Liang et al. Biochar (2026) 8:45
https://doi.org/10.1007/s42773-025-00548-x

Biochar

ORIGINAL RESEARCH
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Micro-nanoscale bone char alters Cd accumulation and rhizosphere functional genes to enhance rice yield and quality

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Abstract

This work investigates the impact of micro-nanoscale bone char (MNBC) soil amendment on Cd-stressed rice over a full life cycle, aiming to develop a sustainable remediation strategy integrating yield improvement and soil health. MNBC was sourced from widely available pork bones in the waste stream, and was generated by pyrolysis at 400 °C and 600 °C, followed by ball-milling to reduce the particle size to micro-nanoscale. A 140-day full-life-cycle experiments was conducted under greenhouse conditions, and soil samples across all the treatments were collected at different growth periods for metagenomic analysis. At harvest, rice grains were sampled for metabolomic analysis. Results showed that treatment with 600 °C MNBC significantly increased grain yield by 49.72%, while 400 °C MNBC increased the effective tiller number by 23.08%, compared to Cd treatment. Both types of MNBCs reduced Cd accumulation in rice tissues, with reductions of 65.0–68.7% in polished rice relative to the Cd treatment. Metabolomic analysis highlights MNBC modulated the nutritional value of the grains, effectively slowing down the biochemical processes of carbohydrates and branched-chain amino acids into simple sugars or polyols in rice grains. In the aerobic phase of soil, the acid-soluble Cd with MNBC treatments decreased by 31.56–35.51% as compared to the Cd treatment. Metagenomic analyses show that MNBC had a significant impact on the microbial communities involved in soil carbon, nitrogen, and phosphorus cycling, such as Actinomycetota, Cyanobacteria, and Gemmatimonadota, as well as on related genes; particularly enhancing the complexity of the phosphorus gene network. Overall, these findings demonstrate the significant potential of MNBC-enabled agriculture practices as a sustainable crop strategy.

Highlights

- Micro/nano-scale bone biochar (MNBC) significantly increased rice yield and tiller number.
- MNBC effectively fixed soil Cd, reduced the Cd bioavailability, and significantly reduced Cd accumulation in rice.
- MNBC positively affected the composition of soil organic matter and microbial communities at different rice growth phases.
- Soil P-cycling genes were highly sensitive to long-term MNBC application.

Keywords Micro-nanoscale bone char, Cadmium, Phosphorus, Grain yield, Metabolites, Soil metagenomics

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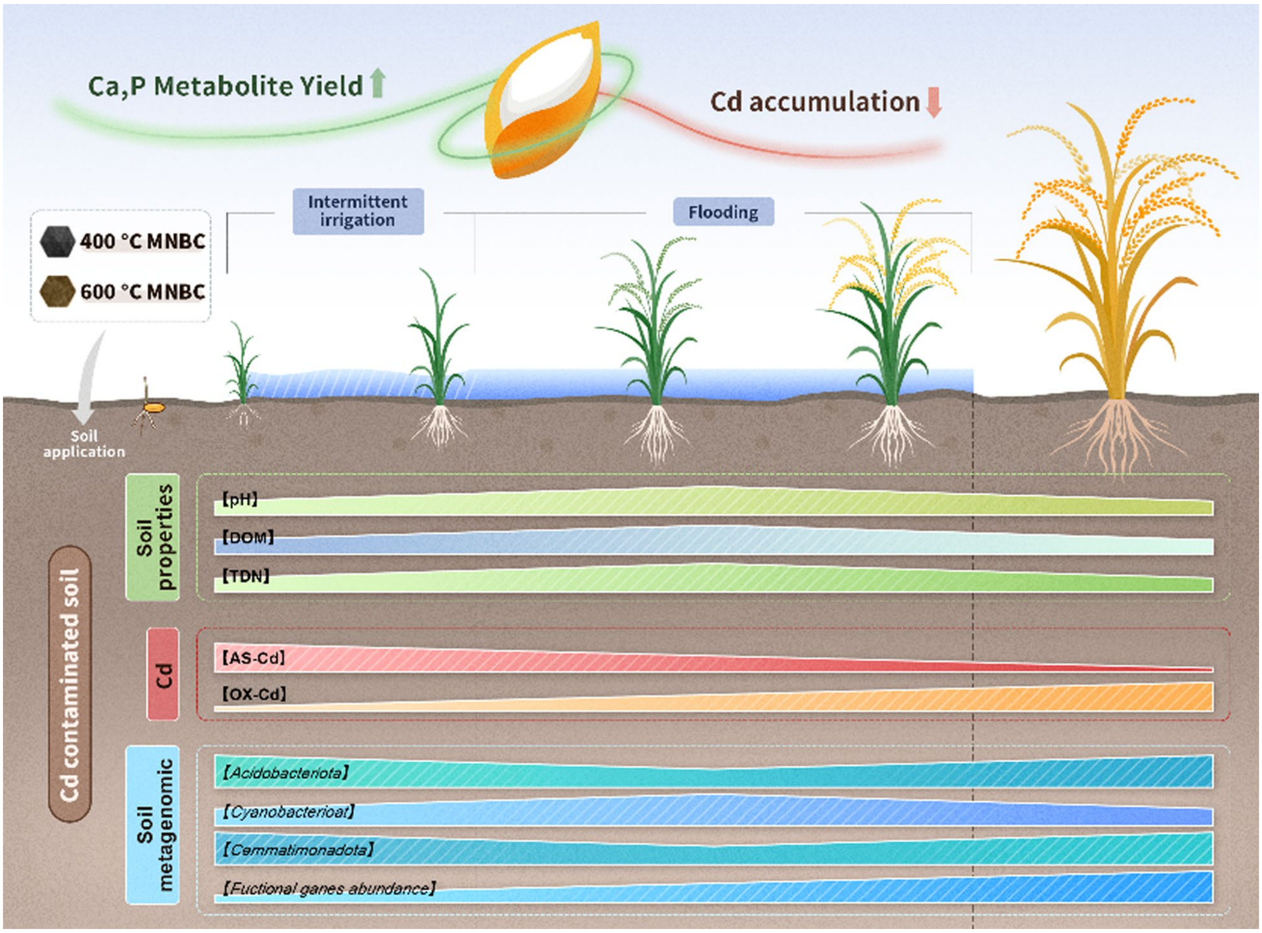
Graphical Abstract



1 Introduction

Due to its high mobility, bioavailability and toxicity, cadmium (Cd) in soil is a major global issue threatening agricultural production, food safety and human health. Rice (*Oryza sativa* L.) cultivated land in over 100 countries has been affected by Cd pollution (Sharif et al. 2014; Rai et al. 2019; Chen et al. 2015). Rice exhibits a stronger physiological affinity for accumulating Cd than other crops, which can lead to the accumulation of dangerous levels of Cd in the grain (Song et al. 2015). According to the Chinese national standard "National Food Safety Standard (GB 2762-2017)", the limit for Cd in polished grains of rice is 0.2 mg/kg. In fact, more than 10 million tons of grains in China are contaminated with Cd annually (Cai et al. 2014). This not only results in food waste, but alsoallows Cd to easily enter the human body through the daily diet, posing a serious threat to human health (Wang et al. 2019). Thus, novel solutions to reduce the content of heavy metals, especially Cd, in rice grains while ensuring agricultural production are significantly needed.

At present, a number of soil amendments are commonly used to achieve the purpose of managing contaminated soil. Biochar has garnered significant interest because its rich porous structure, large specific surface area, and typically alkaline pH can effectively decrease the bioavailability of Cd by changing the speciation of Cd^{2+} in soil (Chen et al. 2019). Another benefit of biochar application is that it can promote the sustainable utilization of excessive green waste such as straw, bagasse, and bone, providing a value-added benefit and promoting the circular economy (Yadav et al. 2023). Previous studies have shown that bone-derived biochar can remove Cd and other toxic elements from aqueous solutions or soils (Xiao et al. 2020; Azeem et al. 2021a, 2022), while simultaneously improving overall soil fertility (Siebers and Leinweber 2013; Warren et al. 2009; Hamilton et al. 2019), microbial diversity (Azeem et al. 2023; Liang et al. 2023), and plant growth (Azeem et al. 2021b; Xiao et al. 2023; Mei et al. 2022).

