



# Negatively charged nano-hydroxyapatite can be used as a phosphorus fertilizer to increase the efficacy of wollastonite for soil cadmium immobilization

Rong Huang<sup>a,b</sup>, Peng Mao<sup>a,c</sup>, Lei Xiong<sup>d</sup>, Guoming Qin<sup>a,c</sup>, Jinge Zhou<sup>a,c</sup>, Jingfan Zhang<sup>a,c</sup>, Zhian Li<sup>a,c</sup>, Jingtao Wu<sup>a,c,\*</sup>

<sup>a</sup> Xiaoliang Research Station for Tropical Coastal Ecosystems, Key Laboratory of Vegetation Restoration and Management of Degraded Ecosystems, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou 510650, China

<sup>b</sup> Institute of Bast Fiber Crops, Chinese Academy of Agricultural Sciences, Changsha, 410221, China

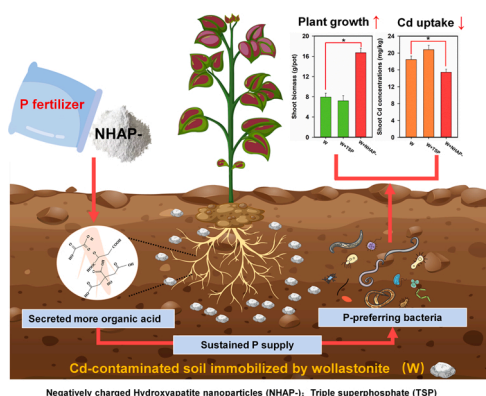
<sup>c</sup> South China National Botanical Garden, Guangzhou 510650, China

<sup>d</sup> Smart Water Affairs Research Center, Shenzhen University, Shenzhen 518000, China

## HIGHLIGHTS

- Soluble P fertilizer reduced the efficacy of wollastonite during Cd immobilization.
- Negatively charged NHAP (NHAP-) amendment provided continuous nutrients to plant.
- Co-application of NHAP- and wollastonite (W) further reduced plant Cd uptake.
- Plants secreted more organic acids to promote plant growth in WNHAP-amended soil.
- The relative abundance of the P-prefering *Bacteroidia* was increased by WNHAP-.

## GRAPHICAL ABSTRACT



## ARTICLE INFO

Editor: Jörg Rinklebe

**Keywords:**  
Cadmium immobilization  
Microbial community  
Nano-hydroxyapatite  
Organic acids  
Wollastonite

## ABSTRACT

Improper application of phosphorus (P) fertilizer during soil cadmium (Cd) immobilization reduces the efficiency of fertilizer and Cd remediation. In this study, we synthesized three types of nano-hydroxyapatite (NHAP) with different surface charges as slow-release P fertilizers during Cd immobilization. We also evaluated the effects of wollastonite application with or without NHAP addition, in comparison with triple superphosphate (TSP) or bulk hydroxyapatite, on Cd accumulation in *Amaranthus tricolor* L. The results showed that adding wollastonite significantly reduced P availability (23.5%) in the soil, but it did not inhibit plant P uptake. In wollastonite-amended soil, the application of negatively/positively charged NHAP significantly increased plant biomass by 643–865% and decreased Cd uptake by 74.8–75.1% compared to the unamended soil as well as showed greater efficiency than those with TSP. This was ascribed to the increased soil pH (from 3.94 to 6.52–6.63) and increased

\* Corresponding author at: Xiaoliang Research Station for Tropical Coastal Ecosystems, Key Laboratory of Vegetation Restoration and Management of Degraded Ecosystems, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou 510650, China.

E-mail address: [wujingtao@scbg.ac.cn](mailto:wujingtao@scbg.ac.cn) (J. Wu).

<https://doi.org/10.1016/j.jhazmat.2022.130291>

Received 17 July 2022; Received in revised form 25 October 2022; Accepted 28 October 2022

Available online 29 October 2022

0304-3894/© 2022 Elsevier B.V. All rights reserved.

abundance of organic acids (including citric acid, malic acid, lactic acid, and acetic acid) secreted by plants. In addition, the P-preferring bacterial class *Bacteroidia* was specific to soils amended with both wollastonite and NHAP-. These results suggest that NHAP- may be an appropriate P fertilizer for soil Cd immobilization using wollastonite.

## 1. Introduction

Heavy metal pollution in soil is becoming a global environmental crisis. Particularly in China, approximately 1/6 of the agricultural land (20 million hectares) is contaminated by heavy metals (including Pb, Zn, Cu, and Cd), which threatens the quality of agricultural products and human health safety (Wang et al., 2019a; Li et al., 2021a). Therefore, it is imperative to identify effective methods to minimize the risk of heavy metal contamination in soils. The principle of in situ immobilization is to add immobilization materials to soil and decrease the mobility and bioavailability of heavy metals through sorption, precipitation, and complexation reactions (Bolan et al., 2014; Wu et al., 2022). This has been widely applied at low- and medium-contamination sites owing to the advantages of low cost, easy operation, environmental friendliness, and agricultural production continuity during remediation.

Wollastonite (W), a silicate mineral that effectively immobilizes soil Pb and Cd, can inhibit the uptake of heavy metals by crops (Mao et al., 2019). It increases soil pH, forms insoluble salts, and improves crop Si supply without negatively affecting crop yield. Because heavy metal immobilization is not exclusive, wollastonite has been reported to simultaneously inhibit the supply of metallic micronutrients (Mao et al., 2019). The application of P fertilizer, an essential agronomic measure for maintaining crop yield and quality, can reduce soil Pb and Zn availability via co-precipitation (Cui et al., 2017). Proper fertilization practices can improve remediation efficiency; however improper fertilization may cause co-precipitation or competitive adsorption of certain substances with heavy metal ions (Ruangcharus et al., 2020). For example, alkaline immobilization materials containing calcium can co-precipitate with phosphate, thus reducing the efficiency of both calcium compounds and phosphate fertilizers (Tang et al., 2022). Through field experiments, Mao et al. (2019) found that phosphate addition reduced the immobilization effect of calcium silicate on soil Cd. Therefore, ensuring the efficiency of both immobilization and phosphate utilization contributes to the remediation of heavy metals in soil.

In recent years, nano-fertilizers have become an emerging field in agricultural and environmental science because their unique small size effect (Xiong et al., 2018b). Nano-hydroxyapatite (NHAP) has a small particle size, low loss rate, and slow-release properties, which can better coordinate the dissolution release of fertilizers with crop uptake (Xiong et al., 2018a; Elsayed et al., 2022). NHAP fertilizers have been widely used for the remediation of heavy metal-contaminated soils (Cui et al., 2018; Zhang et al., 2019). Compared with bulk phosphate fertilizers, NHAP has a higher specific surface area and P availability. In addition, due to the stability of nano-phosphate fertilizers, it also reduces the risk of soil acidification and P leaching (Xiong et al., 2018a). In response to the drawbacks of soluble phosphate in reducing the heavy metal immobilization effect of wollastonite, nano-fertilizers are considered promising materials. Meanwhile, by modifying the surface properties, the migration and transformation behavior of nano-fertilizers can be adjusted (Lee et al., 2013). Ideally, it is feasible to tailor fertilizers to be dual-action fertilizers that can supply nutrients and immobilize heavy metals depending on the soil characteristics and crop type. Xiong et al. (2018a) found that in an acidic Ultisol, the negatively charged NHAP was more migratory and better able to improve the P utilization efficiency of plants owing to repulsion with soil electrical properties. It would be useful to explore the feasibility of NHAP as a new P fertilizer to enhance the immobilization efficiency of wollastonite and further evaluate its impact on soil health.

Microorganisms, as essential components of the soil ecosystem, are

more sensitive to soil disturbance than plants and animals (Jia et al., 2022). The microbial sensitivity of soils to heavy metals is expected to fulfill its potential as an index for monitoring remediation efficiency. Although, wollastonite substantially reduces the availability of Pb, Zn, and Cd, the addition of alkaline calcium silicate can also reduce the available P concentration in soil and the biomasses of fungi and actinomycetes (Wu et al., 2021). Regarding studies on nano-phosphate fertilizers, the addition of nano P fertilizers to soil was found to increase the abundance of *Stenotrophomonas* sp. and *Bacteroides*, and the microbial response was correlated with the particle size of phosphate fertilizers (Cui et al., 2018). Hence, it is recommended that the efficiency of soil Cd immobilization be assessed not only in terms of the chemical effects but also in terms of the biological effects, especially for soil microorganisms (Yang et al., 2021). In addition, during P fertilization, plants change their root morphology and secrete organic acids to intercept P (Reichert et al., 2022). This process affects the physical and chemical conditions of the rhizosphere, which in turn affects the microbial composition (Richardson and Simpson, 2011). However, there is still insufficient information on how nano-phosphate fertilization affects microbial communities during heavy metal immobilization in soils. Herein, analysis of microbial composition and organic acids secreted by roots can help to reveal the mechanisms of nano-P fertilizers.

Most studies have focused on the effect of immobilization materials in reducing plant uptake of heavy metals. However, they neglect the simultaneous immobilization of nutrients, which reduces the efficacy of immobilization and fertilization. In this study, we systematically investigated the effects of NHAPs with different surface charges on Cd and P availability, plant growth, root exudates, and soil microbial community structure in Cd-contaminated soil amended with wollastonite. We hypothesized that NHAP could effectively supply plant nutrients while avoiding simultaneous immobilization with wollastonite due to its slow-release properties, and that the negatively charged modification would be optimal. In conjunction with the premise of adding wollastonite, the specific objectives of this study were (1) to assess the effects of NHAP on the ability to immobilize Cd and maintain P supply and (2) to investigate the mechanisms of using NHAP for increasing the efficacy of wollastonite by analyzing soil microbial structure and root exudate composition. This study systematically evaluated the feasibility and potential of using NHAP as a novel phosphate fertilizer that does not affect the efficacy of immobilization materials. This will provide a theoretical basis and practical guidance for the application of fertilizers during heavy metal remediation.

