes (1), Gemmatimonadota (1), Myxococcota (1). Sphingomonas was the predominant bacterial genus in the BL, BM, and ML, while *norank_f__Geminicoccaceae* was the predominant bacterial genus in CK, BH, MM. *Skermanella* and *Arthrobacter* were the predominant bacterial genus in BL and MM, respectively. The heatmap of bacterial genera separated the samples from different treatments into three groups, with BL, BM and BH together and ML and MM together separated from CK. At the fungal genus level, *Chaetomium*, *Gibberella*, *Schizothecium*, *Alternaria* were the top 4 dominant genera, and their relative abundances together represented approximately 37.6% of all sequences (Figure 2B). The distribution of the genera differed significantly across the different samples and belonged to six phyla, including Ascomycota (25 genera), Basidiomycota (1), Chytridiomycota (1), Mortierellomycota (1), Olpidiomycota (1), unclassified_k__Fungi (1). *Chaetomium* was the predominant bacterial genus in the CK, BL, BM, and ML, *Gibberella* was the predominant bacterial genus in BH and MM. The heatmap of fungal genera separated the samples from different treatments into two groups, with CK, BL, BM and BH together separated from ML and MM together. These results suggest that the effects of organic fertilizer treatment on bacterial and fungal communities are different compared to CK and biochar treatment.

A distinct distinction between soil bacterial and fungal communities under various treatments was made by principal coordinate analysis (PCoA) at the OTU level (Figures 3A,B). Coordinate axes 1 and 2 elucidate 22.82 and 18.33% for bacterial community variation and 18.27 and 17.94% for fungal community variation under different treatments, respectively. The results showed that the soil bacterial and fungal communities in BL, BM, and BH were similar to those in CK. However, the soil bacterial and fungal communities in ML and MM were distinct from those of CK along PCoA1 (Figure 3).

Analysis by LEfSe showed that there were 35 bacterial and 14 fungal taxa enriched ($\alpha < 0.01$, linear discriminant analysis (LDA) score > 2.0) in the six treatments (Figure 4). The bacterial and fungal taxa that were significantly enriched differed between treatments. The index microbes (LDA threshold of 2.0) in the microbial communities

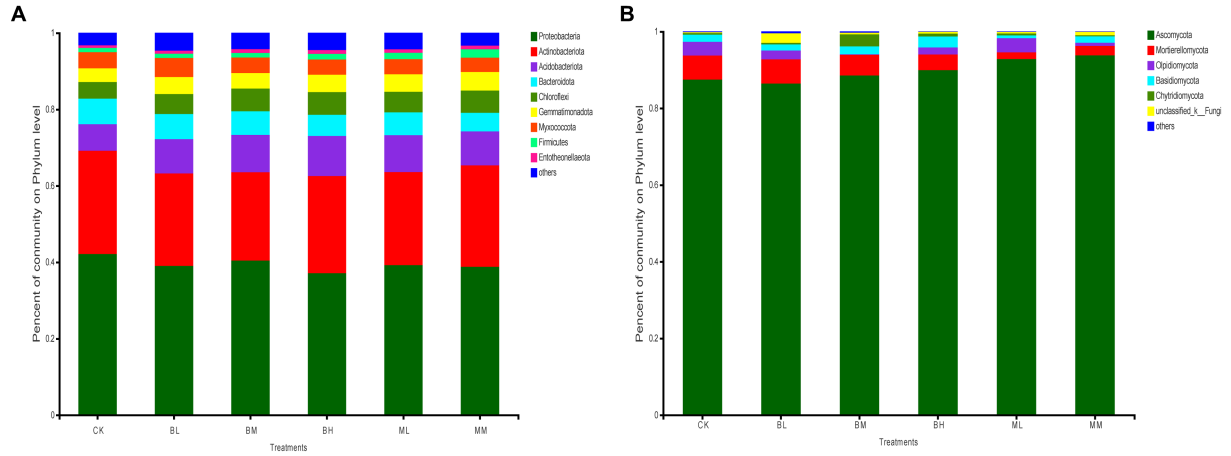

FIGURE 1
The relative abundances of bacteria **(A)** and fungi **(B)** at the phylum level in wheat rhizosphere soil.