Nanotechnology has developed rapidly over the past two decades, largely because nanoscale particles exhibit unique properties relative to their bulk-scale counterparts. Thus, decreasing the size of conventional bulk biochar to micro-nanoscale via mechanical grinding and other green synthesis methods can increase surface area, provide richer surface functional groups and surface-active points, and enhance the adsorption capacity for heavy metals (Ramanayaka et al. 2020). For example, Yue et al. (2019) reported that the adsorption of Cd^{2+} by rice husk derived nanoscale biochar (average particle size of 59 nm) was at least tenfold larger than that of conventional biochar. Pork bone-derived micro-nanoscale biochar increased rice seedling biomass by 15.3%, decreased soil Cd bioavailability by 55%, and promoted the abundance of metal tolerant bacteria (Acidobacteria and Chloroflexi) by 30–40% (Liang et al. 2023). However, little information is available on the effects of ball milling modified biochar on the bioavailability and transport of Cd throughout the full life cycle of rice cultivation.

Water management is another important factor in controlling soil Cd bioavailability (Arao et al. 2009; Tang et al. 2016). For example, soil moisture affects pH and subsequently alters the adsorption of Cd by organic matter, as well as by iron and manganese compounds (Hu et al. 2015). In addition, water status also has an important impact on the activity and diversity of the soil microbiome (Chen et al. 2020). Under flooding conditions, anaerobic bacteria such as sulfate and iron reducing bacteria rapidly increase. These anaerobic bacteria can reduce sulfate and trivalent iron ions in soil to sulfur and divalent iron, respectively, subsequently forming precipitates with Cd (*e.g.*, CdS, $CdFe_2O_4$) that decrease the bioavailability of the heavy metal in soil (Daskalakis and Helz 1992; Shan et al. 2019). Precision water management can effectively balance rice yield and the reduction of Cd absorption and accumulation in the rice grain, thereby ensuring the safe production of agricultural products, and thus merits attention. Therefore, it is particularly important to investigate the water management of rice at different growth phases during biochar amendment, including how water management changes the pH, dissolved organic matter content, and Cd forms in the soil solution.

Importantly, phosphorus (P) is an essential macronutrient for crops (Illakwahhi et al. 2024), but less than 0.1% of soil P exists as bioavailable inorganic (Pi) and organic phosphorus (Po), which is typically insufficient to meet the needs of plants and microorganisms (Cordell et al. 2009; Bünemann 2015). Due to P deficiency, it is estimated that crop productivity in 5.7 billion hectares of worldwide agricultural soil was reduced (Dhillon et al. 2017). However, moisture-driven changes in microbial communities not only participate in Cd immobilization but also enhance P availability through P-cycle-related functional genes (*e.g.*, *phoD*, *pqqC*). Microorganisms play an important role in maintaining and regulating P availability through Pi dissolution and Po mineralization, thereby improving P acquisition by plants (Alori et al. 2017). Therefore, understanding the changes in the rice-grain metabolite profile, soil phosphorus-cycling genes, and soil microbial community composition and structure under a combined scenario of long-term biochar input and soil Cd pollution could serve as a strategy to understand biochar management efficacy at the molecular level.

Here, the effects of micro-nano bone char (MNBC) as a soil amendment on the bioavailability and mobility of Cd in the soil–rice system were investigated. We hypothesized that MNBC can decrease the accumulation of Cd in rice tissues, optimize the nutritional value of grains, and regulate the microbial communities involved in soil elemental cycling. Furthermore, the long-term application of MNBC is expected to improve the yield and quality of rice grown in Cd-contaminated soil. To verify this hypothesis, a 140-day lifecycle experiment was conducted, during which rice plants were grown in 15 mg/kg Cd contaminated soil. The main objectives were (1) to investigate the changes in grain yield and agronomic traits of mature rice treated with 25 g/kg MNBC in Cd-contaminated soil; (2) to determine Cd transformation in the soil-rice system as a function of MNBC; (3) to elucidate the effects of MNBC on rice-grain quality as determined by metabolite profiling; and (4) to investigate the effects of MNBC on Cd forms, soil chemical properties, and functional gene abundance in soils at different growth phases of rice. Overall, this study provides important mechanistic insight for the long-term application of MNBC to improve the yield and quality of rice in Cd-contaminated soil.

2 Materials and methods

2.1 Rice cultivation experiments

MNBC with particle sizes ranging from 30 nm to several millimeters was prepared by pyrolyzing pork bones at 400 °C and 600 °C for 2 h under an N_2 atmosphere, followed by ball-milling (PMQM2, Nanjing, China) (Liang et al. 2023); the synthesis and characterization details are provided in Experiment S1 and Fig. S1. Information on the source and type of rice seeds and soil used for the experiment are presented in Experiment S2. The soil pH value was approximately 4.62, and the soil background values are shown in Table S1. The experiment consisted of four treatments, including control without MNBC and Cd amendments, 15 mg/kg Cd-alone treatment, 15 mg/kg Cd co-treatment with 25 g/kg 400 °C MNBC treatment, and 15 mg/kg Cd co-treatment with 25 g/kg 600 °C MNBC treatment. The experimental design is shown in Table S2. The experiments were conducted at the Ecological Restoration and Carbon Sequestration Research Greenhouse of Guangdong University of Technology, China (23°E2′23″, 113°N23′11″). Following the agricultural management of rice cultivation post transplantation (Honma et al. 2016) (Fig. S2), intermittent irrigation of 20–0–20 mm was implemented during the tillering stage, with soil samples taken corresponding to the facultative anaerobic (FA) phase. From the jointing stage until maturity, irrigation of 50 mm was applied, with soil samples collected corresponding to the anaerobic (AN) phase. Prior to harvest, field drying was conducted, and the harvested dry soil corresponded to the aerobic (AE) phase. Details of the monitored agronomic traits of rice, including spike length, grain weight, effective tillers number, and specific root length (SRL), are provided in Experiment S3.

2.2 Macro- and micro-nutrient content in rice tissues and soil

Briefly, 100 mg of dried plant tissues (including roots, shoots, and grains) or soil were digested in 4 mL of concentrated HNO_3 for 40 min at 115 °C using a graphite furnace reaction system (PT 60, Polytech Instruments Co., LTD., China). After cooling to ambient temperature, 1 mL of 30% (v/v) H_2O_2 was added to the digesta, followed by digestion at 115 °C for 20 min (Liang et al. 2023). The in-planta element content was measured by inductively coupled plasma emission spectrometry (ICP-OES, iCAP 7000, Thermo Scientific, USA) or inductively coupled plasma mass spectrometry (ICP-MS, iCAP RQ, Thermo Scientific, USA), including boron (B), manganese (Mn), copper (Cu), zinc (Zn), iron (Fe), magnesium (Mg), potassium (K), calcium (Ca), phosphorus (P), sulfur (S), and cadmium (Cd). A known standard solution of mixed analytical elements (NCS149160) and certified reference materials of soil powder (GBW07986) were purchased from NCS (NCS Testing Technology, Beijing, China) as the quality assurance/quality control (QA/QC); a known concentration of standard solution was measured every 20 samples.

2.3 Metabolomics analysis in rice grains

Rice grain metabolites were measured and quantified by ultra-high performance liquid chromatography tandem mass spectrometry (UPLC-MS) as previously described with minor modifications (Chen et al. 2013). Briefly, the rice grains were thoroughly ground in liquid nitrogen, then 50 mg freeze-dried tissue was transferred to a 2 mL Eppendorf tube, extracted with 1.2 mL pre-cooled methanol and water (7/3, v/v), and vortexed for 30 s once every 30 min for a total of 6 times. The samples were centrifuged at 12,000 rpm at 4 °C for 3 min. One mL of the supernatant was added to the chromatographic bottle through a 0.22 μm microfilter for subsequent analysis. UPLC-MS analysis was performed on an AB SCIEX ExionLC™ AD system equipped with an Agilent SB-C18 column (1.8 μm, 2.1 × 100 mm), using a mobile phase of 0.1% (v/v) formic acid in water-acetonitrile with gradient elution (starting at 95:5 water:acetonitrile, transitioning to 5:95 over 9 min, then returning to initial conditions). Detection was carried out using a QTRAP mass spectrometer in alternating ESI+/ESI− mode and operated in MRM mode for targeted metabolite quantification. Additional details on operation and analysis parameters are shown in Experiment S4.

