

Effects of *Chlorophytum comosum* Combined with *Pteroceltis tatarinowii* Biochar on Farmland Soil Remediation in Cu-Pb Mining Areas and Lettuce Growth

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Plant-biochar remediation is a promising strategy for heavy metal-contaminated farmland, but its practical value should be evaluated by considering both residual soil risk and the response of subsequently planted crops. In this study, waste-derived *Pteroceltis tatarinowii* Maxim. (PTM) biochar was combined with the non-food accumulator *Chlorophytum comosum* (*C. comosum*) to remediate field-collected Cu-Pb-contaminated soil. *Lactuca sativa* (*L. sativa*) was subsequently planted as a sensitive indicator crop. Among the treatments, the combined application of 5% PTM biochar and *C. comosum* (BC5) produced the strongest overall remediation effect. BC5 improved the growth of *L. sativa*, increased leaf chlorophyll content, decreased malondialdehyde content and antioxidant-enzyme activities, and reduced Cu and Pb uptake and translocation ($P < 0.05$). BC5 also reduced the bioavailable fractions of Cu and Pb in soil and was associated with shifts in bacterial community structure, increased relative abundances of *Bryobacter* and *Vibrionimonas*, and changes in predicted metabolic pathways related to nutrient cycling and stress regulation. These microbial responses may help explain the improvement in soil ecological function and the reduced metal stress in *L. sativa*, although further functional validation is needed. Overall, sequential remediation using PTM biochar and *C. comosum* provides a potential low-cost and eco-friendly approach for reducing residual phytotoxicity and crop metal-uptake risk in Cu-Pb-contaminated farmland.

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INTRODUCTION

Heavy metal contamination in farmland around mining areas poses persistent risks to soil ecological function and crop safety, particularly when Cu and Pb occur together. Plant-biochar combined remediation has attracted increasing attention because it can integrate the immobilization capacity of biochar with the uptake and rhizosphere-regulating functions of plants, while remaining relatively low-cost and environmentally compatible (Wang *et al.* 2021; Wu *et al.* 2024). However, the effectiveness of this strategy depends strongly on the matched selection of biochar feedstock and remediation plant.

Previous studies have shown that biochar can reduce the bioavailability of heavy metals by increasing soil pH, providing adsorption sites, and promoting precipitation or complexation reactions (Kwak *et al.* 2019; Li *et al.* 2019). Calcium-rich or calcium-

modified biochar are especially promising because Ca-containing minerals and functional groups can enhance heavy metal immobilization (He *et al.* 2025). Nevertheless, most studies have focused on soil chemical indicators or single remediation measures, whereas fewer studies have evaluated the combined effects of biochar, remediation plants, soil microbial responses, and the growth and heavy metal uptake of a subsequent sensitive crop. This limits understanding of both remediation mechanisms and the practical value of restored farmland.

Pteroceltis tatarinowii Maxim. (PTM) is a suitable biochar feedstock for this purpose. As a calcium-loving plant, PTM has relatively high Ca content (Chen *et al.* 2011), and biochar derived from calcium-rich biomass may possess abundant surface functional groups, high adsorption capacity, and favorable mineral components for heavy metal retention (Cho *et al.* 2017; Islam *et al.* 2021). In addition, PTM is an important raw material for traditional rice paper production, and waste branches or processing residues can be converted into biochar, providing a potential route for resource utilization of industrial biomass residues (Sun *et al.* 2017).

Chlorophytum comosum (*C. comosum*) was selected as the remediation plant because it combines heavy metal tolerance, accumulation capacity, rapid growth, a well-developed root system, and perennial regeneration. Previous studies have reported its ability to tolerate and accumulate heavy metals such as Cu, Pb, and Cd (Sangsuwan and Prapagdee 2021; Wang *et al.* 2021, 2012; Zia-ur-Rehman *et al.* 2023). These traits allow *C. comosum* to remove metals through plant uptake and repeated harvesting of aboveground tissues, while avoiding the direct food-chain risk associated with cultivating edible crops during the remediation stage.

Lactuca sativa L. (*L. sativa*) was used in the subsequent planting stage as a sensitive edible indicator crop rather than as evidence that contaminated farmland can be used directly for vegetable production. Because lettuce has a shallow root system and is sensitive to Pb and Cd stress (Lin *et al.* 2022), its growth, physiological response, and Cu/Pb accumulation can reflect residual phytotoxicity and potential food safety risks after remediation.

Therefore, this study tested the hypothesis that PTM biochar combined with *C. comosum* can together constitute a synergistic remediation system involving biochar immobilization, plant extraction, and microbial regulation. Specifically, this study aimed to (1) evaluate the effects of PTM biochar and *C. comosum* on soil physicochemical properties and Cu/Pb availability; (2) clarify changes in soil bacterial community structure and predicted metabolic functions; and (3) assess remediation efficiency using the growth, physiological characteristics, and Cu/Pb accumulation of subsequently planted *L. sativa*.

MATERIALS AND METHODS

Test Materials

Test soil

Surface soil (0 to 20 cm) was collected from Cu–Pb-contaminated farmland near the Laoyaling copper tailings in Tongling City. After visible debris was removed, the soil was air-dried, ground, and passed through a 2-mm sieve. Its initial physicochemical properties are summarized in Table 1. Total Cu and Pb exceeded the risk-screening values specified in GB 15618—2018 (GB 15618 2018).

Table 1. Initial Physicochemical Properties of the Experimental Soil

Property	Value	Unit
pH	6.63	—
Electrical conductivity	162	$\mu\text{S cm}^{-1}$
Organic matter	15.1	g kg^{-1}
Total nitrogen	1.08	g kg^{-1}
Total phosphorus	454	mg kg^{-1}
Total potassium	20.6	g kg^{-1}
Available nitrogen	76.1	mg kg^{-1}
Available phosphorus	22.3	mg kg^{-1}
Available potassium	112	mg kg^{-1}
Total Cu	180	mg kg^{-1}
Total Pb	285	mg kg^{-1}

Preparation of test biochar sample

Waste branches of PTM (after peeling) were washed with pure water, dried at 105 °C to constant weight, and pyrolyzed at 500 °C for 2 h under oxygen-limited conditions. After cooling, the product was ground, passed through a 200-mesh sieve, repeatedly washed with deionized water by suction filtration, and dried. The measured properties of the resulting PTM biochar are summarized in Table 2.

Table 2. Physicochemical Properties of PTM Biochar

Property	Value	Unit
pH	7.04	—
Organic matter	52.0	g kg^{-1}
Available nitrogen	5.83	mg kg^{-1}
Available phosphorus	190	mg kg^{-1}
Available potassium	531	mg kg^{-1}
Specific surface area	226	$\text{m}^2 \text{kg}^{-1}$
Ca	4.08	mg g^{-1}
K	2.16	mg g^{-1}
Na	1.27	mg g^{-1}
Mg	1.09	mg g^{-1}
Mn	0.08	mg g^{-1}

Test plants

Seedlings of *C. comosum* were obtained from mother plants; seedlings with aerial roots were cut and soil-cultured for 1 week. After the roots were established, seedlings with similar growth status were selected as test materials. Seeds of *L. sativa* were purchased from Botou Yonghong Seed Co., Ltd.

Experimental Design

Pots (35 cm × 20 cm × 15 cm) were filled with 3.0 kg of test soil. Six treatments were established: CK, no biochar and no *C. comosum*; BCK, no biochar with *C. comosum*; C3 and C5, 3% and 5% (w/w) PTM biochar without *C. comosum*, respectively; and BC3 and BC5, 3% and 5% (w/w) PTM biochar with *C. comosum*, respectively. Each treatment had three independent replicate pots. After biochar and soil were equilibrated for 1 week, three similarly developed *C. comosum* seedlings were planted in each BCK, BC3, and BC5 pot. All pots were maintained at 25 °C, with soil moisture at 60% of maximum field water-holding capacity. Plant and soil samples were collected after 2 months. The 3% and 5% rates were selected based on the effective range reported in our previous PTM-biochar study (Jiang *et al.* 2025).

