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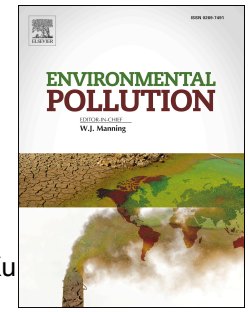

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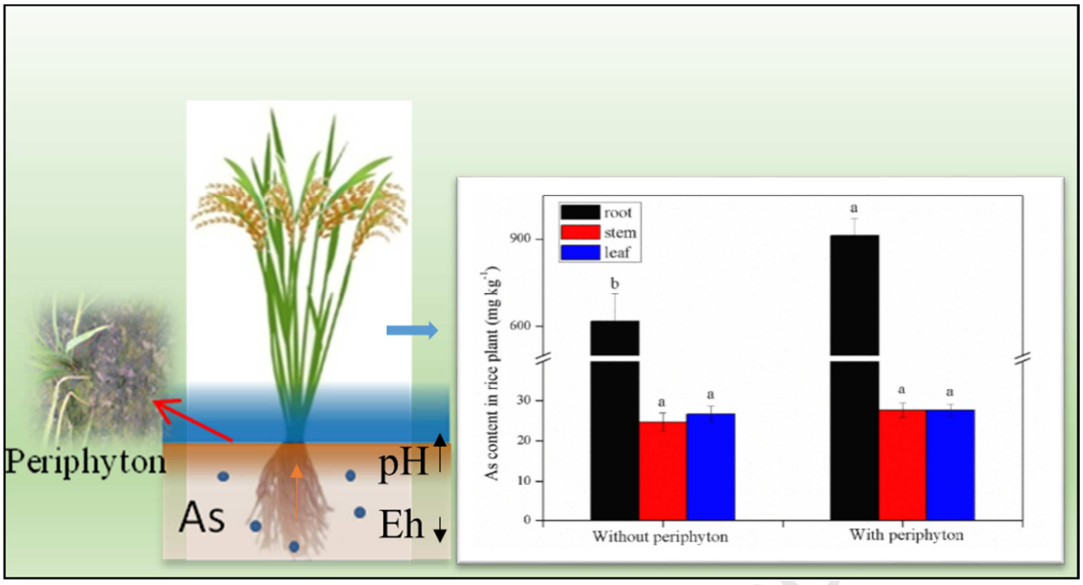

The influence of periphyton on the migration and transformation of arsenic in the paddy soil: rules and mechanisms

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Abstract

Periphyton, composed of algae, bacteria, protozoa, epiphytes, and detritus, is widely distributed on the surfaces of paddy soils. Little is known about the interactions between the periphyton and arsenic (As) in the paddy soil. In the present study, model paddy ecosystems with and without periphyton were set up to explore the effects of periphyton on As migration and transformation in soil. According to the results, periphyton played dual roles in the mobility of As in soil. Periphyton on the surface of paddy soil could significantly increase the mobility and bioavailability of As in soils in the rice tillering stage because of the increased pH and the decreased Eh. The As uptake by rice also increased in the presence of periphyton. However, a significant fraction of the released As was further entrapped by the periphyton, significantly decreasing As concentration in pore water. As biotransformation genes, including *aioA*, *arrA*, *arsC*, and *arsM*, were identified in periphyton, with *arsM* being the most abundant in periphyton and soil. Periphyton significantly decreased *the abundance of aioA*, but increased the abundance of *arsC* in soils. *Cupriavidus* and *Afipia*, which are involved in As(V) cytoplasmic reduction, were significantly increased in the presence of periphyton. Periphyton exerted minor effects on the highly abundant and predominant bacteria but had major effects on the less abundant bacteria in the paddy soil. The results of the present study could facilitate the regulation of As contamination in paddy soil, and enhance our understanding of the role of periphyton in the As biogeochemical cycle.

Capsule: Periphyton increased the bioavailability and plant uptake of As in the paddy soil.

Keywords: Arsenic, paddy soil, periphyton, bioavailability, Arsenic biotransformation gene

1. Introduction

The contamination of paddy fields with arsenic (As), a highly toxic and carcinogenic metalloid, has caused serious environmental challenges globally (Mandal and Suzuki, 2002). Further, the anaerobic conditions in paddy soils could facilitate the release and mobilization of As (Xu et al., 2008). Rice grains have been reported to accumulate high levels of As because rice is grown under flooded conditions (Williams et al., 2007). The toxicity and biogeochemical behavior of As extensively vary among different As species. Pentavalent methylated As is less toxic to animals and human cells than inorganic As, whereas trivalent methylated As is more toxic (Styblo et al., 2000). With respect to inorganic As, arsenate [As(V)] is considerably less toxic than arsenite [As(III)]. As mainly exists in two inorganic species in the paddy soils (Huang and Matzner, 2007; Huang et al., 2011). The mobility and bioavailability of As depend on the As species in the soil (Yamaguchi et al., 2011). Therefore, it is important to understand how As species change in paddy fields.

Various factors influence the bioavailability of As in the paddy soil, such as soil properties, fertilizer and water regimes, and microorganisms (Xu et al., 2008; Yamaguchi et al., 2011). Increasing pH and decreasing Eh facilitate the mobilization of As due to the desorption of As (V) and the reductive dissolution of iron (Fe) oxides/hydroxides, respectively (Masscheleyn et al., 1991). Similarly, flooding facilitates the mobilization of high amounts of As, mainly in the form of As(III), by decreasing the soil redox potential (Li et al., 2009). Microbial-mediated As

metabolism, including As(V) respiratory reduction, As(V) cytoplasmic reduction, and As(III) oxidation and methylation, plays a key role in the biogeochemical cycling of As; therefore, microbes could influence the mobility and toxicity of As directly (Mukhopadhyay et al., 2002). As(III) oxidase genes (*aioA*), respiratory As(V) reductase genes (*arrA*), As(V) reductase genes (*arsC*), and As(III) Sadenosylmethionine methyltransferase genes (*arsM*) have been found in high abundance and diversity in paddy soils in southern China (Zhang et al., 2015). In addition, paddy soil properties, such as pH, total As, and total Fe could influence the abundance and diversity of As metabolism genes (Zhang et al., 2015).

Periphyton is ubiquitous in aquatic and soil ecosystems. Periphyton can tolerate relatively high level of heavy metal toxicity than single-species microbial communities and adapt to diverse environmental conditions by adjusting their community structure (Leguay et al., 2016; Yang et al., 2016a). Extracellular polymeric substance (EPS) plays key roles in biosorption of high-concentration heavy metals by periphyton (Liu et al., 2018; Teitzel and Parsek, 2003). It has been used to remove heavy metals from wastewater through the complexation, ion exchange adsorption, flocculation, precipitation, and adsorption processes (Wu et al., 2012). The As(III) removal efficiency in wastewater using periphyton at As(III) concentrations of 2.0 and 5.0 mg L^{-1} was 96 % and 60%, respectively (Zhu et al., 2018). The combination of calcite with As(III) and -OH and -C=O in the periphyton surfaces plays a vital role in As(III) removal using periphyton (Zhu et al., 2018). In addition to the adsorption of heavy metals, periphyton also has the ability of metal biotransformation. For example,

periphyton (*Cladophora* sp.) from a suburban stream was able to reduce As(V) to As(III) and conversely oxidize As(III) to As(V) (Kulp et al., 2004). There are many studies showing that periphyton plays a key role in production and transfer of neurotoxin methylmercury through Hg methylation and demethylation (Hamelin et al., 2015; Olsen et al., 2016). These studies mainly focused on the periphyton from lakes，streams and other aquatic systems, since periphyton is a direct food for several primary consumers in lotic food webs. Accumulation and biotransformation of heavy metals in periphyton have important implications for the transfer and transport of heavy metals in ecosystems.

However, considering the ubiquitous presence of periphyton in paddy soil, there is a need for more studies on the effect of periphyton on the behavior of heavy metals, particularly As, in paddy soil ecosystems. Periphyton in paddy soil is composed of algae, bacteria, protozoa, metazoans, and epiphytes (Quinlan et al., 2011; Wu et al., 2014). A previous study reported that native periphyton in paddy soils entrapped copper (Cu) and cadmium (Cd) simultaneously, and decreased Cu and Cd accumulation in rice significantly (Yang et al., 2016b). A similar result was observed in soil contaminated with Cd and As (Shi et al., 2017). The presence of periphyton decreased Cd accumulation in rice roots and shoots significantly, while increasing As accumulation, by significantly increasing pH and decreasing Eh (Shi et al., 2017). However, such studies only observed the effects of periphyton on heavy metal accumulation in rice seedlings but did not focus on the microbial mechanisms in soils, especially the changes of functional microbe. Microbes playing important roles in As

metabolism should be considered when explaining the behavior of As in soils. In this study, we explored the influence of periphyton on As bioavailability and its mechanisms from chemical and microbial perspective which were neglected before.

