Phytoextraction Potential of Arsenic And Cadmium And Response of Rhizosphere Microbial Community By Intercropping With Two Types of Hyperaccumulators

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Research Article

Keywords: Intercropping, As and Cd phytoextraction, rhizosphere bacterial microbiota

Posted Date: November 3rd, 2021

DOI: https://doi.org/10.21203/rs.3.rs-888645/v1

Abstract

Intercropping with hyperaccumulators/accumulators is a promising alternative to enhance phytoextraction of heavy metal(loid)s in contaminated soil. In this research, a pot experiment was conducted to evaluate the influences of intercropping As hyperaccumulator *Pteris vittata* L. with Cd hyperaccumulator *Sedum alfredii* Hance or accumulator *Hylotelephium spectabile* (Boreau) H. Ohba on the plant growth, As and Cd phytoextraction, and rhizosphere bacterial microbiota. The results indicated that intercropping can promote the growth of plants. The total biomass of *P. vittata*, *S. alfredii* and *H. spectabile* in intercropping systems was significantly improved by 19.9% - 34.1%, 16.8% and 11.5%, respectively in comparison with corresponding plant monoculture. The As content in rhizoid and frond of *P. vittata* when intercropped with *S. alfredii* was increased by 28.3% and 19.0% ($P < 0.05$), respectively as compared with *P. vittata* monoculture, and this treatment acquired the maximum As and Cd accumulation with 2032 and 397 μg•pot^{-1}, respectively. Intercropping enhanced the soil bacterial community diversity. The genera of *Lysobacter* in *S. alfredii* rhizosphere soil and *Massilia* in *P. vittata* rhizosphere soil had higher abundance in the intercropping system of *P. vittata* and *S. alfredii*. And the significantly positive correlation relationships were found between *Massilia*, *Lysobacter* and plant As content, and *Arthrobacter* with plant Cd content, indicating that they may play important roles in As and Cd phytoextraction. The results suggested that intercropping *P. vittata* with *S. alfredii* could be a potential strategy for phytoextraction of As and Cd from co-contaminated soil.

1 Introduction

Soil polluted with heavy metals (metalloids) has been one of the global environmental challenges due to their persistence and toxicity to animals, inhibition to the growth of plants and microorganisms in the soil environment (Antoniadis et al., 2017). There was more than 10 million km^2 contaminated soil in the world, and about 1.0 million km^2 contaminated soil with approximately 80% of those caused by heavy metal(loid)s has been reported in China (He et al., 2015). Among those heavy metals, high concentrations of cadmium (Cd) and arsenic (As) in soils are frequently reported (Xiao et al., 2008; Huang et al., 2016). China nationwide surveys have been shown that Cd and As ranks the first (7.0%) and third (2.7%) in the percentage of soil samples overrunning the Ministry of Environmental Protection limit among the 8 heavy-metal elements (Ni, Hg, Cu, Pb, Zn, As, Cd, Cr) (Zhao et al., 2015). A document retrieval database with heavy metal contents in soils obtained from 1443 industrial and agricultural sites in China has been reported that As and Cd were determined as the priority control pollutants (Yang et al., 2018). Arsenic and Cd have been the category one carcinogens claimed by the World Health Organization (WHO, 2017). Moreover, agricultural soil polluted with As and Cd damages human health through the food chain (Mu et al., 2019; Kumar et al., 2019). Thus, it is urgent to find an efficient and cost-effective technique for the remediation of As and Cd co-contaminated soil.

Phytoextraction utilizes the hyperaccumulators with the accumulation ability for high content of metal(loid)s in aboveground part to remove pollutants in soil and has been one of the most widespread and alternative phytoremediation techniques (Patra et al., 2020). There are 721 metal/metalloid hyperaccumulator species in the global database with the global records in July 2017 (Reeves et al., 2017). Some heavy metal accumulators with high shoot biomass such as *Linum usitatissimum* L. and *Hylotelephium spectabile* (Boreau) H. Ohba were used to remediate soil (Yang et al., 2018; Guo et al., 2020). However, the majority of hyperaccumulators and accumulators were mainly limited to choose one or two specific metals, which limits their application in phytoremediation of multi-metal(loid)s polluted soils (January et al., 2008). So far, there was little information on As and Cd co-hyperaccumulators, which was a great challenge for phytoextraction of As and Cd in co-contaminated soil due to their different opposite geochemical behavior.

Intercropping, one of the most representative agronomical practice, is helpful for the improvement of the structure of planting system and the efficiency of resource utilization (Bedoussac et al., 2015). Some researchers have reported that intercropping hyperaccumulators with other plant species could improve the tolerance of plants to heavy metal(loid)s and/or enhance the comprehensive phytoextraction efficiency (Desjardins et al., 2018). For example, the intercropping of hyperaccumulator *Sedum plumbizincicola* and moso bamboo (Bian et al., 2017), *Sedum alfredii* and oilseed rape (Cao et al., 2020), *Pteris vittata* L. and *Morus alba* L. (mulberry) (Wan et al., 2017) could simultaneously remediate soil and obtain safe crop products. The higher accumulation of different heavy metals by intercropping with diverse plant species was owing to the effect of root exudates and soil microorganisms on metal speciation and bioavailability in rhizosphere soil (Tang et al., 2017; Yu et al., 2016; Li et al., 2019). In addition, there were positive relationship between soil microorganism response and intercropping plants. For instance, the toxicity of heavy metals to soil microbial community structures could be efficiently alleviated by intercropping herbs with woody plants for phytoremediation of contaminated soil compared with monoculture (Zeng et al., 2019a). Cao et al. (2020) have founded that the variations of bacterial community in rhizosphere soil were very important for Cd phytoextraction in the intercropping system of *Sedum alfredii* and oilseed rape. Hence, when soils contaminated with multiple metals are remediated, intercropping with two or more different heavy metal(loid)s hyperaccumulators/accumulators might be an effective measure to solve this problem (Lin et al., 2018).