## 2. Materials and methods

### 2.1. Synthesis and characterization of three NHAPs

According to Xiong et al. (2018b), NHAP was synthesized by wet chemical deposition. Briefly, we prepared 100 mL of a 0.6 mol/L Ca (NO<sub>3</sub>)<sub>2</sub> solution and 100 mL of a 0.36 mol/L (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub> solution using deionized water and adjusted the pH of the two solutions to 10 using aqueous NH<sub>3</sub>-H<sub>2</sub>O. Next, the (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub> solution was added dropwise to a round bottom flask of Ca(NO<sub>3</sub>)<sub>2</sub> under magnetic stirring at 90 °C for 12 h. After another aging process for 12 h, the white colloidal dispersion of the nanoparticles was centrifuged at 12,000 g. The precipitate was then washed using deionized water three times, and we obtained the unmodified NHAP0.

Subsequently, we prepared diammonium citrate and glycine solutions to modify the NHAP to obtain negative or positive surface charged

NHAP (Li et al., 2011; Lee et al., 2013). In Brief, two 1.2 mol/L solutions were prepared by dissolving 40.5 g of diammonium citrate or 13.5 g of glycine in 100 mL of deionized water. The two modifier solutions were added to the  $\text{Ca}(\text{NO}_3)_2$  solution before adding the  $(\text{NH}_4)_2\text{HPO}_4$  solution. Following the same precipitation, aging, and centrifuging procedure, we obtained the diammonium citrate-modified NHAP as NHAP<sup>-</sup> and the glycine-modified NHAP as NHAP<sup>+</sup>.

Next, the zeta potential of the three NHAPs was examined using dynamic light scattering as described in [Supporting Information](#). We then observed the sample morphology using a scanning electron microscope (SEM) (JEOL JSM-7100 F, Japan) and a transmission electron microscopy (TEM) (JEOL, JEM-1400plus, Japan) operating at an accelerating voltage of 20 kV. The physical phase and crystallinity were characterized using an X-ray diffractometer (Rigaku, SmartLab 9 kW, Japan) with a scanning angle range of 10–80° and a scanning rate of 4°/min. Functional groups were analyzed in the infrared region using a Fourier Transform infrared spectroscopy spectrophotometer (FTIR, Nicolet iS10, Japan) at wave numbers ranging between 400 and 4000  $\text{cm}^{-1}$ .

## 2.2. Soil and plants

Metal-polluted soil was collected from the surface layer (0–20 cm) of croplands in Maba Town, Shaoguan City, Guangdong Province, China (24°38'41"N, 113°35'29"E). The contaminated soil was air-dried and crushed. After crushing, the contaminated soil was sieved through a 1-cm sieve for pot experiment and through a 2-mm sieve for soil basic physicochemical property analysis. Standard procedures determined the basic physicochemical properties of this soil; the results are presented in [Table S1](#).

*Amaranthus tricolor* L., which belongs to the family of *Amaranthaceae*, is a common edible plant in South China. However, it has been reported as a Cd accumulator (Lu et al., 2014; Wu et al., 2021). Hence, it is suitable for detecting rhizosphere microorganisms and magnifying the efficiency of Cd immobilization. The seeds of *A. tricolor* L. were acquired from the South China Botanical Garden (Guangzhou, China). The seedlings were preincubated in nursery pots.

## 2.3. Pot experiment

A pot experiment was conducted in the greenhouse from Sep 15 to Nov 5 (dry season) in the South China Botanical Garden (Guangzhou, China). The region has a humid subtropical climate. The temperature ranged from 19.3 to 27.9 °C, and the relative humidity ranged from 77% to 90% during the experiment. Approximately 3 kg of air-dried soil was placed in each plastic pot (bottom diameter: 12 cm; upper diameter: 24 cm; height: 15.5 cm). In the current study, wollastonite was selected as the immobilization amendment. We initially hypothesized that the small particle size hydroxyapatite would be more effective in supplying plant P in a sustained manner compared to bulk hydroxyapatite [ $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ , HAP] and triple superphosphate [ $\text{Ca}(\text{H}_2\text{PO}_4)_2$  (TSP)], which has been confirmed by [Montalvo et al. \(2015\)](#) and [Xiong et al. \(2018\)](#). Therefore, we used soluble  $\text{Ca}(\text{H}_2\text{PO}_4)_2$  (TSP) and the large grain size HAP as two phosphate control to test the efficacy of NHAPs. Five phosphate fertilizers, including TSP, HAP, unmodified nano-hydroxyapatite (NHAP0), negatively charged nano-hydroxyapatite (NHAP<sup>-</sup>), and positively charged nano-hydroxyapatite (NHAP<sup>+</sup>) were chosen as representative P fertilizers. Immobilization amendment and five P fertilizers were blended into the soil before the pot experiment. The dose of wollastonite was 5 g/kg. The dose of the five P fertilizers was 180 mg P/kg soil, which was similar to the application reported by [Xiong et al. \(2018a\)](#). This resulted in 12 treatments as follows: control (CK), wollastonite (W), P fertilizers only (TSP, HAP, NHAP<sup>-</sup>, NHAP0, and NHAP<sup>+</sup>), wollastonite plus P fertilizers (WTSP, WHAP, WNHAP<sup>-</sup>, WNHAP0, and WNHAP<sup>+</sup>). Once in the pot, the soil water content was adjusted to 70% of its water-holding capacity. The

pots were then left undisturbed for 7 d before three *A. tricolor* seedlings of uniform sizes with four true leaves were transplanted into each pot.

## 2.4. Growth index measurement

The leaf area and relative chlorophyll concentration of *A. tricolor* were measured in the third mature leaf of each plant (at 45 days of cultivation) in each pot (three plants per pot) using a leaf area and SPAD chlorophyll meter (502Plus, Saiyasi, Japan). On the same day, we measured the plant height of each plant in each pot using a rigid ruler, and the average height of each pot was used as the plant height for that treatment.

## 2.5. Plant and soil sample analysis

After growing for 45 d in the pots, the plants were harvested, separated into roots, stems, and leaves, and rinsed using deionized water. Root exudates were determined according to the method of [Chen et al. \(2019\)](#), by mixing 0.4 g of the plant samples and 1.5 mL of deionized water and then shaking for 30 min at 1400 rpm (Thermomixer compact, Eppendorf, Switzerland). The samples were subsequently centrifuged for 5 min at 1,3000 g, and the supernatants were filtered through a 0.22- $\mu\text{m}$  syringe filter. Next, 0.5 mL of the filtered supernatants were analyzed to determine the root organic carbon (ROC), and 0.5 mL of the filtered supernatants were analyzed to identify the low molecular weight organic acids (LMWOAs). The ROC was measured using an elemental analyzer (Multi N/C 3100, Germany). The LMWOAs were measured using a high-performance liquid chromatograph (HPLC) (Agilent 1260 infinity, USA) equipped with a reversed-phase silica C18 column (XSelect HSS T3 250 × 4.6 mm, 5  $\mu\text{m}$ , Water) at 30 °C. We chose 20 mM sodium phosphate buffer at pH 2.73 as the mobile phase, and the flow rate and injection volume were 0.5 mL/min and 10  $\mu\text{L}$ , respectively. The absorbance of the test was 210 nm, and calibration curves were constructed with standard organic acids (e.g., oxalic acid, tartaric acid, formic acid, malic acid, lactic acid, acetic acid, maleic acid, and citric acids) ([Chen et al., 2019](#)). The remaining rinsed plant samples were oven-dried for 72 h at 70 °C, weighed, and ground for later analysis. The plant samples were digested using high-purity  $\text{HNO}_3$ , and the digested samples were diluted to 50 mL using deionized water and then filtered. The Cd concentrations in the digested liquid were analyzed using a flame atomic absorption spectrophotometer (AAS, contraA800, Analytikjena, Germany). Quality control of the digestion procedure and the analysis process was ensured using a standard reference material, GBW10015 (spinach).

After the rhizosphere soil was mixed, a 15 g sample of fresh soil was collected from each pot and placed in a 50-mL centrifuge tube. The tubes were centrifuged at 10,000 rpm for 5 min and maintained at 4 °C before analysis of soil bacteria. The remaining soil from each pot was air-dried and passed through a 2-mm sieve for chemical analysis. The measurement and results of soil basic property, Colwell-P, soil P fractionation, and soil ammonium and nitrate are presented in the [Supporting Information](#).

