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Biochar

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P-modified biochar alters the microbial community in heavy metal-contaminated soils by regulating nutrient supply balance

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Abstract

Phosphorus (P)-modified biochar demonstrates dual capabilities for heavy metal immobilization and soil quality enhancement. However, the underlying mechanism of microbial response to changes in soil properties is still unclear. In this study, P-modified biochar, prepared by co-pyrolysis of apple tree branches with K_3PO_4, was used for removal of heavy metals from the soils near a mining area. Effects of P-modified biochar on the microbial communities in soil were investigated and the key driving factors were identified. Adding P-modified biochar reduced the bioavailable cadmium and lead contents of the soil by 28.21% and 28.64%, respectively, mainly through improved co-precipitation and cation exchange. In turn, the cadmium and lead concentrations in maize grains were reduced by 36.52% and 61.82% respectively. Meanwhile, the richness and diversity of soil bacteria significantly decreased with the addition of P-modified biochar ($P < 0.05$). Microbial multi-trophic ecological network module analysis and partial least squares pathway modeling indicated that biochar changed the capacity of the soil to provide microorganisms with nitrogen and P, requiring the key microbial taxa (modules 1 and 3) to adjust. Modules 1 and 3 played important but opposite functions in the nitrogen and P cycle of the soil. This further led to variations in the composition and structure of microbial communities in soil. Particularly, changes in the bioavailability of heavy metals showed a negligible effect on soil microbial communities. This study emphasizes that P-modified biochar can efficiently reduce soil heavy metal bioavailability and alter the microbial community by regulating nutrient supply balance.

Highlights

- P-modified biochar can effectively remediate heavy metal contaminated soil.
- Bacteria show sensitivity to biochar addition compared to fungal communities.
- Biochar-induced adaptations in key microbial taxa result from nutrient balances.
- Changes in heavy metal bioavailability minimally affect soil microorganisms.

Keywords Heavy metal, P-modified biochar, Nutrient supply, Multi-trophic ecological network, Soil microbial community

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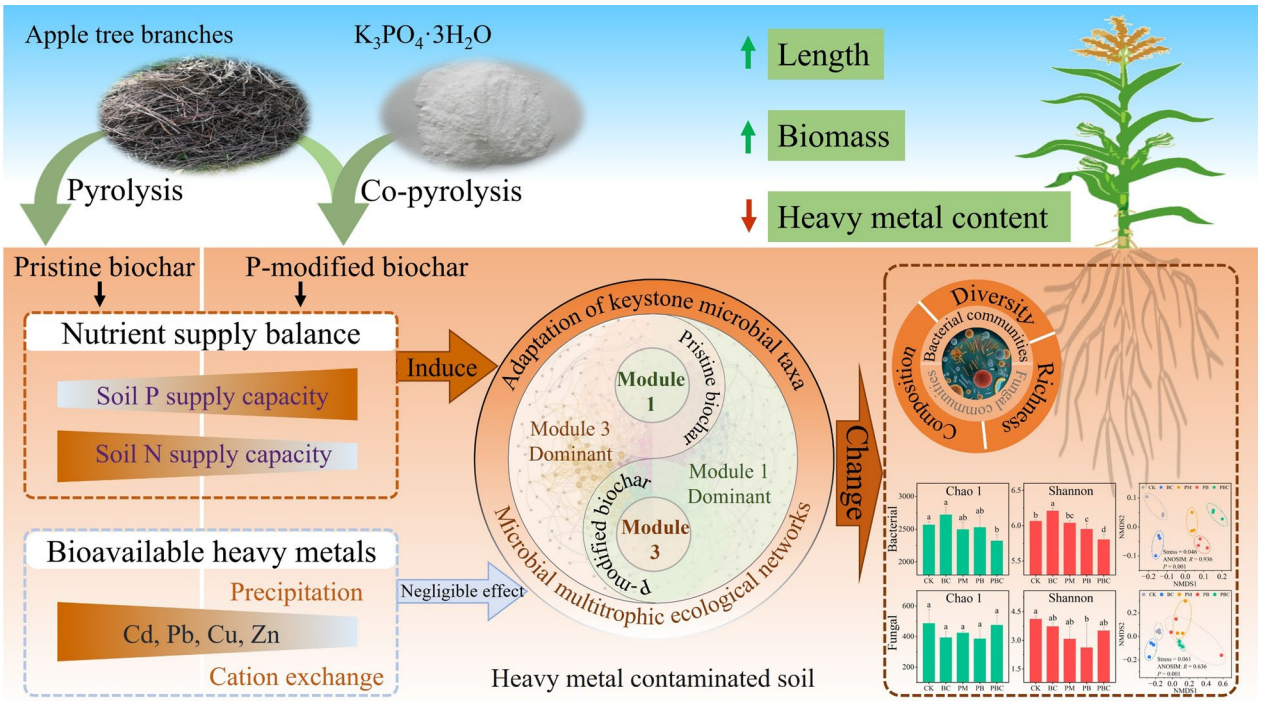
Graphical Abstract



1 Introduction

Heavy metal pollution in agricultural soils is steadily rising along with global industrialization and urbanization (Qi et al. 2022a). Among the heavy metals, cadmium (Cd), lead (Pb), and copper (Cu) are often considered highly dangerous pollutants, as they are difficult to degrade, highly toxic, and may lead to bio-enrichment (Huang et al. 2018). Heavy metals in soil can not only threaten the survival of plants, animals, and humans via the food chain (Duan et al. 2019), but also affect the ecological functions of the soil by stimulating or inhibiting microbial activity (Fan et al. 2021; Yan et al. 2020). Therefore, it is imperative to remediate heavy metal-contaminated soils using cost-effective and efficient technologies (Sun et al. 2024).

Biochar is a carbon-rich material produced by high-temperature pyrolysis of biomass under oxygen-limited conditions (Wang et al. 2022), which is widely used in heavy metal-contaminated soil remediation because of its adsorption properties (Khan et al. 2020). However, the small adsorption capacity of unmodified biochar for heavy metals cannot meet the actual demand, so biochar needs to be modified appropriately to improve its adsorption capacity (Li et al. 2017). Recent studies have shown that phosphorus (P)-modified biochar prepared by co-pyrolysis of P-containing materials and biomass can effectively immobilize heavy metals in the soil through cation exchange and co-precipitation (Gao et al. 2020; Wang et al. 2022, 2023, 2024). Moreover, the co-pyrolysis of P and biomass can also improve the carbon retention and stability of biochar (Gao et al. 2019; Xu et al. 2017). Thus far, the adsorption of a single kind of heavy metal has been the primary focus of research on P-modified biochar. However, the majority of soils are tainted with several heavy metals (Qi et al. 2022a). As a result, further exploration into P-modified biochar is required for the remediation of soils contaminated with multiple heavy metals.

Particularly, microorganisms play an irreplaceable role in preserving the functions and stability of soil ecosystems (Cao et al. 2023) and mitigating environmental stresses and disturbances (Han et al. 2019; Wu et al. 2019). Heavy metal interactions with proteins (enzymes) and metabolic process inhibition are the primary causes of their harmful effects (Wang et al. 2007). Environmental stress caused by heavy metals generally decreases the diversity and activity of soil microbial populations and disrupts the ecological balance of population interactions within the community (Wang et al. 2010). Changes in soil microbial communities are often considered as

early warning of heavy metal contamination (Nigam et al. 2019).

P-modified biochar can greatly affect the nutrient supply capacity, pH, bulk density, and other characteristics of the soil, in addition to decreasing the amount of bioavailable heavy metals in the soil (Li et al. 2020; Wang et al. 2023, 2024). Microbial populations can be influenced by the type, concentration, bioavailability of heavy metals, and physicochemical properties of soil. All these factors directly or indirectly change the soil microbial community, which further affects the ecological functions of the soil (Liu et al. 2020a). However, the effects of P-modified biochar on microbial communities in soils contaminated with heavy metals, as well as the underlying mechanisms, are still unclear.

