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RESEARCH ARTICLE

Compounded chelating agent derived from fruit residue extracts effectively enhances Cd phytoextraction by *Sedum alfredii*

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HIGHLIGHTS

- Cd extractability of eleven kinds of fruit residue extractions was compared.
- The most effective volume ratio of LRE, GLDA and tea saponin in Cd phytoextraction was 15:4:1.
- CPC improved plant growth, Cd phytoextraction performance and soil organic matter content.
- CPC induced less changes in bacterial community composition and had no evident influence on MBC and bacterial α-diversity.

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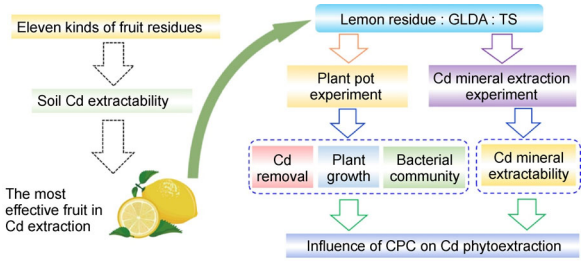
GRAPHICAL ABSTRACT



ABSTRACT

Chelating agent is known as the enhancer for metal phytoextraction; however, there is still a lack of efficient and environmentally sustainable chelators. Here, lemon residue extraction (LRE), prepared from 11 kinds of fruit wastes, was combined with *N*, *N*-bis (carboxymethyl) glutamic acid (GLDA), and tea saponin (T.S.) for the compounded plant-derived chelator (CPC), and their influences on Cd phytoextraction by the hyperaccumulator *Sedum alfredii* was evaluated. Among these fruits, the lemon residue extracted the most significant amount of Cd from the soil. The most effective CPC was at the volume ratio of three agents being 15:4:1 (LRE:GLDA:T.S.). Compared with the deionized water, the solubility of three Cd minerals was increased by 85–256 times, and Cd speciation was substantially altered after CPC application. In the pot experiment, CPC addition caused evident increases in plant shoot biomass, Cd phytoextraction efficiency, and organic matter content compared with EDTA and nitrilotriacetic acid (NTA) application. CPC induced fewer changes in bacterial community composition compared with EDTA and had no pronounced influence on microbial biomass carbon and bacterial α-diversity, suggesting CPC had a subtle impact on the microbiological environments. Our study provides a theoretical base for the reutilization of fruit wastes and the development of environmental-friendly chelator that assists Cd phytoextraction.

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1 Introduction

Soil cadmium (Cd) pollution has become a serious environmental problem around the globe, which poses critical threats to ecosystem service and human health. The variable metal complexes and strong persistence of Cd elements in the soil make their removal from the soil a daunting task (Ehsan et al., 2014; Li et al., 2018). Traditional physicochemical methods, such as washing with acids and chelators, metal stabilization using soil amendments, or landfilling, are not only costly but also detrimental to the soil ecosystem (Meng et al., 2017). Phytoextraction makes use of trace element-(hyper)accumulating plants to extract heavy metals from the soil and concentrate them into the aboveground tissues, and is also found as an environmentally compatible alternative compared to other chemical amendments that can increase the bioavailability and plant uptake of heavy metals (Mahar et al., 2016; Sheoran et al., 2016). However, the general use of the plant-based remediation technique is often restricted as the consequence of the low metal bioavailability and solubility in soils (Sessitsch et al., 2013), resulting in the low phytoextraction efficiency. This problem hinders our ability to efficiently restore the metal-disturbed soils and to formulate sustainable management and remediation policies.

A multitude of studies have been conducted to enhance the metal phytoextraction efficiency through improving the extractability and bioavailability of heavy metals by plants (Barrutia et al., 2010; Sessitsch et al., 2013; Ye et al., 2015; Liu et al., 2017). Among these studies, a variety of amendments (e.g. effective solvents, metal chelators, surfactants) were shown to assist metal phytoextraction by increasing the solubility of the target metals in soil, and thus increasing their bioavailability and accessibility to plant roots and accelerating metal root-to-shoot transfer and accumulation in green plants (Hseu et al., 2013). For example, the addition of traditional chelating agents like ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA), and nitrilotriacetic acid (NTA) could facilitate metal desorption from the soil into soil solution and subsequently enhance metal (e.g., Pb, Zn, Cu) phytoavailability and uptake by plants (Luo et al., 2005; Barrutia et al., 2010; Li et al., 2010b; Chen et al., 2017). Despite a broad understanding of this knowledge, those synthetic chemical chelators that assist heavy metal phytoextraction have received increasing criticisms due to their environmental persistence and potential leakage-induced secondary pollution (Yang et al., 2013). In this regard, the sustainable and eco-friendly chelating agent that contributes to the effective removal of heavy metals from metal-contaminated soils is quite imperative.

Tetrasodium of *N,N*-bis(carboxymethyl) glutamic acid (GLDA), a novel readily biodegradable chelating ligand, has been applied for enhancing the extraction of Cd from soils and industrial sludge samples (Wu et al., 2015; Wang et al., 2016; Guo et al., 2018). These studies showed that a variety of parameters, such as contact time, pH values, the concentration of the chelating agent, stability constant, as well as species distribution of metals, could have an effect on the chelating properties of GLDA. Meanwhile, characterized by low toxicity, GLDA also shows excellent biodegradability in the soil, more than 60% of which can be degraded within 28 days (Kołodyńska, 2011). In accordance, GLDA could be an effective chelating agent that assists heavy metal decontamination when compared with the traditional chemical chelators. However, a large amount of GLDA usage would result in leaching problems (Leštan et al., 2008) as well as high cost, which deserves more attention in future metal remediation studies.

Tea saponin, a natural biosurfactant, is separated from industrial waste oil-tea-cake (Xia et al., 2009). An earlier study has shown that tea saponin can alter soil surface properties through reducing the surface tension (Xia et al., 2009). When applied to the soil, tea saponin can be adsorbed on the soil particles, and the solid-liquid interface would be modified, which may accelerate the separation of metal ions from the soils and increase their solubility and availability. Due to its hydrophobic property, tea saponin exhibited a great potential in the removal of organic contaminants such as PAHs and decabromodiphenyl ether from soils (Xia et al., 2009; Tang et al., 2014). In a study conducted by Liu et al. (2017), who have shown that the application of tea saponin also caused increases in soil enzyme activities and improvement of plant growth. Similarly, recent studies demonstrated that tea saponin was able to facilitate the removal of heavy metals (e.g., Cd, Zn, Cu) from metal-contaminated soils as well (Cay, 2016; Yu and He, 2018). In light of these findings, tea saponin might be a promising amendment that can aid in heavy metals phytoextraction (Cay, 2016).

