The addition of exogenous low-molecular-weight organic acids improved phytoremediation by *Bidens pilosa* L. in Cd-contaminated soil

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**Abstract**

Enhancing the uptake and enrichment of heavy metals in plants is one of the important means to strengthen phytoremediation. In the present study, citric acid (CA), tartaric acid (TA), and malic acid (MA) were applied to enhance phytoremediation by *Bidens pilosa* L. in Cd-contaminated soil. The results showed that by the addition of appropriate concentrations of CA, TA, and MA, the values of the bioconcentration factor increased by 77.98%, 78.33%, and 64.49%, respectively, the translocation factor values increased by 16.45%, 12.61%, and 5.73%, respectively, and the values of the phytoextraction rates increased by 169.21%, 71.28%, and 63.11%, respectively. The minimum fluorescence values of leaves decreased by 31.62%, 0.28%, and 17.95%, while the potential efficiency of the PSII values of leaves increased 117.87%, 2.25%, and 13.18%, respectively, when CA, TA, and MA with suitable concentration were added. Redundancy analysis showed that CA and MA in plants were significantly positively correlated with plant growth, photosynthesis, and other indicators, whereas TA showed a negative correlation with most indicators. Moreover, CA addition could significantly increase the abundances of *Azotobacter*, *Pseudomonas*, and other growth-promoting bacteria, and the abundance values of *Actinophytocola* and *Ensifer* were improved in TA treatments. Therefore, our results demonstrated that low-molecular-weight organic acids could enhance phytoremediation, and exogenous CA could significantly improve the phytoremediation of Cd-contaminated soil by *Bidens pilosa* L.

**Keywords** Phytoextraction · Photosynthesis · Antioxidant system · Speciation distribution · Soil Microbial community

**Introduction**

Soil pollution by the metal heavy cadmium (Cd) has become more serious due to the rapid development of mining, metal smelting, waste treatment, and other industries (Dai et al. 2021). Cd as a permanent and non-biodegradable pollutant can disturb the natural ecosystem balance and is toxic, carcinogenic, mutagenic, and teratogenic at higher concentrations. Additionally, plants can absorb and accumulate large amounts of Cd, posing a serious threat to human health through enrichment in the food chain (St et al. 2021). It has been reported that the average concentration of Cd ranges from 0.24 to 2.9 mg/kg in agricultural soil in China (Huang et al. 2019; Wei and Yang 2019; Yang et al. 2018). The study found that the average Cd concentration was about 9.45 mg/kg in 384 mining areas in China, and the Cd concentration in the highly polluted mining areas was as high as 63.9 mg/kg (Shi et al. 2022). In Japan, the average Cd concentration of the soil was 0.2–0.3 mg/kg (Akira et al. 2004). In the Appalachian Basin of the eastern United States, highest Cd concentration was determined as 130 mg/kg with the average of 24 mg/kg in the Sunbury Shale (Michele et al. 2009; Perkins and Mason 2015). Therefore, there is more and more urgent for removing Cd pollutant from kinds of contaminated soil. Among various methods used to remove Cd from the soil, phytoremediation is currently receiving increased attention (Niu et al. 2021). However, the remediation efficiency cannot be significantly improved since Cd hyper-accumulators such as *Thlaspi arvense*, *L.*, *Sedum sinense*, and *Brassica juncea* have a small biomass and poor adaptability (Gurajala et al. 2019; Pence et al. 2000; Pham et al. 2013). *Bidens pilosa* L., as a Cd hyper-accumulator with the advantage of large biomass production and a short growth cycle, has...
gradually attracted the attention of Chinese researchers in recent years (Dai et al. 2017; Wei and Zhou, 2008).

It was reported that exogenous artificial chelators could increase the bioavailability of Cd ions by activating heavy metal ions in the soil, thereby enhancing the ability of plants to extract heavy metals (Gul et al. 2021). Nowadays, ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA), and ethylene glycol tetra-acetic acid (EGTA) are widely used due to their strong chelating ability to heavy metals such as Cd, copper (Cu), and zinc (Zn) (Sarwar et al. 2017). However, such artificial chelators cannot be degraded in the soil, and there is a risk of environmental contamination due to the high mobility of heavy metals (Sarwar et al. 2017). Therefore, the selection of environmentally friendly and efficient chelators is the key to improving phytoremediation. To avoid these risks, it is recommended to use biodegradable chelating agents such as n,n-bis(carboxymethyl)-l-glutamic acid tetrasodium salt (GLDA) and ethylenediamine disuccinic acid (EDDS), but the high costs limit their application in restoration projects (Guo et al. 2018).

Low-molecular-weight organic acids (LMWOAs), with molecular weight lower than 900 Da and special molecular structure and charging characteristics, have one or more carboxyl groups, and are important parts of soluble organic matter in soil (Adeleke et al. 2016). LMWOAs mainly come from the degradation of plant residues, the synthesis of root exudates, and the metabolism of microorganisms (Agnello et al. 2014). The monobasic, dibasic, and tribasic fatty acids that can be detected in soil solutions mainly include citric acid (CA), tartaric acid (TA), malic acid (MA), oxalic acid, succinic acid, butyric acid, acetic acid, formic acid, lactic acid, fumaric acid, and malonic acid (Sokolova 2020). LMWOAs, with low toxicity and biodegradability, can form chelates with heavy metals in the soil to increase metal mobility, thereby improving the phytoremediation efficiency (Almaroai et al. 2012; Han et al. 2021; Kim et al. 2010). In a previous study, the application of CA could increase sunflower biomass and effectively improve the uptake and translocation of uranium and Cd (Chen et al. 2020b). Similarly, the addition of CA, TA, and oxalic acid could increase the Cu concentration in the plant body of castor beans (Huang et al. 2020), whereas gallic acid could increase the metal content of *Brassica juncea* and induce the removal of Cd, Cu, Zn, and nickel (Ni) from the soil without increasing the leaching risk (Nascimento et al. 2006). These findings suggest that LMWOAs are safe, environmentally friendly, biodegradable, and can be used as reasonable metalchelating agents.