Sequential planting refers to cultivating *C. cosmosum* during the first 60-day remediation phase and subsequently cultivating *L. sativa* for 40 days in the same treated soil. After the first phase, *C. cosmosum* was harvested and the soil in each pot was loosened without replacement. *L. sativa* seeds were surface-disinfected in 0.5% NaClO for 10 min, rinsed several times with distilled water, and blotted dry. Fifty seeds were sown in each pot and thinned to 18 seedlings after germination. Pots were maintained at 25 °C and 60% of maximum field water-holding capacity. *L. sativa* plants were harvested after 40 days for subsequent analyses.

Determination of Plant Indicators and Methods

Morphological indicators

After washing *L. sativa* plants with deionized water, plant height (cm) and root length (cm) were measured using a ruler.

Determination of plant biomass

After washing *L. sativa* plants with deionized water, the surface water was blotted dry with filter paper, and the plants were separated into aboveground (leaf) and underground (root) parts. The fresh weights of aboveground and underground parts were measured using an electronic balance (g). After weighing, the samples were deactivated at 105 °C for 1 h, then dried at 75 °C to constant weight, and the dry weights of roots and leaves were measured separately (g).

Determination of plant physiological indicators

Physiological and biochemical indicators of *L. sativa* were determined as follows: The chlorophyll content of *L. sativa* leaves was determined using the acetone extraction colorimetric method. Briefly, 0.20 g of fresh leaf tissue was homogenized with 10 mL of 80% acetone and extracted in the dark until the tissue became colorless. The absorbance of the extract was measured at 663 nm and 645 nm using a spectrophotometer, with 80% acetone as the blank. Chlorophyll a, chlorophyll b, and total chlorophyll contents were calculated as follows: Chl a = $12.72A_{663} - 2.59A_{645}$; Chl b = $22.88A_{645} - 4.67A_{663}$; total chlorophyll = Chl a + Chl b. The results were expressed as mg/g fresh weight; MDA content by the thio-barbituric acid (TBA) colorimetric method; catalase (CAT) activity by ultraviolet absorption method; peroxidase (POD) activity *via* guaiacol method; and superoxide dismutase (SOD) activity using nitroblue tetrazolium (NBT) reduction method.

Determination of heavy metal contents in plants

Heavy metal contents in *L. sativa* were determined after digestion with the HNO₃-HClO₄ method, using an AA-320N flame atomic absorption spectrophotometer (Shimadzu, Japan).

Genetic indicators

Soil bacterial genomic DNA was extracted using the E.Z.N.A.® Soil Kit (Omega Bio-Tek). The integrity, concentration, and purity of DNA were detected by 1% agarose gel electrophoresis and a Nano Drop 2000 ultra-micro spectrophotometer, respectively. The V1-V9 region of the bacterial 16S rRNA gene was amplified using the universal primer pair 27F (5' -AGAGTTTGATCMTGGCTCAG-3') and 1492R (5' -CRGYTACCTTGTTACGACTT-3'), which has been widely used for near-full-length bacterial 16S rRNA gene amplification (Frank *et al.* 2008; Weisburg *et al.* 1991). This

primer pair was selected because the PacBio Sequel IIe platform allows long-read sequencing of near-full-length 16S rRNA amplicons, providing higher taxonomic resolution than short-read sequencing of partial variable regions (Buetas *et al.* 2024).

Data Processing

To evaluate the heavy metal uptake and mobility in the plants, the bioaccumulation factor (BCF) and translocation factor (TF) were calculated. BCF was determined as the ratio of the heavy metal concentration in the plant tissue to that in the soil. Similarly, TF was calculated as the ratio of the heavy metal concentration in the aboveground parts of the plant to the concentration in the underground parts. All heavy metal contents were expressed in mg/kg. Data processing and statistical analyses were performed using Microsoft Excel 2021 and SPSS 19. All graphs and visualizations were generated using Origin 2024, Python 3.13, and BioRender. Microbial community diversity and composition were analyzed based on 16S rRNA gene sequencing data using standard marker-gene analysis procedures. Alpha-diversity indices and beta-diversity ordination were used to evaluate differences in bacterial richness, diversity, and community structure among treatments (Bolyen *et al.* 2019; Schloss *et al.* 2009). Functional profiles of bacterial communities were predicted from 16S rRNA data using Tax4Fun-based analysis (Abhauer *et al.* 2015).

RESULTS AND ANALYSIS

Effects of PTM Biochar Addition on Soil Properties and Heavy Metal Accumulation by *C. comosum*

As shown in Figs. 1 and 2, the application of PTM biochar exerted a certain impact on soil physical and chemical properties when compared to the control CK. With the increase in biochar application rate, the soil pH in all treated groups showed an overall upward trend and was significantly higher than that in CK ($P < 0.05$), and all treated groups had significantly lower EC than CK ($P < 0.05$). Among them, the BC5 treatment achieved the lowest EC, with a reduction of 17.4%. PTM biochar also affected soil enzyme activities. In the single biochar application groups, the activities of alkaline phosphatase, catalase, and urease increased with the increase in biochar dosage. Specifically, these three enzyme activities peaked in the BC5 treatment, reaching 20.8, 9.11, and 0.81 mg/g·d, respectively. However, except for the BCK group, the sucrase activity in all other groups was significantly lower than that in CK ($P < 0.05$). In the single biochar application groups, the contents of soil organic matter (SOM), total nitrogen (TN), total phosphorus (TP), total potassium (TK), available phosphorus (AP), and available potassium (AK) generally increased with the increase in biochar addition, while the available nitrogen (AN) content decreased. In the PTM biochar - *C. comosum* combined application groups, the contents of SOM, TN, TP, AP, and AK were significantly higher than those in BCK ($P < 0.05$). Additionally, both single applications of PTM biochar and its combined application with *C. comosum* significantly reduced the contents of available copper (Cu) and lead (Pb) in soil ($P < 0.05$). Compared with CK, all treated groups showed a significant decrease in available Cu ($P < 0.05$), with the BC5 treatment achieving the maximum reduction of 38.6%. Except for the BCK group, all other groups had significantly lower available Pb than CK ($P < 0.05$), and the BC5 treatment also had the largest reduction of 30.4%. Different application rates of PTM biochar led to variations in the accumulation of Cu and

Pb in different tissues of *C. comosum*. As illustrated in Fig. 2, compared with BCK, the application of biochar increased the Cu and Pb contents in the leaves of *C. comosum*. The Cu contents in both leaves and roots reached the highest in the BC5 treatment, increasing by 51.4% and 15.6% compared with BCK, respectively. The Pb contents in leaves and roots of all treated groups were significantly higher than those in BCK ($P < 0.05$), with the maximum increases of 50.2% (leaves) and 40.5% (roots) observed in the BC5 treatment. Furthermore, the bioaccumulation factors (BCFs) of Cu and Pb in leaves and roots of *C. comosum* were closely related to the biochar application level. Compared with BCK, all treated groups showed significant differences in the BCFs of Cu and Pb in leaves ($P < 0.05$). The BC5 treatment resulted in the highest BCFs of Cu in both leaves and roots, with increases of 84.0% and 40.6% compared with BCK, respectively. The BCFs of Pb in leaves and roots were also significantly higher than those in BCK ($P < 0.05$), and the maximum BCFs of Pb in the BC5 treatment increased by 115% (leaves) and 89.6% (roots) compared with BCK. Additionally, the translocation factors (TFs) of Cu and Pb in *C. comosum* under the BC5 treatment were significantly higher than those in BCK ($P < 0.05$). In summary, the addition of PTM biochar could increase soil pH, reduce soil EC, improve soil nutrient contents, activate soil enzyme activities, and decrease the available contents of Cu and Pb in soil. Meanwhile, the application of an appropriate amount of PTM biochar could promote the absorption of Cu and Pb by *C. comosum* and enhance the translocation of these heavy metals to the aboveground parts, thereby improving the accumulation and translocation capacities of *C. comosum* for soil Cu and Pb. The soil remediation effect of the PTM biochar - *C. comosum* combined group was superior to that of the corresponding single biochar application group.