## 2.6. Soil DNA extraction, bacterial 16 S amplification, and Hiseq sequencing

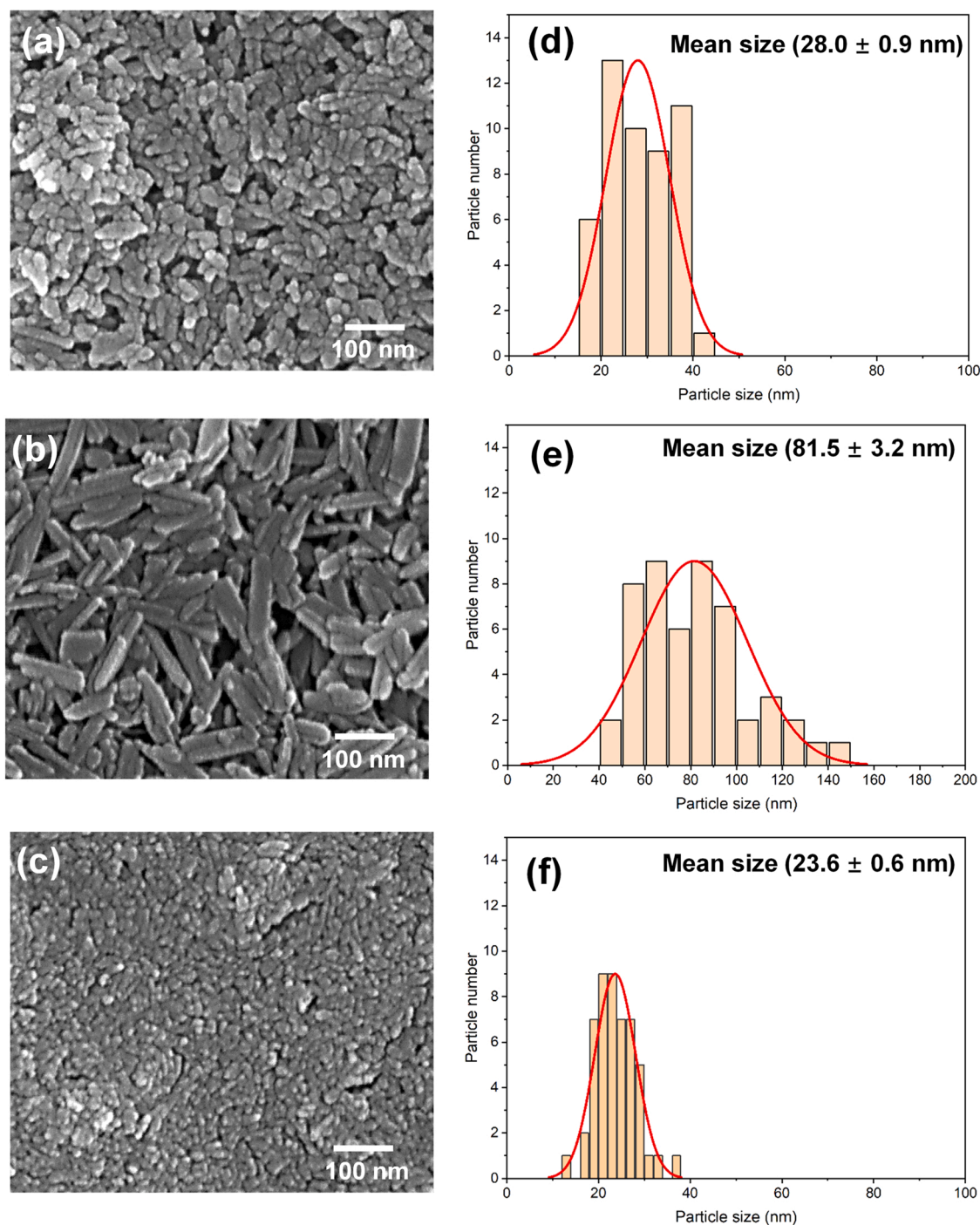
The bacterial community structure was analyzed using high-throughput sequencing of the 16 S rRNA gene. Soil microbial DNA was extracted using an E.Z.N.A.® soil DNA Kit (Omega Bio-Tek, Norcross, GA, U.S.). The DNA quality was checked on a 1% agarose gel. The DNA concentrations and purity were determined with a NanoDrop 2000 UV-vis spectrophotometer (Thermo Scientific, Wilmington, USA). Distinct regions of the 16 S rRNA gene (16 S V3-V4) were amplified with primer pairs 338 F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806 R (5'-GGACTACHVGGGTWTCTAAT-3') by an ABI GeneAmp® 9700 PCR thermocycler (ABI, CA, USA).

A 10-ng quantity of template DNA was amplified in triplicate in a total volume of 20  $\mu$ l that included 4  $\mu$ l of *TransStart* FastPfu buffer, 2  $\mu$ l of 2.5 mM dNTPs, 0.8  $\mu$ l of 5  $\mu$ M forward primer, 0.8  $\mu$ l of a 5  $\mu$ M reverse primer, 0.4  $\mu$ l of *TransStart* FastPfu DNA Polymerase, and 12  $\mu$ l of ddH<sub>2</sub>O. The following amplification conditions were used: initial denaturation at 95  $^{\circ}$ C for 3 min; followed by 27 cycles of denaturation at 95  $^{\circ}$ C for 30 s, annealing at 55  $^{\circ}$ C for 30 s, and extension at 72  $^{\circ}$ C for 45 s; a single extension at 72  $^{\circ}$ C for 10 min, and a final extension at 4  $^{\circ}$ C. The PCR product was extracted from the 2% agarose gel and purified using an AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) according to the manufacturer's instructions using a Quantus™ Fluorometer (Promega, USA).

Purified amplicons were pooled in equimolar concentrations and paired-end sequenced ( $2 \times 300$ ) on an Illumina MiSeq platform (Illumina, San Diego, USA) according to the standard protocols by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China). The raw data have been deposited in the NCBI database under the project numbers PRJNA884889.

## 2.7. Statistical analysis

One-way ANOVA combined with Two-way ANOVA was used to determine statistical significance among treatments with the Tukey post hoc test (version 24.0, IBM SPSS Inc. T). Student's t-test was used to



**Fig. 1.** Scanning electron micrographs of negatively charged NHAP (NHAP<sup>-</sup>) (a), unmodified NHAP (NHAP<sup>0</sup>) (b), and positively charged NHAP (NHAP<sup>+</sup>) (c), and their particle size calculated by ImageJ (d, e, f; N = 50).

examine the difference between two treatments. Pearson correlation heatmap analysis of the relationship between bacterial communities and the selected soil physicochemical properties was conducted by using the “heatmap” package in R. The linear discriminant effect size (LEfSe) analysis was used to discriminate the abundant taxa biomarkers between the groups. Redundancy analysis (RDA) among the group of control, TSP, NHAP-, W, WTSP, and WNHAPO was conducted by using the “vegan, RDA” package in R. It can explore the key influence factor affecting the relationships between soil microbial communities and soil properties. To further test the direct and indirect effects of soil properties, root exudates, and microbial composition on plant growth and Cd uptake, structural equation modeling (SEM) was performed using the AMOS software (version 24, IBM, New York, USA).

### 3. Results and discussion

In the present study, we synthesized three NHAPs with different surface charges and examined their effectiveness in decreasing Cd uptake by *A. tricolor* in combination with wollastonite. We initially assumed that wollastonite limited the use efficiency of soluble phosphate and that using NHAP as a P fertilizer would be a better option. However, in wollastonite-amended soil, wollastonite did not inhibit the supply of P to plants after the addition of P fertilizer. Alternatively, NHAP- and NHAP+ increased the plant growth and decreased Cd uptake more than TSP and bulk HAP did in W-amended soils, which was ascribed to the secretion of high concentrations and diversity of organic acids in the rhizosphere.

#### 3.1. Material characterization and application in soil

According to the method of Xiong et al. (2018b), we successfully synthesized three NHAP fertilizers with surface charges of  $-13.8$ ,  $-1.1$ , and  $+16.7$  mV at a solution pH of 7 (Fig. S1). Using SEM and TEM, the microstructures of the three phosphate compounds were confirmed to be nanosized, with NHAP0 being granular-like and NHAP-/NHAP+ being stick-like (Fig. 1a, b, and c, Fig. S2). The particle sizes were  $28 \pm 0.9$ ,  $81.0 \pm 3.2$ , and  $23.6 \pm 0.6$  nm respectively for NHAP-, NHAP0, and NHAP+ ( $N = 50$ , ImageJ) (Fig. 1). The XRD spectra of these three compounds closely matched those of HAP (JCPDS Code No. 00-064-0738) (Fig. S3). Furthermore, the typical FTIR spectra of these three compounds were characterized by the vibrations of the  $\text{PO}_4^{3-}$  and  $\text{OH}^-$  groups (Fig. S3). As expected, ammonium citrate dibasic-modified NHAP (NHAP-) exhibited weak adsorption signals for the  $\text{C}=\text{O}$  stretch of  $\text{COO}^-$  at  $1360\text{--}1380$   $\text{cm}^{-1}$  (Xiong et al., 2018b). In contrast, glycine-treated NHAP (NHAP+) was characterized by the presence of bands in the  $1410\text{--}1430$   $\text{cm}^{-1}$  region of the FTIR spectra, which were ascribed to  $-\text{NH}_2$  stretching vibrations (Lee et al., 2013; Duan et al., 2021).

To quantitatively evaluate the remediation performance of NHAP and wollastonite, we examined changes in the physical and chemical properties of the Cd-contaminated soils (Table 1). For this acidic vertisol (initial pH of 3.9), the addition of P fertilizer had no effect on soil pH, whereas a slight decrease in Cd availability was found. It is well documented that Cd(II) can substitute Ca(II) in TSP or HAP. Hereafter, precipitation as Cd-P becomes the primary mechanism for Cd immobilization (Yan et al., 2020).

After adding wollastonite, the soil pH values increased significantly from 3.8 to 4.0–6.3–6.6, accompanied by a dramatic reduction in Cd availability ( $p < 0.05$ ). Increasing soil pH enhanced the sorption of heavy metal ions by increasing negatively charged sites, whereas surface charge modification of NHAP had little effect on this aspect. Furthermore, higher soil pH increases  $\text{OH}^-$  or  $\text{HCO}_3^-$  concentrations to precipitate Cd as hydroxides or carbonates (Bolan et al., 2014). However, we should be concerned about the volatilization of  $\text{NH}_3$  under alkaline conditions, as the  $\text{NH}_4^+\text{-N}$  concentration decreased significantly ( $p < 0.05$ ). Interestingly, it should be noted that when wollastonite was

**Table 1**

Effects of different P treatments and wollastonite soil chemical properties after *A. tricolor* L. cultivation of 45 d. Significant differences between means of treatment are indicated by different lowercase letters ( $p < 0.05$ ). Data are shown as mean  $\pm$  SE ( $n = 4$ ). Treatment effects of P fertilizers (P), wollastonite (W), and the interaction of P fertilizers and wollastonite (P X W) are shown using two-way ANOVA.

Treatments	pH	Available Cd mg/kg	N-NH <sub>4</sub> <sup>+</sup>	N-NO <sub>3</sub>
CK	3.94 $\pm 0.2b$	0.97 $\pm 0.06a$	25.13 $\pm 3.7a$	102.15 $\pm 1.7a$
TSP	3.85 $\pm 0b$	0.82 $\pm 0abc$	23.19 $\pm 5.4a$	92.1 $\pm 3a$
HAP	3.81 $\pm 0b$	0.84 $\pm 0.2ab$	25.85 $\pm 3.3a$	105.48 $\pm 2.3a$
NHAP-	3.98 $\pm 0.2b$	0.86 $\pm 0ab$	28.62 $\pm 3.5a$	102.59 $\pm 5a$
NHAP0	3.85 $\pm 0.1b$	0.77 $\pm 0ab$	22.15 $\pm 3a$	88.52 $\pm 9.3a$
NHAP+	3.93 $\pm 0.1b$	0.9 $\pm 0a$	27.92 $\pm 3.4a$	106.18 $\pm 1.8a$
W	6.47 $\pm 0.1a$	0.53 $\pm 0d$	2.43 $\pm 0.1b$	103.85 $\pm 2.8a$
WTSP	6.32 $\pm 0.1a$	0.56 $\pm 0c$	2.54 $\pm 0.1b$	107.48 $\pm 5.8a$
WHAP	6.43 $\pm 0.1a$	0.55 $\pm 0d$	2.52 $\pm 0.1b$	96.06 $\pm 8.7a$
WNHAP-	6.52 $\pm 0.1a$	0.59 $\pm 0cd$	2.65 $\pm 0.2b$	13.93 $\pm 0.7b$
WNHAP0	6.49 $\pm 0.1a$	0.55 $\pm 0cd$	3.96 $\pm 0.4b$	109.35 $\pm 4.8a$
WNHAP+	6.63 $\pm 0a$	0.57 $\pm 0cd$	2.82 $\pm 0.1b$	29.65 $\pm 4.3b$
Analysis of variance (p values)				
P fertilizers (P)	0.37	0.66	0.87	< 0.001
Wollastonite (W)	< 0.001	< 0.001	< 0.001	< 0.001
P X W	0.85	0.54	0.72	< 0.001

Note: 12 treatments are as follows: control (CK), W, P fertilizers only (TSP, HAP, NHAP-, NHAP0, and NHAP+), and W plus P fertilizers (WTSP, WHAP, WNHAP-, WNHAP0, and WNHAP+).

added, the addition of positively or negatively charged NHAP led to a significant decrease in  $\text{NO}_3\text{-N}$ . This decrease was perhaps ascribed to the promotion of plant N uptake or soil denitrification rather than the inhibition of nitrification or  $\text{NO}_3$  leaching.