The objectives of this study were to (i) investigate the mobility and transformation of As in soil-rice systems in the presence of periphyton and (ii) explore the effect of periphyton on the abundance and community composition of As metabolism genes in soil. The results of the present study could not only improve our understanding of the As biogeochemical cycle in paddy ecosystems in the presence of periphyton, but also facilitate the control of the As pollution risk in the paddy soil.

2. Material and methods

2.1. Experimental design and sampling

Pot experiments were conducted in a phytotron with 30°C/25°C day/night temperatures and a 14/10 h day/night period. Each plastic pot of diameter 26.5 cm and height 17.5 cm contained 3.2 kg of soil. The paddy soil was collected in a mining area located in Shangyu, Zhejiang Province, southern China. The total As concentration in this paddy soil was 105.1 mg kg^{-1}. Surface soil was sampled, air dried, and sieved through a 2.0-mm mesh before use. To meet the nutrient requirements for rice growth, chemical fertilizers were added into the soil before seeding. Chemical fertilizers (1.0 g of urea and 0.5 g of monopotassium phosphate [KH_2PO_4]) were added into the soil as base fertilizer. The soil in each pot was maintained in a flooded condition with deionised water, and the surface water depth was approximately 2–3 cm in each pot.

Two treatments were set up. In one treatment, periphyton grew naturally on the surface soil. In another treatment, the surface soil was shaded during the day to prevent the growth of periphyton. Each treatment run four pot replicates.

Rice (Xiushui 03) seeds used in the pot experiments were sterilised with 5% active NaOCl for 15 min, and then rinsed and soaked in deionised water for 24 h. The seeds were uniformly sown on silica sand at a depth of 5 cm. 2–3 layers of quantitative filter paper were placed below the silica sand. Deionised water was poured slowly in the pots to cover the silica sand slightly. The seeds were germinated in a 25°C incubator away from light for three days and then cultivated in an illuminated incubator with a day-night period of 14-10 h and temperature of 25°C for three weeks. Uniform seedlings were selected and then three seedlings were transplanted into each pot. Periphyton was formed on the surface soil after four weeks. The duration of the pot experiment was 40 days, and periphyton grew well at this stage. Soil Eh and pH were determined, and the rice root and shoot, periphyton, and pore water were sampled in the tillering stage.

The whole rice plants were washed with tap water and then with deionised water. The leaves, stems, and roots were separated and dried at 60°C. Surface soil samples of 0- 10 cm were collected from four positions in each pot and mixed. Periphyton was sampled by peeling off from the surface soil using a tweezer. The soil and periphyton samples were freeze-dried before further analyses. All plant, soil, and periphyton samples were ground and sieved through a 2-mm plastic mesh to remove plant residue and detritus, and then ground and passed through another sieve (< 60 mesh). The fresh

154 soil (35 g) was centrifuged for 10 min at *c.* 3100 ×*g* to collect soil pore water (Lomax
155 et al., 2012). Soil water samples were acidified with 6 M HCl to prevent As
156 precipitation and transformation, and passed through a sterilised 0.22-μm filter
157 (Lomax et al., 2012).
158
159 **2.2. Sample analysis**
160 A combined platinum and silver/silver chloride electrode system was used to
161 measure soil Eh at approximately 0.5 cm below the soil surface. The soil pH was
162 determined using a combined electrode inserted to a depth of 3 cm below the soil
163 surface. Soil dissolved organic carbon (DOC) was extracted using water (soil: water
164 =1:5) and DOC concentrations in the filtrates was measured using a Multi N/C
165 TN/TC-analyser (Analytik Jena AG, Jena, Germany).
166 To determine the total As concentrations in plants, soils, and periphyton. 0.2-g
167 samples were digested in an acid mixture of HNO_3, HF, and H_2O_2 (volume ratio =
168 4:2:2) under microwave digestion. An inductively coupled plasma mass spectrometer
169 (ICP-MS) (NEXION300XX, PerkinElmer, Inc., USA) was used to determine the
170 concentrations of As in digestion solution. As content in plants, soils and periphyton
171 were normalized to the dry weight of samples. Certified soil reference materials
172 (GSS-15) with recoveries ranging from 97% to 102% were used for quality assurance.
173 As species in the soil were quantified by HPLC-ICP-MS (PerkinElmer Series
174 200 HPLC and NEXION300XX, ICP-MS). Soil samples (0.2 g) were extracted with
175 10 mL of 2% phosphoric acid solution, and then sonicated for 60 min and centrifuged

for 15 min at 3000 ×*g*. Four species of inorganic and organic As, including As(III), As(V), monomethylarsenate (MMA), and dimethylarsenate (DMA), were separated and quantified. The modified BCR sequential extraction procedure with four steps was used to measure the As fractions in soils (Rauret et al., 1999; Rodriguez et al., 2009). Soil samples (0.5 g) were extracted and As concentrations in the supernatants obtained in each step was determined by ICP-MS. The sum of As fractions determined using the BCR sequential extraction method was consistent with the total As concentration determined by acid digestion with an agreement of 89.19–106.24%.

2.3. Quantitative PCR of *aioA*, *arrA*, *arsC*, and *arsM*

DNA was extracted from periphyton and soil samples (0.5 g fresh weight) using the FastDNA SPIN Kit for Soil (MP Biomedicals, Irvine, CA, USA) according to the manufacturer's instructions. Real-time PCR (qPCR) performed on an iQTM5 Thermocycler (Bio-Rad) was used to determine the copy numbers of the 16S ribosomal RNA (16S rRNA) gene and As metabolism genes including *aioA*, *arrA*, *arsC*, and *arsM*. The PCR system contained 10 μL of 2 × SYBR Premix Ex Taq, 0.8 μL each of 10 μM primer pair, 1 μL of DNA template (10 ng/μL) and 8.2 μL of DNase-free deionised water. The specific primers and cycling parameters used for qPCR are listed in Table S1. The amplification efficiency ranged from 89% to 95% and the correlation coefficients (R^2) of the standard curves were greater than 0.99.

2.4. High-throughput sequencing of *arsM*, *arsC* and 16S rRNA genes

Total genomic DNA was PCR amplified using the primers arsMF1/arsMR2 and amlt-42F/amlt-376R (Jia et al., 2013) for *arsM* and *arsC*. The V3–V4 hypervariable regions of the bacteria 16S rRNA genes were amplified with primers 338F (5' -ACT CCTACGGGAGGCAGCA-3') and 806R (5' -GGACTACHVGGGTWTCTAAT-3') (Lee et al., 2012) using a thermocycler PCR system (GeneAmp 9700, ABI, USA). The PCR was performed in a final volume of 20 μl with 4 μl 5× FastPfu Buffer, 2 μl 2.5-mM dNTPs, 0.8 μl 5 μM of each primer, 0.4 μl FastPfu Polymerase (TransGen Biotech, Beijing, China), 0.2 μl bovine serum albumin (BSA; Takara Biotechnology, Dalian, China), and 10-ng template DNA. The thermal cycling conditions were as follows: 95 °C for 3 min; 27 cycles of denaturation at 95 °C for 30 s, primer annealing at 55 °C for 30 s, and extension at 72 °C for 45 s; followed by a final extension period of 10 min at 72 °C. The amplified DNA was electrophoresed on a 2% agarose gel. Then, the expected bands were extracted from the gels and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) and quantified using QuantiFluor™-ST (Promega, Madison, WI, USA). Purified amplicons were pooled in equimolar concentration and paired-end sequenced (2 ×300) on the Illumina MiSeq platform (Illumina, San Diego, CA, USA) at Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China). The sequence read pre-processing was performed in FLASH. Operational taxonomic units were clustered with a 97% similarity cut-off and chimeric sequences were identified and removed. The taxonomy of each representative *arsM*, *arsC*, and 16S rRNA gene was analysed using the Basic Local

Alignment Search Tool against sequences from the National Center for Biotechnology Information database.

2.5. Terminal restriction fragment length polymorphism (T-RFLP) analysis.

We used terminal restriction fragment length polymorphism (T-RFLP) analysis to investigate the diversity of *aioA*, *arrA*, *arsC* and *arsM* (Zhang et al., 2015). PCR amplifications were performed using the forward primers labelled with 6-carboxyfluorescein. The labelled PCR products were digested using the TaqI restriction enzyme (Takara Bio Inc., Japan) at 65°C for 4 h, and then determined using the ABI PRISM 3130XL Genetic Analyser (Applied Biosystems, USA). The relative abundance of terminal restriction fragments (T-RFs) was calculated by dividing the peak areas of each T-RF with that of the total T-RFs. T-RFs of > 1% were listed.

2.6. Statistical analysis

Significant differences in As concentrations between different treatments were tested using an independent-samples *t*-test in IBM SPSS 22.0 (IBM Corp., Armonk, NY, USA). *P*-values <0.05 were considered significant. The redundancy analysis (RDA) was performed to explore the relationships between the environmental variables and abundance profiles of As metabolism genes using R with the vegan package (Dixon, 2003).