Although many studies suggest that exogenous LMWOAs can promote phytoremediation, there are few studies on whether LMWOAs can promote Cd absorption and accumulation by *Bidens pilosa* L. In particular, most studies ignored the effects of LMWOA addition on microbial community characteristics and soil enzyme activities in phytoremediation, and the effects of LMWOA addition on plant photosynthesis have also been largely ignored. Moreover, the influences of LMWOA concentration in plants on the physiological characteristics (e.g., photosynthesis) and the correlation between the concentration and various indicators are not fully understood. In the present study, we enhanced the capacity of *Bidens pilosa* L. to remediate Cd-contaminated soil by adding exogenous LMWOAs. The main objectives were as follows: (1) to investigate the effects of exogenous LMWOAs on various physiological and biochemical characteristics of *Bidens pilosa* L. in Cd-contaminated soil and to clarify the correlation between the contents of LMWOAs in plants and various physiological indicators; (2) to evaluate whether the application of exogenous LMWOAs can enhance the remediation ability of *Bidens pilosa* L. in Cd-contaminated soil; (3) to explore the effects of exogenous LMWOA addition on the rhizosphere microbial composition and enzyme activities and to analyze the correlation between these factors and the contents of LMWOAs in the roots.

**Materials and methods**

**Soil sample preparation**

The experimental soil was taken from the wheat field in Tongshan County, Xuzhou City, Jiangsu Province, China (N34.18, E117.17). The surface soil (0–20 cm) was sampled consistently, and rocks, plant residues, and other impurities were carefully removed. After drying, the soil was passed through a 2-mm nylon sieve and stored for subsequent experimental use. The physical and chemical properties of the soil were determined and were as follows: total Cd concentration, 0 mg/kg; pH value, 6.19; organic matter, 1.39%; available phosphorus and potassium, 15 and 20 mg/kg, respectively. Subsequently, CdCl$_2$·2.5H$_2$O was added to the soil to simulate Cd contamination. Briefly, the water solution containing Cd was mixed thoroughly with the appropriate amount of soil, and the final Cd concentration of the soil was 32 mg/kg (Sun et al. 2009). The mixed and polluted soil was left for 60 days to balance the aging of soil contaminants. All reagents used in this study were of analytical grade and were purchased from Zhejiang Changqing Chemical Co., Ltd.

**Experimental design**

*Bidens pilosa* L., an annual herb reaching a height of 30–100 cm, is a potential hyper-accumulator of Cd and widely used to remediate Cd-polluted soil in China. The soil prepared as above was used for the pot experiment. First, approximately 1000 g of dry contaminated soil was placed in
a plastic pot with a diameter of 17 cm and a height of 8.5 cm, and a tray was placed at the bottom for leachate reflux to prevent the loss of Cd and nutrients. Subsequently, seeds of Bidens pilosa L. were soaked and disinfected in 70% alcohol for 10 min, followed by washing with purified and sterile water. Eight seeds with the same shape and size were sown in each pot, and the surface layer was covered with 1.5 cm of soil. The plants were cultured in a growth chamber with fluorescent light with an intensity of 200 μmol·m⁻²·s⁻¹ at a photoperiod of 12 h of light and 12 h of dark. The maximum and minimum culture temperatures were 25 and 20 °C, respectively, at a relative air humidity of 65%. At 2 weeks after germination, three seedlings with similar size were kept in each pot. During cultivation, the soil moisture content was maintained at 60%, the soil water holding capacity was about 30%, and water was added every 5 days (40 mL each time).

In the experimental group, CA, TA, MA were used as the three exogenous LMWOAs at concentrations of 1, 5, and 15 mmol/kg, respectively. The LMWOAs were added to the soil in the form of an aqueous solution. In the first sowing, organic acid solution with an appropriate concentration was mixed into the soil. During the entire growth cycle of the plant, a total of three applications of LMWOAs were conducted at a frequency of 15 days. Additionally, Cd-free (CKK) and Cd-contaminated (CK) soils were used as controls with water instead of LMWOAs. Our study involved a total of 11 sets of experiments, each containing five replicates, with a total of 55 pots. After 6 weeks of treatment, plants were harvested for further physiological characterization analysis.

**Measurement of plant physiological characteristics**

The content of chlorophyll in fresh leaves was determined via the anhydrous ethanol extraction method (Parker et al. 2016). The activities of superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) levels of the plant were determined by NBT colorimetry, UV spectrophotometry, and the guaiacol method, respectively (Lu et al. 2009). The content of malondialdehyde (MDA) was measured via the thiobarbituric acid (TBA) method (Heath and Packer 1968). The glutathione (GSH) content was determined using 5,5-dithio-2,2-dinitrobenzoic acid (DTNB) (Foyer and Halliwell 1976), and the total soluble protein (Tsp) were measured by Coomassie Bright Blue (G-250) staining (Maehre et al. 2018). The content of proline (Pro) was determined by the sulfosalicylic acid method (Bananfshah and Soleymani 2021), and the levels of CA, MA, and TA in plant leaves and roots were analyzed using high-performance liquid chromatography (Montiel-Rozas et al. 2016). The content of superoxide anion (O²⁻) and hydrogen peroxide (H₂O₂) was determined by the chloroform extraction method and the potassium iodide method, respectively (Gupta and Seth, 2019). The detailed procedures of all these analytical methods were demonstrated in supplementary materials.