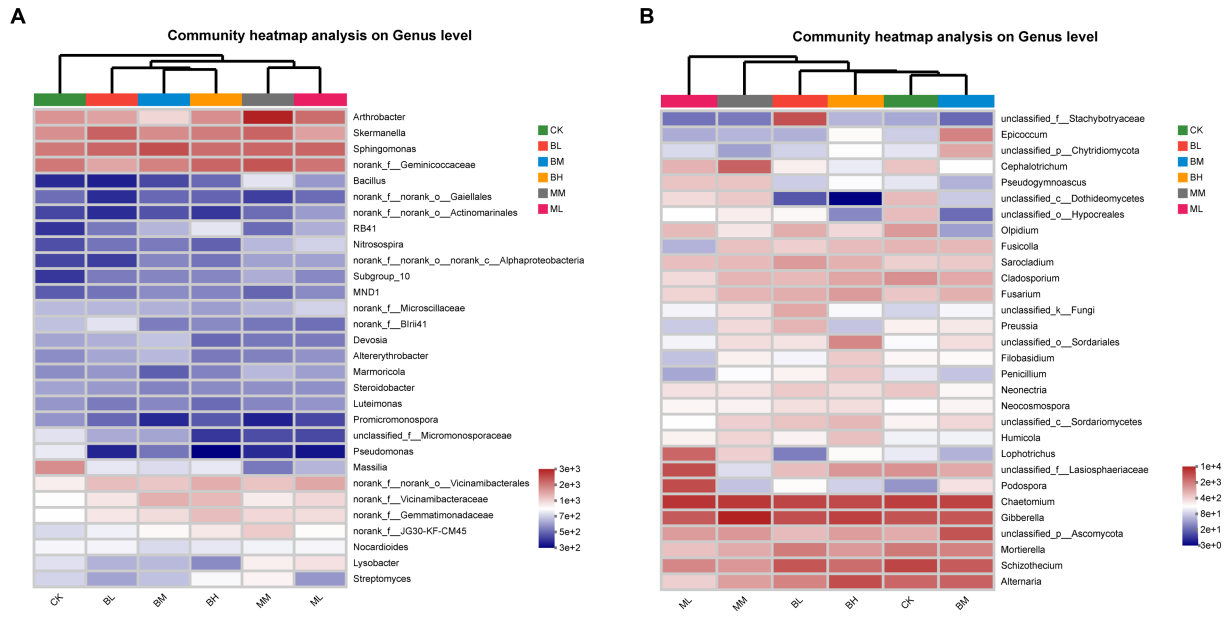

FIGURE 2
Heatmap of the top 30 classified genera of bacteria **(A)** and fungi **(B)** in wheat rhizosphere soil.

relevant to the treatment groups are shown in Supplementary Figures S2A,B, respectively. Of these, the largest number of bacterial species was enriched in the ML treatment with 9, followed by BL with the second largest at 7. The largest variety of fungal species enriched in the BM treatment was the largest at 6; no abundant fungal taxa were identified in the ML treatment.

The Redundancy analysis (RDA) was used to explain the impact of soil parameters on microbial populations at the genus level. These soil indicators elucidated 46.09% of the total variation in the soil bacterial community (RDA1, 30.42%; RDA2, 15.67%; see Figure 5A). It was identified that soil pH, TP, TN, and TOC were the main factors to cause changes in bacterial community variation (Supplementary Table S1). For the fungal communities, RDA1 and RDA2, respectively, explained 16.68 and 14.29% of the total variation (Figure 5B). Furthermore, soil pH, TP and AP were significant factors influencing the rhizosphere fungal community (Supplementary Table S2).

Correlation analysis between soil parameters and bacterial communities showed that Proteobacteria was significant negatively associated with TN ($p < 0.01$) and TOC ($p < 0.05$), while Actinobacteriota was significant positively associated with EC ($p < 0.01$), Acidobacteriota was significant positively associated with the TN ($p < 0.01$) and TOC ($p < 0.05$), Chloroflexi was significantly positively associated with the TN ($p < 0.01$), and Firmicutes was significant positively associated with the M ($p < 0.01$) (Figure 6A). Correlation analysis between soil parameters and fungal communities showed that Olpidiomycota was significantly positively associated with EC ($p < 0.05$), while Basidiomycota was significant positively

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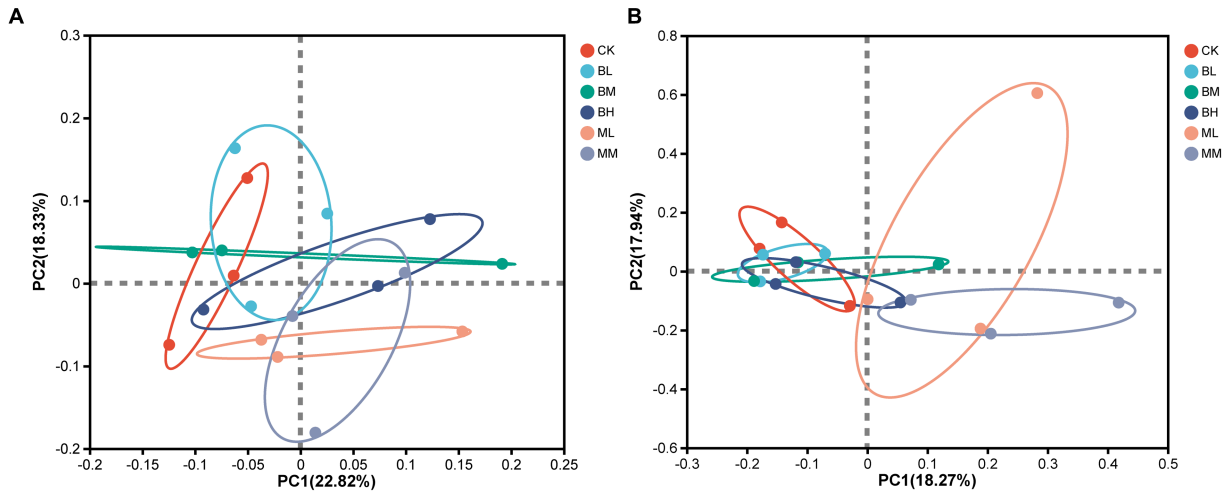

