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# Biochar enhanced phytoremediation efficiency of *Salix* for soil cadmium: the differentiated responses of bacteria and fungi to biochar and rhizosphere effects

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## Abstract

Biochar is suggested to enhance the phytoremediation of cadmium (Cd) via regulating the rhizosphere environment and plant traits in contaminated soil. However, the effect of phosphorus (P)-modified biochar, the rhizosphere effect, and their interaction in improving phytoremediation efficiency of *Salix* for Cd remains unclear. Here, the effects of bamboo biochar, phytic acid-modified biochar, and sodium phytate-modified biochar on soil properties, the microbial community, plant traits, and Cd accumulation of *Salix J1010* in Cd contaminated soil were comparatively and systematically studied. P-modified biochar significantly increased plant growth, Cd accumulation, and its translocation from roots to the aboveground parts of *Salix*. Cd concentration, root biomass, net photosynthetic rate, and rhizosphere microbial community variations were identified as critical predictors for phytoremediation efficiency using random forest models. Rhizosphere bacteria were more influenced by biochar amendment, while the fungi were more influenced by the rhizosphere effects. A key bacterial cluster, with a preference for high soil carbon and P, was further found to stimulate root growth and improve the bioavailability of soil Cd. Collectively, the study revealed differentiated responses of bacteria and fungi to biochar and rhizosphere effects of *Salix*, highlighting the importance of biochar modifications to optimize microbial interactions and enhance the phytoremediation efficiency of *Salix* in Cd-contaminated soils.

## Highlights

- Biochar increased bacterial diversity in the unplanted soil, while reducing the diversity in rhizosphere of *Salix*
- Phytic acid-modified biochar significantly promoted root biomass, translocation, and accumulation of Cd in *Salix J1010*
- A key bacterial cluster with a preference for P is pivotal in enhancing root biomass and Cd bioavailability

**Keywords** Heavy metals, Dendroremediation, Phytic acid, Sodium phytate, Plant-biochar interactions

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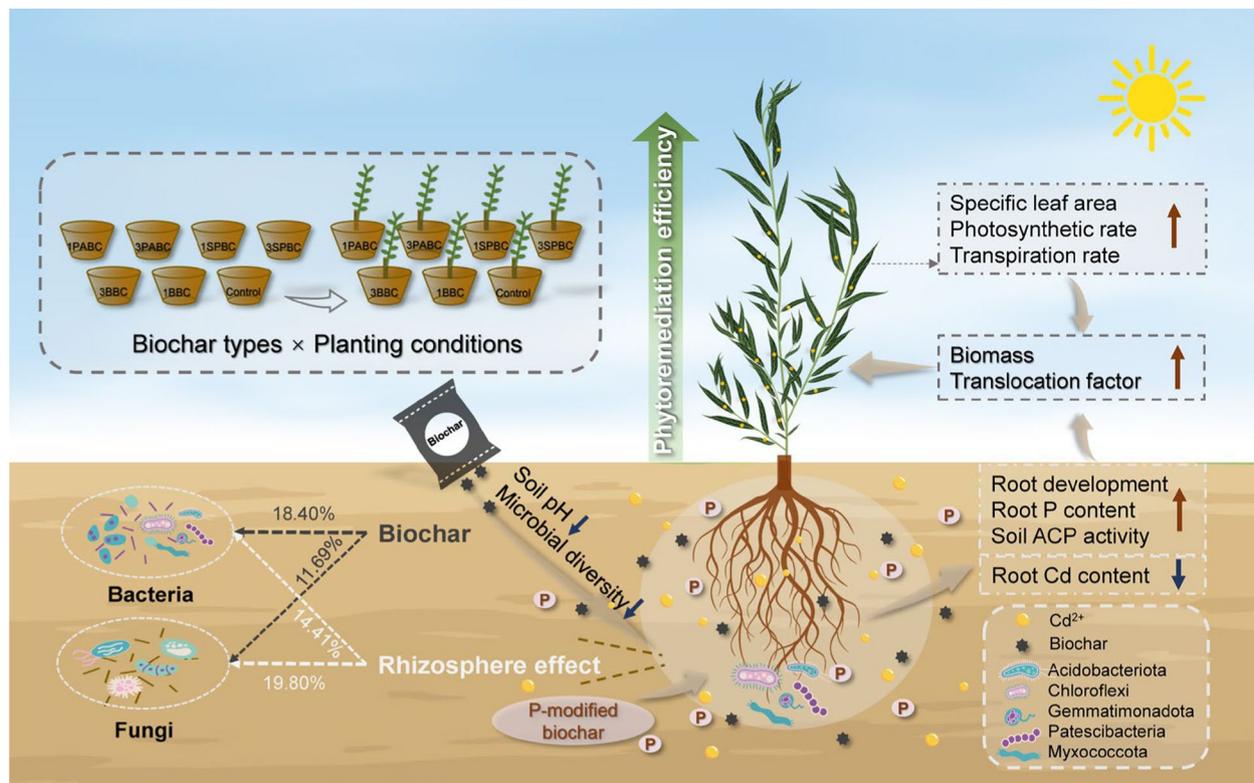
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Graphical Abstract



1 Introduction

Heavy metals (HMs), particularly cadmium (Cd), with high mobility and persistence in the environment, threaten soil, plants, and public health (Sarker et al. 2023; Li et al. 2024a). Phytoremediation is an economical and eco-friendly technology, leveraging the combined actions of plant roots and microbes to immobilize Cd from the soil (Xu et al. 2024; Xiao et al. 2024). Willows (*Salix* spp.) are considered excellent species for phytoremediation owing to their rapid growth, high biomass, deep roots, high adaptability, and easy propagation (Cao et al. 2022). Phytoremediation efficiency primarily depends on plant biomass and the concentration of HMs in plant tissues (Bortoloti et al. 2022; Kumar et al. 2022). Nevertheless, under higher levels of contamination, biomass typically becomes the limiting factor rather than the HMs concentration, as HMs toxicity suppresses plant cell elongation, photosynthesis, and nutrient uptake (Andrej et al. 2019). Thus, it is critical to develop innovative strategies to enhance the resilience and remediation efficiency of plants in contaminated soils.

Biochar, a carbon (C)-dense product of biomass pyrolysis, improves soil quality by increasing nutrients, adsorbing HMs, and adjusting soil pH (Gong et al. 2019). It also promotes root and leaf development, boosting photosynthesis and plant growth, thereby improving plant tolerance to HMs (Xing et al. 2017; Khan et al. 2022). Numerous studies have suggested that biochar reduced HMs concentration in crops like maize, wheat, and rice (Ahmed et al. 2023; Wang et al. 2023; Liang et al. 2023). However, it facilitated HMs transport and accumulation in *Salix* (Li et al. 2022; Xiao et al. 2024). This phenomenon suggested that biochar may affect woody plants differently compared to crops. Yet, the mechanisms regulating Cd transformation, particularly the belowground interactions between biochar and rhizosphere processes, remain poorly understood. Furthermore, unmodified biochar has limited capacity to stably immobilize HMs, prompting the need for further enhancement through modification (Ahmed et al. 2023). Phosphorus (P)-modification can improve the ability of biochar to immobilize HMs due to the increased adsorption sites and functional groups (Zhang et al. 2020a; Wang et al. 2023).