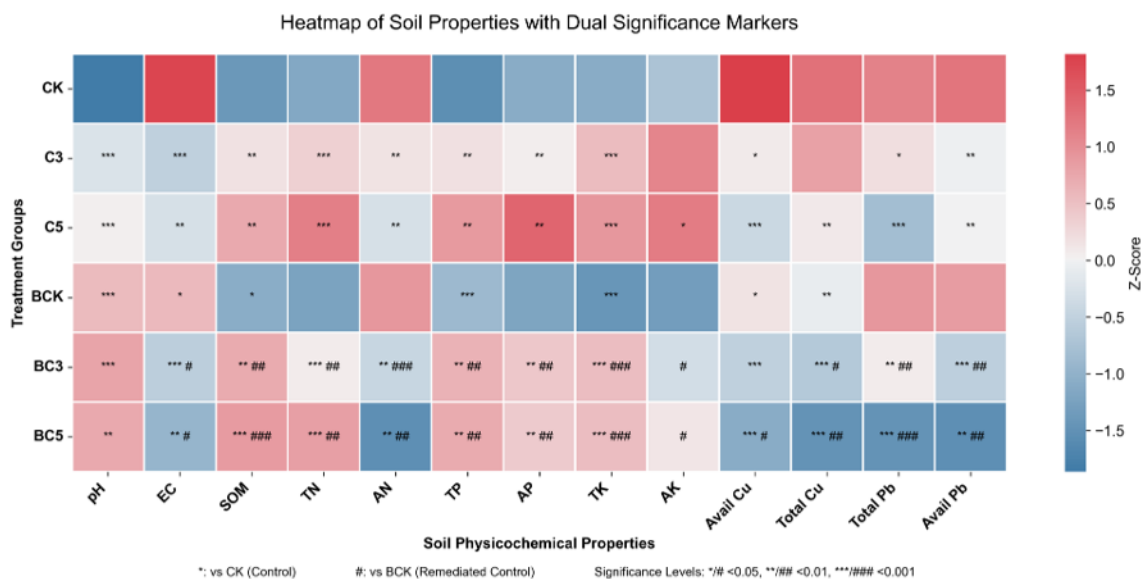


Fig. 1. Z-Score heatmap of soil physicochemical properties in different treatment groups. Red/blue colors denote positive/negative Z-scores; * (vs CK) and # (vs BCK) indicate significance at $P < 0.05$, **/## $P < 0.01$, ***/### $P < 0.001$. **Note:** Abbreviations of soil physicochemical properties in the figure correspond to their full names: EC: Electrical Conductivity, SOM: Soil Organic Matter, TN: Total Nitrogen, TP: Total Phosphorus, TK: Total Potassium, AN: Available Nitrogen, AP: Available Phosphorus, AK: Available Potassium, Available Cu: Soil Available Cu, Available Pb: Soil Available Pb

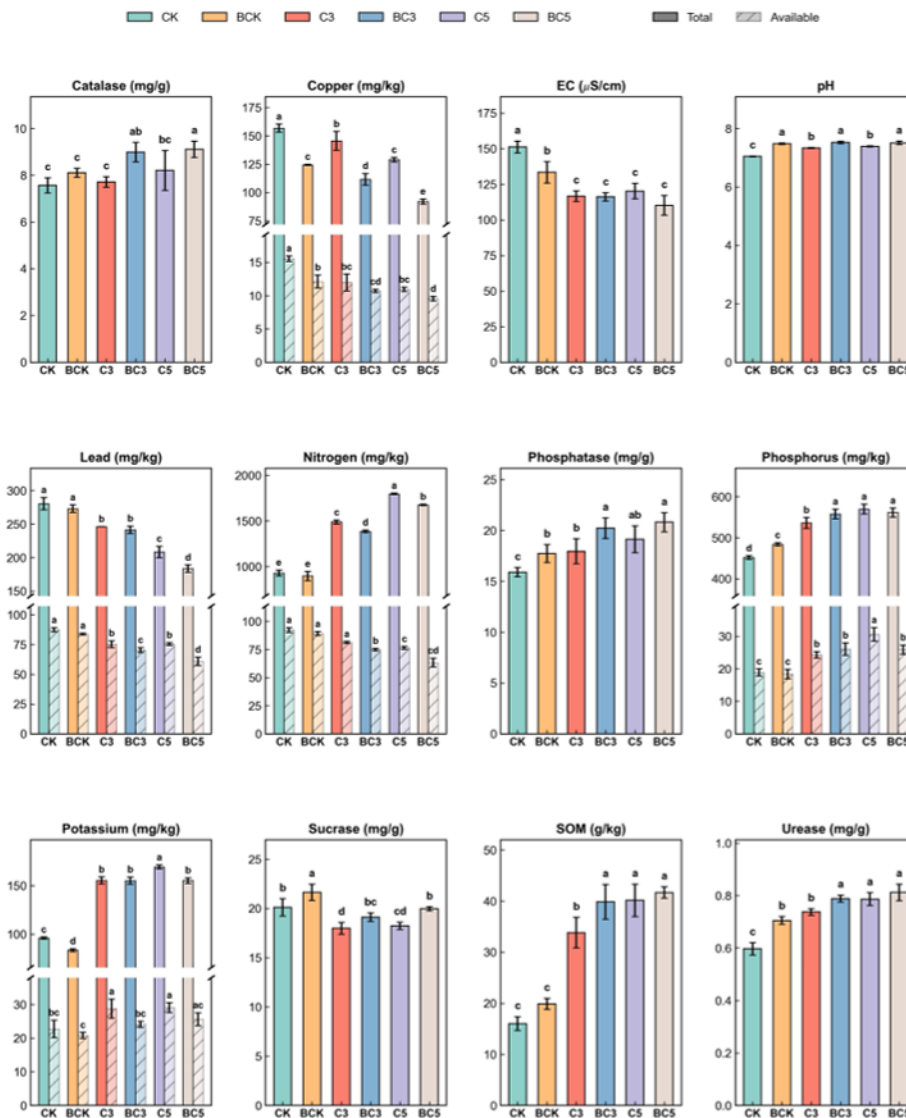


Fig. 2. The effect of PTM biochar application on soil. **Note:** Bars with slanted hatching denote available heavy metal fractions, and solid-filled bars denote total heavy metal fractions.

Effects of Combined Application of PTM Biochar and *C. cosmosum* on *L. sativa* Growth

As shown in Table 3, the application of PTM biochar alone or in combination with *C. cosmosum* affected the growth indicators of *L. sativa* to varying degrees, among which the BC5 treatment showed the most significant increase in all indicators compared with CK ($P < 0.05$). The specific effects were as follows:

In the PTM biochar alone groups (C3 and C5), the plant height and root length of *L. sativa* were higher than those in CK. The plant height in the C3 treatment was significantly higher than that in CK (increase of 49.4%, $P < 0.05$), and the root length in the C5 treatment was significantly higher than that in CK (increase of 23.8%, $P < 0.05$).

