Ecotoxicology and Environmental Safety 192 (2020) 110311

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Microbial inoculant and garbage enzyme reduced cadmium (Cd) uptake in *Salvia miltiorrhiza* (Bge.) under Cd stress

Xuemin Wei, Pei Cao, Gang Wang, Jianping Han*

Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing, 100193, China

ARTICLE INFO

Keywords:
Bioactive compounds
Heavy metal
Herbal quality
Microbial remediation
Rhizospheric microorganisms

ABSTRACT

The uptake and accumulation of cadmium (Cd) in *Salvia miltiorrhiza* (Bge.) negatively affects the quality of its harvested roots, and seriously threatens human health. This study investigates the effect of a microbial inoculant (MI) and garbage enzyme (GE) on Cd uptake, the accumulation of bioactive compounds, and the community composition of microbes in the rhizosphere soil of *S. miltiorrhiza* under Cd stress. *S. miltiorrhiza* seedlings were transplanted to Cd-contaminated pots and irrigated with an MI, GE, a combination of an MI and GE (MIGE) or water (control). The results indicated that treatments with an MI, GE or MIGE can reduce Cd uptake in *S. miltiorrhiza*. The MIGE treatment had greater efficiency in reducing Cd uptake than the control (reduction by 37.90%), followed by the GE (25.31%) and MI (5.84%) treatments. Treatments with an MI, GE and MIGE had no significant impact on fresh and dry root biomass. Relative to the control, the MI treatment had the highest efficiency in increasing the accumulation of total tanshinones (an increase of 40.45%), followed by the GE treatment (40.08%), with the MIGE treatment (9.90%) treatment not having a more favorable effect than the separate application of an MI or GE. The salvianolic acid content for all groups was higher than the standard prescribed by Chinese pharmacopoeia, notwithstanding a slightly lower level in the treated groups relative to the control. In addition, metagenomic analysis indicated changes in the relative abundance of soil microbes associated with the bioremediation of heavy metals. The relative abundances of *Brevundimonas, Microbacterium, Cupriavidus* and *Aspergillus* were significantly greater in the treated groups than in the Control. These results suggest that using MI and GE, either separately or together, may not only improve the quality of *S. miltiorrhiza* but may also facilitate the microbial remediation of soil contaminated with Cd.

1. Introduction

The contamination of agricultural soils with cadmium (Cd) has become a serious global environmental concern. In 2007, the area of Chinese farmland that was contaminated with Cd exceeded 1300 ha, largely due to urbanization, rapid industrialization, mining, smelting, waste disposal, and sewage irrigation (An et al., 2007). Cd is classified as the seventh most toxic substance on the 2017 Substance Priority List of Hazardous Substances by the Agency for Toxic Substances and Disease Registry (2017) due to its high mobility and severe toxicity to organisms, even at low concentrations (Mezynska and Brzoska, 2018). The excessive uptake of Cd by medicinal plants is not only extremely toxic to the plants themselves and associated soil microorganisms but also threatens the consumer safety when it enters the food chain (Gong et al., 2017; Nayak et al., 2015; Zhao et al., 2019). Dghaim et al. (2015) reported that the majority of the 81 samples of seven selected traditional herbs they collected contained unsafe Cd concentrations that exceeded the limits set out by the World Health Organization. The dried root and rhizome of *Salvia miltiorrhiza* (Bge.), called *danshen* in Chinese, is one of the most commonly used herbal medicines in China, largely due to its wide range of pharmacological effects, including immunemodulatory (Wei et al., 2017), anti-inflammatory (Choi et al., 2018), and anti-fibrotic (Zheng et al., 2017) effects. Yan et al. (2012) reported that 5 of the 13 samples of *S. miltiorrhiza* they collected from the main production area in China contained Cd or Cu concentrations above the limits prescribed by the Chinese Pharmacopoeia Committee of P. R. China (2015). Similarly, Meng et al. (2009) indicated that the Cd concentrations of all samples from different production areas exceeded

Abbreviations: GE, garbage enzyme; ICP-MS, inductively coupled plasma mass spectrometry; LDA, linear discriminant analysis; LEfSe, linear discriminant analysis effect size; MI, microbial inoculant; MIGE, combination of microbial inoculant and garbage enzyme; PGPR, plant-growth-promoting rhizobacteria; PCA, principal component analysis; TEM, transmission electron microscopy; SA, salvianolic acid; TTs, total tanshinones

* Corresponding author. No. 151 Malianwa North Road, Haidian District, Beijing, 100193, PR China.
E-mail address: happymyra2007@163.com (J. Han).

https://doi.org/10.1016/j.ecoenv.2020.110311
Received 30 July 2019; Received in revised form 5 February 2020; Accepted 5 February 2020

the limits prescribed by the Chinese Pharmacopoeia Committee of P. R. China (2015) (216.00%–872.67% above the limit). Additionally, Li et al. (2013) indicated that the Cd content in *S. miltiorrhiza* roots exceeded the limits after being grown in Cd-contaminated soil (0.5 $mg{\cdot}kg^{-1}$) for 90 days. Thus, it is well documented that the uptake and accumulation of Cd in *S. miltiorrhiza* grown in polluted soil poses a significant threat to human health.

Soil remediation technologies designed to remove and/or recover heavy metals from polluted environments are becoming increasingly important. Bioremediation is an eco-friendly and cost-effective technology that uses the inherent biological mechanisms of microbes and plants to reclaim environments contaminated by heavy metals (Ojuederie and Babalola, 2017). Although a wide range of methods of bioremediation have been developed, microbial remediation, which employs microorganisms with the proven ability to remediate and tolerate heavy metal toxicity, is attracting increasing levels of attention (Ayangbenro and Babalola, 2017). Microbial inoculants (MIs) consist of one or more species or strains of beneficial microorganisms that promote the bioremediation of toxic compounds, enhance plant growth, and directly or indirectly stimulate microbial activity (Baez-Rogelio et al., 2017; Trabelsi and Mhamdi, 2013). The most commonly used beneficial microorganisms are *Bacillus* species. By virtue of their wide metabolic capability, these microorganisms can play an important role in the functions and processes of a soil ecosystem (Calvo et al., 2016). A range of *Bacillus* species are resistant to and can immobilize Cd, as well as being able to reduce the uptake of metals in plants (Dawkar et al., 2010; Huang et al., 2018). *Bacillus megaterium* H3 can decrease the metal (Pb and Cd) uptake of greens and improved vegetable (*Brassica campestris* L. var. *Aijiaohuang* and *Brassica rapa* L. var. *Shanghaiqing*) qualities in metal-contaminated soils (Wang et al., 2018). The inoculation of *Bacillus cereus* for Cd treatment can reduce Cd uptake and increase antioxidant enzyme activities in rice cultivars (Jan et al., 2019). Garbage enzyme (GE) is an organic solution produced by the fermentation of edible medicinal herbs, fruits, vegetables, or other kitchen waste; water; and brown sugar (Arun and Sivashanmugam, 2017). Many different types of microorganisms are produced during the natural fermentation process, predominantly lactic acid bacteria (such as *Lactobacillus* and *Leuconostoc*) and yeast (such as *Pichia* and *Candida*) (Du et al., 2017). Lactic acid bacteria are generally recognized as safe and are the best known probiotic microorganisms for reducing biocontamination (Elsanhoty et al., 2016). Yeast can also act as an efficient bioremediator for a variety of heavy metals, including Cr, Cu and Cd, largely because it possesses a variety of tolerance mechanisms to metal toxicity (Fernandez et al., 2017). GE is attracting increasing amounts of attention in research communities because it has been shown to reduce pollutant levels in domestic wastewater by removing impurities and bacteria (Arun and Sivashanmugam, 2015, 2018). GE can remove 90% of the oil and grease, 50% of the suspended solids, and 25% of the chemical oxygen demand in palm oil mill effluent (Rasit and Kuan, 2018). GE is also used in agriculture. Li et al. (2016) reported that it can help increase the contents of soil organic matter, total phosphorus, and available phosphorus. Additionally, Zhou et al. (2016) found that spraying it on plants can significantly decrease the Cd content in rice by 47.54–63.08%.

As summarized above, MIs and GE have been successfully used to reduce Cd uptake in some crops. However, few studies have focused on their impacts on medicinal plants such as *S. miltiorrhiza*. Moreover, unlike most food crops, the contents of bioactive compounds in medicinal plants are important indicators that must be paid attention to, beyond Cd uptake because the accumulation of bioactive compounds is directly related to the efficacy and quality of medicinal materials. Here we hypothesized that MIs and GE would help to reduce Cd uptake in *S. miltiorrhiza,* without a negative impact on the accumulation of the bioactive compounds. This study conducted a pot culture experiment to investigate the impacts of MI and GE on Cd uptake, the synthesis of bioactive compounds, and the biomass of *S. miltiorrhiza* roots under Cd stress. In addition, this study characterized the behavior of soil microbial communities under Cd stress to determine the microbial remediation effects of MI and GE on soil contaminated with Cd. Metagenomic analysis was used to investigate the microbial community in the rhizosphere. It was anticipated that this research will help to provide insight into the impact of MI and GE on medicinal plants.