Additionally, it may stimulate the production of root exudates, thus reducing Cd toxicity and alleviate its harmful effects on cellular functions (Dai et al. 2017). The P-modified biochar acts a slow-release nutrients reservoir by gradually releasing P, thereby extending the duration of nutrient availability and supporting sustainable plant growth (Zhang et al. 2022a). These combined effects significantly improve the detoxification efficiency and phytoremediation outcomes. For example,  $K_3PO_4$ -modified biochar increased maize's root diameter and elevated concentrations of Cd and P (Wang et al. 2023). Meanwhile, hydroxyapatite-modified biochar reduced DTPA-extractable Pb and As, while increasing maize biomass by 80% (Ahmed et al. 2023). Most studies have focused on inorganic P-modified biochar, while organic P sources from plants, such as phytic acid (PA) and sodium phytate (SP), have been overlooked. These organic P sources offer a green and eco-friendly alternative (Liu et al. 2022; Xue et al. 2023). Our previous study compared PA- and SP-modified biochar (PABC, SPBC) with unmodified bamboo biochar and found that the physicochemical characteristics and adsorption mechanisms of the biochar changed significantly (Di et al. 2025). PABC has large aggregate surface area, with cation- $\pi$  interactions and mineral precipitation as the main adsorption mechanisms; SPBC has the smallest aggregate surface area, and ion exchange is the main adsorption mechanism (Di et al. 2025). However, the use of PABC and SPBC on phytoremediation capacity of *Salix* for in Cd contaminated soil remains largely unexplored.

The soil microbiome is recognized as a key driver of biogeochemical cycles and phytoremediation. However, HM toxicity in contaminated soils often impairs microbial activity and functions (Wu et al. 2024). Biochar application can effectively mitigate these toxic effects, such as increasing bacterial populations and activity (Li et al. 2024b). Meanwhile, the rhizosphere is a hotspot for microbial activity, largely influenced by root-microbial interactions (Zhang et al. 2022b; Xu et al. 2024). For instance, a marked increase in fungal community richness was observed in the rhizosphere in Cd and Cr polluted soil (Zhang et al. 2024), and greater stability in rhizosphere co-occurrence networks compared to unplanted soil under contamination soils (Xu et al. 2024). Additionally, plants can recruit beneficial microbes such as *Bacillus. sp* and *Burkholderia. sp* to enhance Cd transport within the soil-plant system (Ilyas et al. 2022; Liu et al. 2023). Numerous studies have examined the direct microbial responses to biochar amendment in contaminated soil, whereas their potential functions in regulating phytoremediation under biochar-root interactions remain poorly understood. Rhizosphere microbes, which are essential to phytoremediation, are shaped by both

environmental factors and plant genetics (Xun et al. 2024). Furthermore, previous studies have demonstrated that bacteria respond rapidly to changes in soil nutrient by modulating substrate availability (Wong et al. 2019). In contrast, fungi tend to establish stable and long-term symbiotic relationships with plant roots, playing a critical role in maintaining plant homeostasis and facilitating nutrient acquisition (Yelle et al. 2008). Fungi are generally more influenced by plants, while bacteria are more affected by nutrient inputs (e.g., C and P). Under biochar amendment, such microbial functional divergence may further influence the uptake and translocation of HMs by plants. However, the key microbial taxa driving phytoremediation in this system and their specific roles remain largely unclear.

To address these issues mentioned above, a pot experiment using *Salix × aureo-pendula* cv 'J1010' (*Salix J1010*) was designed to examine the effect of engineered biochar on phytoremediation in high Cd-contaminated soils. We hypothesized that (1) P-modified bamboo biochar would promote Cd accumulation in *Salix J1010*, with biomass being the key determinant, (2) bacteria might be more sensitive to the biochar application and mediate the effect of P-modified biochar on Cd translocation. The specific objectives were: (1) to assess the effectiveness of different biochar types in enhancing Cd phytoremediation by *Salix J1010*; (2) to elucidate the mechanisms influencing Cd translocation and accumulation in *Salix* from the perspectives of biochar, the rhizosphere, and their interactions; (3) to reveal the critical roles of bacteria and fungi in phytoremediation. The findings may offer new insights into the use of P-modified biochar to enhance phytoremediation efficiency, contributing to the advancement of more effective methods for managing Cd contaminated soils.

## 2 Materials and methods

### 2.1 Experimental design

This experiment was conducted in Hangzhou, Zhejiang Province, China (30°03'51"N, 119°57'59"E). The tested soil was classified as Ferralic Cambisols (FAO 2006), with a pH of 4.6. The contents of total carbon (TC), total nitrogen (TN), total phosphorus (TP), and total potassium (TK) were 12 g kg<sup>-1</sup>, 0.8 g kg<sup>-1</sup>, 227 mg kg<sup>-1</sup>, and 6900 mg kg<sup>-1</sup>, respectively. The soil was spiked with CdCl<sub>2</sub>·2.5H<sub>2</sub>O to get a final Cd concentration of 23.6 mg kg<sup>-1</sup>, which is well above the limit of 1.5 mg kg<sup>-1</sup> specified by the Soil Pollution Risk Control Standard (GB15618-2018 2018) for soils with pH < 5.5. When the Cd concentration in agricultural soil exceeds this limit, phytoremediation becomes necessary. The contaminated soil was allowed to equilibrate under natural conditions for four weeks (Gai et al. 2024b).

Three types of bamboo-derived biochar— bamboo biochar (BBC), phytic acid-modified bamboo biochar (PABC), and sodium phytate-modified bamboo biochar (SPBC)— were prepared and utilized in the experiment. Biochars were produced by pyrolyzing bamboo feedstock at 450 °C for 2 h, following our previous methods (Di et al. 2025). Detailed biochar properties are provided in Table S1. *Salix × aureo-pendula* cv 'J1010' (*Salix J1010*), a male clone selected for its rapid growth, high biomass yield, and HM tolerance, was selected for the study based on prior screening of willow genotypes (Cao et al. 2022). One-year-old uniform cuttings were collected from the nursery and cut into lengths of 15 cm for planting.

A greenhouse pot experiment was initiated in April using a completely randomized block design. The biochar treatments were established: (1) Control (without biochar addition); (2) 1% (w/w) bamboo biochar (1BBC); (3) 3% (w/w) bamboo biochar (3BBC); (4) 1% (w/w) phytic acid-modified bamboo biochar (1PABC); (5) 3% (w/w) phytic acid-modified bamboo biochar (3PABC); (6) 1% (w/w) sodium phytate-modified bamboo biochar (1SPBC); and (7) 3% (w/w) sodium phytate-modified bamboo biochar (3SPBC). Cd-contaminated soil was thoroughly mixed with biochar at the specified dry-weight ratio. Then, 1.7 kg of the mixture was placed into each pot with 15 cm inner diameter and 13 cm height (Gai et al. 2024a, b; Xiao et al. 2024). *Salix J1010* cuttings were planted in pots, with one cutting per pot (Planted). The biochar-amended contaminated soil was included to assess soil properties and microbial community variations in the absence of plants (Unplanted) (Fig. S1). Each treatment was replicated five times, totaling 35 pots for planted treatments and 35 pots for unplanted controls. Due to high mortality in the 3SPBC treatment, it was excluded. The experiment proceeded with the remaining six treatments. Further details on the 3SPBC treatment and its analysis are in the Supporting Information (Text S2).

## 2.2 Sample collection

Taking comprehensive consideration of the experimental feasibility, growth patterns of *Salix J1010*, and microbial activity phases, plant samples and rhizosphere soil samples were collected from each treatment after 180 days of cultivation (Li et al. 2021). For planted treatments, the pots were carefully opened to expose the complete soil mass. Rhizosphere soil was collected by gently shaking the plants to remove loosely attached soil, followed by brushing off the soil adhering to root surfaces. These samples were placed in sterile bags for further analysis (Batista et al. 2024). In unplanted pots, three randomly selected vertical soil cores were taken from each pot at depths corresponding to the root zones in the planted pots (Xu et al. 2024). Soil samples from each pot were

homogenized into a composite sample, ensuring meticulous avoidance of cross-contamination. After passing through a 10-mesh sieve, each soil sample was divided into 4 subsamples for different analyses: (a) stored at 4 °C for enzyme activity, (b) stored at −80 °C for microbial sequencing, (c) air-dried for chemical analysis, and (d) further sieved through a 100-mesh after air-drying to determine total and DTPA-extractable Cd content. In total, 6 soil samples (excluding the 3SPBC treatment due to high mortality) were collected, yielding 30 rhizosphere soil samples and 30 unplanted soil samples.