2.4 Cd fractions and soil DOM analysis

Different fractions (acid-soluble, reducible, oxidizable and residual) of Cd and other elements (*e.g.*, S, Ca, Mg, Mn, Fe, Cu, Zn) in soil were extracted using the European Community Extraction Reference Committee (BCR) protocol (Usero et al. 1998). Soil pH was measured using a pH meter (PB-10, Sartorius, Germany) (soil to water = 1:5; w/v), and soil cation exchange capacity (CEC) was measured using a hexamminecobalt trichloride solution-spectrophotometric method (soil to water = 1:2; w/v). The total dissolved organic matter (DOM) and total dissolved nitrogen (TN) contents were determined using a TOC analyzer (TOC-L, Shimadzu, Japan) (Zhang et al. 2020). The chemical composition of DOM was determined by a 3D-EEM spectral fluorescence spectrophotometer (RF-6000, Shimadzu, Japan). Additional details on the DOM extraction and characterization methods are provided in Experiment S5.

2.5 Soil metagenomic analysis

Total soil DNA was extracted using E.Z.N.A.® Viral DNA Kit (Omega Bio-tek, Norcross, GA, USA) according to the manufacturer's instructions. The quality of the extracted DNA was checked by 1% agarose gel electrophoresis. The NanoDrop 2000 (Thermo Fisher Scientific, USA) was used to determine DNA purity and concentration. DNA samples were sequenced using the Illumina sequencing platform with pair-end 150 bp (PE150) mode. Data quality was controlled via FastQC, with adapter trimming and read filtering using Trimmomatic with a quality threshold of Q30. Details of the metagenomic analysis for isolated DNA are provided in Experiment S6.

2.6 Data analysis

A one-way analysis of variance (ANOVA) followed by Duncan's multiple range test was used to compare the significant differences across all the treatments (SPSS 26.0; $p<0.05$). Figures were plotted using Graphpad Prism 8 software and R software (version 4.2.0). The correlation between various factors was determined by the Pearson correlation method.

3 Results and discussion

3.1 Rice biomass and grain yield as affected by MNBC

The phenotypic images of rice plants showed no difference across all the treatments (Fig. 1a–d). However, the size of panicles in the Cd-alone treatment was overtly smaller compared to other treatments (Fig. S3). It is worth noting that select replicates in the Cd-alone treatment appeared to have delayed physiological development. The stem dry weight was significantly decreased by 21.37% in Cd-alone treatment compared to the control (Fig. 1j). Importantly, the addition of 600 °C and 400 °C MNBCs significantly increased the stem dry weight by 35.57% and 18.82%, respectively, relative to the Cd-alone treatment. In addition, 600 °C MNBC improved rice grain development (Fig. 1f; Fig. S3c). The average grain yield per pot in the Cd-alone treatment was 3.58 g, while this was increased by 49.72% with 600 °C MNBC (Fig. 1f). Additionally, the 100-grain weight in the Cd co-treatment with 600 °C MNBC was slightly higher than that in both the untreated control and the Cd-alone treatment (Fig. 1g). The addition of 400 °C MNBC did not increase growth and yield under Cd exposure, although rice heading appeared to be promoted (Fig. 1h; Fig. S3d). Amendment with 400 °C MNBC increased the

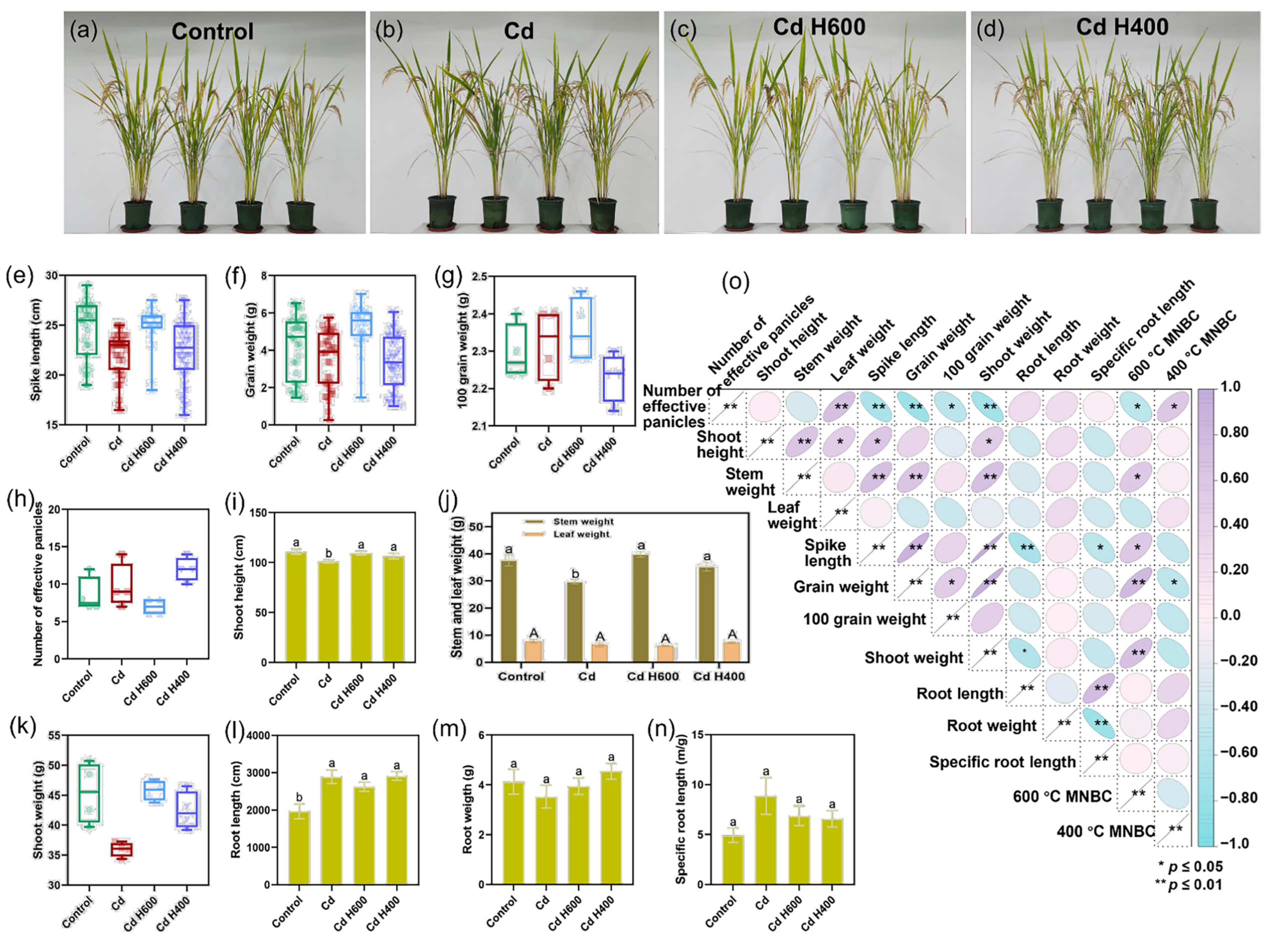


Fig. 1 MNBC effects on rice growth and development in the 15 mg/kg Cd-contaminated soils. **(a–d)** Phenotypic images of rice plants at maturity. **(e–n)** Agronomic traits of Cd-contaminated rice. **(o)** Correlation analysis of agronomic traits of mature rice. Purple ellipses indicate positive correlation coefficients, while blue ellipses indicate negative correlation coefficients. Flatter ellipses indicate larger correlation coefficients, and narrower ellipses to circles indicate smaller correlations. * indicates significant correlation at $p<0.05$, ** indicate significant correlation at $p<0.01$. Rice seedlings were grown in soil amended with different types of MNBCs and allowed to grow for 140 days. The data are mean ± SEM ($n=4$)

