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Root microbiome assembly of As-hyperaccumulator *Pteris vittata* and its efficacy in arsenic requisition

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Summary

The assemblage of root-associated microorganisms plays important roles in improving their capability to adapt to environmental stress. Metal(loid) hyperaccumulators exhibit disparate adaptive capability compared to that of non-hyperaccumulators when faced with elevated contents of metal(loid)s. However, knowledge of the assemblage of root microbes of hyperaccumulators and their ecological roles in plant growth is still scarce. The present study used *Pteris vittata* as a model plant to study the microbial assemblage and its beneficial role in plant growth. We demonstrated that the assemblage of microbes from the associated bulk soil to the root compartment was based on their lifestyles. We used metagenomic analysis and identified that the assembled microbes were primarily involved in root–microbe interactions in *P. vittata* root. Notably, we identified that the assembled root microbiome played an important role in As requisition, which promoted the fitness and growth of *P. vittata*. This study provides new insights into the root microbiome and potential valuable knowledge to understand how the root microbiome contributes to the fitness of its host.

Introduction

Due to their sessile nature, plants generally face various environmental stresses in their immediate environment. Therefore, the growth of plants depends on their ability to rapidly adjust and relieve the adverse impacts of stress (Zelicourt *et al*., 2013; Llorens *et al*., 2019). Existing evidence demonstrated that the assemblage of microbes from the surrounding bulk soils played vital roles in improving stress tolerance and survival of their host plants in a given natural environment (Bulgarelli *et al*., 2013; Hannula *et al*., 2020). Across diverse ecosystems, the assemblies of root microbiomes exhibit consistent trends that improve the capability of plants to adapt to environmental stress (Bulgarelli *et al*., 2012; Edwards *et al*., 2015; Deveau, 2016). A high content of metal(loid)s generally represents one of the most important abiotic stressors that negatively affect the growth and productivity of plants worldwide (Nagajyoti *et al*., 2010). However, an elevated content of metal(loid)s may increase the growth of metal(loid) hyperaccumulators (Fayiga *et al*., 2004; Sun *et al*., 2008). These facts suggest that these two types of plants have disparate adaptive capabilities when faced with elevated contents of metal(loid)s. However, whether this disparity causes the assemblage of soil microbiomes to maintain the fitness of their host plant in soils with an elevated content of metal(loid)s is unknown. Emerging evidence suggests that metal(loid)-induced recruitment in root microbiomes reduce the contents of metal(loid) uptake into non-hyperaccumulator plants via metal biosorption, metal bioaccumulation, metal precipitation, metal complexation, metal reduction and oxidization, and enzymatic metal transformation (Rajkumar *et al*., 2012; Ma *et al*., 2016; Sharma and Archana, 2016). However, few studies investigated the assembly of root microbes of metal(loid) hyperaccumulators and their beneficial roles in metal(loid) requirement. The answer to these questions is important to understand how the root microbiota contributes to the fitness of its hosts.

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Pteris vittata is extraordinary in its ability to tolerate (Cai *et al*., 2019) and hyperaccumulate high levels of arsenic (Ma *et al*., 2001; Lombi *et al*., 2002). Several phytoremediation projects using *P*. *vittata* have also been successfully conducted in As-contaminated sites in China (Wan *et al*., 2016; Chen *et al*., 2018) and the US (Kertulis-Tartar *et al*., 2006; Ebbs *et al*., 2009). Extensive studies demonstrated that *P*. *vittata* evolved effective strategies to obtain As from poorly soluble sources and translocate a large amount of As from the soil to aboveground plant parts (Wang *et al*., 2002; Fayiga *et al*., 2004). Several recent attempts found evidence that root exudate-mediated chemical solubilization was the principal strategy for *P*. *vittata* to acquire As from insoluble $FeAsO_4$ mineral (Tu *et al*., 2004; Liu *et al*., 2016). A growing body of research demonstrated that many rhizosphere bacteria identified from *P*. *vittata* were characterized as As resistant and capable of As(III) oxidation, which improved the efficiency of As uptake (Ghosh *et al*., 2011; Das *et al*., 2017). These studies suggested that root microbes play a crucial role in As uptake in *P*. *vittata*. However, most studies of the root microbiomes of *P*. *vittata* only focused on the microbial compositions in the root or rhizosphere compartment (Ghosh *et al*., 2011; Das *et al*., 2017), and few studies focused systematic attention on roots, rhizospheres and bulk soil compartments. The assembly and succession patterns of root microbiomes from the associated bulk soil to the root compartment are not clear. Are there relationships between plant recruitment and the enrichment of specific soil microorganisms in the *P*. *vittata* root compartment? If so, what do these relationships have to do with As uptake in *P*. *vittata*? Therefore, the present study provided a finer dissection of the microbial composition across root compartments to gain new insights into the microbial assemblage and As requisition mechanisms of *P*. *vittata*.

The elucidation of microbial functional traits is key to reveal the effect of root microbes on plant hosts, but this goal is highly challenging due to the complexity of the microbial structure composition (Bulgarelli *et al*., 2012; Xu *et al*., 2018). Microbiome-level functional studies that aimed to understand the mechanism of assemblage of root microbiome are not widely reported. Current studies identified the abundance of aroA-like and arsC genes from *P*. *vittata* rhizosphere soils (Xiong *et al*., 2010). These studies elucidated As cycling in the rhizosphere compartment in *P*. *vittata* at the molecular level. However, these studies frequently used traditional molecular technologies, such as qPCR and DNA microarray approaches (Xiong *et al*., 2010). Because of the limited availability of primers (Xu *et al*., 2018), little is known about the genes involved in As metabolism in the root compartment of *P*. *vittata*. A shotgun metagenomic sequencing approach was considered a useful tool to provide detailed information of gene function at the molecular level, and it is frequently applied to various communities, such as human, animal, and oceanic microbiomes (Sunagawa *et al*., 2015; Wang and Jia, 2016), and complex soil communities (Ofek-Lalzar *et al*., 2014; Bahram *et al*., 2018). The present study used shotgun metagenomic sequencing and identified the relative abundance of As genes. We also linked the identified genes to their probable role in As requisition of *P*. *vittata*.