Each plant sample was carefully washed with tap water and subsequently separated into roots, stems, leaves, and cuttings. To eliminate surface-bound metal ions, leaves and roots were soaked in solution of Na<sub>2</sub>-EDTA (5 mmol L<sup>−1</sup>) for 20 min (Huang et al. 2024a). After measuring root and leaf traits, samples were initially dried at 105 °C, further dried at 70 °C to a constant weight for biomass determination. The dried material was finely ground using a ball mill (JXFSTPRP-24, Haixin, Shanghai) prior to analysis.

## 2.3 Determination of soil properties and plant traits

Soil pH was measured in a soil-water suspension (1:2.5 w/v) (PHS-3E; Leici, China). TC was measured with a TOC analyzer (Multi N/C 3100; Analytik Jena, Germany), while cation exchange capacity (CEC) was determined through extraction with ammonium acetate solution (Liu et al. 2001). Enzyme activities, including urease (Ure), acid phosphatase (ACP), and beta-glucosidase (β-NAG), were assessed using the sodium hypochlorite-sodium phenol, disodium phenyl phosphate, and nitrophenol colorimetry methods, respectively (Ma et al. 2020). To analyze metals and P, 0.1 g of soil was processed via graphite furnace digestion (ED36, Lab Tech, China) to determine total Cd (TCd), TP, TK, and total sodium (TNa). The bioavailable fraction of Cd (DTPA-extractable Cd) was extracted with a DTPA, calcium chloride, and triethanolamine solution (Rauret et al. 1999). Finally, these elements were quantified using ICP-MS (Agilent 7700x, USA).

During the growing season (August), photosynthetic parameters were measured on leaves using an Li-6400XT system (LI-6400XT; LI-COR, USA). Measurements were taken from 3 leaves per plant (the 7th–9th fully expanded leaves from the top) (Xiao et al. 2024). Leaf and root morphological traits were analyzed using WinRHIZO (Regent Instruments, Canada). Specific leaf area (SLA), specific root length (SRL), specific root area (SRA), and root tissue density (RTD) were determined (Wang et al. 2024). The calculation formulas are provided in Text S3. For plant mineral element analysis, 0.20 g of tissue was digested using HNO<sub>3</sub> and H<sub>2</sub>O<sub>2</sub>

in a graphite digestion furnace (ED36, LabTech, China). The concentrations of Cd and mineral elements (P, K, Ca, Na) were determined using ICP-MS (Agilent 7700x, USA). Quality control included blank samples and certified reference material (GBW07603-GSV-2 for plants and GBW07405a-GSS-5a for soil), with recovery rates ranging from  $95.5 \pm 6.4\%$  to  $101 \pm 8.5\%$ , confirming method accuracy. The calculation methods for bioaccumulation factor (BCF) and translocation factor (TF) are detailed in Text S4.

#### 2.4 Soil DNA extraction and high-throughput sequencing

To evaluate the microbial community composition in soil with biochar and planted with *Salix J1010*, genomic DNA was extracted, followed by high-throughput sequencing of the amplified fragments. The bacterial 16S rRNA gene (V3-V4 region) was amplified with primers 338F (5'-ACTCCTACGGGGGAGGGCAGCA-3') and 806R (5'-TCGGACTACHVGGGTWTCTAAT-3'), and fungal ITS regions were amplified using primers ITS1F (5'-CTTGGTCATTTAGGAAGAAGTAA-3') and ITS2R (5'-GCTGCGTTCTTCATCGATGC-3'). Purified PCR products were analyzed by Shanghai MEIJI Biotechnology Co., Ltd. Sequences were classified and annotated using DADA2 and Bayesian methods, with the SILVA 138 database for bacteria and the UNITE 8.0 database for fungi.

#### 2.5 Statistical analysis

Statistical analyses and data visualization were performed using R software (v4.4.1). One-way ANOVA followed by Tukey's multiple comparison test ( $p < 0.05$ ) was used to evaluate differences in plant traits, while two-way ANOVA with Tukey's test ( $p < 0.05$ ) was applied to assess variations in soil properties among the sample groups.

To compare differences in microbial community structures across biochar types and planting conditions, principal coordinates analysis (PCoA) and PERMANOVA were conducted based on Bray-Curtis distances, using the "vegan", "permute", and "lattice" packages. The random forest model was applied separately to the root BCF, and the Cd accumulation. For each dataset, 1000 out-of-bag trees were generated with 299 random permutations to estimate feature importance using the "rfPermute" algorithm (Archer 2013). Model accuracy was evaluated by predicting the observed accumulation values, yielding an  $R^2$  statistic. Variable importance was quantified based on the increase in mean squared error (%IncMSE), normalized against the total importance to calculate the relative explanatory contribution of each predictor.

Co-occurrence networks of rhizosphere microorganisms were analyzed using the "WGCNA", "psych", "reshape2", and "igraph" packages. After removing samples with zero counts, relative abundances were

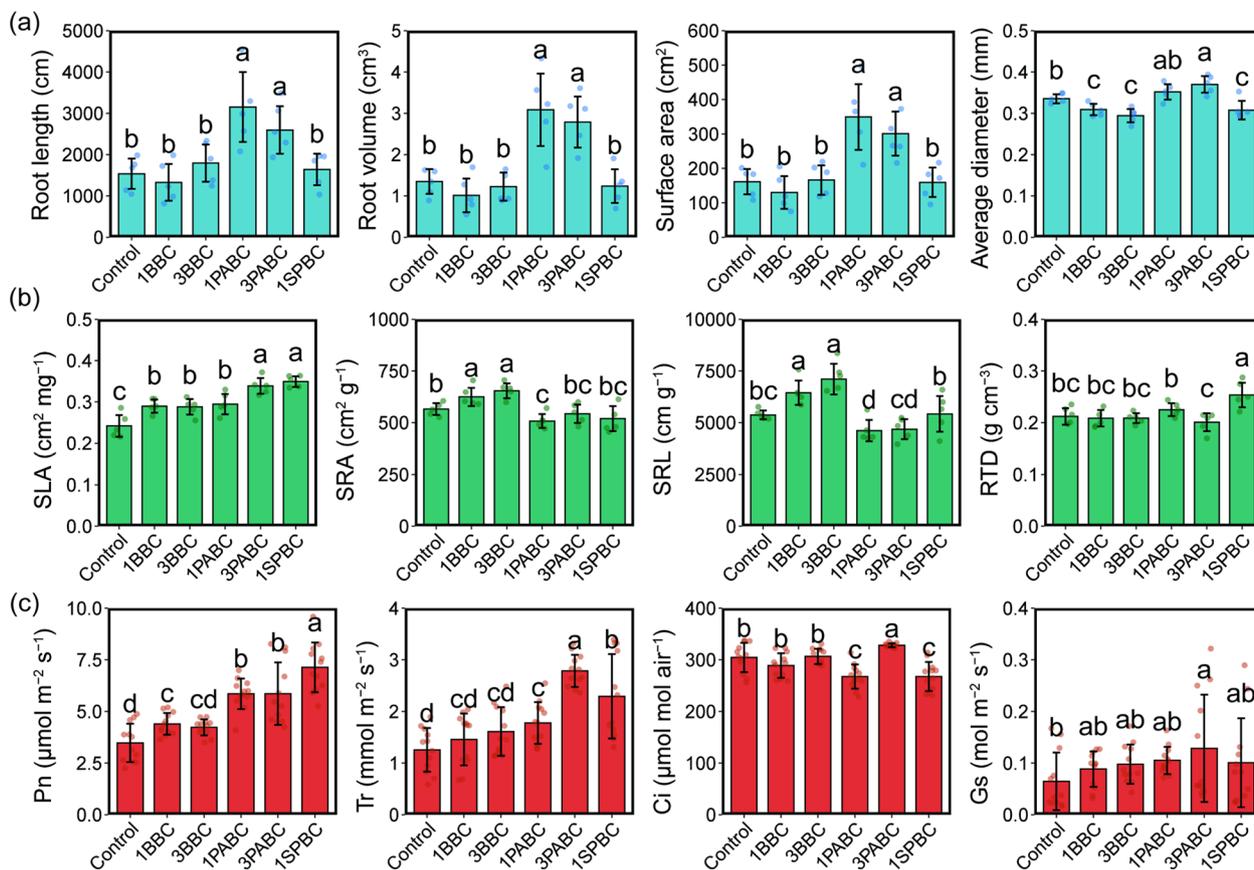
calculated. ASVs with average relative abundances below 0.01% and those present in fewer than one-third of the samples were excluded. Spearman's correlation coefficients between ASVs were calculated using pairwise complete observations. Significant correlations ( $r > 0.60$ ,  $p < 0.001$ , corrected by the Benjamini-Hochberg method) were retained, while others were set to zero. The resulting correlation matrix was used to construct an undirected, weighted co-occurrence network, where nodes represent ASVs and edges reflect significant correlations. Isolated nodes were removed. Taxonomic information from the kingdom to species level was added to nodes for biological interpretation. The network data were exported for visualization and further analysis in Gephi (0.9.2). To identify the environmental preferences of different microbial modules (i.e., ecological clusters), we conducted semi-partial Spearman correlation analyses using the "ggcorrplot" package, examining relationships between the relative abundances of ecological clusters and seven environmental predictors: pH, CEC, TCd, TNa, TK, TP, and TC. Subsequently, we employed the "ggpubr" package to analyze correlations between cluster abundances and soil DTPA-Cd concentration, root traits, and root element contents, aiming to further elucidate the potential mechanism by which key bacterial and fungal clusters influence phytoremediation efficiency.