Arsenic hyperaccumulator *P. vittata* L. has been widely applied for the phytoextraction of As-contaminated soil (Wan et al., 2020). *S. alfredii* Hance was a Cd/Zn hyperaccumulator and was also used for phytoextraction of multiple-metal (Cd, Pb and Zn) soils (Yang et al., 2004; Liang et al., 2019). *Hylotelephium spectabile* (Boreau) H. Ohba, a potential Cd accumulator with high shoot biomass, could make up for a relatively low Cd content in aboveground parts (Zhou et al., 2020; Guo et al., 2020). Several studies about *P. vittata* or *S. alfredii* intercropping with other plant species to remediate only As or Cd contaminated soils have been reported (Wan et al., 2017; Cao et al., 2020). However, there is scarce information about the simultaneous phytoextraction As and Cd from co-contaminated soil by intercropping of As hyperaccumulator and Cd hyperaccumulator. Also, the influences of intercropping on rhizosphere microbial response and their effects on plant performance and heavy metal accumulation still remain unknown (Cao et al., 2020). In this study, a greenhouse experiment was conducted to study the phytoextraction potential of As and Cd in co-contaminated soil by intercropping *P. vittata* with *S. alfredii*/*H. spectabile*. The aims were to 1) investigate the impact of intercropping on plant biomass and physiological response; 2) compare the As and Cd content and accumulation in plants under the different intercropping systems; and 3) determine the changes in rhizosphere microflora and explore the correlation between response of rhizosphere soil microorganisms and heavy metal removal efficiency. The outcome in this study may helpful for establishing hyperaccumulator/accumulator intercropping systems to remediate soil co-polluted by As and Cd.

2 Materials And Methods

2.1 Soil sample and plant seedlings

The tested soils were sampled from a deserted agricultural land of the surface layer (0–20 cm) near a closed As smelting plant in Changde city of Hunan Province, China. After the scrap and plant residues were removed, the soil samples were air-dried, pestled, and passed through a 5 mm nylon sieve for pot experiments. The soil physicochemical properties were pH, 8.10; soil organic matter content, 16.6 $g{\bullet}kg^{-1}$; available content of nitrogen (N), phosphorous (P), and potassium (K) with 59.5, 30.0, and 109 $mg{\bullet}kg^{-1}$, respectively. The total content of As (410 $mg{\cdot}kg^{-1}$) and Cd (3.0 $mg{\cdot}kg^{-1}$) was far higher than the recommended content of As (25 $mg{\cdot}kg^{-1}$) and Cd (0.6 $mg{\cdot}kg^{-1}$) listed in the Risk Control Standard for Soil Contamination of Agricultural Land (GB15618-2018) (pH > 7.5).

Seedlings of *P. vittata* and *H. spectabile* were acquired from a nursery base in Shimen County, Hunan Province and a nursery base in Hebei Province established by the Institute of Geosciences and Resources of the Chinese Academy of Sciences, respectively. The *S. alfredii s*eedlings sampled from Zhejiang Province were planted in the unpolluted farm soil for pot experiments.

2.2 Experimental design and plant incubation

6 kg air-dried experimental soil mixed with the basic fertilizer including 0.27g $CO(NH_2)_2{\bullet}kg^{-1}$, 0.05g $KH_2PO_4{\bullet}kg^{-1}$, and 0.1g $KNO_3{\bullet}kg^{-1}$ soil was placed into a container (30 cm length × 22cm width × 12 cm height). The soil was sprayed with deionized water to maintain at equilibration with 70% water-holding capacity for two weeks. Then, the healthy seedlings with uniform size of *P. vittata*, *S. alfredii*, and *H. spectabile* were transplanted into each pot. The intercropping patterns were listed in Table S1, which included: 1) monoculture treatment of *P. vittata* (PM), *S. alfredii* (SM), and *H. spectabile* (HM) with 4 seedlings, respectively; 2) intercropping of 2 *P. vittata* with 2 *S. alfredii* seedlings (PS) or 2 *H. spectabile* seedlings (PH). The unplanning

soil was used as a control treatment (CK). Each treatment has 4 replicates. The pot experiment was conducted in a greenhouse under the controlled conditions of 14 h photoperiod with photon flux of 260–350 mmol m^{-2}•s^{-1} and a day/night temperature of 30°C/22°C. Deionized water was added into each pot daily to maintain plant's growth.