**Chlorophyll fluorescence parameter measurement**

Chlorophyll fluorescence parameters and fast light response curves were obtained by using an imaging pulse amplitude-modulated fluorometer (IMAG-max/L; Walz, Germany). The samples of the experimental group were adapted to dark conditions for 20 min, and subsequently, three leaves were picked from the plants of each experimental group and placed in a pulse amplitude-modulated fluorometer for measurement. Chlorophyll fluorescence parameters of PSII, including minimum fluorescence (F⁰) and maximum fluorescence (Fₘ), were measured, and the potential efficiency of the PSII (Fₚ/F⁰) and the maximum quantum efficiency of PSII photochemistry (Fₚ/Fₘ) were determined (Liu et al. 2018). The fast light response curves included the actual photochemical quantum yield (Y(II)), adjusting quantum yield for energy dissipation (Y (NPQ)), quantum yield of unregulated energy dissipation (Y (NO)), and the photosynthetic electron transfer rate (ETR) (Kim et al. 2014; Lefebvre et al. 2011); we used the following equations:

\[
F_v = F_m - F_0
\]
\[
F_v/F_0 = (F_m - F_0)/F_0
\]
\[
F_v/F_m = (F_m - F_0)/F_m
\]
\[
Y(II) = \Delta F_v/F'_m = (F'_m - F_t)/F'_m
\]
\[
ERT = PFD \times AF \times \Delta F/F'_{m(MT)} \times FII
\]
\[
Y(NPQ) = 1 - Y(II) - 1/(NPQ + 1 + qL(F_v/F_0 - 1))
\]
\[
Y(NO) = 1/(NPQ + 1 + qL(F_v/F_0 - 1))
\]

**Determination of heavy metal contents**

**Cd determination in plants**

The Bidens pilosa L. plants were harvested in the 10th week of growth for Cd determination. We separated the aboveground from the belowground parts and washed, dried, and weighted the parts in preparation for the Cd determination. Subsequently, the dried tissue was placed in 10 mL 65% HNO₃, and digested on an electric heating plate at 180°C (Lu et al. 2020). After digestion, the mixture was diluted with ultrapure water when the liquid was colorless and transparent, and it was filtered through a 0.22-μm filter membrane before concentration determination. All glassware used in the experiments was soaked in 30% HNO₃ for 24 h and
rinsed with ultrapure water before using. The Cd concentration in plants was determined by flame atomic absorption spectrometry with an FAAS instrument (iCE3300, Thermo Scientific).

**Cd speciation distribution analysis**

The form of Cd in soil was analyzed by the European Community Bureau of Reference (BCR) continuous extraction method (Wu et al. 2016). In the first step, 1.0 g of dry soil was weighed into a 100 mL polypropylene centrifuge tube, added to the CH₃COOH extract, shaken at 26 °C, 250 rpm for 16 h, and then centrifuged for 20 min. The supernatant was collected for the determination of Cd in the weak acid-extracted state. And then ultrapure water was added into the above residue to shake and clean the residue, and the cleaning solution was filtered off to leave the residue. In the second step, NH₂OH·HCl was added to the residue from the previous step and shaken for 16 h. After centrifugation, the supernatant was collected for the determination of oxidizable Cd content, and the remaining operations are the same as the previous step. In the third step, add 30% H₂O₂ (pH = 2.5) to the residue in the previous step, cover with a watch glass and place it in a water bath at 85 °C for digestion for 1 h, then open the lid and continue digestion until the solution is nearly dry (liquid volume ≤ 3 mL). when the liquid is nearly dry, add H₂O₂ and CH₃COONH₄ (pH = 2) for extraction, after centrifugation, collect the supernatant for the determination of oxidizable Cd content, and the remaining operations are the same as the previous step. In the fourth step, transfer the residue from the previous step to a 50-mL polytetrafluoroethylene beaker, add HNO₃, HF and HClO₄ to cover, put it on an electric hot plate for digestion, digest to colorless and clear, and then use ultrapure water to make up the volume. After digestion, the solution was diluted with ultrapure water to a constant volume and filtered through a 0.22-μm filter membrane. The concentration of Cd in the solution was determined by flame atomic absorption spectrometry with an FAAS instrument (iCE3300, Thermo Scientific).

**Parameter calculation**

The Cd bioconcentration factor (BCF) is the ratio of the Cd concentration in Bidens pilosa L. (mg kg⁻¹ dry weight) to the Cd concentration in the soil (mg/kg). The translocation factor (TF) is the ratio of the Cd concentration in the shoots to that in the roots (mg/kg dry weight). The phytoextraction rate (PR) is the ratio of the Cd content in the whole plant to that in the soil (Yang et al. 2020).

**Determinations of soil enzyme activity**

Soil urease, sucrase, protease, catalase, and phosphatase activities were analyzed by the sodium phenol colorimetric, 3,5-dinitrosalicylic acid colorimetric, ninhydrin colorimetric, and potassium permanganate titration and phenyl disodium phosphate colorimetry method, respectively (Ge et al. 2009). The detailed experimental methods for the determination of soil enzyme activities were demonstrated in supplementary materials.

**DNA extraction, 16 s RNA amplification, and high-throughput sequencing**

Microbial DNA was extracted using the E.Z.N.A.® soil DNA Kit (Omega Bio-tek, Norcross, GA, USA), and concentration and purity were determined with a NanoDrop 2000 UV–vis spectrophotometer (Thermo Scientific, Wilmington, USA). The hypervariable region V3–V4 of the bacterial 16S rRNA gene was amplified with primer pairs 338F (5’-ACTCTCTACGGGAGGCAGCAG-3’) and 806R (5’-GGA CTACHVGGGTWTCTAAT-3’) by an ABI GeneAmp® 9700 PCR thermocycler (ABI, CA, USA). Purified amplions were pooled in equimolar ratios and paired-end sequenced on an Illumina MiSeq PE300 platform/NovaSeq PE250 platform (Illumina, San Diego, USA) according to the standard protocols by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China).