Piecewise structural equation modeling (SEM) was applied to analyze how soil properties, plant traits, microbial communities, and treatment factors jointly and indirectly affected the Cd accumulation. The "piecewiseSEM" package in R was utilized for model construction (Lefcheck et al. 2016). A composite SEM was built to analyze relationships among key variables, including treatment effects, soil properties, and plant traits. Model refinement involved eliminating non-significant paths. Model performance was validated using Fisher's C test, AIC, degrees of freedom (df), and  $P$ -values, with  $p > 0.05$  indicating an acceptable fit to the data. The model was interpreted by examining the standardized path coefficients and determining the significance of the direct and indirect effects on total Cd accumulation.

## 3 Results

### 3.1 Plant traits and growth affected by biochar

The PABC treatment significantly increased root length, volume, surface area, and average diameter, while BBC treatment markedly enhanced SRL and SRA of *Salix J1010* (Fig. 1a, b). Meanwhile, biochar significantly boosted specific leaf area (SLA) and P content (Figs. 1b, S2). The net photosynthetic rate ( $P_n$ ) and transpiration rate ( $T_r$ ) were both elevated, with the most notable enhancements observed under P-modified biochar treatments (Fig. 1c). Furthermore,



**Fig. 1** Effects of biochar amendments on photosynthetic and physiological traits of *Salix J1010*. **a** Root morphological traits including root length, volume, surface area, and average diameter; **b** functional traits including specific leaf area (SLA), specific root area (SRA), specific root length (SRL), and root tissue density (RTD); **c** photosynthetic performance parameters including net photosynthetic rate (Pn), transpiration rate (Tr), intercellular CO<sub>2</sub> concentration (Ci), and stomatal conductance (Gs). Different letters indicate significant differences among treatments (n = 5). One-way analysis of variance (ANOVA), followed by Tukey's multiple comparison test ( $p < 0.05$ ), was used to assess significant differences in plant traits

**Table 1** Effects of biochar addition on the biomass of roots, stems, leaves, and cuttings of *Salix J1010*

Treatment	Root biomass (g plant <sup>-1</sup> )	Stem biomass (g plant <sup>-1</sup> )	Leaf biomass (g plant <sup>-1</sup> )	Cutting biomass (g plant <sup>-1</sup> )	Total biomass (g plant <sup>-1</sup> )
Control	0.29 ± 0.07b	0.18 ± 0.05c	0.26 ± 0.04c	3.17 ± 0.34a	3.89 ± 0.36bc
1BBC	0.21 ± 0.08b	0.14 ± 0.06c	0.22 ± 0.02c	3.31 ± 0.54a	3.88 ± 0.6bc
3BBC	0.26 ± 0.08b	0.23 ± 0.1c	0.23 ± 0.08c	2.98 ± 0.4a	3.7 ± 0.54c
1PABC	0.7 ± 0.23a	0.66 ± 0.15a	0.51 ± 0.06a	3.38 ± 0.49a	5.25 ± 0.77a
3PABC	0.56 ± 0.14a	0.44 ± 0.1b	0.4 ± 0.06b	3.15 ± 0.38a	4.55 ± 0.6ab
1SPBC	0.31 ± 0.09b	0.58 ± 0.12a	0.45 ± 0.13ab	3.25 ± 0.66a	4.59 ± 0.84ab

Different letters indicate significant differences (n = 5). One-way analysis of variance (ANOVA), followed by Tukey's multiple comparison test ( $p < 0.05$ ), was used to assess significant differences in plant biomass

P-modified biochar substantially enhanced the overall growth of *Salix J1010*. The 1PABC treatment resulted in the highest biomass in roots, stems, as well as leaves, and total biomass (Table 1), with increases

of 144.36%, 271.82%, 98.50%, and 34.91%, respectively, compared to the Control. The SPBC treatment notably increased the aboveground biomass while showing significant effect on root biomass, and Na content

was significantly elevated. Bamboo-derived biochar also increased the concentrations of K and Mg (except for the root of 1SPBC, cutting of 1BBC and 1SPBC) in *Salix J1010* (Fig. S2).

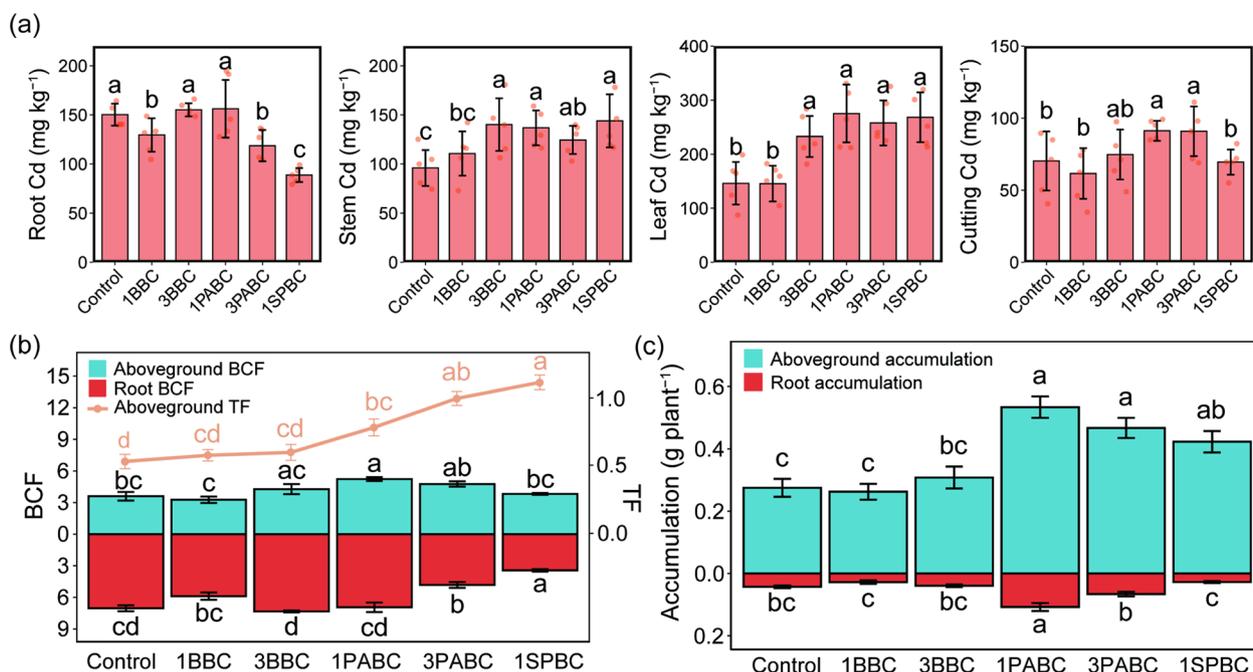
### 3.2 Cd accumulation in plant tissues as affected by biochar