2. Materials and methods

2.1. Pot experiment and plant growth conditions

Soil for the pot trial was collected from an agricultural field located in a suburb of Beijing, China (N 40°2′0″, E 116°16′4″) Soil was taken from the top 0–20 cm and dried in the shade to avoid direct contact with sunlight. Once dry, the soil was passed through a sieve with a mesh size of < 5 mm. The physical and chemical properties of the soil were as follows: pH (7.12), organic matter (10.88 $mg{\cdot}kg^{-1}$), total nitrogen (0.47 $g{\cdot}kg^{-1}$), available nitrogen (20.56 $mg{\cdot}kg^{-1}$), available phosphorus (11.17 $mg{\cdot}kg^{-1}$), and available potassium (74.58 $mg{\cdot}kg^{-1}$). Cd content was also determined (0.12 $mg{\cdot}kg^{-1}$). Individual pots (32.5 cm in diameter × 30 cm in height) were filled with 13 kg soil that had previously been thoroughly blended with cadmium chloride hemi (pentahydrate) ($CdCl_2{\cdot}2.5H_2O$, Haoke Technology Beijing Co. Ltd., China) powder to a final concentration of 200 $mg{\cdot}kg^{-1}$ Shaheen et al. (2018). The soil was equilibrated for 24 h prior to use. Four treatments were featured: control (water irrigation), MI (MI irrigation), GE (GE irrigation), and MIGE (combined irrigation of MI and GE). Three biological replicates were used for each treatment, and one *S. miltiorrhiza* seedling was planted in each pot; each of the seedlings showed uniform growth. Then the seedlings were allowed to grow for 1 month without any form of treatment. Root irrigation with an MI, GE, and MIGE was performed once per month for 4 months. The MI, tianxia diyi jun, was purchased from Chenao Runze Technology (Chenao Runze Technology Co. Ltd., Beijing, China) and was used in accordance with the manufacturer's instructions. The manufacturer states that this product contains effective viable bacteria, including *Bacillus amyloliquefaciens, Bacillus licheniformis* and *Actinomyces bovis*, at a concentration exceeding two billion$\cdot mL^{-1}$. GE was prepared using *Aurantii fructus immaturus* (the dried young fruits of *Citrus aurantium* L.), water, and brown sugar, following a common fermentation principle described previously (Ouyang and Gu, 2017). The microbial community composition of GE was investigated prior to the experiment using a high-throughput sequencing technique. This analysis predominantly identified lactic acid bacteria (such as *Lactobacillus* and *Leuconostoc*) and yeast (such as *Pichia* and *Candida*). Plants were irrigated to maintain 60–70% moisture content of the total water-holding capacity of the soil during the entire cultivation period of 7 months.

2.2. Plant and soil sample collection

Experimental plants were harvested after 7 months, and the fresh and dry biomasses of the roots were determined. The roots were dried in an oven at 45 °C and then ground to a fine powder. At harvest, a rhizospheric soil sample, defined as the soil that was tightly adherent onto the root surfaces, was collected. The plants were removed from the pots, and their roots were gently shaken to remove the loosely adhering soil. Then, soil that tightly adhered to the surface of roots was brushed and pooled into sterile plastic bags. All soil samples were homogenized by being passed through a 2–mm sieve and then being stored at −80 °C to await molecular analysis.

2.3. Transmission electron microscopy observation and the determination of Cd concentrations in S. miltiorrhiza roots

Fresh roots from all experimental groups were imaged using transmission electron microscopy (TEM) to observe Cd uptake. The samples

for TEM were prepared following the methodology described by Zhang et al. (2012). Root apices were prefixed in 2.5% glutaraldehyde for at least 2 h, dehydrated in ethyl alcohol, and then embedded in Spurr's resin. Ultrathin sections (90 nm) were created using an ultramicrotome (Leica Co., EM UC 7, Germany) with a diamond knife and were mounted onto a Cu grid. A TEM (FEI Co., Tecnai G2 20 S–TWIN, USA) with energy dispersive X–ray spectroscopy (EDS) (Oxford, INCA MAX 20, UK) was used at 80 KV to determine whether Cd had entered the root cells. The concentration of Cd in *S. miltiorrhiza* roots was then determined by inductively coupled plasma mass spectrometry (ICP-MS). In brief, the dry samples were ground to a fine powder using an agate mortar and pestle and were digested with a mixture of concentrated plasma pure HNO_3 and H_2O_2 (v/v, 6:1) using a microwave-accelerated reaction system (Speedwave MWS-2, Berghof, Germany). The heating program was as follows: 0–5 min, 25–160 °C; 6–10 min, 160–200 °C and 200 °C for 20 min. Then the digested samples were diluted with ultrapure water and analyzed using ICP-MS (Thermo X7, Waltham, MA, USA) (Rui et al., 2008).

2.4. Determination of bioactive compounds in S. miltiorrhiza

Next, the concentration of 4 main bioactive compounds (salvianolic acid [SA]: salvianolic acid B, and total tanshinones [TTs]: tanshinone I, tanshinone IIA, and cryptotanshinone) in *S. miltiorrhiza* were determined. The samples and standards were prepared in accordance with guidelines set out by the Chinese Pharmacopoeia (Pharmacopoeia Committee of P. R. China, 2015). The standards for salvianolic acid B, tanshinone I, tanshinone IIA, and cryptotanshinone were purchased from the National Institutes for Food and Drug Control, Beijing, China. High-performance liquid-phase (HPLC) –grade acetonitrile (A) (Fisher, Emerson, IA, USA) and Wahaha purified water were used for the HPLC analysis. The other chemicals and solvents used were of an analytical grade and were obtained from Siaopharm Chemical Reagents, Beijing, China. HPLC analysis was carried out on a PerkinElmer HPLC–Class system (Flexar, PerkinElmer, USA.), equipped with a binary solvent delivery pump, an auto sampler, and a photodiode array detector. Chromatographic separation was performed on an Agilent ZORBAX Extend-C18 column (4.6 × 250 mm, 5 μm). For TTs, the mobile phase consisted of A and 0.02% aqueous phosphoric acid (B) with a gradient elution as follows: 0–6 min, 61% A; 6–11 min, 61–71% A; 11–15 min, 71–75% A; 15–22.5 min, 75–90% A; 22.5–30 min, 90-61% A. Finally, the column was reconditioned isocratically with 61% A for 5 min. The flow rate was 1 mL/min, and the photo-diode array (PDA) detection wavelength was set at 270 nm. The injection volume of the sample was 10 μL, and the column temperature was set at 28 °C. For salvianolic acid, the mobile phase consisted of A and 0.1% B. Analysis involved a gradient program (0–22 min and 22% A). The flow rate was 1 mL/min, and the PDA detection wavelength was set at 286 nm. The sample injection volume was 20 μL and the column temperature was set at 28 °C.

2.5. DNA extraction, and polymerase chain reaction (PCR) amplification and sequencing

A soil genomic DNA kit (Tiangen Biotech Beijing Co., China) was used in accordance with the manufacturer's instructions to extract genomic DNA from 0.25 g of each rhizosphere soil sample. The successful extraction and purity of the extracted genomic DNA was checked using 0.8% agarose gel electrophoresis, and then the DNA was stored at −20 °C to await further analysis. For each sample, the 16 S rRNA gene was amplified using the bacterial primers 515 F (5′-GTGC CAGCMGCCGCGG) and 907 R (5′-CCGTCAATTCMTTTRAGTTT) (Xiong et al., 2012). The internal transcribed spacer (ITS) region of the rRNA gene was amplified using the fungal-specific primer pair ITS1F (5′-CTTGGTCATTTAGAGGAAGTAA) and ITS2R (5′-GCTGCGTTCTTCA TCGATGC) (Mueller et al., 2014). PCR amplification and purification were performed as described previously (Rodrigues et al., 2013). The purified PCR products were quantified using a QuantiFluor™-ST system (Promega, USA), and the amplicons were pooled in equimolar ratios for sequencing. The DNA products were paired-end sequenced (2 × 250) on an Illumina HiSeq platform (Shanghai Biozeron Co. Ltd., China) following standard protocols (Caporaso et al., 2012).

2.6. Statistical analysis

Metagenomic data were demultiplexed and quality filtered using QIIME, according to the standard pipeline (Caporaso et al., 2010a, 2010b); after trimming, the FASTQ files were transformed to the fasta format. Sequences with 97% identity were assigned to the same operational taxonomic unit (OTU) using UPARSE (Edgar, 2013). Chimeric sequences were identified and removed using UCHIME. The taxonomic identities of the bacteria and fungi were determined using the Silva (http://www.arb-silva.de) (Quast et al., 2013) and Unite databases (http://unite.ut.ee/index.php) (Koljalg et al., 2013), respectively. Alpha diversity analysis was performed for the bacterial and fungal communities to determine diversity indices, including the Shannon (*H′*) and Chao I indices, using a modified version of the procedure previously described (Schloss et al., 2009). Principal component analysis (PCA) was performed using R software (Version 3.5.1). The linear discriminant analysis (LDA) effect size (LEfSe) (Quagliariello et al., 2018; Segata et al., 2011) was used to further identify bacterial and fungal genera among all OTUs with statistically different abundances in different groups. Differences were considered significant at an LDA score > 2 and $P < 0.05$. The data represent means of three replicates ± standard errors. Statistical analysis was performed using one-way analysis of variance (ANOVA) and an independent samples *t*-test using SPSS 22.0 software (SPSS 21.0 package, Chicago, IL, USA). Values of $P < 0.05$ were considered significant in all statistical analyses.

3. Results

3.1. Root biomass

The effects of MI and GE on *S. miltiorrhiza* growth were determined using root fresh and dry biomasses. There were no significant differences in the fresh and dry root biomass among the MI, GE, and MIGE treatments and the control group (Fig. S1).

3.2. MI and GE application reduced Cd uptake in S. miltiorrhiza roots under Cd stress

TEM was used to investigate the distribution of Cd aggregates in the root cells of *S. miltiorrhiza*. Many dark spots were observed, and the elemental composition of the selected areas for each image was then confirmed by EDS. High Cd concentrations were detected for each of the selected areas. Then, ICP-MS was used to precisely determine the concentrations of Cd in the roots (Fig. 1). The highest Cd contents were observed in the control group, followed by the MI and GE groups. The lowest Cd contents were identified in the MIGE group. The contents of Cd in the MI, GE, and MIGE groups were 5.84%, 25.31%, and 37.90% lower than the control, respectively. The combined MIGE treatment resulted in significantly larger reductions of Cd content in *S. miltiorrhiza* than in the application of an MI and GE separately. Overall, the application of an MI and GE, either separately or in combination, reduced the Cd content in *S. miltiorrhiza*.