Therefore, the current work aims to evaluate the capacity of P-modified biochar to immobilize heavy metals in contaminated soil and investigate the response of the microbial community, as well as the mechanism and driving factor. This study investigated the changes in bioavailable heavy metals and microbial community composition in soil following the application of P-modified biochar, prepared through co-pyrolysis, in a maize pot experiment. The research is based on two hypotheses: (1) P-modified biochar can effectively reduce the bioavailable heavy metal content in compound-polluted soils and the heavy metal content in plant tissues; (2) P-modified biochar changes the composition of soil microbial community mainly by regulating the heavy metal bioavailability in soil. This study provides new insights into microbiology regarding the use of P-modified biochar to remediate soils contaminated with heavy metals.

2 Materials and methods

2.1 Materials

Topsoil (0–20 cm) of an abandoned farm adjacent to a Pb-zinc (Zn) mine in Hanzhong, Shaanxi Province of China, was collected. Samples were air-dried, ground, and sieved (2 mm) for further use in pot experiments. An appropriate amount of soil was ground and sieved (2, 1, and 0.149 mm) for analysis of soil physicochemical properties, which are detailed in Table S1 of Supplementary Material (SI). *The China Soil Environmental Quality Risk Control Standard for Soil Pollution on Agricultural Land* (GB 15618-2018) specifies that the risk screening values for Cd, Pb, Cu, Zn, Cr, and Ni in soil are 0.6, 170, 100, 300, 250, and 190 mg kg^{-1}, respectively (pH > 7.5). Therefore, the obtained heavy metal data showed that the test soil was polluted by Cd, Pb, Cu, and Zn.

Apple tree branches are a rich natural woody material with a high lignin content and low ash and moisture content. This makes them suitable for producing high-quality porous biochar, which is a perfect raw material for charring biomass (Bai et al. 2019; Yang et al. 2020). Biochar from apple tree branches can provide additional binding sites for heavy metal immobilization (Wang et al. 2022). Moreover, it can enhance the physical and chemical characteristics of the soil, giving soil microbes a habitat, resource, and energy source. This can either directly or indirectly improve the microenvironment for microbial survival (Chen et al. 2020; Wang et al. 2020; Zhang et al. 2021). Pristine biochar was prepared by pyrolyzing apple tree branches at 500 °C for 2 h under limited oxygen conditions. To prepare P-modified biochar, apple tree branches were pretreated with K_3PO_4 solution and then pyrolyzed under the same conditions. Preparation and characterization of pristine biochar and P-modified biochar were carried out following the methods described in the previous study (Wang et al. 2022).

2.2 Experimental design

There were five treatment groups, each with three replicates (12 kg soil): (1) CK: with no soil amendment; (2) BC: the soil amended with pristine biochar (1.50%, w/w); (3) PM: the soil amended with mineral fertilizer (0.24% $K_3PO_4{\cdot}3H_2O$, w/w); (4) PB: soil amended with both mineral fertilizer (0.23% $K_3PO_4{\cdot}3H_2O$, w/w) and pristine biochar (1.5%, w/w); (5) PBC: soil amended with P-modified biochar (1.50%, w/w). According to a previous study, available phosphorus (AP) is the total phosphorus (TP) supplied by the amendments to plants (Li et al. 2020). In this study, AP contents in pristine and P-modified biochar were found to be 0.89 and 18.86 mg g^{-1}, respectively. Therefore, an appropriate amount of mineral phosphate fertilizer was added to PM and PB treatments to obtain the same initial AP as in PBC. The amendments were mixed thoroughly with the soil and incubated under dark conditions for 2 months at 65% field water holding capacity.

Several studies have demonstrated high concentrations of heavy metals in maize-producing areas. Maize has a high enrichment capacity for heavy metals. Therefore, maize was used as a test plant in this study. Maize seeds (Zhengdan 958) were sterilized, rinsed, and germinated at 25 °C for 3 days. Fifteen pots (3 replicates × 5 treatments) were set up according to a randomized complete block design, with one maize seedling planted in each PVC pot. All used maize seedlings were at the same growth stage. After planting maize seedlings, soil moisture content was maintained at 65% of the field capacity. Samples of plant tissue and soil were collected at the maturity stage (May 25–October 10). In particular, samples of seeds, roots, stems, and leaves were collected, cleaned, and dried at 65 °C until their mass remained consistent. After determining the dry weight, the dried plant samples were ground and sieved to determine the

heavy metal contents. The soil of each pot was mixed well and partitioned into two sub-samples. One sub-sample was air-dried, ground, and sieved (2, 1, and 0.149 mm) to determine the concentrations of bioavailable and total heavy metals and analyze basic soil properties. Another sub-sample was employed for the analysis of bacterial and fungal community composition.

2.3 Sample measurement

AP content in biochar was extracted by 0.5 M $NaHCO_3$ (pH 8.5) and quantified using the molybdenum antimony colorimetric method (Li et al. 2020; Wang et al. 2023).

The soil was suspended in water (soil/water ratio, 1: 2.5) to record its pH using a pH meter. The cation exchange capacity (CEC) and electrical conductivity (EC) of soil were measured using CH_3COONH_4 extraction and electrode method, respectively. Soil organic carbon (SOC) content was quantified using H_2SO_4–$K_2Cr_2O_7$ oxidation and $FeSO_4$ titration. Soil total nitrogen (TN) content was estimated by the semi-micro Kjeldahl method. Soil total phosphorus (TP) was determined by $HClO_4$–H_2SO_4 digestion and molybdenum antimony colorimetric method using a UV spectrophotometer (UV2450, Shimadzu, Japan) (Wang et al. 2023). Dissolved carbon (DOC) and dissolved nitrogen (DON) contents in soil were extracted by 0.5 M K_2SO_4 and measured by total organic carbon analyzer (TOC-VCPH, Shimadzu, Japan) and continuous flowing analyzer (AA3, SEAL, Germany), respectively (Jones and Willett 2006). The molybdenum antimony colorimetric technique and 0.5 M $NaHCO_3$ (pH 8.5) extraction were used to measure the AP of the soil (Wang et al. 2023). Following the digestion of soil samples with HNO_3–HCl–$HClO_4$, the concentrations of total Cd, Pb, Cu, Zn, Cr, and Ni in soil were determined using flame/graphite furnace atomic absorption spectrometry (PE 900T, Perkin Elmer, USA). To determine the concentration of bioavailable heavy metals, diethylene triamine penta-acetic acid (DTPA) was used for extraction (GB/T 23739-2009). Reference material (GBW07405, IGGE, Institute of Geological Sciences, Chinese Academy of Sciences, Hebei, China) was used as standard. The obtained metal recovery rates ranged from 95% to 105%.

Microbial DNA was extracted from fresh soil using FastDNA SPIN Kit for soil as instructed in the user manual. The NanoDrop2000 spectrophotometer (Thermo Scientific, USA) and gel electrophoresis (1.0% agarose) were used to assess the purity and concentration of the isolated DNA. Fungal ITS region and V3–V4 region of bacterial 16S rRNA gene were amplified by polymerase chain reaction (PCR) using the primers ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3')/ITS2R (5'-GCTGCGTTCTTCATCGATGC-3') and 338F (5'-ACTCCTACGGGAGGCAGCAG-3')/806R (5'-GGACTACHVGGGTWTCTAAT-3'), respectively. PCR products were outsourced to Majorbio Bioinformatics Technology Co., Ltd. (Shanghai, China) for sequencing using the Illumina MiSeq PE300 platform (Illumina, USA). Details of the sequencing process are presented in the Supporting Information (SI).

Following digestion with an acid solution (HNO_3: $HClO_4$ = 4: 1), the quantities of heavy metals in plant tissues were determined using flame/graphite furnace atomic absorption spectrometry (Duan et al. 2022). The standard substance for quality control was reference material (GBW-10012), which was acquired from the National Research Center for Certified Reference Materials to ensure accurate analysis.