3. Results

3.1. Soil properties

The results of soil properties showed that the pH and Eh of the paddy soil were considerably affected by periphyton (Table 1). Soil pH was significantly (P = 0.011) higher in the periphyton treatment and it increased from 6.94 to 7.10 in the presence of periphyton. However, the soil Eh significantly decreased (P = 0.007) in the presence of periphyton, and it decreased from -164.5 to -186.5 mV. Soil DOC was slightly lower in the periphyton treatment. As shown in Table 1, Periphyton had a significant effect on the As concentration in pore water with As content in pore water decreasing significantly (P <0.001) from 283.0 μg L^{-1} to 173.5 μg L^{-1} in the presence of periphyton.

3.2. As species and fractions in soil

Inorganic As was the predominant form of As in the soil, accounting for more than 99% of the total As and only a small amount of MMA was detected in the soil. The As(V) concentration was considerably higher than the As(III) and MMA concentrations, accounting for 76.4% in the soil with periphyton and 73.2% in the soil without periphyton. As illustrated in Figure 1a, periphyton significantly (P = 0.019) influenced the As(III) concentration and percentage in the soil. The percentage of As(III) in the soil increased significantly from 23.1% to 26.2% in the presence of periphyton. Figure 1b shows the percentage of As fractions in the soil determined using the BCR sequential extraction method. Four operationally defined fractions

were isolated, including acid extractable, reducible, oxidizable, and residual, using aqua regia. Residual As accounted for approximately 70% of the As fractions, followed by reducible, acid extractable, and oxidizable As. Figure 1b shows the effect of periphyton on the As fractions and As bioavailability in the soil. Periphyton increased the proportions of acid extractable and reducible As in the soil. The proportion of acid extractable As increased from 13.0% to 14.2% and the proportion of reducible As increased from 13.5% to 14.6% in the presence of periphyton.

3.3. Effect of periphyton on As uptake in different rice parts

Figure 2 illustrates the total As concentrations in the roots, stems, and leaves of rice grown in the soils with or without periphyton. As accumulation in the roots was considerably higher than that in the stems and leaves. Periphyton influenced As uptake by rice roots significantly (P = 0.021). Compared with the treatment without periphyton, the presence of periphyton increased the As content in rice roots from 617.3 to 911.3 mg kg^{-1}, which was equivalent to a 47.6% increase. However, the total As concentrations in rice stems and leaves did not vary significantly between the treatments. The concentration of As in the stems under the periphyton and no-periphyton treatments was 27.6 and 24.7 mg kg^{-1}, respectively. In addition, the total As concentrations in the leaves in both treatments was approximately 27 mg kg^{-1}.

3.4. Entrapment of As by periphyton

In the rice tillering stage, periphyton was formed on the soil surface like a green

mat. The As concentration in periphyton was 272.4±27.5 mg kg^{-1}, with a bioconcentration factor (BCF) of 2.6±0.26. The BCF refers to the ratio of As concentrations in periphyton to that in soils. Scanning electron microscopy was used to observe the morphological characteristics of the periphyton samples. Periphyton in the paddy soils contaminated with heavy metals mainly consisted of a microbial aggregate bound by extracellular polymeric substance (EPS) (Figure 3a). Microalgae dominated the periphyton community and provided a surface for bacteria to grow (Figure 3b-d) (Caires et al., 2018; Li and Brand, 2007; Wu et al., 2016). The Fourier-transform infrared spectra (FTIR) of periphyton revealed three strong and/or broad transmittance bands in the regions of 3384 cm^{-1}, 1650 cm^{-1}, and 1028 cm^{-1} （Figure S1）.The As metabolism genes, including *aioA*, *arrA*, *arsC*, and *arsM*, were detected in the periphyton samples (Figure 3e). *arsM* was the most abundant gene, with a relative abundance of 1.16×10^{-3}, followed by *arrA*, with a relative abundance of 8.43×10^{-5}. Twenty two T-RFs (32, 36, 41, 42, 45, 50, 54, 55, 57, 58, 64, 77, 78, 83, 84, 88, 92, 95, 126, 330, and 344 bp) were detected in the T-RFLP profiles (Figure S2). *aioA* and *arrA* were harboured in more diverse microbes than *arsC* and *arsM* in the periphyton. The 32, 41, and 88-bp T-RFs were dominant in the communities carrying *aioA*. In addition, the 83-bp T-RF had the highest relative abundances in the communities harbouring *arrA*.

3.5. Influence of periphyton on the abundance and diversity of As metabolism genes in soil

arsM was the most abundant gene in paddy soils in the present study (Figure S3), with average relative abundance of 2.5 $\times 10^{-3}$, followed by *aioA*, *arrA* and *arsC*. *aioA* was less abundant in the soil with periphyton than that in the soil without periphyton, with relative abundance of 5.3 $\times 10^{-4}$ in the soil without periphyton and 3.4 $\times 10^{-4}$ in the soil with periphyton. In contrast, *arsC* was more abundant in the soil with periphyton than that in the soil without periphyton, with relative abundance of 7.1 $\times 10^{-6}$ in the soil without periphyton and 8.7 $\times 10^{-6}$ in the soil with periphyton (Figure S3). The phylum-level diversity of *arsC* source bacteria revealed that *Proteobacteria* was the dominant microbe involved in As(V) cytoplasmic reduction, with a relative abundance of 83.70% and 92.43%, in soils with and without periphyton, respectively. *Actinobacteria*, *Proteobacteria*, and *Chloroflexi* were the dominant microbes involved in As(III) methylation, with average relative abundances of 29.95%, 20.77% and 17.66%, respectively (Figure S4). Microbes harbouring *arsC* and *arsM* were distributed in 51 and 110 genera, respectively. STAMP 2.1.3 was used to assess significant differences among microbial communities harbouring *arsC* and *arsM* at the genus level (Parks et al., 2014). With respect to microbes harbouring *arsC*, *Cupriavidus* was significantly (P = 0.039) more abundant in soils with periphyton than in soils without periphyton, with relative abundances of 8.00% and 0.50%, respectively. Besides *Cupriavidus*, the abundance of *Afipia* was also significantly (P = 0.028) increased in the presence of periphyton. According to the RDA results, pH, Eh,

and As species (As(III), As(V), MMA) in soils had dominant effects on *Cupriavidus* abundance (Figure 4a). The DOC and pH were the two primary factors influencing the community diversity of microbes harbouring *arsM*. Among micobes harbouring *arsM*, *Bradyrhizobium* was significantly (P = 0.019) less abundant in soils with periphyton than in soils without periphyton, with relative abundances of 0.73% and 0.98%, respectively.

3.6. Microbial community structure of bacteria in soils influenced by periphyton

Bacterial community structures in soils in the different treatments are presented at the phylum level in Figure 5a. *Firmicutes* was the most abundant bacteria phylum between the two treatments with relative abundances of 28.08% and 25.68% in soils without and with periphyton, respectively, followed by *Proteobacteria* (20.20% vs 21.11%), *Actinobacteria* (19.24% vs 20.25%), and Chloroflexi (14.38% vs 15.09%). The relative abundances of *Chloroflexi*, *Proteobacteria*, *Actinobacteria*, and *Cyanobacteria* were slightly increased in the presence of periphyton. However, the relative abundances of *Firmicutes* and *Planctomycetes* were slightly decreased in the presence of periphyton. A heatmap of bacterial taxonomic groups at the genus level revealed that the most abundant genera were *Clostridium*, *Anaerolineaceae*, *Bacillus*, *Fonticella*, *Heliobacteriaceae*, and *Gaiellales* (Figure S5). In addition, the abundances of *Elev-16S-1332*, *Fonticella*, *SJA-15*, *_KD4-96*, and *Gaiellales* were slightly increased while *Heliobacteriaceae* and *Clostridium* were decreased in the soil with periphyton. The 15 most abundant genera with a significant difference between

348 the periphyton-treated soil and the soil without periphyton based on the Wilcoxon
349 rank sum test are presented in Figure 5b. The abundance of *Thiobacillus* was
350 significantly decreased (P = 0.030) whereas that of other genera such as *Rhodobacter*,
351 *Devosia*, *Patulibacter*, *Romboutsia*, *Halobacillus*, and *Methylobacter* was increased
352 significantly in the presence of periphyton.
353

354 **4. Discussion**

355 **4.1. Effect of periphyton on As mobility and bioavailability**

356 The significantly increased pH and decreased Eh of soils in the treatment with
357 periphyton were expected in the present study (Table 1). Previous studies have
358 demonstrated that periphyton could increase the pH and decrease the Eh of soil (Shi et
359 al., 2017). Periphyton is ubiquitous in paddy soils as a 'green coat' on the soil surface.
360 This coat covering in the soil could decrease oxygen transfer from water to soil (Lu et
361 al., 2016). Microalgae dominated the periphyton community (Figure 3a–d) and
362 photosynthesis by the algae would utilise CO_2 from the water, resulting in an
363 increased soil pH (Wu et al., 2016). Minerals in soils such as Fe (hydr)oxides can
364 adsorb both As(III) and As(V), and the bioavailability of As(V) is relatively low in
365 soil (Goldberg, 2002; Dixit and Hering, 2003). However, As(III) is less strongly
366 adsorbed by Fe (hydr)oxides than As(V), and more rapidly desorbed (Fendorf et al.,
367 2008; Tufano et al., 2008). The pH and Eh of soil are the primary factors influencing
368 As species bioavailability. Increased soil pH facilitates the desorption of As(V) and
369 decreased soil Eh facilitates the reductive dissolution of As(V)-bearing secondary

Fe(III) minerals, and the reduction of As(V) to As (III) (Masscheleyn et al., 1991). Therefore, the increased pH and decreased Eh could explain the increased content of As(III) in soils in the treatment with periphyton（Figure 1a）.

