Ren *et al. Biochar* (2025) 7:119
https://doi.org/10.1007/s42773-025-00535-2

Biochar

ORIGINAL RESEARCH

Open Access

Biochar and microbial synergy: enhancing tobacco plant resistance and soil remediation under cadmium stress

Tianbao Ren[1,2*†], Huilin Feng[1†], Wan Adibah Wan Mahari[3†], Fei Yun[1,2], Maosen Li[1], Nyuk Ling Ma[4], Xianjie Cai[6], Guoshun Liu[1,2], Rock Keey Liew[5] and Su Shiung Lam[3,7,8*]

Abstract

The increasing contamination of soil with heavy metals, particularly cadmium, poses a significant threat to agricultural productivity, especially in tobacco cultivation. The primary objective of this study is to explore the impacts of biochar combined with microorganisms on tobacco resistance and soil remediation under cadmium stress. The experiment consisted of four treatments: namely, G0C0 (no cadmium or biochar added), G1C0 (cadmium 130 mg added, no biochar added), G1C1 (cadmium 130 mg added, 10 g kg^{-1} biochar added), and G1C2 (cadmium 130 mg added, 10 g kg^{-1} biochar added, and 1% microbial inoculant added). The influence of each treatment on tobacco growth and development, cadmium uptake by tobacco, soil biological characteristics, and components of soil microbial communities was investigated. The study revealed that cadmium stress had a negative impact on tobacco net photosynthesis. Notably, biochar was found to be effective in alleviating this effect. The results demonstrated that Apparent Quantum Yield (AQY), Maximum Photosynthetic Rate (Pmax), and Light Saturation Point (LSP) in G1C1 increased by 1.69%, 80.50%, and 30.76%, respectively, compared to G1C0. Similarly, AQY, Pmax, and LSP in G1C2 increased by 3.39%, 86.84%, and 62.35%, respectively. Following cadmium contamination, the levels of soil urease, catalase activity, and microbial biomass nitrogen and carbon were reduced. However, the application of biochar significantly enhanced urease and catalase activities by 12.42–63.89% and 13.03–14.17%, respectively. Additionally, the carbon and nitrogen content of microbial biomass increased by 46.42–76.27% and 21.56–28.12%, respectively, compared to the G1C0 treatment. The combined application of biochar and *Trichoderma* exhibited a synergistic effect, enhancing soil microbial diversity and community structure while increasing the abundance of beneficial genera such as *Mucoromycota, Chaetomium,* and *Bacillus.* Additionally, biochar effectively restricted the upward transport of cadmium in tobacco plants. These findings suggest that co-applying biochar and microorganisms offers a sustainable strategy to enhance tobacco plant resilience under cadmium stress, improve soil ecology, and promote soil remediation in contaminated environments.

†Tianbao Ren, Huilin Feng and Wan Adibah Wan Mahari These authors are Co-first author.

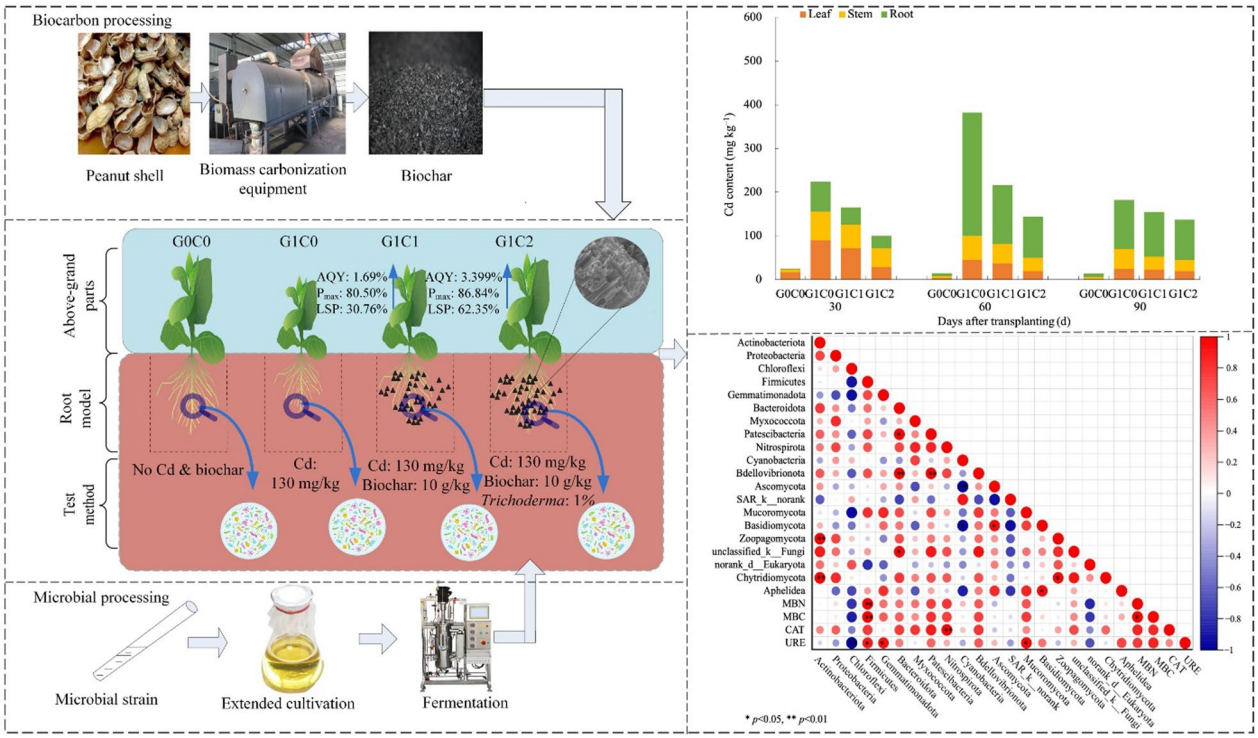
*Correspondence:
Tianbao Ren
biochar2018@henau.edu.cn
Su Shiung Lam
lam@umt.edu.my
Full list of author information is available at the end of the article

Highlights

- Innovative use of biochar and Trichoderma enhances soil health and fertility.
- Biochar enhances the physiological resistance of tobacco under cadmium stress.
- Combining biochar and microorganisms reduces cadmium uptake in tobacco and improves soil microbial diversity.

Keywords Biochar, Cadmium stress, Rhizosphere soil micro-ecology, Micro-ecological balance

Graphical Abstract



1 Introduction

The deterioration of soil quality driven by intensified human activities, such as mining and excessive agrochemical use, has amplified concerns over heavy metal contamination in soils (Zhou et al. 2017; Nirola et al. 2016). Among these heavy metal contaminants, cadmium (Cd) is particularly hazardous due to its high mobility and persistence in the environment, even at low concentrations (Xu et al. 2020). Cd contamination in farmland soils is a growing issue, especially in China, where extensive soil studies have identified Cd as the most prevalent heavy metal pollutant (Zhang et al. 2014; Liu et al. 2020a, b). Plants readily absorb Cd from contaminated soil, leading to its bioaccumulation in edible crops, which poses significant risks to human and animal health through the soil–plant-food chain (Aryal et al. 2016; Wang et al. 2010; Jianrui and Yingming 2015; Zhu et al. 2023). This has raised concerns regarding food safety and public health, particularly in regions with high Cd contamination levels. The presence of other heavy metals and secondary contaminants in the soil can further complicate the bioavailability and toxicity of Cd, highlighting the need for a comprehensive understanding of multi-pollutant interactions. For instance, the presence of lead and arsenic can alter Cd uptake in plants, while mercury contamination may enhance Cd's mobility in soil systems. In addition, secondary contaminants such as persistent organic pollutants, including polycyclic aromatic hydrocarbons and pesticides, can bind with heavy metals, complicating

their environmental behavior and increasing ecological risks. These multi-contaminant interactions can enhance toxicity, reduce soil fertility, and further endanger food safety.

Various techniques have been proposed for the remediation of soils contaminated with heavy metals (Sánchez-Castro et al. 2023). Among these, the immobilization of Cd using soil amendments is regarded as a more cost-efficient and environmentally sustainable alternative compared to other methods such as soil leaching, thermal treatment, microbial remediation, and phytoremediation (Hu et al. 2020). However, the effectiveness of these techniques may be influenced by the presence of secondary contaminants and their interactions with Cd in the soil matrix. Further research is needed to optimize remediation strategies that address multi-pollutant scenarios and minimize the risks associated with heavy metal contamination.

Biochar has been proven effective in mitigating Cd contamination and improving polluted soils (Hong et al. 2019; Yu et al. 2018). Biochar is produced by heating biomass at high temperatures through pyrolysis. It possesses a porous structure that can provide numerous attachment sites for soil microorganisms, improving microbial activity and boosting crop growth and stress resistance (Qi et al. 2022; Yang et al. 2023) Recent studies have delved into the potential of biochar as a solution to mitigate plant and soil contamination caused by excessive heavy metal levels (Kayoumu et al. 2025). Huang et al. (2020a, b) demonstrated that biochar enhances soil microorganism habitat by adsorbing heavy metals and reducing the bioavailability of toxic substances. Meanwhile, Ren et al. (2021)observed that biochar effectively absorbs Cd ions in the soil, leading to a significant reduction of up to 48% in Cd content in leaves. Currently, the most common mechanisms by which biochar alleviates cadmium pollution are ion exchange, co-precipitation, cation π-electron interaction, and complexation. While the precise mechanism remains unclear, these are the most widely accepted explanations according to Hamid et al. (2019). In addition to biochar, biological agents containing functional micro-organisms are also seen as crucial in addressing soil Cd contamination and improving soil microbial ecology conditions (Yu et al. 2022). Meanwhile, *Trichoderma*, a genus of beneficial fungi, has been widely studied for its bioremediation potential, plant growth-promoting abilities, and heavy metal detoxification properties (Zhang et al. 2018). *Trichoderma* species play a crucial role in soil remediation by mitigating heavy metal contamination through multiple biological mechanisms. These fungi exhibit high tolerance to toxic metals such as Cd, lead (Pb), nickel (Ni), and copper (Cu), and can immobilize these metals through biosorption and bioaccumulation, thereby reducing their bioavailability and toxicity in the soil. For instance, *Trichoderma asperellum* has demonstrated a strong ability to scavenge heavy metals, while *Trichoderma atroviride* has been identified for its capability to uptake and immobilize toxic metals, making them valuable in bioremediation strategies (Hoseinzadeh et al. 2017). By improving soil microbial diversity, promoting enzymatic activity, and fostering plant–microbe interactions, *Trichoderma* serves as an effective biological agent for soil remediation, offering an eco-friendly approach to mitigating heavy metal contamination while supporting sustainable agriculture.

Although the individual benefits of biochar and *Trichoderma* fungi in soil remediation are well-documented, their synergistic effects in Cd-contaminated soils remain insufficiently studied. Previous studies have primarily focused on the influence of biochar on soil Cd content, bacterial diversity, and enzyme activity (Ren et al. 2022). However, there is a significant gap in understanding the micro-ecological restoration mechanisms associated with biochar application and the potential advantages of combining biochar with *Trichoderma* to reduce Cd accumulation and toxicity. In this study, tobacco (*Nicotiana tabacum)* was selected as the model plant for investigating Cd accumulation and soil remediation strategies. Unlike common vegetables and staple crops, tobacco is a hyperaccumulator of heavy metals, particularly Cd, and is widely cultivated in Cd-contaminated regions (Chen et al. 2022; da Silva et al. 2016). Due to its high Cd tolerance and accumulation capacity, tobacco serves as an ideal plant for assessing Cd stress responses and remediation efficiency. In addition, tobacco cultivation is economically significant, and excessive Cd accumulation in tobacco leaves poses risks not only to human health through smoking but also to soil sustainability in tobacco-growing areas.

Therefore, this study aims to address these knowledge gaps through the following objectives: (1) evaluate the impact of Cd stress on tobacco plants and soil by analyzing physiological and biochemical stress responses, (2) investigate the effects of using solely biochar and in combination with *Trichoderma* on Cd migration, bioavailability, and accumulation in soil and tobacco plants, (3) evaluate changes in soil microbial communities and plant health following the application of biochar and biochar-*Trichoderma* combinations under Cd stress, (4) elucidate the underlying mechanisms by which biochar and *Trichoderma* influence soil microecology and plant resilience, and (5) determine the overall effectiveness of biochar-based strategies for Cd remediation while recommending future research directions to enhance

ecological restoration efforts. This research seeks to provide comprehensive insights into sustainable soil remediation practices and the practical application of biochar and *Trichoderma* in enhancing soil health and plant resistance in Cd-contaminated environments.