Biochar amendments significantly altered Cd accumulation in *Salix J1010*, particularly with P-modified bamboo biochar (PABC and SPBC) (Fig. 2). Biochar application changed Cd distribution, reducing Cd concentrations in roots (except for 3BBC and 1PABC) and elevating the concentrations of Cd in stems and leaves, thereby promoting Cd translocation to aboveground parts (stems and leaves), increasing the TF by 9.23%–119.14% (Fig. 2a, b). The biochar amendments significantly reduced the root BCF, with 1SPBC recording the lowest value at 48.90% of the Control. In contrast, biochar treatments increased the aboveground BCF, with 1PABC significantly increasing the BCF to 5.23, representing a 45.65% increase relative to the Control (Fig. 2b). Specifically, 1PABC achieved the highest total Cd accumulation of 0.64 g plant<sup>-1</sup>, with 0.53 g in aboveground parts and 0.11 g in roots, representing increases of 94.33% and 151.39%, respectively, compared to the Control (Fig. 2c).

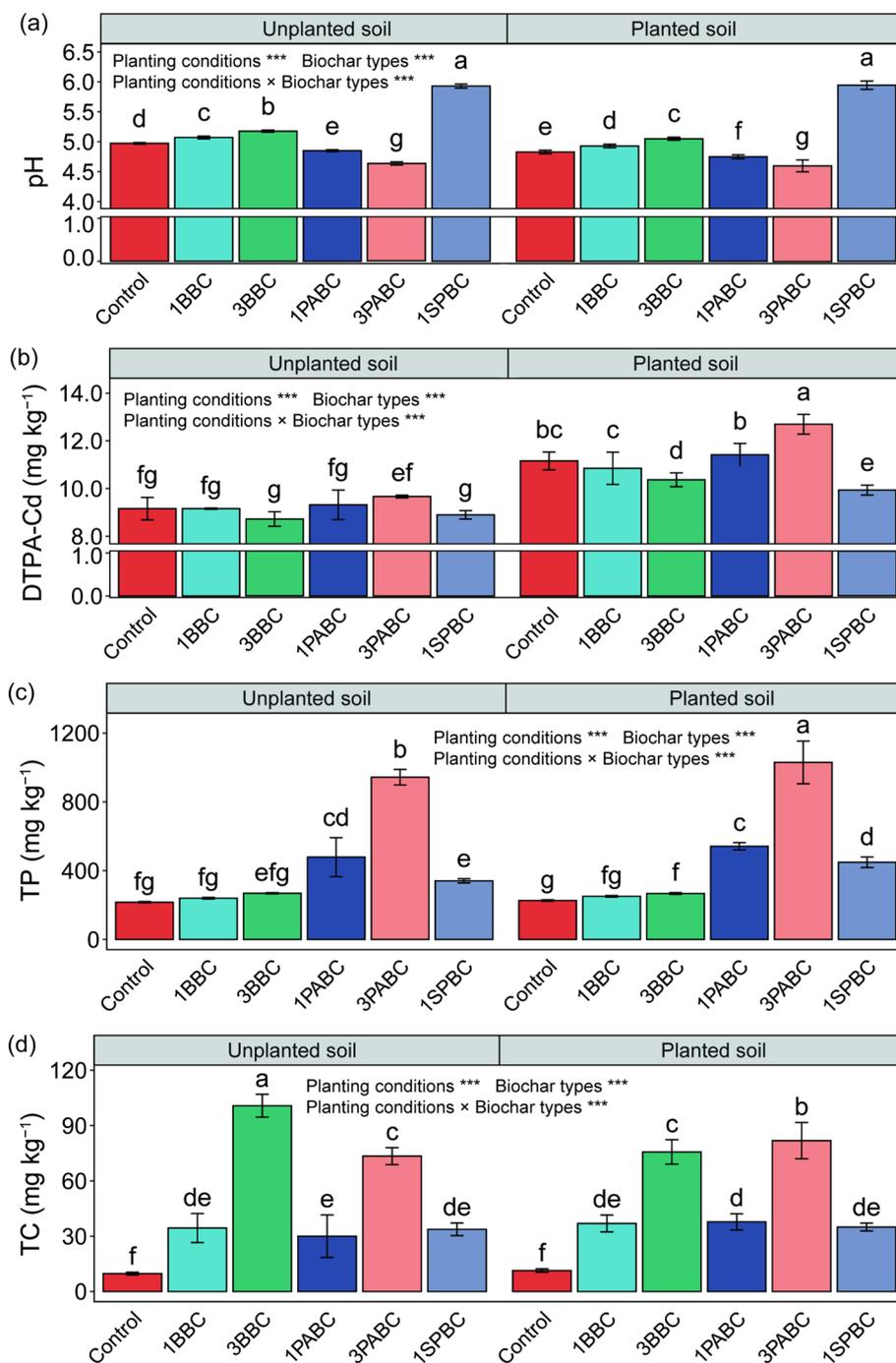
### 3.3 Soil responses to biochar amendment and rhizosphere effect

Biochar and plant had significant effects on soil chemical properties and enzyme activity (Figs. 3, S3, S4). Both BBC and SPBC significantly increased soil pH, whereas PABC reduced it. The rhizosphere soil of *Salix J1010* exhibited lower pH than unplanted soil (Fig. 3a). Biochar addition did not significantly impact on DTPA-extractable Cd in unplanted soil. In planted soil, the 3BBC treatment significantly reduced the DTPA-extractable Cd concentration, while 3PABC increased it compared to the Control. Additionally, DTPA-extractable Cd concentration was higher in planted soil than in unplanted soil (Fig. 3b). The modified biochar treatments significantly increased soil P levels, particularly under the 3PABC treatment. In this case, the P content in the planted and unplanted soil reached 943.51 mg kg<sup>-1</sup> and 1029.4 mg kg<sup>-1</sup>, respectively (Fig. 3c). Further, all biochar treatments increased soil C content, showing a positive correlation with the biochar application rate (Fig. 3d). SPBC treatment increased soil Na content, while the rhizosphere soil of *Salix J1010* exhibited a significant increase in K levels (Fig. S3a, b).

The interactive effect of biochar and the rhizosphere significantly affected microbial diversity. Specifically, the addition of biochar considerably increased bacterial diversity in unplanted soil while decreasing bacterial