#### 3.2. Changes in the P pool in the soil

First, we used the Colwell-P as an indicator of available P (Fig. 2). The five P fertilizers and wollastonite changed the P availability in the soil. After adding TSP, the available P in the soil increased from 15.6 to 182.2 mg/kg. Of particular concern are the adverse outcomes of P beyond the rhizosphere. Despite the high solubility of TSP, a large amount of P runoff from soil leads to low P use efficiency and even environmental pollution (Zhang et al., 2020). Phosphate fertilizer do not volatilize, and are assimilated by plants, adsorbed by soil, and leached from the soil. Alternatively, in a sand column leaching experiment conducted by Xiong et al. (2018b), 72% of P accumulated in the leachate for sand treated with TSP. In contrast, the accumulative P recovered in the leachate remained low ( $< 0.5\%$ ) across the remaining P fertilizer treatments. Therefore, we concluded that the phosphorus utilization efficiency of TSP was comparably low compared to the NHAP. In comparison, HAP and the three forms of NHAP increased the available P to a lesser but significant extent ( $p < 0.05$ ). Across the incubation period, the Colwell-P in the TSP treatment significantly decreased from 182.1 mg/kg after 0 d to 144.9 mg/kg after 45 d ( $p < 0.05$ , Fig. 2). In contrast, although Colwell-P in three NHAP fertilizer treatments were comparably lower compared to TSP treatment after 45 d, their values were higher than their counterpart after 0 d, being 87.7 mg/kg for

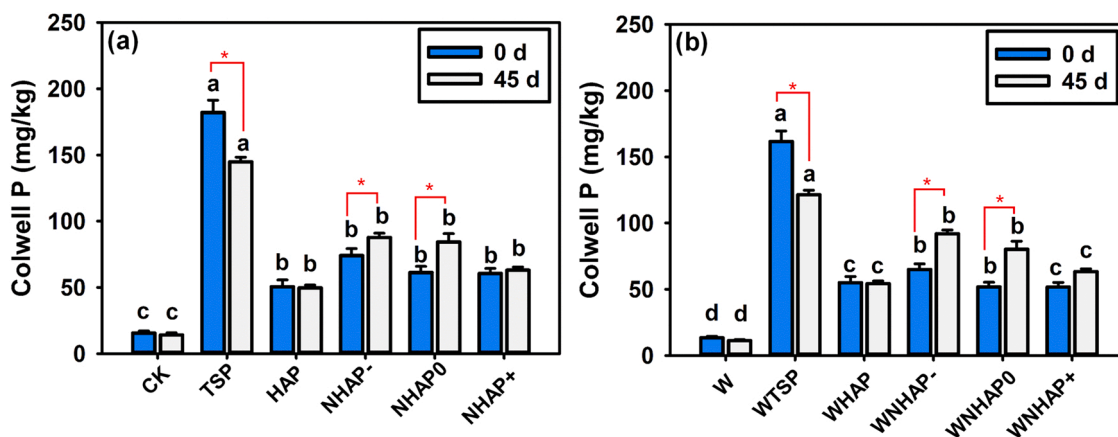


Fig. 2. Changes in Colwell-P after the addition of five different P-containing compounds, including triple superphosphate (TSP), bulk hydroxyapatite (HAP), negatively charged NHAP-, neutral NHAP0, and positively charged NHAP+ in soil without (a) or with (b) wollastonite addition. Soils were incubated for up to 45 d. Different lowercase letters above the error bar indicate significant differences among treatments incubated for 0 d or 45 d ( $p < 0.05$ ). \* denotes a significant difference between the two treatments at  $p < 0.05$  (Student's t-test).

NHAP-, 84.3 mg/kg for NHAP0, and 63.0 mg/kg for NHAP+ (Fig. 2). This difference was confirmed by the slow-release characteristics of HAP and NHAP (Xiong et al., 2018a; Szameitat et al., 2021). Bulk soil P and soil solution phosphate are in a dynamic dissolution equilibrium ( $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2 + 2\text{H}^+ \rightleftharpoons 10\text{Ca}^{2+} + 6\text{PO}_4^{3-} + 2\text{H}_2\text{O}$ ). The capability of soil to replenish P when plant roots consume it controls the availability of soil P (Lambers, 2022). With the extension of the interaction between soil and NHAP, it is expected that more P will be released from NHAP to maintain fast plant growth. After adding wollastonite, Colwell-P in the TSP treatment substantially reduced from 144.9 mg/kg to 121.3 mg/kg. The precipitation of P in soils is highly dependent on soil pH (Hong et al., 2014; Wei et al., 2022). In acidic soils, with increasing pH, those excessive P from TSP precipitates as Ca phosphates ( $3\text{Ca}^{2+} + 2\text{PO}_4^{3-} \rightleftharpoons \text{Ca}_3(\text{PO}_4)_2$ ) (Xiong et al., 2018a). However, that P availability diminishing effects did not exist in soils amended with other P compounds (Fig. 2), confirming that the insoluble fertilizer can maintain their efficiency for a longer term.

We then examined the distribution of P fractions in the soil (Fig. S4). The proportion of resin-P in all treatments was deficient (1.1–11.6%). The P fractions mainly existed in  $\text{NaHCO}_3\text{-P}$  (19.5–54.7%) and  $\text{NaOH-P}$  (21.1–35.6%), representing the high percentage of readily available inorganic P and Fe/Al minerals, respectively. After adding TSP, the proportion of  $\text{NaHCO}_3\text{-P}$  increased while that of  $\text{NaOH-P}$  decreased. Normally, resin-P and  $\text{NaHCO}_3\text{-P}$  are labile P available to plants. Alternatively,  $\text{NaOH-P}$  is associated with Fe/Al minerals (Xiong et al., 2018a). These results were consistent with the high Colwell-P concentrations in soil amended with TSP. In comparison, the shift of proportion from  $\text{NaOH-P}$  to residue-P for the soils to which NHAPs were added provides evidence that NHAPs were highly insoluble. They can serve as a P reservoir in soil for an extended period. Regarding the addition of wollastonite, the proportion of resin-P increased slightly. In theory, silicate and phosphate compete for soil absorption sites to increase P availability. Adding wollastonite shifted the  $\text{NaHCO}_3\text{-P}$  pool to the  $\text{NaOH-P}$  pool. This is consistent with the results of available P, in which P precipitated as Ca phosphates at a high soil pH.

### 3.3. Plant Cd concentration and plant growth

To study the P bioavailability and Cd immobilization capacity of NHAP combined with wollastonite, we planted *A. tricolor* L. in a Cd-contaminated Vertisol for 45 d. P compounds, except TSP, were ineffective in reducing the Cd concentration in the shoots of *A. tricolor* L. due to the original low soil pH (Fig. 2). Triple superphosphate is an acidic salt, whereas HAP is a slightly alkaline salt (Table S2). Rather than

modifying the soil pH, TSP increases the negative charge of the soil surface to increase Cd adsorption by the soil (Ruangcharus et al., 2020). The addition of wollastonite significantly reduced the concentration of shoot Cd by 70.0%. In consistent with these hypotheses, adding TSP slightly diminished the Cd immobilization efficiency of wollastonite. Most experiments have demonstrated that P fertilizers can diminish the immobilization efficacy of alkaline materials on Cd in a paddy soil under flooding conditions (Mao et al., 2019). Theoretically, the application of P fertilizers in Cd-contaminated paddy soil reduces the availability of Fe and Mn, releasing Cd from Fe and Mn oxyhydrogen (Wang et al., 2019b). In combination with NHAP- and NHAP+, we observed an even more pronounced decrease in Cd concentrations by 74.8% and 75.1%, respectively. According to the plant growth promotion effects of these two treatments, the dilution of Cd concentrations may explain the above results.

Furthermore, screening the effects of P fertilizers and wollastonite on the uptake of plant nutrients (such as Cu, Zn, Ca, N and P) is important for evaluating their application feasibility (Fig. S5). The addition of P fertilizers had little effect on the concentrations of Cu and Zn in the plants. Alternatively, we found synchronous immobilization of micronutrients in soils amended with wollastonite, leading to decreased Cu and Zn plant uptake. Similar to promoting plant growth, NHAP- and NHAP+ also slightly increased Zn uptake under the wollastonite treatments. Since both P fertilizers and wollastonite contained Ca, the concentration of Ca in plants increased slightly after applying five P compounds. Wollastonite treatment significantly increased the Ca concentration from 11,529 mg/kg to 23,584–29,161 mg/kg, due to the higher application rate of wollastonite compared to the five P fertilizers (5 g/kg vs. ca. 1 g/kg).

The addition of P fertilizers increased the plant N concentration. Although, the addition of wollastonite reduced the  $\text{NH}_4^+$  in the soil, it did not affect the plant N concentration. For plant the P concentration, in the absence of wollastonite, TSP significantly increased the P concentration to the highest level (approximately 4447 mg/kg, dry mass basis) due to the high P availability of TSP in soil, followed by the plants treated by NHAP0 and those treated by NHAP+. Notably, we initially assumed that adding wollastonite would reduce plants' P concentration. Nonetheless, in this experiment, the P concentration of plants increased significantly after adding wollastonite. This was likely caused by the high soil pH leading to P desorption. Studies have also shown that, because the similar chemical structures of silicate and phosphate, applied silicate can promote the desorption of soil P through competition (Haynes and Zhou, 2018). Thus, in this study, the excessive Ca from wollastonite did not limit the P supply to the plant.