2 Methodology

2.1 Materials

This study was conducted at the Tobacco Research Laboratory of Henan Agricultural University Zhengzhou Campus (34°01′N, 113°49′E). The soil in this study was gathered from the ploughing layer (0–20 cm) of a sandy loam-textured wheat field in Suqiao Town, Xuchang County. The soil used in this study was subjected to air-drying, sterilization, and sieving via a 2 mm mesh screen. Table 1 presents the fundamental features of the soil. In this study, the biochar was procured using a pyrolysis method jointly developed by Henan Biochar Engineering Technology Research Center and the Institute of Tropical Aquaculture and Fisheries of Universiti Malaysia Terengganu, produced through oxygen-limited pyrolytic thermal cracking of peanut shells at temperatures between 380 °C and 400 °C. The biochar has a pH of 8.55, contains 61.97% carbon, and 1.29% nitrogen, and has a specific surface area of 15.89 m^2g^{-1} and an average pore size of 1.85 nm. The microbial inoculant of *Trichoderma harzianum* (Patent deposit number CGMCC23294) required for the experiment was prepared by Henan Biochar Engineering Technology Research Center.

2.2 Design of the test

The experiment consisted of four treatments: G0C0 (no cadmium, no biochar), G1C0 (cadmium 130 mg, no biochar), G1C1 (cadmium 130 mg, 10 g kg^{-1} biochar), and G1C2 (cadmium 130 mg, 10 g kg^{-1} biochar + 1% microbial inoculant). The experiment employed a two-factor completely randomized block design. To ensure reproducibility, all treatments were replicated three times. Each treatment group contained approximately 16 tobacco plants, which ensured sufficient plant availability for biomass sampling at three time points while maintaining experimental integrity. To evaluate the stability and stress resilience of the biochar–microorganism composite under extreme contamination conditions, and to enhance the detectability of treatment effects, a high-Cd stress scenario was simulated. Based on preliminary trials, 130 mg kg^{-1} of exogenous Cd was introduced into the soil, as this concentration triggered the most pronounced physiological responses in plants, thereby facilitating a better elucidation of the biochar–microbe remediation mechanisms. Cd was applied in the form of cadmium nitrate and thoroughly mixed into the soil rather than surface-applied to ensure uniform distribution. The soil was homogenized using mechanical mixing followed by manual stirring before potting. The total soil weight per pot was 16.8 kg.

Soil preparation occurred two weeks before transplanting, during which fertilizers, biochar, and other supplements were incorporated to allow for the appropriate aging of Cd. Analytical-grade fertilizers and exogenous additives were used, including cadmium nitrate, ammonium nitrate, calcium dihydrogen phosphate, potassium sulfate, and tobacco rod charcoal (sieved through a 10-mesh screen). A pure nitrogen amount of 1.16 g per pot was applied, maintaining an N: P_2O_5: K_2O ratio of 1: 1.5: 3. Following the initial fertilizer application, no further top-dressing was conducted, in accordance with the field tobacco leaf management method (GB/T 23221–2008).

2.3 Soil sampling and determination of soil characteristics

2.3.1 Soil sampling

Following 75 days of transplanting, the soil at a depth of 0–10 cm surrounding the pot was compressed and the complete root structure of the tobacco plant was carefully extracted. The roots were delicately shaken to eliminate most of the adhering soil, leaving only a minimal amount attached to the root surface. The root ball was then returned to its original position in the pot and the roots were vigorously shaken to dislodge any remaining soil particles. A 5–10 g soil sample was collected from the 0–10 cm soil layer within the pot, ensuring it was free from debris, plant roots, animal remains, or other contaminants. The sample was then processed by passing it through a 2 mm sieve before being kept in a sterile 10 mL centrifuge tube. To preserve microbial integrity, the soil samples were immediately placed on dry ice and subsequently analyzed for microbial diversity at Shanghai Ouyi Biomedical Technology Co., Ltd.

2.3.2 Soil enzyme activity and soil microbial biomass

Enzyme activity in the soil was determined by the kit provided by Suzhou Keming Biotechnology Co., Ltd. Urea was employed as the substrate and the NH3-N generated

Table 1 Main features of soils

Soil texture	Sampling site	pH	Organic matter (g kg^{-1})	Total N (mg kg^{-1})	Available K (mg kg^{-1})	Available P (mg kg^{-1})
Sandy loam	Xuchang	7.8	12.12	46.79	120.53	11.18

by urea hydrolysis via urease was quantified using indophenol blue calorimetry. The ammonia concentration can be measured by determining the intensity of the blue indophenol formed, which is directly proportional to urease activity. Soil catalase has a characteristic absorption peak at 240 nm. Thus, the solution was tested for optical density at 240 nm after reaction with soil samples containing different levels of S-CAT activity.

The chloroform fumigation extraction technique was employed to quantify soil microbial biomass carbon (MBC) and nitrogen (MBN). Fresh soil samples were treated with chloroform and subsequently extracted with K2SO4. MBC was quantified using a fully automated organic carbon analyzer (Vario TOC, Elemental, Germany), and MBN was measured using a fully automated Kjeldahl nitrogen analyzer. The conversion coefficients of MBN and MBC were 0.54 and 0.45, respectively (Brookes et al. 1985; Vance et al. 1987).

2.3.3 Soil microbial determination

2.3.3.1 Extraction of DNA and amplification of PCR Microbial community DNA was isolated using the E.Z.N.A.® soil DNA kit (Omega Bio-tek, Norcross, GA, U.S.) according to the manufacturer's protocol. The quality of the extracted DNA was assessed using 1% agarose gel electrophoresis, while its purity and concentration were determined with a NanoDrop2000 spectrophotometer.

ABI GeneAmp® 9700 was used for PCR amplification in this study. Bacterial analysis involved amplifying the V3-V4 variable region of the 16S rRNA gene using primers (338F: 5′-ACTCCTACGGGAGGCAGCAG-3′ and 806R: 5′-GGACTACHV GGGTWTCTAAT-3′). For fungal analysis, the V5-V7 variable region of the 18SrRNA gene was targeted using primers (SSU0817F: 5′-TTAGCATGGAATAATRR AATAGGA-3′ and 1196R: 5′-TCTGGACCTGGTGAGTTTCC-3′). PCR amplification commenced with an initial denaturation at 95 °C for 3 min, followed by 27 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and elongation at 72 °C for 30 s. A final elongation step was performed at 72 °C for 10 min. The amplified products were subsequently stored at 4 °C for further analysis.

The PCR reaction mixture for each sample consisted of 4 μL of 5×TransStart FastPfu buffer, 2 μL of 2.5 mM dNTPs, 0.8 μL of each 5 μM upstream and downstream primer, 0.4 μL of TransStart FastPfu DNA polymerase, and 10 ng of template DNA, with a total volume of 20 μL. Each sample was replicated three times.

2.3.3.2 Illumina Miseq sequencing PCR products from each sample were separated using a 2% agarose gel. The targeted bands were then purified with the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA), and the purity of the extracted DNA was confirmed via 2% agarose gel electrophoresis. The concentration of the purified products was quantified using the Quantus™ Fluorometer (Promega, USA). For library preparation, the NEXTFLEX Rapid DNA-Seq Kit was employed, following these steps: (1) adapter ligation, (2) magnetic bead selection to eliminate self-ligated adapters, (3) PCR amplification of the library templates, and (4) recovery of PCR products using magnetic beads. The prepared libraries were then sequenced on the Illumina Miseq PE300 platform by Shanghai Majorbio Co., Ltd.

2.4 Determination of botanical traits

2.4.1 Determination of photosynthetic physiological indicators

The intercellular CO_2 concentration (Ci), net photosynthetic rate (Pn), transpiration rate (Tr), and stomatal conductance (Gs) of tobacco plants were assessed at different growth stages (30-day clump stage, 60-day dome stage, and 90-day mature stage) using the LI-6400 portable photosynthesis system (LI-COR, USA). For each treatment, three strains were chosen and each strain underwent three measurements. The resulting data was analyzed by calculating the average value for each strain, and a rotational order was used to ensure accuracy and consistency in the measurements.

2.4.2 Determination of dry matter weight

Three plants were arbitrarily chosen from each treatment at distinct phases of tobacco plant growth (30-day clump stage, 60-day dome stage, 90-day mature stage). The fresh weight of roots, stems, and leaves was individually measured, followed by subjecting the samples to 105 ℃ for 15 min to halt biological activity. The samples were dried at 65 ℃ until a consistent weight was attained. Finally, the dried samples were weighed to calculate the accumulation of the substances.

2.4.3 Determination of cadmium content in flue-cured tobacco

The dry ashing method specified in the National Food Safety Standard, GB5009.15–2014 (Standardization Administration of China 2014) was used to incinerate the Cd present in tobacco plants. The Cd extract from the ash was evaluated by an inductively coupled plasma atomic emission spectrometer (ICP-AES). The assessment was conducted to determine Cd accumulation, distribution rate, transport coefficient and enrichment coefficient in various parts of tobacco plants, including leaves, stems, and roots. Cd accumulation was estimated by multiplying the Cd content of each plant part by the biomass of the corresponding part. The transport coefficient was

determined by comparing the content of heavy metals in the latter part to that in the former part. The enrichment coefficient was calculated by dividing the heavy metal content in a specific organism part by the soil heavy metal content.

2.5 Data processing

Trimmomatic was employed for quality control of the raw sequencing data. Paired-end reads with low tail quality (less than 20) were merged using FLASH with a 50 bp window size. In addition, bases were trimmed from the rear end of the sequence if the average quality score within the sliding window dropped below 20. Finally, sequences shorter than 50 bp after quality control, along with those containing ambiguous N bases were filtered out. For merging paired reads, a minimum 10 bp overlap length was required and sequences with a maximum of 0.2 mismatch ratio were excluded. The samples were discerned by barcodes and primers located at the initial and end of the sequence. Then the direction of the sequence was modified accordingly. The barcodes were matched exactly and the primers allowed a maximum of 2 mismatches.

Sequences were grouped into operational taxonomic units (OTUs) and chimeras were eradicated using UPARSE software (version 7.1 http://drive5.com/uparse/) with a 97% similarity threshold. RDP classifier (http://rdp.cme.msu.edu/) was employed to assign species classifications to each sequence, with a comparison threshold set at 70% against the Silva database (SSU128). Data processing was performed using Microsoft Excel 2016 and variance analysis was conducted using the least significant difference method in DPS7.0 software.

3 Results

3.1 Effects of remediation on tobacco photosynthesis

3.1.1 Light response curve

As depicted in Fig. 1, the photosynthetic-light response curves of the four treatment groups of tobacco exhibited a similar trend. When photosynthetically active radiation (PAR) was ≤ 1200 (μmol m^{-2} s^{-1}), tobacco Pn (net photosynthetic rate) increased rapidly with increasing PAR. However, as PAR continued to rise, the growth of Pn in each treated tobacco decelerated, gradually approaching the maximum net photosynthetic rate of tobacco. At PAR ≤ 800 (μmol m^{-2} s^{-1}), the net photosynthetic rate of each treatment followed the order: G1C2 > G0C0 > G1C0 > G1C1. The net photosynthetic rate of tobacco was impacted differently by varying levels of Cd within the PAR range of 800 to 1800 (μmol m^{-2} s^{-1}). The G1C2 treatment had the highest net photosynthetic rate, followed by G0C0, G1C0, and G1C1. From PAR 200 to 1200 (μmol m^{-2} s^{-1}), the Pn values

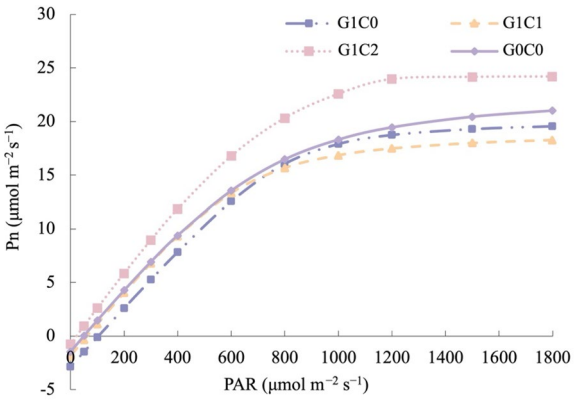


Fig. 1 Effect of Cd stress on the photosynthetic-light response curve of tobacco. The x-axis scale represents photosynthetically active radiation, while the y-axis indicates the net photosynthesis rate. Different sample groups are depicted using columns of distinct colors

of G1C1 decreased by 31.11, 24.25, 21.45, 20.63, 22.93, 25.39, and 27.09% compared to G1C2, respectively. These changes were all dependent on the action of microorganisms, indicating that microorganisms play an important role in antagonizing Cd. These results highlight the negative effects of cadmium on the net photosynthetic rate of tobacco. However, adding biochar in sufficient quantities to bind microorganisms can effectively mitigate this decline and even enhance the net photosynthetic rate of tobacco beyond its original level.

3.1.2 Photosynthetic light-response curve of tobacco

Table 2 shows that the photosynthesis of tobacco changed after the soil was contaminated with Cd. The AQY, Pmax, and LSP of G1C0 were all lower than those of G0C0, with reductions of 15.71%, 44.47%, and 30.24%, respectively. The addition of biochar to soil has the potential to substantially affect photosynthesis. Specifically, it has been observed that higher levels of biochar can lead to increased values of AQY, Pmax, and LSP, even at the same Cd concentration. These values were highest in G1C2, followed by G1C1 and G1C0. The AQY, Pmax, and LSP of G1C1 increased by 1.69%, 80.50%, and 30.76%, respectively, whereas the AQY, Pmax and LSP of G1C2 increased by 3.39%, 86.84%, and 62.35%, respectively, in comparison to G1C0. In summary, it can be inferred that Cd stress can adversely affect photosynthesis in plants. However, the use of biochar can potentially mitigate this stress effect.

