

Article

Periphyton-Associated Arsenic-Transforming Functional Potential at Paddy Soil–Water Interfaces

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Abstract

Arsenic (As) contamination in paddy soils poses a persistent threat to food safety and human health through rice consumption. The environmental fate of As in flooded paddy soils is governed by complex physicochemical and microbially transformations, yet the role of biologically active interfaces remains poorly understood. Periphyton, a ubiquitous and metabolically active biofilm at the paddy soil–water interface, may play a critical role in regulating As speciation and behavior. Herein, we integrated biofilm development, microscopic and elemental characterization, bacterial community profiling, and As-transforming functional gene analysis to elucidate the role of periphyton in As transformation potential. Results showed that periphyton formed a heterogeneous three-dimensional matrix composed of microbial aggregates, extracellular matrix-like materials, and mineral particles. Periphyton-associated As was 2.37 mg kg^{−1} in the irradiated-soil sample and 1.16 mg kg^{−1} in the natural-soil sample, whereas dissolved As in the overlying water was 2.06 and 5.85 µg L^{−1}, respectively. The natural-soil composite showed higher bacterial richness and diversity indices than the irradiated-soil composite, with 2615 versus 1124 observed ASVs and Shannon indices of 8.63 versus 7.22. Proteobacteria and Cyanobacteria dominated both communities. The coexistence of *aioA*, *arrA*, *arsC*, and *arsM* indicated the potential for concurrent As(III) oxidation, As(V) reduction, detoxification, and methylation. In the natural-soil composite, *aioA*, *arrA*, and *arsM* reached 1.84×10^6 , 1.99×10^6 , and 2.63×10^6 copies g^{−1} sample, respectively. These values were 2.4-, 9.8-, and 7.2-fold higher than those in the irradiated-soil composite, whereas *arsC* remained relatively stable. These findings indicate that periphyton provides a structured microbial interface with the genetic potential for multiple As transformation pathways. We propose that paddy periphyton represents a structurally and microbiologically complex interface linking microbial assembly with As-transforming functional potential.

Keywords: paddy soil; periphyton; arsenic; redox processes; pollution control and risk mitigation



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1. Introduction

Arsenic (As) is a toxic and carcinogenic metalloid widely distributed in the environment, posing serious risks to human health upon long-term exposure [1]. Anthropogenic activities, particularly mining and the use of As-containing agrochemicals, can lead to As accumulation in agricultural soils, thereby increasing its uptake by rice [2–4]. Compared with many other cereal crops, rice exhibits a greater capacity to accumulate As from soil [5]. Consequently, As can enter the human food chain through rice consumption, potentially resulting in chronic toxicity and associated health effects [3,5].

As at the soil–water interface can undergo diverse microbial transformations that alter its speciation and, consequently, its mobility and environmental fate [6,7]. These transformations primarily involve As(III) oxidation, As(V) reduction, intracellular detoxification, and As methylation [6,8]. Microbial As(III) oxidation, mediated by the *aio* system including the catalytic gene *aioA*, converts the more mobile As(III) to As(V) [7,9,10]. Conversely, respiratory As(V) reduction uses As(V) as an electron acceptor and produces As(III), with *arrA* serving as a key functional marker [6,11,12]. Intracellular As(V) can also be reduced by the detoxification enzyme encoded by *arsC*, after which the resulting As(III) is exported through cellular As-resistance systems [7,10,12]. In addition, the *arsM*-encoded As(III) methyltransferase mediates As methylation, generating methylated species with potentially greater mobility or the capacity for further volatilization [6,13,14]. These functionally distinct pathways are widely distributed and can coexist within paddy soil microbial communities, as evidenced by the widespread occurrence and high diversity of *aioA*, *arrA*, *arsC*, and *arsM* [6,15,16]. Because these processes exert divergent effects on As speciation and transport, the net fate of As is ultimately governed by their interactions, together with the composition of the microbial community and prevailing environmental conditions.