The tested soil was rich in P, with Colwell-P being 15.6 mg/kg. Hence, the plant growth promotion upon the addition of P fertilizers was not as pronounced as expected (Fig. 3). In the absence of wollastonite, the P fertilizers (except for bulk HAP) significantly increased the plant biomass and plant height ( $p < 0.05$ ). Three kinds of NHAP increased the shoot mass to 6.4–8.8 g per pot and the plant height to 21.1–28.2 cm, with the corresponding values being 1.7 g per pot for plant biomass and 8.9 cm for plant height in the control. TSP was the most favorable fertilizer for plant biomass, while NHAP- was promising for plant height. The addition of wollastonite increased the plant biomass and plant height by 359% and 350%, respectively. As a cheap alkaline and Si-rich material, wollastonite can improve the supply of Si and Ca in the soil while raising the soil pH. In South China, attempts have been made to remediate heavy metal-contaminated farmland, which is generally acidic and lacks Si (Mao et al., 2022). The effect of the combined application of TSP and wollastonite on promoting plant growth was weaker than that of wollastonite alone, whereas in combination with NHAP- or NHAP+, wollastonite could further improve plant biomass ( $p < 0.05$ ). We initially assumed that NHAPs promoted plant growth by the maintenance of plant nutrient uptake because wollastonite normally synchronously immobilized heavy metal and micronutrients (Wu et al., 2016), and excessive P could precipitate with micronutrients in soil (He et al., 2021; Shahid, 2021). However, only Zn concentrations increased slightly in WNHAP- treatments.

The addition of NHAP0 or NHAP+ alone increased the chlorophyll content of the leaves (Fig. 3). It has been reported that wollastonite reduces the bioavailability of Zn and Mn during Cd immobilization, thereby inhibiting the photosynthetic activity of plants (Wu et al., 2016). The immobilization treatment did not reduce the chlorophyll content, indicating that the micronutrients in the soil were sufficient to maintain photosynthesis. Similar to the trend in plant biomass, the addition of NHAP- and NHAP+ to the wollastonite-amended soil further increased the chlorophyll content. For leaf area, five P fertilizers and wollastonite slightly or significantly increased the plant leaf area (Fig. 3). Based on the overall results on the plant biomass, plant height,

chlorophyll content, and leaf area, the combined application of NHAP- and wollastonite was the most promising treatment for plant growth.

### 3.4. Soil microbial community

During soil Cd remediation, the modification of the soil microbial composition is mainly driven by contamination stress and soil nutrient availability (Wu et al., 2021). A total of 37 categories were detected in all the incubated soils (Fig. 4a). Among the 12 treatments, they shared 1496 OTUs. More OTU reads were detected in soils amended with wollastonite than those without wollastonite, increasing from 2947–3325 to 3385–3574 (Fig. 4b). *Actinobacteria*, *Proteobacteria*, *Chloroflexi*, and *Firmicutes* dominated the classified bacterial phyla and accounted for 25.6–33.4%, 18.3–26.2%, 13.7–18.8%, and 11.1–20.7% of all the sequences, respectively (Fig. 4a). Regardless of the addition of wollastonite, the addition of P fertilizers slightly increased the abundance of *Gemmatimonadetes* and *Patescibacteria*. Adding wollastonite slightly increased the relative abundance of *Proteobacteria* and decreased the relative abundance of *Actinobacteria*. Moreover, the addition of wollastonite significantly reduced the abundance of *Firmicutes* and increased the abundance of *Bacteroidetes*. Previous studies have reported that *Firmicutes* were the main bacterial phylum in Cd-contaminated soil controlling the transformation of short-chain fatty acids (Li et al., 2017, 2021b). In contrast, *Bacteroidetes* are intolerant to Cd, and normally thrive after restoration (Liu et al., 2020; Wang et al., 2021). Therefore, as the ecological risk of the heavy metal Cd decreases, the predominant microbial community will shift from *Firmicutes* to *Bacteroidetes*.

We then utilized LefSe analysis combined with LDA to inspect changes in the taxa from phylum to family (Fig. 4c). According to LefSe analysis, seven treatments had specific taxa with LDA scores  $> 4$  (Fig. 4d). The unamended control soil was marked by predominantly containing the *Firmicutes* phylum. The sole wollastonite-amended soil was dominated by the class *Anaerolineae*, assigned to the phylum *Chloroflexi*. Generally, *Chloroflexi* is a well-known oligotrophic bacterium that can thrive in nutrient-limited environments, as has been found

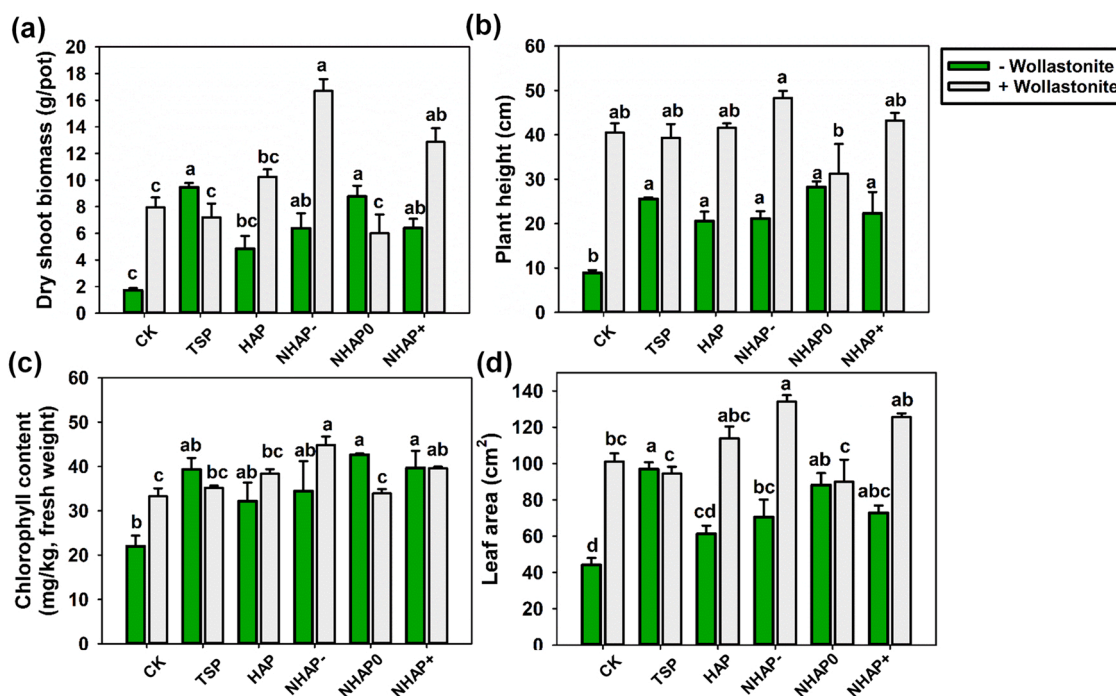


Fig. 3. Effects of different P treatments and wollastonite on the shoot biomass (a), plant height (b), chlorophyll content (c), and leaf area (d) of *A. tricolor* L. grown in soils to which triple superphosphate (TSP), bulk hydroxyapatite (HAP), and nano-hydroxyapatite (NHAP) differing in surface charge, have been added as P fertilizers. Data are means  $\pm$  SE ( $n = 4$ ). Different lowercase letters above the error bar indicate significant differences among treatments with or without wollastonite addition ( $p < 0.05$ ).

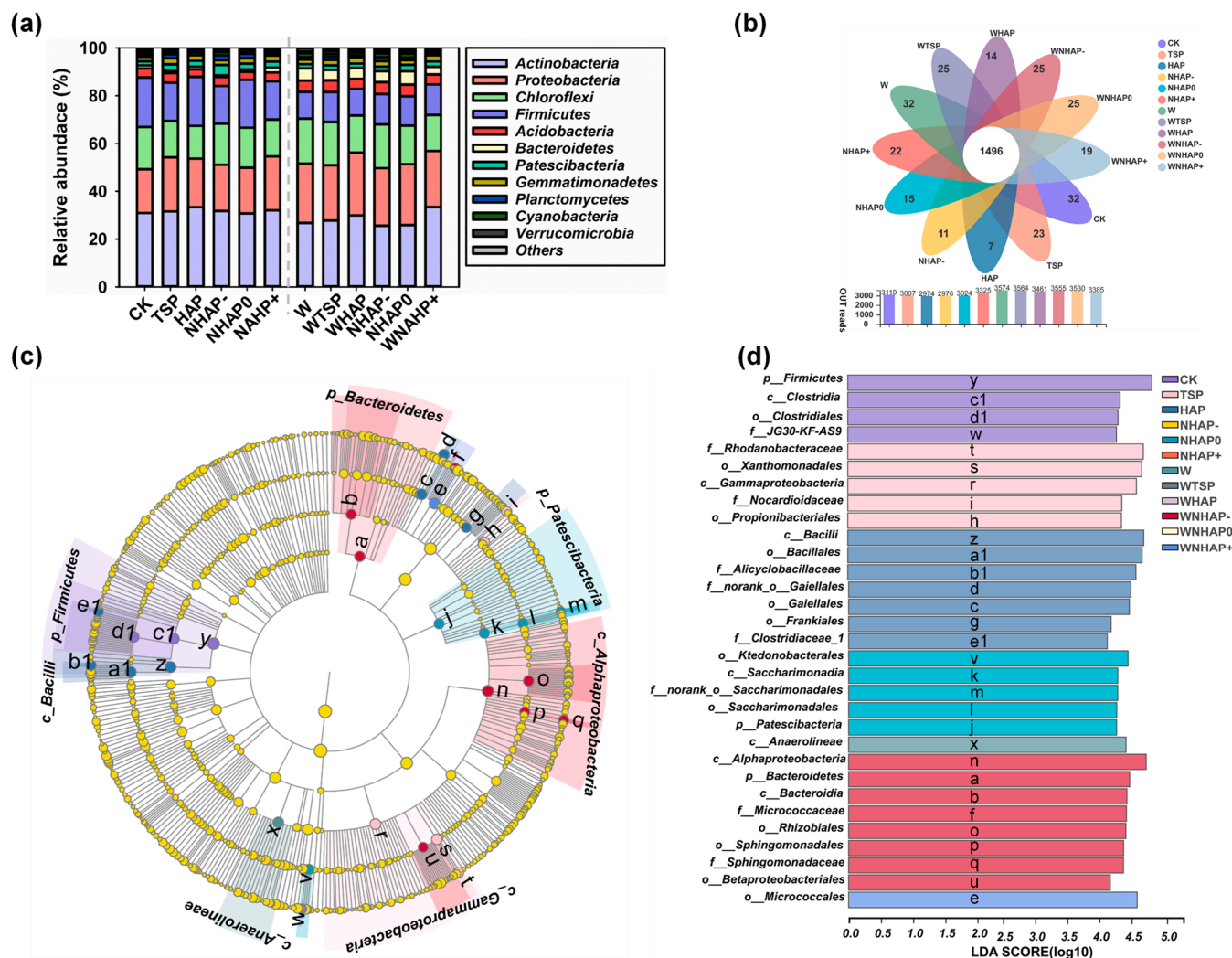


Fig. 4. Effects of different P treatments and wollastonite on rhizosphere microorganism abundance and composition. The relative abundance of the dominant phyla (a). Venn diagram of the shared OTUs (b). LefSe cladogram for the representative taxa (c). LDA scores of the differentially abundant taxa (LDA > 4) (d).

