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Biochar-bacteria partnership based on microbially induced calcite precipitation improves Cd immobilization and soil function

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Abstract

Microbially induced calcite precipitation (MICP) technique utilizes ureolytic bacteria to decompose urea and generate carbonate ions for metal combination. MICP can remediate heavy metal (e.g., Cd) contaminated soils while maintaining or even improving soil functions, but its efficiency in agricultural soil practical application still needs to be enhanced. Here, we constructed a biochar-bacteria (2B) partnership in which biochar provides high nutrition and diverse sorption sites. Using the 2B system, Cd immobilization effectiveness and the underlying mechanism were examined along with the soil properties and soil functions. Results showed that compared to the single biochar and ureolytic bacteria systems, soil Cd mobility was reduced by 23.6% and 45.8% through co-precipitating with $CaCO_3$ as otavite ($CdCO_3$) in the 2B system, whereas soil fertility, bacterial diversity, and richness increased by 11.7–90.2%, 5.4–16.1%, and 6.8–54.7%, respectively. Moreover, the abundances of *Proteobacteria* and *Firmicutes* were enhanced in the 2B system. Notably, *Sporosarcina* and *Bacillus* (*Firmicutes* genus) that carry the *ure*C gene were boosted in the system, further implicating the microbiological mechanism in reducing Cd migration and its bioavailability in soil. Overall, the constructed 2B system was efficient in soil Cd immobilization by strengthening the ureolytic bacteria growth and their nutrient supply in the bacteria-rich soil ecosystem.

Highlights

- A biochar-bacteria (2B) system was constructed based on MICP.
- Cd was immobilized (23.6–45.8%) in the 2B system.
- 2B system facilitated the formation of insoluble otavite ($CdCO_3$).
- Bacteria carrying the ureC gene were boosted to lower the Cd mobility.

Keywords Microbially induced calcite precipitation (MICP), Biochar-bacteria system, Cd immobilization, Carbonate, Nitrogen cycle

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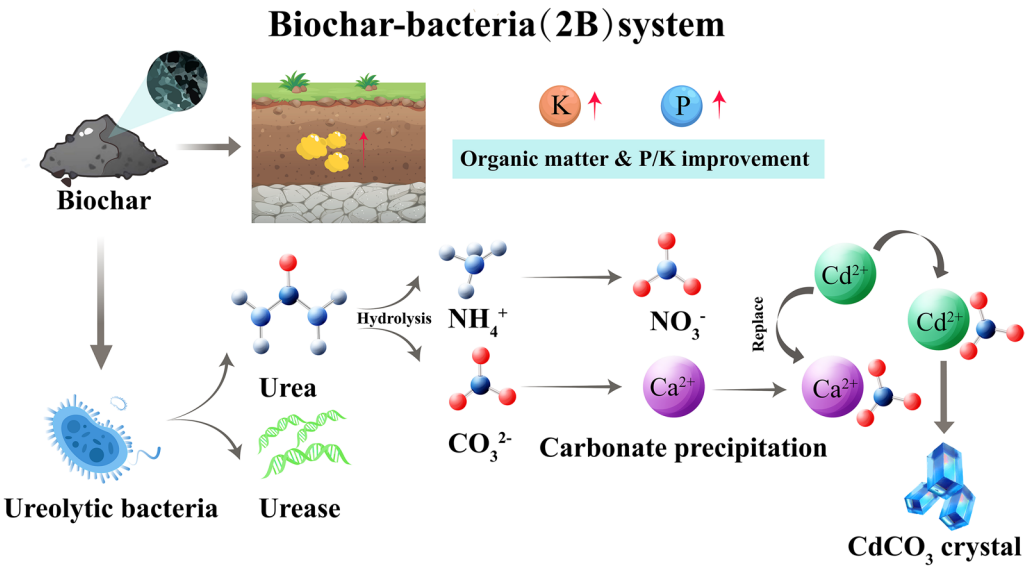
Graphical Abstract



1 Introduction

Agricultural soil contamination by cadmium (Cd) causes considerable health problems for humans via food chain exposure (Zhao et al. 2022a). Due to the no-biodegradability, bioaccumulation, and high toxicity, Cd can cause damage to the human brain, bones, and liver (Ali et al. 2020; Fang et al. 2021; Zhao et al. 2022a). High levels of Cd in soils mostly result from anthropogenic activities such as plating, refineries, and mine discharges (Li et al. 2012). To date, numerous physical and chemical methods have been used to remove Cd from the soil, including excavation and landfill, soil washing, soil flushing, chemical precipitation, and chemical oxidation (Jiang et al. 2019; Ali et al. 2020; Liu et al. 2021b). However, most technologies are disruptive to the surrounding environment and more expensive, making them impractical (Li et al. 2012; Guo et al. 2021). Because of their autotrophy for removing Cd from the soil, bio-immobilization of Cd by microbes is more cost-effective and environmentally benign than these methods (Govarthanan et al. 2019; Cuaxinque-Flores et al. 2020; Álvarez-Ayuso et al. 2021; Qiao et al. 2021; Usmani et al. 2021). Furthermore, it is viewed as a feasible approach for agricultural soil remediation because it does not necessitate the suspension of agricultural activities during the restoration process (Ma et al. 2020). As a result, there has recently been a lot of buzz about Cd immobilization in soils using microbes.

Microbially induced calcite precipitation technology (MICP) has been widely used in the bio-immobilization of heavy metals and is considered an efficient and cost-effective remediation approach (Achal et al. 2012a; Jiang et al. 2019; Naveed et al. 2020; de Oliveira et al. 2021; Graddy et al. 2021; Feng et al. 2022). In the process of MICP, ureolytic bacteria produce urease that hydrolyzes urea into ammonium and carbonate, leading to calcium carbonate ($CaCO_3$) precipitation. Cd^{2+} which has an ion radius close to Ca^{2+} might be incorporated into the calcite crystal by replacement of Ca^{2+}, thus making the immobilized Cd hard to release (Zhao et al. 2015) and slowing Cd transport into the environment (Achal et al. 2012a; Han et al. 2020b; Guo et al. 2021). In addition, the ureolytic bacteria hydrolyze urea into ammonium and carbonate associated with pH elevation, leading to stabilization of heavy metals (e.g., Cd) (Fang et al. 2021; Hu et al. 2021; Qiao et al. 2021). Co-precipitation of heavy metal with carbonate minerals in the MICP process is the dominant mechanism for heavy metals immobilization (Jiang et al. 2019), and the hydrolysis of the substrate via enzymes secreted by ureolytic bacteria is the core reaction of precipitation (Han et al. 2020b; Qiao et al. 2021). Ureolytic bacteria indigenously exist in the natural environment (Jiang et al. 2019; Chung et al. 2020; Han et al. 2020b), and the hydrolysis of urea by bacteria was 1014 times faster in the MICP process than the natural chemical decomposition of urea (de Oliveira et al. 2021). Besides, in the MICP process, ureolytic bacteria do not need external energy for self-growth (Jiang et al. 2019; de

Oliveira et al. 2021; Guo et al. 2021). Thus, MICP has been proven to be a potential green, effective and environmental method for soil remediation with ecological benefits (Guo et al. 2021).