3.3. Effects of MI and GE on the concentration of bioactive compounds in S. miltiorrhiza under Cd stress

The calibration equations, correlation coefficient, and linear ranges for the standard solution, TTs and salvianolic acid B are shown in Table S1. All of the marker substances showed good linearity, with

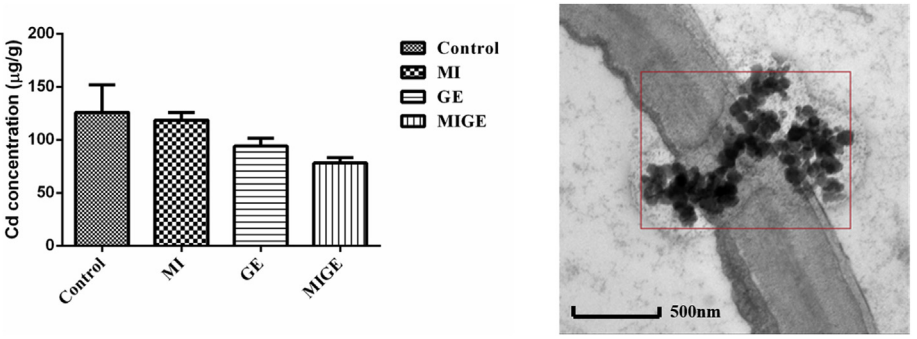

Fig. 1. Concentrations and transmission electron microscopy images of Cd in the roots of *Salvia miltiorrhiza* under Cd stress. The plots labelled Control, MI, GE, and MIGE represent the control, microbial inoculant application, garbage enzyme application, and combined treatment with a microbial inoculant and garbage enzyme, respectively. The values represent the means (± standard errors) of three replicates.

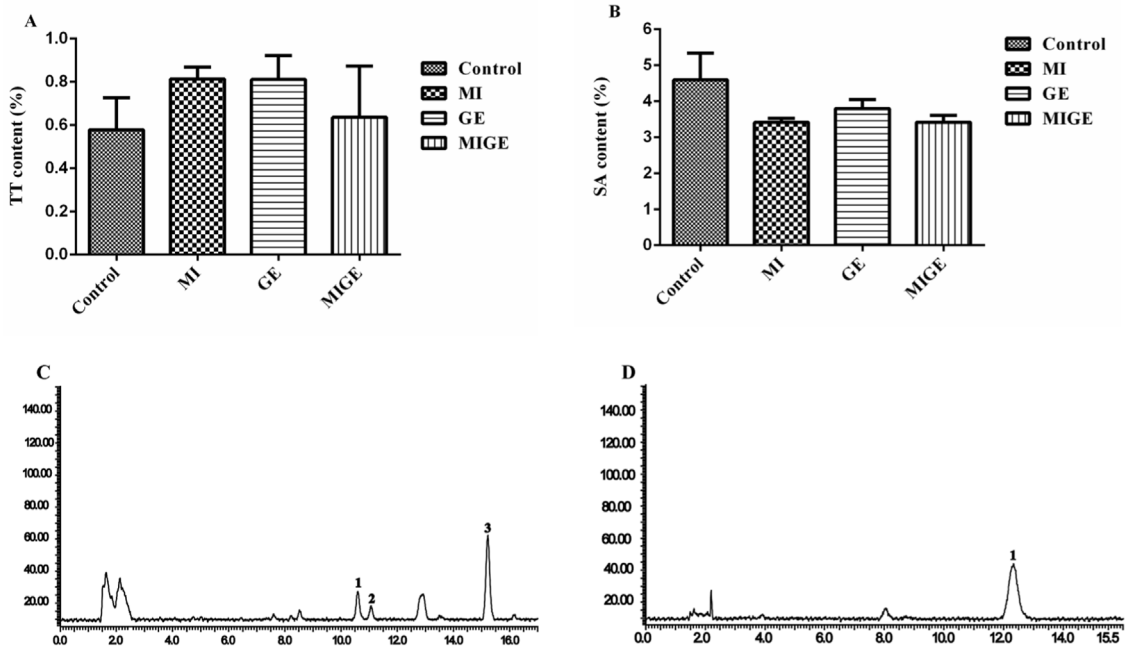

Fig. 2. Total tanshinone (A) and salvianolic acid (B) contents in the roots of *Salvia miltiorrhiza* under Cd stress. (C) High-performance liquid-phase chromatogram of total tanshinones (TTs) and (D) salvianolic acid (SA). The plots labelled Control, MI, GE, and MIGE represent the control, microbial inoculant application, garbage enzyme application, and combined treatment with a microbial inoculant and garbage enzyme, respectively. TT, total tanshinone; SA, salvianolic acid. Values represent the means (± standard errors) of three replicates. The peaks shown are as follows: C: 1, cryptotanshinone; 2, tanshinone I; 3, tanshinone IIA; D: 1, salvianolic acid B.

determination coefficients (R^2) ranging from 0.9994 to 0.9997 in a relatively wide concentration range. The TT content was quantified in terms of cryptotanshinone, tanshinone I, and tanshinone IIA. The chromatograms and contents of salvianolic acid and TTs in the experimental *S. miltiorrhiza* samples are shown in Fig. 2. The highest content of TTs was detected in the MI group, followed by the GE and MIGE groups; the lowest content of TTs was observed in the control group. The contents of TTs in the MI, GE, and MIGE groups were 40.45%, 40.08%, and 9.90% greater than the control group, respectively. The combined MIGE treatment did not result in higher TTs in *S. miltiorrhiza* than the separate MI and GE treatments. The highest salvianolic acid content in *S. miltiorrhiza* roots was observed in the control group, followed by the GE and MIGE groups; the lowest salvianolic acid content was detected in the MI group. All of the samples conformed to the Chinese Pharmacopoeia standard with respect to the content of TTs and salvianolic acid.

3.4. Total bacterial and fungal community structure

In 16s rRNA gene sequencing, a total of 250,261 classifiable bacterial sequences were obtained from 12 soil samples after quality control filtering, with a mean number of 62,565 classifiable sequences per sample (dominant length: 351–400). The alpha diversity of the bacterial microbiome for each sample was estimated using the community richness Chao I and *H′* indices. The results showed that the application of MI and GE, either separately or in combination, led to alterations in soil microbial diversity relative to the control (Fig. 3A and B). Venn diagram analysis indicated that the number of bacterial OTUs shared by the control, MI, GE, and MIGE groups was 2850, and that the numbers of OTUs found exclusively in the control, MI, GE, and MIGE groups were 174, 132, 128, and 234, respectively. PCA revealed differences in the bacterial communities of different groups (Fig. 4A). The second principal component (15.77% contribution) showed that the bacterial communities in the MI and MIGE groups varied from those present in the control and GE groups. The bacterial communities in the MI and MIGE groups were separated from each other. The bacterial communities in the control and GE groups were close to each other and strikingly different from those in the MI and MIGE groups. The bacterial profiles are presented in Fig. 5A. The predominant phyla in all samples were Proteobacteria, Actinobacteria, Acidobacteria, Chloroflexi, Bacteroidetes, Gemmatimonadetes, Planctomycetes, Firmicutes, Verrucomicrobia, and Patescibacteria, with mean abundances of 34.56%, 18.85%, 10.68%, 10.36%, 8.36%, 4.99%, 3.47%, 2.92%, 1.32%, and 0.81%, respectively. The relative abundances of Proteobacteria,

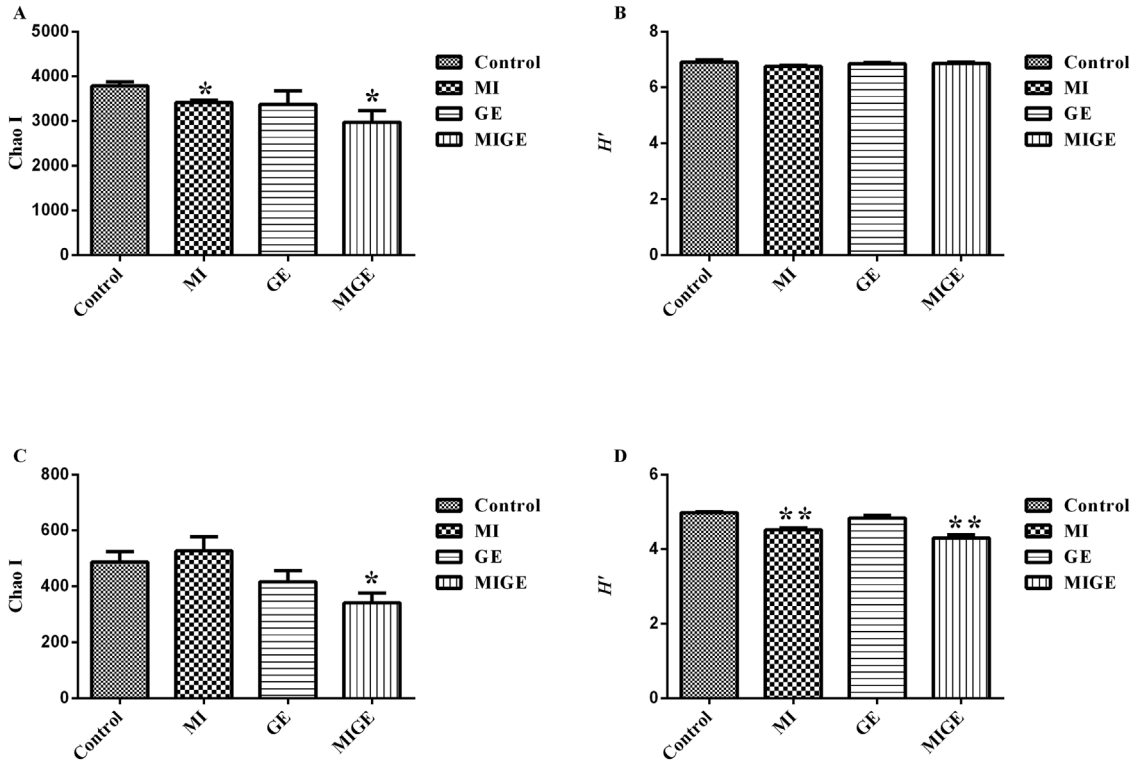

Fig. 3. Bacterial and fungal diversity in the control, MI, GE, and MIGE soils. (A) and (B) show the Chao I and *H′* values for the bacterial community, respectively. (C) and (D) show the Chao I and *H′* values for the fungal community, respectively. All values represent means ± standard errors (n = 3). *Significant differences at the 0.05 level. **Significant differences at the 0.01 level.