3.2 Effects of remediation on tobacco dry matter accumulation

Based on the data presented in Table 3, it can be observed that Cd has a negative effect on the reproductive period of tobacco plants, leading to a constant inhibition of

Table 2 Effects of Cd stress on characteristic parameters of tobacco photosynthetic-light response curve

Treatment	AQY ($\mu mol\ mol^{-1}$)	P_{max} ($\mu mol\ m^{-2}\ s^{-1}$)	R_d ($\mu mol\ m^{-2}\ s^{-1}$)	LCP ($\mu mol\ m^{-2}\ s^{-1}$)	LSP ($\mu mol\ m^{-2}\ s^{-1}$)	R^2
G0C0	0.070	25.86	2.45	32.76	477.34	0.991
G1C0	0.059	14.36	3.53	47.11	332.99	0.996
G1C1	0.060	25.92	3.29	40.67	435.41	0.999
G1C2	0.061	26.83	3.09	39.97	540.61	0.996

Table 3 Effects of Cd stress on dry matter accumulation in tobacco

Days	Treatment	Root	Stem	Leaf	Total accumulation
30 d	G0C0	0.73 ± 0.041ab	0.41 ± 0.019c	3.73 ± 0.158c	4.87 ± 0.213c
	G1C0	0.28 ± 0.029 cd	0.09 ± 0.008d	0.73 ± 0.099f	1.10 ± 0.134ef
	G1C1	0.32 ± 0.012 cd	0.13 ± 0.012d	1.09 ± 0.062de	1.54 ± 0.086de
	G1C2	0.61 ± 0.021bc	0.15 ± 0.022d	1.37 ± 0.069d	1.90 ± 0.092d
60 d	G0C0	8.32 ± 1.02b	15.53 ± 0.99c	55.06 ± 4.69ab	78.91 ± 5.81b
	G1C0	4.73 ± 0.78c	3.49 ± 1.72e	27.27 ± 4.50c	35.49 ± 5.39d
	G1C1	7.22 ± 1.12b	8.99 ± 0.98d	43.79 ± 5.11b	60.00 ± 6.08c
	G1C2	11.88 ± 2.21ab	12.93 ± 2.26c	53.87 ± 4.76ab	78.67 ± 7.21b
90 d	G0C0	27.09 ± 2.12a	41.02 ± 2.78b	88.65 ± 5.09a	156.76 ± 9.99a
	G1C0	8.00 ± 0.59c	11.00 ± 0.77d	29.78 ± 2.49c	48.78 ± 3.85d
	G1C1	9.56 ± 0.52c	21.04 ± 1.76c	52.52 ± 4.15b	83.11 ± 6.43c
	G1C2	14.29 ± 1.87b	29.63 ± 2.15c	58.78 ± 5.45b	102.70 ± 9.47b

their growth. During the growth stages, it was observed that the highest total dry matter accumulation occurred in G0C0, while the lowest accumulation was found in G1C0. Specifically, at the 30th, 60th, and 90th days after tobacco transplantation, the total dry matter accumulation in G1C0 decreased by 77.41%, 55.02%, and 68.88%, respectively, compared to G0C0. On the other hand, G1C2 witnessed a significant reduction of 60.98%, 0.29%, and 34.49%, respectively, in the total dry matter accumulation compared to G0C0. Interestingly, the combination of biochar and microorganisms positively impacted dry matter accumulation in G1C2, as it was considerably higher than in G1C0. This finding suggests that biochar and microorganisms can potentially mitigate the adverse effects of Cd toxicity on tobacco plants.

3.3 Effects of remediation on cadmium in tobacco

Figure 2 shows that during tobacco transplantation, the leaves and stems had higher Cd levels, while the roots registered lower cadmium content. As growth advanced, the Cd concentration in the root area progressively increased, whereas the leaf and stem regions declined. After 60 days of tobacco transplantation, G1C0 roots had the highest Cd content, while G0C0, G1C1, and G1C2 showed significant reductions of 97.51%, 52.05%, and 66.63%, respectively in comparison to G1C0. The Cd concentration in tobacco remained unchanged 90 days after transplantation, indicating that the rate of Cd accumulation in tobacco is relatively stable after 60 days. The study found that biochar caused a notable decline in Cd

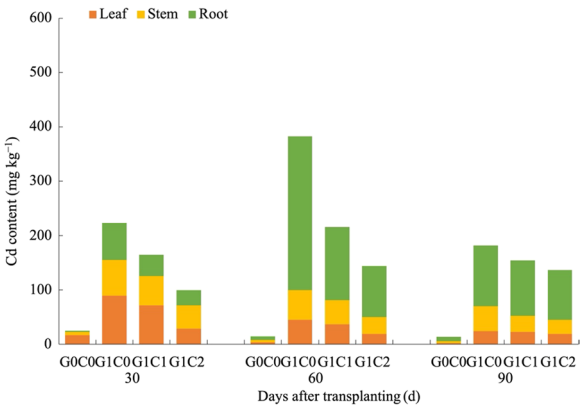


Fig. 2 Characteristics of Cd content in each part of flue-cured tobacco. The abscissa scale stands for the transplantation period, whereas the ordinate signifies the Cd content of each part, and the different colors characterize different parts of tobacco, and the letters below the column are the corresponding sample group names

content in tobacco plants under stress. Specifically, the root parts of G1C1 and G1C2 showed a decrease of 9.64% and 18.15%, respectively, compared to G1C0. The results indicate that biochar has a Cd-binding effect, and its combination with microorganisms can further enhance its effectiveness in reducing Cd content.

3.4 Effects of remediation on Cd enrichment and transport in tobacco

Table 4 displays the differences in bioconcentration and transport coefficients across various Cd treatments. When considering the entire tobacco plant, the pattern of enrichment coefficients for different parts (roots, stems, leaves) in each treatment was similar: G1C2 < G1C1 < G1C0, with G1C2 consistently having the lowest enrichment coefficients. The enrichment coefficients of G1C2 tobacco plants were significantly decreased by 70.89%, 70.18%, 72.52%, 71.91%, and 68.32%, respectively, compared to G1C0. The impact of biochar addition on the Cd enrichment coefficient varies depending on the dosage. When compared to the G1C1 group (no biochar addition), the G1C2 group with increased biochar content and microorganism addition had significantly lower enrichment coefficients of Cd by 32.16%, 36.43%, 16.00%, 10.32%, and 30.64% in different parts of the tobacco plant. Notably, this combination resulted in the smallest enrichment coefficient of Cd in each part of the tobacco plant. Therefore, the enrichment coefficients of Cd in the untreated tobacco plant (G0C0) were not analyzed since it did not receive Cd addition.

The translocation coefficient is a measure of the ability of a plant to transport Cd from underground to aboveground. It is expressed as the ratio of the Cd content of a particular aboveground plant part to that of a particular underground plant part. A higher transport coefficient indicates a greater plant capacity to transport Cd. Among the three treatments (G1C0, G1C1, G1C2) in this study, G1C1 showed the smallest transport index, while G1C2 displayed the lowest translocation index for the two translocation processes from stem to upper leaf and stem to lower leaf (Table 5). On the other hand, G1C0 exhibited the smallest transport index during the three translocation processes of root to lower leaf, root to stem, and stem to central leaf. In summary, biochar is an effective method for inhibiting the transfer of Cd from the roots and stem to the upper parts of the tobacco plant. This results in a decrease in the Cd content in the middle and upper sections of the plant. However, it should be noted that biochar is not effective in blocking the movement of Cd from the roots to the lower leaf and stem parts of the plant. Therefore, the transport coefficient of Cd was not analyzed for G0C0 as it was not added with Cd.

3.5 Effects of remediation on tobacco rhizosphere microbiota

The study obtained 669,023 valid sequences with an average of 111,503 sequences per sample. After uniform sampling, the study detected 496 fungal operational taxonomic units (OTUs) and 9,261 bacterial OTUs. Among all the samples, 77 fungal OTUs and 1,470 bacterial OTUs were shared. Figure 3 provides a visual representation of the findings.

The Simpson and Shannon indices were used to measure the diversity of bacterial communities, whereas the Chao indices were used to measure the richness of bacterial communities. A Coverage index was used to estimate the extent of bacterial community coverage. In Table 6, it was revealed that Cd in soil significantly reduced soil

Table 4 Effects of enrichment coefficients of Cd in various parts of tobacco

Treatment	Root	Stem	Leaf		
			Upper	Middle	Lower
G1C0	7.97 ± 0.37a	2.75 ± 0.09a	10.70 ± 0.43a	15.77 ± 0.72a	48.75 ± 1.59a
G1C1	3.42 ± 0.32b	1.29 ± 0.07b	3.50 ± 0.19b	4.94 ± 0.12b	22.26 ± 1.02b
G1C2	2.32 ± 0.16b	0.82 ± 0.03b	2.94 ± 0.18b	4.43 ± 0.44b	15.44 ± 0.98c

Table 5 Effects of heavy metals in Cd-contaminated soil on the transport coefficients between tobacco plants

Treatment	Root to upper leaf	Root to middle leaf	Root to lower leaf	Root to stem	Stem to upper leaf	Stem to middle leaf	Stem to lower leaf
G1C0	1.343 ± 0.065a	1.980 ± 0.059a	6.118 ± 0.099a	0.346 ± 0.027a	3.884 ± 0.077a	0.175 ± 0.007b	0.057 ± 0.001a
G1C1	1.023 ± 0.019b	1.444 ± 0.073b	6.510 ± 0.240a	0.378 ± 0.031a	2.709 ± 0.099b	0.262 ± 0.014a	0.058 ± 0.001a
G1C2	1.266 ± 0.076a	1.905 ± 0.045a	6.641 ± 0.177a	0.355 ± 0.016a	3.571 ± 0.063a	0.186 ± 0.004b	0.053 ± 0.002a

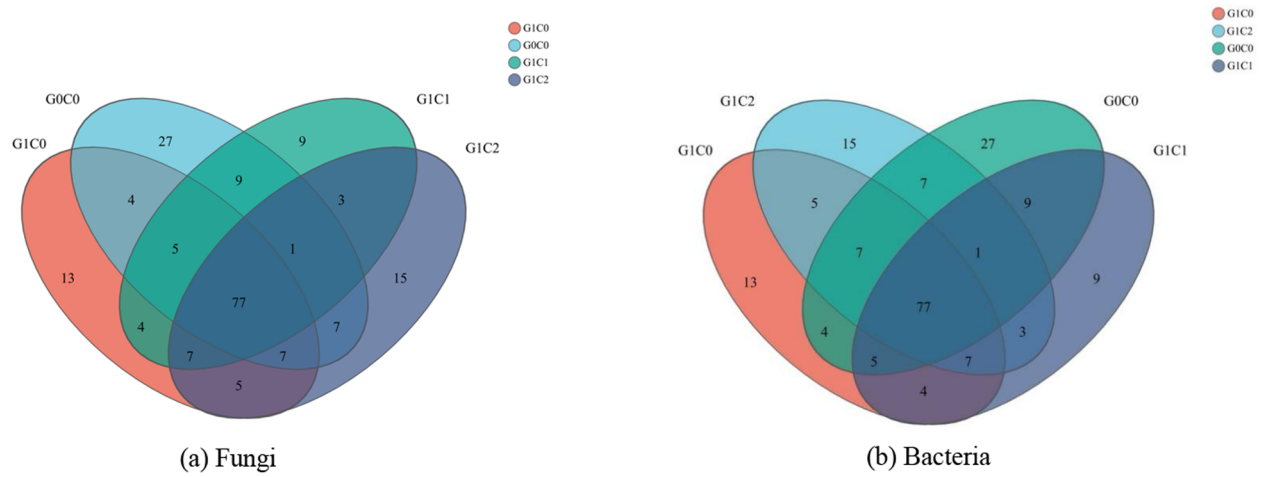


Fig. 3 Sample species Wayne diagram. Different color patches represent various interventions, and the overlapping parts of the two-color patches represent the same number of OTUs

Table 6 Diversity index of microbial communities

Kind	Treatment	Shannon	Simpson	Chao	Coverage
Fungi	G0C0	3.067368	0.092477	137.143	0.999968
	G1C0	2.551152	0.193266	118.500	0.999872
	G1C1	2.803606	0.109857	131.333	0.999855
	G1C2	2.796934	0.118193	131.1666	0.999832
Bacteria	G0C0	6.541458	0.003522	2733.815	0.988888
	G1C0	6.208819	0.005666	2486.901	0.986026
	G1C1	6.402161	0.005104	2875.289	0.985417
	G1C2	6.352537	0.005102	2774.637	0.986795

fungi diversity, as shown by the lowest Shannon index and highest Simpson index values observed in G1C0. Conversely, the application of biochar seemed to mitigate the negative impact of Cd on soil fungal diversity, as signified by higher Shannon index and lower Simpson index values in G1C1 and G1C2. Additionally, the Chao index values showed that Cd contamination significantly decreased soil fungi richness, with the greatest richness observed in G0C0 and the lowest in G1C0. In contrast, biochar was found to elevate the richness of soil fungal communities affected by Cd stress.