FIGURE 3
PCoA analysis of bacterial **(A)** and fungal **(B)** community structures based on Bray-Curtis distance at the OTU level.

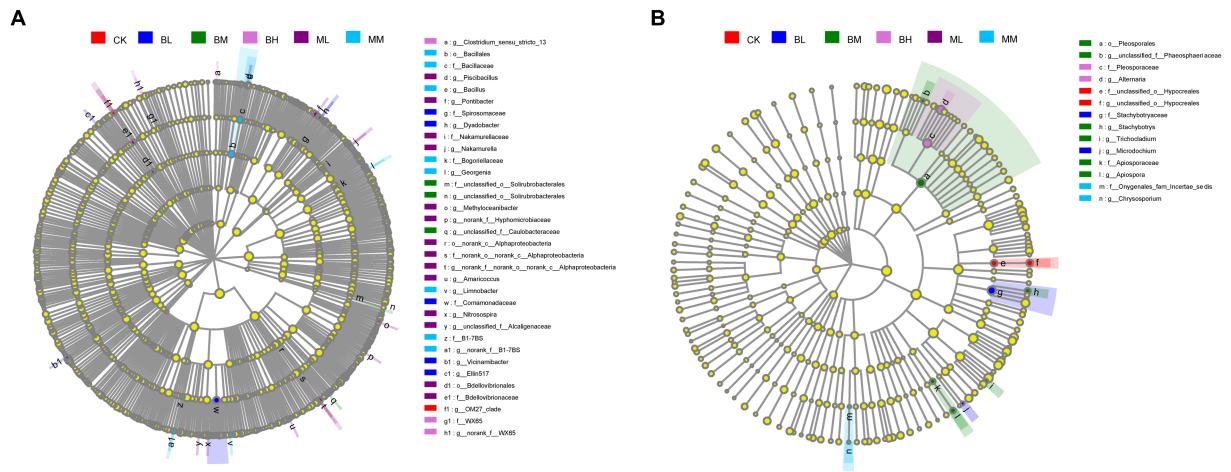

FIGURE 4
LEfSe analysis for **(A)** bacterial and **(B)** fungal community of treatments control.

associated with TC ($p < 0.05$), pH ($p < 0.05$), AP ($p < 0.05$), and AK ($p < 0.01$) (Figure 6B).

3.4. Microbial network analysis

The complexity of the interactions within the microbial communities and soil parameter under biochar and organic fertilizer application was conducted by to assess their topological properties. The results showed that there was a difference between the microbial communities based on the addition of biochar and organic fertilizer (Figure 7). For bacteria, the complexity of organic fertilizer-bacteria was greater than those of biochar-bacteria, indicating that the addition of organic fertilizer increased the complexity of the correlation between bacteria and soil environmental factors (Figures 7A,B). But for fungi, the complexity of biochar-fungi was greater than those of organic fertilizer-fungi, indicating that the addition of biochar increased the complexity of the correlation between fungi and soil environmental factors. For bacteria, the average number of connections per node was higher following organic fertilizer treatment (node average degree = 2.43) than after the biochar treatment (node average degree = 2.07) (Supplementary Table S3). But for fungi, the average number of connections per node was higher following biochar treatment (node average degree = 2.25) than after the organic fertilizer treatment (node average degree = 2.00; Supplementary Table S4). Nodes with the highest connections between environmental parameters and bacteria under biochar and organic fertilizer were TN (9) and TC (13), respectively. While nodes with the highest connections between environmental parameters and fungi under biochar and organic fertilizer were pH (12) and Ec (10), respectively. This result suggests that bacteria and fungi respond differently to biochar and organic fertilizer treatments, and soil parameters have different effects on bacterial and fungal communities.