**Statistical analysis**

For one-way ANOVA, the IBM SPSS Statistics 20 was used and the significance of the treatments was evaluated by Student’s t test (n = 3, p ≤ 0.05). The correlation between various soil enzyme activities, plant physiological indicators, and the concentration of LMWOAs in the plant body was assessed using partial redundancy analysis (RDA) in CANOCO 5 (n = 3, p ≤ 0.05). The Origin 2018 software was used for the drawing of graphs.

**Results**

**Plant growth and biomass production**

Plant leaf area increased with increasing CA concentration and decreased with increasing TA and MA concentrations (Fig. 1). Under Cd stress (CK), root, shoot, and plant biomass decreased by 64.66%, 24.86%, and 39.19%, respectively. After the application of CA at different concentrations, root, shoot, and plant biomass increased significantly compared with CK, namely by 153.96%, 38.39%, and 64.29%, with the addition of 15 mmol/kg CA. However, the
addition of TA and MA at low concentrations significantly facilitated plant growth. Root, shoot, and plant biomass increased by 107.74%, 14.27%, and 31.86%, respectively, when 1 mmol/kg TA was added, and increased by 57.86%, 38.26%, and 45.03%, respectively, after the addition of 1 mmol/kg MA compared with CK. Low concentrations of CA, TA, and MA could also stimulate plant stem growth, which increased by 29.81%, 28.45%, and 49.81%, respectively, under the addition of 1 mmol/kg CA, TA, and MA (Table 1).

Chlorophyll fluorescence parameters and fast light response curves

The addition of LMWOAs with higher concentration could significantly improve the plant chlorophyll content (Fig. S1). The $F_0$ and $F_m$ values under Cd stress increased by 74.63% and 6.33%, respectively, whereas the $F_v/F_m$ and $F_v/F_0$ values decreased by 31.53% and 63.84%, respectively (Table 2). The addition of CA could reduce the values of $F_0$ and $F_m$ and elevate those values.

Table 1 The effects of exogenous LMWOAs addition on the biomass of Bidens pilosa L.

| Treatments | Root dry weight (mg) | Shoot dry weight (mg) | Plant dry weight (mg) | Plant stem length (cm) |
|------------|----------------------|-----------------------|-----------------------|------------------------|
| CKK        | 123.23 ± 9.42 g      | 254.37 ± 7.65 cd      | 377.60 ± 16.30e       | 11.33 ± 0.15bc         |
| CK         | 43.55 ± 11.55ab      | 191.13 ± 17.28a       | 229.6 ± 21.24a        | 10.30 ± 0.1a           |
| CA1        | 78.85 ± 7.00d        | 242.60 ± 14.15c       | 329.35 ± 12.09 cd     | 13.37 ± 0.42f          |
| CA5        | 87.90 ± 11.44e       | 259.20 ± 18.95 cd     | 347.70 ± 35.07d       | 12.53 ± 0.81de         |
| CA15       | 110.60 ± 4.24f       | 264.50 ± 2.97d        | 377.20 ± 7.21e        | 11.73 ± 0.25bc         |
| TA1        | 90.47 ± 10.95e       | 218.40 ± 13.01b       | 302.75 ± 9.12c        | 13.23 ± 0.51ef         |
| TA5        | 53.375 ± 11.30b      | 213.70 ± 6.51b        | 264.15 ± 9.83b        | 11.00 ± 0.62ab         |
| TA15       | 37.7 ± 5.46ab        | 195.05 ± 22.13a       | 230.70 ± 28.00a       | 10.47 ± 0.25a          |
| MA1        | 68.75 ± 1.34 cd      | 264.25 ± 3.61d        | 333.00 ± 4.95 cd      | 15.43 ± 0.15 g         |
| MA5        | 60.57 ± 8.21c        | 224.65 ± 0.21b        | 278.10 ± 20.53b       | 12.07 ± 0.21 cd        |
| MA15       | 34.10 ± 3.96a        | 180.30 ± 5.66a        | 214.40 ± 9.62a        | 11.43 ± 0.55bc         |

CKK is the control with same amount of clean water addition and Cd-free in the soil; CK is the control with same amount of clean water addition and same amount of Cd in the soil; CA1, CA5, CA15 mean the treatment of 1 mmol/kg, 5 mmol/kg, 15 mmol/kg citric acid addition, respectively; TA1, TA5, TA15 mean the treatment of 1 mmol/kg, 5 mmol/kg, 15 mmol/kg tartaric acid addition, respectively; MA1, MA5, MA15 mean the treatment of 1 mmol/kg, 5 mmol/kg, 15 mmol/kg malic acid addition, respectively. These abbreviations in the next table show the same meaning.
of \( F_v/F_m \) and \( F_0/F_0 \). At a dose of 15 mmol/kg CA, the values of \( F_0 \) and \( F_m \) decreased by 31.62% and 39.68%, whereas the \( F_v/F_m \) and \( F_0/F_0 \) values were 1.36 and 2.18 times those of CK. With increasing CA concentrations, the blue color deepened in the CA treatment, whereas the green color deepened in the treatment with TA and MA.