To verify the result of increased As bioavailability in pore water caused by periphyton, we determined the fractions of As in soil, since bioavailability of As also depends on its fractions. Chemical forms of heavy metals in the soil were divided into four fractions, including an acid extractable fraction, a reducible and oxidizable fraction, and a residual fraction (Rauret et al., 1999). Among the four fractions, the acid-soluble and reduced fractions are categorised as unstable, and are bioavailable to plants, whereas oxidation and residual fractions are considered as stable (Roosa et al., 2014). Residual As was the dominant fraction among the four fractions in the present study (Figure 1). In addition, acid extractable and reducible As increased in the presence of periphyton, indicating that periphyton growing on the soil surface could enhance As bioavailability. The As uptake by rice root still increased in the presence of periphyton (Figure 2). The increased uptake of As could be due to the increased As(III) concentrations and the bioavailable As fractions in the soil. Though the periphtyon could increase the As mobility and bioavailability, the As concentration in pore water significantly decreased. This seemingly contradictory phenomenon might be owing to the dual roles of periphyton, since large amounts of As could be entrapped by periphyton. These results suggest that periphyton influence As mobility and bioavailability mainly by increasing soil pH and decreasing soil Eh, and the effect outweighs the As concentrations entrapped by periphyton.

392 **4.2. Possible mechanisms of As entrapment by periphyton**

393 Metals could be rapidly adsorbed and accumulated by periphyton in water
394 (Ancion et al., 2010; Li et al., 2015). Periphyton could also accumulate heavy metals
395 from soils. As concentrations in periphyton that grew in highly As contaminated soil
396 reached 458 mg kg^{-1}, with a BCF value of 1.4 (Shi et al., 2017). The BCF value of As
397 in periphyton in the present study was 2.6, indicating that periphyton had a high
398 tolerance to As stress and could entrap As from the paddy soil. Therefore, the
399 entrapment of As from the paddy soil decreased the As concentrations in pore water
400 (Table 1). To explore the mechanism of As accumulation by periphyton, the functional
401 groups on the surfaces of periphyton were investigated by FTIR Spectrometer. The
402 broad band at 3384 cm^{-1} could be due to vibrations of the -OH and -NH bonds. In
403 addition, the band at 1650 cm^{-1} could represent the absorption peaks of amide I, while
404 the band at 1028 cm^{-1} could have originated from the vibration of polysaccharides
405 (Zivanovic et al., 2007; Leceta et al., 2013). The peaks observed in the FTIR spectra
406 confirmed that EPS could play an important role in As adsorption since it was a major
407 component of the periphyton (Figure 3).

408 To tolerate heavy metal stress, periphyton alter their specific gene expression and
409 metabolism (Koechler et al., 2015). For example, periphyton in wetlands and
410 microbial mats can methylate mercury (Hg) and they are recognised as important
411 environmental sites for Hg methylation (Hamelin et al., 2011; Lin et al., 2013).
412 *Cyanobacteria* was a key component of periphyton in the present study (Figure 3). A
413 previous study reported that the cyanobacterium *Nostoc* sp. PCC 7120 could

414 methylate and demethylate As simultaneously (Xue et al., 2017). As metabolism
415 genes, including *aioA*, *arrA*, *arsC*, and *arsM* were detected in periphyton, with the
416 most abundant being *arsM* (Figure 3e). Therefore, there were numerous functional As
417 microbes in the periphyton. The T-RFLP analysis confirmed As biotransformation
418 microbes in periphyton.
419

420 **4.3. Effect of periphyton on microbial composition and diversity of As**
421 **biotransformation genes and its relationship with As behavior in soils**

422 The decreased abundance of *aioA* and increased abundance of *arsC* indicated a
423 decreased potential for microbial As (III) oxidation and an increased potential for
424 microbial As (V) cytoplasmic reduction in the paddy soils. The results might explain
425 why the concentrations and proportion of As(III) increased in soils while those of As
426 (V) decreased, as microbes have been reported to play key roles in As
427 biotransformation in paddy soils (Meharg and Zhao, 2012). The findings were
428 consistent with the decreased Eh in soils with periphyton. *Cupriavidus* isolated from
429 paddy soils in South China has been demonstrated to perform the As(V) reduction
430 function under neutral anaerobic conditions (Chen et al., 2017). Changes in Eh and
431 pH, therefore, could play a role in the increased abundance of *Cupriavidus* in
432 response to periphyton. *Bradyrhizobium*, which is an aerobic or facultative anaerobic
433 gram-negative bacteria, has been demonstrated to have the ability to limit As
434 translocation and accumulation in plants (Bianucci et al., 2018). Therefore, the
435 decreased *Bradyrhizobium* abundance in the soils with periphyton could be due to

436 decreased Eh. Notably, besides unclassified microbes, *Braddyrhizobium* was the most
437 abundant microbe harbouring *arsC*.
438
439 **4.4. Effect of periphyton on the structure and diversity of bacteria in the soil**
440 *Firmicutes*, *Proteobacteria*, and *Actinobacteria* were predominantly found in the
441 paddy soil contaminated by As, which was consistent with the results of previous
442 studies investigating the diversity of bacterial communities contaminated with heavy
443 metals (Jackson et al., 2005; Xiao et al., 2017). The increased abundance of
444 *Cyanobacteria* could have been due to the dominance of *Cyanobacteria* in the
445 periphyton, which is illustrated in Figure 3. Notably，the relative abundance of
446 *Tectomicrobia* was significantly higher in soil with periphyton than in soil without
447 periphyton, which indicated that *Tectomicrobia* abundance in the soil was easily
448 influenced by periphyton. The largest phylogenetic clades belonging to
449 '*Tectomicrobia*' encompass all '*Entotheonella*' sequences *sensu stricto* and these
450 sequences have mostly been recovered from marine sponges and seawater (Wilson et
451 al., 2014).
452 The slight change in microbial community at the genus level (Figure S5) also
453 indicate that periphyton had a minor effect on the highly abundant and dominant
454 bacteria. Significant differences at the genus level occurred in the bacteria with
455 relatively low abundance (Figure 5b). The significant decrease in the abundance of
456 *Thiobacillus* in the presence of periphyton could be associated with the decrease in Eh
457 in soil. *Thiobacillus* are gram-negative bacteria, growing under aerobic conditions,
458 and they play key roles in metal solubilization (Bosecker, 1997). The abundance of

Rhodobacter, *Devosia*, *Patulibacter*, *Romboutsia*, *Halobacillus*, and *Methylobacter* increased in the presence of periphyton. *Rhodobacter*, belonging to the Alpha-proteobacteria class, is generally found in freshwater or marine environments. Bacterial communities dominated by *Rhodobacter* spp. have been found in streambed sediments exposed to acid mine drainage (Hery et al., 2014). In addition, As-tolerant bacterial strains of *Rhodobacter* and *Devosia* have been isolated and characterised from soils and sediment (Lin et al., 2014; Mu et al., 2016).

4.5. Environmental implications

Periphyton has been used extensively to remove heavy metals from wastewater and contaminated water. In the present study, As bioavailability to rice root significantly increased due to the increased pH and decreased Eh, although periphyton can entrap high amounts of As. In previous studies, periphyton could decrease bioavailability of heavy metals via adsorption or entrapment by periphyton. However, the dispersion of periphyton cells and desorption could release heavy metals into soil, which were overlooked. Therefore, whether periphyton is suitable for the remediation and alleviation of heavy metals in soil requires further investigations. In the present study, we investigated the effect of periphyton on As bioavailability. However, the contamination of As and heavy metals has been observed in paddy soils in Southern China (Fu et al., 2013). While in the present study, we determined the effect of periphyton on As mobility and bioavailability. We did not evaluate the effect of periphyton on other heavy metals such as Cd and Pb. In addition, As metabolism

genes, particularly highly abundant *arsM* and microbes involved in As biotransformation, were detected in periphyton. Thus, the biotransformation of As by periphyton growing in paddy soils remains unclear and requires further investigations.