This article investigated the assemblage of root microbiomes across root compartments and revealed their beneficial roles in As requisition of *P*. *vittata*. We performed amplicon sequencing to study the bacterial and fungal compositions of root, rhizosphere and associated bulk soil samples from five sampling sites. We selected a subset of samples and performed shotgun metagenomic sequencing to study the attributes of microbial functional across the root compartments of *P*. *vittata*. This study provides new insights into the beneficial roles of root microbiomes in the As requisition of *P*. *vittata*, which improves our understanding of how the root microbiota contribute to the fitness of its hosts.

Results

Geochemical conditions

The samples were collected from five sampling sites that contained elevated soil As contents, with values that varied from 556 to 5880 mg/kg. As shown in Fig. Fig. S1, the average content of soil As in root and rhizosphere soils was significantly higher than the associated bulk soils. We used XANES analysis and identified that As(V) was the predominant species (>82%) in the detected soil samples (Fig. 1). The dominance of As(V) in the soil samples reflected the oxidizing environment in the study site. The average contents of TOC and Total C in root samples were significantly higher than the associated bulk and rhizosphere soils. The contents of other parameters, such as P, Total S, Fe and Mn, were not obviously different across root compartments (Table Fig. S1; Fig. Fig. S1).

General information of 16S rRNA amplicon sequencing

Approximately 2.6 M high-quality sequencing reads (176 951 reads per sample on average) were generated for bacteria and ~2.8 M high-quality sequencing reads (181 150 reads per sample on average) were generated for fungi composition. We clustered 34 816 and 7182 OTUs at 97% similarity for bacteria and fungi respectively (Table S2). We identified the differences in microbial diversity, including the observed OTUs, Chao1, Simpson,

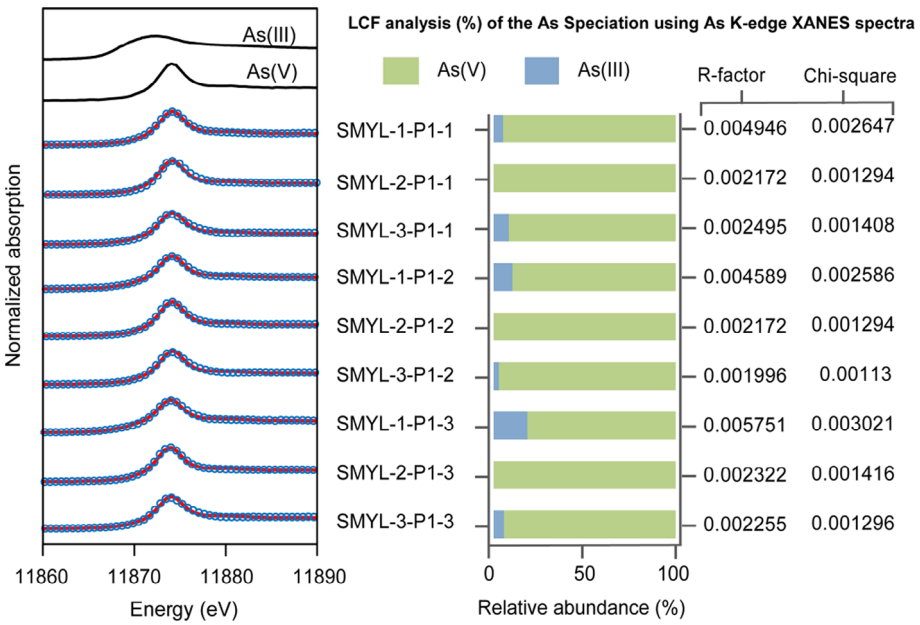

Fig 1. Determination of the valent states of nine selected soil samples using As K-edge XANES spectra (including As standard references of As(III) ($NaAsO_2$) and As(V) ($Na_2HAsO_4{\cdot}7H_2O$)). XANES data and linear combination fitting (LCF) spectra are shown using black dots and red lines, respectively. The fitted As species, R-factor and Chi-square were also shown in this figure. [Color figure can be viewed at wileyonlinelibrary.com]

ACE and Shannon diversity indices, between the root compartments of *P. vittata*. Notably, all of the microbial indices demonstrated similar distributional patterns, and the values decreased from root to bulk soil (Fig. S2). The results of bacterial taxonomic classification at the phylum level demonstrated that *Proteobacteria*, *Chloroflexi*, *Bacteroidetes*, *Actinobacteria* and *Acidobacteria* largely predominated the *P. vittata* root, rhizosphere and associated bulk soil communities and accounted for 73% 71% and 69% of the pyrosequencing reads respectively. For fungal taxonomic classification of phyla, *Ascomycota*, *Mortierellomycota*, *Basidiomycota* and *Chytridiomycota* predominated in root, rhizosphere and bulk soil communities and accounted for 71% 70% and 80% of the reads, respectively (Fig. S3). Notably, the dominant bacterial and fungal phyla did not show significant discrimination between the root, rhizosphere and bulk soil samples. Therefore, fine detailed information of the microbial communities was needed to identify the response of bacteria and fungi between root compartments.

Taxonomical composition of the P. vittata *root microbiome*

To dissect the bacterial and fungal diversification between root, rhizosphere and root compartments (Supplementary file Dataset S1 for bacteria and Dataset S2 for fungi), we used a linear model analysis to identify OTUs that were significantly enriched in each root compartment. This approach identified three distinct subcommunities in root (Root OTUs), rhizosphere (Rhizosphere OTUs) and bulk soil (Soil OTUs) samples. For bacteria, the enriched OTUs gradually reduced from 27 Soil OTUs to 13 Root OTUs and 8 Rhizosphere OTUs (Fig. 2A). Taxonomic assignments at the phylum level revealed that the Root OTUs primarily consisted of *Gemmatimonadetes*, *Proteobacteria* and *Chloroflexi*. Rhizosphere OTUs were dominated by bacteria belonging to *Proteobacteria*, *Chloroflexi* and *Bacteroidetes*. Soil OTUs were affiliated to bacteria belonging to *Cyanobacteria*, *Planctomycetes* and *Proteobacteria*. For fungi, the enriched OTUs gradually declined from 12 Rhizosphere OTUs to 9 Bulk OTUs and 5 Root OTUs (Fig. 2B). Unlike the bacterial community, the fungal communities showed distinctive different fungal communities between root compartments. Taxonomic assignments at the phylum level revealed that the Root OTUs were dominated by *Chytridiomycota* and *Ascomycota*, and Rhizosphere OTUs consisted of *Basidiomycota* and *Ascomycota*. The Soil OTUs were enriched by *Ascomycota*.