After 120 d cultivation, plants in each pot were harvested and divided into rhizoids and fronds for *P. vittata*, shoots and roots for *S. alfredii* and *H. spectabile*. The plant samples were clean washed and weighed as fresh biomass. Some fresh leaves/pinna (for *P. vittata*) was placed in 4°C for the analysis of malondialdehyde (MDA) and soluble protein contents. The rest plant parts were de-enzymized for 30 min at 105°C, dried at 65°C until a constant weight. The samples were ground and screened by a 1 mm nylon sieve for further determination. Besides, the rhizosphere soil samples from each pot were collected as described by Wang et al. (2018). The 7 rhizosphere soil samples were recorded as PM, SM and HM (monoculture treatment), PS-P (*P. vittata* in PS treatment), PS-S (*S. alfredii* in PS treatment), PH-P (*P. vittata* in PH treatment), PH-H (*H. spectabile* in PH treatment). These soil samples were used for the analysis of pH, available As and Cd content, and microbial community.

2.3 Soil and plant sample analysis

Soil physiochemical properties were measured following the methods of Lu (2000). The content of soluble protein in fresh leaves/pinna was measured using coomassie brilliant blue G-250 and bovine serum albumin, and then counted and presented as mg•g^{-1} FW (fresh weight) (Aitken and Learmonth, 2009). The MDA content was analyzed according to the thiobarbituric acid (TBA) reaction following the method of Velikova et al. (2000).

The plant and soil samples were digested in the acid mixture of HNO_3:$HClO_4$ (5:1) (Yang et al., 2012) and mixture of HNO_3–H_2O_2 (USEPA, 1996) to determine total As and Cd content, respectively. Blank and standard reference materials of plant (GBW-07603) and soil (GBW-08303) were used to assess the quality control of samples analysis. The available As and Cd content in soil was extracted using $NaHCO_3$ solution (Woolson et al., 1971) and the diethylene-triaminepenta acetic acid (DTPA) solution (Lindsay and Norvell, 1978), respectively. The Cd and As contents in digested and extracted solutions were measured through the inductive coupled plasma-optical emission spectrometer (ICP-OES, Thermo, USA) and hydrogen generation-atomic fluorescence spectrometer (AFS-2202E, Haiguang Instrument Company of Beijing, China), respectively.

The DNA extraction from the rhizosphere soil samples was performed by the method of Moffett et al. (2003) and detected using 1.0% agarose gel electrophoresis. The V3-V4 regions of the bacterial 16S rRNA gene were amplified with the polymerase chain reaction, which was performed using the 341F/806R primer set. All PCR products were recovered and purified with 2% agarose gel and AxyPrepDNA Gel Extraction Kit (Thermo Scientific, USA), eluted with Tris-HCl buffer solution, and detected with QuantiFluor™ -ST (Promega, USA) blue fluorescence quantitative system. Purified amplicons were sequenced according to an Illumina platform (Illumina Inc., San Diego, CA, USA) at Novogene Co., Ltd, Beijing, China.

2.4 Statistical analysis

The translocation factor (TF) of As and Cd in plant was counted as TF = As or Cd content in shoots(fronds)/As or Cd content in root (rhizoid) (Liang et al., 2019). The As and Cd phytoextraction efficiency was described as the plant metal accumulations, which are based on the biomass of root (rhizoid) and shoot (frond) multiplied by As and Cd content in corresponding plant part. The data were analyzed by Microsoft Excel 2016 and shown as the mean values ± standard deviations. All statistical analyses were conducted using SPSS 18.0 software. One-way analysis of variance (ANOVA) with Duncan's test at the significance level of $P < 0.05$ was applied for the comparison of differences in plant growth, As and Cd uptake among different intercropping treatments.

The Majorbio cloud platform was used for online data analysis of diversity gene sequencing. Venn diagram was drawn to highlight similarities between the monoculture and intercropping treatments. Community bar plot was drawn to show the relative abundance of microorganisms. Heat map analysis was performed using Heml 1.0. Redundancy analysis (RDA) was applied to analyze the correlation between the bacterial communities and selected soil environmental factors or the phytoremediation parameters, various R packages (http://www.r-project.org).

3 Results And Discussion

3.1 Plant growth, MDA, and soluble protein content in leaf / pinna

The plant growth was positively affected by intercropping treatment (Fig. 1). Compared with monoculture treatment (PM), the rhizoid and frond biomass of *P. vittata* was enhanced by 20.1% and 13.2% for PS treatment, 34.1% and 24.3% for PH treatment, consequently, the total biomass was significantly increased by 19.9% and 34.1%, respectively ($P < 0.05$). The shoot biomass of *S. alfredii* in PS treatment was significantly improved by 21.1%, and that of *H. spectabile* from PH treatment improved by 11.5% as compared with SM and HM, respectively ($P < 0.05$). This is consistent with previous results that intercropping could protect neighboring companion plants and enhance plant growing on soil contaminated with heavy metals (Wan et al., 2017; Zeng et al., 2019b; Cao et al., 2020). This may be contributed to the improvement of the rhizosphere environment where the soil As efficiently extracted by *P. vittata* (Wan et al., 2017). Hong et al (2017) confirmed that different plant species show different temporal and spatial resource requirements, so intercropping can obtain essential growth resources more conveniently than monoculture plant.