5. Conclusions

In this study, we explored the influence of periphyton on the migration and transformation of As in the paddy soil system and its mechanisms. The presence of periphyton could increase the mobility and bioavailability of As in soils owing to the increased pH and decreased Eh of the soil. However, periphyton could also entrap a large amount of As, finally resulting in a significant decrease of As concentrations in pore water. As metabolism genes, including *aioA*, *arrA*, *arsC*, and *arsM*, were identified in the periphyton and soil, with *arsM* being the most abundant. The abundance of *aioA* was lower, while the abundance of *arsC* was higher in the treatment with periphyton than that in the treatment without periphyton. The increased abundance of *Cupriavidus* and *Afipia* harbouring *arsC* in the soil with periphyton might be responsible for the increased bioavailability of As. Periphyton had negligible effect on the highly abundant and predominant bacteria in soils. However, significant differences were observed in the relatively less abundant bacteria owing to the composition of periphyton and the changed properties of the soil. The results of the present study enhanced our understanding of the role of periphyton in the As biogeochemical cycle. However, the adsorption and biotransformation of As in various fractions of periphyton should be explored in the future.

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References:

Ancion, P.Y., Lear, G., Lewis, G.D., 2010. Three common metal contaminants of urban runoff (Zn, Cu & Pb) accumulate in freshwater biofilm and modify embedded bacterial communities. Environ. Pollut. 158, 2738-2745.

Bianucci, E., Godoy, A., Furlan, A., Peralta, J.M., Hernandez, L.E., Carpena-Ruiz, R.O., Castro, S., 2018. Arsenic toxicity in soybean alleviated by a symbiotic species of *Bradyrhizobium*. Symbiosis 74, 167-176.

Bosecker, K., 1997. Bioleaching: Metal solubilization by microorganisms. Fems Microbiol. Rev. 20, 591-604.

Caires, T.A., Lyra, G.d.M., Hentschke, G.S., Pedrini, A.d.G., Sant'Anna, C.L. and de Castro Nunes, J.M. 2018. Neolyngbya gen. nov (*Cyanobacteria*, *Oscillatoriaceae*): A new filamentous benthic marine taxon widely distributed along the Brazilian coast. Mol. Phylogene. Evol. 120, 196-211.

Chen, P., Li, X., Li, F., 2017. Effects of Nitrate Reduction and Ferrous Oxidation on Microbial Arsenate Reduction by Cupriavidusmetallidurans Paddy-2. Ecol. Environ. Sci. 26, 328-334.

Dixit, S., Hering, J.G., 2003. Comparison of arsenic(V) and arsenic(III) sorption onto iron oxide minerals: Implications for arsenic mobility. Environ. Sci. Technol. 37,

525 4182-4189.

526 Fendorf, S., Herbel, M.J., Tufano, K.J., Kocar, B.D., 2008. Biogeochemical processes
527 controlling the cycling of arsenic in soils and sediments. In *Biophysico-Chemical*
528 *Processes of Heavy Metals and Metalloids in Soil Environments*, ed. A Violante,
529 PM Huang, GM Gadd, pp. 313–38. Hoboken, NJ: Wiley

530 Fu, J.J., Zhang, A.Q., Wang, T., Qu, G.B., Shao, J.J., Yuan, B., Wang, Y.W., Jiang,
531 G.B., 2013. Influence of E-Waste Dismantling and Its Regulations: Temporal
532 Trend, Spatial Distribution of Heavy Metals in Rice Grains, and Its Potential
533 Health Risk. Environ. Sci. Technol. 47, 7437-7445.

534 Goldberg, S., 2002. Competitive adsorption of arsenate and arsenite on oxides and
535 clay minerals. Soil Sci. Soc. Am. J. 66, 413-421.

536 Hamelin, S., Amyot, M., Barkay, T., Wang, Y., Planas, D., 2011. Methanogens:
537 Principal Methylators of Mercury in Lake Periphyton. Environ. Sci. Technol. 45,
538 7693-7700.

539 Hamelin, S., Planas, D. and Amyot, M. 2015. Mercury methylation and
540 demethylation by periphyton biofilms and their host in a fluvial wetland of the St.
541 Lawrence River (QC, Canada). Sci. Total Enviro. 512, 464-471.

542 Hery, M., Casiot, C., Resongles, E., Gallice, Z., Bruneel, O., Desoeuvre, A., Delpoux,
543 S., 2014. Release of arsenite, arsenate and methyl-arsenic species from
544 streambed sediment affected by acid mine drainage: a microcosm study. Environ.
545 Chem. 11, 514-524.

546 Huang, J.H., Hu, K.N., Decker, B., 2011. Organic arsenic in the soil environment:

547 Speciation, occurrence, transformation, and adsorption behavior. Water, Air, Soil
548 Pollut. 219, 401-415.
549 Huang, J.H., Matzner, E., 2007. Mobile arsenic species in unpolluted and polluted
550 soils. Sci. Total Environ. 377, 308-318.
551 Jackson, C.R., Dugas, S.L., Harrison, K.G., 2005. Enumeration and characterization
552 of arsenate-resistant bacteria in arsenic free soils. Soil Biol. Biochem. 37,
553 2319-2322.
554 Jia, Y., Huang, H., Zhong, M., Wang, F.-H., Zhang, L.-M., Zhu, Y.-G., 2013.
555 Microbial Arsenic Methylation in Soil and Rice Rhizosphere. Environ. Sci.
556 Technol. 47, 3141-3148.
557 Koechler, S., Farasin, J., Cleiss-Arnold, J., Arsene-Ploetze, F., 2015. Toxic metal
558 resistance in biofilms: diversity of microbial responses and their evolution. Res.
559 Microbiol. 166, 764-773.
560 Kulp, T.R., Hoeft, S.E. and Oremland, R.S. 2004. Redox transformations of arsenic
561 oxyanions in periphyton communities. Appl. Environ. Microbio. 70, 6428-6434.
562 Leceta, I., Guerrero, P., de la Caba, K., 2013. Functional properties of chitosan-based
563 films. Carbohydr. Polym. 93, 339-346.
564 Lee, C.K., Barbier, B.A., Bottos, E.M., McDonald, I.R. and Cary, S.C. 2012. The
565 Inter-Valley Soil Comparative Survey: the ecology of Dry Valley edaphic
566 microbial communities. Isme J. 65, 1046-1057.
567 Leguay, S., Lavoie, I., Levy, J.L., Fortin, C., 2016. Using biofilms for monitering
568 metal contamination in lotic ecosystems: The protective effects of hardness and

569 ph on metal bioaccumulation. Environ. Toxicol. Chem. 35, 1489-1501.

570 Li, R.Y., Stroud, J.L., Ma, J.F., McGrath, S.P., Zhao, F.J., 2009. Mitigation of
571 Arsenic Accumulation in Rice with Water Management and Silicon Fertilization.
572 Environ. Sci. Technol. 43, 3778-3783.

573 Li, T., Lin, G.Y., Podola, B., Melkonian, M., 2015. Continuous removal of zinc from
574 wastewater and mine dump leachate by a microalgal biofilm PSBR. J. Hazard.
575 Mater. 297, 112-118.

576 Li, Z. and Brand, J. 2007. Leptolyngbya nodulosa sp nov (Oscillatoriaceae), a
577 subtropical marine cyanobacterium that produces a unique multicellular structure.
578 Phycologia 46(4), 396-401.

579 Lin, H.Z., Yue, Y.H., Lu, J.C., Zhao, G.C., Yang, P.S., 2014. Variation in
580 composition and relative content of accumulated photopigments in a newly
581 isolated Rhodobacter capsulatus strain XJ-1 in response to arsenic. J. Environ.
582 Sci. Health Part A-Toxic/Hazard. Subst. Environ. Eng. 49, 1493-1500.

583 Lin, T.Y., Kampalath, R.A., Lin, C.-C., Zhang, M., Chavarria, K., Lacson, J., Jay,
584 J.A., 2013. Investigation of Mercury Methylation Pathways in Biofilm versus
585 Planktonic Cultures of Desulfovibrio desulfuricans. Environ. Sci. Technol. 47,
586 5695-5702.

587 Liu, J.Z., Wang, F.W., Wu, W.T., Wan, J.J., Yang, J.L., Xiang, S.L. and Wu, Y.H.,
588 2018. Biosorption of high-concentration Cu (II) by periphytic biofilms and the
589 development of a fiber periphyton bioreactor (FPBR). Bioresour. Technol. 248,
590 127-134.

591 Lomax, C., Liu, W.J., Wu, L.Y., Xue, K., Xiong, J.B., Zhou, J.Z., McGrath, S.P.,
592 Meharg, A.A., Miller, A.J., Zhao, F.J., 2012. Methylated arsenic species in plants
593 originate from soil microorganisms. New Phytol. 193, 665-672.
594 Lu, H., Wan, J., Li, J., Shao, H., Wu, Y., 2016. Periphytic biofilm: A buffer for
595 phosphorus precipitation and release between sediments and water.
596 Chemosphere 144, 2058-2064.
597 Mandal, B.K., Suzuki, K.T., 2002. Arsenic round the world: a review. Talanta 58,
598 201-235.
599 Masscheleyn, P.H., Delaune, R.D., Patrick, W.H., 1991. Effect of redox potential and
600 ph on arsenic speciation and solubility in a contaminated soil. Environ. Sci.
601 Technol. 25, 1414-1419.
602 Meharg, A.A., Zhao, F.J., 2012. Biogeochemistry of Arsenic in Paddy Environments.
603 Arsenic & Rice. Springer Science & Business Media Press: Dordrecht 2012; pp
604 71-101.
605 Mu, Y., Zhou, L.L., Zeng, X.C., Liu, L., Pan, Y.F., Chen, X.M., Wang, J.N., Li, S.H.,
606 Li, W.J., Wang, Y.X., 2016. Arsenicitalea aurantiaca gen. nov., sp nov., a new
607 member of the family Hyphomicrobiaceae, isolated from high-arsenic sediment.
608 Int. J. Syst. Evol. Microbiol. 66, 5478-5484.
609 Mukhopadhyay, R., Rosen, B.P., Pung, L.T., Silver, S., 2002. Microbial arsenic: from
610 geocycles to genes and enzymes. Fems Microbiol. Rev. 26, 311-325.
611 Olsen, T.A., Brandt, C.C. and Brooks, S.C. 2016. Periphyton Biofilms Influence
612 Net Methylmercury Production in an Industrially Contaminated System. Environ.