To further obtain finer detailed information about the microbial communities among root compartments (Supplementary file Dataset S3 for bacteria and Dataset S4 for fungi), we compared bacterial and fungal genera that were significantly enriched in bulk (Soil genera), root (Root genera) and rhizosphere soils (Rhizosphere genera). For bacteria, the enriched genera gradually decreased from soil (14 genera) to root (9 genera) and rhizosphere compartments (7 genera) (Fig. 3A). Notably, the dominant genera enriched in root were *Gemmatimonas*, *Lysobacter*, *Opitutus* and *Nitrospira*. The genera enriched in rhizosphere soils were *Gemmatimonas* and *Nitrospira*, and bulk soils were

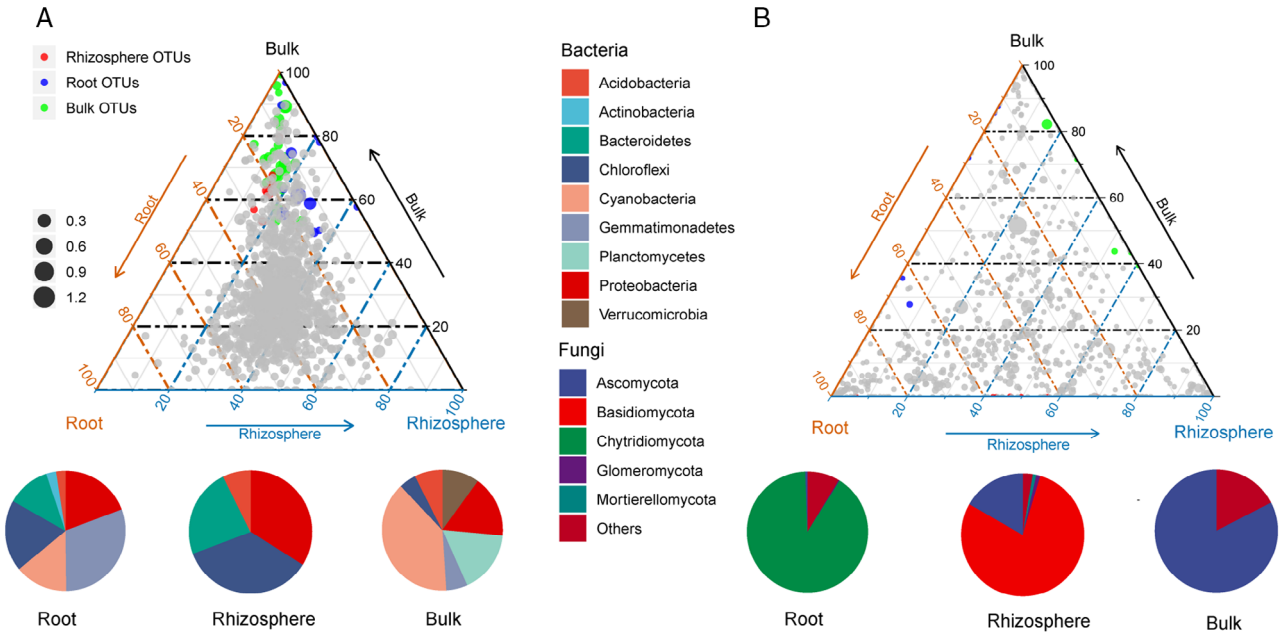

Fig 2. Distribution pattern of dominant OTUs and the taxonomy composition (phylum) across Root, Rhizosphere, and Bulk soil for (A) bacterial and (B) fungal communities. [Color figure can be viewed at wileyonlinelibrary.com]

significantly enriched with *Flavobacterium*, *Bellilinea* and *Cellvibrio*. Fungi exhibited a total of 190 genera, and the enriched genera gradually declined from 10 Soil genera to 9 Root genera and 6 Rhizosphere genera (Fig. 3B). Notably, the dominant fungi genera enriched in root soils were *Spizellomyces*, *Eocronartium*, *Penicillium* and *Glomus*. Fungi genera enriched in rhizosphere soils were *Spizellomyces*, *Eocronartium*, *Penicillium*, *Verticillium* and *Glomus*. Bulk soils were significantly enriched in *Articulospora*, *Cladosporium* and *Paraphoma*.

The metabolic potential of the P. vittata *rhizosphere microbiome*

The core root microbiome of most plants was recently defined based on taxonomic markers (Bulgarelli *et al*., 2012; Edwards *et al*., 2015). However, a growing body of studies addressed the critical role of microbial functional traits on the distributional pattern of root microbiomes (Lundberg *et al*., 2012; Bulgarelli *et al*., 2013; Bulgarelli *et al*., 2015; Xu *et al*., 2018). The present study selected a subset of samples to demonstrate the microbial functional traits of the *P. vittata* root, rhizosphere, and associated bulk soils using shotgun metagenomics sequencing approaches. This understanding enabled us to better define core rhizosphere microbial functional traits between *P. vittata* root compartments. A total of 37.17% of the unigenes (1 557 367 of 4 189 426) was annotated via blasting against the KEGG Orthology (KO) database, and 9041 KOs were obtained. These KOs were primarily involved in 4 KEGG *level 1* and 23 KEGG *level 2* pathways (Fig. 4). Notably, metabolism was identified as the major pathway due to selective enrichment of amino acid metabolism, carbohydrate metabolism, energy metabolism, and metabolism of cofactors and vitamins. The pathways of membrane transport and signal transduction involved in environmental information processing were the dominant core functional traits. We further identified nutrients and metal-related genes. The results showed that the enrichment of carbon-related genes primarily consisted of dicarboxylate-hydroxybutyrate cycle (DC/4-HB), Arnon-Buchanan cycle, rTCA, coenzyme F421 hydrogenase (frhABDG) and methane monooxygenase (pmoABC) (Fig. S4). The nitrogen-related genes primarily consisted of ammonium transporter (nrgA), nitrate/nitrite transport system substrate-binding protein (nrtABCD), nitrate reductase/nitrite oxidoreductase (narGHIJ) and nitrite reductase [NAD(P)H] (nirBD) (Fig. S5). The As-related genes primarily consisted of arsenic resistance transcriptional regulator (arsR), arsenate reductase (ARSC, arsC), arsenite transporter, ACR3 family (ACR3) and arsenite oxidase (aoxAB) (Fig. 5).