2.4 Calculations

The bioconcentration factor (BCF) and translocation factor (TF) were determined to evaluate the capacity of the maize to absorb heavy metals from the soil and move them from the roots to the grains (Antoniadis et al. 2017; Chitimus et al. 2023).

$$BCF = \frac{Plantrootheavymetalconcentration}{Soilheavymetalconcentration} \quad (1)$$

$$TF = \frac{Plantgrainheavymetalconcentration}{Plantrootheavymetalconcentration} \quad (2)$$

2.5 Statistical analysis

SPSS 20.0 (IBM Inc., USA) was employed for statistical analyses. One-way analysis of variance (ANOVA) and Duncan's tests were employed to determine the significance of differences ($P < 0.05$). Pearson correlation analysis was performed in SPSS 20.0 to ascertain the relationships among the microbial community traits, cumulative relative abundance of modules, and soil physiochemical parameters. The Majorbio Cloud platform (https://cloud.majorbio.com) was employed for bioinformatic analysis of sequence data. The relative abundance of each soil microbial taxon was determined as the proportion of its abundance to the total microbial community abundance. Mothur v1.30.1 was used to determine the Chao1 and Shannon indices (alpha-diversity) of microorganisms based on the operational taxonomic units (OTUs). The "vegan" package in R 4.2.0 was employed to perform non-metric multidimensional scaling (NMDS) and analysis of similarities (ANOSIM) based on the Bray-Curtis distance. The "Hmisc" package in R 4.2.0 was employed to build microbial multitrophic ecological networks based on Spearman's correlations. The networks were visualized using Gephi. Furthermore, the

"pheatmap" package in R 4.2.0 and GraPhlAn software were used for classification and visualization, respectively. Details of the network construction process are provided in the supplementary materials. A partial least squares pathway model (PLS-PM) was established in R 4.2.0 using the 'plspm' package to examine the potential pathways involved in the response of microbial taxa to changes in soil characteristics and heavy metal bioavailability. Other figures were generated using Origin 2024 (OriginLab, USA), and data were presented as mean ± standard deviation (SD).

3 Results

3.1 Impact of biochar on soil physicochemical characteristics and bioavailability of heavy metals in soil

It is evident from Table S2 that the BC treatment increased the soil moisture content by 13.47%, while PBC decreased it by 44.71% compared to CK. The application of both biochar amendments resulted in a significant increase in soil pH compared to the CK. Compared to that in BC, soil pH was considerably higher in the PBC group ($P<0.05$). Furthermore, SOC content increased significantly after PBC, PB, and BC treatments, with SOC content in BC and PB groups being significantly more than that in the PBC ($P<0.05$). The TN concentration of the soil increased significantly with BC, PB, and PBC treatments, whereas it significantly decreased with PM treatment ($P<0.05$). The soil TP content was considerably higher in PBC, PB, and PM compared to that in BC and CK ($P<0.05$). Similarly, soil C/N was considerably higher in PBC, PB, and BC than in PM and CK ($P<0.05$). In comparison to the other three groups, the BC and PB treatments caused a significant increase in soil C/P. The soil C/P in BC was nearly twice as high as that in CK ($P<0.05$), while PM and PBC caused a significant decline in soil C/P ($P<0.05$). Highest soil N/P was observed in BC ($P<0.05$). Soil N/P decreased significantly ($P<0.05$) as a result of PB, PM, and PBC treatments; in PBC, soil N/P was almost twice as low as in CK. Soil DOC level was considerably higher in BC, PM, and PB, than in PBC and CK ($P<0.05$). Soil DON in BC was significantly higher ($P<0.05$) than that in PM, PB, and PBC. On the other hand, PM, PB, and PBC treatments led to a significant increase in soil AP, in comparison to BC and CK, with PBC treatment leading to the highest soil AP ($P<0.05$).

DTPA-extracted heavy metal contents in soil were considered the bioavailable heavy metal contents (Cd, Zn, Pb, and Cu) in this study. The soil of the BC group demonstrated significantly lower DTPA-Cd content than CK ($P<0.05$) (Table 1). PM and PB had significantly lower levels of DTPA-Pb and DTPA-Cd than CK and BC ($P<0.05$). The DTPA-Cd content in PBC soil was significantly lower than that in CK, PB, and BC, while its DTPA-Pb content was considerably lower than that in the other four groups. Furthermore, DTPA-Zn content in PBC was significantly less than that in PB and CK ($P<0.05$).

Table 1 Concentrations of bioavailable heavy metals in soil under different treatments (mg kg^{-1})

Treatment	DTPA-Cd	DTPA-Pb	DTPA-Cu	DTPA-Zn
CK	0.39 ± 0.01 a	26.19 ± 1.54 a	13.31 ± 0.25 a	162.54 ± 6.19 a
BC	0.36 ± 0.01 b	26.21 ± 0.78 a	13.20 ± 0.45 a	157.42 ± 6.12 ab
PM	0.30 ± 0.02 cd	21.38 ± 0.65 b	13.32 ± 0.35 a	160.60 ± 8.64 ab
PB	0.30 ± 0.00 c	20.83 ± 1.28 b	13.33 ± 0.63 a	165.00 ± 6.40 a
PBC	0.28 ± 0.00 d	18.69 ± 0.81 c	12.59 ± 0.43 a	149.77 ± 1.55 b

DTPA-Cd, DTPA-Pb, DTPA-Cu, and DTPA-Zn indicate Cd, Pb, Cu, and Zn extracted by DTPA, respectively. CK, with no soil amendment; BC, soil amended with pristine biochar; PM, soil amended with mineral fertilizer; PB, soil amended with both mineral fertilizer and pristine biochar; PBC, soil amended with P-modified biochar. The lower-case letters indicate significant differences among different treatments via ANOVA followed by Duncan's post-test ($P<0.05$)

3.2 Plant growth and heavy metal content in plant organs

The dry weight and height of plant samples collected from each treatment group are presented in Table S3. Plant height increased in both BC and PBC treatments, with PBC plants significantly taller than CK ones ($P<0.05$). Meanwhile, PBC and BC treatments resulted in an increase in the dry weights of all four plant tissues compared to CK. The dry weights of stems, grains, and leaves of maize in BC were significantly higher than those in CK ($P<0.05$), while the dry weights of these plant organs in PBC were considerably higher than those in BC and CK ($P<0.05$).

Figure S1 shows the heavy metal concentrations in various organs of maize. Compared to the CK, Cd concentrations in maize roots, stems, and leaves in the BC group were reduced by 35.00%, 32.24%, and 15.37%, respectively ($P<0.05$). In the PBC group, Cd concentrations in maize roots, stems, leaves, and grains decreased by 51.73%, 57.03%, 26.38%, and 36.52%, respectively. Maize roots and stems in BC showed significantly lower Pb concentrations compared to those in CK ($P<0.05$). Similarly, Pb concentrations in maize stems, roots, grains, and leaves in the BC group were decreased by 37.46%, 17.94%, 15.77%, and 7.39%, respectively, compared to the CK. In the PBC group, Pb concentrations in maize roots, stems, leaves, and grains reduced by 27.64%, 62.82%, 8.73%, and 61.82%, respectively, relative to CK. However, the decrease in Pb content in leaves was not significant. Fig. S1 shows that the BC treatment significantly decreased

the amount of Cu in the roots and leaves of maize ($P<0.05$), with the Cu content in the roots, leaves, and grains being 13.46%, 50.90%, and 11.70% lower, respectively. The Cu content of maize roots, stems, leaves, and grains decreased by 21.92%, 60.07%, 60.62%, and 36.93%, respectively, as a result of PBC treatment ($P<0.05$).

The BCF and TF of maize for the four heavy metals are presented in Table S4. The BC treatment significantly reduced the BCF of maize for Cd (0.996), Cu (0.436) and Zn (0.096), and PBC treatment significantly reduced the BCF of maize for Cd (0.725), Pb (0.103), Cu (0.388) and Zn (0.090), and the BCF of maize to Cd in the PBC treatment was significantly smaller than that in the BC treatment ($P<0.05$). Moreover, the TF of maize for Cd and Zn was significantly reduced by BC treatment, and the TF of maize for Pb was significantly reduced by PBC treatment.