613 Sci. Technol. 50, 10843-10850.

614 Parks, D.H., Tyson, G.W., Hugenholtz, P., Beiko, R.G., 2014. STAMP: statistical
615 analysis of taxonomic and functional profiles. Bioinformatics 30, 3123-3124.

616 Quinlan, E.L., Nietch, C.T., Blocksom, K., Lazorchak, J.M., Batt, A.L., Griffiths, R.,
617 Klemm, D.J., 2011. Temporal Dynamics of Periphyton Exposed to Tetracycline
618 in Stream Mesocosms. Environ. Sci. Technol. 45, 10684-10690.

619 Rauret, G., Lopez-Sanchez, J.F., Sahuquillo, A., Rubio, R., Davidson, C., Ure, A.,
620 Quevauviller, P., 1999. Improvement of the BCR three step sequential extraction
621 procedure prior to the certification of new sediment and soil reference materials.
622 J. Environ. Monit. 1, 57-61.

623 Rodriguez, L., Ruiz, E., Alonso-Azcarate, J., Rincon, J., 2009. Heavy metal
624 distribution and chemical speciation in tailings and soils around a Pb-Zn mine in
625 Spain. J. Environ. Manage. 90, 1106-1116.

626 Roosa, S., Wattiez, R., Prygiel, E., Lesven, L., Billon, G. and Gillan, D.C. 2014.
627 Bacterial metal resistance genes and metal bioavailability in contaminated
628 sediments. Environ. Pollut. 189, 143-151.

629 Shi, G.L., Lu, H.Y., Liu, J.Z., Lou, L.Q., Tang, X.J., Wu, Y.H., Ma, H.X., 2017.
630 Periphyton growth reduces cadmium but enhances arsenic accumulation in rice
631 (Oryza sativa) seedlings from contaminated soil. Plant Soil 421, 137-146.

632 Styblo, M., Del Razo, L.M., Vega, L., Germolec, D.R., LeCluyse, E.L., Hamilton,
633 G.A., Reed, W., Wang, C., Cullen, W.R., Thomas, D.J., 2000. Comparative
634 toxicity of trivalent and pentavalent inorganic and methylated arsenicals in rat

635 and human cells. Arch. Toxicol. 74, 289-299.

636 Teitzel, G.M. and Parsek, M.R. 2003. Heavy metal resistance of biofilm and
637 planktonic Pseudomonas aeruginosa. Appl. Environ. Microbio. 69, 2313-2320.

638 Tufano, K.J., Reyes, C., Saltikov, C.W., Fendorf, S., 2008. Reductive Processes
639 Controlling Arsenic Retention: Revealing the Relative Importance of Iron and
640 Arsenic Reduction. Environ. Sci. Technol. 42, 8283-8289.

641 Williams, P.N., Villada, A., Deacon, C., Raab, A., Figuerola, J., Green, A.J.,
642 Feldmann, J., Meharg, A.A., 2007. Greatly enhanced arsenic shoot assimilation
643 in rice leads to elevated grain levels compared to wheat and barley. Environ. Sci.
644 Technol. 41, 6854-6859.

645 Wilson, M.C., Mori, T., Ruckert, C., Uria, A.R., Helf, M.J., Takada, K., Gernert, C.,
646 Steffens, U.A.E., Heycke, N., Schmitt, S., Rinke, C., Helfrich, E.J.N.,
647 Brachmann, A.O., Gurgui, C., Wakimoto, T., Kracht, M., Crusemann, M.,
648 Hentschel, U., Abe, I., Matsunaga, S., Kalinowski, J., Takeyama, H., Piel, J.,
649 2014. An environmental bacterial taxon with a large and distinct metabolic
650 repertoire. Nature 506, 58-+.

651 Wu, Y.H., Liu, J.Z., Lu, H.Y., Wu, C.X., Kerr, P., 2016. Periphyton: an important
652 regulator in optimizing soil phosphorus bioavailability in paddy fields. Environ.
653 Sci. Pollut. Res. 23, 21377-21384.

654 Wu, Y.H., Xia, L.Z., Yu, Z.Q., Shabbir, S., Kerr, P.G., 2014. In situ bioremediation of
655 surface waters by periphytons. Bioresour. Technol. 151, 367-372.

656 Wu, Y.H., Li, T.L., Yang, L.Z., 2012. Mechanisms of removing pollutants from

aqueous solutions by microorganisms and their aggregates: A review. Bioresour. Technol. 107, 10-18.

Xiao, E.Z., Krumins, V., Xiao, T.F., Dong, Y.R., Tang, S., Ning, Z.P., Huang, Z.Y., Sun, W.M., 2017. Depth-resolved microbial community analyses in two contrasting soil cores contaminated by antimony and arsenic. Environ. Pollut. 221, 244-255.

Xu, X.Y., McGrath, S.P., Meharg, A.A., Zhao, F.J., 2008. Growing rice aerobically markedly decreases arsenic accumulation. Environ. Sci. Technol. 42, 5574-5579.

Xue, X.M., Yan, Y., Xiong, C., Raber, G., Francesconi, K., Pan, T., Ye, J., Zhu, Y.G., 2017. Arsenic biotransformation by a cyanobacterium *Nostoc* sp PCC 7120. Environ. Pollut. 228, 111-117.

Yamaguchi, N., Nakamura, T., Dong, D., Takahashi, Y., Amachi, S., Makino, T., 2011. Arsenic release from flooded paddy soils is influenced by speciation, Eh, pH, and iron dissolution. Chemosphere 83, 925-932.

Yang, J.L., Liu, J.Z., Wu, CX.., Kerr, P.G., Wong, P.K., Wu, Y.H., 2016a. Bioremediation of agricultural solid waste leachates with diverse species of Cu (II) and Cd (II) by periphyton. Bioresour. Technol. 221, 214-221.

Yang, J.L., Tang, C.L., Wang, F.W., Wu, Y.H., 2016b. Co-contamination of Cu and Cd in paddy fields: Using periphyton to entrap heavy metals. J. Hazard. Mater. 304, 150-158.

Zhang, S.Y., Zhao, F.J., Sun, G.X., Su, J.Q., Yang, X.R., Li, H., Zhu, Y.G., 2015. Diversity and Abundance of Arsenic Biotransformation Genes in Paddy Soils

679 from Southern China. Environ. Sci. Technol. 49, 4138-4146.

680 Zhu, N.Y., Zhang, J.H., Tang, J., Zhu, Y., Wu, Y.H., 2018. Arsenic removal by

681 periphytic biofilm and its application combined with biochar. Bioresour. Technol.

682 248, 49-55.

683 Zivanovic, S., Li, J., Davidson, P.M., Kit, K., 2007. Physical, mechanical, and

684 antibacterial properties of chitosan/PEO blend films. Biomacromolecules 8,

685 1505-1510.