Bacteroidetes, Firmicutes, and Patescibacteria were greater in the treatment groups (MI, GE, and MIGE) than in the control group, to varying degrees, although the opposite was true for Chloroflexi and Planctomycetes. These results provide important insights into shifts in the structure of bacterial communities in response to MI and GE under Cd stress.

ITS sequencing identified a total of 260,404 classifiable fungal sequences from the 12 soil samples after quality control filtering, with a mean of 65,101 classifiable sequences per sample (dominant length: 201–250 bp). Next, the Chao I and *H′* indices in the rhizosphere soil fungal microbiome under different treatments were analyzed. The results showed that when applied separately or in combination, MI and GE made significant contributions to variations in soil microbial diversity (Fig. 3C and D). Venn diagram analysis revealed that the control, MI, GE, and MIGE groups shared 326 fungal OTUs, while 109, 152, 75, and 72 OTUs were featured exclusively in the control, MI, GE, and MIGE groups, respectively. The results of the PCA revealed a difference in fungal communities from different groups (Fig. 4B). Similarly, the fungal communities in the MI and MIGE groups were strikingly different from other groups. The first principal component (31.55% contribution) differentiated the fungal communities of MIGE group from those of the GE and Control groups. Taxonomic information at the

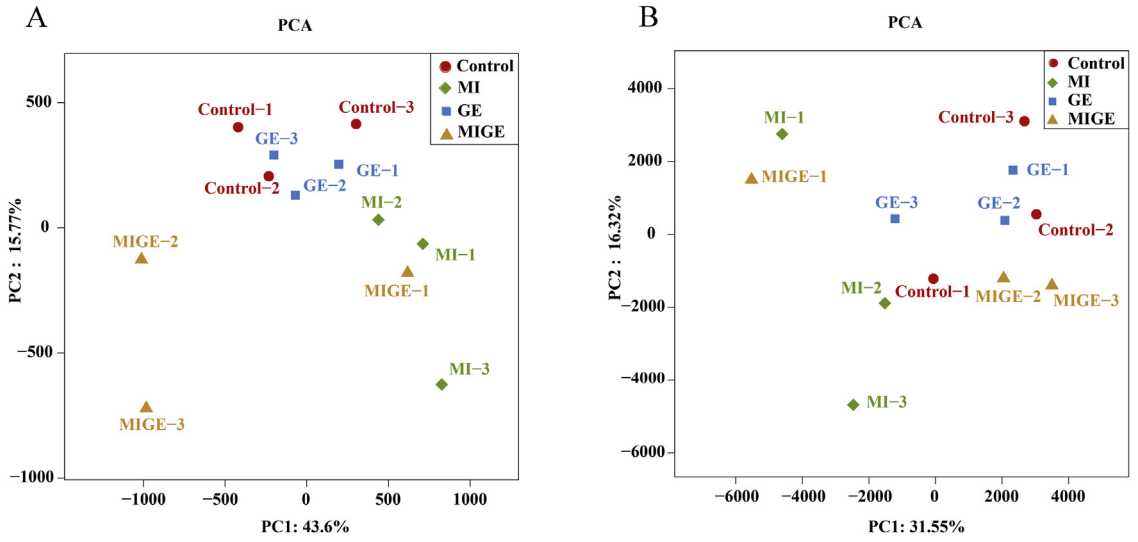

Fig. 4. Principal component analysis (PCA) plot based on the bacterial (A) and fungal (B) community from the control, MI, GE, and MIGE soil samples. The scatter plot is of principal coordinate 1 (PC1) vs. principal coordinate 2 (PC2).

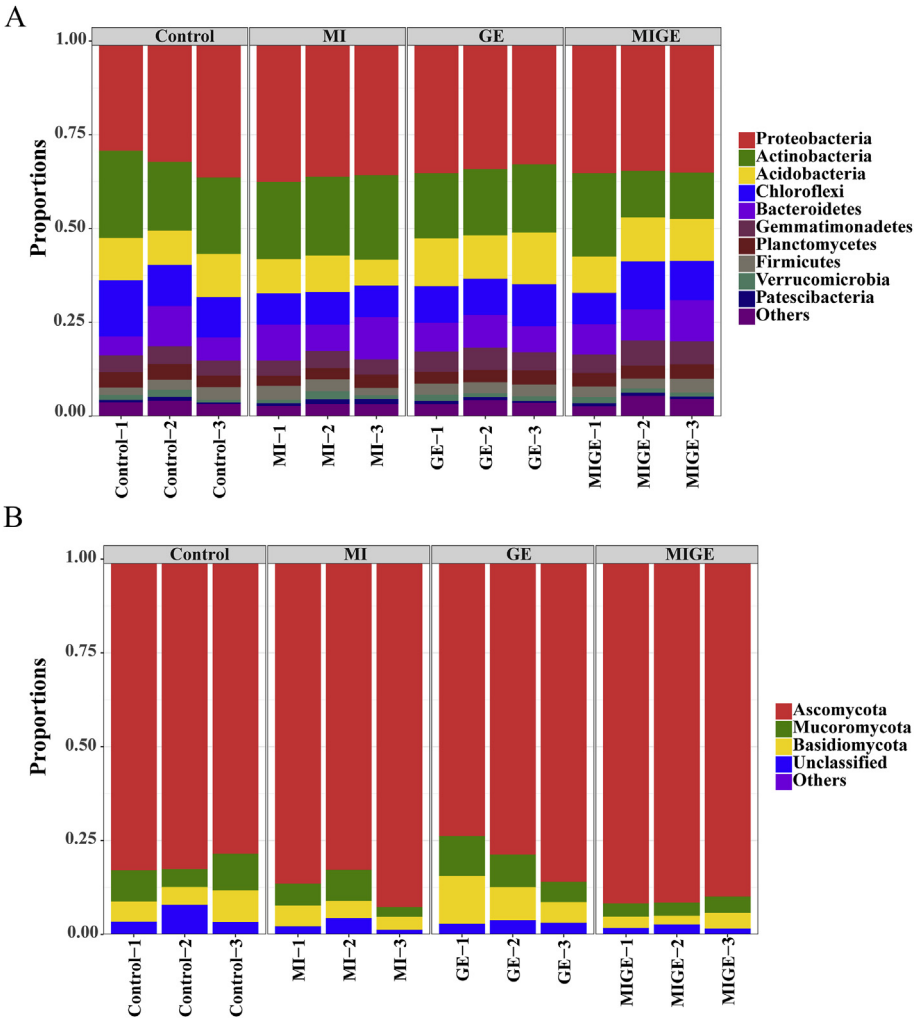

Fig. 5. Taxonomic classification of bacterial (A) and fungal (B) reads retrieved from the control, MI, GE, and MIGE soils at the phylum level. The bar marked Others represents the relative abundance of all other phyla that are not specifically listed.

phylum level is shown in Fig. 5B. The predominant phyla across all samples were Ascomycota, Mucoromycota, and Basidiomycota accounting on average for 84.81%, 6.30%, and 5.73% of the total population of all soil samples, respectively. Compared to the control groups, the relative abundances of Ascomycota, Mucoromycota, and Basidiomycota showed fluctuation in the MI, GE, and MIGE samples. The highest relative abundance for Ascomycota was identified in the combined MIGE group, followed by the Control and MI groups; the lowest relative abundance for Ascomycota was observed in the GE group. The highest relative abundances of both Mucoromycota and Basidiomycota were detected in the GE group, followed by the control and MI groups, and the lowest relative abundances for both Mucoromycota and Basidiomycota were detected in the combined MIGE group.

3.5. Relative abundances of specific bacterial and fungal genera

The LEfSe algorithm was used to identify bacterial genera from all OTUs, showing statistically different abundances between different groups (Fig. 6). At the genus level, the groups that were more abundant in the MI samples than in the control samples and the relative abundances were *Blastococcus* (52.32%), *Brevundimonas* (264.06%), *Microbacterium* (86.96%), *Pseudonocardia* (118.52%), *Qipengyuania* (114.61%), *Friedmanniella* (600.00%), and *Actinomycetospora* (77.78%). The relative abundances of *Devosia*, *Cupriavidus*, *Pseudactinotalea*, *Afipia*, and *Pelagibacterium* in the MIGE samples to the control samples were 64.14%, 199.15%, 500.00%, 272.55%, and 1762.50% greater, respectively. In addition, *Rhizobacter*, *Nordella*, and *Neorhizobium* were more abundant in the control samples than in the MIGE samples. The soil samples in the treated groups (MI, GE, and MIGE) featured significant changes in the relative abundances of specific bacterial genera. In addition, the mean relative abundance of *Bacillus* in the MI group was 21.21% greater than in the control group, and the mean relative abundance of *Leuconostoc* in the GE group was 45.45% greater than in the control group.

LEfSe analysis also detected fungal genera that showed significant differences in abundance between different soil samples (Fig. 7). *Neonectria* (70.02%) and *Pseudombrophilain* (114.43%) were more abundant in the GE group than in the control group. The relative abundance of *Aspergillus* was 177.94% greater in the MIGE group than in the control group. However, *Cylindrocarpon* and *Monodictys* were more abundant in the control than in the treated groups. These results indicated that MI, GE, and MIGE led to significant differences in the relative abundance of specific fungi genera from the abundance in the control. Likewise, the mean relative abundance of *Candida* in the GE group was 611.11% greater than in the control group.