The α diversity index of bacterial communities exposed to Cd-contaminated soil was similar to that of fungal communities. Notably, the Simpson index sequence of fungal communities was observed to be G0C0 < G1C2 < G1C1 < G1C0, while the Shannon index sequence was G1C0 < G1C2 < G1C1 < G0C0. These results suggest that soil contamination with Cd caused a significant reduction in the α diversity of bacterial communities. However, the utilization of biochar has the potential to ameliorate this situation. Among the bacterial communities studied, G1C1 showed the highest species richness while G1C0 showed the lowest diversity as measured by the Chao index. All samples were sequenced to a coverage of over 98.54%, indicating that the sequencing depth was sufficient for further analysis.

3.6 Effects of remediation on tobacco rhizosphere microbial communities

3.6.1 Soil flora structure at the phylum level

As described in Fig. 4, the dominant species of soil fungal communities were primarily *Ascomycota*, *SAR_k__norank*, *Mucoromycota*, *Basidiomycota*, and *Zoopagomycota*. The study results demonstrate that G1C0 exhibited the highest relative abundance of *Ascomycota*, with an increase of 4.81% compared to G0C0. This finding suggests a slight increase in *Ascomycota* relative abundance in the soil following Cd addition. However, *Ascomycota* relative abundance decreased by 20.38% in G1C1 and 0.26% in G1C2 compared to G1C0. These results indicate that biochar can potentially reduce *Ascomycota* abundance in the soil. Additionally, G1C0 exhibited the lowest relative abundance of Mucoromycota among the treatments. However, G0C0, G1C1, and G1C2 showed an increase of 108.49%, 4.06%, and 273.51%, respectively, as compared to G1C0. Although there was no substantial variance in *Basidiomycota* relative abundance between G0C0 and G1C0, a noticeable difference was observed between G1C1 and G1C2. Specifically, the relative abundance of G1C1 was 95.74% lesser than that of G1C2.

As shown in Fig. 4, soil bacterial communities predominantly comprise *Actinobacteria*, *Proteobacteria*, *Chloroflexi*, *Acidobacteria*, and *Firmicutes*. The relative abundance of *Actinobacteria* on G0C0 and G1C0

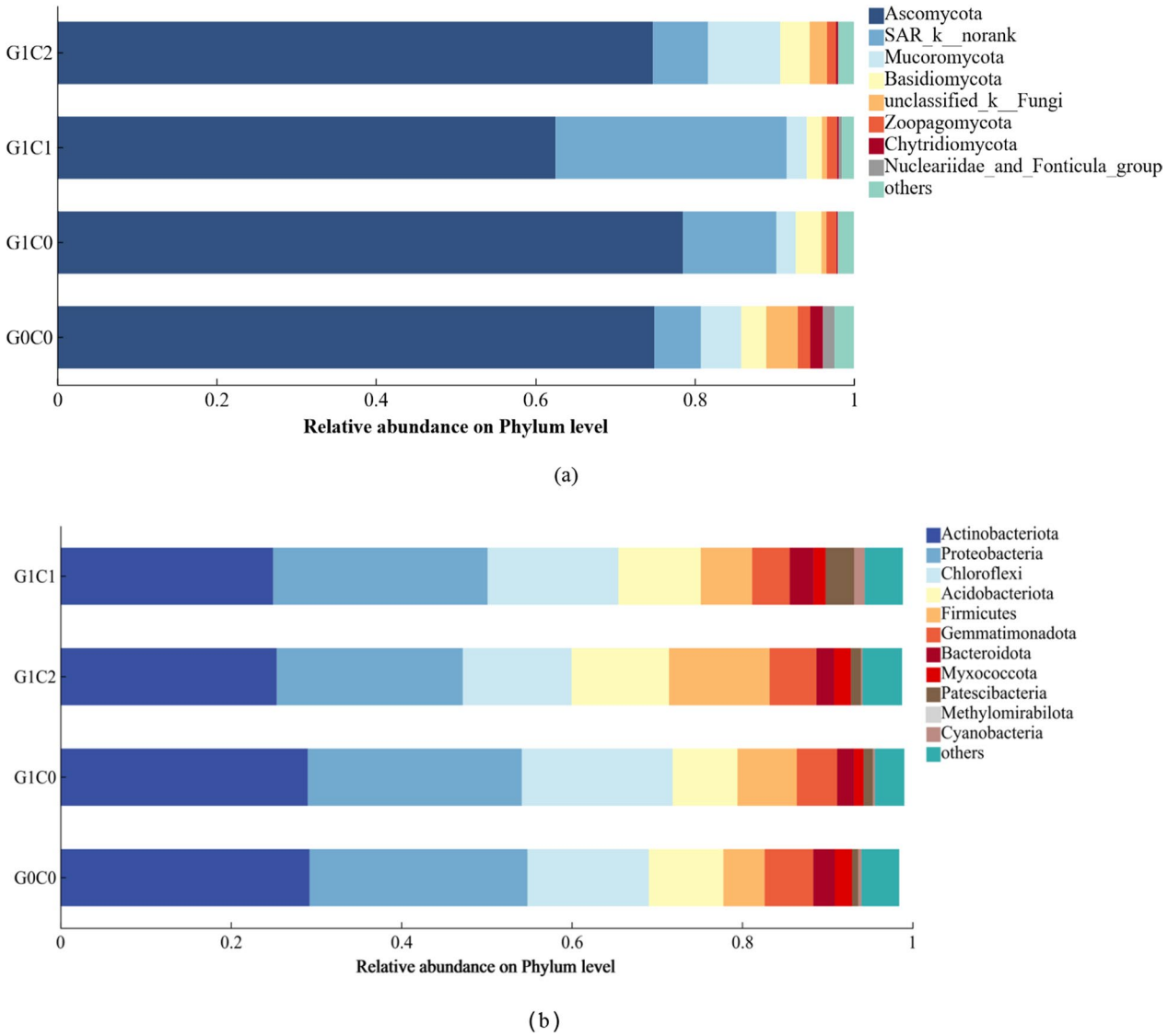


Fig. 4 Composition of fungal (a) and bacterial (b) community species at phylum level. The horizontal axis represents the species proportion, while the vertical axis represents different sample groups. Different species are denoted by columns of various colors, with the length of the columns indicating the proportion of each species

was markedly higher than that of G1C1 and G1C2. The addition of Cd did not significantly modify the relative abundance of *Actinobacteria* in the soil, but adding biochar reduced its abundance. The relative abundance of *Actinobacteria* in G0C0 and G1C0 showed a significant increase of 15.16% and 14.33%, respectively, compared to G1C1. These findings suggest that biochar addition can decrease the abundance of *Actinobacteria* in the soil, while Cd does not substantially impact its relative abundance. The treatment G1C2 recorded the lowest relative abundance of *Proteobacteria*, while the highest was observed in G0C0. Notably, the relative abundance of *Proteobacteria* in G1C2 was reduced by 17.08% compared to G0C0.

Conversely, the relative abundance of *Proteobacteria* in G1C0 and G1C1 did not exhibit significant differences compared to G0C0. Furthermore, the highest relative abundance of *Chloroflexi* was observed in the G1C0 treatment, while the lowest was recorded in G1C2. This suggests that the relative abundance of *Chloroflexi* increased when Cd was introduced into the soil, but biochar addition inhibited bacterial growth. The relative

abundance of *Chloroflexi* treated with G1C1 and G1C2 decreased by 27.85% and 15.31%, respectively, compared with G1C0. These findings suggest that the presence of Cd in soil impacts the relative abundance of *Proteobacteria* and *Chloroflexi*, and that adding biochar can mitigate this effect.

3.6.2 Soil microbiota structure at the genus level

Figure 5(a) shows the visible fungal community species at the genus level, revealing the dominant fungal species in the soil, including *Chaetomium*, *unclassified_f__Colpodea*, and *unclassified_c__Sordariomycetes*. Among these species, *Chaetomium* exhibited the highest abundance in G1C0 treatment, accounting for 38% of the total. The present study compared the impacts of G0C0, G1C1, and G1C2 treatments on *Chaetomium* relative abundance in soil samples. The results showed that G0C0 and G1C2 treatments accounted for 23% of the relative abundance, while G1C1 contributed 17%. The relative abundance of Chaetomium in G0C0, G1C1 and G1C2 decreased by 39.72%, 56.01%, and 40.63%, respectively, compared to G1C0. These findings suggest that Cd pollution leads to a decline in the relative abundance of *Chaetomium* in soil, but adding biochar can help mitigate this problem.

Figure 5(b) shows the visible bacterial community species at the genus level, highlighting the dominant bacteria in the soil, including *norank_f__JG30-KF-CM45*, *Bacillus*, *norank_f__norank_o__Vicinamibacterales*, *Arthrobacter*, and *Streptomyces*. In *norank_f__JG30-KF-CM45*, the four treatments accounted for the abundance of G0C0 (23%), G1C0 (32%), G1C1 (23%), and G1C2 (22%). The abundance of different *Bacillus* species was analyzed in a soil sample contaminated with Cd. The results indicated that Cd contamination led to variations in *Bacillus* species abundance levels. Specifically, the abundance shares of G0C0, G1C0, G1C1, and G1C2 were 17%, 14%, 48%, and 20%, respectively. These findings suggest that Cd contamination can significantly impact the microbial community in soil, potentially affecting soil health and plant growth. However, biochar has been shown to restore the abundance of these bacterial genera to normal levels.

3.7 Effects of remediation on soil microbial and soil enzyme activity

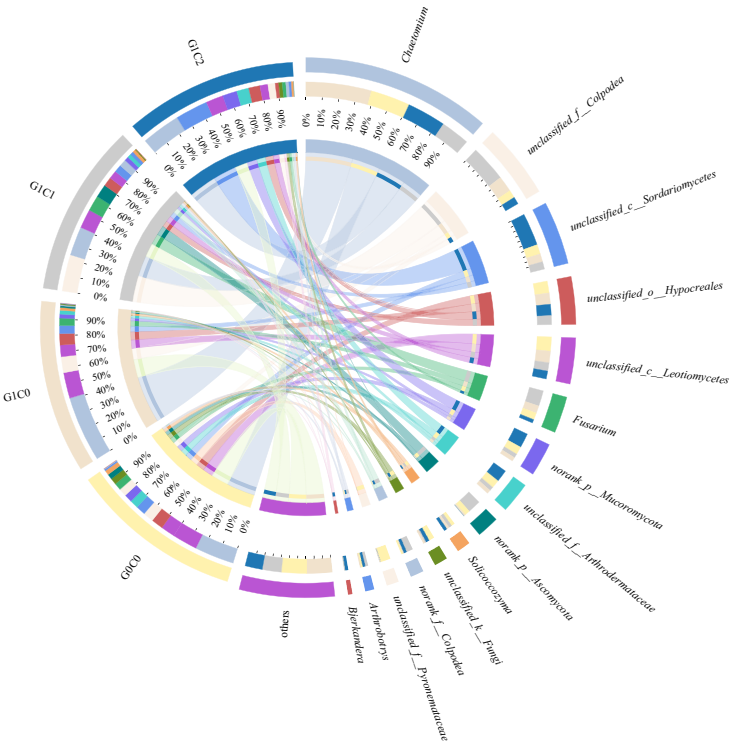
Soil microbial carbon was significantly reduced after Cd contamination. The results indicate a significant decline of 32.48% in soil microbial carbon content compared to G0C0 (Fig. 6). However, an increase in soil microbial carbon content was observed in both G1C1 and G1C2, by 46.42% and 76.27%, respectively, when compared to G1C0. It is imperative to note that G1C2 exhibited a substantial increase of 19.01% compared to G0C0. These findings suggest that biochar can effectively enhance soil microbial carbon content and mitigate Cd pollution. In addition, the combination of biochar with microorganisms showed the most significant improvement in soil microbial carbon.

Figure 7 shows the application of biochar and microorganisms to soil contaminated with Cd, resulting in a significant increase in soil microbial nitrogen. Specifically, the soil microbial nitrogen of G0C0, G1C1, and G1C2 increased by 18.24%, 21.56%, and 28.12%, respectively, compared to G1C0. Notably, the increase in soil microbial nitrogen from applying biochar and microorganisms was significantly different from the soil microbial biomass nitrogen of G1C2. These findings suggest that although Cd contamination negatively affects soil microbial nitrogen, applying biochar and microorganisms can mitigate these effects and even enhance soil microbial nitrogen levels.