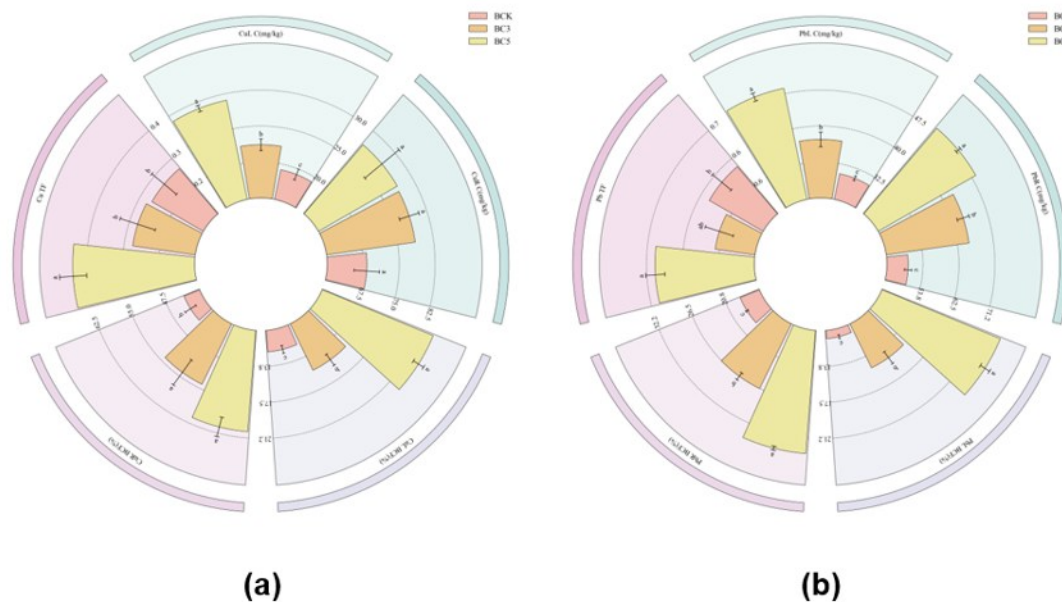


Fig. 3. (a, b): The effects of different treatments on the absorption and transport of heavy metals (copper and lead) by *Chlorophytum comosum*. Note: CuL C: Copper content in leaves of *C. comosum*; CuR C: Copper content in roots of *C. comosum*; CuL BCF: Bioaccumulation factor of Cu in leaves of *C. comosum*; CuR BCF: Bioaccumulation factor of Cu in roots of *C. comosum*; Cu TF: Translocation factor of Cu; PbL C: Lead content in leaves of *C. comosum*; PbR C: Lead content in roots of *C. comosum*; PbL BCF: Bioaccumulation factor of Pb in leaves of *C. comosum*; PbR BCF: Bioaccumulation factor of Pb in roots of *C. comosum*; Pb TF: Translocation factor of Pb

Table 3. Effects of Different Treatments on Growth Indicators of *L. sativa*

Treatment	Fresh Weight (g)		Dry Weight (g)		Plant Height (cm)	Length of Root (cm)
	Foliage	Root	Foliage	Root		
CK	2.72±0.350b	0.19±0.016c	0.14±0.017b	0.02±0.003c	7.90±1.150c	8.00±0.500c
C3	3.49±0.410a	0.23±0.030ab	0.19±0.023a	0.03±0.004b	11.80±1.150ab	8.47±0.750bc
C5	3.46±0.133a	0.23±0.008b	0.19±0.004a	0.03±0.002b	10.03±0.750bc	9.90±0.460ab
BCK	2.80±0.289b	0.19±0.020c	0.15±0.007b	0.03±0.002c	10.03±0.950bc	8.13±0.700c
BC3	3.74±0.411a	0.24±0.022ab	0.21±0.033a	0.03±0.004b	10.65±1.690b	9.83±1.530ab
BC5	4.06±0.547a	0.27±0.025a	0.22±0.022a	0.04±0.002a	13.50±1.320a	10.37±0.710a

Note: Different lowercase letters in the same column indicate significant differences among treatments ($P < 0.05$). The same applies below.

The fresh weight and dry weight in all biochar-alone treatments were significantly higher than those in CK ($P < 0.05$), with the maximum increase observed in the C3 treatment: 28.2% (leaf fresh weight), 25.0% (root fresh weight), 25.7% (root dry weight), and 32.3% (leaf dry weight). In the combined application groups of PTM biochar and *C. comosum* (BC3 and BC5), all growth indicators of *L. sativa* were better than those in BCK, with significant differences in key indicators ($P < 0.05$). The BC5 treatment showed the most significant effects and the largest increases: plant height increased by 34.6%, root

length increased by 27.5%, and compared with BCK, the fresh weight and dry weight increased by 44.7% (leaf fresh weight), 46.0% (root fresh weight), 45.7% (leaf dry weight), and 46.1% (root dry weight), respectively.

The above results indicate that the promoting effect of the PTM biochar – *C. comosum* combination on *L. sativa* growth was better than that of PTM biochar alone, and the BC5 treatment had the best application effect.

Effects of Combined Application of PTM Biochar and *C. comosum* on Chlorophyll and MDA Contents in *L. sativa* Leaves

Table 4 shows the effects of different treatments on chlorophyll and MDA content in *L. sativa* leaves. Compared with CK, planting *C. comosum* significantly increased the chlorophyll content in *L. sativa* leaves ($P < 0.05$). When PTM biochar was applied alone, the chlorophyll content in *L. sativa* leaves in all treatments was higher than that in CK; among them, the chlorophyll content in the C5 treatment was significantly higher than that in CK (increase of 21.8%, $P < 0.05$). In the combined application groups of PTM biochar and *C. comosum*, the chlorophyll content in *L. sativa* leaves in all treatments was significantly higher than that in BCK ($P < 0.05$), with the maximum increase observed in the BC5 treatment (24.7%).

Both the application of PTM biochar alone and in combination with *C. comosum* reduced the MDA content in *L. sativa* leaves to varying degrees. In the PTM biochar alone groups, the MDA content in *L. sativa* leaves in all treatments was significantly lower than that in CK, with the maximum reduction (38.1%) observed in the C5 treatment. In the combined application groups, the MDA content in *L. sativa* leaves in all treatments was lower than that in BCK, and the difference reached a significant level in the BC5 treatment ($P < 0.05$).

In summary, the promoting effect on chlorophyll content and the reducing effect on MDA content in *L. sativa* leaves in the PTM biochar – *C. comosum* combination groups were better than those in the PTM biochar alone groups, and the BC5 treatment had the best effect on *L. sativa* leaves.

Table 4. Effects of Different Treatments on Chlorophyll and MDA Contents in Lettuce Leaves

Treatment	Chlorophyll (mg/g)	MDA (umol/uFW)
CK	0.38±0.010d	0.08±0.004a
BCK	0.47±0.029c	0.05±0.008bc
C3	0.39±0.004d	0.06±0.008b
BC3	0.50±0.010b	0.05±0.003cd
C5	0.47±0.015c	0.05±0.001cd
BC5	0.59±0.015a	0.04±0.005d

Effects of Combined Application of PTM Biochar and *C. comosum* on Antioxidant Enzyme Activities in *L. sativa* Leaves

Figure 4 shows the effects of different treatments on antioxidant enzyme activities in *L. sativa* leaves. The application of PTM biochar alone or in combination with *C. comosum* affected the antioxidant enzyme activities in *L. sativa* leaves to varying degrees,

among which the BC5 treatment showed the most significant decrease in all indicators compared with CK ($P < 0.05$). The specific effects are as follows:

When PTM biochar was applied alone, the activities of POD and CAT in *L. sativa* leaves in all treatments were significantly lower than those in CK ($P < 0.05$), with reductions of 55.1% to 56.7% and 26.8% to 30.4%, respectively. Additionally, the SOD activity in *L. sativa* leaves in all treatments was significantly lower than that in CK ($P < 0.05$), with the maximum reduction (60.8%) observed in the C5 treatment.

In the combined application groups of PTM biochar and *C. comosum*, the activities of POD, SOD, and CAT in *L. sativa* leaves in all treatments were significantly lower than those in BCK ($P < 0.05$), and the maximum reductions were observed in the BC5 treatment: 62.9% (POD), 70.0% (SOD), and 26.1% (CAT), respectively.

Collectively, the reducing effects on POD, SOD, and CAT activities in *L. sativa* leaves in the PTM biochar – *C. comosum* combination groups were higher than those in the PTM biochar alone groups.