Discussion

Assembly of the root microbial community of P. vittata

The assemblage of root-associated microbes from surrounding bulk soils has widely been reported in prior

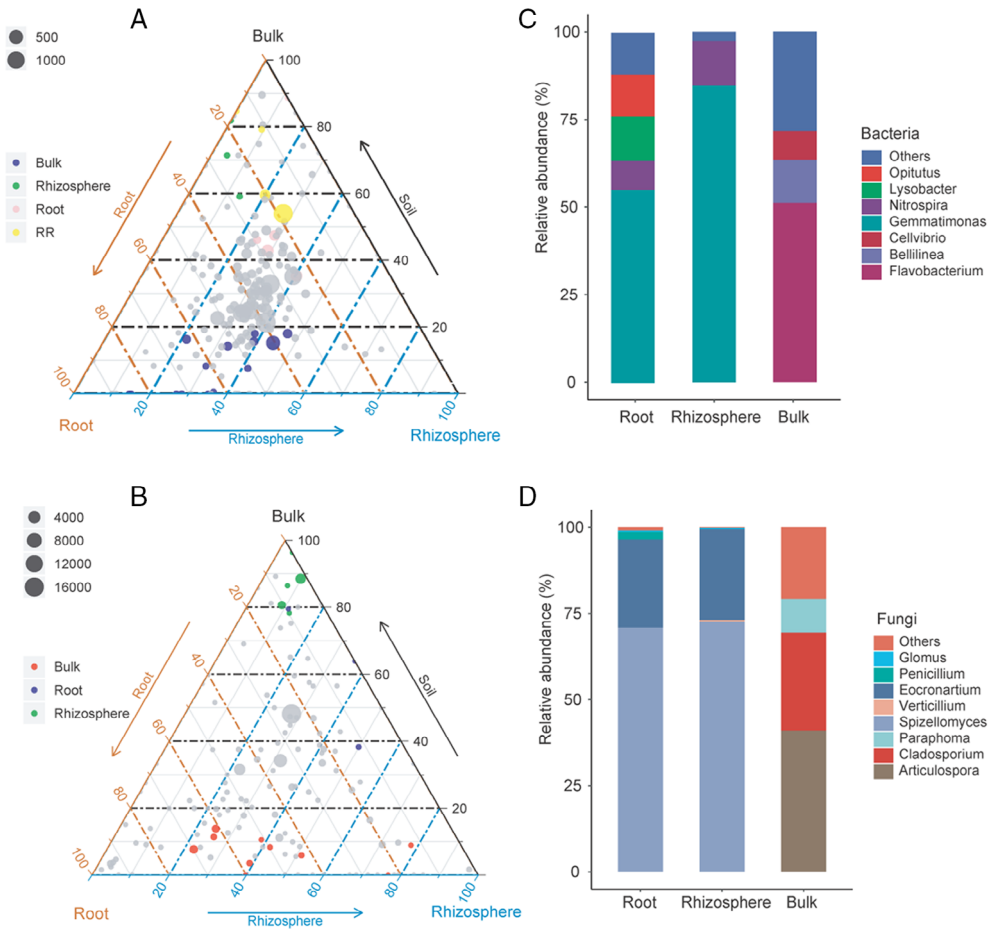

Fig 3. Distribution pattern of dominant genera and the taxonomy composition across Root, Rhizosphere and Bulk soil for (A) bacterial and (B) fungal communities. [Color figure can be viewed at wileyonlinelibrary.com]

studies (Bulgarelli *et al*., 2012; Edwards *et al*., 2015). It is reasonable to propose that a distinct microbial structure may be detected in *P. vittata* root, rhizosphere and associated bulk soils. However, the distribution pattern across root compartments in *P. vittata* was not known. We examined the taxonomic features of root, rhizosphere and associated bulk soils to gain insight into the assembly of root microbiomes in *P. vittata*. We found a decreasing gradient in bacterial and fungal diversity from the root to the bulk soils (Fig. S2). The linear model analysis identified that the dominant OTUs and genera were divided into three distinct microbial sub-communities that thrived at the root–soil interface (Fig. 2). These results suggest that the structure of the microbiome shifted across three distinct root compartments in *P. vittata*. Notably, similar distribution patterns were identified in previous studies of *Arabidopsis halleri*, *Elymus mollis*, rice, wheat (Likar *et al*., 2008), *Rehmannia glutinosa* and pea, which suggests that the assembly of root microbiomes is common across plant species. Recent evidence recognized that the assemblage of the root microbiome constituents was primarily attributed to their lifestyles (Leff *et al*., 2015), which is consistent with our observations. For example, the identified root- and rhizosphere-associated phyla *Proteobacteria*, *Bacteroidetes* and *Chloroflexi* (Fig. 2) are fast growth bacteria with the ability of copiotrophs to use a variety of carbon sources (Ai *et al*., 2015; Leff *et al*., 2015). Bulk soil-associated phyla of *Planctomycetes* and *Cyanobacteria* (Fig. 2) are slow growth bacteria that do not use a variety of carbon sources (Mager and Thomas, 2011; Leff *et al*., 2015). In addition, the identified root-associated fungal phylum Chytridiomycota is prevalent in terrestrial ecosystems (Spatafora *et al*., 2016), with the ability to use various carbohydrates (Gleason *et al*., 2011). Therefore, our results suggest that the assemblage of microbial consortia across the root interface improves the ability of host plants to better fill the root-colonized niche (Bulgarelli *et al*., 2015; Edwards *et al*., 2015).