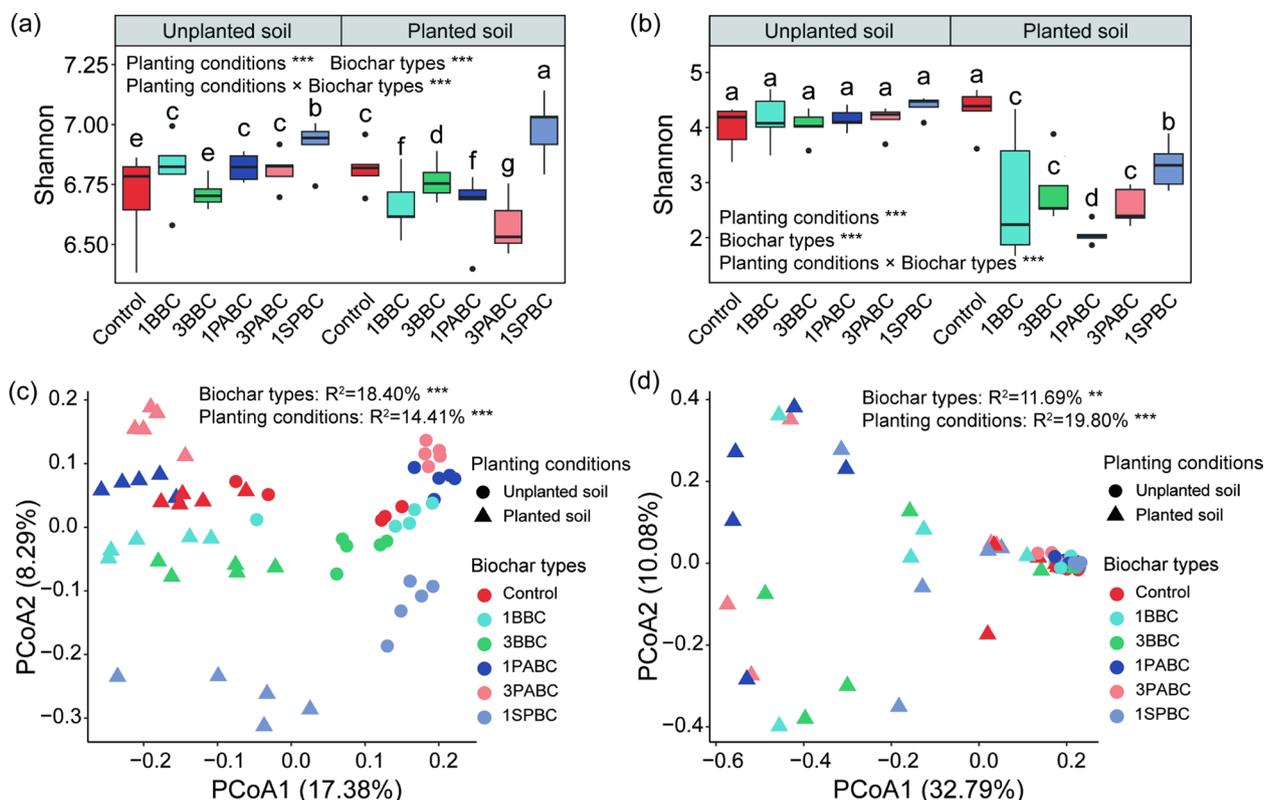
**Fig. 2** Effects of biochar amendments on Cd distribution patterns and phytoremediation efficiency in *Salix J1010*. **a** Cd concentrations in roots, stems, leaves, and cuttings of *Salix J1010*; **b** bioconcentration factor (BCF) in aboveground parts and roots, and translocation factor (TF); **c** Cd accumulation in aboveground parts and roots. Different letters indicate significant differences (n = 5). One-way analysis of variance (ANOVA), followed by Tukey’s multiple comparison test (p < 0.05), was used to assess significant differences in plant traits



**Fig. 3** Interactive effects of biochar types and planting conditions on soil properties. *TP* soil total P concentration, *TC* soil total C concentration. Different letters indicate significant differences (n = 5). Two-way ANOVA (\*\*\*p < 0.001) and Tukey's test (p < 0.05) were used to evaluate significant differences in soil properties among the sample groups

diversity in the rhizosphere soil, except for 1SPBC (Fig. 4a). Regarding fungal communities, biochar addition significantly decreased their diversity in rhizosphere soil, whereas it did not affect fungal diversity in unplanted soil (Fig. 4b). The variations in microbial

communities were regulated by the interactive effect of biochar and the rhizosphere, though with differing responses between bacterial and fungal communities. Adonis analyses revealed that biochar addition exerted a stronger influence on bacterial communities (18.40%),



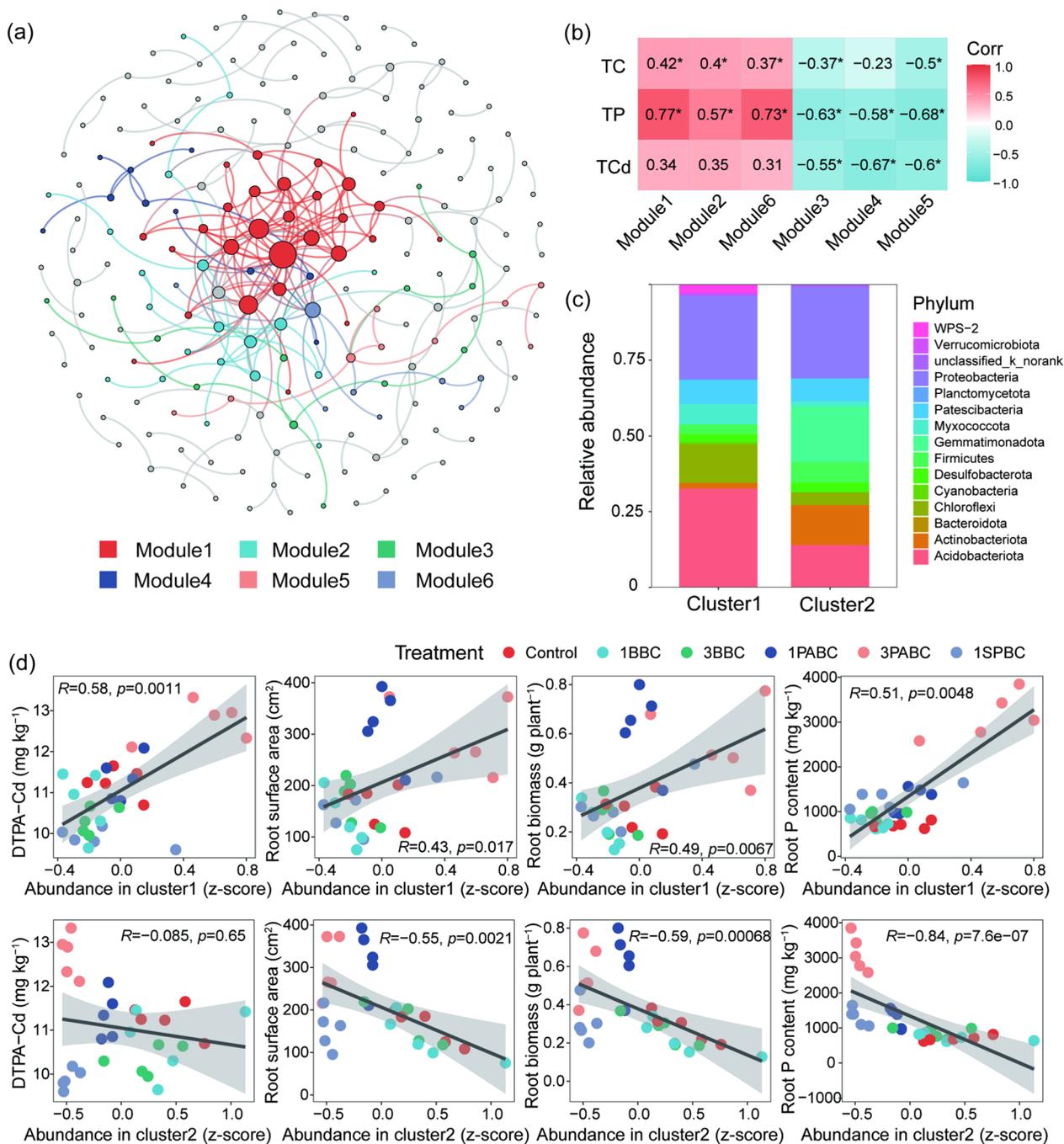
**Fig. 4** Interactive effects of biochar types and planting conditions on soil microbial diversity and community variations. **a** Bacterial shannon index, **b** fungal shannon index, **c** principal coordinates analysis (PCoA) and PERMANOVA of bacterial community (Bray-Curtis distance); **d** principal coordinates analysis (PCoA) and PERMANOVA of fungal community (Bray-Curtis distance). R<sup>2</sup> values represent the explanation ratio, and asterisks (\*) denote statistically significant correlations (\**p*<0.05, \*\**p*<0.01, \*\*\**p*<0.001)

whereas fungal communities were more affected by rhizosphere effects (19.80%) (Fig. 4c, d). Biochar generally increased Ure activity while reducing β-NAG activity, and PABC significantly enhanced ACP activity. The roots of *Salix J1010* impacted these enzyme activities, Ure activity decreased, whereas ACP activity significantly increased, with the highest ACP activity observed under the 3PABC treatment (Fig. S4).