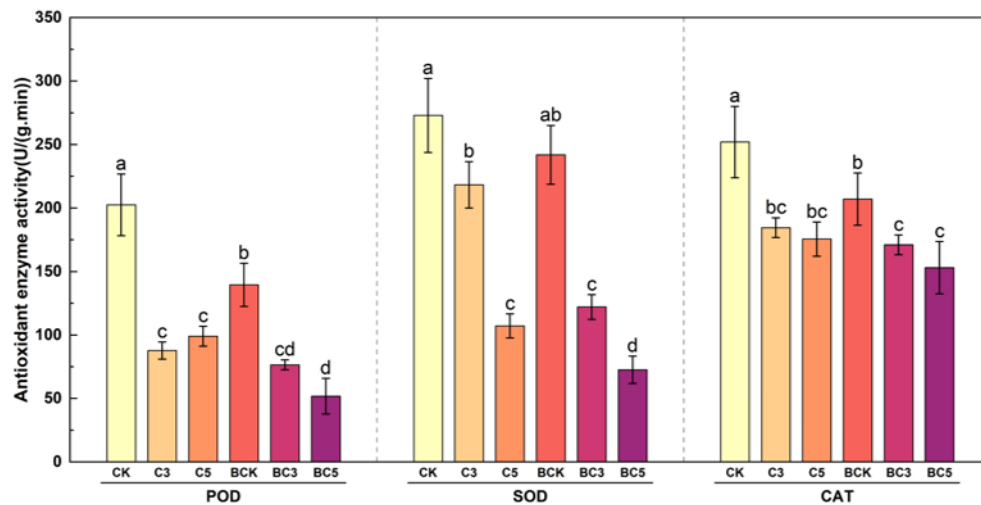


Fig. 4. Effects of different treatments on the activities of antioxidant enzymes in lettuce leaves

Effects of Combined Application of PTM Biochar and *C. comosum* on Cu and Pb Accumulation Characteristics of *L. sativa*

As shown in Fig. 5, the application of PTM biochar alone or in combination with *C. comosum* affected the absorption and translocation of heavy metals by *L. sativa* to varying degrees, among which the BC5 treatment had the most significant effect on all indicators compared with CK ($P < 0.05$). The specific effects are as follows:

In the PTM biochar alone groups, except for the Pb BCF and Pb TF in *L. sativa* roots, all other indicators were significantly lower than those in CK ($P < 0.05$), with the maximum reduction observed in the C5 treatment: 51.9% (Cu in leaves), 34.7% (Cu in roots), 39.0% (Pb in leaves), and 30.3% (Pb in roots). The Cu BCF in *L. sativa* leaves and roots decreased by 41.6% and 20.6% compared with CK, respectively; the Pb BCF in *L. sativa* leaves decreased by 17.8%; and the Cu TF decreased by 26.6% compared with CK.

In the combined application groups of PTM biochar and *C. comosum*, all indicators related to Cu and Pb accumulation in *L. sativa* were lower than those in BCK, with the BC5 treatment showing the most significant effect ($P < 0.05$). Except for the maximum reduction in Pb BCF in *L. sativa* roots observed in the BC3 treatment (32.0%), the maximum reductions in other indicators were observed in the BC5 treatment: 58.2% (Cu

in leaves), 41.5% (Cu in roots), 76.8% (Pb in leaves), and 49.0% (Pb in roots). The Cu BCF in leaves and roots decreased by 43.4% and 20.8%, respectively; the Pb BCF in leaves decreased by 65.5%; and the Cu TF and Pb TF decreased by 28.1% and 54.5%, respectively.

Overall, the PTM biochar – *C. comosum* combination groups showed more significant heavy metal immobilization effects.

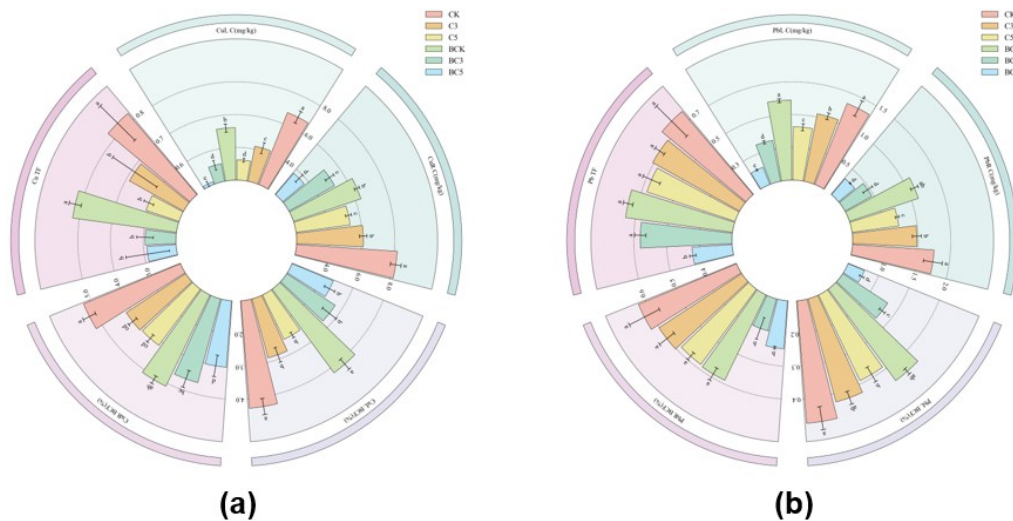


Fig. 5. Effects of different treatments on the absorption and translocation of heavy metals: ((a) Cu; and (b) Pb) by *Lactuca sativa* L. Note: CuL C: Cu content in leaves; CuR C: Cu content in roots; CuL BCF: Cu bioaccumulation factor in leaves; CuR BCF: Cu bioaccumulation factor in roots; Cu TF: Cu translocation factor; PbL C: Pb content in leaves; PbR C: Pb content in roots; PbL BCF: Pb bioaccumulation factor in leaves; PbR BCF: Pb bioaccumulation factor in roots; Pb TF: Translocation factor of Pb

Effects of Combined Application of PTM Biochar and *C. comosum* on Soil Bacterial Diversity

Due to the better enhancement effect of the combination of 5% PTM biochar and *C. comosum* on the remediation of Cu-Pb co-contaminated soil, the CK, BCK, C5, and BC5 treatments were selected for 16S rRNA gene sequencing of soil microorganisms.

α-Diversity analysis

α-Diversity is used to analyze the richness and diversity of microbial communities within samples. The Chao1 index and ACE index are used to evaluate bacterial community richness (higher values indicate more abundant species), while the Simpson index reflects bacterial community diversity (higher values indicate higher diversity). As shown in Table 5, the Chao1 index and ACE index of all treatments were higher than those of CK, indicating that the bacterial community richness was improved in all treatments, but there was no significant difference among treatments ($P > 0.05$). The indices of the four treatments were the highest in the BCK treatment, and slightly lower in the C5 and BC5 treatments, indicating that the addition of biochar inhibited the growth of some bacteria, and thus planting *C. comosum* alone had a better effect on improving the richness and diversity of soil bacteria. In general, the addition of PTM biochar had no significant effect on soil bacterial richness but reduced soil bacterial diversity.

A Venn diagram (Fig. 6) was used to analyze the similarity, specificity, and overlap of OTU (operational taxonomic unit) composition among groups. The total number of OTUs in CK, BCK, C5, and BC5 were 4124, 4223, 4117, and 4207, respectively. The number of unique OTUs in each treatment was 66, 112, 69, and 70, accounting for 1.36%, 2.30%, 1.42%, and 1.44% of the total OTUs, respectively. The number of shared OTUs among all treatments was 3253, accounting for 66.89% of the total OTUs.