Existing evidence recognizes that the microbial function traits involved in root–microbe interactions play critical roles in the assembly of root microbiomes

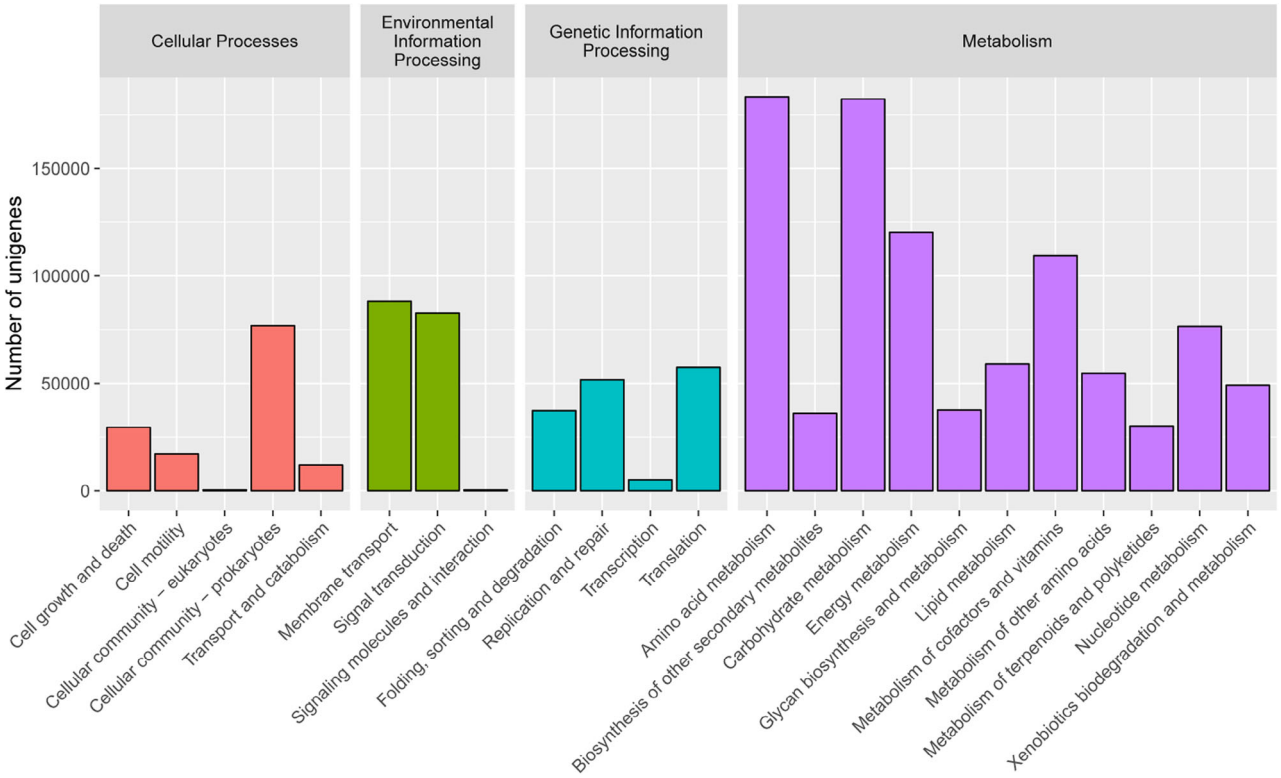

Fig 4. The dominant metabolic potential pathways of the *Pteris vittata* rhizosphere microbiome annotated via blasting against the KEGG Orthology (KO) database (involved in 4 KEGG *level 1* and 23 KEGG *level 2* pathways). [Color figure can be viewed at wileyonlinelibrary.com]

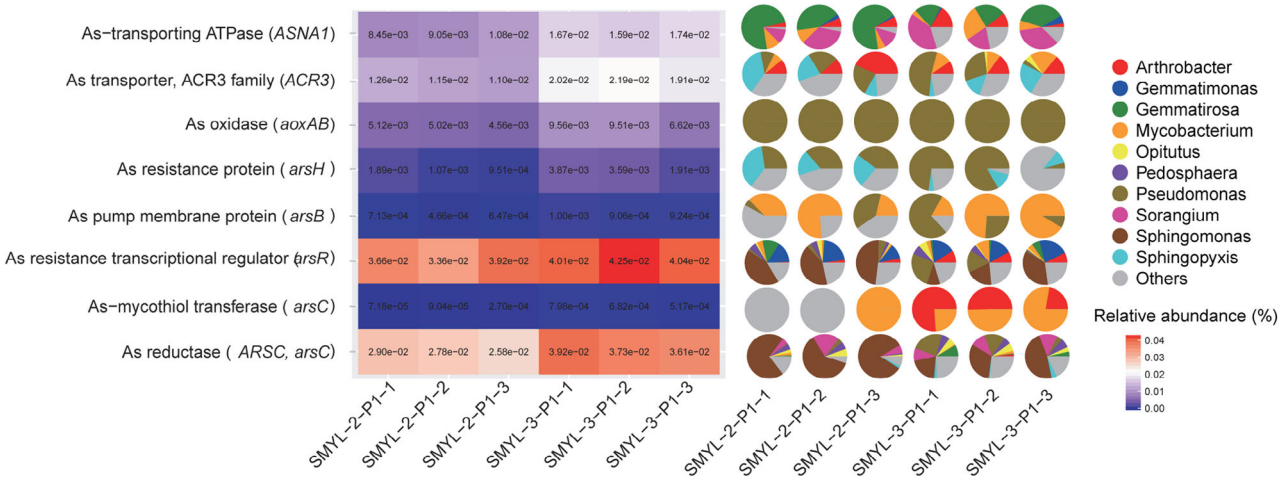

Fig 5. Distributional pattern of As metabolic potential genes and their potential hosts (genus level). [Color figure can be viewed at wileyonlinelibrary.com]