### Cd uptake by the plants

Metal accumulation was evaluated to reveal the effects of LMWOAs on Cd uptake in *Bidens pilosa* L. The Cd accumulated in roots and shoots increased gradually with the application of CA, TA, and MA from low to high concentrations. The Cd concentrations in roots and shoots were 75.22 and 109.49 mg/kg in the treatment of CK (Fig. 3a, b). The Cd concentrations in roots were 109.65, 152.07, and 108.07 mg/kg, and that in shoots were 186.38, 161.94, and 155.30 mg/kg, respectively, when 15 mmol/kg CA, TA, and MA were added. The transport, absorption, and enrichment of Cd by *Bidens pilosa* L. varied greatly with the application of LMWOAs. The TF value increased continuously with increasing CA concentrations, with an increase by 16.45% with the addition of 15 mmol/kg CA (Table 3). However, the TF value increased by 12.61% and 5.73%, respectively, with the addition of 1 mmol/kg TA and MA and decreased by 27.03% and 1.55%, respectively, after the application of 15 mmol/kg TA and MA. The PR value increased gradually with increasing CA concentrations and was 169.21% higher in the treatment with the addition of 15 mmol/kg CA. Higher PR values were found in the plants treated with 5 mmol/kg TA and MA, increasing by 71.28% and 63.11%, respectively. The BCF value increased continuously with increasing CA, TA, and MA concentrations, with 77.98%, 78.33%, and 64.49% higher levels, respectively, with the addition of 15 mmol/kg.

### Speciation distribution of Cd in the soil

The acid-soluble, reducible, oxidizable, and residual states of Cd accounted for 38.60%, 12.73%, 12.89%, and 35.79% significantly affected the speciation distribution of Cd in the soil and enhanced the proportions of the acid-soluble state. The proportions of acid-soluble Cd in the soil increased by 10.26%, 6.78%, and 6.58%, respectively, whereas those of

| Treatments | \( F_0 \) | \( F_m \) | \( F_v/F_m \) | \( F_0/F_0 \) |
|------------|------------|------------|---------------|---------------|
| CKK        | 0.067 ± 0.004f | 0.237 ± 0.028ab | 0.739 ± 0.014f | 2.832 ± 0.208f |
| CK         | 0.117 ± 0.001a | 0.252 ± 0.021a | 0.506 ± 0.012abc | 1.024 ± 0.047ab |
| CA1        | 0.083 ± 0.001e | 0.180 ± 0.018g | 0.672 ± 0.001e | 1.999 ± 0.071d |
| CA5        | 0.093 ± 0.001d | 0.179 ± 0.005g | 0.683 ± 0.015e | 2.033 ± 0.059de |
| CA15       | 0.080 ± 0.004e | 0.152 ± 0.006h | 0.690 ± 0.016e | 2.231 ± 0.174e |
| TA1        | 0.117 ± 0.001a | 0.353 ± 0.001c | 0.511 ± 0.010bc | 1.047 ± 0.040ab |
| TA5        | 0.125 ± 0.002b | 0.393 ± 0.023d | 0.480 ± 0.008ab | 0.869 ± 0.062ac |
| TA15       | 0.133 ± 0.003c | 0.416 ± 0.012d | 0.440 ± 0.002d | 0.787 ± 0.006c |
| MA1        | 0.096 ± 0.002d | 0.187 ± 0.002eg | 0.533 ± 0.054c | 1.159 ± 0.239b |
| MA5        | 0.114 ± 0.003a | 0.225 ± 0.005bf | 0.477 ± 0.019ab | 0.915 ± 0.068ac |
| MA15       | 0.120 ± 0.003a | 0.266 ± 0.006ef | 0.473 ± 0.002ad | 0.898 ± 0.007ac |
Fig. 2 The effects of exogenous LMWOAs addition on chlorophyll fluorescence parameters of *Bidens pilosa* L. a \(F_{v}/F_{m}\) image; b photosynthetic electron transfer rate (ETR); c actual photochemical quantum yield (Y(II)); d adjusting quantum yield for energy dissipation (Y(NPQ)); e quantum yield of unregulated energy dissipation (Y(NO))
the reducible state decreased by 3.00%, 2.97%, and 0.95%. The proportions of the residual state decreased by 8.00%, 4.07%, and 5.86%, respectively, when 15 mmol/kg CA, TA, and MA were added. However, no significant difference was found in the proportions of oxidizable Cd with the application of LMWOAs.

Soil bacterial community structure

To explore the influences of the three LMWOAs on the rhizosphere bacterial composition under Cd stress, bacterial 16Sr DNA was sequenced in the experiments treated with 15 mmol/kg CA, TA, and MA. The Chao, Ace, and Shannon index values of the rhizosphere bacterial communities decreased by 9.79%, 8.39%, and 3.16%, respectively, under Cd stress (Table 4). The application of 15 mmol/kg CA, TA, and MA reduced the diversity and abundance of rhizosphere bacteria, and the inhibitory effect of CA was the weakest. The values of the Chao, Ace, and Shannon indices decreased by 6.39%, 5.38%, and 4.28%, respectively with CA addition, and decreased by 21.85%, 21.59%, and 11.28%, respectively, with TA application. When MA was added, they decreased by 11.68%, 10.57%, and 5.15%, respectively. The percentages of Proteobacteria, Bacteroidoia, and Gemmatimonadota of rhizosphere bacteria decreased by 10.16%, 2.78%, and 2.21%, whereas those of Acidobacteriota and Chloroflexi increased by 8.96% and 4.84%, respectively, under Cd stress (Fig. 5a). With the application of LMWOAs, the proportions of Proteobacteria and Bacteroidota increased significantly, whereas those of Acidobacteriota, Chloroflexi, and Gemmatimonadota decreased compared to CK.