MICP process plays a central role in the N cycle because the reaction of MICP produces an amount of NH_4^+ and NH_3 (Han et al. 2020b; Zhao et al. 2022b), which are beneficial for maintaining soil fertility. Therefore, MICP is a promising pathway for agricultural soil remediation (Qiao et al. 2021). Nowadays, MICP has been broadly employed in engineering, such as sand consolidation, soil stabilization, heavy metal passivation, crack healing in cement-based materials, CO_2 sequestration, erosion mitigation, and dust suppression (Achal et al. 2012b; Phillips et al. 2016; Cuaxinque-Flores et al. 2020; Fang et al. 2021). However, the practical application of MICP for agricultural soil remediation is very limited because it may make soil coagulation, enhance mineral crystallization, increase soil strength, and reduce porosity and permeability (Jiang et al. 2019; Chung et al. 2020; Cuaxinque-Flores et al. 2020; Proudfoot et al. 2022), which may affect the soil properties, and tend to be disruptive to soil functions, thus not conducive to soil cultivation. Besides, contaminated soil usually suppresses microorganism growth because of the nutritional deficiency and high toxicity of pollutants (Ma et al. 2020). Thus, we need to develop a feasible strategy to overcome these defects so that the MICP can be highly efficient for heavy metal remediation for agricultural soils.

Biochar has high internal and external porosity which can conserve plentiful nutrients, thus making itself an efficient matrix for soil amelioration and bacteria thrive (Phillips et al. 2022; Ok et al. 2021; Woods et al. 2006; Ali et al. 2020). In addition, biochar can serve as a platform for bioremediation (Chen et al. 2020). Therefore, the combination of biochar and bacteria can be supportive for both plant growth and Cd immobilization (Li et al. 2022). Although Cd bio-immobilization using MICP has been studied extensively (Zhu et al. 2016; Do et al. 2020; Fang et al. 2021), no research has been conducted on the combined effect of biochar and MICP on soil fertility, soil functions, and Cd immobilization. Therefore, we combined bacteria and biochar as the prevalent soil ameliorant to develop a simple strategy to overcome the disadvantages of MICP, and modify the MICP-based Cd bio-immobilization pathway.

Based on the theory of MICP, we constructed a biochar-bacteria (2B) system, and verified its high efficiency in Cd immobilization in soils, together with the effects on soil properties and functions. In particular, Cd-contaminated paddy soil was used in this study. Ureolytic bacteria (i.e., *Sporosarcina pasteurii*), and 0 or 1% biochar (w/w) were applied to the soil, and the mixtures were incubated for 20 days. Finally, we also used the 16S rRNA method to evaluate the short-term effects on soil microbial community structure to make sure that there are no adverse effects on critical soil functions.

2 Materials and methods

2.1 Ureolytic bacteria cultivation

A ureolytic bacterium *Sporosarcina pasteurii* (S. *pasteurii*) was used for Cd bio-immobilization because it can produce a large amount of urease (de Oliveira et al. 2021; Fang et al. 2021). S. *pasteurii* was purchased from China General Microbiological Culture Collection Center. The ureolytic bacterium was cultivated in standard 250 mL Erlenmeyer culture flasks containing 125 mL of Tris-YE growth medium (20 g L^{-1} yeast extract, 10 g L^{-1} $(NH_4)_2SO_4$, 15 g L^{-1} Tris, and 1 g 24 mL^{-1} urea) (Liu et al. 2021a). The bacterial cultures were aerobically incubated at 30 °C for 48 h with shaking (130 rpm). The optical density was then measured using UV–visible spectroscopy (V-730, Japan) to assess bacterial growth. The intensity of the bacterium was 1.52×10^8 cell mL^{-1} for further use.

2.2 Soil and biochar preparation and characterization

The Cd-contaminated soil used for the incubation experiment was collected from the top layer (0–20 cm) of a paddy field located in Mianzhu, China (31°25′ N, 104°15′ E). Soil samples were air-dried, milled, and sieved to < 2 mm for incubation and characterization.

Biochar was derived using rice straw by pyrolysis. The raw material was oven-dried at 60 °C until a constant weight was obtained, ground to pass through a 0.15-mm sieve, and then pyrolyzed at 300 °C in a muffle furnace for 2 h with limited air. To keep bacteria thriving, the temperature of pyrolysis was 300 °C when the polysized biochar has a large number of functional groups. The biochar was then cooled and ground to pass through a

Table 1 The properties of soil and biochar

Properties	Soil	Biochar
pH	6.73 ± 0.78	10.60 ± 0.92
Organic matter (g kg^{-1})	20.63 ± 0.05	556.5 ± 24.3
Cation exchange capacity (cmol(+) kg^{-1})	6.40 ± 0.16	13.15 ± 2.09
Total nitrogen (g kg^{-1})	1.18 ± 0.09	4.15 ± 0.15
Total phosphorus (g kg^{-1})	0.23 ± 0.00	1.16 ± 0.08
Total potassium (g kg^{-1})	1.74 ± 0.01	7.15 ± 0.25
Total cadmium (mg kg^{-1})	2.74 ± 0.72	-
BET surface area (m^2 g^{-1})	-	1.69 ± 0.01

"-": not determined. Data are mean ± SD (n = 3)

100-mesh size before use. The properties of soil and biochar are shown in Table 1.

2.3 Experiment setup

To prevent MICP from negatively affecting the soil properties and disrupting the soil environment, *Sporosarcina pasteurii* (S. *pasteurii*) strain was used for the bioremediation of Cd in soil, and biochar was used to maintain soil fertility and function. The S. *pasteurii* and biochar system was referred to as the biochar-bacteria (2B) system. We directly applied extra strain and biochar to the polluted soil for creating a simple and workable pathway. The steps of the experiments are listed below.

The incubation experiment was conducted in beakers and each beaker contained 100 g of air-dried soil. After that, 0.694 mg urea and 1.25 mg $CaCl_2$ were added to all soils to activate the MICP process (Li et al. 2014). One gram of biochar (1%, w/w) and 10 mL of bacterium solution were added to the soil and thoroughly mixed (BSW), but no bacteria were introduced to the controlled soil (BCK). Additionally, soil with 1% biochar (SW) and soil with no amendment (CK) were included. Each treatment was conducted in triplicate. The experiment was operated in an incubator under 25 °C with 12/12 h day/night light. During the incubation, soil samples were watered every day to keep a 60% water-holding capacity. After 20 days of incubation, soil samples were collected and then air-dried or kept at −4 °C or −80 °C before analyses. Air-dried soil was used for chemical analysis; fresh soils kept at −4 °C were used for the determination of ammonium (NH_4^+-N), nitrate (NO_3^--N), and enzyme activities; and fresh soils kept at −80 °C were used for DNA extraction.