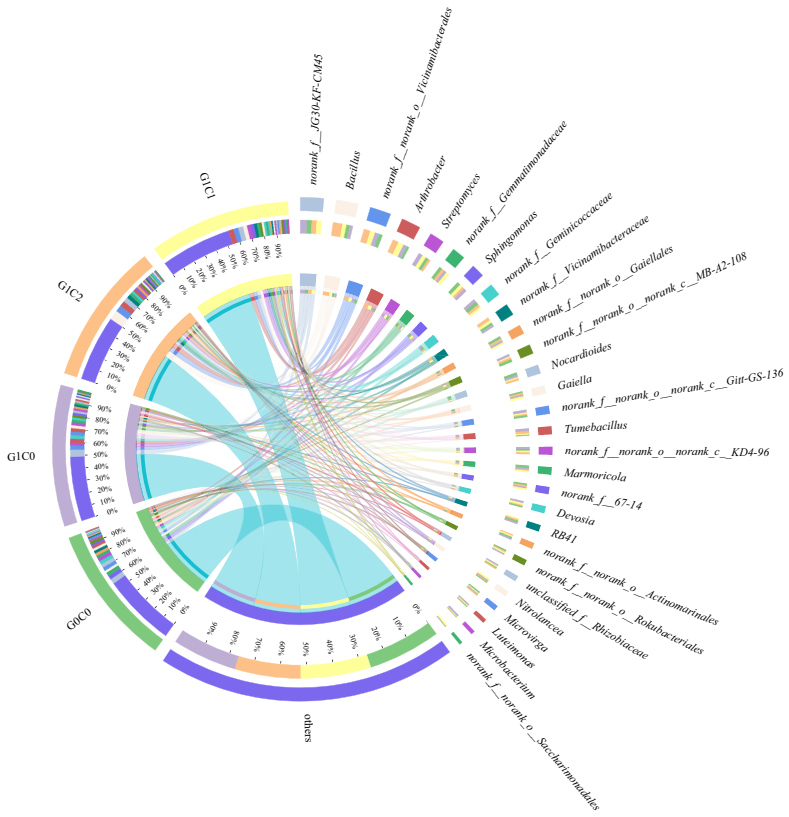
Figure 8 depicts that the soil sample G1C0 exhibited the lowest urease activity, which was 13.34% less than that of G0C0. This observation suggests a significant reduction in soil urease activity due to Cd contamination. However, the urease activities of G1C1 and G1C2 showed a noteworthy increase of 12.42% and 63.89%, respectively, compared to G1C0. Notably, the urease activity in G1C2 exceeded that of G0C0, indicating that biochar can enhance urease activity and restore it in Cd-contaminated soils. In summary, biochar may be an effective tool to remediate cadmium-contaminated soils by enhancing urease activity. The results presented in Fig. 9 demonstrate that the catalase activity of G0C0 was 18.11% higher than that of G1C0. This observation implies that Cd contamination would significantly reduce soil peroxidase activity. However, the soil catalase activities of G1C1 and G1C2 increased significantly by 13.03% and 14.17%, respectively, compared to G1C0. Overall, this finding suggests that biochar in Cd-contaminated soil can help restore catalase activity.

3.8 Correlation analysis of soil microorganisms with soil enzyme activity and microbial biomass under different treatments

Table 7 reveals a significant correlation between the relative abundance of the top 20 microorganisms at the phylum level and the amount of soil microbial carbon, nitrogen, and enzyme activity. It was observed that *Firmicutes* exhibited a positive association with microbial biomass nitrogen, microbial biomass carbon, and urease activity. In fact, *Firmicutes* emerged as the microbial class with the strongest correlation with soil microbial biomass and enzyme activity. In addition, the results indicate that urease activity is significantly associated with microbial community composition. Specifically, *Chloroflexi* negatively correlated with urease activity,



(a)



(b)

Fig. 5 Visible circle diagram of fungi (a) and bacterial (b) community species at genus level. The width of the bars from each genus indicates the relative abundance of that genus in the sample. Different color blocks represent different microorganisms

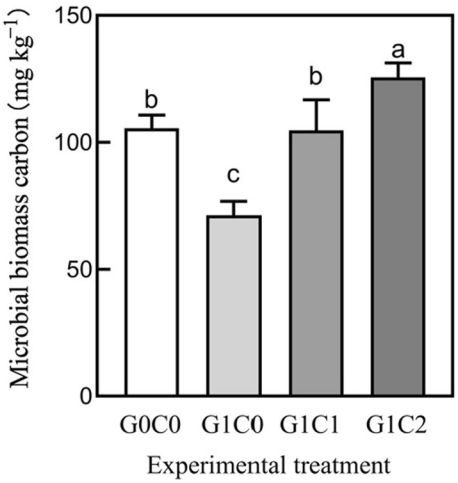


Fig. 6 Effects of biochar on soil microbial biomass carbon

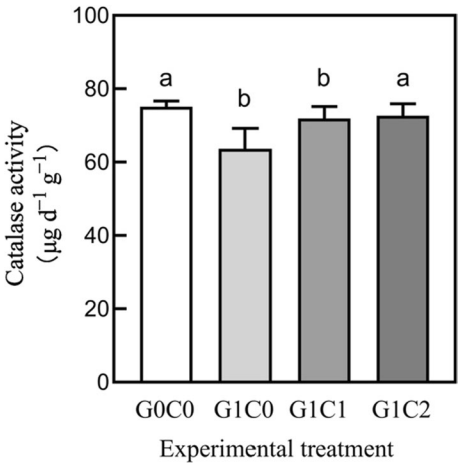


Fig. 9 Effect of biochar on soil catalase activity

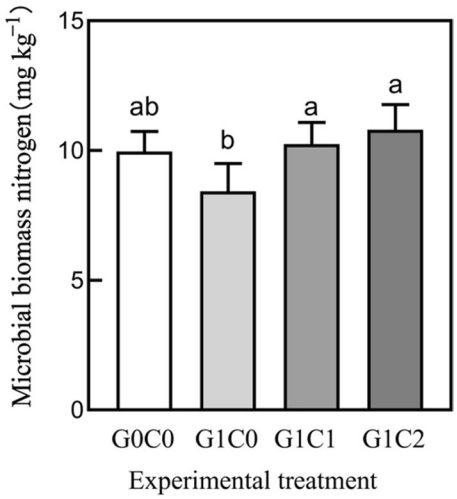


Fig. 7 Effects of biochar on soil microbial biomass nitrogen

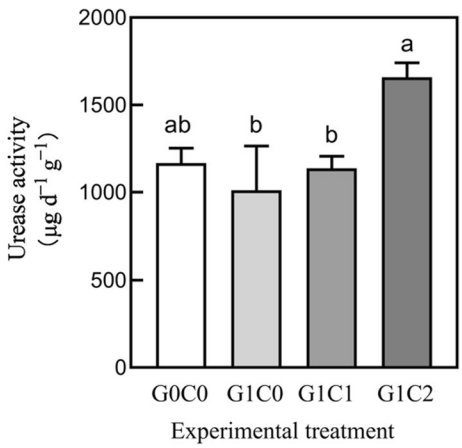


Fig. 8 Effect of biochar on soil urease activity

while *Gemmatimonadota*, *Mucoromycota*, and *Firmicutes* demonstrated a positive correlation. These findings underscore the pivotal role that urease activity plays in shaping microbial community dynamics, as shown in Fig. 10.

4 Discussions

4.1 Impact of remediation on tobacco physiological indicators under cadmium contamination

Tobacco is widely recognized for its capacity to absorb Cd from the soil. However, excessive Cd uptake can significantly impair tobacco growth, photosynthetic efficiency, and overall physiological function while also posing serious health risks through smoking (Rosén et al. 2012). A recent study revealed that Cd contamination in the soil negatively affects tobacco photosynthesis, resulting in a substantial reduction in AQY, Pmax, and LSP (Nie et al. 2019). However, the application of biochar has been found to mitigate this adverse effect. To mitigate Cd uptake in tobacco, biochar and microbial agents are widely utilized due to their ability to immobilize heavy metals in soil, alter Cd bioavailability, and enhance plant stress tolerance. Biochar, produced through biomass pyrolysis, contains abundant oxygen-containing functional groups and a highly porous structure, which contribute to Cd immobilization by various mechanisms, including ion exchange, complexation, precipitation, and electrostatic interactions (Li et al. 2021). These properties enable biochar to adsorb and retain Cd, thereby limiting its mobility in the soil and reducing its uptake by plant roots. Furthermore, biochar improves soil physicochemical properties, such as pH and cation exchange capacity, which further restrict Cd solubility and uptake by plants.

Microbial agents, particularly *Trichoderma* species, also play a crucial role in reducing Cd absorption

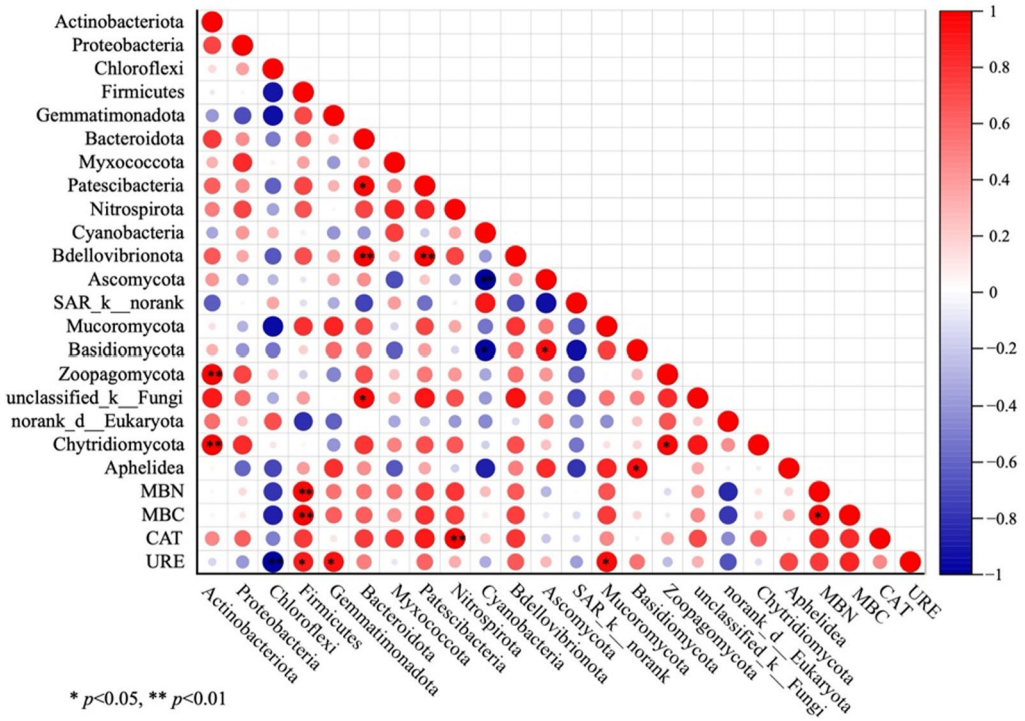


Fig. 10 Correlation analysis of microbial communities and environmental factors. The visualization of the relationship between the microbial community and environmental factors is achieved using a color gradient in the color block. The color column on the right side of the figure represents the numerical values corresponding to the color gradient. The symbol * indicates significance at $p < 0.05$, while ** indicates significance at $p < 0.01$

by tobacco. These beneficial microorganisms promote plant resistance to heavy metal stress through multiple mechanisms. The microbes produce organic acids and extracellular enzymes that bind to Cd ions, forming stable complexes that prevent Cd from entering plant roots (Babu et al. 2014; Li et al. 2021). In addition, microbial biofilms enhance root protection by creating a physical barrier against Cd uptake. Some microbes also induce the formation of plant metallothioneins and phytochelatins, which help detoxify Cd within plant tissues, reducing its harmful effects (Devi et al. 2022). Moreover, biochar provides a conducive environment for microbial activity by offering a stable habitat and improving soil moisture and nutrient retention (Karnwal et al. 2024), further enhancing the efficiency of microbial Cd immobilization.

The combined application of biochar and microorganisms has been shown to be more effective than biochar alone in mitigating Cd stress in tobacco. The use of biochar significantly decreases Cd accumulation in different tobacco plant tissues, lowering the Cd enrichment index and restricting its translocation from roots to upper leaves, roots to middle leaves, and stems to upper leaves. These findings are consistent with previous studies by Shar et al. (2025), which highlight the effectiveness of biochar-microbe interactions in Cd remediation. This study also found that Cd concentrations were consistently higher in roots than in above-ground tissues. This phenomenon is primarily attributed to the suppression of xylem loading under elevated Cd conditions, which impairs Cd translocation efficiency to aerial organs. Sequestration of Cd within root vacuoles further restricts its mobilization to above-ground tissues, thereby contributing to the alleviation of Cd toxicity in shoots.