The content of MDA in pinna of *P. vittata* from the intercropping system of PS and PH was significantly dropped by 15.3% and 17.3% ($P < 0.05$) compared with PM, respectively (Table 1). The result was in consistent with former findings that intercropping with *Solanum nigrum* or *Solanum photeinocarpum* significantly decreased the MDA content in eggplant in comparison to monoculture (Tang et al., 2017). The reason may be related to that intercropping alleviates the oxidative stress of metal(loid)s to decrease the degree of leaf lipid peroxidation and promote the *P. vittata* growth (Du et al., 2020). The soluble protein is beneficial to reduce water loss and maintain the main function of cellular membranes stressed by heavy metals (Pan et al., 2018). Intercropping treatments of PS and PH significantly increased the soluble protein content of *P. vittata* compared with PM ($P < 0.05$) (Table 1), which was consistent with the result of higher *P. vittata* biomass from the intercropping system (Fig. 1). The difference in the leaf MDA and protein content of *S. alfredii* and *H. spectabile* between intercropping and monoculture treatment was not significant, indicating that intercropping has no obvious influence on their physiological response.

3.2 Arsenic and Cd uptake by plants

3.2.1 Uptake and transport of As and Cd

The As content in rhizoid and frond of *P. vittata* from intercropping treatments was significantly increased by 10.9 ~ 28.3% ($P < 0.05$) in contrast with PM, and the PS treatment showed the highest As content in rhizoid (245 mg•kg^{-1}) and frond (1339 mg•kg^{-1}) of *P. vittata*. The Cd content in root and shoot of *S. alfredii* in PS treatment was also significantly enhanced by 13.3% and 25.2% in comparison to monoculture, respectively ($P < 0.05$) (Table 2). Similar studies have been confirmed that As content in *P. vittata* rhizoid was significantly improved when intercropping with *Morus alba* and *Broussonetia papyrifera* L. (Wan and Lei, 2018; Zeng et al., 2019b), and Cd content in *S. alfredii* intercropped with pakchoi was significantly improved (Ma et al., 2020). These results may be due to the interactions of root exudates which increased the heavy metal phytoavailability in rhizosphere soil (Yang et al., 2006). Unfortunately, the content of Cd in *P. vittata* frond and that of As in *S. alfredii* shoot was very low with the maximum value of 2.00 mg•kg^{-1}and 19.0 mg•kg^{-1}, respectively. This may be related to that most hyperaccumulators strongly accumulate specific metals (Mahar et al., 2016). The difference in the TF_{shoot} value of As for *P. vittata* and Cd for *S. alfredii* between intercropping and corresponding monoculture treatment was not significant. The content of Cd in root and shoot of *H. spectabile* from intercropping treatment were sightly decreased, while shoot As content was significantly increased compared with HM (Table 2). Though the content of Cd and As in *H. spectabile* was low, the TF_{shoot} value of Cd was high than 1.0, suggesting that it was effective in Cd uptake. Our previous study has shown that the maximum Cd content in shoots of *H. spectabile* grown in 5 mgCd•L^{-1} solution reached up to 603 mg/kg with the TF_{shoot} value of 5.62 (Zhou et al., 2020). This may be owing to low soil Cd content in this study (Yang et al., 2018).

3.2.2 Arsenic and Cd accumulation in plants

The As accumulation in fronds of *P. vittata* was significantly enhanced by 27.5% and 23.4% ($P < 0.05$) after intercropping with *S. alfredii* and *H. spectabile* compared with PM, respectively (Table 3). Similarly, the Cd accumulation in *S. alfredii* from PS treatment was significantly increased by 14.6% ($P < 0.05$) in comparison to monoculture. Previous studies also have been reported that intercropping can enhance As accumulation in *P. vittata* intercropped with wood species of *Morus alba* L. or *Broussonetia papyrifera* L. (Zeng et al., 2019b). Cao et al. (2020) have demonstrated that co-planting with oilseed rape can improve Cd phytoextraction of *S. alfredii* due to reducing intra-species competition for nutrients and water. The As accumulation of *H. spectabile* from PH treatment was significantly higher than monoculture ($P < 0.05$). It is possible that more root exudates (organic acids) secreted between intercropped plants, which could increase the phytoavailable As and Cd contents in rhizosphere soil and promote heavy metal extraction by corresponding plant (Kim et al., 2013, Li et al., 2019). Generally, the As accumulation from PS and PH treatments were close with 2065 and 1988 $\mu g \bullet pot^{-1}$, respectively, and the Cd accumulation for PS (397 $\mu g \bullet pot^{-1}$) was far higher than that from PH (19.0 $\mu g \bullet pot^{-1}$) treatment, suggesting that the Cd removal was more effective when *P. vittata* intercropped with *S. alfredii* than *H. spectabile*. Thus, intercropping of *P. vittata* and *S. alfredii* may be more effective in simultaneous phytoextraction As and Cd in co-contaminated soil.