2.4 Soil property analyses

0.01 M $CaCl_2$ was used to extract soil-soluble Cd (Lu et al. 2017). The speciation of the Cd fractions was determined using a modified Community Bureau of Reference (BCR) sequential extraction method, which defines Cd in four chemical forms, i.e., acid exchangeable fraction, reducible fraction, oxidizable fraction, and residual fraction (Rauret et al. 1999; Wan et al. 2017). HAC, $NH_2OH{\cdot}HCl$, H_2O_2, and HCl: HNO_3 (V/V, 7.0:2.3) were used as chemical reagents for each batch of extractions. Cd concentration in the extracts of each step was determined with inductively coupled plasma mass spectrometry (ICP-MS, 7700, PerkinElmer, USA). To determine the crystalline phase of precipitated samples, fresh soil samples were freeze-dried, and then characterized by X-ray diffractometer (Bruker, Germany).

Soil pH was measured in a 1:25 soil: water (w/v) ratio using a pH meter. Organic matter (OM) was quantified by the $K_2Cr_2O_7$ wet oxidation method (Walkley et al. 1934). Total nitrogen (TN) was measured using Kjeldahl method (Kammann et al. 2015). Total phosphorus (TP) was estimated by colorimetry procedure after H_2SO_4-$HClO_4$ digestion (Murphy et al. 1962). Total potassium (TK) was measured using a Flame photometer (JK-FP6510, China) after H_2SO_4-$HClO_4$ digestion. NH_4^+-N and NO_3^-- N concentrations were extracted using 2 M KCl at 1:25 soil: water (w/v) ratio by shaking at 150 r min^{-1} at 25 °C for 45 min, and then were measured through a continuous flow analyzer (SAN + +, Holland) (Bello et al. 2021). Available phosphorus (AP) was estimated by colorimetry procedure after 0.5 M $NaHCO_3$ extraction (Murphy et al. 1962). Available potassium (AK) was extracted by 1 M NH_4OAC (pH = 7.0) and then measured using a flame photometer (JK-FP6510, China).

The activities of soil enzymes (i.e., urease, dehydrogenase, and phosphatase) were evaluated to reflect soil qualities and functions. Urease activity was measured by indophenol blue method (Kandeler et al. 1988), and the activity of urease was expressed as mg g^{-1} 24 h^{-1}. Dehydrogenase activity was measured spectrophotometrically at 492 nm by triphenylformazan (TPF) method, and the result was expressed as μg TPF g^{-1} h^{-1} (Benefield et al. 1977). Phosphatase activity was measured spectrophotometrically at 400 nm (van Aarle et al. 2010), and the result was expressed as mg g^{-1} 24 h^{-1}.

2.5 16S rRNA

Soil total DNA was extracted from fresh soil using a DNA extraction Kit (E.Z.N.A.® soil DNA Kit, Omega Bio-Tek, Norcross, GA, U.S.). Soil DNA was then amplified. 16S rRNA gene was amplified with primers 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) by thermocycler PCR system (GeneAmp 9700, USA). Amplification was performed in triplicate using 20 μL mixtures, which contained 4 μL PCR buffer, 2 μL of 2.5 mM dNTPs, 0.8 μL of each primer (5 μM), 0.4 μL of FastPfu Polymerase, and 10 ng of template DNA. The PCR amplification conditions were: 3 min of denaturation at 95 °C, followed by 27 cycles of 30 s at 95 °C and 30 s for annealing at 55 °C, 45 s for elongation at 72 °C, and a final extension at 72 °C for 10 min. The PCR products were conditioned for sequencing through Illumina's high-throughput sequencing platform. Alpha diversity and richness indices were calculated to estimate the microbial diversity within an individual sample.

2.6 QA/QC and data analysis

For quality control, sample blanks and standard reference materials (GBW07428) were included when determining Cd concentration in the soil samples. The recovery rate from standard materials for Cd was 80–120%. Statistical

analysis was performed using IBM SPSS Statistics 22.0 software. Data were analyzed by two-way analysis of variance ANOVA and the means were compared by Duncan method at the 0.05 level. Principal component analysis (PCA) and redundancy analysis (RDA) of the correlations between the environmental factors and the microbial groups were performed using Canoco 4.5 software.

3 Results

3.1 Effect of different treatments on Cd mobility

Compared with single biochar and ureolytic bacteria systems, using the 2B system reduced $CaCl_2$ extractable Cd in soil by 23.6% and 45.8% (Fig. 1A). The notable decrease in the reducible Cd ($F=19.393$, $p=0.002$) and increase in the residual Cd ($F=6.049$, $p=0.039$) were examined after adding bacteria and biochar (Fig. 1B), respectively. When compared to the single ureolytic bacteria system, the 2B system reduced reducible Cd by 14.9% while increasing residual Cd by 16.7% ($p<0.05$).

As illustrated in Fig. 2, the calcite peaks (31.1°) were observed in all soils. Other crystalline polymorphs of calcium carbonate such as vaterite and aragonite were also present in all soil samples. The peaks at 23.4°, 36.4°, 43.8°, 61.2° and 72.9° in samples confirmed that Cd existed in soil primarily as $CdCO_3$. Calcite (22.3°) and $CdCO_3$ (43.8° and 61.2°) were confirmed to be present in all soils, with more calcite and $CdCO_3$ showing in BSW than in other treatments.

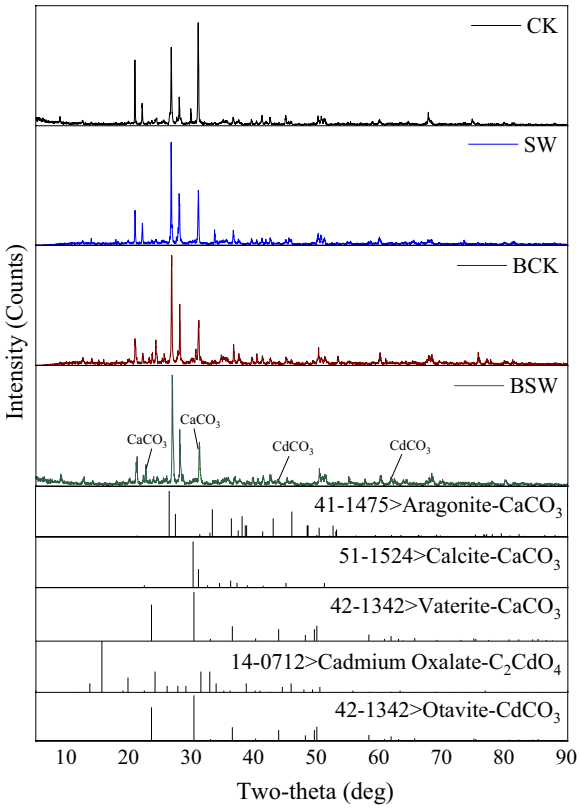


Fig. 2 XRD patterns of the soil samples. CK: control; SW: soil treated with 1%-biochar; BCK: soil treated with S. *pasteurii*; BSW: soil treated with S. *pasteurii* and 1%-biochar

3.2 Effect of different treatments on soil properties and enzymatic activities

Bacteria addition increased soil pH ($F=8.849$, $p=0.020$). Both biochar and bacteria increased soil OM ($F_{biochar}=8.344$, $p=0.020$; $F_{bacteria}=6.644$, $p=0.022$), TP ($F_{biochar}=13.889$, $p=0.010$; $F_{bacteria}=5.763$, $p=0.049$), and NH_4^+-N ($F_{biochar}=11.213$, $p=0.010$; $F_{bacteria}=874.687$, $p=0.000$). Compared with single biochar and ureolytic bacteria systems, the 2B system enhanced TK ($F=9.500$, $p=0.015$).