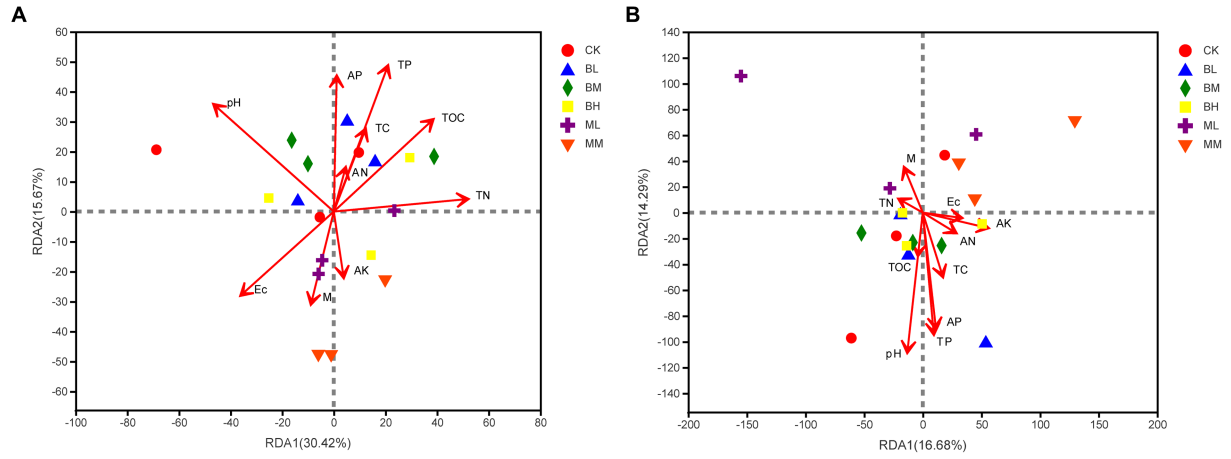

FIGURE 5
RDA of the relationships between soil bacterial **(A)** and fungal **(B)** community with soil properties.

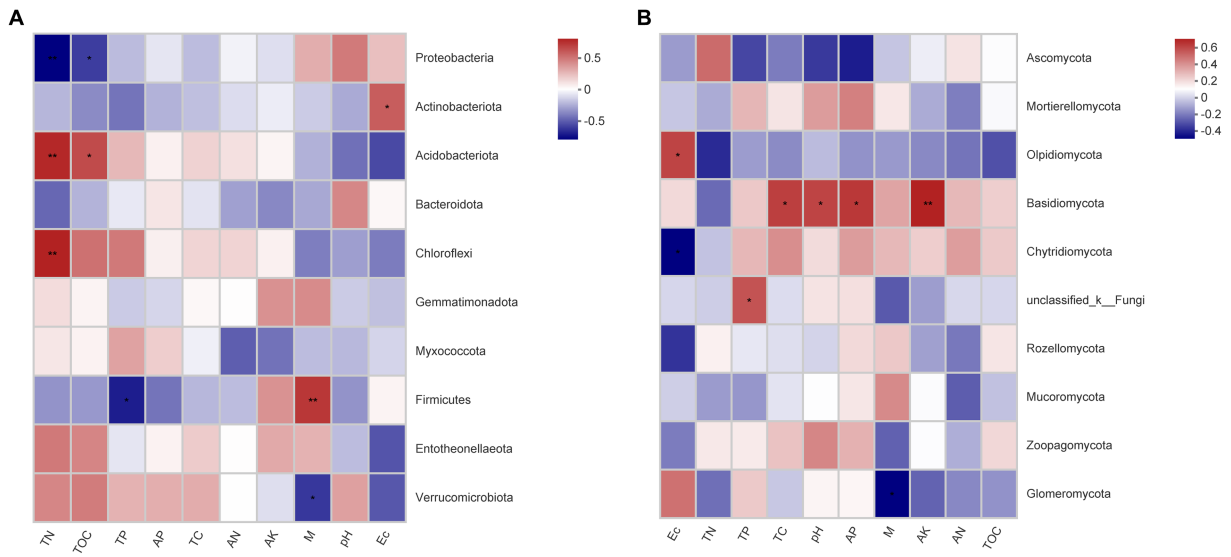

FIGURE 6
Heatmap of the Spearman correlation between the predominant **(A)** bacterial and **(B)** fungal phyla and environmental variables. *Correlation is significant at the 0.05 level (two-tailed). **Correlation is significant at the 0.01 level (two-tailed).

To investigate the potential interactions between bacteria and fungi in wheat soil after the application of biochar and organic fertilizer, we constructed a correlation network of microbial communities (Figure 8). Supplementary Table S5 provides an overview of several significant topological properties of this microbial community correlation network. It was observed that the number of nodes and edges in the bacterial and fungal correlation network was higher for the organic fertilizer application compared to the biochar application. Additionally, a greater number of positive correlated edges were found in the organic fertilizer application, whereas the biochar application exhibited the opposite trend. Regarding the biochar application, 91.66% of the bacterial nodes were affiliated with Proteobacteria (44%), followed by Actinobacteriota (28%) and Acidobacteriota (16%). As for the fungal nodes, 75% belonged to Ascomycota. On the other hand, for the organic fertilizer application, 77.78% of the bacterial nodes were affiliated with Proteobacteria (40.74%), Actinobacteriota accounted for 22.22%, and Acidobacteriota for 14.81%. Furthermore, 79.17% of the fungal nodes remained within the Ascomycota group.