A large number of fruit wastes such as sugarcane bagasse, pineapple, and banana residues, citrus peel are generated during the agricultural and industrial production processes (Deng et al., 2012; Liu et al., 2008). A majority of these fruit wastes were used to feed animals and/or were dumped into landfills or oceans because of the high expense of other disposal forms. On the other hand, the role of some valuable properties of fruit wastes, such as being a natural resource of many low molecular weight organic acids (LMWOAs) and bioactive substances, in the regeneration of metal-polluted soils have been completely neglected. Moreover, the use of the natural chelating agents in the remediation of heavy metal-polluted soils and/or in the improvement of soil quality has been rarely explored. An earlier study showed that combined application of chemical chelator (nitrilotriacetic acid; NTA) and biosurfactant (alkyl polyglucoside; APG) could significantly promote Pb and Cd accumulation by plants (Hu et al., 2017). Analogous to previous research findings, it is possible that combining GLDA (natural chelator) and tea saponin (natural biosurfactant) with fruit residues would significantly increase the availability and solubility of metals in soil, which can help develop the novel degradable metal chelators that can elevate the phytoextraction efficiency and increase the usage of natural resources. To our knowledge, only a few studies have

evaluated the effect of the compounded chelating agents on the behavior and fate of heavy metal in soils and also the metal phytoextraction efficiency.

Sedum alfredii, an important Zn/Cd co-hyperaccumulator, is recognized as a model plant species for metal phytoxtraction research in contaminated soils (Yang et al., 2004; Guo et al., 2021). Our preliminary researches have provided evidence for the feasibility of enhancing Cd phytoextraction by *S. alfredii* through the single application of chemical chelators or surfactants (Liu et al., 2007; Wu et al., 2007). In this study, a pot experiment was conducted to provide insights into the effect of compounded plant-derived chelator (CPC) on Cd phytoextraction performance, soil properties, microbial characteristics, and plant growth. The aim of this study was to: (i) select an optimal fruit residue to act as the main component of CPC; (ii) explore the optimal mixing volume ratios of CPC for Cd mineral or soil extractability; (iii) evaluate the effect of CPC on Cd phytoextraction and microbiological status with the plantation of *S. alfredii*.

2 Materials and methods

2.1 Soil collection and preparation

The typical natural metal polluted soil was collected from an old Pb/Zn mining area in Quzhou, Zhejiang province, China (about 118°56′E, 29°17′N), and its physicochemical properties have been detailed in our previous study (Luo et al., 2017). Topsoil samples (0–20 cm) were collected, air-dried, and sieved through a < 2-mm mesh. The experimental soil with a pH of 7.1 contained 13.87% of clay, 20.82% of silt and 65.31% of sand, and 0.58 mg kg^{-1} total nitrogen, 0.68 mg kg^{-1} total phosphorus, 3.75 mg kg^{-1} total potassium, and 25.72 g kg^{-1} organic matter. In determining the heavy metal content, the soil contained 40.55 mg kg^{-1} total Cd, 3564.42 mg kg^{-1} total Zn, and 7514.37 mg kg^{-1} total Pb.

2.2 Selection of plant-derived activation substances and soil Cd extraction

Eleven kinds of fruit wastes were chosen according to the composition and concentration of their reported low molecular weight organic acids (LMWOAs) and other natural organic compounds (Nour et al., 2010; Pavloušek and Kumšta, 2011), and these fruits were also used to prepare the fruit residues in our recent study (Ning et al., 2019). The fruit residues used in the present study were as follows: grapefruit (*Citrus maxima* (Burm) Merr.), pineapple (*Ananas comosus*), grape (*Vitis vinifera* L.), orange (*Citrus sinensis*), lemon (*Citrus limon* (L.) Burm. F.), tangerine (*nobis Tangerine*), kiwi (*Actinidia chinensis Planch*), apple (*Malus pumila*), tomato (*Lycopersicon esculentum* Mill.), kumquat (*Fortunella margarita* (Lour.) Swingle) and carambola (*Averrhoa carambola* L.). The 11 fruits were selected depending on their annual yield and content of plant-derived substances (Buekens and Huang, 1998; Wu et al., 2007). The fruit wastes used were collected from the local markets and were strictly selected only those with a uniform appearance. After transported to the laboratory, the residue and peel of the collected fruits were washed 2–3 times using distilled water and mixed with distilled water at the mass ratio of 1:5, and the mixtures were mechanically homogenized by using a crusher. The mixtures were then diluted with the distilled water at the volume ratio of 1:10 and centrifuged at 5000 × *g* for 10 min to obtain the supernatants for the subsequent analyses.

A soil Cd extraction experiment, aimed to assess the efficiency of different fruit residues in extracting Cd from the soil, was performed as the following procedures: 1.00 g of soil was added into 20 mL of the 11 kinds of fruit extractions (soil-to-water ratio of 1:20) respectively, and the suspension was shaken for 12 h (25°C, 200 oscillations min^{-1}) and then centrifuged at 5000 r min^{-1} for 15 min. The supernatant was filtered through a 0.45-μm Millipore filter for Cd determination. The Cd activation ability of fruit residue extraction was represented as the concentration of Cd in the solution. The total concentration of Cd in extraction solution was determined by inductively coupled plasma-atomic emission spectroscopy (ICP-AES) (Shimadzu ICPS-7510; Shimadzu, Tokyo, Japan).

2.3 Preparation of the compounded plant-derived chelator (CPC)

After obtained the lemon residue extraction (LRE), GLDA (0.7%) and tea saponin (T.S.; 4%) were combined with LRE. GLDA solution with a solid content of 47% and a density of 1.40 g cm^{-3} (Akzo Nobel Chemicals (Ningbo), Co., Ltd., China), and tea saponin (75%) was obtained from the Quality Department of Hangzhou Choisun Tea Sci-Tech Co., Ltd. (Zhejiang, China). LRE was the main functional component used for the synthesis of compounded plant-derived chelator, accounting for 2/3 of the total volume. We established such a ratio of volume (2/3) because of the economic cost and the efficacy of Cd extraction, with a comparable amount of Cd extracted from the soil being detected between the pure LRE and the 2/3 volume LRE solution (data not shown). A series of the volume ratio of LRE, GLDA and tea saponin were set as follows: 15:4:1, 15:3:2, 15:2.5:2.5, 15:2:3, and 15:1:4. Subsequently, the steps described in Section 2.2 were followed to determine the optimal ratio of CPC by the Cd extraction abilities., All experiments were operated in triplicate to ensure repeatability.