The proportions of the genera *Ensifer*, *Ramlibacter*, *Devosia*, and *Pseudoxanthomonas* in the rhizosphere soil were reduced due to Cd stress, whereas they were enhanced by the application of LMWOAs (Fig. 5b). Interestingly, the
abundances of *Actinophytocola*, *Azotobacter*, *Pseudomonas*, and *Ramlibacter*, which were extremely low in the CKK and CK treatments, were high in the treatments with LMWOA addition. The three LMWOAs had different impacts on the bacterial genera in the rhizosphere. The addition of CA could significantly increase the abundances of *Azotobacter* and *Pseudomonas*, whereas the abundance levels of *Actinophytocola* and *Ensifer* were improved in the TA treatments.

**Redundancy analysis**

The CA, TA, and MA levels in *Bidens pilosa* L. varied with the addition of exogenous LMWOAs, and the contents in the plant leaves were determined (Fig. S2). The antioxidant levels (CAT, SOD, GSH), osmotic adjustment substance levels (Tsp), and oxidative damage index levels (MDA, $H_2O_2$, $O_2^-$, Pro) of *Bidens pilosa* L. were also measured after exogenous LMWOA addition (Fig. S3, S4, S5). The correlation between LMWOAs in plants and the related parameters was described by redundancy analysis (Fig. 6a). In the RDA diagram, axis 1 explained 57.02% of the variation of the physiological index, and axis 2 explained 18.25% of the variation. In the photosynthetic system, CA and MA addition was significantly negatively correlated with $F_m$ and $F_o$ and positively correlated with $F_v/F_m$ and $F_v/F_o$. In the antioxidant system, CA and MA addition was significantly positively correlated with SOD and GSH and negatively correlated with MDA, $H_2O_2$, Pro, and $O_2^-$. In the osmotic regulation system, CA and MA additions were significantly positively correlated with total soluble protein levels.

The contents of CA, MA, and TA in plant roots and rhizosphere soil enzyme activity (sucrase activity, urease activity, protease activity, phosphatase activity, catalase activity) were also determined (Fig. S6, S7), and there were correlations between LMWOAs in plant roots and soil enzyme activities and bacterial abundance (Fig. 6b). In the RDA diagram, axis 1 explained 59.25% of the variation in the physiological index, and axis 2 explained 13.83% of this variation. The addition of CA and MA was positively correlated with protease, urease, and phosphatase levels, whereas TA addition was positively correlated with sucrose levels. The application of CA, TA, and MA could reduce catalase activity in the soil. Based on Pearson’s correlation heat map analysis, LMWOAs and soil enzymes had similar effects on bacterial phyla and genera (Fig. 6c, d), and it can be concluded that CA, MA, and protease have similar effects on bacterial communities. The effect of TA on the bacterial community was similar to those of urease, phosphatase, and sucrase.

**Discussion**

**Exogenous LMWOAs improve photosynthesis and antioxidant activity under Cd stress**

Cadmium ions (Cd$^{2+}$) in the soil, when entering the plant body, can inhibit chlorophyll synthesis, weaken photosynthesis, produce a large amount of reactive oxygen species (ROS), and cause lipid peroxidation, cell inactivation, and other irreversible damage (Ashraf and Harris 2013). In previous studies, the levels of Chla, Chlb, and Caro were increased in *Phytolacca americana* (Liu et al. 2015) and *Brassica juncea* (Kaur et al. 2017a) with CA addition, which is in agreement with our finding, most likely because CA can inhibit the expression of the chlorophyll catabolism enzyme (CHLASE) and promote the expression of phytoene synthase (PSY) (Chen et al. 2020a). The lower value of Y(NO) illustrates the stronger ability of plants to dissipate too much light energy. In the present study, the increased $F_v/F_m$, $F_o/F_m$, and Y(NO) values under Cd stress indicated that Cd caused irreversible damage to the PSI reaction center, whereas exogenous CA addition significantly reduced the $F_o$, $F_m$, and Y(NO) values. This might explain why CA in plants can be used as a chelating agent to fix Cd in vacuoles and to limit the transport in the cytoplasm (Danijela et al. 2019), which can protect the photosynthetic reaction center of the chloroplast from damage by ROS. Additionally, exogenous CA can also mitigate ROS damage in plants by increasing the content of antioxidants such as GSH, polyphenols, and total flavonoids (Kaur et al. 2017b), and our results also showed that the addition of appropriate CA levels could significantly enhance SOD activity, GSH, as well as Tsp and Pro contents (Fig. S3).

In our study, the MA content in the plant body was positively correlated with the values of $F_v/F_m$ and $F_v/F_o$ at a
certain concentration, and MA addition could also significantly increase the plant chlorophyll content (Fig. S1), most likely because the application of exogenous MA can promote the transport of more magnesium ions (Mg\(^{2+}\)) to the plant leaves (Feng et al. 2012). According to a previous study, magnesium (Mg) is the central atom in chlorophyll and plays a crucial role in chlorophyll biosynthesis (Tränkner et al. 2018). In addition, MA in the plant body could also produce carbon dioxide through malate dehydrogenation (MDH) decarboxylation, mediate carbon fixation, and then participate in photosynthesis (Chen et al. 2020c). These mechanisms of action promote an increase in the chlorophyll content, consequently facilitating strengthening photosynthesis. Moreover, MA also had a significantly positive correlation with CAT, SOD, and other antioxidant enzyme activities since the addition of MA could promote the expression levels of Cu/Zn-SOD, POD1, CAT1, and other antioxidant genes in leaves (Guo et al. 2017), which could increase the contents of antioxidant enzymes in plants.

In addition, low concentrations of TA could facilitate photosynthesis and antioxidant abilities, which is consistent with the findings that the chloroplast structure of *Salix Variegata* was intact, the number of chloroplasts was significantly increased, and the activity of antioxidant enzymes was stimulated after the application of TA under heavy metal stress (Chen et al. 2020a). The antioxidant properties of plants are increased when cichoric acid, a derivative of TA with biological activity, forms complexes with metal cations (Świderski et al. 2020). Exogenous TA can improve plant antioxidant activity by producing cichoric acid under heavy metal stress.