Building upon these observations, a comparative evaluation of G1C2 (biochar + microorganisms) and G1C1 (biochar only) treatments further illustrates the added value of microbial inoculation in enhancing physiological and biochemical responses in tobacco under cadmium stress. The co-application of biochar and microorganisms (G1C2) demonstrated notable improvements in various physiological and biochemical indicators of tobacco plants compared to the biochar-only treatment (G1C1), thus confirming the additional benefits conferred by microbial inoculation. In particular, the G1C2 group exhibited higher AQY, Pmax, and LSP, indicating enhanced photosynthetic efficiency under cadmium stress. Compared to G1C1, these parameters in G1C2 increased by 1.70%, 3.51%, and 24.18%, respectively,

demonstrating the microorganisms' role in further alleviating the inhibitory effects of cadmium on photosynthesis. Similarly, total dry matter accumulation in G1C2 reached 102.70 g at the 90-day growth stage, which was approximately 23.6% higher than that in G1C1, suggesting that the addition of microorganisms supported more vigorous plant growth under stress conditions. Furthermore, the G1C2 treatment resulted in lower cadmium content in tobacco tissues and reduced enrichment and translocation coefficients, confirming that microorganisms not only improved plant physiological performance but also contributed to reduced cadmium uptake and mobility within the plant. Enhanced soil enzymatic activities (urease and catalase) and greater microbial biomass carbon and nitrogen levels were also observed in G1C2, reflecting a more active and healthier rhizosphere environment due to microbial presence. The microbial inoculation further enriched the soil microbial community structure, as evidenced by increased fungal and bacterial diversity, including the proliferation of beneficial genera such as *Mucoromycota*, *Chaetomium*, and *Bacillus*. These results collectively support the conclusion that microorganisms significantly contribute to the overall effectiveness of the biochar-based remediation strategy and should be considered an integral component of sustainable soil restoration approaches under heavy metal stress.

4.2 Effects of different treatments on soil microorganisms under cadmium contamination

The high toxicity of Cd metal in soil can cause severe cellular damage, DNA destruction, and a reduction in the diversity of soil microbial communities (Xie et al. 2016). Excessive toxicity may result in microbial death (Mitra et al. 2018; Qin et al. 2019). A recent study discovered that fungal and bacterial community diversity decreased after soil was contaminated with Cd (Azadi and Raiesi 2021; Xu et al. 2023). Fang et al. (2020) confirmed that the abundance of fungal and bacterial species in cadmium-contaminated soil was significantly lower than in cadmium-free soil.

Similarly, Xu et al. (2020) examined the effects of excess cadmium on microbial enzyme activity, finding that cadmium contamination disrupts gene regulation, impairs microbial metabolic pathways, induces cell death, and alters the population and structure of soil microbial communities. Specifically, Cd contamination in the soil significantly alters the structure of the soil microbial community, resulting in a decline in the abundance of dominant bacteria such as *Mucoromycota*, *Chaetomium*, and *Bacillus*. Furthermore, the study found that the variation and stability of soil microbial communities increase significantly when soil Cd pollution reaches a certain level, ultimately affecting the structure of rhizosphere microbial communities (Gao et al. 2025). Overall, this study highlights the need for effective measures to mitigate Cd contamination in soil to preserve the health of soil microbial communities and maintain the ecological balance of soil ecosystems.

Nevertheless, soil Cd contamination can be remedied by adding soil amendments, with biochar being a cost-effective method (Zhang et al. 2024). Compared to other soil amendments, biochar has been proven to be more effective in improving soil fertility and microbial activity while reducing heavy metal bioavailability. Research (Yan and Liu 2020) has shown that when exposed to similar Cd pollution conditions, the Shannon index and Chao index of G1C1 and G1C2 are significantly greater than those of G1C0, signifying that biochar can improve microbial diversity and community richness in Cd-contaminated soil. This finding is consistent with previous studies that reported increased microbial diversity in biochar-amended soils (He et al. 2020; Hossain et al. 2020). However, unlike previous research that primarily focused on the physical adsorption of heavy metals by biochar, our study uniquely demonstrates that the combination of biochar with microorganisms further enhances the relative abundance of dominant bacterial species such as Mucoromycota, Chaetomium, and Bacillus. This is because biochar has substantial porosity and desirable physicochemical properties that allow it to absorb, co-precipitate, and form compounds with heavy metal, such as Cd (He et al. 2020; Hossain et al. 2020; Pokharel et al. 2020). As a result, biochar alters the spatial distribution of heavy metals in soil, reduces their bioavailability, mitigates their toxicity, and minimizes the damage caused to soil microorganisms by Cd (Liu et al. 2020a, b; Huang et al. 2020a, b). Unlike other studies that mainly focused on the impact of biochar on heavy metal speciation, our research provides comprehensive insights into the combined effects of biochar and microorganisms on soil microbial communities. These findings underscore biochar's promise in remediating Cd-contaminated soil and provide new insights into its interplay with soil microorganisms. Specifically, the results demonstrate that soil Cd contamination disrupts gene regulation, impairs microbial metabolic pathways, triggers apoptosis, and alters the population dynamics and community structure of soil microorganisms.

4.3 Effects of biochar and microbial combination on soil enzyme activity under cadmium contamination

Biochar has been found to have a dual effect on soil quality. In addition to mitigating Cd toxicity, it also enhances soil enzyme activity and increases soil microbial biomass carbon and nitrogen content (Max et al. 2011; Ball et al. 2010). Soil contamination with Cd is known to cause a

decline in urease and catalase activities. This is primarily because cadmium tends to occupy the active center of the enzyme or forms stable complexes with the thiol, amine, and carboxyl groups of the enzyme molecule, which subsequently results in competitive inhibition of the substrate. Moreover, soil heavy metals hinder the proliferation and reproduction of microorganisms in the soil, leading to decreased enzyme synthesis, secretion, and enzyme activity (Fang et al. 2020).

The soil catalase activities of G1C1 and G1C2 were found to have increased significantly by 13.03% and 14.17%, respectively, when compared to G1C0. Similarly, the urease activities of G1C1 and G1C2 showed considerable increase by 12.42% and 63.89%, respectively, compared to G1C0. These observations suggest that biochar utilization has led to higher urease and catalase activities (Feng et al. 2020). The combination of biochar and microbial agents enhances soil microbial diversity, leading to a more pronounced improvement in soil enzyme activity. This effect can be explained through microbial ecology theory, where biochar provides a habitat for beneficial microorganisms, fostering microbial community stability and diversity. Increased microbial diversity promotes more efficient nutrient cycling by accelerating the decomposition of organic matter and the transformation of essential nutrients. In addition, biochar could help to stabilize microbial populations by improving soil structure and water retention, creating a favorable environment for microbial activity. The biochar-microbial approach also mitigates the negative impact of Cd contamination on soil enzyme activity. This finding aligns with a study by Majewska and Hanaka (2025), which demonstrated that heavy metal pollution alters microbial metabolic communities and inhibits urease and catalase enzyme activity. The observed effects can be attributed to the interaction between Cd and soil enzyme–substrate complexes, which can cause denaturation of enzyme proteins, interaction with active protein groups, and affect microbial cell synthases, ultimately leading to decreased soil enzyme activity (Xu et al. 2020). Overall, the use of biochar and microbial agents can be an effective approach to mitigating the adverse effects of heavy metal contamination on soil enzyme activity.

Biochar has been shown to improve soil organic matter content and increase the amount of stable carbon components. It provides more substrate for enzymatic reactions, regulates soil water and fertilizer balance, and creates favourable conditions for enzymatic reactions (Wang et al. 2019). The increase of microbial agents can improve soil physicochemical properties and introduce numerous active strains. When exogenous microorganisms are added, they further enhance soil nutrient cycling and improve soil enzyme activity. The combined effect of biochar and microbial agents can significantly improve soil quality and promote healthy plant growth.

4.4 Mechanism of biochar and microbial combination to microbial control in cadmium-contaminated soil

The analysis of soil enzyme activity, soil microbial biomass carbon and nitrogen, and soil microorganisms showed a strong correlation between soil urease and soil microorganisms. Specifically, microbial biomass carbon, nitrogen, and catalase were found to be related to certain dominant bacteria phyla such as *Gemmatimadota* and *Firmicutes*. These microbial groups play key roles in nutrient cycling, particularly in the transformation of organic matter and nitrogen, which directly influences soil fertility and plant growth under Cd stress (Mukherjee et al. 2022; Zhou et al. 2024). The enrichment of these beneficial microbial taxa suggests that biochar, in combination with microbial inoculants, enhances microbial-driven nutrient transformation, thereby promoting a more resilient soil environment.

Biochar, with its large surface area, high porosity, and oxygen-containing functional groups, creates a favorable microhabitat for microbial colonization and enhances microbial metabolic activity (Fig. 11). The interaction between Cd and biochar probably occurs through adsorption, co-precipitation, and complexation, leading to a reduction in Cd bioavailability and toxicity (Yuan et al. 2020). By limiting Cd mobility in the soil, biochar indirectly supports microbial survival and enzymatic functions, as heavy metal stress often inhibits microbial diversity and activity. Biochar also adsorbs soil enzyme reaction substrates, facilitating multi-enzyme system activity that enhances nutrient cycling, particularly in nitrogen and carbon transformations. Increased urease activity, for instance, suggests an accelerated nitrogen mineralization process, making nitrogen more available for plant uptake, which is critical for tobacco biomass accumulation and stress tolerance.

In addition, the observed increase in *Gemmatimonadota* and *Firmicutes* abundance in biochar-treated soils aligns with their known ecological functions in organic matter decomposition, heavy metal detoxification, and soil aggregation. *Gemmatimonadota* has been reported to have a positive correlation with urease activity, indicating its role in nitrogen cycling and microbial-mediated Cd detoxification (Gong et al. 2024; Mukherjee et al. 2022). Similarly, *Firmicutes* were positively associated with microbial biomass carbon and nitrogen, reflecting their contribution to carbon turnover, organic matter decomposition, and microbial resilience under Cd stress. By improving soil microbial community structure and enzymatic activity, biochar-based amendments reduce Cd phytotoxicity, enhance nutrient availability, and

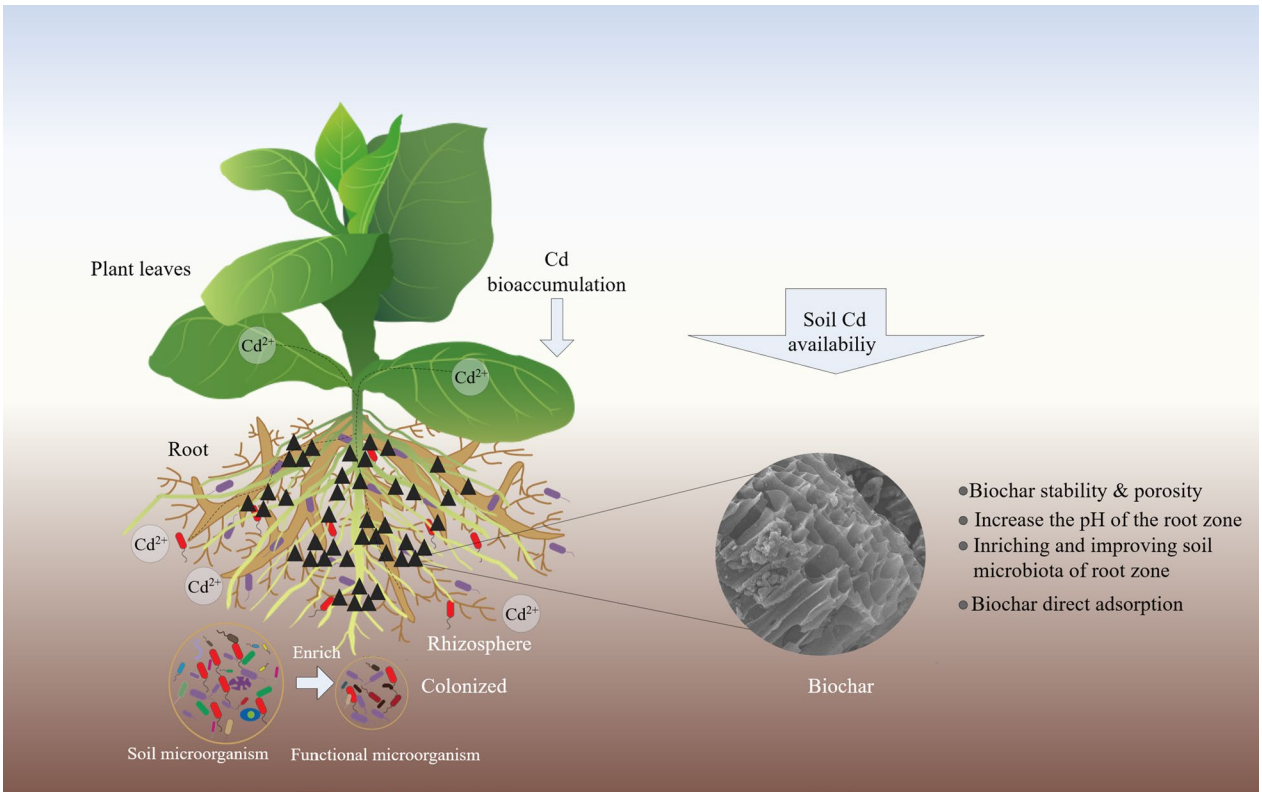


Fig. 11 Mechanism of biochar and microbial combination to reduce Cd stress. The black solid triangle represents biochar, and colorful cells represent soil microorganisms

promote a balanced rhizosphere microecology, ultimately supporting tobacco root development, nutrient uptake, and stress resistance.