2.4 Cd minerals extraction experiment

The mineral extraction experiment was performed to assess the efficacy of CPC on Cd minerals solubility. Three Cd minerals of sulfide, carbonate, and hydroxide (McLean Reagent Co., Ltd., Shanghai, China) that exhibit extremely low solubility in water were used. Treatments of two doses of

CPC (5 mL CPC, 15 mL CPC) were set, and equivalent deionized water was added to each Cd mineral as control. 2.0 g of the mineral was equilibrated with 20 mL of extractant with two doses (containing 5 and 15 mL of CPC) in 50-mL polyethylene centrifuge tubes. The suspensions were shaken on a reciprocal shaker at 200 rpm and 25°C for 4 h and then centrifuged at 5000 × *g* for 30 min and filtered for determination.

2.5 Pot experiment

2.5.1 Soil preparation

1.5 kg of the air-dried soil was transferred into a plastic pot (25 cm × 30 cm). Four uniform seedlings of *Sedum alfredii* plants were transferred to each pot after 45 days of pretreatment in hydroponics and grown in a greenhouse with a daily 14-h photoperiod and 10-h dark period, day/night temperature of 30°C/20°C, and relative humidity of 60%. Soils were watered every two days to 60% of water holding capacity by deionized water.

2.5.2 Plant harvest and analysis

After 10 and 20 days of transplantation, the CPC, EDTA, and NTA were added to the pots. Four treatments were established: (i) C.K.: soil with deionized water; (ii) CPC: addition of 15 mL CPC and 5 mL deionized water per pot per time; (iii) EDTA: addition of 5 mmol kg^{-1} EDTA per pot per time; (iv) NTA: addition of 5 mmol kg^{-1} NTA per pot per time. The pH of CPC solutions was adjusted to soil pH (~7.1) to maintain the fundamental property of the soil matrix. The plants were harvested after 35 days of plant incubation, similar to our recent report (Ning et al., 2019). The shoots were harvested by detaching from the root-shoot junction, and the roots were shaken gently to remove the attached soils. Plants were rinsed with distilled water 5 times, oven-dried at 70°C for 24 h to a constant weight. The dry plant samples were subsequently grounded and passed through a 1.0 mm sieve. Plant samples of 150 mg were digested with 4 mL HNO_3 and 1 mL H_2O_2 and diluted to 15 mL with distilled water, and analyzed for Cd.

2.5.3 Soil analysis

Soil adhering to the roots was considered as the rhizosphere soil, which was sampled from all pots at the time of harvest according to our previous study (Luo et al., 2017). The sampled soil was air-dried and sieved through a 0.8 mm nylon mesh. 2.0 g of soil samples were used for Cd extraction using the modified BCR (European Community Bureau of Reference) sequential extraction procedure (Luo and Christie 1998). Fresh soil was analyzed for the water-soluble Cd by extracting it with deionized water (w/v 1:5). The concentration of Cd in the soil extraction solution and the digested plant samples in 2.5.2 were determined by ICP-OES.

2.6 DNA extraction, 16S rRNA gene sequencing, and analyses

Total microbial DNA was extracted from the soil samples using the PowerSoil® DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA, USA) according to the manufacturer's instructions. Extracted DNA was subjected to quality check using NanoDrop 2000 spectrophotometer (Thermo Scientific, Waltham, USA) and gel electrophoresis (1.5% agarose). Amplicon libraries were prepared from two independent PCR reactions. During the first PCR run, the bacterial 16S rRNA gene of the V3-V4 region was amplified using the primer pairs of S-D-Bact-0341-b-S-17 (5′-CCTACGGGNGGCWGCAG-3′) /S-DBact-0785-a-A-21 (5′- GACTACHVGGGTATCTAATCC') (Klindworth et al., 2013). The PCR conditions were as follow: 3 min at 95°C for, then 25 cycles of 30 s at 95°C, 30 s at 55°C, followed by 45 s at 72°C and holding at 4°C. PCR reactions were carried out in triplicate. In the second PCR run, each sample was tagged with a unique dual-index barcode. The thermal cycling programs were as follow: 3 min at 95°C for, then 8 cycles of 30 s at 95°C, 30 s at 55°C, followed by 45 s at 72°C and holding at 4°C. PCR products of each sample were pooled and then purified with magnetic bead (Beckman, USA). The purified PCR products were size-checked and quantified with an Agilent 2100 Bioanalyzer (Agilent, USA). Amplicons were then pooled in equimolar ratios and were combined into one pooled sample and sequenced on Illumina Hiseq platform (Illumina, USA).

Raw sequence data was processed using the QIIME 2 (v. 2020.6) pipeline to perform the initial steps of bacterial community characterization. These steps include demultiplexing, quality control (Q.C.), taxonomy assignments, sequence alignments and computing pairwise community dissimilarities. The DADA2 method (Callahan et al., 2016) was used to trim the primer pairs, perform Q.C. of sequences, and assemble quality-filtered reads into error-corrected ASVs, which represent unique bacterial taxa and reveal cryptic diversity. A minimum of 25 bp of overlap was required for the read merging step. The script '*data2 denoise-paired*' in QIIME2 was used to perform DADA2, which is a pipeline for detecting and correcting (where possible) Illumina amplicon sequence data. The quality control process filters any phiX reads and chimeric sequences. Taxonomic classification of the assembled ASVs was conducted by training the naive Bayes classifier against the Silva 138 99% OTUs (Operational Taxonomic Units) full-length reference sequences. Alpha-diversity indexes of the observed ASVs and Shannon and unweighted UniFrac (UUF) distance metric were calculated in QIIME 2 platform. Bacterial diversity was estimated using the normalized ASV table by rarefying to 5000 reads per sample.

2.7 Statistical analysis

All data were presented as the means of three replicates together with standard deviation (S.D.) and statistically

analyzed using the R software (v. 3.6.0). Differences in the amounts of the extracted Cd, plant biomass, soil properties, and bacterial alpha-diversity indexes among treatments were determined using the one-way analysis of variance (ANOVA) with *post hoc* by LSD's multiple range tests at $P < 0.05$. Permutational Multivariate Analysis of Variance (PERMANOVA) was conducted to test the significance of the effect of each factor on the bacterial community composition using 'adonis' function in 'vegan' package (Philip, 2003). A difference in the relative abundance among soils added with different additives was conducted with the *Kruskal–Wallis* with post hoc by Dunn Test. The extraction ability and phytoextraction efficiency were calculated by following equations:

$$\begin{aligned}&\text{Extraction ability}\\ &= (\text{the Cd concentration in extraction solution}\\ &\quad - \text{the Cd concentration extracted by deionized water})\\ &\quad /\text{the total Cd concentration in the soil} \times 100\%.\\ &\text{Phytoextraction efficiency}\\ &= (\text{the mass of Cd concentrated in the shoot}\\ &\quad /\text{the mass of the total Cd in the soil}) \times 100\%.\end{aligned}$$