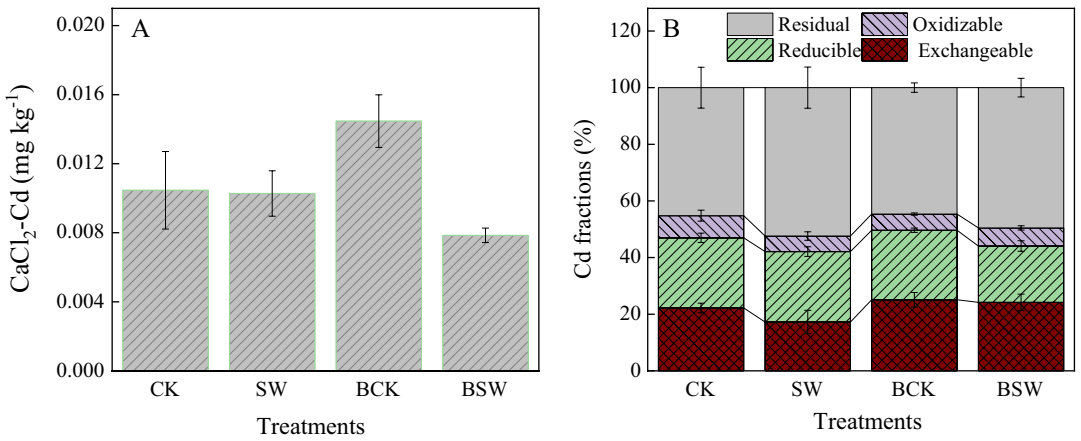


Fig. 1 $CaCl_2$-Cd concentration (**A**) and the fraction of Cd (**B**) in soils between the treatments. CK: control; SW: soil treated with 1%-biochar; BCK: soil treated with S. *pasteurii*; BSW: soil treated with S. *pasteurii* and 1%-biochar. Error bars represent the standard deviation of the means (n = 3)

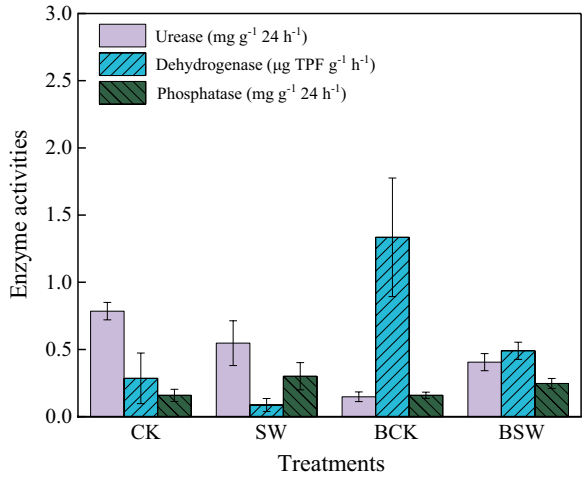


Fig. 3 Soil enzyme activities in soils under different treatments. CK: control; SW: soil treated with 1%-biochar; BCK: soil treated with S. *pasteurii*; BSW: soil treated with S. *pasteurii* and 1%-biochar. Error bars represent the standard deviation of the means (n = 3)

Notably, SW, BCK, and BSW treatments caused a dramatic increase of NH_4^+-N (82.5%, 98.0%, and 98.3%) as compared with CK. Biochar addition increased NO_3^--N content (F = 33.942, $p = 0.000$), while bacteria addition significantly reduced NO_3^--N (F = 56.914, $p = 0.000$). Biochar addition increased AK (F = 94.994, $p = 0.000$), while it did not affect AP. However, CEC and TN did not significantly differ between the treatments.

Bacteria addition reduced urease by 81.1% and 25.9% in non-biochar and biochar soils (F = 48.551, $p = 0.000$), respectively (Fig. 3). Biochar addition inhibited dehydrogenase activity (F = 13.728, $p = 0.006$), while bacteria addition increased dehydrogenase activity (F = 26.656, $p = 0.001$). Biochar significantly increased phosphatase activity (F = 11.210, $p = 0.010$). However, bacteria addition and the 2B system did not affect phosphatase activity.

3.3 Effect of different treatments on soil bacterial diversity and community

The alpha diversity of bacteria is shown in Table 2. As compared with CK, BCK reduced Chao1, Faith_pd, Shannon, and Simpson by 48.7%, 38.2%, 23.5%, and 2.3% ($p < 0.05$), respectively. Both SW and BCK significantly decreased Shannon (15.7–23.5%) and Simpson (2.3–2.7%) as compared with CK ($p < 0.05$). As compared with single biochar and ureolytic bacteria systems, the 2B system had higher Chao1 (6.8–54.7%), Faith_pd (3.4–42.0%), and Shannon (5.4–16.1%).

The PCA plot showed that soil bacterial beta diversity was significantly influenced by biochar and bacteria addition (Fig. 4A). RDA analysis was performed for all soil factors and the top 14 phyla. Specifically, urease, TP, and NH_4^+-N were the main factors shifting bacteria community, explaining 47.5% (F = 8.0, $p < 0.05$), 42.4% (F = 6.6, $p < 0.05$) and 41.9% (F = 6.5, $p < 0.05$) of the total variation, respectively (Fig. 4B).

3.4 Effect of different treatments on soil bacterial community

The dominant phyla in soil under different treatments were *Proteobacteria* (28.5–63.4%), *Actinobacteria* (11.3–34.1%), *Firmicutes* (3.0–19.3%), *Chloroflexi* (2.4–11.5%), and *Gemmatimonadetes* (1.9–7.4%), which accounted for more than 80% of the total (Fig. 5A). The abundances of *Proteobacteria* and *Firmicutes* significantly increased by 27.0–93.0% (F = 50.244, $p = 0.000$) and 10.9–45.0% (F = 10.267, $p = 0.013$) in biochar treated soil as compared with biochar-free soil. The relative abundance of *Firmicutes* in bacteria-treated soils was 3.4–4.5 times higher than that in biochar-free soils (F = 149.922, $p = 0.000$). *Actinobacteria* (F = 31.873, $p = 0.000$) and *Chloroflexi* (F = 23.244, $p = 0.001$) were significantly reduced after adding bacteria. Besides, *Firmicutes* (31.0–77.8%), and *Nitrospirae* (25.7–68.5%) showed significant increases in the 2B system as compared with single biochar and ureolytic bacteria systems.

These genera dominated the community with *Bacillus* composing 2.0–7.5% of the total community, followed by *Sporosarcina* at 0.2–6.4%, *Lysobacter* at 2.0–5.9%, *Chelativorans* at 0–10.7%, and *Arthrobacter* at 0.3–6.2%, respectively. The relative abundances of *Bacillus* (37.7–62.6%), *Sporosarcina* (22.6–97.8%), *Arthrobacter* (23.0–89.5%), and *Nitrospira* (24.4–64.5%) were much more in 2B system than in the single biochar and ureolytic bacteria systems.