4. Discussion

Although many researches have explored the effects of biochar and organic fertilizer in agricultural fields, only a few have conducted saline–alkali field experiments (Yao et al., 2017; Gu et al., 2022a, 2022b, 2023). Biochar amendment and application of organic fertilizer generally changes soil quality, such as pH, soil organic carbon, and nutrient content (Lehmann et al., 2011; Luo et al., 2014; Yao et al., 2017). In reports from Yao et al. (2017), the addition of high

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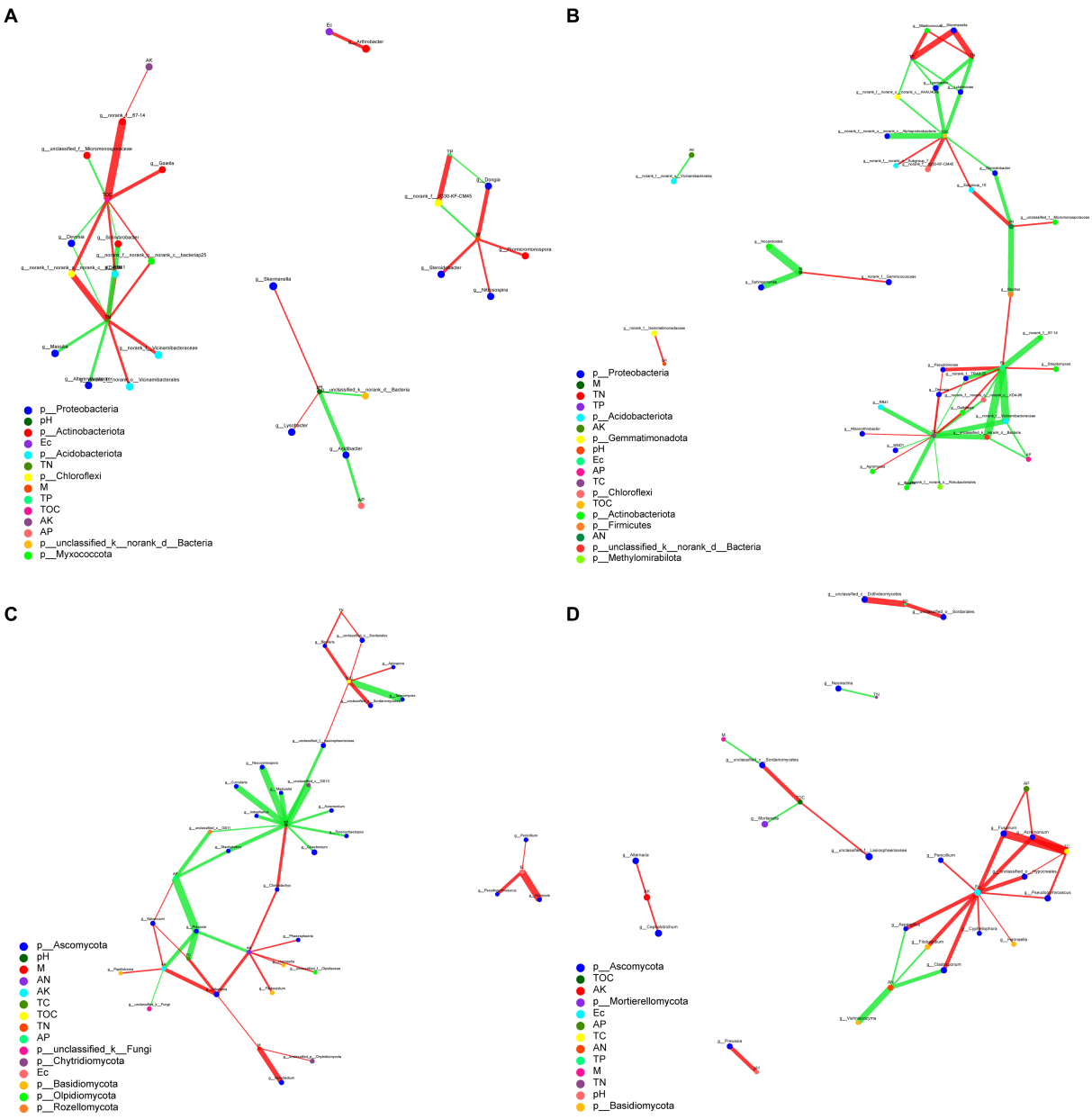

FIGURE 7
Correlation network analysis of environmental parameters and microbial communities **(A)** Biochar-bacteria. **(B)** Organic fertilizer-bacteria. **(C)** Biochar-fungi. **(D)** Organic fertilizer-fungi.