3 Results and discussion

3.1 Comparison of Cd extracted from soil by different fruit residue extractions

The amount of Cd extracted from metal-contaminated soil by different fruit residue extracts ranged from 3.72 to 35.60 mg kg^{-1} (Fig. 1A). Among the 11 kinds of fruits, the lemon residue extracted the largest amount of Cd (35.60 mg kg^{-1}) from the soil, and carambola extracted the lowest amount (3.72 mg kg^{-1}), with the amount of Cd extracted with these two fruit residues differing by 9.6 times. After the lemon residue, Kiwi extracted 22.83 mg kg^{-1} of Cd from the soil, which was significantly ($P < 0.05$) higher than that extracted by pineapple (18.02 mg kg^{-1}) and tomato (17.77 mg kg^{-1}) residues, followed by Grapefruit, Kumquat, and orange (Fig. 1A). Moreover, the characteristics of soil Cd activation efficiency among different fruit residues resemble the amount of Cd extracted by distinct residues (Fig. 1B). Therefore, lemon residue can be served as a good resource of plant-derived substances to facilitate Cd phytoextraction by plants and was thus selected as the optimum candidate to combine into the compounded activation agent for subsequent analyses.

The differences in Cd extraction efficiency among the 11 types of fruit residues could be chiefly related to the differences in LMWOA content. A previous study examining the amount of LMWOA in lemon juice by HPLC showed that the LMWOAs were composed of oxalic (0.094 g L^{-1}), tartaric (0.073 g L^{-1}), malic (1.465 g L^{-1}), lactic (1.545 g L^{-1}), citric tartaric (73.936 g L^{-1}) and ascorbic (0.718 L^{-1}) (Nour et al., 2010). It was also suggested that citric acid could constitute as much as 8% of the dry weight of citrus fruits and 97% of the total acids (Penniston et al., 2008; González-Molina et al., 2010). Other fruit residues were shown to contain less LMWOA as compared with lemon residue. For example, the grape residue contained the lowest Pb concentration in soil extraction solution and was found to harbor less amount of LMWOAs: citric (0.067 g L^{-1}), tartaric (4.58 g L^{-1}), and malic (2.33 g L^{-1}) (Pavloušek and Kumšta, 2011). A previous study involving evaluating the influence of LMWOAs (including citric acid, malic acid, and oxalic acid) on metal-contaminated soils has suggested that the LMWOA can alter the speciation and therefore increase Cd solubility and even bioavailability in soil (Liu et al., 2008). Of the 11 evaluated fruit residue extractions, citric acid was known to effectively facilitate Cd phytoextraction by plants (Gao et al., 2010). As a ternary acid, citric acid has a more potent ability to mobilize heavy metals in soil compared with the monoprotic and binary acids, as more hydroxyl and carboxylic groups can complex with a larger

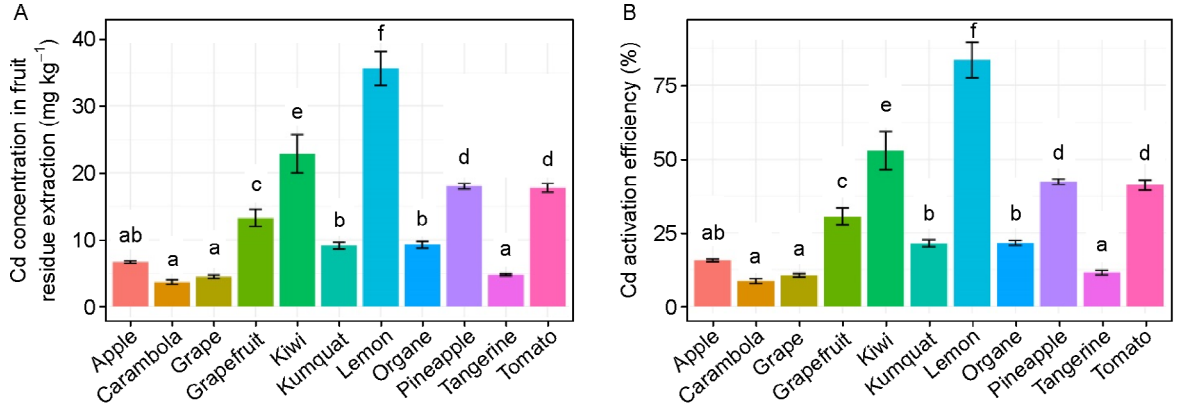

Fig. 1 The amount of Cd activated (A) and Cd activation efficiency (B) in polluted soil by fruit residue extraction prepared from 11 kinds of fruit. Data are in the form of mean±S.D. (n = 3). Different letters indicate significant differences between residues (P < 0.05).

amount of heavy metals and result in more mobilized metal ions (Jiang et al., 2012; Li et al., 2010b). Similarly, the high molecular weight organic acids, e.g. citric acid, are able to attract and/or chelate more metals due to their larger surface area and the more negative charge with respect to the lighter acid such as oxalic and malic acids (Jiang et al., 2012). Therefore, it can conclude that the Cd extraction ability of fruit residue extraction can be closely related to the amount of LMWOAs, which harbor a higher molecular weight may exhibit more potent extraction ability.

3.2 Combination of CPC and its Cd extractability

Although the lemon residue exhibits a strong ability to extract Cd from soil in our experimental study, it still failed to meet the extraction efficiency required in the practical field study. The biodegradable chelating ligand of GLDA and biosurfactant of tea saponin have been proven as effective additives that increase the solubility and phytoavailability of various heavy metals in soil, suggesting their promising functions that aid in metal phytoextraction (Wang et al., 2016; Wu et al., 2015). In this regard, lemon residue extraction (LRE), GLDA (0.7%), and tea saponin (4%) were proportionally mixed into the compounded plant-derived chelator (CPC) at the four-volume ratios (15:3:2, 15:2.5:2.5, 15:2:3, and 15:1:4), and their ability to extract Cd from the soil was also compared with 0.08 mol L^{-1} EDTA and pure LRE (Fig. 2). The highest Cd extractability with CPC was 64.67% at the volume ratio of 15:4:1. The Cd extractability by CPC at the volume ratio of 15:4:1 was 18.7% higher, and that by EDTA was 48.3% significantly higher ($P < 0.05$) than that by the single LRE, respectively (Fig. 2), indicating an evident improvement in Cd activation efficiency in the mixture of LRE with GLDA and tea saponin. Specifically, EDTA and LRE extracted 77.67% and 49.07% Cd from the soil, and CPC at the volume ratios of 15:3:2, 15:2.5:2.5, 15:2:3 and 15:1:4 removed 48.31%, 34.65%, 31.53%, and 22.46% from the soil, respectively. This result demonstrates that Cd extractability declines with the decrease of GLDA proportion and the increase of tea saponin proportion when conditioned for the proportion of LRE in CPC.