4 Discussion

MICP is known as an effective remediation strategy for remediating heavy metal contaminated soil (Zhu et al. 2016; Han et al. 2020; Zhao et al. 2022b). However, the practical application of MICP for agricultural soil remediation is very limited because it reduces soil ecosystem function. To address the issue that the practical application of MICP may affect the soil properties and tend to be disruptive to the soil environment, we constructed a biochar-bacteria (2B) system based on the theory of MICP and verified the efficiency of the 2B system in Cd immobilization in soils, together with the effects on soil properties and functions.

4.1 The efficiency of the 2B system in Cd immobilization

When compared with single biochar and ureolytic bacteria systems, the 2B system significantly reduced $CaCl_2$

Table 2 Changes in the soil properties under different treatments

Treatments	pH	Cation exchange capacity (cmol(+) kg^{-1})	Organic matter (g kg^{-1})	Total nitrogen (g kg^{-1})	Total phosphorus (g kg^{-1})	Total potassium (g kg^{-1})	NH_4^+-N (mg kg^{-1})	NO_3^--N (mg kg^{-1})	Available phosphorus (mg kg^{-1})	Available potassium (mg kg^{-1})
CK	6.64 ± 0.03b	7.69 ± 0.39a	25.3 ± 2.2b	1.31 ± 0.05a	0.23 ± 0.01b	1.89 ± 0.03a	1.29 ± 1.42c	497 ± 31b	15.1 ± 1.9b	19.1 ± 7.5c
SW	7.00 ± 0.19ab	6.35 ± 1.18a	27.2 ± 0.9ab	1.43 ± 0.04a	0.27 ± 0.22ab	0.59 ± 1.03b	7.76 ± 4.38c	630 ± 29a	16.5 ± 1.3b	65.9 ± 4.1a
BCK	7.84 ± 0.53a	6.14 ± 0.33a	26.9 ± 2.1ab	1.45 ± 0.21a	0.25 ± 0.03ab	1.29 ± 0.05ab	63.7 ± 3.48b	172 ± 42c	19.3 ± 1.2a	27.7 ± 3.0bc
BSW	7.65 ± 0.95ab	6.65 ± 0.75a	30.4 ± 0.0a	1.36 ± 0.09a	0.29 ± 0.01a	1.84 ± 0.13a	75.3 ± 4.06a	438 ± 102b	17.1 ± 0.7ab	33.4 ± 2.5b
Analysis of variance										
Biochar (B)	0.077	0.930	8.344*	0.038	13.889*	1.525	11.213*	33.942***	0.352	94.994***
Bacteria (B)	8.849*	2.106	6.644*	0.227	5.763*	1.191	874.687***	56.914***	9.861*	19.634**
B × B (2B)	0.743	4.649	0.783	2.506	0.003	9.500*	3.252	3.779	5.478	58.054***

CK: control; SW: soil treated with 1%-biochar; BCK: soil treated with S. *pasteurii*; BSW: soil treated with S. *pasteurii* and 1%-biochar. Data are mean ± SD (n = 3). Different letters indicate significant differences at $p < 0.05$ level. *, ** and *** indicate significant at $p < 0.05$, 0.01, and 0.001, respectively

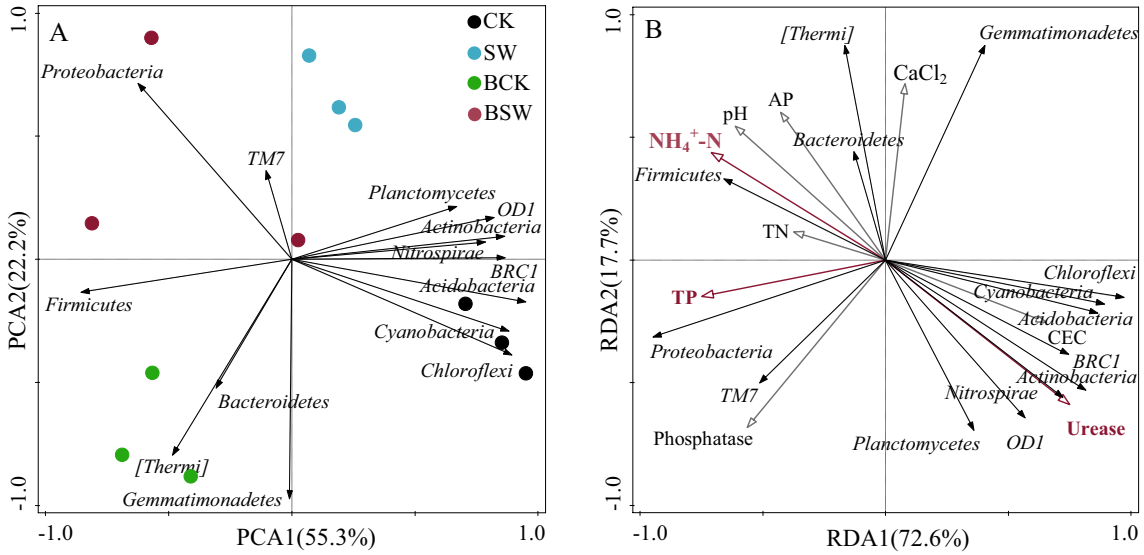


Fig. 4 The PCA plots of the beta diversity of soil bacteria (**A**) and redundancy analysis (RDA) of the correlations between bacterial communities and environmental parameters (**B**). CK: control; SW: soil treated with 1%-biochar; BCK: soil treated with S. *pasteurii*; BSW: soil treated with S. *pasteurii* and 1%-biochar

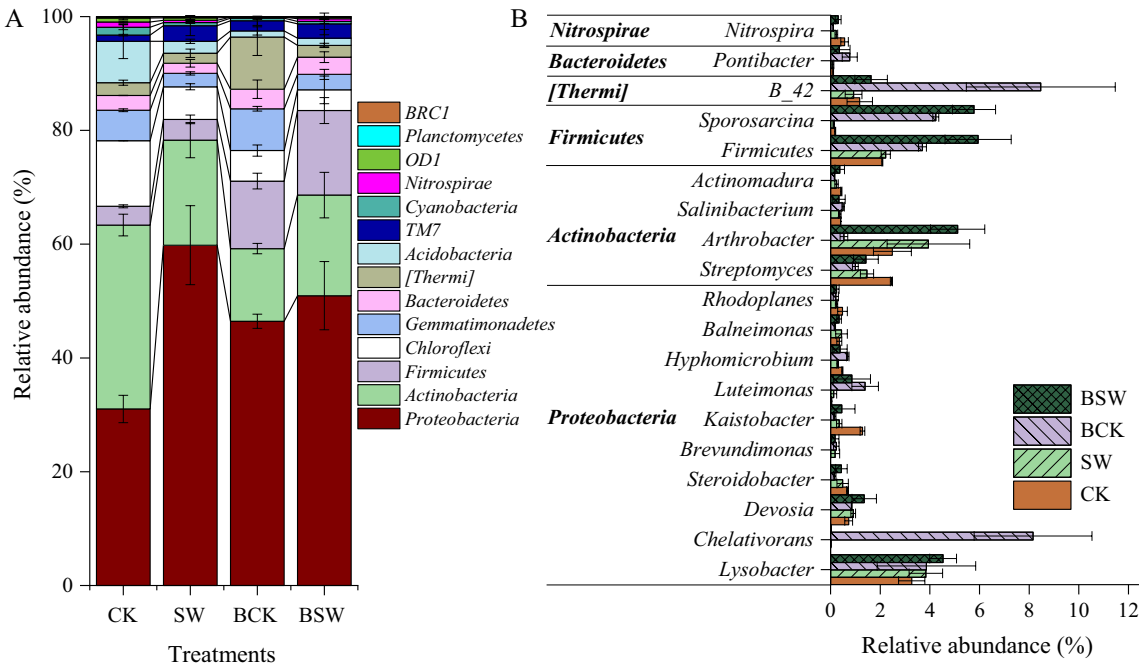


Fig. 5 Relative abundance of bacterial community composition at the phylum (**A**) and genus (**B**) levels. CK: control; SW: soil treated with 1%-biochar; BCK: soil treated with S. *pasteurii*; BSW: soil treated with S. *pasteurii* and 1%-biochar. Error bars represent the standard deviation of the means (n = 3)