3.3 Soil pH, available contents of As and Cd after remediation

The rhizosphere soil pH was slightly varied from 7.84 to 7.99 (Fig. 2), which was coincided with previous findings that soil pH under the intercropping of *P. vittata* and castor bean slightly altered (Yang et al., 2017), suggesting that acidification is not the mobilization mechanism of soil metals in present study (Liang et al., 2019). The available As content in PS-S rhizosphere soil was significantly higher than that from unplanted soil (CK) and other planting treatments except for PS-P and PH-H rhizosphere soil. The available Cd content in SM and PS-P rhizosphere soil was higher than that in HM, PH-P and PH-H rhizosphere soil, indicating that intercropping *P. vittata* and *S. alfredii* could enhance As and Cd mobility in soil (Fig. 2). Organic acids secreted by hyperaccumulators could facilitate the transformation of heavy metals to an exchangeable fraction thus indirectly resulting in high phytoremediation efficiency (Zu et al., 2020). Previous researches have been confirmed that organic acids like oxalic acid as a predominant root exudate of both *S. alfredii* and *P. vittata* could trigger soil As and Cd availability and enhance uptake by plant (Tao et al., 2016; Das et al., 2017; Liang et al., 2021). Also, Xia et al. (2018) have reported that the secretion components in soil from intercropping systems with *Conyzacanadensis*, *Cardaminehirsuta*, and *Cerastiumglomeratum* are significantly more complex than those from monoculture treatments, which have effects on heavy metal accumulation. Therefore, further research is needed to investigate organic acids in the intercropping system of *P. vittata* and *S. alfredii*. Additionally, the available As content in HM rhizosphere soil and that of Cd in PH-H rhizosphere was lower than that from other treatments, which might explain the results of lower As and Cd content in *H. spectabile* (Table 3).

3.4 Soil microbial diversity and community structure

3.4.1 soil bacterial community diversity

Generally, intercropping enhanced the soil bacterial community diversity. The bacterial α-diversity indices of ACE, Chao 1 and Shannon in intercropping treatments were significantly more than in SM and HM (Table S2), which could help to maintain the stability of soil microbial structure, indicating that these corresponding species could be intercropped. There were 590 OTUs common to CK and planting treatments (Fig. S1a). The PM, SM and PS treatments shared higher OTUs of 646, and 632 OTUs common to PM, HM, and PH treatments was found (Fig. S1b, S1c). Though the unique OTUs in CK and planting treatments was very low, which from intercropping treatments were higher than monoculture. This agreed with former research that the microbial composition from the intercropping system of *P. vittata* with *M. alba* or *B. papyrifera* was more complex (Zeng et al., 2019a). Different plant has distinct rhizosphere soil condition due to its own spectrum and specificity of root exudates (Deng et al., 2018). Bian et al. (2021) have found that acetic acid, malic acid, and n-hexadecanoic acid were closely correlated with multiple bacterial species in rhizosphere for intercropping system of Moso bamboo with *Sedum plumbizincicola*.

3.4.2 Bacterial community structure

The phylum *Proteobacteria* was the dominant bacterial community in all treatments, accounting for 31.0-36.4% of total phyla in soil (Fig. 3a). The abundance of *Proteobacteria* was increased in intercropping system compared with monoculture, and that in *S. alfredii* rhizosphere soil of PS treatment was highest. Similar result has been reported that *Proteobacteria* predominated in bacterial phyla with more than 31% relative abundances from intercropping treatment with Moso bamboo and S*edum* *plumbizincicola* (Bian et al., 2021). *Proteobacteria* was important in resistance to metal(loid)s toxicity and significantly correlated with soil nutrients such as carbon, nitrogen, phosphorus contents (Zhang et al., 2016; Zhao et al., 2019). These results suggested that *Proteobacteria* could help plants increase the environmental quality and intensify the biological function of metal(loid) contaminated soil, in turn the presence of plants could provide a better living condition for microorganisms. Additionally, the other predominant phyla included *Actinobacteria*, *Chloroflexi*, *Acidobacteria*, *Bacteroidetes* and *Gemmatimonadete*, which was in consistent with prior research reported by Chen et al. (2018), and they are important for the microbial community reconstruction in metal(loid)-contaminated soil (Zhai et al., 2020).

The genera *Massilia*, *Lysobacter* and *Sphingomonas*, belonging to the phylum *Proteobacteria*, could adapt to extreme soil conditions (Yang et al., 2019; Jiao et al., 2019). *Massilia*, *Sphingomonas*, *Lysobacter*, *arthrobacter* and *norank_c__Subgroup_6* with an average abundance more than 1% were the main genera in soil after phytoremediation (Fig. 3b). *Lysobacter* can resist the pathogens by generating extracellular enzymes and affect the bacterial behaviors (Expósito et al., 2015; Feng et al., 2019), and *Massilia* could effectively enhance soil P mobilization (Zheng et al., 2017). In the present study, *Massilia* in *S. alfredii* rhizosphere soil and *Lysobacter* in *P. vittata* rhizosphere soil of PS intercropping system was the richest genus. Zhang et al. (2018) have found that intercropping of *Morus alba* L. and *Medicago sativa* L. had a positive impact on bacterial taxa with soil nutrients cycling such as *Bacillus*, *Bradyrhizobium* and *Sphingomonas* as compared to monoculture. The results may be related to that root exudates of different cropping treatments may alter the bacterial community (Li et al., 2016).