3.3 Response of soil microorganisms

3.3.1 Alpha diversity

The diversity and richness of microbial community in soil samples were assessed by alpha diversity analysis (i.e., Shannon and Chao1 indices) (Fig. 1). The Chao1 index of bacteria significantly decreased after PBC treatment. The bacterial Chao1 index in PBC was significantly lower than that in BC ($P<0.05$) (Fig. 1a), indicating a reduction in soil bacterial richness following the addition of P-modified biochar. The soil in BC demonstrated a significantly higher Shannon index ($P<0.05$) of bacteria compared to that in CK (Fig. 1b), which indicated an increase in the diversity of bacteria in the soil after the addition of BC. Furthermore, in comparison to CK, Shannon indices of soil bacterial community in PBC were significantly lower ($P<0.05$) (Fig. 1b), indicating a decrease in bacterial diversity after the amendment of soil with P-modified biochar. BC and PBC treatments demonstrated no significant effect on the fungal alpha diversity in soil (Fig. 1d and e).

3.3.2 Beta diversity

The variations in the composition of soil microbial communities among various treatment groups were studied using NMDS. The treatments significantly changed the structure and composition of bacterial (Fig. 1c) and fungal (Fig. 1f) communities ($P=0.001$). Soil bacterial

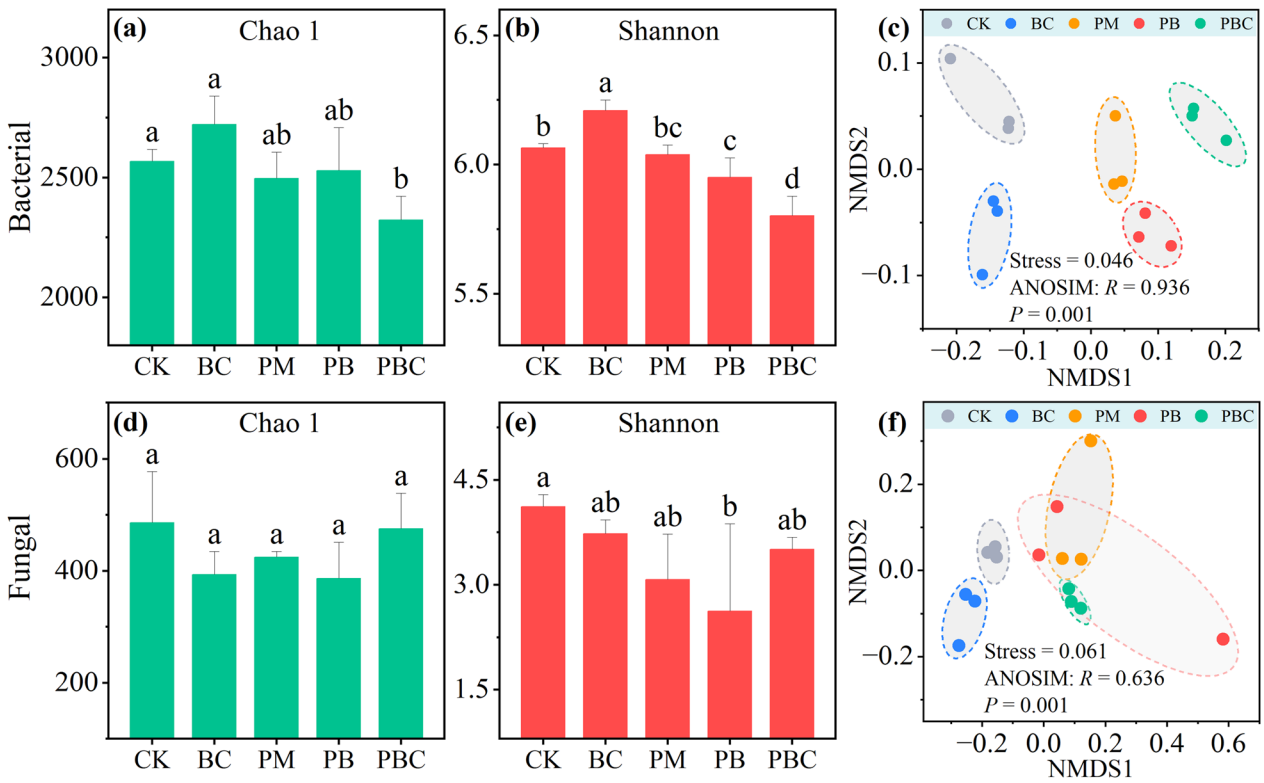


Fig. 1 Alpha and beta diversities of soil microbes under different treatments. *Note* Effects of different treatments on alpha diversity of the bacterial ((**a**) and (**b**)) and fungal ((**d**) and (**e**)) communities in the soil. The non-metric multidimensional scaling (NMDS) analysis plots of bacterial communities (**c**) and fungal communities (**f**). CK, control; BC, pristine biochar; PM, mineral fertilizer; PB, mineral fertilizer + pristine biochar; PBC, P-modified biochar. The lower-case letters indicate significant differences among different treatments via ANOVA followed by Duncan's post-test ($P<0.05$)

communities in the treatment groups were significantly different than those in CK, with no intersect of confidence range between the treatments (Fig. 1c). PBC, PB, and PM groups interacted with one another for soil fungal communities; however, the fungal communities in PBC and BC differed greatly from those in CK (Fig. 1f). Microbial community composition (beta-diversity) was represented by the first axis of NMDS analysis (NMDS1).

3.3.3 Soil microbial community composition

A total of 14 bacterial phyla were detected in different treatment groups (Fig. S2a), including Proteobacteria, Actinobacteriota, Acidobacteriota, Chloroflexi, Gemmatimonadota, Firmicutes, Bacteroidota, Cyanobacteria, Myxococcota, Patescibacteria, Nitrospirota, Verrucomicrobiota, Methylomirabilota, and Deinococcota. Among these phyla, Actinobacteriota, Proteobacteria, Chloroflexi, Acidobacteriota, and Gemmatimonadota were the dominant phyla. A total of 17 bacterial genera were detected (Fig. S2b) with relative abundance > 1%. A total of 6 fungal phyla were observed (Fig. S2c), including Basidiomycota, Ascomycota, Chytridiomycota, Mortierellomycota, Olpidiomycota, and Glomeromycota. Among these phyla, Ascomycota, Basidiomycota, Mortierellomycota, and Chytridiomycota were observed as the dominant phyla. A total of 23 fungal genera (relative abundance > 1%) were detected in the soil (Fig. S2d).

3.4 Keystone taxa of microbes in the soils of different treatment groups

To further explore the relationships between keystone microbial taxa and soil properties, a multitrophic network of microbes was established (Fig. 2). Four major microbial taxa (modules 1–4) constituted the network; they accounted for 21.60%, 18.94%, 18.64%, and 14.79% of all network nodes, respectively (Fig. 2b). Module 1 comprised 63% bacteria and 37% fungi, while module 2 contained 86% bacteria and 14% fungi. Likewise, module 3 was predominantly composed of 98% bacteria and 2% fungi (Fig. 2c). The three microbial taxa corresponding to modules 1–3 showed the highest number of nodes (Fig. 2b) and cumulative abundance (Fig. 2d).

In comparison to CK, cumulative relative abundances of module 1 in BC and PBC groups were significantly higher ($P < 0.05$) and lower ($P < 0.01$), respectively, with cumulative relative abundance of module 1 in PBC being considerably less than that in BC ($P < 0.001$) (Fig. 2d). Whereas, cumulative relative abundances of modules 2 and 3 increased significantly after PBC treatment, in comparison to CK ($P < 0.05$) and BC ($P < 0.01$) treatments. There was no significant difference between the cumulative abundances of modules 2 and 3 in the CK and BC groups (Fig. 2d).

Heatmaps and GraPhlAn were employed to display the abundances of the microbial taxa associated with modules 1 and 3 (Figs. 3, S3, and S4). Application of P-modified biochar resulted in a reduction in the relative abundance of most genera in module 1 (Fig. S3), while the abundance of most genera in module 3 increased (Fig. S4). Acidobacteriota, Actinobacteriota, and Proteobacteria were the main bacterial phyla in module 1, which included 2, 6, and 7 bacterial genera, respectively (Fig. 3). Ascomycota was the main fungal phylum in module 1, including 12 fungal genera (Fig. 3). Actinobacteriota and Proteobacteria were the dominant bacterial phyla in module 3, which included 3 and 5 bacterial genera, respectively (Fig. 3). Ascomycota was the dominant fungal phylum in module 3, which included 5 fungal genera, namely *Fusarium*, *Gibberella*, *Neocosmospora*, *Penicillium* and *Sarocladium* (Fig. 3).