Table 5. α -Diversity Indices of Soil Bacterial Communities

Group	Chao1	ACE	Simpson
CK	3705 $\pm$ 98a	3731 $\pm$ 80a	0.99666 $\pm$ 0.00014a
BCK	3881 $\pm$ 154a	3882 $\pm$ 115a	0.99687 $\pm$ 0.00014a
C5	3816 $\pm$ 90a	3820 $\pm$ 76a	0.99665 $\pm$ 0.00019a
BC5	3830 $\pm$ 49a	3776 $\pm$ 68a	0.99607 $\pm$ 0.00009b

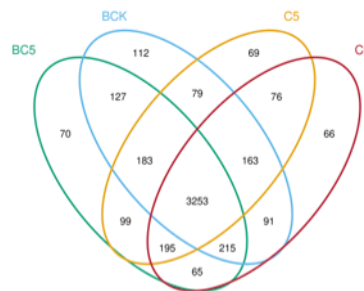


Fig. 6. Dilution curve and OTU number of soil bacterial communities

β -Diversity analysis

As shown in Fig. 7, principal coordinate analysis (PCoA) was performed at the OTU classification level to evaluate the effects of PTM biochar on the β -diversity of soil bacterial communities. Based on the PCoA ordination plot and permutational multivariate analysis of variance (PERMANOVA) statistical results, a refined analysis was conducted to explore the individual and combined effects of *C. comosum* and biochar on soil microbial community structure. The core conclusions are as follows:

Analysis of individual effects of single factors (C. comosum or Biochar): Comparison between the CK and BCK groups revealed no significant effect of *C. comosum* cultivation alone on the microbial community structure. This was supported by the clustered distribution of sample points in the PCoA plot, with high overlap along the PC1 and PC2 axes. These results indicate that sole cultivation of *C. comosum* did not significantly alter the microbial community structure, suggesting that the plant itself does not exert a substantial regulatory effect on the microbial community in this experimental system. In contrast, a highly significant regulatory effect of biochar alone on the community structure was observed when comparing the CK and C5 groups. The sample points of CK and C5 were clearly separated along PC1 (the primary axis of variation, explaining 46% of total variance), with C5 samples shifting positively and CK samples distributing negatively along PC1. These findings demonstrate that the sole addition of biochar can drastically alter the microbial community structure and act as a core driver of community variation. This is further supported by the PERMANOVA result ($R^2 = 63.37\%$), indicating that biochar was the primary contributor to the observed group differences.

Analysis of interactive effects of combined factors (C. comosum + Biochar): No significant difference was detected between the C5 and BC5 groups, as evidenced by the clustered distribution of sample points without obvious separation in the PCoA plot. This suggests that the combined application of *C. comosum* and biochar did not produce additional synergistic or antagonistic effects — *i.e.*, *C. comosum* could neither enhance nor weaken the regulatory effect of biochar on the microbial community, and the final community structure remained dominated by biochar.

The key factor driving changes in soil microbial community structure was biochar, whose sole application induced highly significant differences, whereas *C. comosum* cultivation alone had no significant effect. The regulatory capacity of biochar was so strong that even when combined with *C. comosum*, it retained its dominant influence on the microbial community.

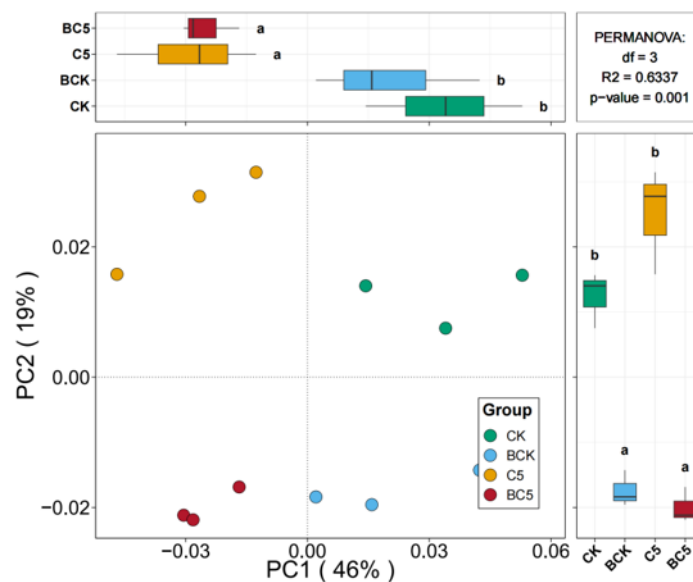


Fig. 7. PCoA Analysis of soil bacterial community structure

Effects of combined application of PTM biochar and C. comosum on soil bacterial community structure

A total of 35 bacterial phyla were detected in the four treatments (CK, BCK, C5, BC5). This study presents the top 10 dominant phyla in terms of relative abundance in each treatment. The main dominant phyla in all treatments were Acidobacteriota, Proteobacteria, Actinobacteriota, Chloroflexi, Bacteroidota, and Planctomycetota (relative abundance > 5%), accounting for 80.8% to 83.7% of the total relative abundance of soil bacteria.

As shown in Fig. 8, a total of 396 bacterial genera were detected in all treatments. This study presents the top 10 dominant genera in terms of relative abundance in each treatment. The main dominant genera in all treatments were *RB41*, *Sphingomonas*, *MND1*, *Nitrospira*, *Vibrionimonas*, and *Bryobacter*.

Compared with CK, the relative abundance of *Sphingomonas* was significantly reduced in all treatments ($P < 0.05$); the relative abundance of *MND1* was increased in BCK and BC5, with a significant increase in BCK ($P < 0.05$); although the relative abundance of *MND1* was decreased in C5, the difference was not significant ($P > 0.05$). Except for BCK, the relative abundance of *Nitrospira* was decreased in C5 and BC5, with a significant decrease in BC5 ($P < 0.05$); the relative abundance of *Bryobacter* was

significantly increased in C5 and BC5 ($P < 0.05$); the relative abundance of *Vibrionimonas* was increased in all treatments, with a significant increase in BC5 ($P < 0.05$); there was no significant difference in the relative abundance of *RB41* among all treatments ($P > 0.05$). These results indicate that the application of biochar, planting *C. comosum*, or their combination did not affect the composition of the main dominant genera in the soil but changed their relative abundance.

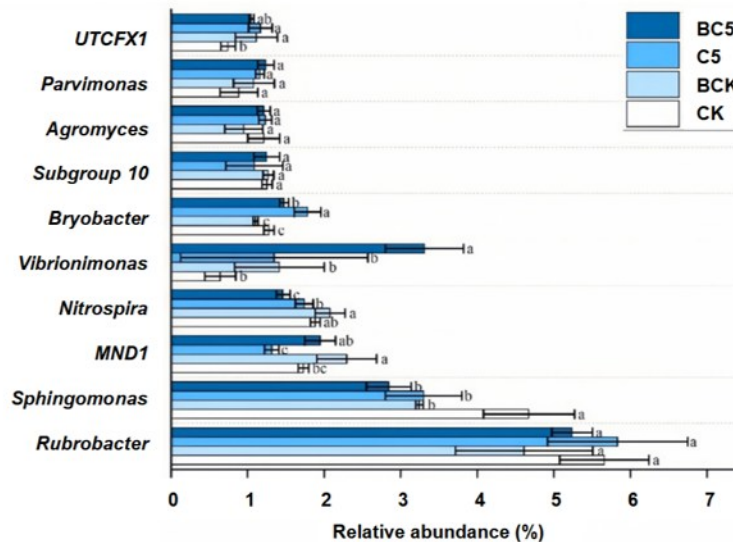


Fig. 8. Relative abundance of soil bacteria at the genus level

Effects of Combined Application of PTM Biochar and *C. comosum* on Metabolic Functions of Soil Bacterial Communities

The metabolic functions of soil bacterial communities under different treatments were predicted using Tax4Fun. Rows in the figure represent KEGG pathways, columns represent samples, and the color of the color blocks represent the difference in the relative abundance of KEGG pathways among samples. The top 35 functions in terms of abundance and their abundance information in each sample were selected to draw a heatmap, and clustering was performed from the perspective of functional differences (Fig. 9). Based on the Tax4Fun prediction results, the metabolic function difference between BC5 and CK was the largest. The differences between each treatment (BCK, C5, BC5) and CK are as follows:

- (1) The metabolic functions of BCK and CK had the highest overall similarity, and they shared most core metabolic pathways, such as Purine metabolism, Pyrimidine metabolism, Two-component system, Oxidative phosphorylation, and Glycolysis/Gluconeogenesis. Compared with CK, BCK lacked 7 pathways, including Pyruvate metabolism, Amino sugar and nucleotide sugar metabolism, Biosynthesis of antibiotics, Biosynthesis of secondary metabolites, Biosynthesis of amino acids, Carbon fixation pathways in prokaryotes, Ribosome, and Biofilm formation - *Pseudomonas aeruginosa*. The newly added metabolic pathway in BCK was Glycine, serine and threonine metabolism.
- (2) Compared with CK, C5 lacked 7 pathways, including Pyruvate metabolism, Amino sugar and nucleotide sugar metabolism, Biosynthesis of antibiotics, Biosynthesis of secondary metabolites, Biosynthesis of amino acids, Carbon fixation pathways in prokaryotes, Ribosome, and Biofilm formation - *Pseudomonas aeruginosa*. The newly

added metabolic pathways in C5 were mainly related to fatty acid metabolism and carbon metabolism, including Fatty acid degradation, Carbon metabolism, Propanoate metabolism, Butanoate metabolism, Bacterial chemotaxis, Glycine, serine and threonine metabolism, and Valine, leucine and isoleucine degradation.