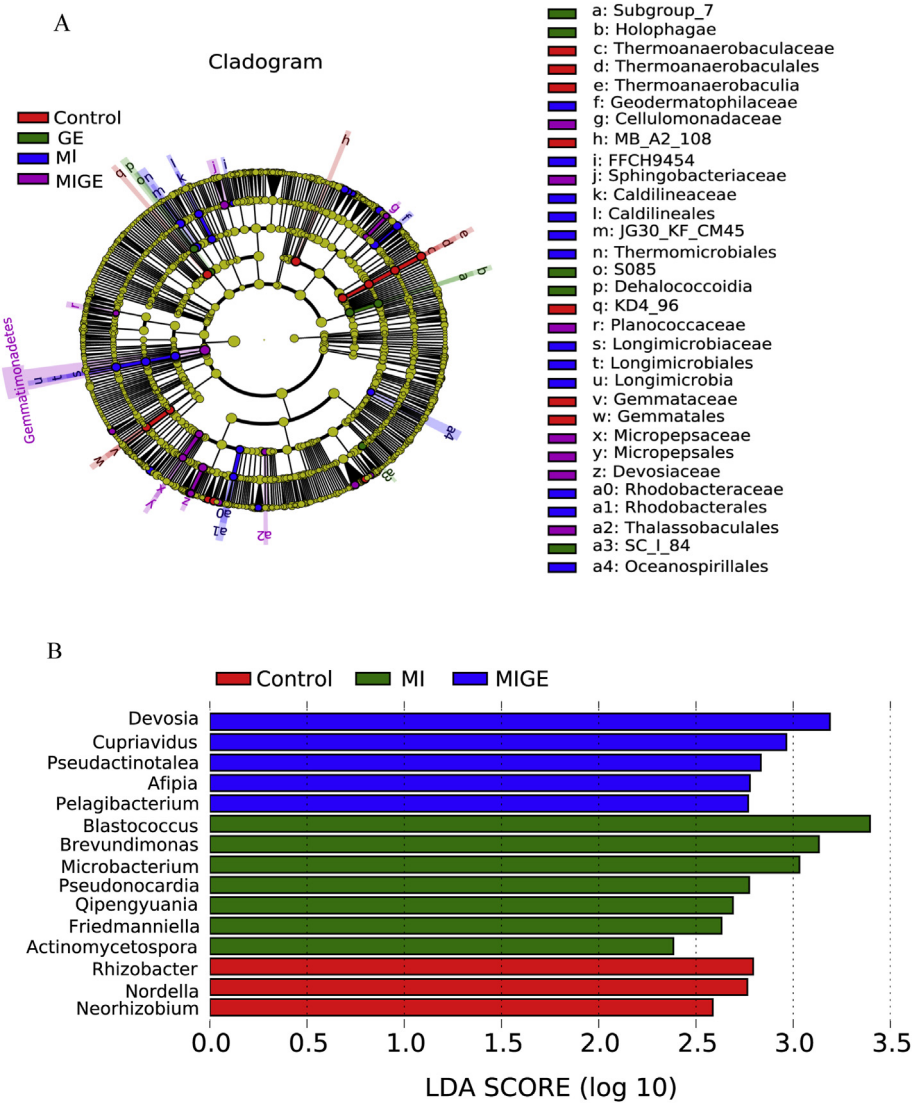

Fig. 6. Linear discriminant analysis effect size analysis of bacterial taxa for the control, MI, GE and MIGE soils. (A) Cladograms showing significantly enriched bacterial taxa (from the phylum to the genus level). (B) Bar chart showing linear discriminant analysis (LDA) scores for the bacterial taxa. Significance was defined as $P < 0.05$ and LDA score > 2.0.

4. Discussion

4.1. Potential of MI and GE to improve S. miltiorrhiza quality

This study found that MI and GE reduced Cd uptake in *S. miltiorrhiza* roots and that a combined treatment of MI and GE was more effective than either was separately. Many beneficial microorganisms, such as strains from the genera *Lactobacillus, Pichia*, and *Bacillus* (*B. amyloliquefaciens* and *B. licheniformis*), are contained in the MI and GE used in this research, and many of those microorganisms show the potential bioremediation ability of Cd. *Bacillus amyloliquefaciens* SAY09 can increase cadmium resistance in plants by activating auxin-mediated signaling pathways (Zhou et al., 2017). The extracellular polysaccharides extracted from isolated *B. licheniformis* NSPA5 show the strong biosorption ability of Cd and have potential application to the treatment of Cd-contaminated waters (Shameer, 2016). *Pichia hampshirensis* 4Aer can remove Cd by surface adsorption as well as intracellular accumulation, as was confirmed by Fourier transform infrared spectroscopy, EDS, scanning electron microscopy, and TEM (Khan et al., 2016). Kirillova et al. (2017) assessed the resistance and bioremediation ability of *Lactobacillus* strains to Cd and proposed a metabolism-dependent accumulation mechanism for Cd removal. Therefore, in this study, the reduction of Cd uptake in *S. miltiorrhiza* roots would mainly be ascribed to the inoculation of these beneficial microorganism. At present, GE is mainly used in polluted wastewater treatment due to its ability to remove impurities, harmful sludge, and bacteria. For example, Rasit and Kuan (2018) indicated that GE solution with 10–15% dilution applied to palm oil mill effluent can remove 50% of the chemical oxygen demand, 25% of the suspended solids, and 90% of the oil and grease. However, few reports have focused on the application of GE to the treatment of agricultural soil that is contaminated with heavy metals. To the best of our knowledge, this study is the first report on the effects of GE on the reduction of heavy metal uptake in medicinal plants.

Previous studies have reported that the inoculation of beneficial microorganisms enhances the biomass production of crops and vegetables (Assainar et al., 2018). However, in this study, MI and GE did not significantly affect root biomass in *S. miltiorrhiza*. This difference may be caused by the experimental cycle (this study was a life-cycle study), differences in treatments (such as heavy metal stress), differences in dosage and application frequency of inoculants, or other factors. In addition, the aboveground biomass should also be included in further study of *S. miltiorrhiza*. Importantly, *S. miltiorrhiza* is well-known

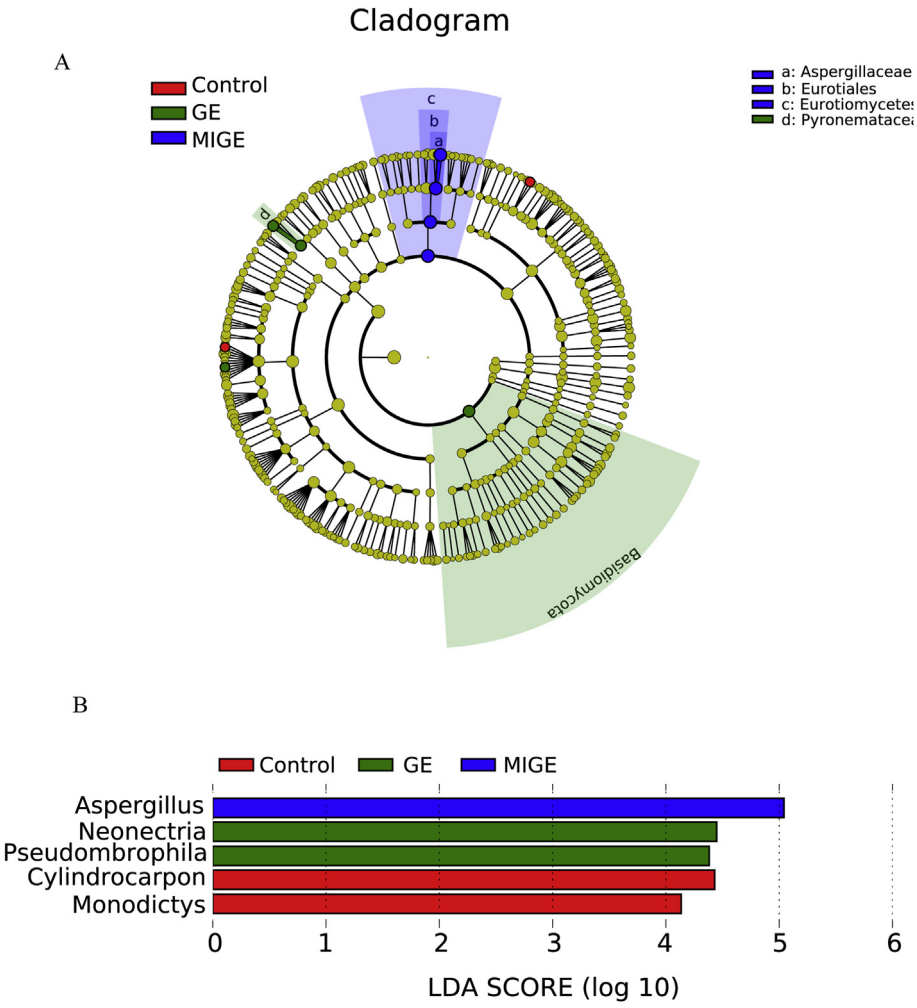

Fig. 7. Linear discriminant analysis effect size analysis of fungal taxa in the control, MI, GE, and MIGE soils. (A) Cladograms showing significantly enriched fungal taxa (from the phylum to the genus level). (B) Bar chart showing the linear discriminant analysis (LDA) score of fungal taxa. Significant differences are defined as $P < 0.05$ and LDA score > 4.0.

globally as a medicinal plant, so the effects of MI and GE on the accumulation of bioactive compounds is a focal point of concern. This study showed that MI and GE treatments improved the synthesis of TTs by 40.45% and 40.08%, respectively. The synthesis of secondary metabolites in medicinal plants is closely related to microbes, including rhizospheric bacteria and fungi. These microbes, especially endophytic fungi, may trigger a complex network of reactions that lead to the biosynthesis and accumulation of secondary metabolites in medicinal plants. For example, Zhai et al. (2018) indicated that *Chaetomium globosum* D38 could significantly stimulate the secondary metabolism in *S. miltiorrhiza* and increase the production of tanshinones, especially dihydrotanshinone I and cryptotanshinone. Microbes can also affect the accumulation of active compounds in medicinal plants by influencing the expression of genes related to the secondary metabolite biosynthetic pathway. For example, it was found that the transcriptional activity of genes involved in the tanshinone biosynthetic pathway was significantly greater under treatment of polysaccharide fraction from the endophytic fungus *Trichoderma atroviride* (Ming et al., 2013). The effects of microbes on plants, such as promoting plant growth, the accumulation of secondary metabolites, and plant protection, do not necessarily result from a direct effect of the inoculated strains and may be related to the induction or repression of resident microbial populations (Trabelsi and Mhamdi, 2013). Although the above-mentioned strains were not directly inoculated in this experiment, the beneficial microorganisms in MI and GE may trigger a series of complex reactions through direct effects on the soil community and/or indirect effects mediated by the plant–microbes system. This may be the mechanism by which MI and GE have favorable effects on tanshinone accumulation in *S. miltiorrhiza*.