effective tiller number per pot by 23.08% beyond that of the Cd-alone treatment. These findings agree with Xu et al. (2024), who reported that in 0.86 mg/kg Cd-contaminated soil, the application of 5% peanut-corn stalk (1:3, w/w) co-pyrolyzed biochar decreased the grain Cd level to as low as 0.11 mg/kg and the biomass of rice roots, stems, and leaves increased by 58.29%, 66.39%, and 92.86%, respectively, compared to the treatment without biochar. Similarly, Liu et al. (2020) observed that the addition of 1% cotton straw biochar resulted in rice yield increases of 45.45%, 30.63%, 22.20%, and 12.98% in the soil amended with 0, 1, 4, and 8 mg/kg Cd, respectively. For the current study, a detailed discussion of the differences in the specific root length (SRL) with MNBC and the results of correlation analysis of agronomic traits are presented in Text S1. Overall, Cd exposure decreased biomass and grain yield, but MNBC amendment significantly alleviated the Cd stress, increasing the number of tillers, biomass and grain yield. Similarly, in a field study under water-saving irrigation conditions, the addition of 40 t/ha rice straw-derived biochar increased the effective panicle number, grain setting rate (filled grain number/grain number), and yield by 17.179%, 14.04%, and 15.79%, respectively, compared to the rice field without biochar application (Chen et al. 2021a).

3.2 Cd accumulation and translocation in rice as affected by MNBC

The Cd content in Cd-alone-treated polished grains exceeded the standard value (0.2 mg/kg, National Food Safety Standard, GB 2762-2017) by 12-fold. Both 600 °C and 400 °C MNBC significantly reduced the accumulation of Cd in rice tissues. Specifically, in the rice grains, the Cd content in hulls, bran and polished grains were reduced by 75.0% and 74.5%, 66.7% and 63.7%, and 68.7% and 65.0% in the treatment with 600 °C and 400 °C MNBC, respectively, when compared to the Cd-alone treatment (Fig. 2a). Rice hulls are important photosynthetic organs during grain development, while the bran is a mixture of seed coat and aleurone layer (Ren et al. 2023). Generally, the pattern of Cd distribution in rice tissues was in a decreasing order of roots > stems > leaves > hull > bran > polished grain regardless of the MNBC addition (Fig. 2a, b). During the jointing and grain-filling phases, rice exhibits a rapid growth rate, with an active root system and increased demand for nutrients, thereby enhancing the likelihood of Cd absorption (Huang et al. 2022). Consequently, the soil Cd content during the anaerobic phase corresponding to the jointing, heading, and grain-filling stages of rice was found to be relatively low (Fig. 2c). With MNBCs, Cd was effectively fixed in the soil, particularly in the aerobic phase; the 400 °C MNBC significantly increased the total soil Cd content by 17.33% compared to the metal-alone controls (Fig. 2c). This can be attributed to the MNBC being enriched with C, P and Ca, yielding a hydroxyapatite-like structure that promotes the transformation of Cd from the acid-soluble to residual fractions, and reduces the bioavailability of Cd in soil (Liang et al. 2023). Thus, Cd transport to the aboveground parts of the rice was inhibited. Additionally, the large specific surface area of biochar, along with its abundant organic carbon and surface functional groups, likely contributed to Cd immobilization in soil (Azeem et al. 2022; Li et al. 2016). A detailed analysis of the effects of MNBC on essential elements in rice tissues is presented in Text S2. Overall, MNBC amendment efficiently fixed Cd in soil and significantly reduced the accumulation of Cd in rice tissues, thereby alleviating the Cd stress.

3.3 MNBC affects metabolomic profile of rice grains

Rice typically contains approximately 80–85% starch, 4–10% protein, and 1% lipid, as well as various vitamins and minerals (Balindong et al. 2018). These components make rice an important source of energy and a nutritious food (Tong et al. 2019). Carbohydrates are a group of energy storage substances that can respond to environmental stresses (Sen et al. 2020). With regard to the carbohydrate metabolism (Fig. 3c), the primary carbohydrates were disturbed in the Cd-alone treatment, indicating that rice might enhance energy metabolism to defend against heavy metal stress. However, the addition of 400 °C MNBC decreased the levels of D-glucuronate, xylitol, D-arabitol, D-xylonate, and ribitol in the interconversions of pentoses and glucuronate; and decreased methylmalonic acid and 3-hydroxypropanoic acid in the pyruvate metabolism as compared to the Cd-alone treatment. Additionally, the levels of citrate, succinate, fumarate, and malate in the citric acid cycle (TCA cycle) in the Cd-treated grains were all decreased as affected by 400 °C MNBC, suggesting that MNBC effectively slowed the metabolism of grain carbohydrates into simple sugars or polyols. Although 600 °C MNBC increased the levels of these compounds in carbohydrate metabolism, their amounts were still lower than those in the Cd-alone treatment (Table S3). A number of studies have reported that when rice and wheat are subjected to osmotic or salt stress under low moisture conditions, the polyol content increases (Majee et al. 2004; Sebastian and Prasad 2019). Grains subjected to biotic stress accumulated a large number of intermediates associated with the citric acid cycle (such as citrate, succinate, fumarate, and malate), resulting in a gradual decline in rice quality (Lee et al. 2019). Similarly, 400 °C MNBC treatment downregulated the degradation of valine, leucine, and isoleucine (pathway) in the amino acid metabolism compared to