3.5 Relationships of soil keystone microbial taxa with properties, bioavailable heavy metals, and microbial communities in soil

Results of Pearson correlation analysis (Table 2) revealed highly significant correlations of module 1 with TP, C/P, N/P, DON, AP, DTPA-Cd, DTPA-Pb, and DTPA-Cu ($P < 0.01$). Similarly, significant ($P < 0.05$) or highly significant ($P < 0.01$) correlations of module 3 were observed with pH, TP, N/P, DON, AP, DTPA-Cd, and DTPA-Pb. On the other hand, there were no significant correlations between module 2 and soil properties. There were also no significant correlations between module 4 and most of the soil properties (Table 2). Overall, most of the microbial taxa (modules 1 and 3) demonstrated significant or even highly significant correlations with soil TP, C/P, N/P, DON, AP, DTPA-Cd, and DTPA-Pb (Fig. S5). Therefore, modules 1 and 3 were considered as the keystone taxa of soil microbes in this study.

Based on the Pearson correlation analysis, the cascading relationships of biochar treatment with soil properties, heavy metal bioavailability, and soil keystone microbial taxa were further analyzed by PLS-PM. The findings demonstrated that biochar directly affects the fundamental physicochemical characteristics of soil (i.e., soil pH, moisture, TP, C/P, N/P, DON, and AP) and bioavailability of Cd, Pb, and Cu. The findings also demonstrated that biochar indirectly affects keystone microbial species (modules 1 and 3) by controlling the DON and AP contents in soil (Fig. 4a). Furthermore, keystone microbial taxa were most affected overall by soil DON and AP concentrations (0.884) (Fig. 4b). Biochar addition significantly affected the bioavailability of heavy metals.

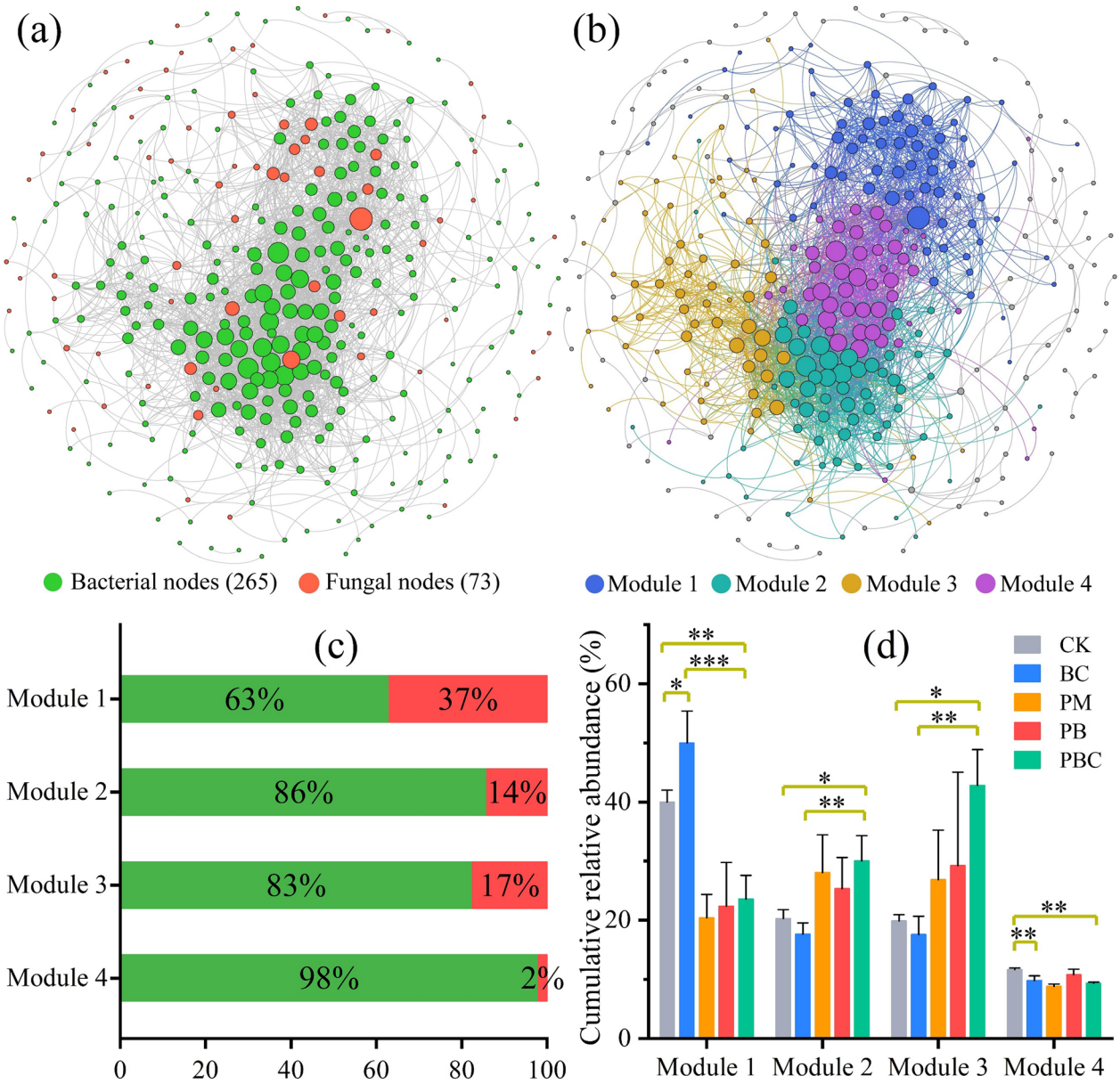


Fig. 2 Keystone microbial taxa. Note: (**a**) Multitrophic network constructed including bacterial and fungal genera, microbial kingdoms in the network indicated by colors of node; (**b**) Microbial taxa constructed based on multitrophic network (module 1 – 4), Modules in the network indicated by colors of node; (**c**) Microbial composition in module 1 – 4; (**d**) Cumulative relative abundance of modules in different samples. Asterisks indicate statistically significant differences (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$). CK, with no soil amendment; BC, soil amended with pristine biochar; PM, soil amended with mineral fertilizer; PB, soil amended with both mineral fertilizer and pristine biochar; PBC, soil amended with P-modified biochar

However, this change did not have any significant effect on the keystone microbial taxa (Fig. 4).

Linear regression analysis demonstrated that the cumulative abundances of modules 1 and 3 were significantly ($P < 0.05$) or highly significantly ($P < 0.01$) correlated with the Chao1, Shannon, and NMDS1 indices of bacterial community (Fig. 5). Meanwhile, the cumulative abundance of module 1 showed significant ($P < 0.05$) or highly significant ($P < 0.01$) relationships with the Shannon and NMDS1 indices of fungi community (Fig. 5).