(Xu *et al*., 2018). We found that microbial functions across the root and soil interface primarily involved known root–microbe interactions, such as amino acid metabolism (Stuttmann *et al*., 2011), carbohydrate metabolism (Rohel *et al*., 2001), energy metabolism (Hampp and Schaeffer, 1999), metabolism of cofactors and vitamins, pathways of membrane transport and signal transduction (Haas *et al*., 2002; Popp and Ott, 2011), xenobiotics biodegradation and metabolism Folding (Crowley *et al*., 1997), and translation (Ren *et al*., 2013). These results are consistent with a prior study by Hassani *et al*. (2018), who found that the evolution of plant–microbe interactions was logically linked to their selection in roots and rhizospheres. Notably, we

identified that many core functional traits were involved in the degradation of various compounds, such as leucine, aromatic compounds, fatty acids, benzoate, lysine, aminobenzoate, limonene, pinene, geraniol, chlorobenzene, glycosaminoglycan and xylene (Fig. S6). These diverse compounds were often identified as root exudates and released by plants (Rovira, 1969), which indicates that the root microbiome may be logically mediated via root exudates of *P. vittata*. This finding broadly supports the fact that the root microbiome acquired various simple carbon and nitrogen sources from root exudation (Bai *et al*., 2015) and would not need to invest in the biosynthesis of these compounds. These results are consistent with the fact that the assembly of the root microbiome was attributed to their lifestyle (Mager and Thomas, 2011; Leff *et al*., 2015), in which fast growth bacteria, such as *Proteobacteria*, *Bacteroidetes* and *Chloroflexi*, were enriched in the root and rhizosphere microbiomes, and photosynthetic microorganisms, such as *Cyanobacteria*, were depleted in the *P. vittata* root and rhizosphere microbiome (Fig. 2). Taken together, our results provide solid evidence that the assemblage of soil microbes of *P. vittata* is based on lifestyle.

Root microbiome facilitates As requisition of P. vittata

In bioavailable As-limited habitats, such as the mined soil studied here, the ability of *P. vittata* to acquire As from poorly soluble sources is an important trait that determines its fitness in these habitats. The roles that the established root microbiome played in As acquisition and accumulation in *P. vittata* were not well elucidated. We demonstrated that the assembled bacterial and fungal genera inhabiting the root and rhizosphere compartments of *P. vittata* were reportedly involved in As cycling. For example, the bacterial genera *Gemmatimonas*, *Lysobacter* and *Nitrospira* are tolerant to high levels of As and are widely identified in As-contaminated environments, such as soil (Sun *et al*., 2019), rivers (Halter *et al*., 2011; Leon *et al*., 2018) and tailing dumps (Xiao *et al*., 2016a). *Opitutus* and *Penicillium* were identified as arsenate-reducing bacteria and are widely reported in As-contaminated soils (Xiao *et al*., 2016c), activated sludge and coastal sediments (Cai *et al*., 2013). Notably, Chen *et al*. (2007) identified that a member of *Glomus*, an indicator genus of the root compartment, improved arsenic tolerance in plants via enhancing plant phosphorus uptake. Therefore, our study raises the intriguing possibility that the selective enrichment of root microbial taxa may be involved in As cycling and promote *P. vittata* growth in bioavailable As-limited environments.

To further examine the responses of root microbiome to As stress and their roles in As uptake, we used shotgun metagenomic sequencing to analyse As metabolic pathways, including resistance and redox transformation in the root compartments of *P. vittata*. As-resistance genes were identified as the most abundant As metabolic genes, which is consistent with a prior study by Cai *et al*. (2013). The relative abundance of As-resistance genes increased with the soil As content (Fig. 6). These patterns were reasonable because the prevalence of As-resistance genes is primarily due to continuous exposure

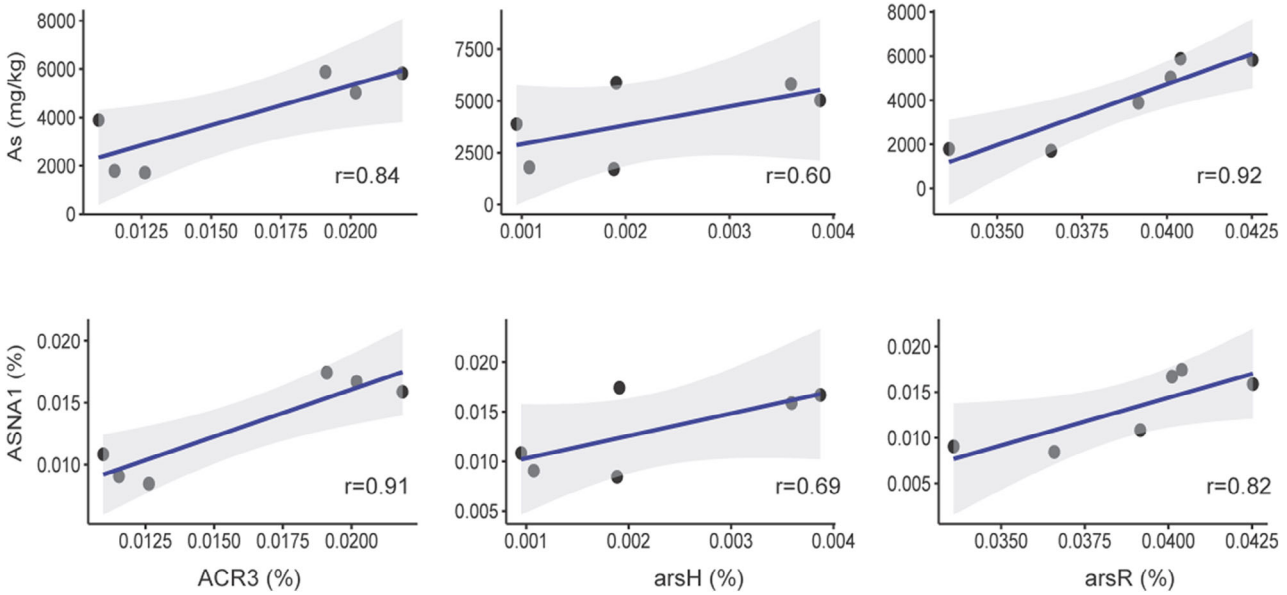

Fig 6. Spearman's linear relationships between the relative abundance of arsenic resistant genes and As content and the relative abundance of ASNA1 ($p < 0.05$). [Color figure can be viewed at wileyonlinelibrary.com]

to high As levels (Ben *et al*., 2018). Notably, we identified that these As-resistance genes significantly correlated with As-transporting ATPase (ASNA1) (Fig. 6). As efflux may be an effective pathway for As detoxification, even at the expense of more energy by As(III) transportation of ATPase (Li *et al*., 2017). Existing evidence recognized that the accumulation of As-resistance genes efficiently mobilized As from soils (Cao *et al*., 2003), which promotes As requisition in *P. vittata*. Because root exudates, such as carboxylate, play a critical role in the distribution of As resistance genes in the rhizosphere and root soils (Xiong *et al*., 2010), it is reasonable to propose that plant roots recruit microbes with As resistance genes to promote As uptake by and translocation into the *P. vittata* root.