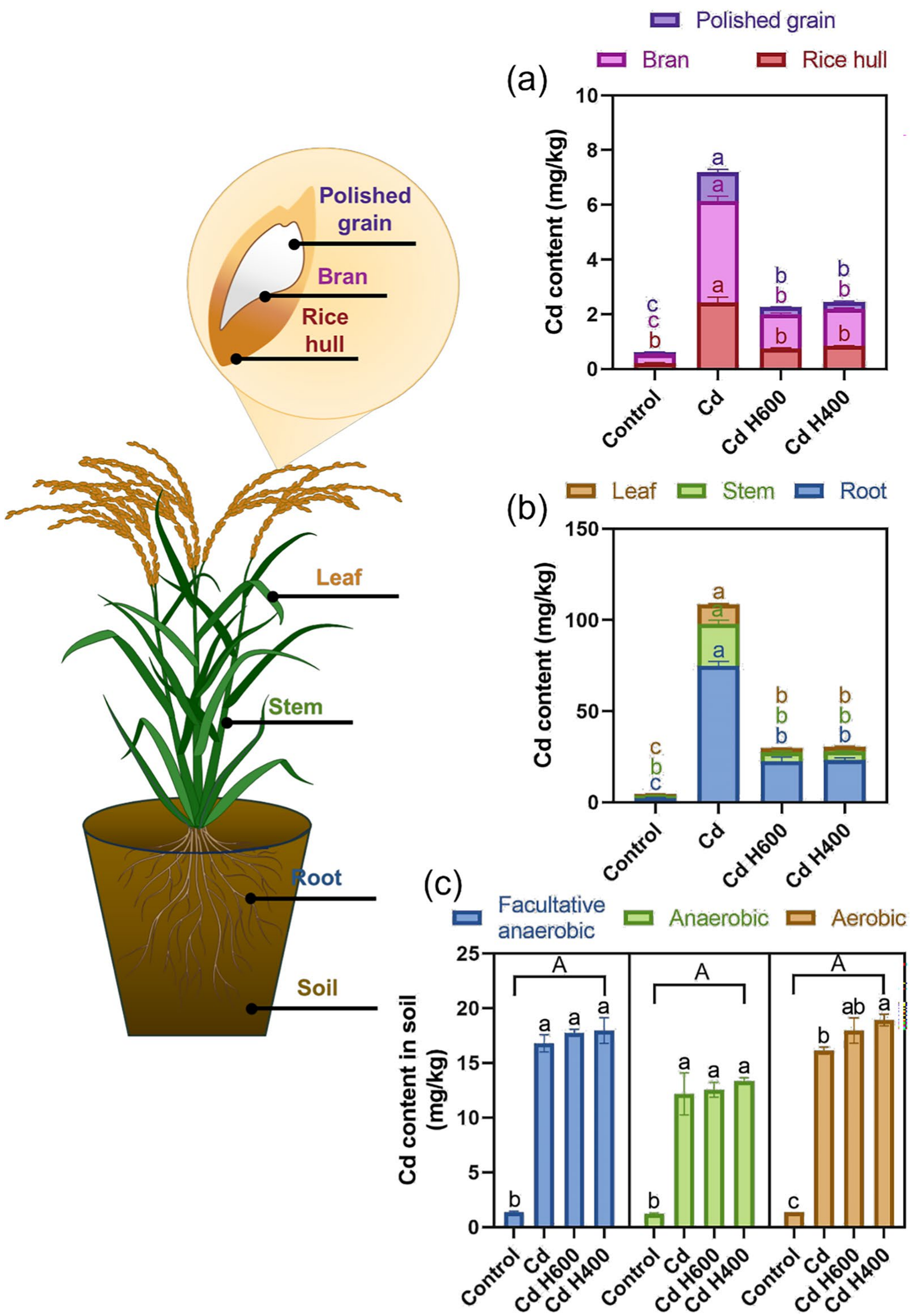


Fig. 2 Effect of micro/nano bone char on Cd distribution in grains **(a)**, rice tissues **(b)** and soils **(c)**. Data represent the mean ± SEM ($n = 4$). Different lowercase letters indicate significant differences among different treatments at the same phase, while the uppercase letters represent significant differences between different phases ($p < 0.05$, ANOVA with a multiple comparison test)

the Cd-alone treatment (Fig. 3c; Fig. S10; Text S3). Additionally, in lipid metabolism pathway (Fig. 3c; Fig. S10), 600 °C MNBC treatment significantly enriched the metabolic pathway of linoleic acid metabolism as compared to the untreated control. A more detailed analysis of the effects of MNBC on amino acid and lipid metabolism, as well as the metabolism of are provided in Text S3. Overall, the application of MNBC not only alleviated the alleviation of Cd stress but also increased the nutritional value of the grains, which could provide an important positive impact on human health.

3.4 MNBC alters Cd fractions and soil chemical properties

MNBC amendment decreased the proportion of the acid-soluble Cd (AS-Cd) compared to the Cd-alone treatment (Fig. 4a). In the aerobic phase, the AS-Cd proportion in the 600 °C and 400 °C MNBC treatments decreased by 35.51% and 31.56%, respectively, while the proportion of reducible Cd (OX-Cd) increased by 84.74% and 75.02%, respectively. MNBC increased the soil pH to approximately 6.49, and under the anaerobic phase, the soil pH across all the treatments increased significantly (Fig. 4b), due to the enriched mineral content, including alkaline cations and carbonates, in the biochar (Liang et al. 2023; Gul et al. 2015; Yuan et al. 2011). Similarly, Yu et al. (2016) reported that in the anaerobic phase, the pH of acidic soils increased to neutral as iron oxides were reduced and H^+ was consumed, whereas the pH decreased again in the aerobic phase, possibly due to the oxidation of ferrous ions producing H^+. Therefore, the soil moisture content can affect the pH of paddy soil, as well as the types and concentrations of major cations. For phosphorus (P), the addition of both MNBCs significantly increased the soil total P (TP) content, which was almost 3.82-fold that of the control (Fig. 4c) and notably reduced the proportion of residual P (EXC-P) by 30% as compared to the control (Fig. 4d), indicating that MNBC could enhance the availability of P for crops, providing a sustainable P management strategy to improve crop production. Notably, in the aerobic phase, 400 °C MNBC exhibited the greatest increase in the acid-soluble P (AS-P) proportion, with values being 397.16% higher than those in the Cd-alone treatment (Fig. 4d). This might be due to the fact that MNBC pyrolyzed at 400 °C contained more soluble phosphates than that produced at 600 °C. With increasing pyrolysis temperature, the levels of H_2O-P and $NaHCO_3$-P in biochar gradually decrease, leading to the conversion of P into more stable forms (Liang et al. 2023; Han et al. 2018; Wu et al. 2012). Regarding other elements, Mg, Fe, and Zn were primarily deposited in soil as the residual form which is difficult for plants to utilize during different growth phases (Fig. S11), while Ca, S, Mn and Cu were largely deposited in soil as more plant available forms. MNBC also significantly increased the total dissolved nitrogen (TDN) content in the soil (Fig. S12b), which could be due to the high proportion of nutrients, such as N and P, in bone-derived biochar, which could subsequently improve the nutrient levels in the soil (Warren et al. 2009). Azeem et al. (2021a) reported that the addition of bulk cow bone biochar (500 °C BCB) at a 10% application rate improved the quality of Cd and Zn-contaminated mining soils, while simultaneously decreasing the proportion of AS-Cd by 19%. Importantly, in comparison with BCB, the 2.5% MNBCs application in this study not only more effectively increased TP content in soil, but also was more efficient at promoting Cd fixation. This phenomenon may be attributed to the larger specific surface area, smaller particle size, and stronger negative charge of MNBCs (Liang et al. 2023), which allow nutrients to be retained on their surfaces for a longer duration, leading to slow release and greater adsorption of Cd^{2+}. The soil DOM content also increased significantly, with DOM bioavailability increasing with MNBC application (Fig. 4e; Fig. S12d). Interestingly, the highest fluorescence peak in the 600 °C MNBC treatment appeared in region IV (microbial degradation byproduct analogs) in the anaerobic phase (Fig. 4f; Fig. S13). This may be due to increased microbial activity in the water, which decomposes more organic matter and produces more degradation by-products. A more detailed analysis of the effects of MNBC on three-dimensional fluorescence spectra of DOM is presented in Text S4. Overall, MNBC could improve soil properties, alter the composition and structure of soil organic carbon, thereby enhancing the ability of soil microorganisms to utilize carbon sources.

(See figure on next page.)

Fig. 3 Effect of micro/nano bone char on rice grain metabolite. **(a)** Score plot of the PLS-DA of metabolites in rice grains with the MNBCs treatments. **(b)** The richness index was calculated according to following formula: Richness index = (the proportion of this class metabolites in the top 20 differential metabolites)/(the proportion of this class metabolites in total identified metabolites). **(c)** Key metabolic network diagrams in rice grains under different exposure conditions, including carbohydrate metabolism, amino acid metabolism, lipid metabolism, and cofactor and vitamin metabolism. The heatmap represents the ratio of average metabolite expression in different comparisons (Log_2FC), with positive values indicating upregulation and negative values indicating downregulation; The six boxes from left to right in each row represent Cd vs. Control, Cd H600 vs. Cd, Cd H400 vs. Cd, Cd H600 vs. Control, Cd H400 vs. Control, Cd H400 vs. Cd H600.