concentrations biochar in soil has been shown to significantly improve pH and TN. During this research, we found that the biochar and organic fertilizer caused no significant changes in pH and TN, while the TOC content showed significant change, this is like the findings of Gao et al. (2021). This was due to the high background pH of the soil (7.95) for which biochar application resulted in only a slight increase (8.05–8.07), but this result was non-statistically significant ($p > 0.05$). We found in this research that the EC value of the soil decreases with the addition of biochar and organic fertilizer. Previous study has also showed a similar decrease in soil Ec values when various organic additives were used to remediate the soil (Tejada et al., 2006). Soil AP and AK improved with the addition of biochar, which agreed with Oladele et al. (2019). This may be because some nutrients are produced by the pyrolysis of the biochar during its manufacture, and because the porous structure of the biochar causes it to adhere to more functional groups, which increases its ability to adsorb soil nutrients (Ameloot et al., 2013; Zhang et al., 2019).Organic fertilizer is known to contain nitrogen, phosphorus, and potassium. However, for this research, the addition of organic fertilizer only increased the content of AK, even with a reduced AP content. It is well known that biochar and organic fertilizers can induce variations in the soil environment, such as soil nitrogen, potassium, phosphorus levels, and pH (Sun et al., 2020). The differences between study results may be due to a variety of factors, such as the climate, soil properties, fertilizer, and plants grown (Assefa and Tadesse, 2019).Therefore, many factors need to be considered when analyzing the changes in soil properties.

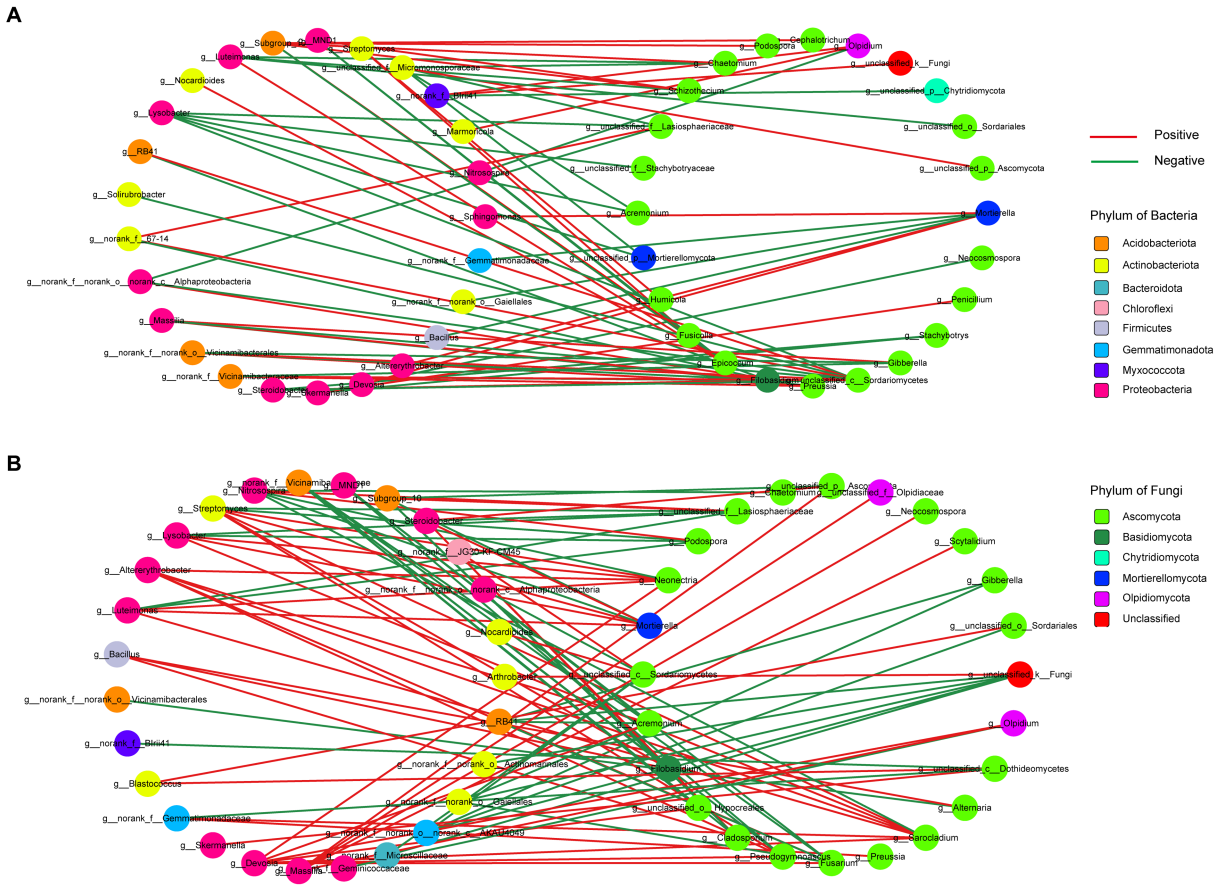

FIGURE 8
Correlation network analysis of microbial communities. Node color represents phylum classification. Edge color corre- sponds to positive (red) and negative (green) correlation, and the edge thickness is equivalent to the correlation values. **(A)** The network between biochar. **(B)** The network between organic fertilizer.