### 3.4 Rhizosphere bacterial clusters and their potential functions as affected by biochar

Random forest model analysis indicated that the rhizosphere bacterial and fungal communities were key predictors for the accumulation of Cd in *Salix J1010* (R<sup>2</sup>=0.72) (Fig. S5). Networks for rhizosphere bacteria and fungi were constructed to further examine the connection between the rhizosphere microbial community and phytoremediation capacity. These networks were divided into six main modules, which were subsequently organized into two major clusters based on their environmental preferences (Figs. 5a, S6). Cluster 1 of the rhizosphere bacterial community structure

predominantly congregated in soils characterized by high levels of Cd, C, and P (Fig. 5b), primarily comprising taxa such as Acidobacteriota (g: *Acidobacteriales*, *Solibacter*, *Koribacter*), Chloroflexi (g: *Anaerolineaceae*, *KD4-96*), Patescibacteria (g: *Parcubacteria*, *Micropepsaceae*, *Patescibacteria*), Myxococcota (g: *Haliangium*), and Gemmatimonadota (g: *Gemmatimonas*) (Fig. 5c). This cluster exhibited a strong positive correlation with soil DTPA-extractable Cd, root surface area, root biomass, and root P content (Fig. 5d). In contrast, cluster 2 displayed significant negative correlations with these variables (Fig. 5d). Meanwhile, cluster 1 of the rhizosphere fungal community structure mainly aggregated in soils with high levels of Cd, Na, and pH (Fig. S6b), predominantly including fungi from Ascomycota (g: *Melnicomyces*, *Devriesia*, *Exophiala*, *Coniosporium*) and Mortierellomycota (g: *Mortierella*) (Fig. S6c). However, this cluster did not show significant correlations with DTPA-extractable Cd and plant traits (Fig. S6d).

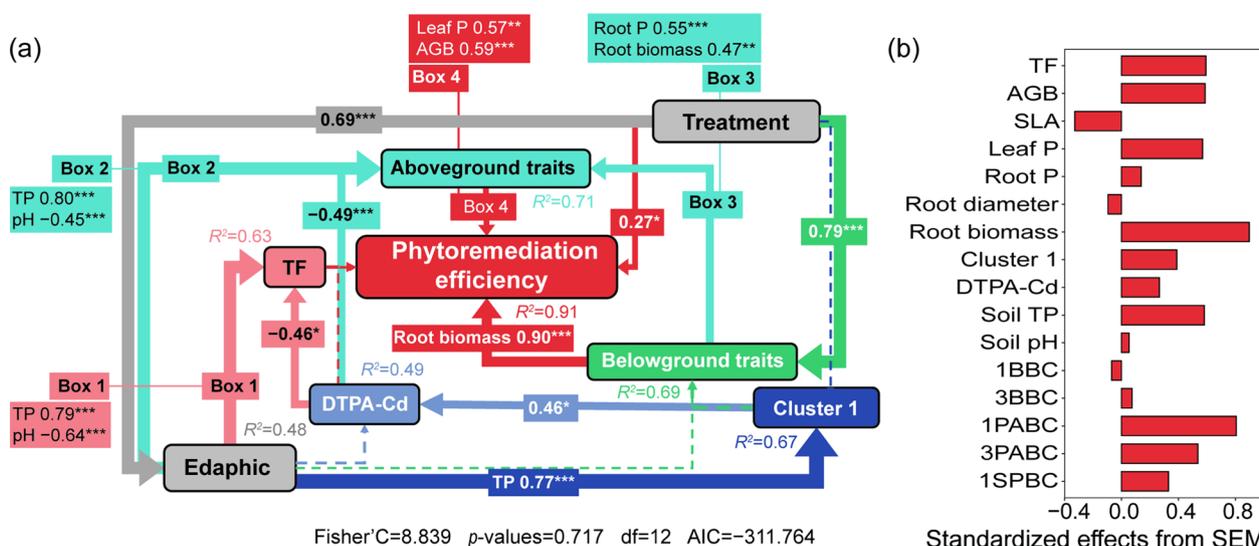


**Fig. 5** Bacterial co-occurrence network and their potential functions. **a** Rhizosphere bacterial network; **b** environmental preferences of major modules (TP, soil total P concentration; TC, soil total C concentration; TCd, soil total Cd concentration); **c** composition of clusters; **d** correlation analysis of clusters with DTPA-extractable Cd (DTPA-Cd), and root traits (root surface area, root biomass, and root P content). R indicates the strength and direction of correlations, while *p* denotes the significance level

### 3.5 The important factors regulating phytoremediation capacity

The SEM was employed to investigate the direct and indirect mechanisms regulating the phytoremediation

capacity of *Salix J1010* (Fig. 6a). The SEM explained 91% of the variation in phytoremediation capacity, and root biomass was the key trait determining phytoremediation efficiency. Biochar application, particularly P-modified biochar, notably enhanced the soil P



**Fig. 6** Structural equation model on factors regulating phyto remediation efficiency. **a** Structural equation model. Arrows are colored according to their targets, with arrow width proportional to the path coefficient. Solid arrows indicate significant effects (Significant level: \*\*\* $p < 0.001$ , \*\* $p < 0.01$ , \* $p < 0.05$ ), while dash arrows represent non-significant effects. Values of Fisher's C with  $P$  in the model were  $> 0.05$ , representing acceptable goodness of fit for the constructed model. **b** Standardized total effect (STE) of the model. *TF* translocation, *AGB* aboveground biomass, *SLA* specific leaf area, *DTPA-Cd* DTPA-extractable Cd, *Cluster 1* ecological clusters of bacteria. Phyto remediation efficiency is represented by total accumulation of *Salix J1010*

content, which in turn activated a specific rhizosphere bacterial group (Cluster 1). Cluster 1 stimulated root growth and significantly improved Cd bioavailability, further enhancing phyto remediation efficiency. Furthermore, the increase in soil TP positively affected the nutritional status of *Salix J1010*, as evidenced by the increased root and leaf P content, ultimately enhancing Cd translocation.

## 4 Discussion

### 4.1 Effects of biochar and rhizosphere on soil properties

Biochar amendment significantly increased soil P concentration. P-modified biochar, functioning like slow-release fertilizers, gradually releases P, raising soil P levels (Ahmed et al. 2023). The P-modified biochar contained 1.18–1.58% P by total elemental content (Di et al. 2025). Furthermore, it promoted organic P mineralization, improving P bioavailability, and significantly enhanced ACP activity, particularly in the rhizosphere soil (Ahmed et al. 2023; Hossain et al. 2020). The increase in soil pH and nutrients is strongly associated with the characteristics of biochar (He et al. 2021). Biochar is generally alkaline, as observed with BBC and SPBC, while PABC exhibited acidic properties due to the modification by phytic acid, leading to diverse impacts on soil pH (Di et al. 2025). The organic acids reduced the rhizosphere soil pH and enhanced Cd mobilization (Xie et al. 2024). However, the increased bioavailable Cd ( $12.70 \text{ mg kg}^{-1}$ )

did not harm the health of *Salix J1010*. Xiao et al. (2024) proposed that *Salix* could grow well in soils with bioavailable Cd up to  $35 \text{ mg kg}^{-1}$ . In fact, 3PABC, which had the highest bioavailable Cd concentration, significantly promoted photosynthesis and growth. Root and leaf biomass increased by 93.10% and 53.85%, respectively, compared to the Control.