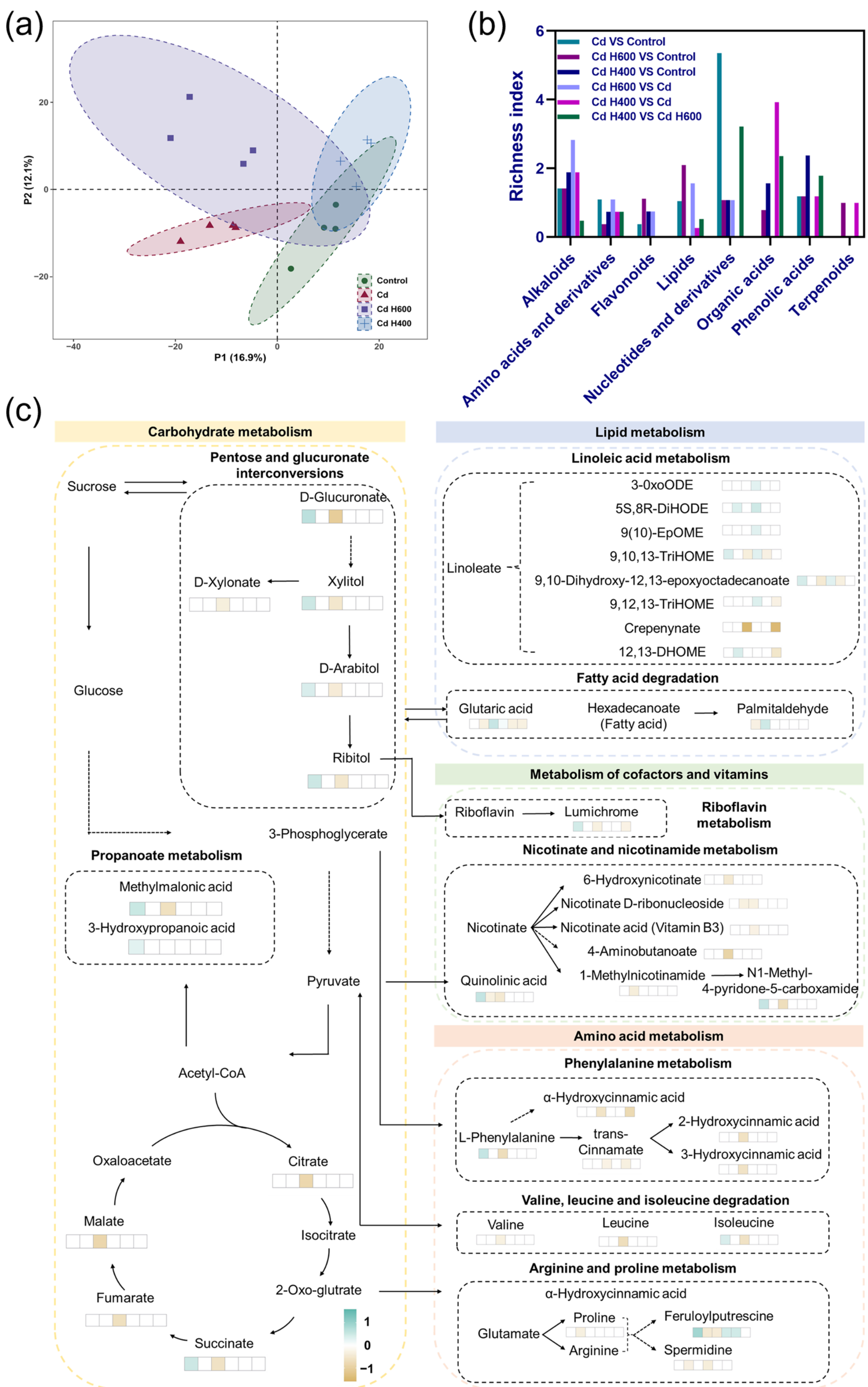


Fig. 3 (See legend on previous page.)

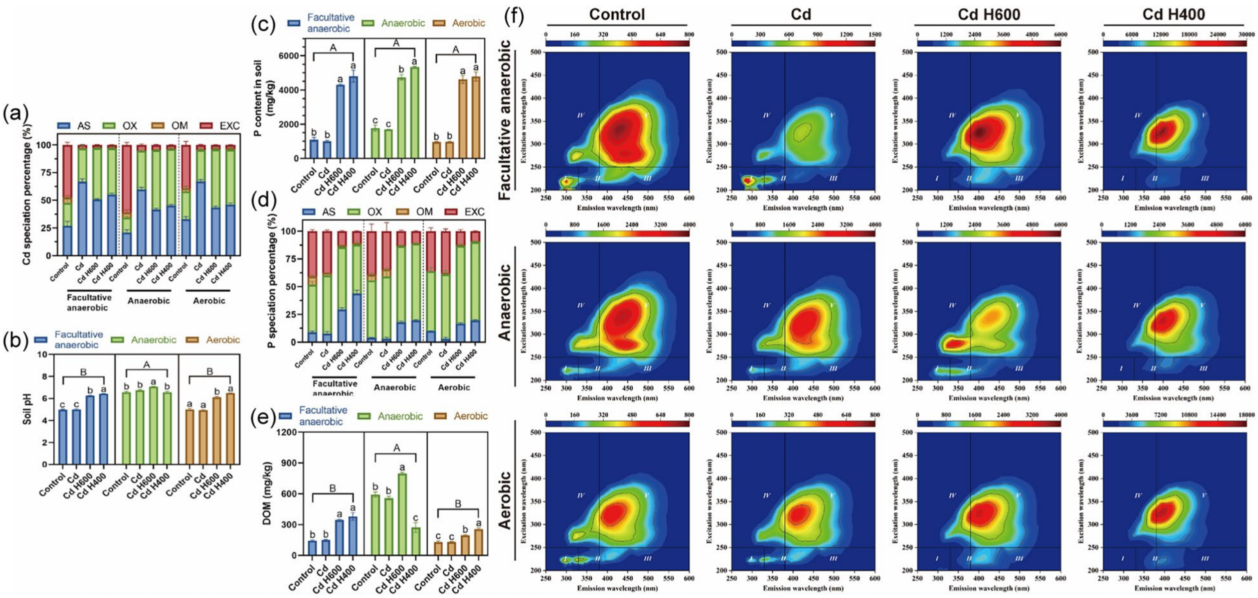


Fig. 4 Effect of micro/nano bone char on Cd fractions and soil chemical properties at different rice growth phases. Soil Cd percentage in each fraction **(a)**, soil pH **(b)**, soil P content **(c)**, P percentage in each fraction **(d)**, dissolved organic matter content **(e)**, three-dimensional fluorescence spectra of dissolved organic matter **(f)** extracted from the control and Cd-contaminated soils. AS: acid-soluble speciation; OX: reducible speciation; OM: oxidizable speciation; EXC: residual speciation. Five groups represent different organic carbon components. I: tyrosine protein; II: tryptophan protein; III: fulvic acid; IV: microbial by-product analogues; V: humic acid. Data represent the mean ± SEM ($n = 4$). Different lowercase letters indicate significant differences among different treatments at the same phase, while the uppercase letters represent significant differences between different phases ($p < 0.05$, ANOVA)

3.5 MNBC alters soil microbial community at different rice growth phases

The dominant bacterial phyla in soil were largely the same across all treatments, consisting of Pseudomonadota and Acidobacteriota (Fig. 5c; Text S5). Specifically, Acidobacteriota are acidophilic bacteria, and exhibit higher relative abundance during facultative anaerobic and aerobic phases as compared to anaerobic phase. The relative abundance of Acidobacteriota was reduced with MNBC due to the pH increases noted above. Interestingly, during the aerobic phase, the relative abundance of Actinomycetota significantly increased, especially in both MNBC treatments, where the abundance of Actinomycetota reached the highest levels. Actinomycetota play a crucial role in carbon cycling (Zheng et al. 2016). In the anaerobic phase, significant differences were noted in the relative abundance of Bacteroidota in the Cd-alone treatment (Fig. 5f); additionally, the relative abundance of Cyanobacteriota notably increased, with 216.73% and 106.91% greater in the 600 °C and 400 °C MNBC treatments, respectively, compared to Cd-alone treatment (Fig. 5c, f). The horizontal heatmap of the top ten soil bacterial genera shows that in the aerobic phase, the relative abundance of Pseudanabaena and SCGC-AG-212-J23 significantly decreased (Fig. 5d). Cyanobacteriota have a strong adaptability, allowing them to survive and reproduce in hypoxic or flooded environments (Chen et al. 2021b). The increased abundance of Pseudanabaena and SCGC-AG-212-J23 under anaerobic conditions might contribute to improving nitrogen supply in the soil, enhancing soil aeration and drainage, thereby boosting plant growth potential (Nawaz et al. 2024). SCGC-AG-212-J23 is known to exhibit high levels of tolerance in heavy metal-contaminated environments (Ghosh and Das 2018). The current work shows that SCGC-AG-212-J23 has a high relative abundance in the Cd-alone treatment under both facultative and anaerobic phases, while 600 °C and 400 °C MNBC decreased its relative abundance significantly by 64.97% and 33.06% at the facultative anaerobic phase, 43.01% and 27.96% at the anaerobic phase, respectively, again suggesting that MNBCs improved the health of Cd-contaminated soils. The relative abundance of Acidobacteriota, Actinomycetota, and Gemmatimonadota are correlated with soil P levels, and play vital roles in maintaining soil health and ecological balance (Jiao et al. 2023; Sui et al. 2022). In the aerobic phase, MNBC addition increased the relative abundance of Actinomycetota while decreasing that of Acidobacteriota and Gemmatimonadota, possibly due to an increase in the unstable P fraction in the