With respect to the Cd mineral extraction experiment, the addition of 5 mL CPC extracted significantly ($P < 0.05$) greater amount of Cd from the three different Cd minerals than the addition of water, and the addition of 15 mL CPC extracted a significantly greater amount of Cd than did 5 mL CPC (Fig. 3). Application of 5 mL and 15 mL CPC extracted 85-fold and 256-fold higher Cd from CdS than did the control and a similar

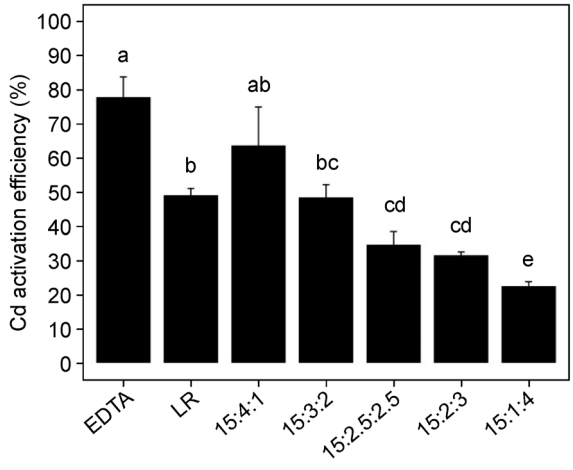

Fig. 2 The Cd extract ability of EDTA, lemon reside and compounded activation agents with five volume radios (15:4:1, 15:3:2, 15:2.5:2.5, 15:2:3, 15:1:4). Data are in the form of mean± S.D. (n = 3). Different letters indicate significant differences between distinct additives ($P < 0.05$).

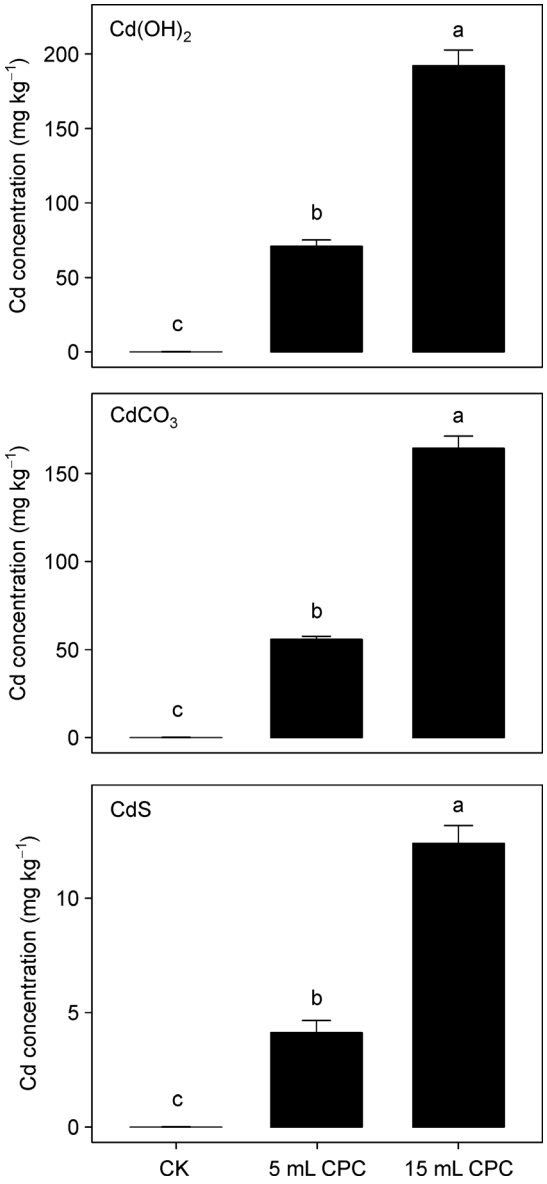

Fig. 3 The Cd extracted by compounded activation chelators from three Cd minerals ($Cd(OH)_2$, $CdCO_3$, CdS) at two addition dosages. Data are in the form of mean±S.D. (n = 3). Different letters indicate significant differences between distinct CPC ($P < 0.05$).

trend was also detected for $CdCO_3$ and $Cd(OH)_2$ (Fig. 3). The results suggest that GLDA is likely a promising chelator that can assist heavy metal phytoextraction, and its Cd activation ability can be affected by the two factors: the concentration of H^+ and free metals in soil solution (Li et al., 2016). In our study, the H^+ dissociated from LMWOA can effectively promote metal ion-ligand complexing reaction and increase the bioavailability of heavy metal in soil solution (Wang et al., 2016). Meanwhile, as a ternary acid, Cd in solution has more opportunity to be chelated and, thus, generating soluble Cd-LMWOA complexes and facilitating Cd removal from soils or minerals (Yang et al., 2006). When the concentration of H^+ was high in the soil solution, the complexing form of GLDA would be shifted from $Hglda^{3-}$ to $glda^{4-}$, which can be easier to form the soluble chelator ligand with Cd. Of note, the proportion of LRE present in CPC at different volume ratios remained stable, and thus the differences in Cd extractability of different CPC mixtures may be more related to the concentration of the chelating agent in the extraction solution. In this study, the tea saponin mixed in CPC could change soil surface properties by reducing the surface tension and increase Cd bioavailability and accessibility to plant roots (Xia et al., 2009). In tea saponin, its functional groups like hydroxyl groups, ester groups, and a variety of polycyclic organic structures can form negatively charged complexes with Cd^{2+} and prevent free Cd from binding to the cation exchange sites on the soil particles (Nowack et al., 2006; Duquène et al., 2009), and a greater amount of Cd^{2+} can be easily combined with GLDA and replace the H^+ of its hydroxylic and carboxyl groups, resulting in more H^+ being released into soil solution and more Cd being mobilized from the soil (Duquène et al., 2009). Collectively, the proportional mixture consisting of LRE, GLDA, and tea saponin can act synergistically to enhance Cd extraction from soil and facilitate metal decontamination, with the optimal volume ratio of these three components being 15:4:1.