extractable Cd in soil by 23.6% and 45.8%, implying that the 2B system reduced soluble Cd. Moreover, using the 2B system can reduce reducible Cd while increasing residual Cd, thus converting soil Cd from reducible to residual fraction (Ma et al. 2020). The data indicated that Cd was reduced to a state with lower mobility, and stable mineralization occurs with soluble Cd becoming inherently stable phases, which can be explained by the biochar promoting MICP process and leading to Cd mineralization. The 2B system provided more nutrients for

carbonate precipitation, leading to higher efficiency for Cd immobilization (Li et al. 2022). Besides, Cd can inhibit bacteria growth even at low concentrations (Chen et al. 2020), while biochar addition protected bacteria against Cd (de Oliveira et al. 2021), thus promoting MICP. Our study was consistent with the findings of Li et al. (2022), who reported that biochar addition effectively immobilized Cd in soil when using strain TZ5 as immobilizing bacteria. Therefore, the 2B system was able to immobilize a larger amount of soluble Cd into insoluble Cd through carbonate precipitation.

Detailed XRD patterns of the soil samples were sufficient enough to confirm the carbonate precipitation and mineral formation (Fig. 2). The calcite peaks (31.1°) were observed in all soils, indicating that MICP occurred. The peaks at 23.4°, 36.4°, 43.8°, 61.2° and 72.9° in samples suggested that the MICP process immobilized Cd mainly in the form of $CdCO_3$ and S. *pasteurii* was able to sequester Cd along with calcium carbonate biomineralization (Zhao et al. 2022b). The formation of $CdCO_3$ was caused by the isomorphic substitution of Ca by Cd (Zhu et al. 2016; Chen et al. 2019). Cd incorporation into biologically synthesized calcite was also confirmed in other studies (Zeng et al. 2021; Lyu et al. 2022). Calcite (22.3°) and $CdCO_3$ (43.8° and 61.2°) were presented in all soils, with more calcite and $CdCO_3$ showing in BSW than in other treatments. This suggested that the 2B system was successful in promoting bioremediation, accelerating Cd precipitation, and lowering soluble Cd in soil (Peng et al. 2020).

4.2 Performance of the 2B system in regulating soil properties

Soil properties are imperative for evaluating agricultural soil remediation performance. Meanwhile, the bioremediation process is strongly affected by soil properties (Usmani et al. 2021). Thus, soil properties after bioremediation should be understood (Table 3). Soil OM (11.7–12.9%), TP (13.7–15.2%), TK (30.1–67.7%), and NH_4^+-N (18.2–90.2%) were significantly improved in the 2B system, as compared with single biochar and ureolytic bacteria systems, which confirmed that the 2B system significantly improved the soil fertility of Cd-contaminated soil. Soil properties such as OM, TP, and TK can be enhanced by biochar addition, which has been extensively studied in recent years (Agegnehu et al. 2016b; Yan et al. 2022; Bello et al. 2021). Because biochar is a carbon-rich material, it is not surprising thats the 2B system had higher OM, which was consistent with previous reports (Agegnehu et al. 2016a; Plaza et al. 2016). 2B system also significantly increased TP but did not affect AP, which may account for the lower availability of P within biochar. Previous studies have demonstrated that after pyrolysis at > 200℃, soluble P in biochar was reduced due to the formation of Ca-Mg-P crystallization (Cao et al. 2010; Bekiaris et al. 2016). Unlike P, the availability of K was not decreased after pyrolysis as K is the most mobile element, therefore, the 2B system increased TK.

Further, bacteria addition increased soil pH and NH_4^+-N, while significantly reducing NO_3^--N, which supported the assumption that the MICP process was enhanced by bacteria addition. Ureolytic bacteria hydrolyze urea into ammonium associated with pH elevation, which has been previously described (Fang et al. 2021; Hu et al. 2021; Qiao et al. 2021). Similarly, NH_4^+-N increased in soils with biochar addition as compared with biochar-free soil, indicating that biochar also promoted the MICP process. Biochar addition increased NO_3^--N probably due to its high absorption capacity. Notably, SW, BCK, and BSW treatments caused a dramatic increase of NH_4^+-N as compared with CK, indicating that all treated soils can stimulate the MICP process and produce NH_4^+-N, with the 2B system being more successful at doing so. Besides, the higher levels of NO_3^--N (60.7%) in the BSW treatment compared to the BCK treatment suggested that the 2B system enhanced NH_4^+-N content and generated a significant amount of NO_3^--N, which encouraged nitrification. Overall, the 2B system was shown to be more suited than single biochar and ureolytic bacteria systems for bioremediation.

Table 3 Bacterial diversity and richness indexes in soils under different treatments

Treatments	Chao1	Faith_pd	Shannon	Simpson
CK	1462 ± 100a	87.9 ± 3.0a	9.46 ± 0.11a	0.997 ± 0.000a
SW	1086 ± 103ab	74.6 ± 8.9ab	7.97 ± 0.53b	0.970 ± 0.017b
BCK	750 ± 60b	54.3 ± 5.7b	7.24 ± 0.24b	0.974 ± 0.005b
BSW	1161 ± 247ab	77.2 ± 11.3ab	8.40 ± 0.66ab	0.987 ± 0.007ab
Analysis of variance				
Biochar (B)	0.034	0.867	0.318	1.207
Bacteria (B)	11.852*	9.238*	9.463*	0.261
B × B (2B)	18.028*	12.454*	20.728**	10.023*

CK: control; SW: soil treated with 1%-biochar; BCK: soil treated with S. *pasteurii*; BSW: soil treated with S. *pasteurii* and 1%-biochar. Data are mean ± SD (n = 3). Different letters indicate significant differences at $p < 0.05$ level. *, ** and *** indicate significant at $p < 0.05$, 0.01, and 0.001, respectively

4.3 Performance of the 2B system in maintaining soil functions

Urease, dehydrogenase, and phosphatase are extracellular enzymes and are involved in soil biochemical reactions and nutrient cycles such as N, C, and P cycles, which reflect soil functions (Ma et al. 2020; Álvarez-Ayuso et al. 2021). Therefore, the activities of urease, dehydrogenase, and phosphatase after bioremediation were analyzed.