It was shown that either biochar or fertilizer can change the structure of the soil microbial community and crop performance (Tao et al., 2020; Gao et al., 2021). Biochar can provide a habitat for microbiota through the provision of organic compounds, further mediating changes in microbial population structure and abundance (Gao et al., 2021), and on the other hand, toxic substances such as polycyclic aromatic hydrocarbons (PAHs) present in biochar can adversely affect soil microbial populations, which can lead to alterations in the composition of the fungal community and reductions in its diversity (Zheng et al., 2016; Sheng and Zhu, 2018). In this study, the richness of soil bacterial and fungal communities were not significantly different after biochar treatment compared to CK, but the overall trend showed an increase both in richness and diversity of soil bacterial and fungal communities with the biochar applied (Table 2), and there was a significant increase ($p < 0.05$) in diversity of soil bacterial community compared to CK under the BM, which were consistent with Yao et al. (2017) and Gao et al. (2021). Tao et al. (2020) showed that the application of organic fertilizer had a significantly higher effect on bacterial richness and diverstiy than CK, but reduced the richness and diversity of fungal species. Sun et al. (2020) also found the same fungal results as Tao et al. (2020). Chen et al. (2021) discovered that organic fertilizer application significantly enhanced the richness and diversity of fungal species, but it had no significant impact on bacteria. Our research results on bacteria were consistent with Chen et al. (2021), while fungi were consistent with Tao et al. (2020). Differences in the effects of organic fertilizer application on bacteria and fungi may be caused by differences in crops, soil conditions, types of organic fertilizer and application methods.

Effects of biochar application on the composition and structure of soil microbial communities have been frequently reported (Zheng et al., 2016; Gao et al., 2021). In this study, the predominant phyla of bacterial community in the wheat soil were Proteobacteria, Actinobacteria, Acidobacteria, Bacteroidota, and Chloroflexi, which fitted to the findings by other researchers also observed in wheat soil (Meng et al., 2019). However, the dominant fungus in this study is the Ascomycota, which is different from the Zygomycota reported by Meng et al. (2019), but consistent with Chen et al. (2021) and Zheng et al. (2016). Yao et al. (2017) found that relative abundances of Acidobacteria were decreased, while the abundances of Chloroflexi were increased with biochar addition. Khodadad et al. (2011) reported that biochar amendment decreased the bacterial diversity but increased the relative abundance of bacterial phyla Actinobacteria and Gemmatimonadetes. Our results indicated that Acidobacteria, Chloroflexi, and Gemmatimonadetes increased with biochar addition, especially BH, whereas Actinobacteria decreased. Wolna-Maruwka

et al. (2021) discovered that organic fertilizer increased the abundence of Actinobacteria and Firmicute, but decreased Acidobacteria, which is partially consistent with our results that Gemmatimonadetes and Firmicutes increased with organic fertilizer addition. Acidobacteriota was significantly positively correlated with TOC ($p < 0.05$) content, it can also partially explain the significant increase in TOC of biochar application compared to CK. Both Ascomycota (copiotrophic) and Basidiomycota (oligotrophic) play important roles in plant growth by improving nutrition and defense against pests (Meng et al., 2019). Yao et al. (2017) reported that biochar addition increased the relative abundance of Ascomycota and decreased the relative abundance of Basidiomycota compared to CK. In this study, the abundance of Ascomycota increased with addition content of biochar and organic fertilizer, but this has not achieved the remarkable level. Basidiomycota, which was significantly positively correlated with the TC ($p < 0.05$), pH ($p < 0.05$), AP ($p < 0.05$), and AK ($p < 0.01$), also increased with biochar addition and decreased with organic fertilizer addition without statistically significant. This may be one of the reasons why the TC, AP, AK of biochar, especially BH, is higher than those of organic fertilizers. Gao et al. (2021) found that biochar addition significantly increased the relative abundance of Mortierellomycota when compared to CK. In contrast, in this study, whether applying biochar or organic fertilizer, Mortierellomycota significantly decreased, especially when applying organic fertilizer. Differences above may be caused by differences in environment and soil conditions, or types of organic fertilizer and biochar.