3.3 Effects of CPC application on plant growth and Cd uptake by *Sedum alfredii*

Sedum alfredii exhibited strong tolerance to soil Cd toxicity (40.55 mg kg^{-1} of total Cd in the soil), and no visible symptoms of phytotoxicity were observed at the harvest time. Shoot biomass of *S. alfredii* was significantly ($P < 0.05$) higher in soil added with CPC relative to soils receiving other amendments, and *S. alfredii* grew in soils amended with NTA and EDTA had significantly ($P < 0.05$) lower shoot biomass than that in the control soil (Table 1). *S. alfredii* grown in the soils added with CPC and NTA and control soils had comparable root biomasses that were significantly ($P < 0.05$) higher than that in the EDTA-added soil. *S. alfredii* grown in the soil with EDTA showed the highest concentration of shoot Cd, which was significantly ($P < 0.05$) greater than those in the other soils (Table 1). The shoot Cd concentration in *S. alfredii* grown in the soil added with CPC and NTA was significantly ($P < 0.05$) increased by 43.2% and 34.7% as compared with the control soil, respectively. Also, the addition of CPC markedly ($P < 0.05$) increased the amount of Cd accumulated (25.68 mg pot^{-1}) in shoot biomass and phytoextraction efficiency (42.21%) compared with the other treatments (Table 1). Collectively, these results suggested that the application of CPC efficiently promoted the growth of *S. alfredii* and improved Cd phytoextraction efficiency, but EDTA addition exhibited an opposite trend.

There is a consensus that the efficiency/success of phytoextraction depends largely on metal availability to the roots, the ability of the plant to take up, and plant biomass (Sessitsch et al., 2013). Numerous studies have demonstrated that the addition of some chemical chelating agents can help elevate plant-uptake of metals, but it also brings some adverse effects to plant growth (Table 1), even the biodegradable GLDA (Tandy et al., 2006; Hseu et al., 2013; Anwar et al., 2017). Conversely, in our study, the addition of CPC significantly increased plant shoot biomass, the amount of extracted Cd, and phytoextraction efficiency relative to the control soil, suggesting that CPC can provide a beneficial effect for the growth of *S. alfredii*. Here the beneficial aspects of CPC on plant growth might be associated with the component of LRE and tea saponin. For example, it has been reported that tea saponin addition can promote plant growth by improving the soil texture and ploughability (Ye et al., 2015). The LMWOAs (primarily by citric acid) in CPC can help reduce Cd phytotoxicity and improve plant Cd tolerance by chelating with Cd (Yang et al., 2006; Ehsan et al., 2014), with the addition of tea saponin further accelerating this process (Duquène et al., 2009). Several studies have also

Table 1 Influence of the different chelators on plant biomass, Cd concentrations, Cd accumulated in shoot and phytoextraction efficiency in the pot experiment.

Treatment	Biomass (FW g pot^{-1})		Cd concentration (mg kg^{-1})		Cd extracted (mg pot^{-1})	Phytoextraction efficiency (%)
	Shoot	Root	Shoot	Root		
CK	19.24±1.49b	0.84±0.13a	608.92±61.82c	70.01±4.98c	11.77±0.93b	19.35±1.04b
15 mL CPC	29.26±1.48a	1.31±0.16a	872.17±33.89b	137.52±23.82ab	25.68±0.54a	42.21±3.54a
NTA	16.67±1.33c	0.88±0.27a	820.11±56.87b	122.73±23.86b	13.70±0.25b	22.52±3.45b
EDTA	16.31±1.75c	0.55±0.35b	950.57±74.84a	150.17±34.09a	15.56±0.35b	25.58±2.95b

Data are means±S.D. ($n = 3$). Different letters indicate significant differences between treatments ($P < 0.05$). FW, fresh weight.

reported that the application of citric acid in metal-polluted environments could significantly improve plant growth (Gao et al., 2010; Ehsan et al., 2014; Sabir et al., 2014). Besides, LRE has been shown to be rich in exchangeable K^+; once applied into soil, LRE may improve plant nutrient status and, thus the growth of the plant (Hassan et al., 2008). It is known that EDTA can destroy the root's physiologic barriers that control metal uptake by plants and cause an excessive accumulation of metals within the plant, more than its potential, and thus causing toxic effects on plant roots (Vassil et al., 1998). In summary, CPC can be the efficient chelating agent enhancing plant Cd accumulation and conferring plant growth benefits and exhibits great potential to substitute the traditional chelators in metal phytoremediation, such as EDTA and NTA.

Table 2 shows that application of CPC had no pronounced effect ($P > 0.05$) on soil pH, available N, available P, available K, and MBC compared with the control treatment. In contrast, the addition of CPC significantly increased soil O.M. content compared with the addition of NTA and EDTA, and increased the O.M. content by 10.9% relative to the control treatment. CPC, NTA and EDTA addition significantly ($P < 0.05$) decreased the soluble Cd concentration compared with the control treatment. The relatively higher O.M. and MBC caused by CPC addition might be due to the presence of excess LMOWAs in fruit residue extracts, which contain high amount of O.M. and organic carbon that can provide microbial growth with available carbon resource (Pavloušek and Kumšta, 2011). In total, the positive effect of tea saponin and LMWOA might have alleviated the phytotoxicity of GLDA and Cd on plant growth and synergistically enhanced the growth and Cd accumulation of *S. alfredii*.

3.4 Effect of CPC on Cd speciation in the soil

Soil Cd speciation was also analyzed by BCR sequential extraction method after harvested the plants, and the results of each fraction of Cd in different treatments are listed in Fig. 4. The distribution of different forms of Cd in extraction solution followed this order: reducible>acid-soluble>oxidizable>residual. In general, the addition of CPC into the soils reduced the reducible, acid-soluble, and total Cd contents, and such reduction was more evident as the CPC application dose increased from 5 mL to 15 mL. In brief, the addition of 15 mL CPC led to a 70.5% decrease in acid-soluble Cd relative to the control treatment (Table 2), and the reducible Cd in soils added with 5 mL and 15 mL CPC were reduced by 17.2% and 15.3%, respectively (Fig. 4). These results could be explained by Cd movement in soil layers induced by the application of different amendments. Similarly, an earlier study showed that EDTA could cause additional Cd loss in the surface soil by enhancing leaching (Zhao et al., 2010). Apart from the BCR forms, the water-soluble Cd, which is rich with toxic Cd^{2+} , can be directly accessible and absorbed by plant roots (Lux et al., 2010). Metal concentration in plants depends mainly on metal availability in soils, and the ability of the plant to intercept and take up metal and metal concentrations within the plant positively correlate with soil metal availability (Vivas et al., 2006). However, metals in soils are generally bound to organic and inorganic soil components, or alternatively, present as insoluble precipitates (Abou-Shanab et al., 2006). In the pot experiment, the addition of CPC prompted the release of available Cd (Fig. 4), which may be related to the increased Cd mobility and availability by the presence of LMWOAs, GLDA, and tea saponin. Among the four Cd forms assessed in this study, water-soluble and acid-soluble Cd are characterized by high bioavailability and mobility, and these two forms were also promoted by CPC (Mońko and Wacławek, 2004; Li et al., 2010a). The other decreased Cd form was the reducible form, which is usually immobile, but showed the potential bioavailable in certain conditions (Banat et al., 2005). This information indicated that CPC could effectively improve the bioavailability and mobility of Cd in soil, which could facilitate Cd uptake and accumulation by *S. alfredii*.