in wollastonite-amended soil (Cui et al., 2022). The *Anaerolineae* class of *Chloroflexi* appears to specialize in the catabolism of carbohydrates in conjunction with hydrogenotrophic methanogens (Sutcliffe et al., 2018). Notably, several branches of biomarkers existed in the soil amended with both NHAP- and wollastonite. For example, the taxonomic branches of *Bacteroidetes* (phylum)–*Bacteroidia* (class) and *Alphaproteobacteria* (class)–*Sphingomonadales* (order)–*Sphingomonadales* (family) were underrepresented. In contrast to *Anaerolineae*, *Bacteroidia* is a copiotrophic class that generally thrives in P-rich soils and has a beneficial function in seedling nutrient use and growth (Dong et al., 2021; Zhang et al., 2021). In addition, *Sphingomonadaceae* is also a plant growth-promoting taxon that can secrete dehydrogenase and preferentially colonizes the rhizosphere (Gong et al., 2021; Tang et al., 2020). These specific taxa confirmed the plant growth-promoting effect of WHAP- treatment.

### 3.5. Plant root organic acid

Several studies have highlighted that plants can promote the uptake of heavy metals and the acquisition of nutrients by secreting low molecular weight organic acids (Qiao et al., 2022). In all treatments, a total of five low molecular weight organic acids were detected (Fig. 5). Oxalic acid and citric acid were the principal components of all the soils. After adding wollastonite, the concentration of oxalic acid decreased

significantly, whereas that of citric acid increased significantly. In fact, citric acid is considered to have a stronger capacity to increase metal availability to plants compared to oxalic acid (Huang et al., 2020). The *A. tricolour* L. we used is a Cd accumulator. This plant tends to excrete more low molecular weight organic acids, especially for citric acid, to increase Cd availability in alkaline soils, as resulting from adding wollastonite (Huang et al., 2020). Moreover, malic acid was detected only in the treatments with wollastonite, where the soil pH and the plant biomass increased. Plants commonly secrete more organic acids in alkaline and pH-neutral soils (Xiong et al., 2018a). Importantly, the application of the three NHAPs promoted the secretion of oxalic acid and citric acid in wollastonite-amended soil. In addition, two new organic acids, lactic and acetic acid, were produced. This can be explained by the development of the rhizosphere, which induces the secretion of organic acids in the rhizosphere to acquire more nutrients.

Organic acids in the root exudates were closely related to a variety of rhizosphere bacteria (phylum communities with a relative abundance > 1%) (Fig. 5b). The oxalic acid and organic N concentrations of root exudates were negatively correlated with the bacterial phyla *Acidobacteria*, *Armatimonadetes*, *Bacteroidetes*, *Chlamydiae*, *Cyanobacteria*, *Dadabacteria*, *Dependentiae*, *Fibrobacteres*, *Nitrospirae*, *Proteobacteria*, and *Rokubacteria* and positively correlated with *Actinobacteria*, *Firmicutes*, and *Patescibacteria*. However, the opposite trend was observed for other organic acids, including the total amount of organic carbon in the root



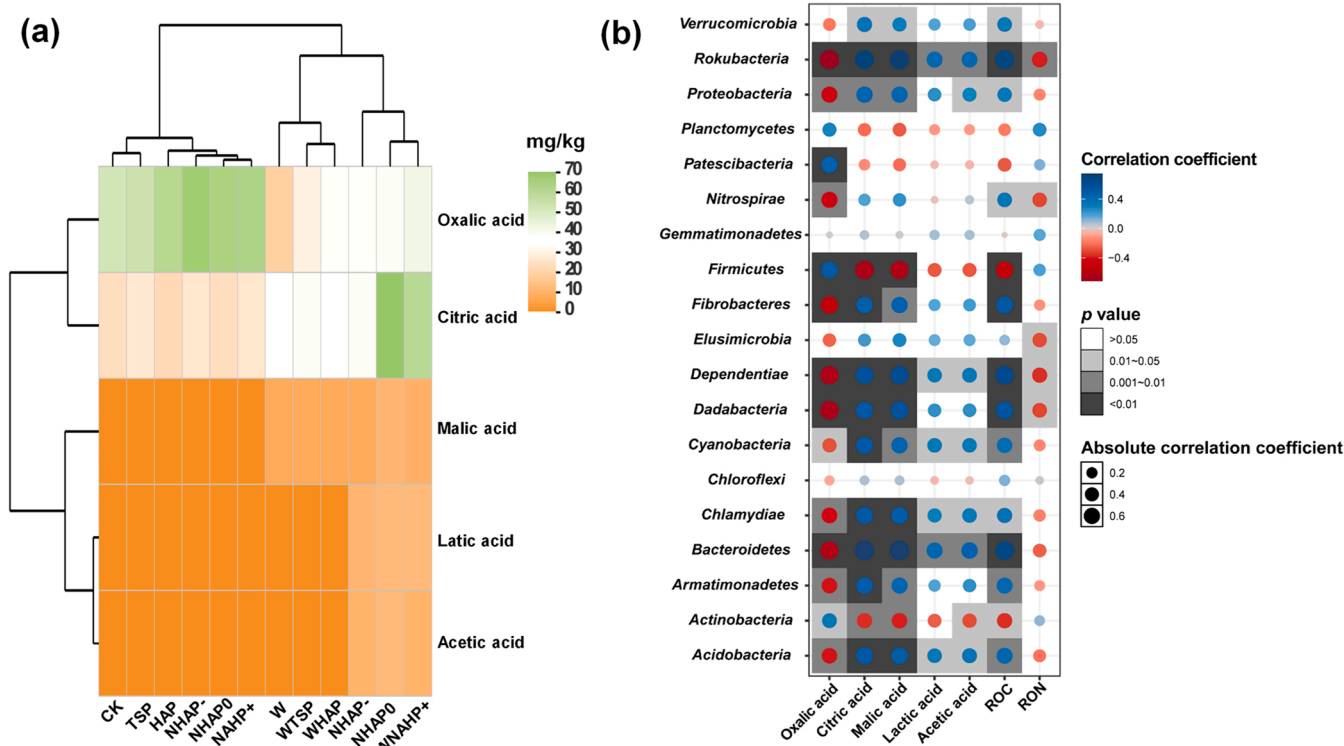


Fig. 5. Effects of different P treatments and wollastonite on organic acid secreted by roots. Heatmap analysis of changes in the root organic acid (a) Correlations between organic acids and the relative abundance of dominant phyla (b).

exudates. According to the relationship between root exudates and soil properties, the changes in root exudate composition were highly controlled by soil pH and available Cd, N, and P (Fig. S6). We used RDA to reveal the relationships between the selected soil physicochemical properties and the soil bacterial community (Fig. S7). Cadmium availability, resin-P, NaHCO<sub>3</sub>-P, NaOH-P, soil pH, and all the test root organic acids control the differences among the treatments along RDA1. They are also the key factors in modifying the soil microbial community (Huang et al., 2020). In our study, the abundances of *Actinobacteria* and *Firmicutes* were positively correlated with the concentrations of soil

available Cd, NaHCO<sub>3</sub>-P, and oxalic acid; whereas they were negatively correlated with pH, resin-P, NaOH-P, and the remaining four organic acids. However, the opposite trend was found for *Bacteroidetes*, *Proteobacteria*, *Chloroflexi*, and *Acidobacteria*. In summary, the addition of wollastonite predominantly influenced the overall microbial community because it substantially increased soil pH and the organic acids secreted by the plants.

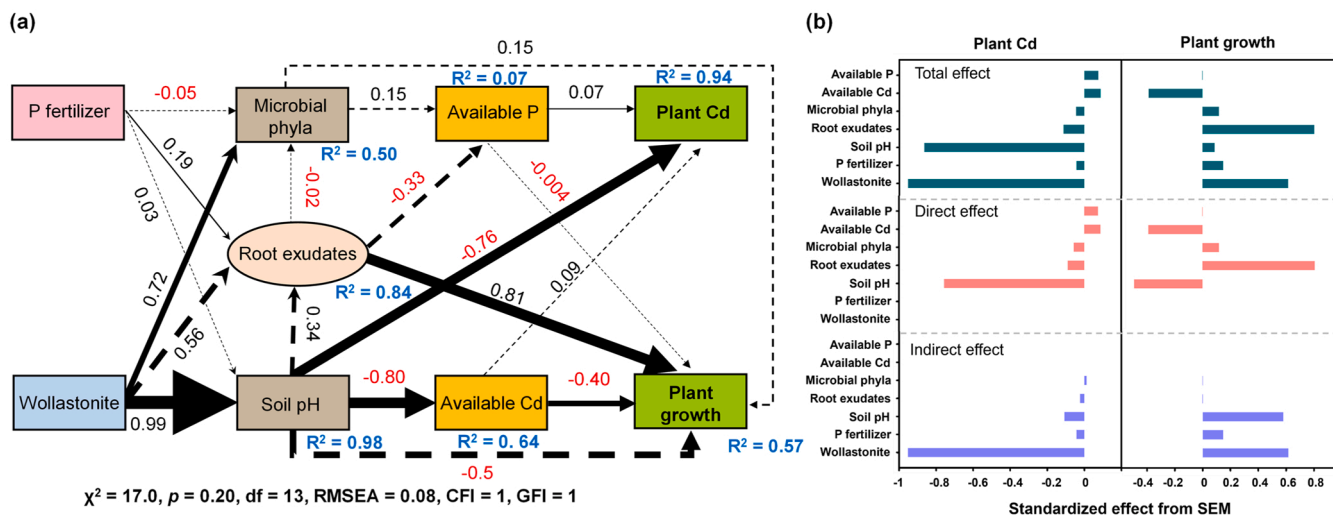


Fig. 6. Structural equation modeling indicating how soil properties, root exudates, and microbial composition affect plant growth and Cd uptake in P fertilizer- and wollastonite-amended soils (a), with standardized effects shown in panel (b). Solid lines denote significant relationships and dashed lines denote non-significance. The numbers and width on the arrows are the standardized path coefficients. The R square values of response variables represent the proportion of variation explained by relationships with other variables. The overall fitness of the model is listed at the bottom.