The abundance of *norank_f__JG30-KF-CM45* increased with the increase of biochar and organic fertilizer concent, and was positively correlated with TP and TOC in biochar and organic fertilizer treatments, respectively, which suggested that *norank_f__JG30-KF-CM45* may be beneficial for saline alkali land remediation. This result was consistent with Shen et al. (2022), who thought that *norank_f__JG30-KF-CM45* was beneficial genus that might potentially promote tobacco growth. Yao et al. (2022) reported that biochar addition inhibited nitrification in salt-affected irrigation-silting soil by shifting the community structures of AOB and reducing the relative abundance of dominant functional ammonia-oxidizers, such as *Nitrosospira*. In this study, the addition of biochar had no effect on the abundance of *Nitrosospira* while the addition of organic fertilizer significantly increased the abundance of *Nitrosospira*, which was consistent with the result of Lin et al. (2018). Furthermore, we also found that the relative abundance of *Bacillus* increased with biochar and organic fertilizer application rate, but only organic fertilizer application reached significant level. *Bacillus* species are commercially marketed as biopesticides, biofertilizers, and soil amendments (Cao et al., 2011). So the increase abundance of *Bacillus* with biochar (Yao et al., 2017) and organic fertilizer (Tao et al., 2020) addition would be benefit for soil physical and chemical properties. Organic fertilization reduced the relative abundances of some pathogenic genera detected in our study, such as *Alternaria*, *Gibellulopsis*, and *Cladosporium*. A similar effect of organic fertilizers was reported in Semenov et al. (2022) and Lu et al. (2020). In addition, organic amendments have been proposed as a strategy for the management of plant diseases caused by soil-borne pathogens, the suppressive effect is likely related to an increase of pathogen-antagonistic fungi, since the organic fertilizers may act as an alternative C source for the antagonists (Semenov et al., 2022). *Cladorrhinum*, a potential biological control agent, increased under long-term application of manure (Semenov et al., 2022), *Trichoderma*, which are known as biocontrol agents against plant pathogens and opportunistic avirulent plant symbionts, which can be parasites and antagonists of many phytopathogenic fungi, thus protecting plants from disease (Vinale et al., 2008), and *Humicola*, a potential antagonists for the biological control of plant diseases (Ko et al., 2011), all of above genera increased with biochar and organic fertilizer application in our study. Sordariomycetes, which was positive correlation with TOC under biochar and organic fertilizer application in this study, also increased with biochar and organic fertilizer application, and this result was consistent with Ding et al. (2017) and Yu et al. (2018). In a word, biochar and organic fertilizer application in our study had led to an increase in some beneficial and biological control bacteria, as well as a decrease in pathogenic bacteria, which contributes to soil remediation in saline alkali soils.

As it is well known, soil microorganisms do not exist in isolation, but rather coexist and jointly construct complex ecological correlation networks, leading to different important and complex interactions, including but not limited to competition, commensalism, and mutualism (Duan et al., 2021). Therefore, a comprehensive understanding of the interactions between bacteria and fungi is also crucial for improving soil system services (Zhu et al., 2022). In this study, we found that the interactions among organic fertilizer treatments formed more connections than that of biochar treatments, in the meantime, both of the content of TOC and TC were higher under biochar application compared to the organic fertilizer application, which was consistent with De Menezes et al. (2017), who thought fungi-bacteria correlations were stronger in low soil organic matter soils. Highly connected taxonomic groups in the co-occurrence network typically significantly influence microbial community structure and function irrespective of their abundance across time and space (Banerjee et al., 2018). In our study, the nodes with the highest connections were *Sarocladium*, *Devosia*, and *Streptomyces* in organic fertilizer application, with a degree of 7, and *Unclassified_c__Sordariomycetes* in biochar application, with a degree of 9, indicating that these genera were relatively active in the interaction network between bacteria and fungi under biochar and organic fertilizers application, and whether they have an impact on soil physicochemical properties and plant growth deserves further study.

5. Conclusion

This study provides evidence that the application of biochar and organic fertilizer has different effects on soil physicochemical properties and microbial community structure. The application of biochar and organic fertilizer significantly reduced Ec and increased nutrient content of saline-alkali soils of the YRD. pH and TP were crucial contributors in regulating the bacterial and fungal community distribution. *Sphingomonas, norank_f__Geminicoccaceae*, and *Skermanella* were the dominant bacteria genera in biochar application, while *Arthrobacter* and *Sphingomonas* were the most abundant bacteria genera in organic fertilizer application. Whether biochar or organic fertilizer application, *Chaetomium* and *Gibberella* were the most dominant fungal genera. Microbial network analysis showed that bacterial and fungal communities responded differently to biochar and organic fertilizer treatments. Compare to biochar treatments, organic fertilizer treatment increasing the complexity of bacterial communities and decreasing the complexity of fungal communities.

The results of the current study provides evidence that biochar and organic fertilizer treatments affect microbial communities differently and provides new insights to remediation of saline-alkali land of the YRD. The combined application of biochar and organic fertilizer and its long-term effects need further study.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary material.

Author contributions

ML contributed to the conception of the study and wrote the manuscript. YG and XG also contributed to the conception of the study. CC, HZ, and JL contributed to field investigation and sample acquisition. ZW, XL, and KY performed the experiments and data analyses. NS contributed to the editing and revising of the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2023.1250453/full#supplementary-material

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