Overall, these findings suggest that the application of biochar and *Trichoderma* not only mitigates the toxic effects of Cd but also enhances soil microbial functions that are essential for sustainable soil health and plant productivity. The integration of microbial ecology theory in this study provides insights into the mechanisms by which biochar stabilizes heavy metals, supports microbial-mediated nutrient transformations, and strengthens plant–microbe interactions in Cd-contaminated soils. This comprehensive approach highlights the potential of biochar-microbial combinations as a sustainable remediation strategy for heavy metal-polluted agricultural lands.

5 Potential applications of biochar-microbial remediation

In addition to the biological and physiological improvements observed in this study, it is essential to assess the practical feasibility and economic viability of the proposed remediation strategy. The results clearly demonstrate that the co-application of biochar and Trichoderma significantly improves plant physiological performance, soil enzymatic activity, and microbial diversity under cadmium stress. Specifically, the G1C2 treatment (biochar + microorganisms) resulted in marked enhancements in photosynthetic efficiency, with Pmax increasing by 86.84%, alongside substantial gains in dry matter accumulation—more than double that observed in the cadmium-only treatment group (G1C0). Furthermore, cadmium accumulation in tobacco tissues was notably reduced. These findings indicate that the synergistic treatment outperforms both biochar alone (G1C1) and untreated controls, confirming its high efficacy in restoring soil quality and promoting plant health in cadmium-contaminated environments.

Beyond performance, the cost estimation of this approach also supports its practical relevance. Biochar production costs are influenced by feedstock type, pyrolysis conditions, and production scale. When using peanut shells (an abundant agricultural residue), the estimated cost breakdown includes a raw material cost of approximately 220–260 CNY/ton, an operational cost of 180–200 CNY/ton, and a selling price in the range of 830–1020 CNY/ton. The microbial inoculant (*Trichoderma spp.*) used in this study was produced by the

Henan Biochar Engineering Technology Research Center. While precise costs are not publicly disclosed, Trichoderma-based products are typically affordable due to low cultivation and formulation costs, particularly when scaled up for agricultural applications.

Compared to conventional remediation techniques such as chemical leaching, soil excavation, or phytoremediation, the biochar–microbial synergy offers a more sustainable and cost-effective alternative. It allows for in-situ treatment, avoiding soil removal and reducing labor and transportation costs. This method not only limits the environmental footprint associated with remediation activities but also enhances long-term soil fertility, microbial balance, and plant resilience. Conventional methods, though sometimes faster in effect, are often cost-prohibitive, disruptive to soil structure, and unsustainable for large-scale agricultural use. In contrast, the biochar–microbial strategy integrates well into existing farming systems with minimal disruption, making it a highly adaptable solution for long-term soil health restoration.

The combined use of biochar and microbial agents thus presents a promising strategy for remediating Cd-contaminated soils, offering both environmental and agricultural benefits. This approach can be applied in farmlands affected by heavy metal pollution, particularly in regions where Cd contamination poses a risk to food security and agricultural sustainability. The ability of biochar to immobilize Cd, enhance soil fertility, and promote microbial diversity makes it a valuable tool for restoring contaminated soil ecosystems. Furthermore, microbial agents such as *Trichoderma* contribute to metal detoxification, plant stress resistance, and soil microbial balance, further enhancing the efficiency of remediation (Altaf et al. 2024). These combined effects not only reduce Cd bioavailability in the soil but also help protect crops from heavy metal stress, ensuring improved plant growth and productivity in contaminated environments. Compared to conventional remediation techniques, such as chemical soil washing or excavation, biochar-based strategies are more sustainable, environmentally friendly, and compatible with agricultural systems. Moreover, this method does not require the removal of contaminated soil, making it a practical in-situ approach that can be integrated into existing agricultural practices with minimal disruption to farming activities.

From an economic perspective, the use of biochar derived from agricultural waste offers a cost-effective solution for soil remediation. Since biochar can be produced from crop residues, wood chips, and animal manure, its widespread availability reduces dependence on expensive soil amendments (Patel and Panwar 2024). Additionally, microbial agents, while requiring initial investment, offer long-term benefits by enhancing soil microbial ecosystems, reducing fertilizer dependency, and improving crop yields (Tripathi et al. 2020). To ensure large-scale implementation, future research should focus on cost–benefit analyses, optimization of biochar-microbial formulations, and regulatory frameworks to support its adoption.

6 Conclusion

The presence of Cd has been found to impede growth, reduce leaf photosynthesis, and hinder dry matter aggregation throughout the tobacco growth cycle. Cd concentration in tobacco leaves is the highest during the pre-growth stage, while it becomes more concentrated in the roots during the intermediate and final stages. This study shows that the combination of biochar and microbes enhances tobacco growth, effectively reduces Cd accumulation, and limits its translocation within the plant. Beyond its detrimental effects on plant physiology, Cd contamination significantly disrupts soil health by inhibiting soil urease and catalase performance, decreasing the amounts of soil microorganisms, carbon and nitrogen, diminishing soil microbial diversity and community richness, and altering the soil flora structure. Consequently, the abundance of dominant bacteria such as *Mucoromycota*, *Chaetomium*, and *Bacillus* was reduced.

The application of biochar alleviates these negative effects by enhancing tobacco's physiological resilience to Cd stress and mitigating its impact on soil quality. Biochar also improves soil enzymatic activity, carbon and nitrogen composition in microbial biomass, and soil microbial diversity. Applying biochar also promotes the growth of beneficial microorganisms such as *Gemmatimonadota* and *Firmicutes*, which help maintain soil health and productivity. In addition to biochar's role, microbial agents play a crucial role in inhibiting Cd uptake by tobacco. *Trichoderma* contribute to Cd immobilization by producing organic acids, extracellular enzymes, and biofilms that bind Cd ions, reducing their bioavailability and uptake by plant roots. Microbial activity also improves soil aggregation and nutrient availability, creating a more favorable environment for plant growth under Cd stress.

The present study highlights the potential of biochar and *Trichoderma* for enhancing soil and plant quality under Cd pollution. Notably, biochar has certain desorption properties, which warrant further investigation into biochar and Cd migration and infiltration behavior under surface runoff, such as irrigation and rainfall. Furthermore, it is crucial to consider the interaction mechanism and environmental ecological consequences of biochar and Cd in the water and the interface between soil and

surroundings from the perspective of soil microorganisms. Consequently, additional research is needed to uncover these aspects and comprehensively understand the potential benefits and drawbacks of biochar and fungal agents for soil and plant health under adverse environmental conditions.

Acknowledgements
This work is supported by the Ministry of Higher Education (MOHE), Malaysia under Higher Institution Centre of Excellence (HICoE) Phase-2 research grant awarded to the Institute of Tropical Aquaculture and Fisheries (AKUATROP), University Malaysia Terengganu. The authors also acknowledge Henan Agricultural University and Sohar University under a Memorandum of Understanding (MOU) for Research Collaboration with Universiti Malaysia Terengganu.

Author contributions
All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Tianbao Ren, Huilin Feng, Wan Adibah Wan Mahari, and Fei Yun. The first draft of the manuscript was written by Huilin Feng. Maosen Li, Nyuk Ling Ma, Xianjie Cai, Guoshun Liu, Rock Keey Liew contributed in writing, review and editing the manuscript. Su Shiung Lam and Tianbao Ren contributed in supervision, project administration, writing, review, editing and validating the manuscript.

Funding
The research leading to these results received funding from the Ministry of Higher Education (MOHE), Malaysia under Higher Institution Centre of Excellence (HICoE) Phase-2 research grant awarded to the Institute of Tropical Aquaculture and Fisheries, Universiti Malaysia Terengganu , Key Research and Development Projects of Henan Tobacco Company (2022611027, 2024411000240036), Shanghai Tobacco Group Science and Technology Project (K2024-1-054P) and Key Science and Technology Project of Sichuan China Tobacco Industry Co., Ltd. (yl202212).

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

Declarations

Competing interests
Su Shiung Lam is an AE of the journal Biochar, but he was not involved in the peer-review or handling of the manuscript. The authors have no other competing interests to disclose.

Author details
[1]Tobacco College of Henan Agricultural University, Zhengzhou 450002, China. [2]Henan Biochar Technology Engineering Laboratory/Henan Biochar Engineering Technology Research Center, Zhengzhou 450002, China. [3]Higher Institution Centre of Excellence (HICoE), Institute of Tropical Aquaculture and Fisheries (AKUATROP), Universiti Malaysia Terengganu, Kuala Nerus, 21030 Terengganu, Malaysia. [4]BIOSES Research Interest Group, Faculty of Science & Marine Environment, Universiti Malaysia Terengganu, Kuala Nerus 21030, Malaysia. [5]Center for Global Health Research (CGHR), Saveetha Medical College, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai, India. [6]Shanghai Tobacco Group Co, Ltd, Shanghai 200082, China. [7]Henan Province Engineering Research Center for Biomass Value-added Products, School of Forestry, Henan Agricultural University, Zhengzhou 450002, China. [8]Faculty of Engineering, Sohar University, PO Box:44, PC:311 Sohar, Oman.

Received: 17 October 2024 Revised: 24 September 2025 Accepted: 9 October 2025
Published online: 19 November 2025

References

Altaf M, Ilyas T, Shahid M, Shafi Z, Tyagi A, Ali S (2024) Trichoderma inoculation alleviates Cd and Pb-induced toxicity and improves growth and physiology of Vigna radiata (L.). ACS Omega 9:8557–8573. https://doi.org/10.1021/acsomega.3c10470

Aryal R, Nirola R, Beecham S, Sarkar B (2016) Influence of heavy metals in root chemistry of Cyperus vaginatus R. Br: a study through optical spectroscopy. Int Biodeterior Biodegrad. https://doi.org/10.1016/j.ibiod.2016.04.008

Azadi N, Raiesi F (2021) Biochar alleviates metal toxicity and improves microbial community functions in a soil co-contaminated with cadmium and lead. Biochar 3:485–498. https://doi.org/10.1007/s42773-021-00123-0

Babu AG, Shim J, Bang K-S, Shea PJ, Oh B-T (2014) Trichoderma virens PDR-28: a heavy metal-tolerant and plant growth-promoting fungus for remediation and bioenergy crop production on mine tailing soil. J Environ Manage 132:129–134. https://doi.org/10.1016/j.jenvman.2013.10.009

Ball PN, Mackenzie MD, Deluca TH, Montana WEH (2010) Wildfire and charcoal enhance nitrification and ammonium-oxidizing bacterial abundance in dry montane forest Soils. J Environ Qual 39(4):1243–1253. https://doi.org/10.2134/jeq2009.0082

Brookes P, Lan D et al (1985) Chloroform fumigation and the release of soil nitrogen: a rapiddirect extraction method to measure microbial biomass-nitrogen in soil. Soil Biol Biochem 17:837–842. https://doi.org/10.1016/0038-0717(85)90144-0

Chen L, Yang W, Yang Y, Tu P, Hu S, Zeng Q (2022) Three-season rotation of chicory–tobacco–peanut with high biomass and bioconcentration factors effectively remediates cadmium-contaminated farmland. Environ Sci Pollut Res 29:64822–64831. https://doi.org/10.1007/s11356-022-20400-0

da Silva CP, de Almeida TE, Zittel R, de Oliveira Stremel TR, Domingues CE, Kordiak J, de Campos SX (2016) Translocation of metal ions from soil to tobacco roots and their concentration in the plant parts. Environ Monit Assess 188:663. https://doi.org/10.1007/s10661-016-5679-3

Devi R, Behera B, Raza MB, Mangal V, Altaf MA, Kumar R, Kumar A, Tiwari RK, Lal MK, Singh B (2022) an insight into microbes mediated heavy metal detoxification in plants: a review. J Soil Sci Plant Nutr 22:914–936. https://doi.org/10.1007/s42729-021-00702-x

Fang D et al (2020) Effects of Superparamagnetism nanomaterials on microorganisms and enzymes in Cd contaminated paddy soil. Environ Sci. https://doi.org/10.13227/j.hjkx.202007303

Feng H, Xu C, He H (2020) Effect of Biochar on Soil Enzyme Activity & Bacterial Community and Its Mechanism. Environ Sci. https://doi.org/10.13227/j.hjkx.202005285

Fang DD Zhang LZ, Wang Q (2020) Effects ofsuper paramagnetic nanomaterials on soil microorganisms and enzymes in cadmium-contaminated paddy fields. Environ Sci 42(03):1523–1534. https://doi.org/10.13227/j.hjkx.202007303

Gao W, Li Y, Luo J, Wang Y, Gao W, Liu X, Li T (2025) Soil cadmium pollution decreases phosphorus-mineralizing microbial diversity and reduces phosphorus availability. Environ Pollut 371:125960. https://doi.org/10.1016/j.envpol.2025.125960

Gong X, Xu L, Langwig MV, Chen Z, Huang S, Zhao D, Su L, Zhang Y, Francis CA, Liu J, Li J, Baker BJ (2024) Globally distributed marine Gemmatimonadota have unique genomic potentials. Microbiome 12:149. https://doi.org/10.1186/s40168-024-01871-4

Hamid Y, Tang L, Hussain B, Usman M, Yang X (2019) Organic soil additives for the remediation of cadmium contaminated soils and their impact on the soil-plant system: a review. Sci Total Environ 707:136121. https://doi.org/10.1016/j.scitotenv.2019.136121