3.5 Influence of CPC, EDTA, and NTA on the bacterial community

The influence of CPC, EDTA, and NTA on bacterial communities and microbial properties was further evaluated. PCoA of the UUF distance metric revealed that bacterial communities differed significantly among the soils complemented with different additives ($R^2 = 0.353$, $P = 0.016$; Fig. 5A). The CPC-added soil samples clustered more closely with the control soil samples than with the other soil samples, and the former two soil samples were differentiated from the EDTA- and NTA-added soil samples along the first principal coordinate (PCo1). These results suggest that the addition of EDTA and NTA caused distinct effects on the composition of the bacterial community, and the community composition was more

Table 2 Influence of the different chelators on soil physicochemical properties, soluble Cd concentration and microbial biomass carbon (MBC).

Treatment	pH	OM (g kg^{-1})	Soluble Cd (mg kg^{-1})	MBC (mg kg^{-1})	Available N (mg kg^{-1})	Available P (mg kg^{-1})	Available K (mg kg^{-1})
CK	7.90±0.13c	19.23±0.93ab	0.78±0.18a	121.91±20.78a	54.21±3.41a	29.41±2.63a	164.92±19.14a
15 mL CPC	7.82±0.04c	21.33±1.53a	0.23±0.042b	112.90±20.06a	48.42±4.36a	30.84±2.28a	124.63±16.14a
NTA	8.38±0.07a	18.13±0.35bc	0.28±0.03b	112.21±11.08a	51.26±6.18a	21.84±3.16a	134.54±16.95a
EDTA	8.16±0.05b	16.01±1.71c	0.16±0.02b	90.04±9.01b	49.87±3.05a	34.21±2.76a	153.22±10.91a

Data are means±S.D. ($n = 3$). Different letters indicate significant differences between treatments ($P < 0.05$). OM, organic matter.

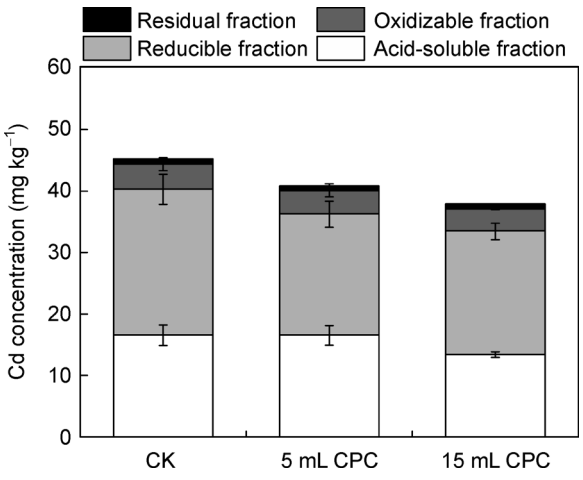

Fig. 4 Distribution of total Cd concentrations found in the four chemical fractions (acid-soluble fraction, reducible fraction, oxidizable fractionand residual fraction) in the plant-cultivated soils under three treatments (CK, 5 mL and 15 mL CPC). Data are means±S.D. (n = 3).

affected by EDTA and NTA than by CPC addition (Fig. 5A). This could be due to the more significant chemical interference effect of EDTA and NTA on soil physicochemical properties and further on the bacterial community diversification in the direct and/or indirect manners (Grčman et al., 2001; Chen et al., 2017). The significantly lower MBC in the soil with EDTA than in the control and soil with CPC partially supported this finding (Table 2), which hints at a more substantial interference effect of EDTA on the biological status of the evaluated soil. Our observation is in keeping with a previous finding showing that bacterial, fungal, and actinomycete abundance in planted soil was significantly depressed by EDTA application, with decreases of 40.4%, 49.8%, and 27.8%, respectively, compared with populations in the control soil (Grčman et al., 2001). Besides, another study reported that EDTA was more toxic to soil bacteria and actinomycetes than was NTA, which was attributable to the higher concentrations of DTPA-extractable Cd, and Zn in EDTA added soil than in the NTA added to soil (Chen et al., 2017). The addition of NTA caused an increase in the relative abundance of Bacteroidetes than the addition of CPC and EDTA, and a significant ($P < 0.05$, Kruskal–Wallis test) increase compared with the control treatment (Figs. 5b, 6). This result was consistent with the representatives of the genera *Vibrio* and *Flavobacterium* (from Bacteroidetes) in the bacterial isolate collections derived from the soils added with NTA (Hunter et al., 1986), indicating an adaptation of members of Bacteroidetes to NTA-amended soil. In contrast,