3.5 Relationship between rhizosphere microecological characteristics and phytoextraction efficiency

The redundancy analysis (RDA) showed that the abundance of *Massilia*, *Arthrobacter* and *Lysobacter* were positively correlated with $NaHCO_3$-As and DTPA-Cd content in rhizosphere soil (Fig. 4a). Moreover, significant correlations were found between the abundance of *Massilia* and *Arthrobacte* and Cd content, the abundance of *Lysobacter* and As content in plant tissues, indicating that they may effectively promote plant Cd and As uptake (Fig. 4b). This was in accordance with the findings reported by Rojjanateeranaj et al. (2017). Previous studies also have confirmed that some types of microorganisms such as *Arthrobater* and *Bacillus* could enhance the phytoremediation efficiency through alleviating metal toxicity to plant (Ma et al., 2016). Therefore, rhizosphere associated microorganisms could play a crucial in regulating phytoremediation, and further studies on the application of the critical genus of microorganisms in phytoremediation with intercropping of *P. vittata* and *S. alfredii*/ *H. spectabile* are warranted.

4 Conclusions

Intercropping of *P. vittata* with *S. alfredii* / *H. spectabile* can effectively alleviate the oxidative stress of As and Cd on plant growth in contaminated soil. The total biomass of *P. vittata*, *S. alfredii* and *H. spectabile* in intercropping systems was significantly improved compared with monoculture by decreasing MDA content and increasing soluble protein content in leaves/pinna. Intercropping of *P. vittata* and *S. alfredii* could simultaneously obtain greater As and Cd accumulation than monoculture and intercropping *P. vittata* with *H. spectabile*. The Venn diagram, heatmap, and RDA analysis showed that the relative abundance of *Massilia* and *Lysobacter* in plant rhizosphere were the richest, and significantly correlated with plant Cd and As content from intercropping system of *P. vittata* with *S. alfredi*. Thus, intercropping of *P. vittata* and *S. alfredii* was a promising strategy to simultaneously phytoextract As and Cd co-contaminated soil.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analysed during this study are included in this published article [and its supplementary information files].

Competing interests

All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

Funding

This work was sponsored by the Environment Production Scientific Research Project of Hunan Province ([2019] 0011) and National Natural Science Foundation of China (Grant No. 41771532), the Fundamental Research Funds for the Central University of Central South University (No. 2019zzts509).

Author contributions

All authors contributed to the study conception and design. Xiaohui Wang and Cong Zhou: Conceiving and designing the experiments; Xiaohui Wang, Cong Zhou and Xiaoyan Wang: Performing the experiments; Xiaohui Wang and Cong Zhou: Analyzing the data and writing original draft; Xiyuan Xiao, Zhaohui Guo and Chi Peng: reviewing and editing, funding acquisition, project administration. All authors commented on previous versions of the manuscript, and all authors read and approved the final manuscript.

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Tables

Table 1
The content of MDA and soluble protein in leaves (pinna for P. vittata) of plants from monoculture and intercropping treatments

Plants	Treatments	MDA content (nmol/g FW)	soluble protein content (mg/g FW)
P. vittata	PM	7.52 ± 0.47a	10.6 ± 0.80c
	PS	6.37 ± 0.16b	11.0 ± 0.37b
	PH	6.22 ± 0.55b	12.4 ± 1.00a
S. alfredii	SM	1.01 ± 0.01a	4.65 ± 0.27a
	PS	1.06 ± 0.08a	4.59 ± 0.49a
H. spectabile	HM	2.04 ± 0.33a	4.60 ± 0.38a
	PH	1.98 ± 0.07a	4.48 ± 0.96a

Different letters within the same column indicate significant differences ($P < 0.05$) between the intercropping treatment and corresponding monoculture. Values are shown as means ± SD (n=4).

Table 2 The As, Cd content and transfer factor in *P. vittata*, *S. alfredii*, *H. spectabile* from monoculture and intercropping treatments

Plants	Treatments	Root (rhizoid) content (mg•kg^{-1})		Shoot (frond) content (mg•kg^{-1})		Transfer factor ($TF_{shoot(frond)/root(rhizoid)}$)	
		As	Cd	As	Cd	As	Cd
P. vittata	PM	191±5.89c	1.75±0.35a	1125±118c	0.50±0.20a	5.89±0.61a	0.31±0.13a
	PS	245±34.7a	2.00±0.04a	1339±63.0a	0.33±0.13a	5.47±0.70a	0.29±0.00a
	PH	213±32.4b	1.41±0.12a	1248±171b	0.44±0.11a	5.85±0.74a	0.30±0.10a
S. alfredii	SM	19.5±1.14a	286±33.7b	3.16±1.20a	362±51.8b	0.16±0.06a	1.26±0.18a
	PS	19.0±0.53a	324±60.1a	2.90±0.77a	416±60.4a	0.21±0.03a	1.31±0.27a
H. spectabile	HM	23.5±2.26a	5.18±0.71a	4.07±0.23b	14.7±3.46a	0.21±0.10a	2.58±0.31a
	PH	21.7±2.32a	4.43±1.15b	5.49±1.13a	11.9±3.25b	0.25±0.04a	2.33±0.58a

Different letters within the same column indicate significant differences (*P* < 0.05) between the intercropping treatment and corresponding monoculture. Values are shown as means ± SD (n=4).