Urease, a key enzyme in MICP, involves the urea hydrolysis to produce ammonia that leads to Cd immobilization (Li et al. 2014; Álvarez-Ayuso et al. 2021), which is dominantly secreted by ureolytic bacteria (Li et al. 2022). In our study, bacteria addition significantly reduced urease (Fig. 3), which was consistent with previous (Ma et al. 2020; Tu et al. 2020). The decrease in urease was probably due to the consumption of urease after bioremediation. After the addition of biochar, the reduced percentage of urease gradually decreased, suggesting the adverse effects can be mitigated by adding biochar. The data demonstrated that the MICP process was improved by adding biochar to the soil, which was consistent with previous findings of Li et al. (2022), who used corncob as an additive and found that urease activity was improved by corncob addition.

A strong negative relationship between urease and $CaCl_2$-Cd ($r = -0.512$, $p < 0.05$) implied that urease was involved in the MICP process and Cd immobilization. As mentioned above, the 2B system led to a lower reducible Cd, but a higher residual Cd content. The data also supported the assumption that biochar promoted the passivation of Cd by stimulating ureolytic microbes to create urease and CO_3^{2-}, which then converted soluble Cd to stable Cd (Zhu et al. 2016; Peng et al. 2020). Using the 2B system, the concentration of urease in the soil released from bacteria was significantly increased, increasing carbonate content and converting solution soluble Cd to state Cd (Fig. 1). Besides, urease is not only related to Cd immobilization but also affects the N cycle (Peng et al. 2020). The increase of NH_4^+-N in soil could support this point.

Dehydrogenase is an important indicator for assessing soil microbial activation (Peng et al. 2020). Bacteria addition increased dehydrogenase, which was mainly due to a higher number of ureolytic bacteria, and then promoted the MICP process. MICP process led to the release of OH^- and then increased pH, increasing dehydrogenase activity (Zhu et al. 2016; Peng et al. 2020). However, biochar addition inhibited the bacteria from secreting dehydrogenase, which corresponded to the previous study (Peng et al. 2020). The decrease in dehydrogenase was likely caused by biochar absorption, which decreased the amount of substrate that was available for bacteria to release dehydrogenase (Li et al. 2022).

Phosphatase catalyzes organic phosphomonoesters' hydrolysis to form soluble P (Li et al. 2022). Biochar addition significantly increased phosphatase, suggesting that biochar addition was beneficial to the P cycle. Nonetheless, we did not observe an increase in AP in biochar-treated soil (Table 1), which might be attributed to Cd chelation. A previous study reported that soil soluble P in the soil can chelate heavy metals to immobilize form (Li et al. 2022), thus depletion of soil AP seems to be the primary reason for the limited increase of AP. The increase of residual Cd in biochar-treated soil (Fig. 1B) can support the point. In addition, as mentioned above, soluble P in biochar was limited, leading to a negligible change in AP.

4.4 Diversity of bacteria to soil factors

The alpha analysis reflects the richness and diversity of bacterial communities (Chen et al. 2019). Shannon and Simpson were significantly lower in SW and BCK treatments compared to CK, suggesting that bacterial richness and diversity were decreased after the MICP process even in biochar and bacteria-treated soils, which was consistent with previous findings (Peng et al. 2020; Lyu et al. 2022). The addition of biochar and bacteria triggered the MICP process, promoted the growth of specific taxonomic groups of bacteria such as ureolytic bacteria, and thus decreased the diversity and richness of bacteria in the soil. Further, as compared with single biochar and ureolytic bacteria systems, the 2B system had higher Chao1, Faith_pd, and Shannon, indicating higher bacterial diversity and richness. It also showed that, compared with CK, α-diversity indices (i.e., Chao1, Faith_pd, and Shannon) were reduced in BCK treatment, while they were increased in BSW treatment as compared with BCK ($p < 0.05$). The data indicated that bacterial diversity and richness were decreased after the MICP process (Peng et al. 2020; Lyu et al. 2022), while biochar addition could alleviate this negative effect, and then increased microbial community diversity and richness, which was consistent with previous results (Mierzwa-Hersztek et al. 2016; Chen et al. 2019; Jiang et al. 2019; Álvarez-Ayuso et al. 2021; Li et al. 2022). The increases in microbial diversity and richness in the 2B system might be explained in terms of the following aspects: (1) habitat effect and (2) nutrient supply (Li et al. 2022). Biochar had a large specific surface area, which was conducive to the attachment of microorganisms (Li et al. 2022). The high number of microbes secreted more urease to promote the MICP process, which meant biochar induced the increase of bacterial diversity and was beneficial for Cd immobilization (Fig. 1) (Chen et al. 2019). In addition, biochar contains sufficient nutrient elements, which might be involved in the microbially induced carbonate precipitation (Li et al. 2022), thus promoting Cd immobilization. Furthermore, soil microbial biomass directly affects soil enzymes, thus the enhancement of richness and diversity in the 2B system resulted in the improvement of activities of soil enzymes, and therefore there were no adverse effects on soil functions. Together, the 2B system improved bacterial variety and richness, enhancing soil

functions and preserving soil production. Additionally, this stimulated the MICP process and immobilized Cd.

The PCA plot showed that soil bacterial beta diversity was significantly influenced by biochar and bacteria addition (Fig. 4A), which was consistent with the changing trends in the composition and alpha diversity of soil bacterial communities. Soil physicochemical variables have been previously described to be the major factors for soil microbial dynamics and structure (Bello et al. 2021; Hu et al. 2021). The bacterial variations in soil samples were caused by the nutrients cascade and competition as well as habitat supply (Ma et al. 2020; Bello et al. 2021). RDA analysis showed that urease, TP, and NH_4^+-N were the main drivers shaping and controlling bacteria species. This result supported the findings of Hu et al. (2021), who reported that available N and urease showed a significant correlation with bacterial composition and diversity in soils.

4.5 Community structure of bacteria

The bacterial community had significant changes (Fig. 5A), indicating its sensitivity to environmental conditions. The relative abundance percentage analysis of microorganisms showed that *Proteobacteria*, *Actinobacteria*, and *Firmicutes* were the most dominant phyla in all samples, which was consistent with previous reports (Hu et al. 2021; Koner et al. 2022).

Proteobacteria can survive in harsh conditions and become dominant in heavy metal contaminated agricultural soil since they can tolerate higher heavy metal concentrations and utilize multiple C and N sources in a complex survival mode, which has been reported previously (Hu et al. 2021). As such, it was not surprising that *Proteobacteria* had the highest abundance in our study. The abundance of *Proteobacteria* significantly increased in biochar-treated soil as compared with biochar-free soil, which may be related to its habit. *Proteobacteria* prefer a eutrophic environment (Hu et al. 2021). Biochar contains plentiful nutrients (C, N, P, and K), which exerted beneficial effects on *Proteobacteria* growth.