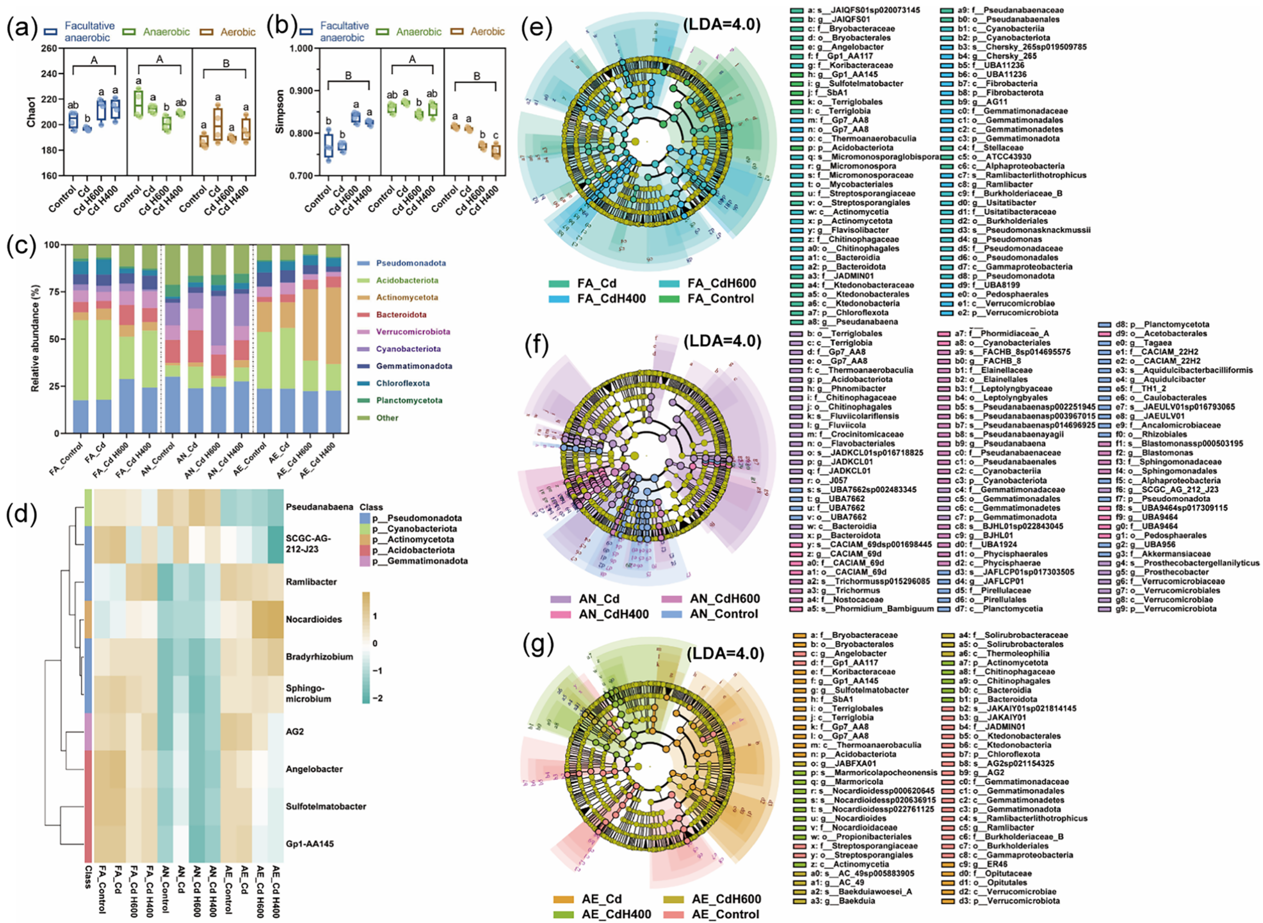


Fig. 5 Effect of micro/nano bone char on the soil microbial community at different rice growth phases. Chao and Simpson index of soil bacterial community (**a**, **b**), the relative abundance of bacteria at the phyla (**c**), top 10 bacteria at genera levels (**d**), and Cladograms of LEfSe showing bacterial indicator taxa (**e**–**g**). The filled circles from inside to outside indicate the taxonomic levels with phylum, class, order, family, genus, and species. Circles or nodes in different colors represent a significant association. Different lowercase letters indicate significant differences among different treatments at the same phase, while the uppercase letters represent significant differences between different phases ($p < 0.05$, ANOVA)

soil. Thus, the current study demonstrates that after a 140-day cultivation period, MNBC could still directly and/or indirectly promote the conversion of insoluble to soluble P (Fig. 4c, d).

3.6 Soil metagenomics at different rice growth stages as a function of MNBC

To analyze the effects of long-term application of MNBC on rhizosphere functional genes at different rice growth phases, a metagenomic analysis was conducted. The total explained variance by two main coordinate axes exceeded 60%, which can well explain the variability of soil microbial gene expression in different groups in the PCoA analysis (Fig. 6a–c; Text S6). In the untreated control and Cd-alone treatment, significant changes in the relative abundances of *purH*, *adk*, *spoT*, and *ppx* were observed in different growth phases; these genes are associated with the biosynthesis of ATP and GTP (Zeng et al. 2022), indicating that under changing anaerobic/aerobic conditions or Cd stress, soil microbes Likely adapted to these conditions by enhancing purine metabolism. Similarly, Yang et al. (2024) found that microbes promoted Cd fixation in paddy soil by upregulating the purine metabolism during the process of inducing carbonate precipitation. Purine and pyrimidine metabolism were the most abundant pathways regulating P turnover in this nutrient cycle, followed by transporters and two-component systems (Fig. 6d), indicating that the P demand in the soil