Periphyton is a ubiquitous and structurally complex biological assemblage in aquatic ecosystems, forming attached biofilms on submerged surfaces in environments such as paddy fields, wetlands, and drainage channels. Originally conceptualized as an assemblage of organisms attached to or associated with submerged substrates, periphyton comprises phototrophic, chemotrophic, and heterotrophic microorganisms, together with mineral particles, detritus, and extracellular polymeric substances (EPSs) [17,18]. Depending on the colonized substrate and growth habitat, periphyton can occur as epiphyton, epipelon, epilithon, or metaphyton. In flooded paddy systems, these components form a heterogeneous three-dimensional matrix rather than a simple algal layer, providing microsites for microbial colonization, metabolite exchange, and interactions between biological and mineral phases [16,19–23]. This complex architecture underpins the important ecological functions of periphyton, including photosynthetic oxygen production, nutrient cycling, and the retention and transformation of dissolved and particulate substances. In particular, EPS-rich matrices provide abundant reactive functional groups and binding sites for trace elements, thereby facilitating the adsorption and accumulation of As and associated mineral phases [20,24–26]. Meanwhile, the coexistence and metabolic interactions of phototrophic and heterotrophic microorganisms promote the exchange of carbon and other metabolites and contribute to the regulation of microbial community assembly and biogeochemical processes [16,22,23]. Through photosynthesis, respiration, organic matter production, and microbial metabolism, periphyton can further modify local pH, redox conditions, dissolved organic carbon, and oxygen availability at the soil–water interface, thereby regulating the mobility, speciation, and transformation of As [27–29]. Periphyton should therefore be regarded as an active biogeochemical interface in which biofilm architecture, microbial metabolism, solute exchange, and mineral–surface interactions are tightly coupled, collectively shaping As cycling at the paddy soil–water interface.

Specifically, the structural complexity and metabolic diversity of periphyton make it a potentially important regulator of As speciation and transformation at the paddy soil–water interface. Its EPS-rich biofilm matrix provides abundant reactive surfaces and binding sites that can concentrate and retain As, while simultaneously creating spatially heterogeneous microhabitats for closely associated phototrophic and heterotrophic microorganisms [16,24]. Beyond As adsorption and accumulation, these structural and microbial characteristics can influence As transformation by regulating local physicochemical conditions and microbial metabolic processes. For example, periphyton-mediated changes in pH and redox conditions can promote As mobilization from soil, whereas part of the released As may subsequently be retained within the biofilm matrix [16]. Periphyton can also enhance As release and methylation at the soil–water interface through coupled alterations in dissolved organic carbon and microbial activity [22]. More recently, trophic interactions between phototrophic and heterotrophic microorganisms have been shown to regulate *arsM*-harboring communities and, consequently, the production of methylated As species [30]. Collectively, these findings suggest that periphyton does not exert a unidirectional effect on As behavior; rather, it can simultaneously influence As retention, mobilization, and species transformation through coupled physicochemical and microbial processes. However, previous studies have largely focused on net As mobility, bioavailability, or individual transformation pathways. The coexistence of multiple As-transforming functional genes and the potential relationships among opposing pathways remain poorly understood in periphyton [16,22]. Therefore, the main objectives are to elucidate the development and structural organization of periphyton at the paddy soil–water interface under contrasting soil and illumination conditions; to compare periphyton-associated bacterial communities and As-transforming functional potential between the two soil backgrounds; and to integrate biofilm structure, elemental associations, microbial communities, and As-related functional genes to evaluate the potential role of periphyton in As cycling.