Firmicutes can adapt to harsh conditions, and most ureolytic microorganisms belonged to *Firmicutes* (Yin et al. 2021; Koner et al. 2022). In our study, the relative abundance of *Firmicutes* in all soils was 3.2–16.5%, indicating that ureolytic microorganisms could survive in an environment with Cd contamination, which had the potential for the use of Cd biomineralization (Hu et al. 2021). Meanwhile, the relative abundance of *Firmicutes* in bacteria soils was 3.4–4.5 times higher than that in the soils without bacteria, indicating that ureolytic microorganisms were inoculated successfully. Additionally, biochar addition significantly increased *Firmicutes* abundance by 10.9–45.0%, which implied that biochar addition was an effective way to promote *Firmicutes* growth, thus promoting heavy metal biomineralization.

Actinobacteria and *Chloroflexi* were significantly reduced in bacteria-treated soil, indicating that the MICP process had adverse effects on soil microorganisms, which has been reported (Lyu et al. 2022). Besides, *Nitrospirae* showed significant increases in the 2B system as compared with single biochar and ureolytic bacteria systems, suggesting that the 2B system played important role in the N cycle.

4.6 Construction of the biochar-bacteria (2B) system and the immobilization mechanism

To further reveal the mechanism of Cd immobilization by MICP, the genera ranked in the top 19 in terms of abundance were analyzed (Fig. 5B). The result showed that *Bacillus* was the dominant genus, followed by *Sporosarcina*, *Lysobacter*, *Chelativorans*, and *Arthrobacter*. Species identified as significantly positively correlated with NH_4^+-N ($r = 0.953$, $p < 0.01$) and negatively correlated with NO_3^--N ($r = -0.509$, $p < 0.05$) belonged to phylum *Firmicutes*. Most of the ureolytic genera that can produce urease belong to this phylum in soil (Hu et al. 2021). Therefore, the ureolytic genera in *Firmicutes* such as *Bacillus* and *Sporosarcina* can carry the *ureC* gene and have a high urease-producing ability (Graddy et al. 2021; Yin et al. 2021), which can immobilize heavy metals by secreting urease that decomposes urea and produce CO_3^{2-} that binds with Cd to form precipitates, resulting in the Cd immobilization in soil (Han et al. 2020a; Hu et al. 2021). In the 2B system, the abundances of *Firmicutes* were enhanced, which again suggested that the 2B system stimulated ureolytic genera growth and accelerated the MICP process. It is worth noting that species *Bacillus* and *Sporosarcina* had significant positive correlations with NH_4^+-N with the correlation coefficients being 0.871 and 0.986 ($p < 0.01$), implying that these genera were important functional genera that could be involved in the MICP process. *Bacillus* and *Sporosarcina* were the dominant genera in all soils, indicating that ureolytic genera were inoculated successfully.

Sporosarcina pasteurii (S. *pasteurii*) belongs to the *Sporosarcina* genus. *Sporosarcina* dominated the community in soils treated with bacteria, making up 4.1–6.4% of the total community, which was 22.7–46.7 times more than it was in soils not treated with bacteria, demonstrating the efficacy of the inoculation of the bacteria. Cd mineralization by S. *pasteurii* caused steady mineralization, which lowered the bioavailability of Cd in agricultural soil and reduced Cd toxicity. It was also obvious from Fig. 5B that *Sporosarcina* and *Bacillus* had high abundances in all soils, and they were significantly increased in the 2B system when compared with single biochar and ureolytic

bacteria systems. Thus, it can be concluded that the 2B system not only enhanced *Sporosarcina* but also favored other ureolytic microorganism growth, such as *Bacillus*, which was consistent with previous studies (Chen et al. 2019; Li et al. 2022). In addition, the *Bacillus* genus is regarded as metal resistant bacteria (Ma et al. 2020), which can promote P solubilization (Pattnaik et al. 2020). Soil soluble P can chelate Cd to immobilize Cd (Ma et al. 2020), thus reducing Cd availability. Thus, the increase of *Bacillus* was also favorable for Cd immobilization.

Besides, *Arthrobacter* is also recognized as a Cd-resistant bacterium (Ma et al. 2020), which was also increased in the 2B system when compared with single biochar and ureolytic bacteria systems. Further, *Nitrospira* belongs to the *Nitrospirae* genera. Both *Nitrospira* and *Nitrospirae* are involved in nitrification because they are correlated positively with NO_3^--N with values of correlation coefficient of 0.526 ($p < 0.01$) for *Nitrospirae* and 0.517 ($p < 0.01$) for *Nitrospira*, respectively. Increases in the abundance of *Nitrospira* and *Nitrospirae* in the 2B system suggested that biochar promoted nitrification.

Overall, the 2B system increased bacterial richness and diversity and increased *Sporosarcina* and *Bacillus* abundance, thus promoting the MICP process. Thus, the constructed 2B system is an easy and feasible pathway to remediate the Cd-contaminated soil. Besides, the 2B system also mediated the N cycle by altering *Nitrospira* growth. The overall reactions involved in biomineralization in the 2B system mainly included the following processes: (1) urea hydrolysis produced NH_4^+-N and CO_3^{2-}; (2) Cd coprecipitated with CO_3^{2-} by replacing Ca; and (3) NO_3^- was produced. Using the proposed system, Cd can be transformed into carbonate-bound and this process was enhanced (Ma et al. 2020). In the process, a large amount of NH_4^+-N led to NO_3^--N production and promoted nitrification.

5 Conclusion

Based on the theory of MICP, we constructed a biochar-bacteria (2B) system, and verified its high efficiency in Cd immobilization in soils, together with the effects on soil properties and functions. Soil Cd mobility was reduced through co-precipitating with $CaCO_3$ as otavite ($CdCO_3$) in the 2B system. Using the 2B system, soil fertility, urease, bacterial diversity, and richness were significantly improved. Urease, TP, and NH_4^+-N were the main drivers shaping and controlling bacteria species. Moreover, in the 2B system, the abundances of *Proteobacteria* and *Firmicutes* were enhanced. *Sporosarcina* and *Bacillus* belonging to the *Firmicutes* genera and carrying the *ure*C gene were further boosted, explaining the microbiological mechanism in reducing soil Cd bioavailability. Thus, the 2B system promoted the passivation of Cd by stimulating ureolytic microbes to create urease and CO_3^{2-}, which then converted soluble Cd to stable Cd. Besides, the 2B system enhanced NO_3^--N content by increasing *Nitrospira* growth. The overall reactions involved in biomineralization in the 2B system mainly included the following processes: (1) urea hydrolysis produced NH_4^+-N and CO_3^{2-}; (2) Cd coprecipitated with CO_3^{2-} by replacing Ca; and (3) NO_3^- was produced. Taken together, the constructed 2B system is efficient in Cd immobilization by strengthening the ureolytic bacteria growth and nutrient supply. However, more research is required to enhance the 2B system's performance in remediating Cd-contaminated agricultural soil as well as its practical use.

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Author contributions
MX: Conceptualization, formal analysis, methodology, experimental operation, visualization, writing—original draft. JM: Investigation. XZ: Writing—review and editing. GY: Data curation, writing—review and editing. LL: Writing—review and editing. CC: Analysis, writing—review and editing. CS: Formal analysis, writing—review and editing. JW: Resources, supervision. PG: Writing—review and editing. DG: Writing—review an editing. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets analyzed in the current study are available from the corresponding author upon reasonable request.

Declarations

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

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