(3) The BC5 treatment had the largest difference in metabolic functions from CK and the most abundant metabolic pathways. BC5 had the most significant difference in metabolic functions from CK among all treatments; it not only included the core metabolic pathways of C5 but also had 8 unique pathways (not involved in CK) added based on C5, further expanding the metabolic range. These pathways included Glyoxylate and dicarboxylate metabolism, Fatty acid biosynthesis, Benzoate degradation, Sulfur metabolism, ABC transporters, Quorum sensing, Microbial metabolism in diverse environments, and Butanoate metabolism.

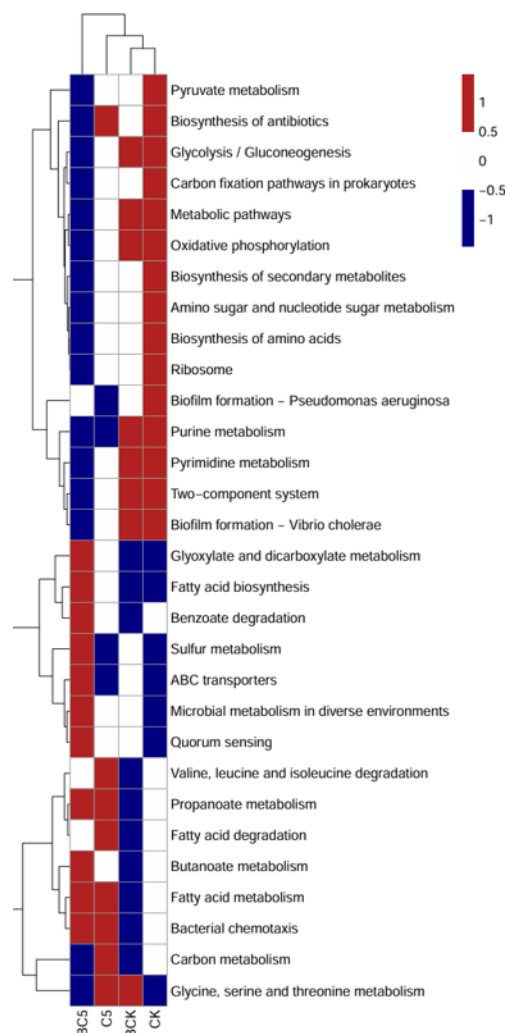


Fig. 9. Main metabolic functions of soil bacterial communities

DISCUSSION

Under the BC5 treatment (combined application of 5% PTM biochar and *C. cosmosum*), the growth indicators and photosynthetic parameters of lettuce were

significantly superior to those of other treatment groups, while the stress resistance indices and heavy metal accumulation were remarkably reduced. This optimal remediation effect is not driven by a single factor, but a synergistically driven comprehensive effect of three components: the physicochemical adsorption by the PTM biochar, the heavy metal enrichment of *C. comosum*, and the optimization of soil microbial community structure and function. These three components form a mutually reinforcing virtuous cycle, collectively enhancing the remediation efficiency of soil contaminated with combined copper (Cu) and lead (Pb) pollution.

As a typical calciphilous plant, the biochar derived from the carbonization of PTM waste branches inherently possesses three core advantages: high calcium content, abundant surface functional groups, and large specific surface area (Chen *et al.* 2011; Cho *et al.* 2017; Islam *et al.* 2021), enabling efficient heavy metal immobilization without additional calcium modification. On one hand, PTM biochar is weakly alkaline (pH 7.04), and its application significantly increases the pH of contaminated soil. This promotes the hydrolysis of Cu^{2+} and Pb^{2+} ions to form insoluble hydroxides or carbonate precipitates, thereby reducing the content of bioavailable heavy metals in the soil. Under the BC5 treatment, the bioavailable Cu and Pb in the soil decreased by 38.6% and 30.4%, respectively, which is consistent with the research conclusions of Jiang *et al.* (2025) on heavy metal immobilization by PTM biochar. In addition to direct metal immobilization, PTM biochar may have indirectly influenced the soil microbial community by modifying soil pH, nutrient availability, and microhabitat conditions. Previous studies have shown that biochar can alter bacterial community composition, and that these responses may depend strongly on biochar feedstock and soil properties (Zhang *et al.* 2021). Biochar amendment has also been reported to affect interactions among soil N and P species, plant uptake, and nutrient-cycling genes (Li *et al.* 2023). In the present study, the BC5 treatment was associated with shifts in bacterial community structure and increased relative abundances of *Bryobacter* and *Vibrionimonas*. These changes may help explain the improved soil ecological function and lettuce growth, but they should be interpreted as potential microbial mechanisms rather than direct causal evidence.

Chlorophytum comosum, a typical heavy metal-accumulating plant, exhibits strong tolerance and absorption capacity for Cu and Pb (Wang *et al.* 2012; Wu *et al.* 2013; Zia-ur-Rehman *et al.* 2023). Its core roles in the remediation process are reflected in two aspects: first, direct enrichment and removal of heavy metals. Under the BC5 treatment, the Cu contents in the leaves and roots of *C. comosum* increased by 51.4% and 15.6%, respectively, compared with the single *C. comosum* treatment (BCK), while the Pb contents increased by 50.2% and 40.5%, respectively. Additionally, the translocation factor of heavy metals to the aboveground parts significantly increased, providing a feasible approach for the continuous removal of heavy metals through harvesting the aboveground biomass. Second, regulation of the rhizosphere microenvironment *via* root exudates. The organic acids secreted by the roots of *C. comosum* can moderately activate insoluble heavy metals in the soil, improving the adsorption efficiency of biochar for heavy metals, while the secreted phytol alleviates heavy metal toxicity to the plant itself, further enhancing its enrichment capacity (Yu *et al.* 2019). Notably, the presence of *C. comosum* did not directly alter the core structure of the soil microbial community (PCoA analysis showed high aggregation of BCK and CK samples), but it provided a rhizosphere microhabitat for microorganisms. Its exudates, acting as “signal substances”, recruit functional bacteria such as *Bryobacter* and *Vibrionimonas* to colonize the rhizosphere, enhancing the plant's

stress resistance (Babikova *et al.* 2013; Trivedi *et al.* 2020) and serving as a bridge for the regulatory role of microorganisms.