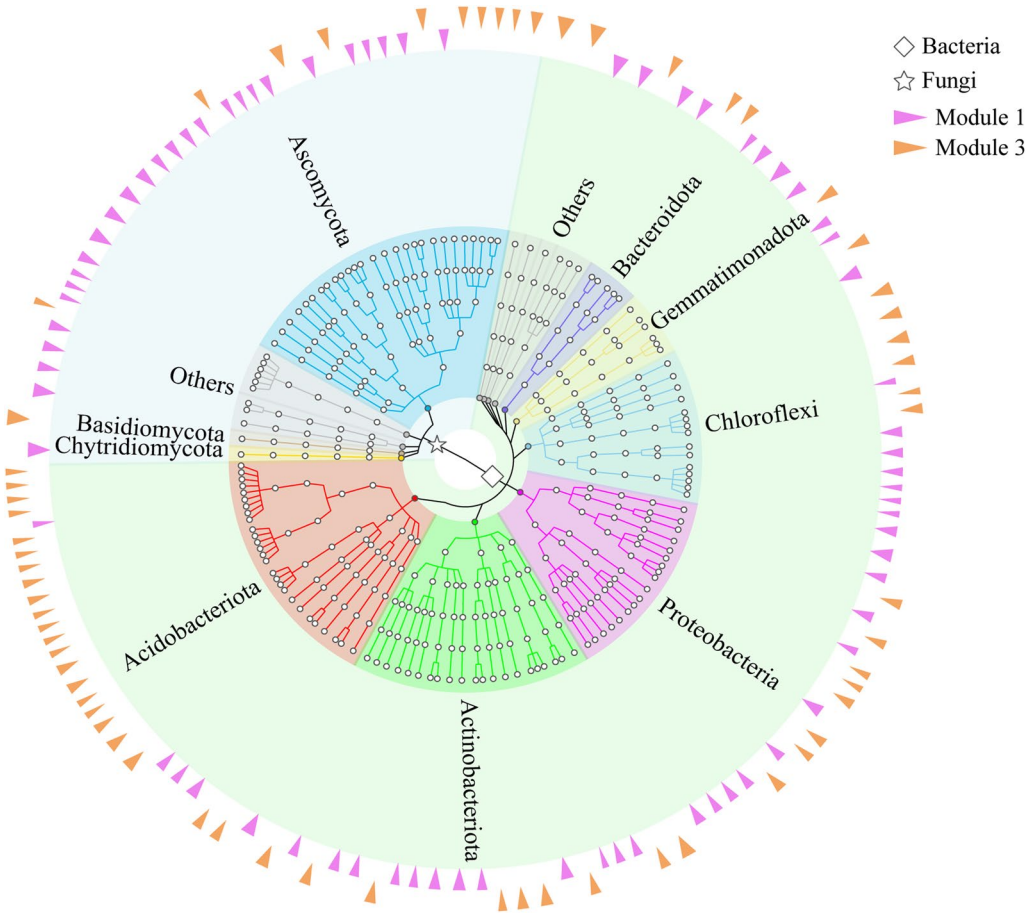


Fig. 3 Taxonomic classification of microbes in modules 1 and 3. *Note*: Inner ring: microorganisms in module 1, outer ring: microorganisms in module 3. Kingdom: ◇, Bacteria; ☆, Fungi

4 Discussion

4.1 Mechanisms of heavy metal immobilization by biochar

DTPA-extracted heavy metals represent the fraction of heavy metals that are readily bioavailable for plant uptake. The study demonstrated that adding biochar significantly decreased the amounts of DTPA-Pb and DTPA-Cd in the soil (Table 1). Pristine biochar also significantly reduced the BCF of maize for Cd, Cu, and Zn (Table S4). This might be a result of the alkaline biochar increasing the pH of the soil and encouraging heavy metal precipitation (Table S2). On the other hand, porous structure, large specific surface area, and numerous functional groups enable the biochar to rapidly adsorb the free heavy metals from soil and effectively immobilize them on its surface (Ren et al. 2019). DTPA-Cu, DTPA-Cd, DTPA-Pb, and DTPA-Zn contents significantly decreased with the addition of P-modified biochar; the decreases in DTPA-Cd, DTPA-Pb, and DTPA-Zn reached highly significant levels ($P < 0.001$). DTPA-Pb and DTPA-Cd contents in the PBC group were considerably less than those in the BC group ($P < 0.001$) (Table 1). Furthermore, P-modified biochar significantly reduced the BCF of maize for Cd, Pb, Cu, and Zn, with the BCF for Cd being significantly lower than that observed in the BC treatment (Table S4). Previous adsorption and incubation experiments confirmed that P-modified biochar efficiently immobilizes soil heavy metals mainly through co-precipitation of phosphate with heavy metals and cation exchange mechanism (Fig. S6) (Wang et al. 2022, 2023, 2024). Meanwhile, alkaline conditions are more favorable for the formation of heavy metal precipitation (Qiu et al. 2022). The results showed that the PBC treatment not only increased the soil pH, but also led to higher TP and AP contents (Table S2), which created favorable conditions for the precipitation of heavy metals such as $M_3(PO_4)_2$, $M_5(PO_4)_3OH$, and $M_5(PO_4)_3Cl$ (M stands for Cu, Zn, Pb, and Cd) (Kim et al. 2015). This finding is consistent with previous studies that reported a significant improvement in the immobilization of Zn, Cd, Pb, and Cu in soil by P-modified biochar (Gao et al. 2020; Zhang et al. 2019).

P-modified biochar reduced the concentration of heavy metals in plants (Fig. S1), by reducing the concentration

Table 2 Pearson correlations between cumulative relative abundance of different modules and soil properties

Soil property	Cumulative relative abundance							
	Module 1		Module 2		Module 3		Module 4	
Moisture	**0.763**	**	−0.156	ns	**−0.651**	**	**0.576**	*
pH	−0.413	ns	0.160	ns	**0.584**	*	**−0.701**	**
EC	**0.730**	**	−0.332	ns	**−0.590**	*	**0.690**	**
CEC	−0.156	ns	0.033	ns	−0.091	ns	**0.622**	*
SOC	0.251	ns	0.143	ns	0.062	ns	0.087	ns
TN	0.442	ns	0.105	ns	−0.162	ns	0.302	ns
TP	**−0.668**	**	0.263	ns	**0.779**	**	−0.446	ns
C/N	0.117	ns	0.155	ns	0.234	ns	−0.022	ns
C/P	**0.706**	**	−0.123	ns	−0.439	ns	0.252	ns
N/P	**0.850**	***	−0.251	ns	**−0.678**	**	0.458	ns
DOC	0.058	ns	0.219	ns	−0.179	ns	**−0.566**	*
DON	**0.779**	**	−0.250	ns	**−0.565**	*	0.082	ns
AP	**−0.714**	**	0.308	ns	**0.783**	**	−0.450	ns
DTPA-Cd	**0.810**	***	−0.313	ns	**−0.590**	*	**0.590**	*
DTPA-Pb	**0.839**	***	−0.305	ns	**−0.757**	**	0.339	ns
DTPA-Cu	**−0.796**	***	0.455	ns	0.284	ns	0.052	ns
DTPA-Zn	−0.494	ns	0.335	ns	−0.078	ns	−0.007	ns

Bold values indicate statistically significant correlations between indicators

EC, electrical conductivity; CEC, cation exchange capacity; SOC, soil organic carbon; TN, total nitrogen; TP, total phosphorus; C/N, SOC/TN; C/P, SOC/TP; N/P, TN/TP; DOC, dissolved organic carbon; DON, dissolved organic nitrogen; AP, available phosphorus; DTPA-Cd, DTPA-Pb, DTPA-Cu, and DTPA-Zn indicate Cd, Pb, Cu, and Zn extracted by DTPA, respectively. Asterisks indicate a significant correlation between indicators (*, $P<0.05$; **, $P<0.01$; ***, $P<0.001$)