However, a combined treatment of MI and GE (MIGE) led to only a 9.90% greater synthesis of TTs than Control group, meaning that the combined treatment did not have a more favorable effect. This supports previous studies indicating that a combination of inoculants will not necessarily produce an additive or synergic effect, but rather a competitive one, on plant protection and plant growth promotion. The combination of *Bacillus subtilis* and *Azospirillum brasilense* had no synergistic or comparable effects on plant biomass in their single applications (Felici et al., 2008). A co-inoculation of *Phaseolus vulgaris* with two indigenous rhizobia strains showed no additive effect on plant growth promotion and nitrogen turnover processes but rather a reduced efficacy (Trabelsi et al., 2011). Therefore, the results of this study were in agreement with these previous researches. The application of MIs in agriculture is promising, and the number of MI products has been steadily increasing, as it has become known that MIs can reduce the use of chemical fertilizers and pesticides. However, in the absence of research to confirm that co-inoculation has additive or synergic effects, it is not recommended to use MI combinations casually. Among MI, GE, and MIGE, GE was found to be the most effective for reducing Cd uptake and simultaneously increasing the accumulation of tanshinones. Thus, GE could be used in Cd-contaminated farmland to improve the

quality of the *S. miltiorrhiza* grown there and to ensure consumer safety. MIs and GE are most effective for increasing tanshinones and reducing Cd uptake, respectively, so specific needs should be evaluated. However, studies under real field conditions (not just pot experiments) are needed to validate these inferences.

In addition, the contents of salvianolic acid were slightly lower after MI, GE, and MIGE treatments than in the control, but they were higher than the standard prescribed by Chinese pharmacopoeia. The biosynthetic pathways of phenolic acids and tanshinones are very different, so one biotic elicitor may not affect the accumulation of two secondary metabolites. For example, SmMYB36, a novel R2R3-MYB transcription factor, enhances tanshinone accumulation but decreases phenolic acid content in the hairy roots of *S. miltiorrhiza*. (Ding et al., 2017). In addition, phenolic acids and tanshinones possess different chemical properties. Tanshinones have much stronger antimicrobial activity than phenolic acids, with DT-I and CT being the strongest antimicrobial components among the tanshinones in the hairy roots of *S. miltiorrhiza*. These may explain why, in the present study, MI and GE were associated with dramatically greater biosynthesis of tanshinones but lower accumulations of salvianolic acid.

4.2. Use of MIs and GE is an effective microbial remediation strategy

MI and GE are two types of bioremediation-based agents, which are attracting increasing levels of research attention. To evaluate the bioremediation effects of MI and GE, this study used high-throughput sequencing to determine the response of the rhizosphere microbial community to the application of MI and GE. The results of this study showed that MI and GE treatments not only had an effect on the microbial community structure but also on microbial abundance. LEfSe analysis further revealed that the relative abundance of some genera (including bacteria and fungi) was significantly greater in soils treated with MI, GE, or MIGE than in the control group, including *Brevundimonas*, *Cupriavidus*, *Rhizobacter*, *Aspergillus*, *Microbacterium*, *Pseudonocardia*, and *Neonectria*. This study found that many of these genera were associated with the bioremediation of heavy metals, such as *Brevundimonas*, *Microbacterium*, *Cupriavidus*, and *Aspergillus*. The use of an MI increased the relative abundance of *Brevundimonas* and *Microbacterium* by 264.06% and 86.96%, respectively. The combined treatment with an MI and GE had relative abundances of *Cupriavidus* and *Aspergillus* that were 199.15% and 177.94% greater than the control, respectively. A number of previous studies have reported that isolates from the genera *Brevundimonas* possess Cu resistance and the potential for phytoremediation of soils polluted by Cu (Kepenek et al., 2019; Rathi and Nandabalan, 2017). Fierros-Romero et al. (2017) reported that *Microbacterium liquefaciens* is a nickel-vanadium-resistant bacterium than can remove nickel and vanadium from liquid media. According to Jiang et al. (2017), *Cupriavidus* strains are tolerant of Cd up to a maximum concentration of 200 $mg{\cdot}L^{-1}$. Recent research has also reported that strains of the *Aspergillus* genus are Cd-resistant and are an eco-friendly and highly efficient biosorbent material for the removal of Cd from aqueous solutions (Li et al., 2017; Mahmoud et al., 2017). The increasing abundance of these beneficial microorganisms (*Brevundimonas*, *Microbacterium*, *Cupriavidus*, and *Aspergillus*) confirmed the effective remediation effects of MI and GE in soil contaminated by Cd. We suggest that these effects were driven by improvements in micro-ecology. The present study indicated that the application of MI and GE should be considered to be effective microbial remediation strategies for soils contaminated with Cd. Microorganisms play a key role in the remediation of environments contaminated with heavy metal due to their ability to sequester, precipitate or change the oxidation state of numerous heavy metals (Ojuederie and Babalola, 2017). However, several studies have confirmed the negative influences of heavy metal pollution on soil microorganisms (Etesami, 2018; Guo et al., 2017; Wang et al., 2007). For example, Chen et al. (2014) demonstrated that heavy metal pollution (such as with Cd, Cu, and Pb) reduces the abundance, diversity, and activity of microbes. Consequently, bioremediation has become an innovative and environmentally friendly technique for the removal and recovery of heavy metal ions from polluted areas, capitalizing on the activities of algae, bacteria, fungi, or plants (Manasi et al., 2018).

It has been extensively reported that microbial-based inoculants, such as plant-growth-promoting rhizobacteria (PGPRs), have the capacity to stimulate plant growth by increasing nutrient uptake and that heavy metal-resistant bacteria or fungi can be efficient bioremediators of metals because they possess numerous mechanisms that allow them to endure metal toxicity (Naz et al., 2016). The microbial-based inoculant used in present study, *tianxia diyi jun*, predominantly consists of a range of PGPRs, including *B. amyloliquefaciens*, *B. lincheniformis*, and *Actinomyces bovis*. Even though microbial-based inoculants with *Bacillus* spp. have been commercialized, little research has been conducted on their potential use in the cultivation of medicinal plants. This study provides important insights into the shifts created in the rhizosphere microbial community and Cd content of roots in a medicinal plant (*S. miltiorrhiza*) in response to an MI and in 4 effective components. The predominant microorganisms detected in the GE samples were lactic acid bacteria and yeast, which supports the research published by Du et al. (2017) on the microbial diversity of the papaya enzyme. However, the composition of the microbial community at the species level in using GE has not been identified, and it is essential to identify effective strains that have heavy metal tolerance and a high capacity for the removal of heavy metals.

Although the results of this study clearly show that GE treatment reduces the concentration of Cd in roots, further research is needed to verify bioremediation effects in soils contaminated with other heavy metals or in other soils with different physical and chemical properties.

5. Conclusions

The application of an MI and GE, either separately or in combination, can reduce Cd uptake by 5.84–37.90% and increase tanshinone accumulation by 9.90–40.45% in *S. miltiorrhiza*. In addition, the relative abundance of specific microbial genera that have a high capacity for heavy metal removal significantly increased. Thus, it appears that MI and GE can be applied in Cd-contaminated farmland to improve the quality of *S. miltiorrhiza* and facilitate microbial remediation.

Author contributions

Jianping Han designed and supervised the study, and Xuemin Wei conducted the experimental work and drafted the manuscript. All of the authors contributed to the writing of the manuscript and approved the final manuscript.

Funding

This work was supported by the National Natural Science Foundation of China [No.81673552] and the CAMS Innovation Fund for Medical Sciences [CIFMS, 2016–I2M–3–016].

Declaration of competing interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecoenv.2020.110311.

References

Agency for Toxic Substances and Disease Registry, 2017. Priority List of Hazardous Substances. https://www.atsdr.cdc.gov/spl/, Accessed date: 19 July 2019.

An, H., Zheng, W., Gao, Y., 2007. Research progress in cadmium toxicity. J. Environ. Health 24, 739–742. https://doi.org/10.3969/j.issn.1001-5914.2007.09.036.

Arun, C., Sivashanmugam, P., 2015. Identification and optimization of parameters for the semi-continuous production of garbage enzyme from pre-consumer organic waste by green RP-HPLC method. Waste Manag. 44, 28–33. https://doi.org/10.1016/j.wasman.2015.07.010.

Arun, C., Sivashanmugam, P., 2017. Study on optimization of process parameters for enhancing the multi-hydrolytic enzyme activity in garbage enzyme produced from preconsumer organic waste. Bioresour. Technol. 226, 200–210. https://doi.org/10.1016/j.biortech.2016.12.029.

Arun, C., Sivashanmugam, P., 2018. Enhanced production of biohydrogen from dairy waste activated sludge pre-treated using multi hydrolytic garbage enzyme complex and ultrasound-optimization. Energy Convers. Manag. 164, 277–287.

Assainar, S.K., Abbott, L.K., Mickan, B.S., Whiteley, A.S., Siddique, K., Solaiman, Z.M., 2018. Response of wheat to a multiple species microbial inoculant compared to fertilizer application. Front. Plant Sci. 9, 1601. https://doi.org/10.3389/fpls.2018.01601.

Ayangbenro, A.S., Babalola, O.O., 2017. A new strategy for heavy metal polluted environments: a review of microbial biosorbents. Int. J. Environ. Res. Publ. Health 14. https://doi.org/10.3390/ijerph14010094.

Baez-Rogelio, A., Morales-Garcia, Y.E., Quintero-Hernandez, V., Munoz-Rojas, J., 2017. Next generation of microbial inoculants for agriculture and bioremediation. Microb Biotechnol 10, 19–21. https://doi.org/10.1111/1751-7915.12448.

Calvo, P., Watts, D.B., Kloepper, J.W., Torbert, H.A., 2016. The influence of microbial-based inoculants on N_2O emissions from soil planted with corn (Zea mays L.) under greenhouse conditions with different nitrogen fertilizer regimens. Can. J. Microbiol. 62, 1041–1056. https://doi.org/10.1139/cjm-2016-0122.