### 3.6. Structural equation modeling

To delineate the Cd immobilization mechanisms, we employed structural equation modeling to quantify the complex interactions among soil pH, root exudates (including five root organic acids), microbial composition (including the top 10 microbial abundances at the phylum level), soil available P and Cd, plant Cd uptake and plant growth (represented by plant biomass, plant height, chlorophyll content, and leaf area) (Fig. 6). The nonsignificant  $X^2$  test ( $p = 0.2$ ), the low root mean square error of approximation (RMSEA = 0.08), and the high fit index (CFI > 0.99 and GFI > 0.99) indicated that the model fits rationally.

Based on the structural equation modeling, soil pH, rather than soil P and microbial properties, exerts a dominant effect on plant Cd uptake and plant growth, explaining 98% of the variation in the overall model (Fig. 6). The results showed that the addition of wollastonite significantly increased the soil pH, changed the soil microbial community structure and available Cd, reduced plant Cd uptake, and promoted plant growth. We initially hypothesized that soil properties would influence plant Cd uptake and growth directly and indirectly by affecting plant root exudates and microbial composition. However, it seems that the promoted plant growth in treatments amended with both wollastonite and NHAPs was not ascribed to the promoted nutrient uptake. The root exudates also have a weak negative direct effect on plant Cd uptake and a strong positive direct effect on plant growth after accounting for the impact of soil pH. Under the joint application of wollastonite and phosphate fertilizer, plants secreted higher concentrations and more types of organic acids to further promote plant growth and reduce Cd uptake.

Overall, using currently-available P fertilizer, only 10–20% of the P is actually assimilated by plants (Holford, 1997), and the remaining is transformed into forms that are of low availability or released into the environment. The aggravating eutrophication caused by P fertilization also cost the community high spending every year (Dodds et al., 2009). In our study, NHAP synthesis by wet precipitation is the most widely used due to its low cost and simplicity (Xiong et al., 2018a). NHAP may be suitable as a new form of P fertilizer with a slower and more sustained release rate to maintain plant growth, and the potential to reduce the adverse effects on the environment.

## 4. Conclusion

The conventional soluble P fertilizers diminish the Cd immobilization efficiency of wollastonite. In addition, the over-application of soluble P fertilizers results in low P efficiency and adverse environmental outcomes. Therefore, the design of novel P fertilizers is urgently needed. In the current study, we investigated the effects of adding surfaced modified NHAPs on the uptake of Cd by *A. tricolour* L. during Cd immobilization using wollastonite. The amendment of wollastonite led to a conspicuous reduction of plant Cd uptake, whereas the application of TSP diminished that effect. Alternatively, the addition of NHAP in wollastonite-amended soil not only further promoted plant growth but also further reduced plant Cd uptake. This plant growth-promoting effect of NHAP was confirmed by the two beneficial branches of *Bacteroidetes* (phylum)–*Bacteroidia* (class) and *Alphaproteobacteria* (class)–*Sphingomonadales* (order)–*Sphingomonadaceae* (family). In addition, the application of the three NHAPs promoted the secretion of oxalic acid and citric acid in wollastonite-amended soil. Two new organic acids, lactic and acetic acid, were produced. Further studies are required to explore the appropriate use of NHANP as a P fertilizer in pH-neutral flooded soil, where phosphate decreased the efficacy of wollastonite due to the reduction in Mn or Fe availability.

## CRedit authorship contribution statement

**Rong Huang:** Investigation, Methodology, Validation, Writing – original draft. **Peng Mao:** Investigation, Methodology, Validation. **Lei Xiong:** Investigation, Methodology, Validation, Funding acquisition. **Guoming Qin:** Investigation, Methodology, Writing – review & editing. **Jinge Zhou:** Investigation, Methodology, Writing – review & editing. **Jingfan Zhang:** Investigation, Methodology, Writing – review & editing. **Zhian Li:** Investigation, Methodology, Validation, Writing – review & editing. **Jingtao Wu:** Investigation, Methodology, Validation, Writing – review & editing, Funding acquisition, Supervision.

## Declaration of Competing Interest

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

## Data availability

The authors do not have permission to share data.

## Acknowledgments

The work was financially supported by R & D Program of Guangdong Provincial Department of Science and Technology (2018B030324003), the National Science Foundation of China (31902115), R&D Program of Bureau of Science and Information Technology of Guangzhou Municipality (201903010022), China Postdoctoral Science Foundation (E11511BH01), and Central Public-interest Scientific Institution Basal Research Fund (1610242022002).

## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.jhazmat.2022.130291](https://doi.org/10.1016/j.jhazmat.2022.130291).

## References

- Bolan, N., Kunhikrishnan, A., Thangarajan, R., Kumpiene, J., Park, J., Makino, T., Kirkham, M.B., Scheckel, K., 2014. Remediation of heavy metal(loid)s contaminated soils - To mobilize or to immobilize? *J. Hazard. Mater.* 266, 141–166.
- Chen, S.M., Waghmode, T.R., Sun, R.B., Kuramae, E.E., Hu, C.S., Liu, B.B., 2019. Root-associated microbiomes of wheat under the combined effect of plant development and nitrogen fertilization. *Microbiome* 7, 136.
- Cui, H.B., Yi, Q.T., Yang, X., Wang, X.M., Wu, H.J., Zhou, J., 2017. Effects of hydroxyapatite on leaching of cadmium and phosphorus and their availability under simulated acid rain. *J. Environ. Chem. Eng.* 5, 3773–3779.
- Cui, H.B., Shi, Y., Zhou, J., Chu, H.Y., Cang, L., Zhou, D.M., 2018. Effect of different grain sizes of hydroxyapatite on soil heavy metal bioavailability and microbial community composition. *Agr. Ecosyst. Environ.* 267, 165–173.
- Cui, J.W., Zhu, R.L., Wang, X.Y., Xu, X.P., Ai, C., He, P., Liang, G.Q., Zhou, W., Zhu, P., 2022. Effect of high soil C/N ratio and nitrogen limitation caused by the long-term combined organic-inorganic fertilization on the soil microbial community structure and its dominated SOC decomposition. *J. Environ. Manag.* 303, 114155.
- Dodds, W., Bouska, W.W., Eitzmann, J.L., Pilger, T.J., Pitts, K.L., Riley, A.J., Schloesser, J.T., Thornbrugh, D.J., 2009. Eutrophication of US freshwaters: analysis of potential economic damages. *Environ. Sci. Technol.* 43, 12–19.
- Dong, H.Y., Sun, H.Y., Fan, S.X., Jiang, L.L., Ma, D.R., 2021. Rhizobacterial communities, enzyme activity, and soil properties affect rice seedling's nitrogen use. *Agron. J.* 113, 633–644.
- Duan, P.J., Liu, X.N., Liu, B.H., Akram, M., Li, Y.W., Pan, J.W., Yue, Q.Y., Gao, B.Y., Xu, X., 2021. Effect of phosphate on peroxymonosulfate activation: Accelerating generation of sulfate radical and underlying mechanism. *Appl. Catal. B Environ.* 2 (298), 120532.
- Elsayed, A.A.A., El-Gohary, A., Taha, Z.K., Farag, H.M., Hussein, M.S., AbouAitah, K., 2022. Hydroxyapatite nanoparticles as novel nano-fertilizer for production of rosemary plants. *Sci. Hortic.* 295, 110851.
- Gong, W.J., Niu, Z.F., Wang, X.R., Zhao, H.P., 2021. How the soil microbial communities and activities respond to long-term heavy metal contamination in electroplating