Table 3
Arsenic and Cd accumulation in plants under monoculture and intercropping treatments

Plant	Treatments	Heavy metal accumulation(μg•plant^{-1})	
		As	Cd
P. vittata	PM	772 ± 90.3c	1.17 ± 0.29a
	PS	953 ± 163b	0.93 ± 0.13a
	PH	984 ± 84.3a	1.30 ± 0.33a
S. alfredii	SM	3.06 ± 0.23a	151 ± 15.4b
	PS	3.22 ± 0.13a	173 ± 9.81a
H. spectabile	HM	8.06 ± 1.17b	8.14 ± 1.30a
	PH	10.1 ± 1.80a	8.22 ± 1.81a

Different letters within the same column indicate significant differences (*P* < 0.05) between the intercropping treatment and corresponding monoculture. Values are shown as means ± SD (n=4).

Figures

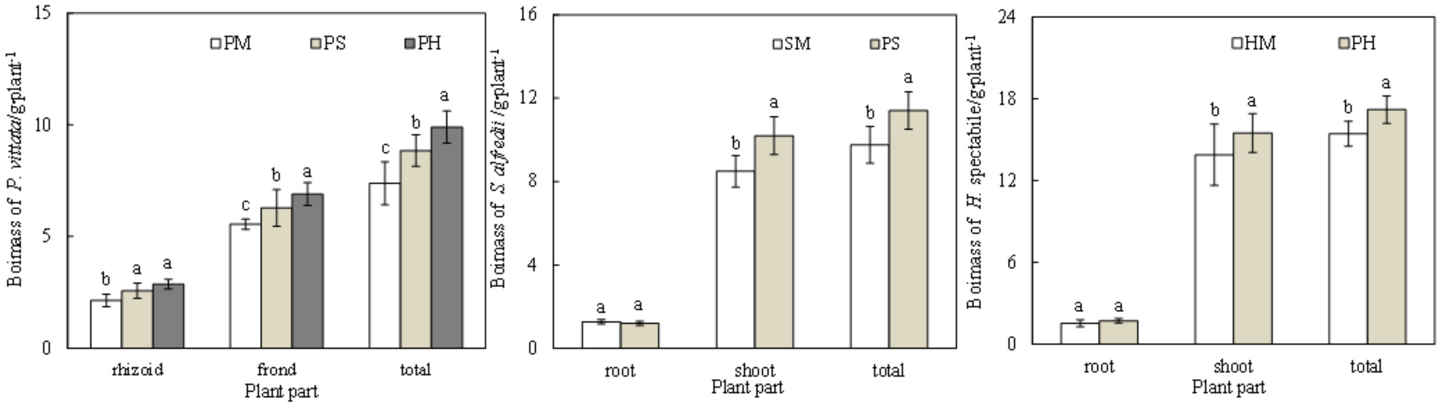


Figure 1

Biomass of P. vittata, S. alfredii and H. spectabile from monoculture and intercropping treatments

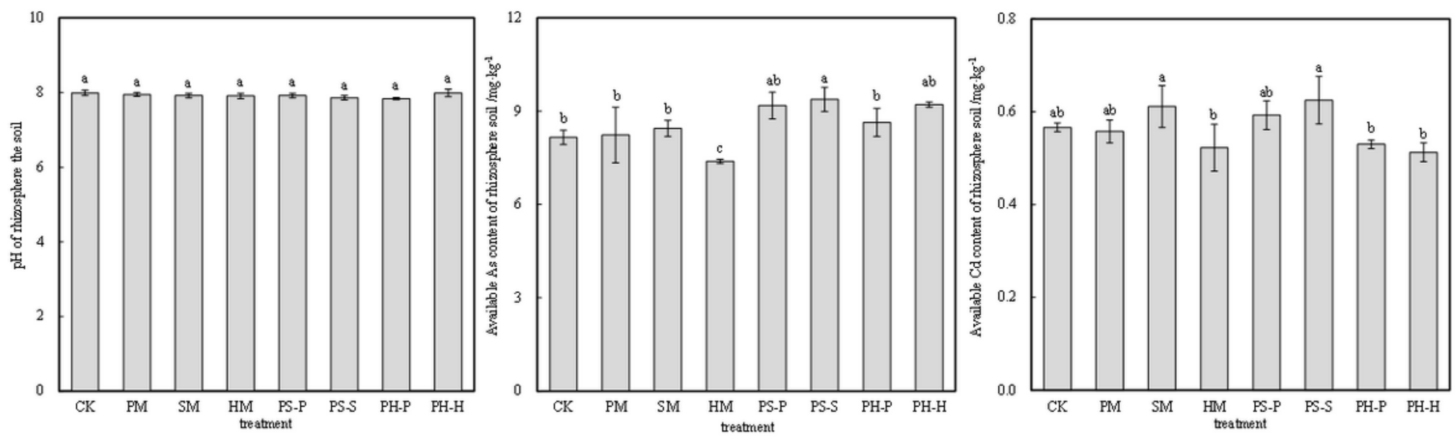


Figure 2

Soil pH, available As and Cd content in rhizosphere soil after phytoremediation under different treatments.