The soil microbial community is a key link connecting the soil environment and plant responses (Yang *et al.* 2021). Although the BC5 treatment did not significantly change the bacterial community richness (Chao index and ACE index showed no differences from CK), it remarkably reshaped the community structure and the abundance of dominant genera, and activated unique metabolic pathways, providing biological regulatory support for the remediation effect. In terms of community structure, the BC5 treatment significantly increased the relative abundances of *Bryobacter* and *Vibrionimonas* ($P < 0.05$). *Bryobacter* is closely associated with soil phosphorus cycling (Zhou *et al.* 2019; Wang *et al.* 2024), and its increased abundance can promote the release of available phosphorus in the soil, providing nutrients for lettuce growth and chlorophyll synthesis. It also participates in the transformation and immobilization of heavy metals, further reducing the bioavailability of soil heavy metals. Meanwhile, the BC5 treatment significantly decreased the abundance of *Sphingomonas*, a typical plant growth-promoting bacterium. The reduction in its abundance may be related to the decreased soil heavy metal toxicity caused by biochar, which reduces the plant's dependence on “growth-promoting bacteria” (Xu *et al.* 2020), indirectly reflecting the improvement of the soil microenvironment. In terms of metabolic functions, Tax4Fun prediction results showed that the BC5 treatment had the greatest difference in metabolic pathways from CK, covering the core pathways of the single biochar application (C5) and adding 8 unique pathways. Among them, the activation of the sulfur metabolism pathway can promote the transformation of sulfur in the soil, and sulfur is a key element for chlorophyll synthesis (requiring glucosinolates) and the production of antioxidant substances (*e.g.*, glutathione). This directly explains the physiological mechanisms underlying the significant increase in lettuce chlorophyll content (24.7% increase) and the significant decrease in SOD activity (70.0% decrease) under the BC5 treatment. In addition, sulfur-based compounds can undergo coordination reactions with heavy metal ions to form insoluble precipitates, thereby alleviating the toxic effects of heavy metals on plants (Courbot *et al.* 2004; Kieu *et al.* 2015; Walker *et al.* 2021). The enhancement of the ABC transporter pathway enables microorganisms to actively transport free Cu^{2+} and Pb^{2+} ions in the soil into microbial cells or extracellular polymers for immobilization (Park *et al.* 2012; Song *et al.* 2014), which is consistent with the results that the Cu contents in lettuce leaves and roots decreased by 58.2% and 41.5%, respectively, and the Pb contents decreased by 76.8% and 49.0%, respectively. The quorum sensing pathway promotes the proliferation of functional bacterial populations through signal transmission between microorganisms (Fuqua *et al.* 1994; Zhang *et al.* 2024; Niu *et al.* 2025), strengthening their regulatory roles in soil nutrient cycling and heavy metal immobilization. In addition, the unique pathways added by the BC5 treatment, such as fatty acid biosynthesis and benzoate degradation, further improve the efficiency of soil organic matter decomposition and nutrient transformation, providing a more suitable soil environment for plant growth.

Therefore, the microbial results provide a possible biological explanation for the observed remediation effects. The improvement of soil properties by PTM biochar may have created more favorable conditions for specific bacterial groups, while *C. comosum* provided a rhizosphere environment for plant-microbe interactions. These microbial shifts were consistent with the reductions in available Cu and Pb and the improved physiological status of *L. sativa*. However, because microbial functions were inferred from community composition and predicted pathways, further metagenomic, transcriptomic, or functional-

gene validation is needed to confirm the causal roles of these microorganisms in heavy metal immobilization and nutrient cycling.

“Biochar Adsorption—Plant Enrichment—Microbial Regulation”, is the core advantage of the BC5 treatment that lies in the construction of a “three-level synergistic” remediation system (Fig. 10).

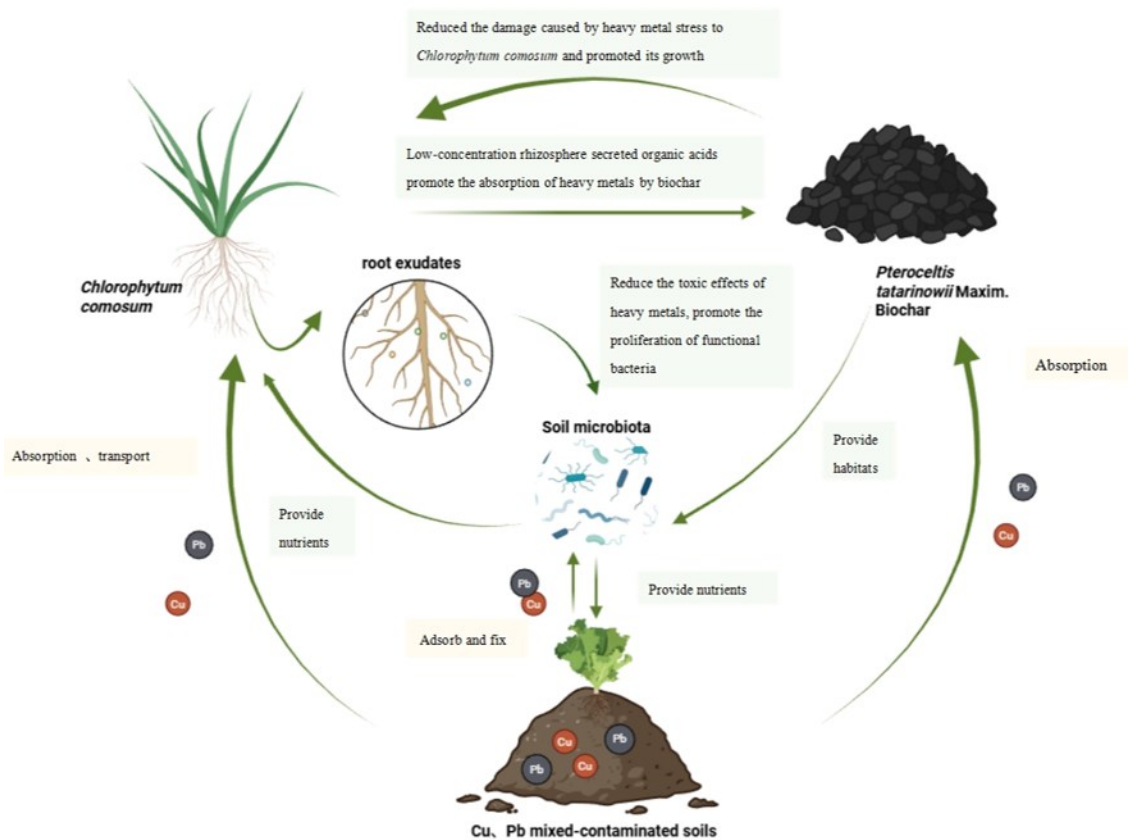


Fig. 10. Multi-level synergistic system of biochar adsorption—*C. comosum* transportation—microbial regulation

Each component does not act in isolation but mutually reinforces and progresses hierarchically:

1: PTM biochar provides the “foundation support” for the entire system. It immobilizes part of the heavy metals through physicochemical effects, reducing soil heavy metal toxicity; meanwhile, it improves soil physicochemical properties and provides carbon sources and nutrients, thus creating suitable conditions for the growth of *C. comosum* and microbial proliferation.

2: *Chlorophytum comosum* serves as the “enrichment core” of the system; it directly absorbs bioavailable heavy metals in the soil through its roots, further reducing heavy metal concentrations; its root exudates not only activate part of the heavy metals to improve its own enrichment efficiency but also recruit functional microorganisms to colonize the rhizosphere, strengthening the microbial regulatory effect.

3: Microorganisms offer “functional enhancement” for the system; through community structure reshaping and metabolic pathway activation. They first improve the efficiency of soil nutrient cycling, promoting the growth and heavy metal tolerance of *C.*

comosum; second, they assist in immobilizing heavy metals through active transport, element transformation, and other methods, reducing the risk of secondary pollution caused by biochar desorption, while improving soil ecological functions. This synergistic system effectively compensates for the shortcomings of single remediation technologies: although single biochar application can immobilize heavy metals, it lacks a continuous removal pathway. Although single *C. comosum* planting can enrich heavy metals, its enrichment efficiency is limited by soil heavy metal toxicity; the participation of microorganisms provides an “amplification effect” for the roles of the first two. Consequently achieving the comprehensive remediation goal of “heavy metal immobilization—enrichment removal—soil function restoration”.

CONCLUSIONS

The combined application of 5% *Pteroceltis tatarinowii* Maxim. (PTM) biochar and *Chlorophytum comosum* (BC5) produced the strongest remediation effect in Cu-Pb co-contaminated farmland soil. This treatment improved soil properties, reduced the bioavailability of Cu and Pb, enhanced the accumulation and translocation of Cu and Pb by *C. comosum*, and promoted the growth and physiological status of subsequently planted *L. sativa*. Meanwhile, BC5 reduced Cu and Pb accumulation and translocation in *L. sativa* and reshaped the soil bacterial community, with increased relative abundances of *Bryobacter* and *Vibrionimonas* and changes in predicted metabolic pathways related to nutrient cycling and stress regulation. These findings suggest that sequential remediation using PTM biochar and *C. comosum* is a promising low-cost and eco-friendly strategy for reducing residual phytotoxicity and crop metal-uptake risk in Cu-Pb-contaminated farmland.

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Declaration of Interest Statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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