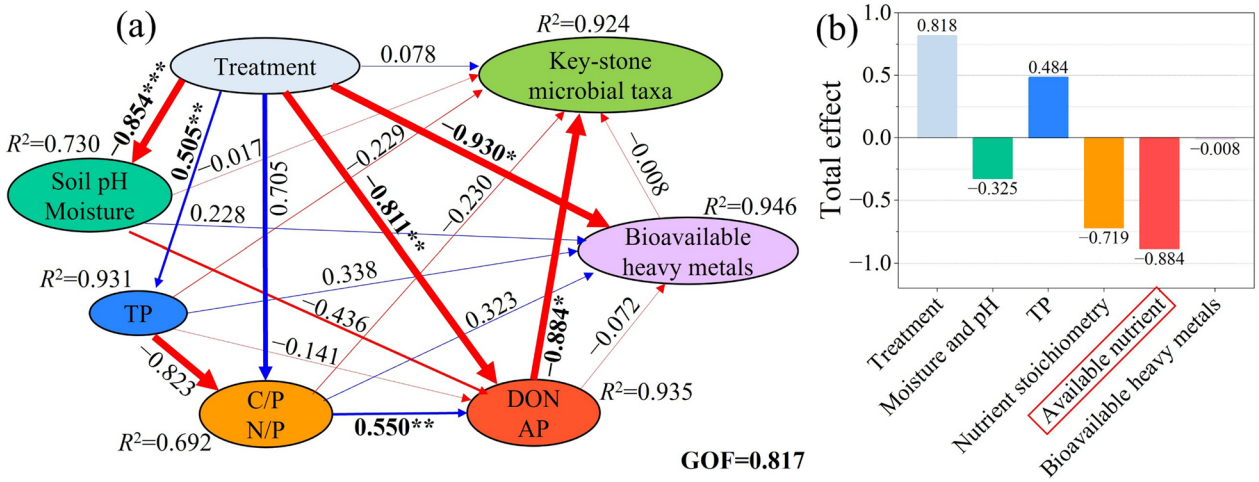


Fig. 4 Cascading relationships of keystone microbial taxa with soil properties and bioavailable heavy metal contents. *Note*: Partial least squares path modelling (PLS-PM) disentangling major pathways of the effects of soil properties and bioavailable heavy metals on soil keystone microbial taxa (**a**); as well as the total effects of each variable on soil keystone microbial taxa (**b**). Red and blue arrows indicate negative and positive flows of causality, respectively; R^2 indicates the dependent variable variance explained by the model; Numbers on the arrow indicate significant standardized path coefficients (*: $P<0.05$; **: $P<0.01$). Treatment includes CK (with no soil amendment), BC (soil amended with pristine biochar), PM (soil amended with mineral fertilizer), PB (soil amended with both mineral fertilizer and pristine biochar), and PBC (soil amended with P-modified biochar); TP, total phosphorus; C/P, soil organic carbon/TP; N/P, total nitrogen/TP; DON, dissolved organic nitrogen; AP, available phosphorus; bioavailable heavy metals includes DTPA-Cd, DTPA-Pb, and DTPA-Cu; keystone microbial taxa includes module 1 and 3

of DTPA-extracted heavy metals in soil and controlling the uptake of heavy metals by plant roots and stems (Ahmad et al. 2018; Zhang et al. 2019). P-modified biochar improves soil fertility by providing more N, P, and K nutrients than P fertilizer or pristine biochar alone (Table S2). The improved soil fertility further results in

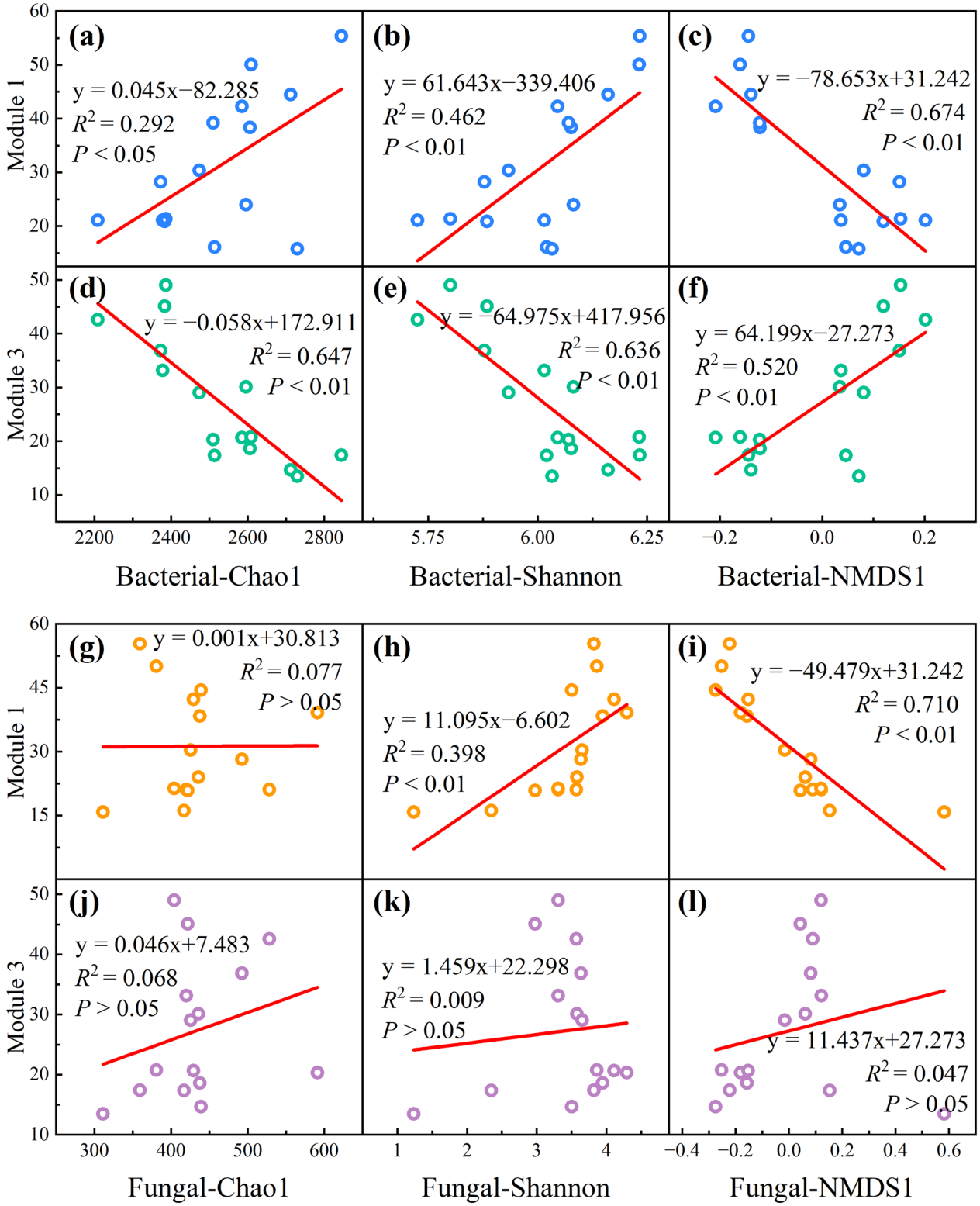


Fig. 5 Linear regression analyses of soil keystone microbial taxa and microbial community indices

enhancement in plant biomass. Higher biomass reduces the heavy metal content in plants through dilution, thus indirectly increasing plant resistance to heavy metal toxicity (Liu et al. 2020b). Moreover, P availability reduces the accumulation of heavy metals in plants and inhibits their transport from roots to stems beyond a certain threshold limit (Jia et al. 2020; Liu et al. 2019). However, this threshold limit varies with soil physicochemical properties and plant species. In turn, plants also regulate the bioavailability of heavy metals in soil by secreting organic acids, enzymes, and other substances through their roots, particularly during the rapid growth phase (Liu et al. 2019).

4.2 Microbial response to biochar addition into soils pollutes with heavy metals

The stability and functionality of the soil ecosystem depend on soil microbes, which also help to reduce external environmental pressures and disturbances (Wu et al. 2019). In this study, biochar addition caused significant changes in soil physicochemical properties and heavy metal bioavailability (Tables 1 and S1), which further led to variations in microbial communities in soil (Wu et al. 2008). BC and PBC treatments demonstrated significant effects on the richness and diversity of bacterial communities in soil (Fig. 1a and b). However, changes in the richness and diversity of fungi were not statistically significant (Fig. 1d and e). Pristine biochar increased the diversity and richness of bacteria in the soil to a certain extent through the rapid degradation of soluble components of biochar in a short period (Table S1), providing ample sources of material and energy to bacteria. Furthermore, the porous structure and large specific surface area of biochar provided a good habitat for bacteria, thus facilitating the growth and development of bacteria (Qi et al. 2022b). However, the addition of P-modified biochar caused a decrease in the diversity and richness of bacteria in soil (Fig. 1a and b). This may be attributed to two reasons. Firstly, P-modified biochar significantly increased the soil pH (Table S2), which was unfavorable for the growth of some bacteria (Liu et al. 2021). Secondly, P-modified biochar caused an evident increase in AP and TP contents in the soil (Table S2), therefore disrupting the nutrient balance in the soil, which led to a reduction in bacterial richness and diversity (Wang et al. 2024). Previously, Liu et al. (2020a) also reported changes in the structure of the soil bacterial community and reduced bacterial richness and diversity after the addition of P fertilizer into the soil. Similarly, Wang et al. (2015) showed a significant reduction in the alpha diversity of soil bacteria due to soil amendment with modified biochar. These findings suggest that the effects of biochar on microbial communities are driven by a variety of factors including biochar type and properties of soil.