686 Table 1 Properties of soil treated soil with and without periphyton. Data are expressed
687 as mean ± standard deviation (n = 3). Different letters indicate the significant
688 differences between the treatments. The results with *P*-values <0.05 were considered
689 significant. Eh: redox potential; DOC: dissolved organic carbon

	pH	Eh (mV)	DOC (mg kg^{-1})	Pore water As (µg L^{-1})
Without periphyton	6.94±0.03 b	-164.5±10.9 a	499.1±50.0 a	283.0±31.2 a
With periphyton	7.10±0.08 a	-186.5±1.7 b	446.6±21.5 a	173.5±16.0 b

690

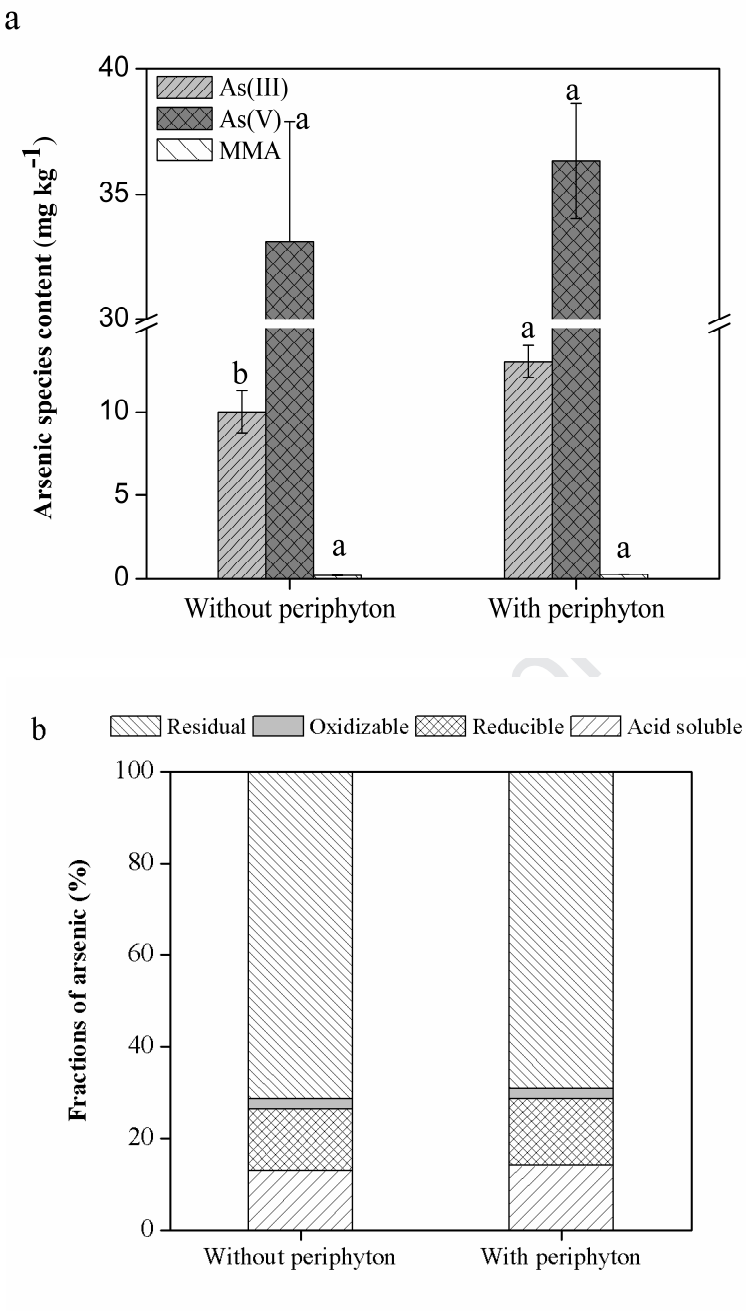


Figure 1. Concentrations of three As species (a) and percentages of four As fractions (b) in the two soil treatments. Different letters indicate significant differences between treatments. The results with *P*-values <0.05 were considered significant.

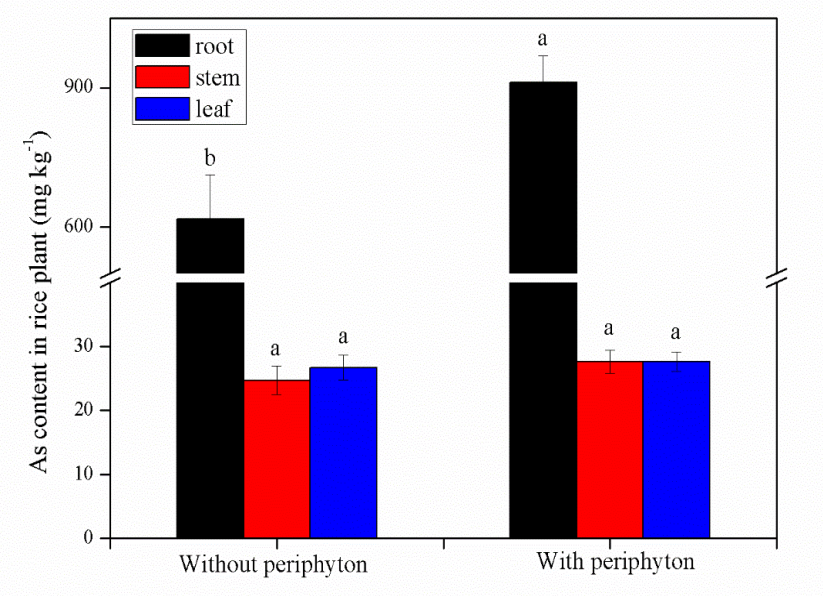


Figure 2. Total As concentrations in different parts of rice in periphyton and the no periphyton treatments. Data are expressed as mean ± standard deviation (n = 4). Different letters indicate significant differences between treatments. The results with *P*-values <0.05 were considered significant.

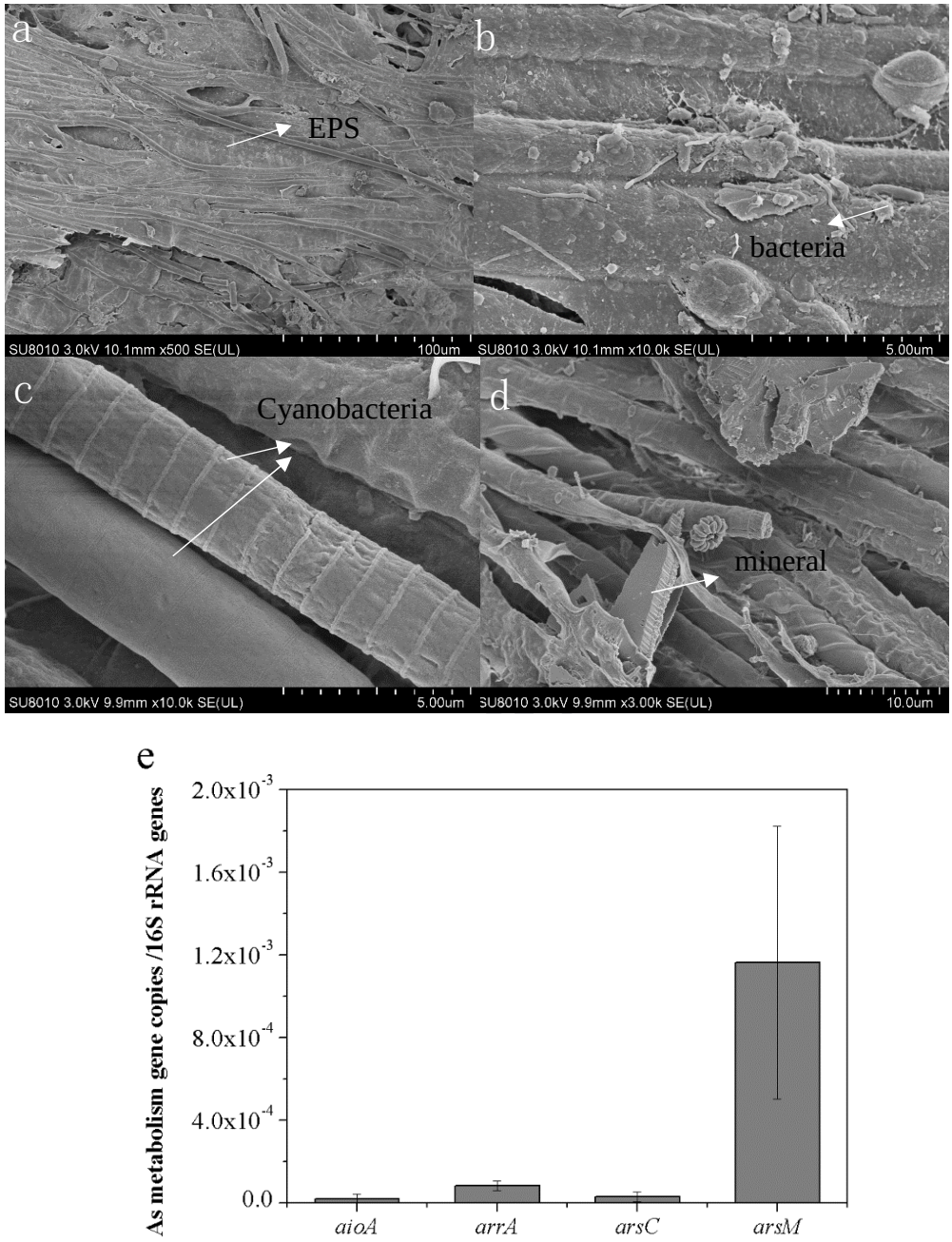


Figure 3. The morphological characteristics of periphyton in the paddy soil observed under a scanning electron microscopy (a-d), and the relative abundances of As metabolism genes in periphyton based on the qPCR analysis (e).