Therefore, the addition of appropriate concentrations of CA, TA, and MA could increase plant chlorophyll content, reduce the damage to the photosynthetic reaction center, and enhance photosynthesis. This approach could also improve the activities of SOD, CAT, and POD and release a variety of antioxidant substances (GSH, Tsp, Pro), which interact with H\(_2\)O\(_2\) to inhibit the production of O\(^{2-}\) and strengthen the plant’s antioxidant capacity (Hawrylak-Nowak et al. 2015).

**Exogenous LMWOAs promote the absorption of Cd by plants**

The Cd\(^{2+}\) absorbed by plant roots are gradually transported to the aboveground plant parts via transpiration and accumulate in the plant body (Naem et al. 2018; Schwalbert et al. 2021). Our results showed that the CA, TA, and MA concentrations in plant leaves and roots were significantly increased with the addition of exogenous LMWOAs (Fig. S2, S6). The application of LMWOAs at appropriate doses could increase the TF and BCF values and promote Cd accumulation in the plant body (Table 3); this was obvious in the treatments with CA and MA addition. First, CA and MA in plants, combined with metal ions, can form negatively charged or uncharged chelates, and the negatively charged xylem of plants has a low adsorption of chelates, which can promote the transfer of metal ions to plant leaves (Wilfried 1999). Therefore, adequate CA and MA addition could promote the transportation of metal ions from belowground to aboveground plant parts. Second, Cd\(^{2+}\) in the plant body can activate the antioxidant defense system, and cell wall fixation, cell membrane transport, cytoplasmic chelation, and vacuolar separation are performed (Gallego et al. 2012). The Cd\(^{2+}\) and LMWOAs in the cytoplasm can form Cd-organic acid chelates, which can be moved to the vacuole through the transporter; regional separation of heavy metals is achieved and toxicity is reduced (Panchal et al. 2021). In a previous study, exogenous CA addition could enhance detoxification by increasing the contents of metal chelate GSH and the metal-binding protein phytochelatin (Ehsan et al. 2014). The expression level of the transporter plays a key role in the cell membrane transport of metals (Gallego et al. 2012). In a similar study, exogenous CA could also increase the expression of Cd transport genes such as *NRAMP5*, *MTP1*, and *HMA1* by 6.96, 2.82, and 2.28 times, respectively, and MA could significantly promote the expression levels of *NRAMP5* and *PCS1* in leaves of *Salix variegata* Franch (Zhang et al. 2020). Therefore, CA and MA addition could increase metal transportation by mediating the expression of transporters. However, the application of exogenous TA significantly reduced the gene expression levels of *MTP1*, *HMA3*, *MT2B*, and other transporter genes (Zhang et al. 2020). Our results also showed that the TF value gradually decreased with the addition of high concentrations of TA, inhibiting the transport of Cd from plant roots to shoots and consequently leading to Cd accumulation in the roots.

In our redundancy analysis, a significantly positive correlation was found between PR and plant biomass (Fig. 6a, b). Moreover, PR increased by 169.21%, and root and shoot biomass increased by 153.96% and 38.39%, respectively, with the addition of 15 mmol/kg CA. However, plant biomass was decreased with the addition of high concentrations of TA and MA. Based on previous results, CA, TA, and MA...
around roots can promote plant growth by providing available ion compounds and depolymerizing high-molecular-weight humus (Chen et al. 2020d). Also, CA can mobilize external nutrients, supply more carbon to plant roots, and improve N₂ fixation (Wang et al. 2019). Additionally, CA in the plant can serve as a substrate for the synthesis of endogenous hormones, amino acids, fatty acids, and other metabolites that protect the plant from stress and promote plant growth (Tahjib-Ul-Arif et al. 2021).

In the present study, CA, TA, and MA addition could promote the transformation of Cd in the soil and enhance the acid-soluble state (Fig. 4). The form of heavy metals in the...
soil determines the extent to which plants can absorb them, and for Cd, the weak acid extraction state represents the form with the strongest activity and bioavailability (Wang et al. 2016). Previous studies demonstrated that LMWOAs might play different roles in controlling the process of adsorption, transport, leaching, and release of heavy metal ions in environmental media (Geng et al. 2020; Ghasemi-Fasaei et al. 2020). The addition of LMWOAs improved the Cd migration efficiency in the process of heavy metal leaching and adsorption in saturated porous media. Intuitively, the addition of LMWOAs can promote the deposition of more positively charged metal ions on the dielectric due to electrostatic attraction (Zhang et al. 2022). However, the transport enhancement effects may largely attribute to the molecules of LMWOAs, which have different amounts of hydroxyl and carboxyl groups (−COOH and −OH) negative charge and these will be complexed with metal cations to form stable aqueous non-adsorbing metal-LMWOA complexes (Kong et al. 2018). These complexes are more fluid, and have a higher bioavailability, which can improve the concentration of Cd in the root (Diarra et al. 2021). Therefore, the enhancement of heavy metal migration largely depends on the differences in molecular structure (i.e., functional group abundance and type) and the complexing capability of the LMWOAs (Zhang et al. 2019). Our results showed that the content of Cd with weakly acid-extracted state in the soil and the Cd content in the plant body was highest with the addition of CA, because CA, with the most significant effect on metal leaching, had three −COOH groups and one −OH oxygen-containing functional group (Zhang et al. 2019). It was also reported that much higher Cd release from contaminated soils was detected in the addition of CA than TA, acetic acid, and oxalic acid, which was consistent with our findings (Ghasemi-Fasaei et al. 2020). Additionally, the addition of exogenous LMWOAs can also improve pH value in soil, which can dissolve and release metal ions co-precipitated with carbonate minerals, and increase heavy metal mobility (Agnello et al. 2014; Ma et al. 2020). Therefore, the addition of LMWOAs can enhance the amount of absorbable Cd$^{2+}$ ions in Cd-contaminated soil, and then promote phytoextraction efficiency.