The As redox transformation genes were widely identified in As-contaminated environmental settings (Cai *et al*., 2013; Ghosh *et al*., 2015; Xiao *et al*., 2016c) and played an important role in As uptake in *P. vittata*. The present study identified the arsC gene, which is involved in the detoxification reduction of the As(V) pathway, as the dominant As redox transformation gene. The prevalence distribution of the arsC gene was also identified in As-contaminated environments, such as paddy soil (Sun *et al*., 2019) and mine fields (Xiao *et al*., 2016c). A prior study by Oremland *et al*. (2005) showed that the arsC gene reduced As(V) within the cytoplasmic membrane and subsequently excreted As(III) via the ArsAB efflux pump. Researchers discovered a general consistence between the arsC and 16S rRNA genes (Xiao *et al*., 2016c). We identified that the arsC gene sequences were primarily from *Sphingomonas* at the genus level, which is consistent with prior studies that identified this gene in diverse As-contaminated environments, including rivers (Escalante *et al*., 2009), soils (Jackson *et al*., 2005), tailing dumps (Wu *et al*., 2018) and mining waste rocks (Casas-Flores *et al*., 2015). Because the relative abundance of arsC increased with soil As contents (Fig. 7A), it is reasonable to suppose that the microbial-mediated As(V) reduction was prevalent in root compartments of *P. vittata*. Because the relative abundance of *Sphingomonas* decreased with As content (Fig. 7B), the arsC-mediated As reduction was inhibited with elevated As content. This conclusion was partially supported by the results of the XANES analysis, which found that the proportion of As(III) was relatively low in all samples (Fig. 1). These results suggest that the assemblage of root microbiomes with the arsC gene decrease microbial-mediated As reduction in root and rhizosphere compartments. This pattern was reasonable because the uptake efficiency of As(III) was relatively low compared to As(V) in *P. vittata* root.

Because As(V) was the dominant species in root and rhizosphere samples (Fig. 1), the relative abundance of As oxidative genes was considered dominant in As redox transformation genes. However, the As oxidative gene aoxAB exhibited relatively low abundance in this study compared with that of As reductive genes, which is similar to the case in acid mine sewage (Majzlan *et al*., 2014) and As-contaminated paddy soils (Zhang *et al*., 2015). Although the abundance of aioAB was relatively low, these genes played a critical role in As oxidation. A recent study demonstrated that the presence of the aoxAB gene primarily originated as a resistance mechanism to convert the more toxic As(III) to the less toxic As(V) (Páez-Espino *et al*., 2009). The aoxAB gene was identified as a heterodimer that contained Fe and molybdenum as part of the catalytic unit (Ellis *et al*., 2001), and it was proposed as a valuable functional marker gene for As(III) oxidation (Páez-Espino *et al*., 2009). The aoxAB gene is widely distributed in diverse microbes in As-contaminated environments (Kang *et al*., 2012; Sun *et al*., 2017). The aoxAB gene was affiliated with *Pseudomonas* at the genus level, which was dominant in root microbiomes and contributed significantly to the oxidation of As(III) (Jia *et al*., 2014). Notably, the relative abundance of *Pseudomonas* was enriched in root and rhizosphere soils compared to the associated bulk soils. This fact suggests that microbial-mediated As oxidation was more frequent in root and rhizosphere soils compared to

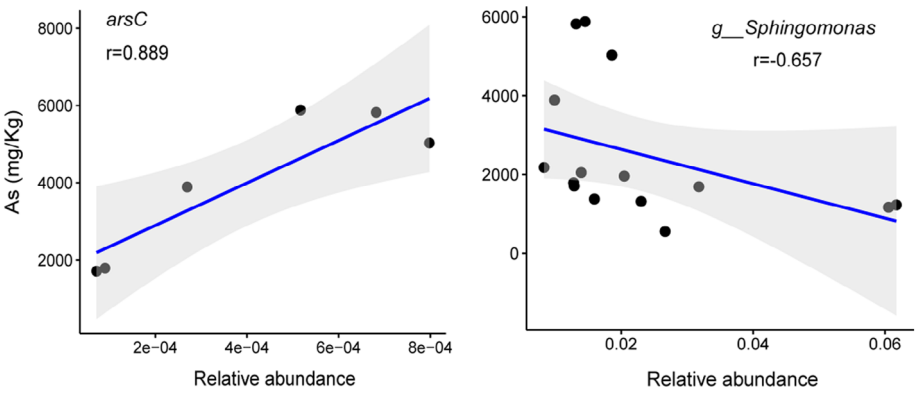

Fig 7. Spearman's linear relationships between As content and the relative abundance of arsC and *Sphingomonas* ($p < 0.05$). [Color figure can be viewed at wileyonlinelibrary.com]

the associated bulk soil. Notably, the existing evidence demonstrated that As(III) oxidation mediated by aoxAB was generally combined with nitrate reduction and that the energy produced served to fix CO_2 (Oremland and Stolz, 2003). Our study identified that the KOs of aoxAB significantly correlated with the KOs involved in nitrate reduction and CO_2 fixation (Fig. S7), which suggests that microbial-mediated As oxidation is commonly coupled with nitrate reduction and carbon fixation in root compartments when *P. vittata* grows in the soils with oligotrophic and elevated As content. Microbial-mediated As(III) oxidization may be important for As uptake by *P. vittata* roots since As(V) is the predominant valence state that may be taken up by *P. vittata* roots (Wang *et al*., 2002).

Conclusion

The assemblage of root-associated microorganisms plays important roles in improving their capability to adapt to environmental stress. We chose *P. vittata* as a model plant to study the microbial assemblage and As requisition mechanisms in the roots of a metal(loid) hyperaccumulator. We demonstrated that the assemblage of root microbiomes from bulk soil to root compartment was attributed to their lifestyles, and they were primarily involved in root–microbe interactions in *P. vittata* root. We also identified that the assembled root microbiome played an important role in As requisition, which promoted the fitness and growth of *P. vittata*. The identification of core taxa and functional traits of the As hyperaccumulator of *P. vittata* is important to understand how the root microbiomes contribute to the fitness of their hosts.