Caporaso, J.G., Bittinger, K., Bushman, F.D., DeSantis, T.Z., Andersen, G.L., Knight, R., 2010a. PyNAST: a flexible tool for aligning sequences to a template alignment. Bioinformatics 26, 266–267. https://doi.org/10.1093/bioinformatics/btp636.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Pena, A.G., Goodrich, J.K., Gordon, J.I., Huttley, G.A., Kelley, S.T., Knights, D., Koenig, J.E., Ley, R.E., Lozupone, C.A., McDonald, D., Muegge, B.D., Pirrung, M., Reeder, J., Sevinsky, J.R., Turnbaugh, P.J., Walters, W.A., Widmann, J., Yatsunenko, T., Zaneveld, J., Knight, R., 2010b. QIIME allows analysis of high-throughput community sequencing data. Nat. Methods 7, 335–336. https://doi.org/10.1038/nmeth.f.303.

Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Huntley, J., Fierer, N., Owens, S.M., Betley, J., Fraser, L., Bauer, M., Gormley, N., Gilbert, J.A., Smith, G., Knight, R., 2012. Ultra-high-throughput microbial community analysis on the Illumina Hiseq and Miseq platforms. ISME J. 6, 1621–1624. https://doi.org/10.1038/ismej.2012.8.

Chen, J., He, F., Zhang, X., Sun, X., Zheng, J., Zheng, J., 2014. Heavy metal pollution decreases microbial abundance, diversity and activity within particle-size fractions of a paddy soil. FEMS Microbiol. Ecol. 87, 164–181. https://doi.org/10.1111/1574-6941.12212.

Choi, H.G., Tran, P.T., Lee, J.H., Min, B.S., Kim, J.A., 2018. Anti-inflammatory activity of caffeic acid derivatives isolated from the roots of Salvia miltiorrhiza Bunge. Arch Pharm. Res. (Seoul) 41, 64–70. https://doi.org/10.1007/s12272-017-0983-1.

Dawkar, V.V., Jadhav, U.U., Tamboli, D.P., Govindwar, S.P., 2010. Efficient industrial dye decolorization by Bacillus sp. VUS with its enzyme system. Ecotoxicol. Environ. Saf. 73, 1696–1703. https://doi.org/10.1016/j.ecoenv.2010.07.002.

Dghaim, R., Al, K.S., Rasool, H., Ali, K.M., 2015. Determination of heavy metals concentration in traditional herbs commonly consumed in the United Arab Emirates. J Environ Public Health 2015, 973878. https://doi.org/10.1155/2015/973878.

Ding, K., Pei, T., Bai, Z., Jia, Y., Ma, P., Liang, Z., 2017. SMMYB36, a novel R2R3-MYB transcription factor, enhances tanshinone accumulation and decreases phenolic acid content in *Salvia miltiorrhiza* hairy roots. Sci. Rep. 7, 5104. https://doi.org/10.1038/s41598-017-04909-w.

Du, L., Liu, Y., Jiao, Y., Ma, L., Xiao, D., Guan, Y., 2017. Polymerase chain reaction-denaturing gradient gel electrophoresis analysis of microbial diversity of papaya enzyme preparation during natural fermentation. Mod Food Sci Tech 33, 80–87. https://doi.org/10.13982/j.mfst.1673-9078.2017.8.013.

Edgar, R.C., 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nat. Methods 10, 996–998. https://doi.org/10.1038/NMETH.2604.

Elsanhoty, R.M., Al-Turki, I.A., Ramadan, M.F., 2016. Application of lactic acid bacteria in removing heavy metals and aflatoxin B1 from contaminated water. Water Sci. Technol. 74 (3), 625–638. https://doi.org/10.2166/wst.2016.255.

Etesami, H., 2018. Bacterial mediated alleviation of heavy metal stress and decreased accumulation of metals in plant tissues: mechanisms and future prospects. Ecotoxicol. Environ. Saf. 147, 175–191. https://doi.org/10.1016/j.ecoenv.2017.08.032.

Felici, C., Vettori, L., Giraldi, E., Forino, L.M.C., Toffanin, A., Tagliasacchi, A.M., Nuti, M., 2008. Single and co-inoculation of *Bacillus subtilis* and *Azospirillum brasilense* on *Lycopersicon esculentum*: effects on plant growth and rhizosphere microbial community. Appl. Soil Ecol. 40, 260–270. https://doi.org/10.1016/j.apsoil.2008.05.002.

Fernandez, P.M., Martorell, M.M., Blaser, M.G., Ruberto, L., de Figueroa, L., Mac, C.W., 2017. Phenol degradation and heavy metal tolerance of Antarctic yeasts. Extremophiles 21, 445–457. https://doi.org/10.1007/s00792-017-0915-5.

Fierros-Romero, G., Wrosek-Cabrera, J.A., Gomez-Ramirez, M., Pless, R.C., Rivas-Castillo, A.M., Rojas-Avelizapa, N.G., 2017. Expression changes in metal-resistance genes in Microbacterium liquefaciens under nickel and vanadium exposure. Curr. Microbiol. 74, 840–847. https://doi.org/10.1007/s00284-017-1252-8.

Gong, B., Nie, W., Yan, Y., Gao, Z., Shi, Q., 2017. Unravelling cadmium toxicity and nitric oxide induced tolerance in Cucumis sativus: insight into regulatory mechanisms using proteomics. J. Hazard Mater. 336, 202–213. https://doi.org/10.1016/j.jhazmat.2017.04.058.

Guo, H., Nasir, M., Lv, J., Dai, Y., Gao, J., 2017. Understanding the variation of microbial community in heavy metals contaminated soil using high throughput sequencing. Ecotoxicol. Environ. Saf. 144, 300–306. https://doi.org/10.1016/j.ecoenv.2017.06.048.

Huang, F., Wang, Z.H., Cai, Y.X., Chen, S.H., Tian, J.H., Cai, K.Z., 2018. Heavy metal bioaccumulation and cation release by growing Bacillus cereus RC-1 under culture conditions. Ecotoxicol. Environ. Saf. 157, 216–226. https://doi.org/10.1016/j.ecoenv.2018.03.077.

Jan, M., Shah, G., Masood, S., Shinwari, K.I., Hameed, R., Rha, E.S., Jamil, M., 2019. Bacillus cereus enhanced phytoremediation ability of rice seedlings under cadmium toxicity. BioMed Res. Int. 2019, 8134651. https://doi.org/10.1155/2019/8134651.

Jiang, J., Pan, C., Xiao, A., Yang, X., Zhang, G., 2017. Isolation, identification, and environmental adaptability of heavy-metal-resistant bacteria from ramie rhizosphere soil around mine refinery. 3 Biotech 7, 5. https://doi.org/10.1007/s13205-017-0603-2.

Kepenek, E.S., Gozen, A.G., Severcan, F., 2019. Molecular characterization of acutely and gradually heavy metal acclimated aquatic bacteria by FTIR spectraoscopy. J. Biophot. 12, e201800301. https://doi.org/10.1002/jbio.201800301.

Khan, Z., Rehman, A., Hussain, S.Z., 2016. Resistance and uptake of cadmium by yeast, *Pichia hampshirensis* 4AER, isolated from industrial effluent and its potential use in decontamination of wastewater. Chemosphere 159, 32–43. https://doi.org/10.1016/j.chemosphere.2016.05.076.

Kirillova, A.V., Danilushkina, A.A., Irisov, D.S., Bruslik, N.L., Fakhrullin, R.F., Zakharov, Y.A., Bukhmin, V.S., Yarullina, D.R., 2017. Assessment of resistance and bioremediation ability of lactobacillus strains to lead and cadmium. Internet J. Microbiol. 2017, 9869145. https://doi.org/10.1155/2017/9869145.

Koljalg, U., Nilsson, R.H., Abarenkov, K., Tedersoo, L., Taylor, A.F., Bahram, M., Bates, S.T., Bruns, T.D., Bengtsson-Palme, J., Callaghan, T.M., Douglas, B., Drenkhan, T., Eberhardt, U., Duenas, M., Grebenc, T., Griffith, G.W., Hartmann, M., Kirk, P.M., Kohout, P., Larsson, E., Lindahl, B.D., Lucking, R., Martin, M.P., Matheny, P.B., Nguyen, N.H., Niskanen, T., Oja, J., Peay, K.G., Peintner, U., Peterson, M., Poldmaa, K., Saag, L., Saar, I., Schussler, A., Scott, J.A., Senes, C., Smith, M.E., Suija, A., Taylor, D.L., Telleria, M.T., Weiss, M., Larsson, K.H., 2013. Towards a unified paradigm for sequence-based identification of fungi. Mol. Ecol. 22, 5271–5277. https://doi.org/10.1111/mec.12481.

Yang, Q., Li, F.Z., Li, S.S., Wang, Y., Yang, R.L., Du, B., Zhao, M.H., 2016. Study on improving Soil Organic Matter and Phosphorus by Garbage Enzymes. Environ. Sci. Surv. 35, 65–69. https://doi.org/10.3969/j.issn.1673-9655.2016.05.015.

Li, X., Wang, S., Guo, L., Huang, L., 2013. Effect of cadmium in the soil on growth, secondary metabolites and metal uptake in *Salvia miltiorrhiza*. Toxicol. Environ. Chem. 95, 1525–1538. https://doi.org/10.1080/02772248.2014.887717.

Li, X., Gitau, M.M., Han, S., Fu, J., Xie, Y., 2017. Effects of cadmium-resistant fungi Aspergillus aculeatus on metabolic profiles of bermudagrass [Cynodondactylon (L.) Pers.] under Cd stress. Plant Physiol. Biochem. 114, 38–50. https://doi.org/10.1016/j.plaphy.2017.02.014.

Mahmoud, M.E., El, Z.G., Farag, A., Abdelwahab, M.S., 2017. Assessment of heat-inactivated marine Aspergillus flavus as a novel biosorbent for removal of Cd (II), Hg (II), and Pb (II) from water. Environ. Sci. Pollut. Res. Int. 24, 18218–18228. https://doi.org/10.1007/s11356-017-9323-8.

Manasi, Tibrewal, A., Rajesh, N., Rajesh, V., 2018. Identification and Characterization of the microbial communities found in electronic industrial effluent and their potential for bioremediation. Ecotoxicol. Environ. Saf. 164, 379–387. https://doi.org/10.1016/j.ecoenv.2018.08.018.