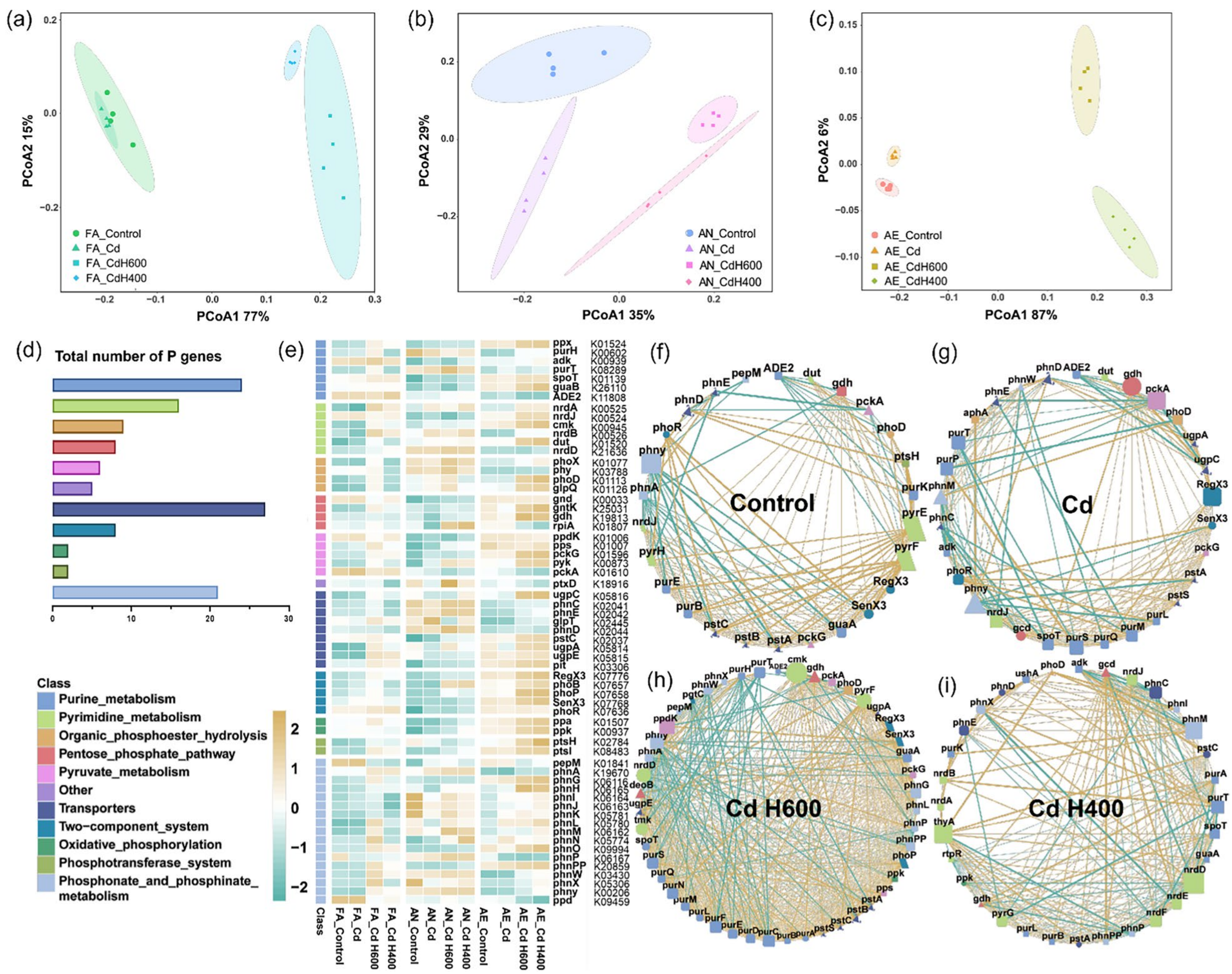


Fig. 6 Phosphorus cycle in Cd-contaminated soil with micro/nano bone char based on metagenomic sequencing. **(a–c)** The PCoA analysis of soil microbial genes with micro/nano bone char under 15 mg/kg Cd contamination during facultative anaerobic, anaerobic, and aerobic phases. **(d)** The number of phosphorus genes detected in each metabolic process of the phosphorus cycle. **(e)** Normalized heatmap showing changes in gene abundance related to phosphorus release across different groups. Changes of functional genes related to phosphorus release in microbial genes summarized in KO genes and KEGG pathway. **(f–i)** Occurrence network analysis showing of genes involved in soil phosphorus cycle with micro/nano bone char under 15 mg/kg Cd contamination, from **(f)** to **(i)** are the control, Cd-alone, Cd H600, and Cd H400 treatments, respectively. The node size shows the intensity of the links with other nodes (genes). Different shapes of nodes represent the genes involved the 10 phosphorus cycling processes. The colored line shows the correlation of the two nodes, with the brown line for the positive relationship and the blue line for the negative relationship

was high. The P assimilation capacity in soil is largely dependent on P cycling genes such as *phoR*, *phoB*, *pstSCAB*, *phoD* and *phoA*, which are influenced by environmental P inputs (Liu et al. 2023; Rawat et al. 2021). For instance, an increase in *phoB* abundance can indicate a higher availability of P in the environment (Liang et al. 2020). However, under low P conditions, microbes may also regulate the expression of transport system genes such as *pstSCAB* through the two-component *phoR* and *phoB* system to adapt to P deficiency (McCloskey et al. 2018). Importantly, the application of 400 °C and 600 °C MNBC significantly increased the relative abundance of *phoB* and *phoR* (Figs. S20 and S21), indicating the presence of abundant available P in the soil, which might aid microbial growth, promote root development in plants, and ultimately enhance crop growth and yield. The addition of MNBCs increased the topological parameters of graph density and node degree compared to the control and Cd-alone treatment, significantly enhancing the correlation among a number of important genes (Fig. 6f–I; Table S5). Additionally, MNBC enhanced the role of several key Pi solubilization-related genes (such as *phoB* and *phoR*) in the overall gene network (increasing node degrees), further demonstrating that MNBC could improve Pi solubilization capacity. Overall, MNBC significantly enhanced the microbiome's ability to solubilize, mineralize, and immobilize P, effectively regulating the P cycle. Interestingly, both MNBCs increased the abundance of genes related to assimilatory sulfate reduction and the transformation of organic and inorganic sulfur, such as *cysCKSLJ*. Moreover, MNBC primarily increased the abundance of specific genes related to carbon decomposition, aerobic respiration, and carbon fixation (*e.g.*, *bglX*, *coxABC*, *rbcL*), while inhibiting methanogenesis-related genes. Thus, MNBC mainly enriched taxa with carbon decomposition functions such as Actinomycetota (enhancing the functional potential of genes like *bglX* and *coxABC*), and increased the abundance of *rbcL* gene through taxa such as Cyanobacteriota during the anaerobic phase to strengthen carbon fixation, forming a synergistic "decomposition-fixation" pathway (Fig. 5c; Fig. S24). A more detailed assessment of the effects of MNBC on the P, S, cand C cycling pathways in Cd-contaminated soil is presented in Text S7–S8. Overall, MNBCs not only increased soil available S, aiding plants in synthesizing essential amino acids and proteins to promote plant growth, but also increased soil available C, by regulating C metabolism pathways, inhibiting methanogenesis, and enhancing microbial carbon fixation.

4 Environmental implications

The current study shows that the application of MNBC significantly increased rice yield, decreased Cd accumulation in rice edible tissues, improved soil health and promoted the soil phosphorus cycle. Given the global impact of Cd contaminated soils and the strong ability of rice to absorb Cd, these findings are highly significant (Muthayya et al. 2014; Normile 2008). The choice of appropriate amendments is a critical component of sustainable and effective soil remediation strategies. Due to the wide range of sources of pork bones in the waste stream, their conversion to biochar is a beneficial approach for green waste reuse. Importantly, compared to biochar derived from other raw materials, bone char contains higher quantities of key nutrients such as calcium and phosphorus, which can better improve soil fertility and provide nutrients for plant growth (Siebers and Leinweber 2013; Liang et al. 2023). From the perspective of energy conservation and cost control, reducing particle size to manage the use rate is also an effective strategy for utilization (Chen et al. 2014). A cost-benefit analysis showed that the application of MNBC (720 kg/ha) incurred a cost of 3600 CNY/ha. However, under soil Cd pollution, it increased rice yield by 18% and reduced Cd content in polished rice by 68%, resulting in a net benefit of 60,215.47 CNY/ha. Overall, the findings of this study enhance the understanding of the underlying mechanisms by which MNBC promotes the growth and development of rice under cadmium stress, and highlight the significant potential of this strategy for promoting food safety and security in sustainable agriculture. At a later stage, MNBC can be applied to actual farmlands. By combining multi-omics technologies, the connection between the response of microbial communities during the transformation of heavy metal forms and MNBC will be further investigated to develop plant-friendly and efficient application strategies.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1007/s42773-025-00548-x.

Additional file 1.

Additional file 2.

Author contribution

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Funding

This project was supported by the Basic Science Center Project of the National Natural Science Foundation of China (52388101), National Natural Science Foundation of China (52261135625, 42307286, 42107022), the Program for Guangdong Introducing Innovative and Entrepreneurial Teams (2019ZT08L213), the MAECI-NSFC Italy-China Joint Research Program 2023-2025, Project FUNCHARS (PGR02026), and the USDA Hatch Program (MAS00616).

Data availability

Data are available upon reasonable request.

Declarations

Competing interests

Baoshan Xing is an editor of the journal Biochar, and he was not involved in the peer-review or handling of the manuscript. The authors declare no competing financial interest.

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Received: 22 April 2025 Revised: 14 October 2025 Accepted: 6 November 2025
Published online: 09 February 2026

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