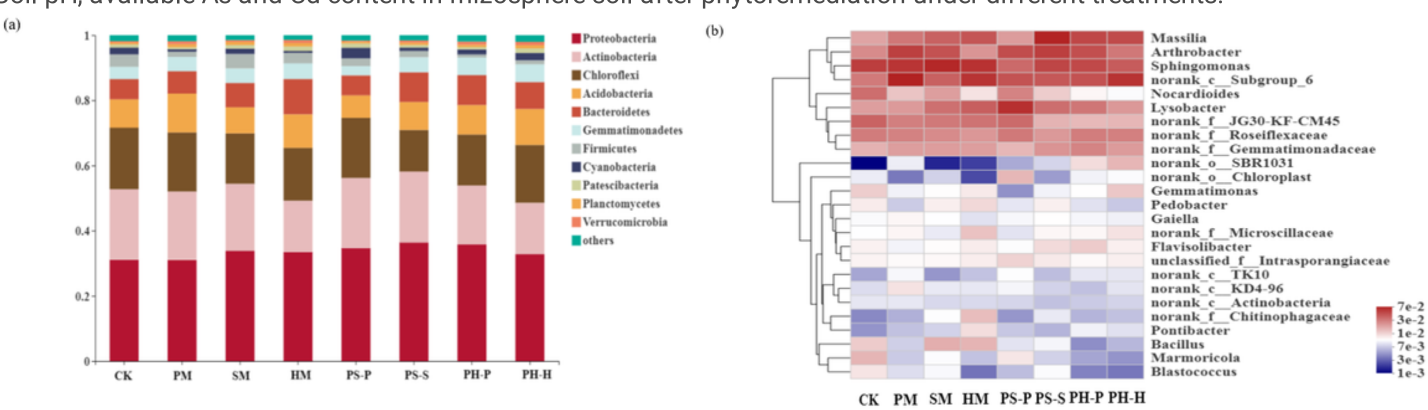
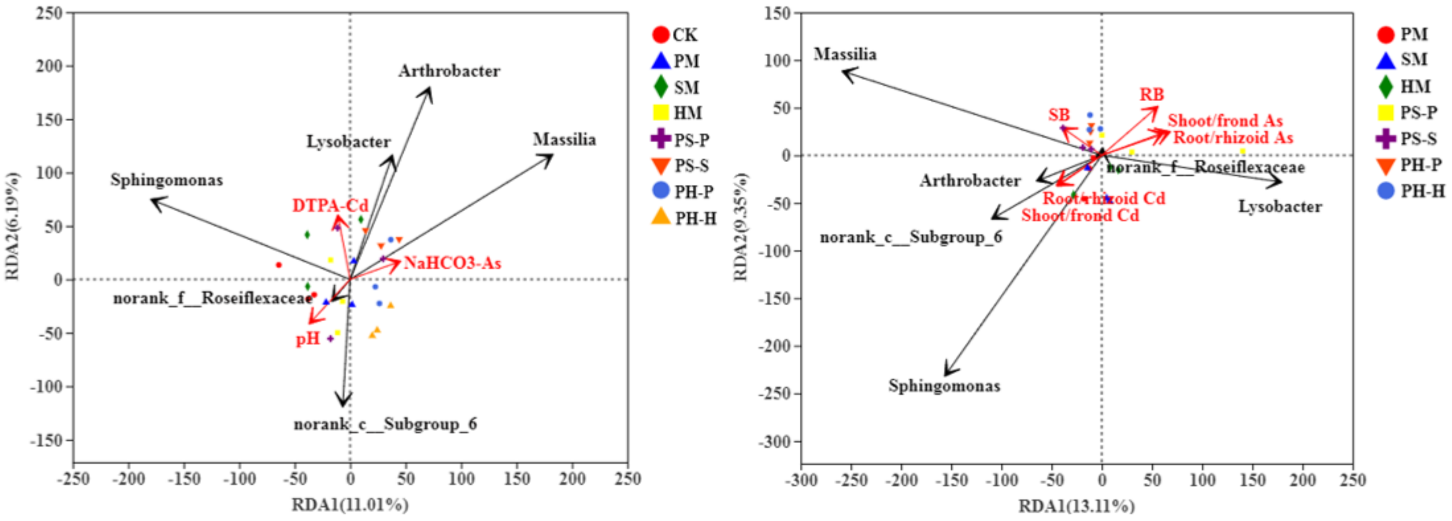


Figure 3

Soil bacterial taxonomic distribution: relative abundance of predominant phyla (a) and community heatmap analysis at genus level (b) under different treatments.



(a) (b)

Figure 4

(a) Redundancy analysis (RDA) of the soil pH, available As and Cd content and bacterial communities at genus level, (b) RDA of the phytoremediation parameters and bacterial communities at genus level. NaHCO3-As, soli available As content; DTPA-Cd, soli available Cd content; SB, shoot/frond biomass; RB, root/rhizoid biomass; Shoot/frond As, As content in shoot/frond; Shoot/frond Cd, Cd content in shoot/frond; Root/rhizoid As, As content in root/rhizoid; Root/rhizoid Cd, Cd content in root/rhizoid.

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