Meng, M., Chen, T., Li, J., Ma, Z., Jia, W., Jia, M., 2009. Determination of Pb, Cd, As, Hg, Cu in radix Salviae miitiorrhizae. Tianjin J Tradit Chin Med 26, 248–249.

Mezynska, M., Brzoska, M.M., 2018. Environmental exposure to cadmium-a risk for health of the general population in industrialized countries and preventive strategies. Environ. Sci. Pollut. Res. Int. 25, 3211–3232. https://doi.org/10.1007/s11356-017-0827-z.

Ming, Q., Su, C., Zheng, C., Jia, M., Zhang, Q., Zhang, H., Rahman, K., Han, T., Qin, L., 2013. Elicitors from the endophytic fungus trichoderma atroviride promote *Salvia miltiorrhiza* hairy root growth and tanshinone biosynthesis. J. Exp. Bot. 64, 5687–5694. https://doi.org/10.1093/jxb/ert342.

Mueller, R.C., Paula, F.S., Mirza, B.S., Rodrigues, J.L., Nusslein, K., Bohannan, B.J., 2014. Links between plant and fungal communities across a deforestation chronosequence in the Amazon rainforest. ISME J. 8, 1548–1550. https://doi.org/10.1038/ismej.2013.253.

Nayak, A.K., Raja, R., Rao, K.S., Shukla, A.K., Mohanty, S., Shahid, M., Tripathi, R., Panda, B.B., Bhattacharyya, P., Kumar, A., Lal, B., Sethi, S.K., Puri, C., Nayak, D., Swain, C.K., 2015. Effect of fly ash application on soil microbial response and heavy metal accumulation in soil and rice plant. Ecotoxicol. Environ. Saf. 114, 257–262. https://doi.org/10.1016/j.ecoenv.2014.03.033.

Naz, T., Khan, M.D., Ahmed, I., Rehman, S.U., Rha, E.S., Malook, I., Jamil, M., 2016. Biosorption of heavy metals by Pseudomonas species isolated from sugar industry. Toxicol. Ind. Health 32, 1619–1627. https://doi.org/10.1177/0748233715569900.

Ojuederie, O.B., Babalola, O.O., 2017. Microbial and plant-assisted bioremediation of heavy metal polluted environments: a review. Int. J. Environ. Res. Publ. Health 14. https://doi.org/10.3390/ijerph14121504.

Ouyang, C., Gu, L., 2017. Experiment on the control effect of garlic enzyme on two common diseases of strawberry in greenhouse. Anhui Agri. Sci. Bull. 23, 73–74. https://doi.org/10.16377/j.cnki.issn1007-7731.2017.23.033.

Pharmacopoeia Committee of P. R. China, 2015. Pharmacopoeia of the People's Republic

of China. China Medical Science Press, Beijing., China, pp. 76–77.
Quagliariello, A., Del, C.F., Russo, A., Reddel, S., Conte, G., Lopetuso, L.R., Ianiro, G., Dallapiccola, B., Cardona, F., Gasbarrini, A., Putignani, L., 2018. Gut microbiota profiling and gut-brain crosstalk in children affected by pediatric acute-onset neuropsychiatric syndrome and pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections. Front. Microbiol. 9, 675. https://doi.org/10.3389/fmicb.2018.00675.
Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glockner, F.O., 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 41, D590–D596. https://doi.org/10.1093/nar/gks1219.
Rasit, N., Kuan, O.C., 2018. Investigation on the influence of bio-catalytic enzyme produced from fruit and vegetable waste on palm oil mill effluent. Environ Earth Sci 140, 012015. https://doi.org/10.1088/1755-1315/140/1/012015.
Rathi, M., Nandabalan, Y.K., 2017. Copper-tolerant rhizosphere bacteria-characterization and assessment of plant growth promoting factors. Environ. Sci. Pollut. Res. Int. 24, 9723–9733. https://doi.org/10.1007/s11356-017-8624-2.
Rodrigues, J.L., Pellizari, V.H., Mueller, R., Baek, K., Jesus, E.C., Paula, F.S., Mirza, B., Hamaoui, G.S., Tsai, S.M., Feigl, B., Tiedje, J.M., Bohannan, B.J.M., Nusslein, K., 2013. Conversion of the Amazon rainforest to agriculture results in biotic homogenization of soil bacterial communities. Proc. Natl. Acad. Sci. U. S. A. 110, 988–993. https://doi.org/10.1073/pnas.1220608110.
Rui, Y.K., Shen, J.B., Zhang, F.S., Yan, Y., Jing, J.Y., Meng, Q.F., 2008. Determination of heavy metal elements in KCl fertilizer by ICP-MS. Spectrosc. Spectr. Anal. 28, 2428–2430. https://doi.org/10.3964/j.issn.1000-0593(2008)10-2428-03.
Schloss, E.P., Flanagan, D.M., Culler, C.L., Wright, A.L., 2009. Some hidden costs of faculty turnover in clinical departments in one academic medical center. Acad. Med. 84, 32–36. https://doi.org/10.1097/ACM.0b013e3181906dff.
Segata, N., Izard, J., Waldron, L., Gevers, D., Miropolsky, L., Garrett, W.S., Huttenhower, C., 2011. Metagenomic biomarker discovery and explanation. Genome Biol. 12 (6), R60. https://doi.org/10.1186/gb-2011-12-6-r60.
Shaheen, S., Ahmad, R., Mahmood, Q., Mubarak, H., Mirza, N., Hayat, M.T., 2018. Physiology and selected genes expression under cadmium stress in *Arundo donax* L. Int. J. Phytoremediation 20, 1162–1167. https://doi.org/10.1080/15226514.2018.1460312.
Shameer, S., 2016. Biosorption of lead, copper and cadmium using the extracellular polysaccharides (EPS) of *Bacillus* sp. from solar salterns. 3 Biotech 6, 194. https://doi.org/10.1007/s13205-016-0498-3.
Trabelsi, D., Mhamdi, R., 2013. Microbial inoculants and their impact on soil microbial communities: a review. BioMed Res. Int. 2013, 863240. https://doi.org/10.1155/2013/863240.
Trabelsi, D., Mengoni, A., Ammar, H.B., Mhamdi, R., 2011. Effect of on-field inoculation of Phaseolus vulgaris with rhizobia on soil bacterial communities. FEMS Microbiol. Ecol. 77, 211–222. https://doi.org/10.1111/j.1574-6941.2011.01102.x.
Wang, Y., Shi, J., Wang, H., Lin, Q., Chen, X., Chen, Y., 2007. The influence of soil heavy metals pollution on soil microbial biomass, enzyme activity, and community composition near a copper smelter. Ecotoxicol. Environ. Saf. 67, 75–81. https://doi.org/10.1016/j.ecoenv.2006.03.007.
Wang, Q., Zhang, W.J., He, L.Y., Sheng, X.F., 2018. Increased biomass and quality and reduced heavy metal accumulation of edible tissues of vegetables in the presence of Cd-tolerant and immobilizing *Bacillus megaterium* H3. Ecotoxicol. Environ. Saf. 148, 269–274. https://doi.org/10.1016/j.ecoenv.2017.10.036.
Wei, W.J., Zhou, P.P., Lin, C.J., Wang, W.F., Li, Y., Gao, K., 2017. Diterpenoids from Salvia miltiorrhiza and their immune-modulating activity. J. Agric. Food Chem. 65, 5985–5993. https://doi.org/10.1021/acs.jafc.7b02384.
Xiong, J., Liu, Y., Lin, X., Zhang, H., Zeng, J., Hou, J., Yang, Y., Yao, T., Knight, R., Chu, H., 2012. Geographic distance and pH drive bacterial distribution in alkaline lake sediments across Tibetan Plateau. Environ. Microbiol. 14, 2457–2466. https://doi.org/10.1111/j.1462-2920.2012.02799.x.
Yan, H., Feng, H., Huang, W., Li, H.C., Feng, C.Q., 2012. Evaluation for heavy metal pollution of soil and herb from the main producing area of *Salvia miltiorrhiza* bge. In China. Chinese Agr Sci Bul 28, 288–293. https://doi.org/10.3724/SP.J.1011.2011.00468.
Zhai, X., Luo, D., Li, X., Han, T., Zheng, C., 2018. Endophyte chaetomium globosum d38 promotes bioactive constituents accumulation and root production in *Salvia miltiorrhiza*. Front. Microbiol. 8, 2694. https://doi.org/10.3389/fmicb.2017.02694.
Zhang, P., Ma, Y., Zhang, Z., He, X., Zhang, J., Guo, Z., Tai, R., Zhao, Y., Chai, Z., 2012. Biotransformation of ceria nanoparticles in cucumber plants. ACS Nano 6, 9943–9950. https://doi.org/10.1021/nn303543n.
Zhao, X., Huang, J., Lu, J., Sun, Y., 2019. Study on the influence of soil microbial community on the long-term heavy metal pollution of different land use types and depth layers in mine. Ecotoxicol. Environ. Saf. 170, 218–226. https://doi.org/10.1016/j.ecoenv.2018.11.136.
Zheng, J., Ma, L.T., Ren, Q.Y., Hu, Y., Bai, Y., Bian, H., Zhang, Y., Zhou, Y.C., Yang, M.H., 2017. Anti-fibrotic effects of Salvia miltiorrhiza and Ligustrazine Injection on LX-2 cells involved with increased N-myc downstream-regulated gene 2 expression. Chin. J. Integr. Med. 23, 923–928. https://doi.org/10.1007/s11655-016-2640-9.
Zhou, Z.Y., Tang, W.B., Ming, X.Q., Zhang, L.K., Zhang, G.L., Lei, B., Peng, S., Deng, H.B., Yi, G.L., 2016. Effects of spraying plant enzymes "jinhe miao" and "garbage enzyme" on yield and cadmium content of rice. China Agr Tech Exten 32, 50–52. https://doi.org/10.3969/j.issn.1002-381X.2016.06.023.
Zhou, C., Zhu, L., Ma, Z., Wang, J.F., 2017. *Bacillus amyloliquefaciens* SAY 09 increases cadmium resistance in plants by activation of auxin-mediated signaling pathways. Genes 8, 173. https://doi.org/10.3390/genes8070173.