- contaminated site. *Microorganisms* 9, 362.
- Haynes, R.J., Zhou, Y.F., 2018. Competitive and noncompetitive adsorption of silicate and phosphate by two acid Si-deficient soils and their effects on P and Si extractability. *Soil Sci. Plant Nutr.* 64, 535–541.
- He, H.H., Wu, M.M., Su, R., Zhang, Z.K., Chang, C., Peng, Q., Dong, Z.G., Pang, J.Y., Lambers, H., 2021. Strong phosphorus (P)-zinc (Zn) interactions in a calcareous soil-alfalfa system suggest that rational P fertilization should be considered for Zn biofortification on Zn-deficient soils and phytoremediation of Zn-contaminated soils. *Plant Soil* 461, 135–135.
- Holford, I., 1997. Soil phosphorus: its measurement, and its uptake by plants. *Soil Res* 35, 227–240.
- Hong, C.O., Owens, V.N., Kim, Y.G., Lee, S.M., Park, H.C., Kim, K.K., Son, H.J., Suh, J.M., Kim, P.J., 2014. Soil pH effect on phosphate induced cadmium precipitation in arable soil. *B. Environ. Contam. Tox* 93, 101–105.
- Huang, R., Dong, M.L., Mao, P., Zhuang, P., Paz-Ferreiro, J., Li, Y.X., Li, Y.W., Hu, X.Y., Netherway, P., Li, Z.A., 2020. Evaluation of phytoremediation potential of five Cd (hyper)accumulators in two Cd contaminated soils. *Sci. Total Environ.* 721, 137581.
- Jia, T., Liang, X.X., Guo, T.Y., Wu, T.H., Chai, B.F., 2022. Bacterial community succession and influencing factors for *Imperata cylindrica* litter decomposition in a copper tailings area of China. *Sci. Total Environ.* 815, 152908.
- Lambers, H., 2022. Phosphorus acquisition and utilization in plants. *Annu. Rev. Plant Biol.* 73, 17–42.
- Lee, W.H., Loo, C.Y., Zavgorodniy, A.V., Rohanizadeh, R., 2013. High protein adsorptive capacity of amino acid-functionalized hydroxyapatite. *J. Biomed. Mater. Res. A* 101, 873–883.
- Li, B.X., Bao, Y.X., Xu, Y.N., Xie, S.G., Huang, J., 2017. Vertical distribution of microbial communities in soils contaminated by chromium and perfluoroalkyl substances. *Sci. Total Environ.* 599, 156–164.
- Li, C.C., Zhao, L.P., Han, J.J., Wang, R.F., Xiong, C.D., Xie, X.Y., 2011. Synthesis of citrate-stabilized hydrocolloids of hydroxyapatite through a novel two-stage method: A possible aggregates-breakdown mechanism of colloid formation. *J. Colloid Interf. Sci.* 360, 341–349.
- Li, X., Li, X.F., Li, Y.Y., Dai, X.Z., Zhang, Q.Z., Zhang, M., Zhang, Z.Q., Tao, Y., Chen, W.C., Zhang, M.X., Zhou, X.Y., Yang, S., Ma, Y.Q., Zhran, M., Zou, X.X., 2021b. Improved immobilization of soil cadmium by regulating soil characteristics and microbial community through reductive soil disinfestation. *Sci. Total Environ.* 778, 146222.
- Li, Z.M., Liang, Y., Hu, H.W., Shaheen, S.M., Zhong, H., Tack, F.M.G., Wu, M.J., Li, Y.F., Gao, Y.X., Rinklebe, J., Zhao, J.T., 2021a. Speciation, transportation, and pathways of cadmium in soil-rice systems: A review on the environmental implications and remediation approaches for food safety. *Environ. Int.* 156, 160749.
- Liu, W., Li, Y.L., Feng, Y., Qiao, J.C., Zhao, H.W., Xie, J.X., Fang, Y.Y., Shen, S.G., Liang, S.X., 2020. The effectiveness of nanobiochar for reducing phytotoxicity and improving soil remediation in cadmium-contaminated soil. *Sci. Rep.* 10, 858.
- Lu, H.P., Zhuang, P., Li, Z.A., Tai, Y.P., Zou, B., Li, Y.W., McBride, M.B., 2014. Contrasting effects of silicates on cadmium uptake by three dicotyledonous crops grown in contaminated soil. *Environ. Sci. Pollut. Res* 21, 9921–9930.
- Mao, P., Zhuang, P., Li, F., McBride, M.B., Ren, W.D., Li, Y.X., Li, Y.W., Mo, H., Fu, H.Y., Li, Z.A., 2019. Phosphate addition diminishes the efficacy of wollastonite in decreasing Cd uptake by rice (*Oryza sativa* L.) in paddy soil. *Sci. Total Environ.* 687, 441–450.
- Mao, P., Wu, J.T., Li, F., Sun, S., Huang, R., Zhang, L.L., Mo, J.M., Li, Z.A., Zhuang, P., 2022. Joint approaches to reduce cadmium exposure risk from rice consumption. *J. Hazard. Mater.* 429, 128263.
- Montalvo, D., McLaughlin, M.J., Degryse, F., 2015. Efficacy of hydroxyapatite nanoparticles as phosphorus fertilizer in andisols and oxisols. *Soil Sci. Soc. Am. J.* 79, 551–558.
- Qiao, D.M., Han, Y., Zhao, Y.L., 2022. Organic acids in conjunction with various oilseed sunflower cultivars promote Cd phytoextraction through regulating micro-environment in root zone. *Ind. Crop. Prod.* 183, 114932.
- Reichert, T., Rammig, A., Fuchslueger, L., Lugli, L.F., Quesada, C.A., Fleischer, K., 2022. Plant phosphorus-use and -acquisition strategies in Amazonia. *N. Phytol.* 234, 1126–1143.
- Richardson, A.E., Simpson, R.J., 2011. Soil microorganisms mediating phosphorus availability. *Plant Physiol.* 156, 989–996.
- Ruangcharus, C., Kim, S.U., Hong, C.O., 2020. Mechanism of cadmium immobilization in phosphate-amended arable soils. *Appl. Biol. Chem.* 63, 36.
- Shahid, M., 2021. Effect of soil amendments on trace element-mediated oxidative stress in plants: Meta-analysis and mechanistic interpretations. *J. Hazard. Mater.* 407, 124881.
- Sutcliffe, B., Chariton, A.A., Harford, A.J., Hose, G.C., Stephenson, S., Greenfield, P., Midgley, D.J., Paulsen, I.T., 2018. Insights from the Genomes of Microbes Thriving in Uranium-Enriched Sediments. *Microb. Ecol.* 75, 970–984.
- Szameitat, A.E., Sharma, A., Minutello, F., Pinna, A., Er-Rafik, M., Hansen, T.H., Persson, D.P., Andersen, B., Husted, S., 2021. Unravelling the interactions between nano-hydroxyapatite and the roots of phosphorus deficient barley plants. *Environ. Sci. Nano* 8, 444–459.
- Tang, L., Hamid, Y., Zehra, A., Sahito, Z.A., He, Z.L., Khan, M.B., Feng, Y., Yang, X.E., 2020. Mechanisms of water regime effects on uptake of cadmium and nitrate by two ecotypes of water spinach (*Ipomoea aquatica* Forsk.) in contaminated soil. *Chemosphere* 246, 125798.
- Tang, X.X., Liu, M.J., Sheng, J.D., Chai, Z.P., Feng, G., Chen, B.L., 2022. Inorganic phosphorus transformation and phosphorus adsorption-desorption properties of soil in a cotton field under mulched drip irrigation in Xinjiang: a four-year field assessment. *Front. Environ. Sci.* 10, 874902.
- Wang, J., Wang, P.M., Gu, Y., Kopittke, P.M., Zhao, F.J., Wang, P., 2019a. Iron-manganese (oxyhydro)oxides, rather than oxidation of sulfides, determine mobilization of Cd during soil drainage in paddy soil systems. *Environ. Sci. Technol.* 53, 2500–2508.
- Wang, P., Chen, H.P., Kopittke, P.M., Zhao, F.J., 2019b. Cadmium contamination in agricultural soils of China and the impact on food safety. *Environ. Pollut.* 249, 1038–1048.
- Wang, Y.L., Xu, Y.M., Huang, Q.Q., Liang, X.F., Sun, Y.B., Qin, X., Zhao, L.J., 2021. Effect of sterilization on cadmium immobilization and bacterial community in alkaline soil remediated by mercapto-palygorskite. *Environ. Pollut.* 273, 116446.
- Wei, L.L., Chen, S., Cui, J.Y., Ping, H.X., Yuan, C.P., Chen, Q., 2022. A meta-analysis of arable soil phosphorus pools response to manure application as influenced by manure types, soil properties, and climate. *J. Environ. Manag.* 313, 115006.
- Wu, J.T., Dumat, C., Lu, H.P., Li, Y.W., Li, H.Q., Xiao, Y.H., Zhuang, P., Li, Z.A., 2016. Synergistic improvement of crop physiological status by combination of cadmium immobilization and micronutrient fertilization. *Environ. Sci. Pollut. Res.* 23, 6661–6670.
- Wu, J.T., Zhou, Q.Q., Huang, R., Wu, K.J., Li, Z.A., 2021. Contrasting impacts of mobilisation and immobilisation amendments on soil health and heavy metal transfer to food chain. *Ecotoxicol. Environ. Saf.* 209, 111836.
- Wu, Y.Q., Yang, H.M., Wang, M., Sun, L., Xu, Y.M., Sun, G.H., Huang, Q.Q., Liang, X.F., 2022. Immobilization of soil Cd by sulphydryl grafted palygorskite in wheat-rice rotation mode: A field-scale investigation. *Sci. Total Environ.* 826, 154156.
- Xiong, L., Wang, P., Hunter, M.N., Kopittke, P.M., 2018a. Bioavailability and movement of hydroxyapatite nanoparticles (HA-NPs) applied as a phosphorus fertiliser in soils. *Environ. Sci. Nano* 5, 2888–2898.
- Xiong, L., Wang, P., Kopittke, P.M., 2018b. Tailoring hydroxyapatite nanoparticles to increase their efficiency as phosphorus fertilisers in soils. *Geoderma* 323, 116–125.
- Yan, Y.B., Li, Q., Yang, J.J., Zhou, S.Y., Wang, L.J., Bolan, N., 2020. Evaluation of hydroxyapatite derived from flue gas desulphurization gypsum on simultaneous immobilization of lead and cadmium in contaminated soil. *J. Hazard. Mater.* 400, 123038.
- Yang, Y., Huang, Y., Tang, X., Li, Y., Liu, J.N., Li, H.Y., Cheng, X., Pei, X.J., Duan, H.R., 2021. Responses of fungal communities along a chronosequence succession in soils of a tailing dam with reclamation by *Heteropogon contortus*. *Ecotoxicol. Environ. Saf.* 218, 112270.
- Zhang, G.L., Chu, X.B., Zhu, H.Y., Zou, D.S., Li, L.C., Du, L.S., 2021. The response of soil nutrients and microbial community structures in long-term tea plantations and diverse agroforestry intercropping systems. *Sustainability* 13, 7799.
- Zhang, W.H., Sun, R.B., Xu, L., Liang, J.N., Zhou, J., 2019. Assessment of bacterial communities in Cu-contaminated soil immobilized by a one-time application of micro-/nano-hydroxyapatite and phytoremediation for 3 years. *Chemosphere* 223, 240–249.
- Zhang, Z.W., Yuan, W.Y., Li, P.Z., Song, Q.B., Wang, X.Y., Xu, W.T., Zhu, X.F., Zhang, Q.W., Yue, J.W., Bai, J.F., Wang, J.W., 2020. Mechanochemical immobilization of lead contaminated soil by ball milling with the additive of Ca(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub>. *Chemosphere* 247, 125963.