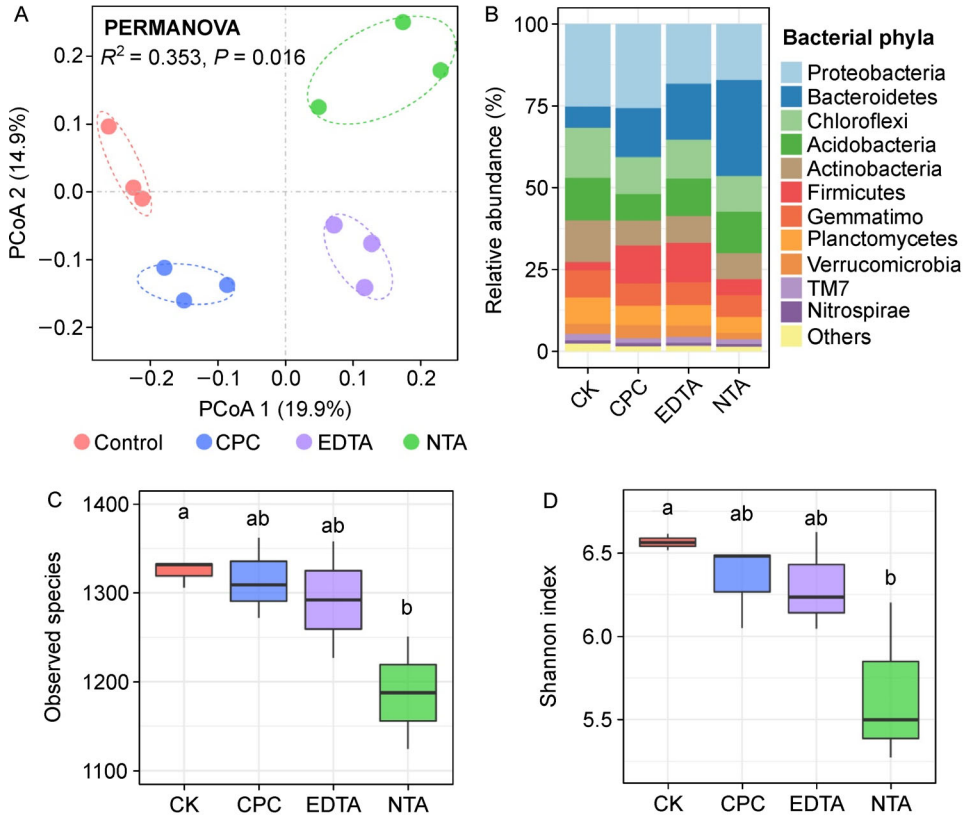

Fig. 5 Influence of CPC, EDTA and NTA on the bacterial community composition and diversity. (A) PCoA of unweighted UniFrac distance metric highlighting the effect of additives on bacterial community composition. (B) Influence of CPC, EDTA and NTA on the relative abundance of the abundant bacterial phyla. Impacts of different additives on alpha-diversity indexes of observed ASVs (C) and Shannon diversity (D). Gemmatimo., Gemmatimonadetes.

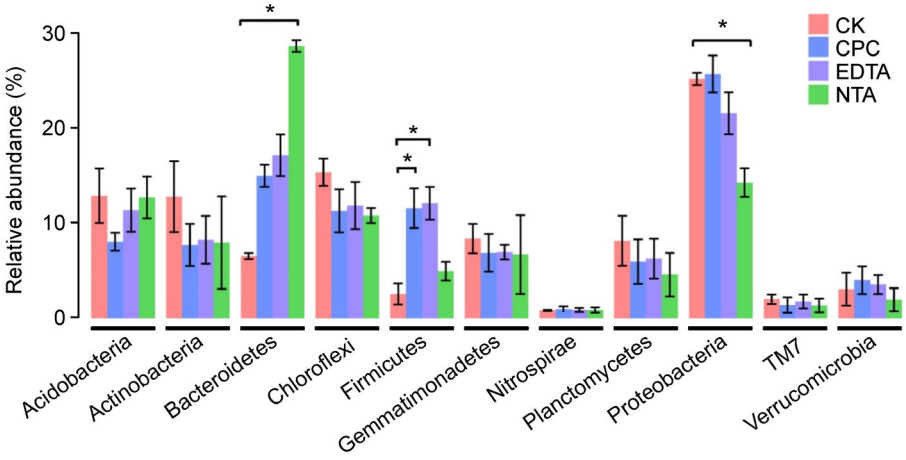

Fig. 6 Relative abundance of abundant soil microbial groups at the phylum level in the control soil and soils receiving different additives, e.g., CPC, EDTA, NTA, as detected by bacterial 16S rRNA gene profiling. Data are presented as means±S.D. (n = 3). The P-values were calculated using the *Kruskal-Wallis* with post hoc by Dunn Test; *, $P < 0.05$. CK, control treatment.

the addition of NTA led to a significantly ($P < 0.05$) decrease in the relative abundance of Proteobacteria relative to the control treatment ($P < 0.05$). These results demonstrated that Proteobacteria and Bacteroidetes exhibited an opposite responsive pattern to NTA addition despite they are characterized as copiotrophic soil bacteria, that is, they compete successfully only when organic resources are abundant (Fierer et al., 2007), suggesting that Proteobacteria are susceptible to NTA addition. The addition of CPC and EDTA resulted in a significant ($P < 0.05$) increase in the relative abundance of Firmicutes compared with the control treatment. A previous study also observed an evident increase in the abundance of *Clostridium* in the biological reactors (Cai et al., 2019). Firmicutes are also known as the copiotrophic soil bacteria, and thus they may be dominant under high organic carbon conditions (Fierer et al., 2007), such as the CPC-added soil. Moreover, the addition of different additives caused slight decreases in the abundance of Actinobacteria, Gemmatimonadetes, Planctomycetes compared with the control treatment (Figs. 5B, 6), indicating that members of these bacterial phyla are sensitive to the addition of xenobiotics. Similarly, the influence of CPC on the bacterial community can also be related to GLDA, tea saponin, and the LMWOA in fruit residue, with each of the components playing a distinct role in influencing the bacterial community structures. Furthermore, the addition of NTA resulted in the pronounced ($P < 0.05$) decrease in the observed species and Shannon diversity indexes (Fig. 5C, D), pointing out that bacterial alpha-diversity was more resistant than the bacterial community structure to the addition of particular additives in the current study. The possible explanation can be related to the short plant growth period (35 days) and the relatively less application times (two times in total) of additives, leading to the insufficient interaction duration between the amendments and microbiological environments. Considering the well-known niche heterogeneity of soil environment, a more protracted experimental investigation is required, and it should include consecutive incubation periods in order to reveal a trend, thus providing deeper insights into the influence of different additives on the structure, diversity, and function of microbial assemblies.

4 Conclusions

We demonstrate that the compounded plant-derived chelators are attractive amendments improving Cd phytoextraction performance by hyperaccumulator *Sedum alfredii* that induce few adverse effects on the soil nutrient status and microbial properties. Our results showed that LRE extracted the most significant amount of Cd from the polluted soil among 11 types of fruit residues due to its unique citric acid content. Therefore, LRE can be the optimal plant-derived component for the synthesis of CPC. When the three components (LRE:GLDA: tea saponin) were at the volume ratio of 15:4:1, CPC showed the most potent Cd extraction ability in soil and solubilized the highest amount of Cd from Cd minerals relative to other volume ratios. The pot experiment revealed that the addition of CPC resulted in a significant increase in shoot biomass, Cd phytoextraction efficiency, and organic matter content compared with the addition of EDTA and NTA, suggesting that the addition of CPC is beneficial to Cd phytoextraction by *S. alfredii*. Besides, CPC addition induced less variation in bacterial community composition and relative abundance of the dominant bacterial phyla and had no significant effect on MBC and bacterial α-diversity when compared with the control soil. This study revealed that plant-derived chelating agent arising from lemon residue is the eco-friendly and sustainable amendment that efficiently enhances Cd phytoextraction without leading to notable environmental risks. Results of our work provide essential bases for promoting the reutilization of waste resources to synthesize efficient and environmental-sustainable chelators that improve phytoextraction efficiency.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgments

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