4.3 Key drivers of the changes in soil microbial community

Based on the microbial co-occurrence network (Fig. 2b), four key microbial taxa were identified to further investigate the mechanism behind the regulation of soil microbes by biochar under compound heavy metal pollution. These taxa not only indicated habitat heterogeneity, diversity selection mechanism, and phylogenetic relationships but also played important roles in regulating soil ecology, nutrient cycling, and maintaining the stability of microbial community (Pan et al. 2022). Biochar treatment significantly changed the cumulative relative abundance of these four microbial taxa in soil (Fig. 2d). The taxa corresponding to modules 1 and 3 were defined as the key microbial taxa in this study, based on their highest cumulative relative abundance and significant correlations with soil physicochemical properties and bioavailable heavy metals (Fig. 2d and Table 2). Linear regression analysis showed that the structure of the bacterial community and the abundance of microorganisms in modules 1 and 3 were significantly correlated (Fig. 5). Therefore, it was concluded that biochar affected the abundance, diversity, and structure of bacterial communities by altering the abundance of key microbial taxa.

PLS-PM analysis demonstrated that soil DON and AP contents were the primary driving factors of the changes in key microbial taxa (modules 1 and 3), followed by elemental stoichiometric ratios (Fig. 4). Module 1 cumulative relative abundance was negatively and positively correlated with soil P (including TP and AP) and DON, respectively, whereas module 3 cumulative relative abundance was positively and negatively correlated with soil P and DON respectively (Table 2). Thus, modules 1 and 3 played important but opposite roles in N and P cycling in soil (Pan et al. 2022). Pristine biochar caused relative P deficiencies by disrupting the balance of soil elements via increasing the C/P, N/P, and DON, in soil (Table S2). As a result, pristine biochar forced the soil microbial communities to promote P activation and adapt to elemental imbalances by increasing the cumulative abundance of module 1 (Fig. 2). This finding was strongly supported by the strong negative correlation of module 1 with soil TP and AP contents and a very strong positive correlation with soil N/P (Table 2). Most of the bacteria in module 1 belonged to Actinobacteriota and Proteobacteria phyla, while most of the fungi in this module belonged to Ascomycota (Figs. 3 and S3). These microbes play important roles in soil microbial ecology by decomposing the organic compounds in soil to release nutrients for plants and participating in various biogeochemical cycles in the soil (Xu et al. 2012). DOC and C/N were significantly

positively correlated with Actinobacteriota, but DON and N/P were negatively correlated with Protobacteria. Meanwhile, Ascomycota exhibited highly significant negative correlations with TP and AP. These correlations confirmed the close relationships between the microbial taxa of module 1 and soil nutrient cycling (Fig. S5). However, PBC treatment caused the relative deficit of N by significantly increasing soil TP and AP contents while decreasing soil DON, N/P, and C/P (Table S2), thereby promoting an increase in the cumulative relative abundance of microbial taxa corresponding to module 3 that were able to use other N sources to improve N supply and maintain soil ecological balance (Fig. 2). The significant negative relationship between DON and the cumulative relative abundance of module 3 microorganisms, as well as the positive correlations of module 3 microbes with soil TP and AP contents, supported this conclusion (Table 2). Most of the bacteria in module 3 belonged to Actinobacteriota and Proteobacteria, while the fungi belonged to Ascomycota (Figs. 3 and S4). Actinobacteriota, Proteobacteria, and Ascomycota showed a very strong positive relationship with soil TP and a very strong negative relationship with N/P. Furthermore, Actinobacteriota and Ascomycota showed a significant negative correlation with DON (Fig. S5). Moreover, the presence of N-cycling-related microbial genera in module 3 (e.g., *Mesorhizobium, Ellin6067*, etc.) further supported these findings (Fig. S4).

Additionally, PLS-PM results suggested that the cumulative effect of bioavailable heavy metals on key microbial taxa (modules 1 and 3) was almost negligible (Table 2). However, it is worth noting that the cumulative abundance of module 1 was higher in the CK and BC groups that showed high heavy metal availability. On the other hand, the cumulative abundance of module 3 was greater in PM, PB, and PBC groups that showed low availability of heavy metals (Fig. 2d). There are two possible explanations for these changes. Firstly, the difference in the resistance of different microorganisms to heavy metals led to corresponding changes in the soil microbial community structure (Hong et al. 2015), including a reduction in the abundance of some metal-sensitive bacteria and an increase in the abundance of metal-resistant bacteria (Zhu et al. 2023). Secondly, the P content in BC and CK groups was low despite the relatively high levels of bioavailable heavy metals, whereas the decrease in bioavailable heavy metals was simultaneously followed by the increase in P content in PBC, PB, and PM groups (Tables 1 and S1). This indicated that the changes in soil nutrient levels may significantly affect the microorganisms. The significant correlation between bioavailable heavy metals and the key microbial taxa might only be a coincidence. PLS-PM results suggest that the second explanation is more reasonable (Fig. 4). In most cases, bioavailable heavy metals exhibited negative effects on soil microbes (Wang et al. 2021). Heavy metals not only cause toxicity to microorganisms, inducing structure changes in organisms via breaking single and double strands or altering the bases (Wood et al. 2016), but also reduce the utilization of carbon by microorganisms and inhibit microbial activity (Kaplan et al. 2014). In particular, heavy metals directly affect the microbial community structure at high concentrations. Compared to fungi, bacteria are more sensitive to heavy metals (Wood et al. 2016). However, several studies have demonstrated the positive effects of heavy metals on organisms (Carvalho et al. 2020; Fan et al. 2021). This effect may be because of hormesis, which is characterized by the presence of toxicological thresholds, promoted by low-doses, and inhibited by high doses of heavy metals (Fan et al. 2021). Similar to the present study, some studies reported no significant relationship between heavy metals and microbial community characteristics (Grandlic et al. 2006; Zhu et al. 2013). Gillan et al. (2005) reported that soil microbial biomass and diversity were not related to heavy metal content. Similarly, Grandlic et al. (2006) showed no impact of Pb on bacterial communities. This may be partly due to the low concentration or bioavailability of heavy metals in the research sites. Furthermore, changes in soil properties, such as SOC, nutrient levels, pH, etc. may also lead to changes in microbial community (Hu et al. 2021; Yang et al. 2023). This study found that the effects of changes in heavy metal bioavailability were obscured by the effects on microbial communities of changes in the soil nutrient supply.

5 Conclusions

P-modified biochar can significantly reduce soil heavy metal bioavailability compared to unmodified biochar, mainly through co-precipitation and cation exchange mechanisms, thereby reducing heavy metal uptake by plants. Meanwhile, the application of biochar disrupts the native balance of soil N and P supply. Key microbial taxa (modules 1 and 3) that have opposing but crucial functions in the cycling of soil N and P had to adapt. This ultimately led to alterations in the structure and composition of microbial communities in the soil. Particularly, changes in heavy metal bioavailability demonstrated minimal effect on the soil microbial community. This finding provides new microbiological insights into the application of P-modified biochar in heavy metal-contaminated soils. Overall, this study indicates that P-modified biochar has excellent remediation ability for heavy metal contaminated soils. However, because of the complicated environmental circumstances in the

field, more field remediation studies are still required to confirm the immobilizing effect of P-modified biochar on heavy metals.

Abbreviations

ANOSIM	Analysis of similarities
AP	Available P
BCF	Bioconcentration factor
Cd	Cadmium
CEC	Cation exchange capacity
Cu	Copper
DOC	Dissolved carbon
DON	Dissolved nitrogen
DTPA	Diethylene triamine penta-acetic acid
EC	Electrical conductivity
NMDS	Non-metric multidimensional scaling
P	Phosphorus
Pb	Lead
PLS-PM	Partial least squares pathway model
SOC	Soil organic carbon
TN	Total nitrogen
TF	Translocation factor
TP	Total phosphorus
Zn	Zinc

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1007/s42773-025-00495-7.

Additional file1 (DOCX 5801 KB)

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Data availability
The datasets used or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests
The authors have no relevant financial or non-financial interests to disclose.

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