2. Materials and Methods

2.1. Periphyton Collection and Biofilm Cultivation

Periphyton was collected from the soil surface of a rice field at the South China Botanical Garden, Guangzhou, China (23°10′53.8716″ N, 113°21′55.2312″ E). During sampling, periphyton attached to the soil surface was gently detached using sterile forceps. Multiple sampling points with comparable growth conditions, including illumination, temperature, and periphyton color, were selected and pooled to obtain a representative sample, thereby minimizing the effects of spatial heterogeneity. The collected periphyton was rinsed three times with deionized water to remove loosely attached soil particles and other impurities and subsequently transferred to BG11 medium for enrichment and biomass expansion. BG11 medium was prepared by adding 1 mL L^{−1} of K₂HPO₄ (40 g·L^{−1}), Na₂CO₃ (20 g·L^{−1}), CaCl₂·2H₂O (36 g·L^{−1}), MgSO₄·7H₂O (75 g·L^{−1}), citric acid (6 g·L^{−1}), ammonium iron(III) citrate (6 g·L^{−1}), and Na₂EDTA (1 g·L^{−1}) stock solutions, together with 4 mL L^{−1} of NaNO₃ stock solution (375 g·L^{−1}) and 1 mL L^{−1} of A5 trace-element solution. The A5 solution contained H₃BO₃ (2.86 mg·L^{−1}), MnCl₂·4H₂O (1.86 mg·L^{−1}), Na₂MoO₄·2H₂O (0.39 mg·L^{−1}), CuSO₄·5H₂O (0.08 mg·L^{−1}), ZnSO₄·7H₂O (0.22 mg·L^{−1}), and Co(NO₃)₂·6H₂O (0.05 mg·L^{−1}). The periphyton was maintained under static conditions in a temperature-controlled illuminated chamber under suitable temperature and light conditions. Its growth was monitored periodically throughout the cultivation period. Fresh BG11 medium was replenished as needed based on changes in medium color and biomass to maintain adequate nutrient availability and stable periphyton growth. The resulting enriched periphyton culture was subsequently used as the inoculum for the paddy soil microcosm experiments.

2.2. Paddy Soil Microcosm Incubation

We collected paddy soil from As-contaminated farmland in Shantou, Guangdong Province. The soil had a pH of 7.67 and an organic matter content of 35.2 g kg^{-1} . The total As concentration was 14.31 mg kg^{-1} . Measured total As concentrations in the initial soil, overlying water, and periphyton are summarized in Table S3. An aliquot of the As-contaminated paddy soil was sterilized by Co-60 γ -irradiated at a dose of 28–50 kGy by a commercial irradiation facility. Gamma irradiation was used to alter the soil microbial background, although associated physicochemical changes could not be excluded. After air drying, the soil was passed through a 10-mesh sieve. Each 90 mm Petri dish received 20 g of soil and 40 mL of ultrapure water. Stable periphyton obtained from the preceding BG11 enrichment culture was inoculated into the soil microcosms using sterile forceps. We established microcosms with natural As-contaminated soil and Co-60 γ -irradiated As-contaminated soil. Normal illumination was used to allow periphyton formation and growth, whereas light exclusion served as a visual reference for assessing the light dependence of macroscopic periphyton development. The chamber followed a 12 h light and 12 h dark photoperiod with an illumination intensity of 3000 lux. We prepared three replicate dishes for each treatment. For downstream analyses, we pooled biofilms from the three replicate dishes into one composite sample. Macroscopic biofilm development under both illuminated and light-exclusion conditions was recorded on days 7 and 14. After 15 d, the normally illuminated biofilms were selected for subsequent SEM-EDS, 16S rRNA sequencing, and qPCR analyses. We removed the overlying water, collected the surface biofilm with sterile forceps, and sampled the upper 0 to 2 mm soil layer from the natural and irradiated soil microcosms. Samples were stored at -80°C until analysis.