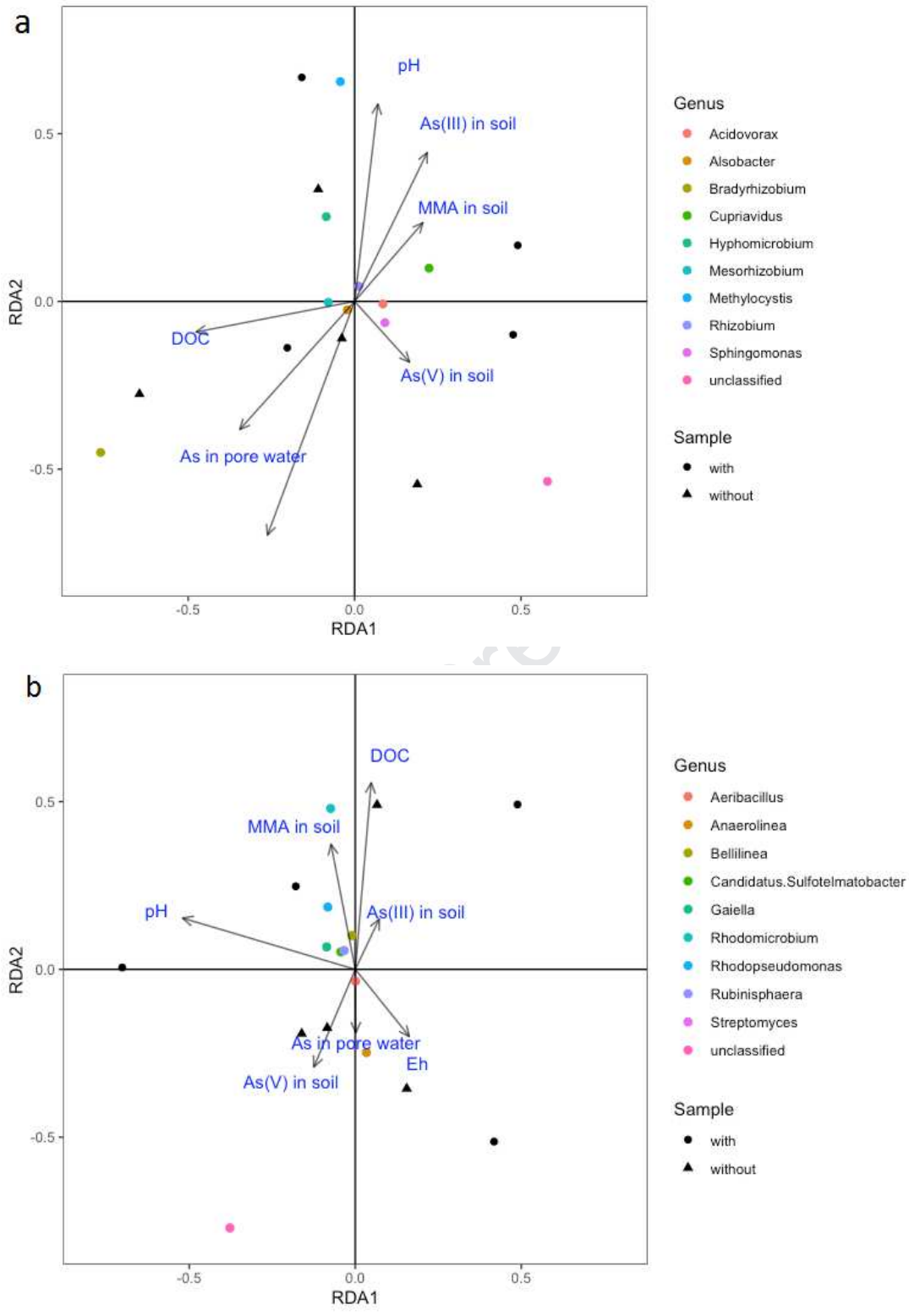
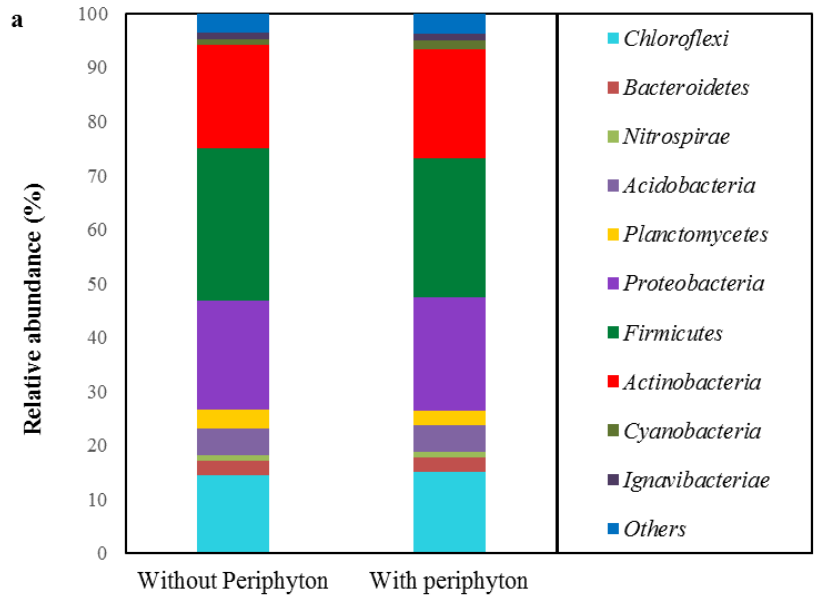


Figure 4. Redundancy analysis (RDA) ordination plots to analyse the relationship between soil properties and community structure of As biotransformation genes, a: *arsC*; b: *arsM*.



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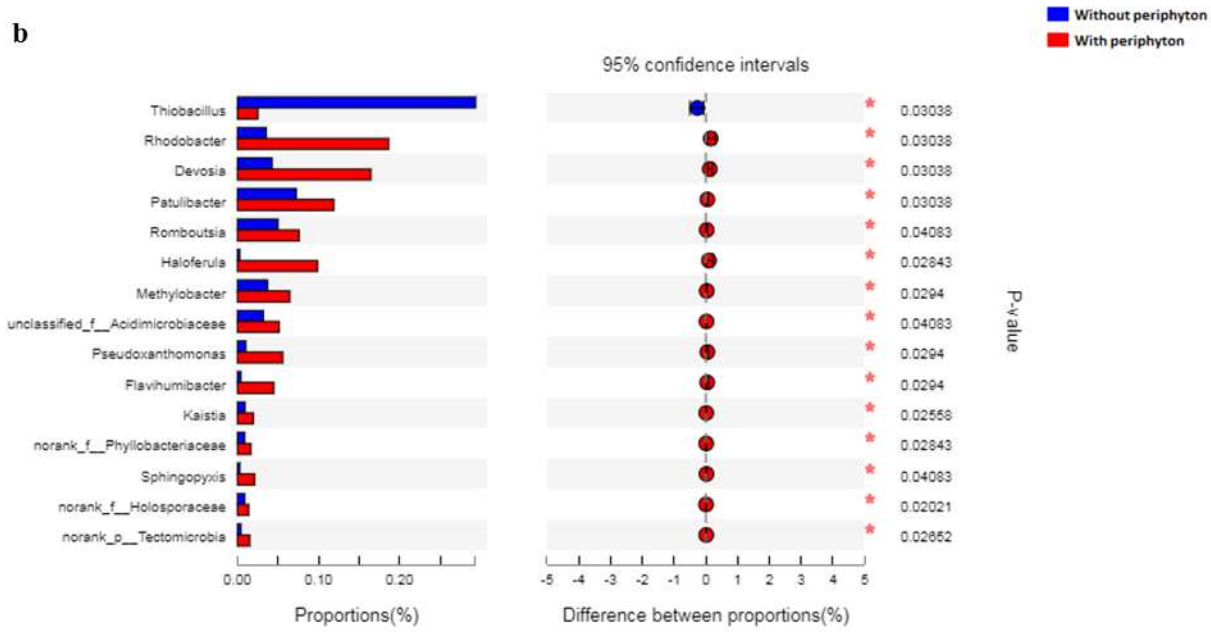

711

712 **Figure 5.** Relative abundance of above 1 % for different phyla in the soils under
713 different treatments in the rice tillering stage. Others include bacteria with a relative
714 abundance of < 1% (a). Top 15 abundant genera with significant differences between
715 the two soil treatments based on the Wilcoxon rank sum test (b).

Highlights

- Periphyton increased the bioavailability of As in paddy soil.
- Further entrapment of As by periphyton reduced the As concentration in pore water.
- As uptake by rice increased in the presence of periphyton.
- As biotransformation genes (*aioA*, *arrA*, *arsC*, *arsM*) were identified in periphyton.
- *Cupriavidus* and *Afipia* harbouring *arsC* increased in the presence of periphyton.

Author Statement

Ting Guo: Conceptualization, Methodology, Software, Investigation, Writing – original draft, Writing – review & editing; **Yujie Zhou:** Methodology, Writing – original draft; **Songcan Chen:** Software, Data curation, Writing- Reviewing and Editing; **Haiying Lu:** Data curation, Writing- Reviewing and Editing; **Yan He:** Supervision, Validation, Writing – review & editing; **Xianjin Tang:** Conceptualization, Project administration, Funding acquisition, Supervision, Resources; **Jianming Xu:** Supervision, Resources, Validation, Writing – review & editing.

Conflict of Interest

All authors have approved the manuscript and agree with its submission to ***Environmental Pollution***. We declare that there are no conflicts of interest to this work.