**Exogenous LMWOAs increase soil enzyme activities and change microbial communities under Cd stress**

Soil enzymes are sensitive biological indicators of environmental changes, and enzymes used to evaluate soil quality are divided into two categories (Deng et al. 2015). The first category contains extracellular hydrolase, which is mainly involved in biological metabolism and nutrient cycling. Our results showed that the activities of protease, urease, sucrase, and phosphatase in the soil were enhanced after the application of LMWOAs (Fig. S7), which was consistent with the results reported elsewhere (Huang et al. 2020). In addition, the enhancement in soil enzyme activity also explained the increased plant growth. The second category directly participates in the detoxification of heavy metals and mainly includes catalase, polyphenol oxidase, and dehydrogenase (Du et al. 2021). Catalase not only catalyzes the decomposition of hydrogen peroxide to reduce toxicity, but it also transforms metal ions from a high to a low toxic state and fixes them on the cell surface (Yang et al. 2016). Based on our results, the catalase activity in soil was decreased with the addition of LMWOAs, since LMWOAs changed the availability of Cd ions and the microorganisms would consume more oxidoreductase to capture or precipitate metal ions (Tang et al. 2020).

In addition to enzyme activities, soil physical and chemical properties also play important roles in life processes, soil ecosystems, and plant health (Abdul Khalil et al. 2015). The extent to which these physicochemical properties change beyond their respective thresholds have significant impacts on soil ecosystems, and ultimately affect the efficiency of phytoremediation. The application of LMWOAs to contaminated soil can change soil pH, promote the increase of soluble heavy metal concentration, and improve the bioavailability of metal ions (Evangelou et al. 2006). Meanwhile, it was reported that the complexation of metals and LMWOAs can strongly alter the fixation of metals by soil organic matter, oxides, and clays, and increase the total amount of dissolved cations in the soil (Ammar et al. 2009). Thus, such properties can be used in phytoremediation and minimize the environmental risk.

LMWOAs as plant root exudates play a key role in the composition of the rhizosphere microbial community (Sasse et al. 2018). Our results showed that CA, TA, and MA addition could change soil diversity and abundance, and the application of CA significantly increased the levels of *Proteobacteria*, *Actinobacteria*, and *Bacteroidota* (Fig. 5). Also, CA addition could effectively enhance the activity of soil protease (Fig. S7), and a significant positive correlation was detected between protease activity and CA concentration in plant roots. Proteases in the soil can hydrolyze large proteins into bioavailable amino acids, which can be taken up by *Proteobacteria*, *Actinobacteria*, and *Bacteroidota*, increasing their abundance levels (Lin et al. 2021). At the genus level, the addition of exogenous CA and TA could significantly increase the levels of *Azotobacter* and *Ensifer*, which were positively correlated with proteases and urease in the soil. Urease can promote the transfer of organic nitrogen to ammonia nitrogen in the soil (Lin et al. 2021). Therefore, CA and TA addition can affect the form of nitrogen and provide more nitrogen sources for the growth of *Azotobacter* and *Ensifer* through increasing soil urease activity. Moreover, the abundance levels of *Pseudomonas* and *Pseudoxanthomonas*, which...
are also plant growth-promoting rhizobacteria (PGPR), were increased with the addition of exogenous CA. As reported previously, PGPRAs can synthesize phosphatase, siderophores, and antibiotics to enhance plant tolerance to Cd toxicity (Drehe et al. 2018). Therefore, the abundance of PGPRs was greatly improved after the addition of exogenous LMWOAs, providing suitable conditions for plant growth and heavy metal speciation activation.

Conclusions

LMWOAs can form ligands combined with heavy metals by changing their form in soil, and change the absorption of heavy metals by plants, which provide scientific basis for enhanced phytoremediation in heavy metal-contaminated soil. This study demonstrates the application potential of LMWOAs to enhance the phytoremediation of Cd-contaminated soil. The addition of LMWOAs, at reasonable doses, could significantly facilitate the growth and development of Bidens pilosa L., enhance photosynthesis efficiency, and reduce oxidative damage. The application of exogenous LMWOAs also improved soil enzyme activity, impacted the soil microbial community and the speciation distribution of Cd in the soil, and enhanced the absorption and accumulation of Cd by Bidens pilosa L. The addition of CA with the appropriate doses of 15 mmol/kg was most significant for the comprehensive strengthening phytoremediation by Bidens pilosa L. to removal Cd in contaminated soil.

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Author contribution All authors contributed to the study conception and design. Material preparation, data collection, and data supervision were performed by Qing Yang, Junting Xie, Zhiguo Fang, and Huijun Liu. The first draft of the manuscript was written by Qing Yang, and was reviewed and edited by Zhiguo Fang. The contributions of the authors are as follows:

Qing Yang: investigation, conceptualization, methodology, formal analysis, original draft writing.
Junting Xie: data curation, original draft preparation and revision.
Huijun Liu: chart making, original draft revision, data supervision.
Zhiguo Fang: supervision, writing—review & editing, project administration, funding acquisition.

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

Declarations

Ethics standards The research was free of potential conflicts of interest and did not involve human participants and/or animals, and all investigators were informed.

Consent to publish I have not submitted my manuscript to a preprint server before submitting it to Environmental Science and Pollution Research. All the authors have read and agreed upon the contents of this manuscript, and the content of the manuscript did not and will not be published by any other journals.

Competing interests The authors declare no competing interests.

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