He J, Li Y, Qi H, Li H, Zhang W (2020) Biochar amendment changed soil-bound fractions of silver nanoparticles and ions but not their uptake by radish at an environmentally-relevant concentration. Biochar 2(3):307–317. https://doi.org/10.1007/s42773-020-00061-3

Hong M, Zhang L, Tan Z, Huang Q (2019) Effect mechanism of biochar's zeta potential on farmland soil's cadmium immobilization. Environ Sci Pollut Res 26(12):19738–19748. https://doi.org/10.1007/s11356-019-05298-5

Hoseinzadeh S, Shahabivand S, Aliloo AA (2017) Toxic metals accumulation in *Trichoderma asperellum* and *T. harzianum*. Microbiology 86:728–736. https://doi.org/10.1134/S0026261717060066

Hossain MZ, Bahar MM, Sarkar B, Donne SW, Ok YS, Palansooriya KN, Kirkham MB, Chowdhury S, Bolan N (2020) Biochar and its importance on nutrient dynamics in soil and plant. Biochar 2:379–420. https://doi.org/10.1007/s42773-020-00065-z

Hu Y, Zhang P, Yang M et al (2020) Biochar is an effective amendment to remediate Cd-contaminated soils—a meta-analysis. J Soils Sediments 20:3884–3895. https://doi.org/10.1007/s11368-020-02726-9

Huang J, Lai Y, Weng B et al (2020a) Effects of Biochar from peanut hull on bacterial community structure in cadmium contaminated vegetable garden soil. Chinese J Appl Environ Biol 26(05):1115–1128. https://doi.org/10.19675/j.cnki.1006-687x.2019.10005

Huang L, Wei L, Liu X et al (2020b) Effects of Biochar on the migration and accumulation of lead and cadmium in soil plant system. J Agro-Environ Sci 39(10):2205–2216. https://doi.org/10.1016/j.chemosphere.2021.130344

Jianrui L, Yingming X (2015) Immobilization of Cd in paddy soil using moisture management and amendment. Environ Sci Pollut Res Int. https://doi.org/10.1007/s11356-014-3788-5

Karnwal A, Martolia S, Dohroo A, Al-Tawaha ARMS, Malik T (2024) Exploring bioremediation strategies for heavy metals and POPs pollution: the role of microbes, plants, and nanotechnology. Front Environ Sci. https://doi.org/10.3389/fenvs.2024.1397850

Kayoumu M, Wang H, Duan G et al (2025) Interactions between microbial extracellular polymeric substances and biochar, and their potential applications: a review. Biochar 7:62. https://doi.org/10.1007/s42773-025-00452-4

Kolton M, Meller Harel Y, Pasternak Z, Graber ER, Elad Y, Cytryn E (2011) Impact of biochar application to soil on the root-associated bacterial community structure of fully developed greenhouse pepper plants. Appl Environ Microbiol 77(14):4924–4930

Li Y, Yu H, Liu L, Yu H (2021) Application of co-pyrolysis biochar for the adsorption and immobilization of heavy metals in contaminated environmental substrates. J Hazard Mater 420:126655. https://doi.org/10.1016/j.jhazmat.2021.126655

Liu G, Meng J, Huang Y, Dai Z, Tang C, Xu J (2020a) Effects of carbide slag, lodestone and biochar on the immobilization, plant uptake and translocation of As and Cd in a contaminated paddy soil. Environ Pollut. https://doi.org/10.1016/j.envpol.2020.115194

Liu, Y,. Qi, M,. Wang, M,. et al (2020) Effects of different exogenous selenium on the growth and cadmium absorption of chinese cabbage in cadmium contaminated soil. Environ Sci 1–8

Majewska M, Hanaka A (2025) Biochar in the Bioremediation of Metal-Contaminated Soils. Agronomy 15:273. https://doi.org/10.3390/agronomy15020273

Mitra S, Pramanik K, Ghosh PK, Soren T, Sarkar A, Dey RS, Pandey S, Maiti TK (2018) Characterization of Cd-resistant Klebsiella michiganensis MCC3089 and its potential for rice seedling growth promotion under Cd stress. Microbiol Res 210:12–25. https://doi.org/10.1016/j.micres.2018.03.003

Mukherjee S, Sarkar B, Aralappanavar VK, Mukhopadhyay R, Basak BB, Srivastava P, Marchut-Mikołajczyk O, Bhatnagar A, Semple KT, Bolan N (2022) Biochar-microorganism interactions for organic pollutant remediation: challenges and perspectives. Environ Pollut 308:119609. https://doi.org/10.1016/j.envpol.2022.119609

Nie XX, Zhang ZY, Huang YH, Feng JY, Zhang ZY, Yang L (2019) Effects of simultaneous biochar and nitrogen fertilizer application on the photosynthesis and Cd uptake of sorghum. J Agro-Environ Sci 38(12):2749–2756

Nirola R, Megharaj M, Saint C, Aryal R, Thavamani P, Venkateswarlu K, Naidu R, Beecham S (2016) Metal bioavailability to Eisenia fetida through copper mine dwelling animal and plant litter, a new challenge on contaminated environment remediation. Int Biodeterior Biodegrad. https://doi.org/10.1016/j.ibiod.2016.03.007

Patel MR, Panwar NL (2024) Evaluating the agronomic and economic viability of biochar in sustainable crop production. Biomass Bioenerg 188:107328. https://doi.org/10.1016/j.biombioe.2024.107328

Pokharel P, Ma Z, Chang SX (2020) Biochar increases soil microbial biomass with changes in extra- and intracellular enzyme activities: a global meta-analysis. Biochar 2(1):65–79. https://doi.org/10.1007/s42773-020-00039-1

Qi X, Xiao S, Chen X, Ali I, Gou J, Wang D, Zhu B, Zhu W, Shang R, Han M (2022) Biochar-based microbial agent reduces u and cd accumulation in vegetables and improves rhizosphere microecology. Soc Sci Electron Publ 436:129147. https://doi.org/10.1016/j.jhazmat.2022.129147

Qin X, Huang Q, Liu Y, Zhao L, Xu Y, Liu Y (2019) Effects of sepiolite and biochar on microbial diversity in acid red soil from southern China. Chem Ecol 35(9):846–860

Ren T, Chen N, Wan Mahari WA, Xu C, Feng H, Ji X, Yin Q, Chen P, Zhu S, Liu H, Liu G, Li L, Lam SS (2021) Biochar for cadmium pollution mitigation and stress resistance in tobacco growth. Environ Res 192:110273. https://doi.org/10.1016/j.envres.2020.110273

Ren T, Feng H, Xu C, Xu Q, Fu B, Azwar E, Wei Y, Lam SS, Liu G (2022) Exogenous application and interaction of biochar with environmental factors for improving functional diversity of rhizosphere's microbial community and health. Chemosphere 294:133710. https://doi.org/10.1016/j.chemosphere.2022.133710

Rosén K, Eriksson J, Vinichuk M (2012) Uptake and translocation of 109Cd and stable Cd within tobacco plants (Nicotiana sylvestris). J Environ Radioactivity 1(113):16–20. https://doi.org/10.1016/j.jenvrad.2012.04.008

Sánchez-Castro I, Molina L, Prieto-Fernández MÁ, Segura A (2023) Past, present and future trends in the remediation of heavy-metal contaminated soil-Remediation techniques applied in real soil-contamination events. Heliyon 9:6. https://doi.org/10.1016/j.heliyon.2023.e16692

Shar AG, Zhang L, Lu A, Ahmad M, Saqib M, Hussain S, Zulfiqar U, Wang P, Zhang L, Rahimi M (2025) Unlocking biochar's potential: innovative strategies for sustainable remediation of heavy metal stress in tobacco plants. Scientifica 2025:6302968. https://doi.org/10.1155/sci5/6302968

Standardization Administration of China (2014). GB 5009.15–2014 National food safety standard: Determination of chloramphenicol in foods. China National Standards Press

Tripathi AN, Meena BR, Pandey KK, Singh J (2020) Microbial bioagents in agriculture: current status and prospects. In: Rakshit A, Singh H, Singh A, Singh U, Fraceto L (eds) New frontiers in stress management for durable agriculture. Springer, Singapore

Vance E, Brookes P et al (1987) An extraction method for measuring soil microbial biomass C. Soil Biol Biochem 19:703–707. https://doi.org/10.1016/0038-0717(87)90052-6

Wang L, Liu F et al (2010) Characteristics of heavy metal release from lead-zinc slag under acidic leaching and its impact on plant seedling growth. Chinese J Ecol 29(06):1121–1126. https://doi.org/10.1007/s11356-022-20280-4

Wang R, Fu W, Wang J, Zhu L, Wang L, Wang J, Ahmad Z (2019) Application of rice grain husk derived biochar in ameliorating toxicity impacts of cu and zn on growth, physiology and enzymatic functioning of wheat seedlings. Bulletin Environ Contam Toxicol. https://doi.org/10.1007/s00128-019-02705-y

Xie Y, Fan J, Zhu W, Amombo E, Lou Y, Chen L, Fu J (2016) Effect of heavy metals pollution on soil microbial diversity and bermudagrass genetic variation. Front Plant Sci. https://doi.org/10.3389/fpls.2016.00755

Xu J, Wang M, Wang M et al (2020) Research progress on biological toxicity of cadmium pollution in soil. Asian J Ecotoxicol. https://doi.org/10.7524/AJE.1673-5897.20200424002

Xu M, Ma J, Zhang XH, Yang G, Long LL, Chen C, Song C, Wu J, Gao P, Guan DX (2023) Biochar-bacteria partnership based on microbially induced calcite precipitation improves Cd immobilization and soil function. Biochar 5(1):20. https://doi.org/10.1007/s42773-023-00222-0

Xu M, Ma J, Zhang X-H, Yang G, Long L-L, Chen C, Song C, Wu J, Gao P, Guan D-X (2023) Biochar-bacteria partnership based on microbially induced calcite precipitation improves Cd immobilization and soil function. Biochar 5:20. https://doi.org/10.1007/s42773-023-00222-0

Yan S, Liu G (2020) Effect of increasing soil carbon content on tobacco aroma and soil microorganisms. Phytochem Lett 36:42–48. https://doi.org/10.1016/j.phytol.2020.01.011

Yang J, Dai X, Zhang Y et al (2023) Biochar improves root growth and nitrogen metabolism in tobacco through physiological pathways modulation. Rhizosphere 33:101011. https://doi.org/10.1016/j.rhisph.2024.101011

Yu L, Yu M, Lu X, Tang C, Liu X, Brookes PC, Xu J (2018) Combined application of biochar and nitrogen fertilizer benefits nitrogen retention in the rhizosphere of soybean by increasing microbial biomass but not altering microbial community structure. Sci Total Environ 640–641:1221–1230. https://doi.org/10.1016/j.scitotenv.2018.06.018

Yu X, Yan MC, Cui Y, Liu Z (2022) Effects of Co-application of Cadmium-immobilizing bacteria and organic fertilizers on houttuynia cordata and microbial communities in a cadmium-contaminated field. Front Microbiol 12:809834. https://doi.org/10.3389/fmicb.2021.809834

Yuan S, Hong M, Li H, Ye Z, Gong H, Zhang J, Huang Q, Tan Z (2020) Contributions and mechanisms of components in modified biochar to adsorb cadmium in aqueous solution. Sci Total Environ 1(733):139320. https://doi.org/10.1016/j.scitotenv.2020.139320

Zhang X, Zhang X et al (2014) Study on the enrichment and spatial distribution of heavy metals in agricultural soils in China. Enviornmental Science 35(02):692–703

Zhang X, Li X, Yang H, Cui Z (2018) Biochemical mechanism of phytoremediation process of lead and cadmium pollution with *Mucor circinelloides* and *Trichoderma asperellum*. Ecotoxicol Environ Saf 157:21–28. https://doi.org/10.1016/j.ecoenv.2018.03.047

Zhang Y, Gao S, Jia H, Sun T, Zheng S, Wu S, Sun Y (2024) Passivation remediation of weakly alkaline Cd-contaminated soils using combined treatments of biochar and sepiolite. Ecol Process 13:3. https://doi.org/10.1186/s13717-023-00469-2

Zhou R, Liu X, Luo L, Zhou Y, Wei J, Chen A, Tang L, Wu H, Deng Y, Zhang F (2017) Remediation of Cu, Pb, Zn and Cd-contaminated agricultural soil using a combined red mud and compost amendment. Int Biodeterior Biodegradation 118:73–81. https://doi.org/10.1016/j.ibiod.2017.01.023

Zhou J, Zhang R, Wang P, Gao Y, Zhang J (2024) Responses of soil and rhizosphere microbial communities to Cd-hyperaccumulating willows and Cd contamination. BMC Plant Biol 24:398. https://doi.org/10.1186/s12870-024-05118-0

Zhu M, Liu Y, Bai H, Zhang W et al (2023) Integrated physio-biochemical and RNA sequencing analysis revealed mechanisms of long non-coding RNA-mediated response to cadmium toxicity in wheat. Plant Physiol Biochem. https://doi.org/10.1016/j.plaphy.2023.108028