Biochar enhanced bacterial diversity in unplanted soil, while reducing it in the rhizosphere soil. Similarly, fungal diversity also decreased in the rhizosphere. In unplanted soil, biochar likely provided a suitable habitat and additional nutrients, promoting microbial colonization and increasing bacterial diversity (Palansooriya et al. 2019). Conversely, the interaction between plant root exudates and biochar alters root-derived compounds in the rhizosphere, fostering the proliferation of specific microbial taxa, thereby reducing bacterial and fungal diversity in the rhizosphere (Ceja-Navarro et al. 2021). Bacteria and fungi exhibit distinct responses to biochar and the rhizosphere effect. The biochar exerted a more pronounced influence on bacterial communities, due to its highly porous structure and abundance of functional groups that enhance nutrient adsorption and retention. These characteristics promoting rapid bacterial metabolism and growth (Palansooriya et al. 2019). In contrast, fungal communities were more significantly influenced by rhizosphere effect, as fungi primarily root-derived C and form symbiotic relationship with plant roots, facilitating

nutrient exchange (Chen et al. 2013). This aligns with our second hypothesis. Wong et al. (2019) found similar results, showing that bacterial communities are more responsive to environmental factors, whereas fungal communities maintain a closer association with plant.

#### 4.2 Influence of biochar on phytoremediation efficiency

Biochar can immobilize HMs in soil and alter their distribution in plants (Liang et al. 2023). Interestingly, biochar led to an increase in Cd concentrations in the above-ground tissues of *Salix J1010*. Consistently, similar trends were observed in other willow genotypes, such as *Salix jiangsuensis* "172" and *Salix psammophila* (Li et al. 2022; Gai et al. 2024b; Xiao et al. 2024). These findings contrast with those observed in crop species (e.g. maize and wheat), likely due to differences in soil HM concentrations and transport mechanisms. Woody plants have well-developed root systems and are usually grown in higher polluted levels, which may limit biochar's adsorption. They also have stronger transport and storage capacities than crops, giving them an advantage in long-distance transport and HMs sequestration (Ahmed et al. 2023; Wang et al. 2023). Consistent with our first hypothesis, our results indicated that P-modified biochar enhances the phytoremediation of *Salix J1010*. P, as an essential nutrient for plant physiological processes, plays a critical role in these interactions (Huang et al. 2024b). P-modified biochar can promote xylem cell formation and vascular efficiency, enhancing translocation to aboveground parts while alleviating root toxicity (Wu et al. 2018). Furthermore, P-induced detoxification via metal-binding proteins and phytochelatin aids in Cd sequestration and translocation (Shi et al. 2015; Yang et al. 2020). In addition to the increase in transpiration and biomass from P-modified biochar, these factors jointly enhanced the phytoremediation efficiency of *Salix J1010*. The single P treatment increased Cd concentration in ryegrass by 16.67% (Huang et al. 2024b). Our results showed that P-modified biochar improved Cd concentration in the leaves by 89.67%, which implies the critical role of C-P coupling effect in phytoremediation. Additionally, Cd accumulation was higher under 1% P-modified biochar than under 3% biochar, which is consistent with Huang et al. (2024b), likely due to the fact that excessive P may reduce Cd uptake via the formation of insoluble Cd-P complexes. Moreover, Na<sup>+</sup>-rich biochar (e.g., SPBC) induced osmotic stress that hinders root growth and reduces phytoremediation efficiency (Cheng et al. 2020). Therefore, when preparing or using SPBC, the Na<sup>+</sup> content it contained must be taken into account. In contrast, BBC increased SRL and SRA but had limited remediation potential, likely due to P deficiency that induced fine root development (Stock

et al. 2021). Nonetheless, a deeper understanding of the regulatory mechanisms mediating plant responses to P-modified biochar is essential to fully clarify its role in adaptation and detoxification.

#### 4.3 Key factors regulating phytoremediation efficiency

The root plays a central role in phytoremediation by mediating plant-soil interactions. Although Cd primarily accumulates in the aboveground parts, root biomass remains a crucial determinant of phytoremediation efficiency, as it increases the surface area for nutrient and metals uptake (Shang et al. 2024). In *Salix J1010*, efficient Cd translocation from root to shoot further mitigates root toxicity (Wang et al. 2020). Bacteria play a more dominant role than fungi in enhancing phytoremediation efficiency, primarily through actively modifying the rhizosphere environment by secreting chelating agents and metabolites, thereby enhancing nutrient availability for roots (Venturi and Keel 2016; Wang et al. 2020). Our results suggest that P-modified biochar improves soil fertility by releasing P, which supports plant nutrition, and stimulates root growth. It activates a key bacteria cluster that facilitates nutrient mobilization and enhances plant resilience, forming a positive feedback loop between root development and microbial activity (Zhang et al. 2020b). Notably, Cluster 1 of bacteria, predominantly found in soil with high levels of Cd, C, and P, showed significant activity. This bacteria cluster is primarily composed of Acidobacteriota, Chloroflexi, Gemmatimonadota, Patescibacteria, and Myxococcota. These taxa are well-adapted to stressful environments and contribute to nutrient cycling. Specifically, Acidobacteriota are recognized for their HM resistance, while Chloroflexi and Gemmatimonadota contribute significantly to organic P mineralization and uptake (Hug et al. 2013; Guo et al. 2017; Zhang et al. 2020b; Bao et al. 2020). Patescibacteria exhibit strong migratory abilities within soil and are closely linked to functional genes responsible for the transport of various HMs, suggesting their role in facilitating the transfer of HMs from the bulk soil to the rhizosphere (Tian et al. 2022). Myxococcota contribute to organic matter decomposition and the regulation of microbial community dynamics (Yu et al. 2024; Feng et al. 2024). The potential functions need further confirmation through metagenomics. Together, nutrient enrichment, microbial activation, and enhanced root traits synergistically improve Cd uptake, highlighting P-modified biochar as a promising amendment for sustainable soil remediation. Compared to unmodified biochar combined with commercially available slow-release fertilizers (e.g. Osmocote, chitosan-coated, polymer-coated fertilizers, or silica-coated fertilizers), P-modified biochar combines the advantages of both, with a synergistic effect

that enhances its ability to adsorb HMs. The combination of unmodified biochar with slow-release fertilizers generally has limitations such as poor stability or non-degradability of coating materials, and weaker adsorption of HMs (Kim et al. 2017; Lebrum et al. 2020; Wu et al. 2025). Therefore, P-modified biochar offers distinct advantages for long-term nutrient management and soil remediation. However, a broader remediation potential in the field requires further studies involving heterogeneous contamination, various soil types, and different hydrological conditions. Additionally, long-term monitoring is essential to assess the stability of P-modified biochar and its potential ecological risks under field conditions.

## 5 Conclusion

P-modified biochar enhanced the phytoremediation capacity of *Salix × aureo-pendula* cv 'J1010' for Cd in soil by increasing the translocation of Cd from roots to aboveground tissues and boosting plant biomass. Bacteria exhibited a more sensitive response to biochar amendment than fungi. The stimulation of bacterial clusters in the rhizosphere promoted root growth, increased Cd bioavailability, and subsequently improved the translocation of Cd. These results underscore the potential of integrating P-modified biochar with *Salix J1010* as a sustainable strategy to improve phytoremediation efficiency for Cd-contaminated soils. The synergistic effect of substrate improvement and phytoremediation enhances nutrient cycling and resilience to contamination. This provides a cost-effective, eco-friendly alternative to chemical methods, reducing long-term remediation costs and facilitating ecological restoration in agricultural soils that exceed HM limits.

## Supplementary Information

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Supplementary material 1.

## Author contributions

Dongliu Di: Methodology, experiment, formal analysis, visualization, manuscript drafting, and revision. Shaokun Wang: Methodology, formal analysis, supervision, and manuscript review. Xu Gai: Supervision and manuscript review. Jiang Xiao: Conceptualization, methodology, and supervision. Haoran Li: Experiment and formal analysis. Guangcai Chen: Conceptualization, supervision, manuscript review, and funding acquisition.

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## Data availability

Data availability All data generated or analyzed during this study are included in this published article (and its supplementary information files). The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

## Declarations

### Competing interests

The authors declare no competing interests.

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