Experimental procedures

Study area and sampling

The samples were obtained from an abandoned As smelting factory in Hunan Province, China. Ongoing smelting activities resulted in serious As contamination in this region. *Pteris vittata* spontaneously grew in the studied regions. We used a randomized field design and chose five sampling sites. At each sampling site, we chose *P. vittata* plants with similar heights to collect their roots, rhizospheres and associated bulk soils. Bulgarelli *et al*. (2012) previously described the sampling protocol. For each sample, a total of three pseudo-replicates (0–15 cm depth) of ~20 g soil were obtained and mixed as a composite soil sample. All samples were transported to the laboratory with ice packs (4 °C). Each sample was divided into two groups based on their use: one part for DNA extraction was stored at −40 °C, and the second part for chemical analyses was stored at 4 °C.

Chemical and As speciation analyses

Soil samples were freeze-dried before chemical analysis. The samples were thoroughly ground using a mortar and pestle and passed through a sieve with 200-mesh. We measured total sulfur and total carbon directly using an elemental analyser (vario MACRO cube, Elementar, Hanau, Germany). The sample was completely digested using concentrated HF and HNO_3 (1:5, vol./vol.) (Edgell, 1989). We used ICP-MS (Agilent, 7700x, California, USA) to measure the contents of trace elements and some macro-elements (Wang *et al*., 2019). Internal standards (Rh, 500 μg/L) and certified reference materials (SLRS-5, National Research Council, Canada) were used to increase the reliability of ICP-MS measurements. The Chinese soil reference GBW07310 was used for the quality control of sample treatments (Xiao *et al*., 2016b).

Arsenic valence state in select soil samples was characterized by detecting the As K-edge (11 867 eV) of XAS spectra (beamline BL01C1) at the National Synchrotron Radiation Research Center in Taiwan. The procedures were proposed in our previous studies (Cui *et al*., 2013; Cui *et al*., 2018). Generally, the ground sample was sealed between two layers of Kapton tape at the beamline. The sample was analysed via the collection of fluorescence signals using a Lytle detector at room temperature. Changes of As species caused by the beamline were not found during analysis. The XANES results of As valance state were performed using a linear combination fit procedure with the Athena program in the IFEFFIT computer package (Ravel and Newville, 2005).

Analyses of bacterial and fungal communities using Illumina MiSeq sequencing

To obtain total genomic DNA, we extracted 0.25 g soil using the FastDNA® spin kit (MP bio, Santa Ana, USA) following the manufacturer's protocol. The purity and concentration of the extracted DNA were tested. The extracted DNA was stored at −80 °C before further analysis. For bacterial analysis, the V4-V5 of 16S rRNA amplicons was amplified using the primer pair 515f/907r (Kuczynski *et al*., 2012). For fungal analysis, we amplified the internal transcribed spacer region 2 (ITS2) using primer pairs (ITS1F and ITS2R/ITS4 and 5.8R′). The 16S rRNA and ITS2 amplicons were sequenced in the Illumina MiSeq platform at the Ecogene Bioinformatics Company (Shenzhen, China). For bacteria analysis, we used FLASH to merge the paired-end reads (Magoč and Salzberg, 2011). After merging, the reads were assigned to each sample based on barcodes. The raw reads were filtered using QIIME (V1.7.0) following the criteria proposed by Bokulich *et al*. (2013). After comparison with the GOLD database, chimeric sequences were

discharged using UCHIME (Haas *et al*., 2011). We clustered operational taxonomic units (OTUs) using UPARSE with 97% similarity. To obtain the information of phylogenetic taxonomy for each sample, we assigned the OTUs using the RDP classifier and the Greengenes database (Wang *et al*., 2007). For fungi analysis, we also used FLASH to merge paired-end reads (Magoč and Salzberg, 2011). We removed low-quality reads (*Q* score >25) and trimmed all sequences to the same length. Using the UNITE database (version 7.1), the merged fungal reads were assigned and clustered into OTUs with a 97% similarity cutoff (Kõljalg *et al*., 2013).

Shotgun metagenomic sequencing and gene analysis

The present study selected six samples for shotgun metagenomic analysis. Shotgun metagenomic sequencing was performed using an Illumina PE150 (Illumina Inc.) at the Ecogene Bioinformatics Company (Shenzhen, China). Clean data were obtained by preprocessing the raw data using Readfq (V8, https://github.com/cjfields/readfq). The clean data were assembled and analysed in MEGAHIT software (v1.0.4-beta). The assembled Scaftigs were interrupted from the N connection, and Scaftigs without N remained (Mende *et al*., 2012; Nielsen *et al*., 2014). To obtain PE reads, we used Bowtie2.2.4 software to compare the clean data from all samples with each Scaffold. We used blast for the Unigenes with the KEGG database (Version 201609, http://www.kegg.jp/kegg/) using DIAMOND software (V0.9.9) (Minoru *et al*., 2014).

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Fig. S1. Distribution pattern of geochemical parameters across Root, Rhizosphere, and Bulk soils.

Fig. S2. The relative abundance of alpha diversity indices across Root, Rhizosphere, and Bulk soils for bacterial and fungal communities.

Fig. S3. The relative abundance of taxonomic composition (phylum level) across Root, Rhizosphere, and Bulk soils for bacterial and fungal communities

Fig. S4. Distributional pattern of carbon metabolic potential genes and their potential hosts (order level).

Fig. S5. Distributional pattern of nitrogen metabolic potential genes and their potential hosts (order level).

Fig. S6. The dominant organic acid metabolic and degradation potential pathways of the *Pteris vittata* rhizosphere microbiome.

Fig. S7. Spearman's linear relationships between the relative abundance of aoxAB and nitrate reductase and carbon fixation ($p < 0.05$).

Table S1. Detail information of geochemical parameters across all samples.

Table S2. Detail information of microbial composition for bacteria and fungi.

Appendix S1: Supporting information

Appendix S2: Supporting information

Appendix S3: Supporting information

Appendix S4: Supporting information