2.3. Determination of Total Arsenic

Total As in soil, overlying water, and periphyton was determined by atomic fluorescence spectrometry (AFS), with sample pretreatment adjusted according to matrix type. Soil samples were air-dried, ground, and passed through a 100-mesh sieve. An aliquot of 0.50 g was digested with 10 mL HCl-HNO₃ (1:1, *v/v*) in a boiling-water bath for 2 h, following GB/T 22105.2-2008 [31]. For periphyton, 0.20 g of freeze-dried and ground material was microwave-digested with 4 mL HNO₃, 2 mL HF, and 2 mL 30% H₂O₂. After digestion, the solution was concentrated by acid evaporation and diluted to 50 mL with deionized water. Overlying-water samples were filtered and acidified with HCl before analysis. Prior to AFS measurement, As(V) was reduced with thiourea–ascorbic acid, followed by hydride generation with KBH₄ under acidic conditions. Procedural blanks and calibration standards were included for quality control. The method detection limit specified by GB/T 22105.2-2008 for soil As was 0.01 mg kg^{-1} .

2.4. Morphological and Elemental Characterization

For SEM–EDS analysis, biofilm pieces ($<1 \text{ cm}^2$) were fixed in 2.5% glutaraldehyde at 4°C for 12 h, followed by three 15-min rinses with 0.1 mol L^{-1} phosphate buffer (pH 7.0). The samples were then post-fixed with 1% osmium tetroxide for 1–2 h and dehydrated through a graded ethanol series (30%, 50%, 70%, 80%, 90%, and 95%), followed by two changes of absolute ethanol. The dehydrated samples were treated sequentially with a 1:1 (*v/v*) mixture of ethanol and isoamyl acetate for 30 min and isoamyl acetate for 1 h or overnight, followed by critical-point drying and coating prior to SEM observation. EDS was used to characterize the elemental composition of selected regions of the periphyton matrix.

2.5. Bacterial Community Analysis

Approximately 0.2–0.5 g of periphyton was homogenized using a TissueLyser-48 tissue grinder prior to DNA extraction. Total genomic DNA was extracted using the MagBeads FastDNA Kit for Soil (MP Biomedicals, Santa Ana, CA, USA) according to the manufacturer's instructions. DNA quality and concentration were assessed by 0.8% agarose gel electrophoresis and NanoDrop NC2000 spectrophotometry (Thermo Fisher Scientific, Wilmington, DE, USA), respectively. The V3–V4 region of the bacterial 16S rRNA gene was amplified using primers 338F (5'-ACTCCTACGGGAGGCAGCA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'). Sample-specific barcodes were incorporated for multiplexed sequencing. Raw reads were processed using QIIME2 (version 2024.5), with cutadapt for primer removal and DADA2 for quality filtering, denoising, paired-end merging, chimera removal, and amplicon sequence variant (ASV) generation. Taxonomic classification was performed against the SILVA database (Release 138). Alpha-diversity metrics, including observed ASVs, Chao1, Shannon, Simpson, Pielou's evenness, and Good's coverage, were calculated, and bacterial community composition was compared at the phylum level. Community profiles were obtained from one composite sample for each soil treatment.

2.6. Arsenic Transformation Gene Analysis

The same composite periphyton samples were used for absolute quantitative PCR (qPCR). DNA was extracted using an Omega DNA extraction kit (M5635-02; Omega Bio-tek, Norcross, GA, USA) according to the manufacturer's instructions. Four As-related functional genes, *aioA*, *arrA*, *arsM*, and *arsC*, were quantified using SYBR Green-based qPCR. Primer sequences and references are provided in Table S1 [32–35]. Absolute quantification was performed using plasmid standard curves prepared by 10-fold serial dilution. The amplification efficiencies ranged from 89.89% to 92.59%, with correlation coefficients (*R*) of 0.9916–0.9966. qPCR was performed using AceQ qPCR SYBR Green Master Mix (Vazyme Biotech, Nanjing, China) on a LightCycler 480 II system (Roche Diagnostics, Mannheim, Germany). The reaction program consisted of 95 °C for 5 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 30 s. A blank control was included during primer optimization to check for possible contamination. Amplification specificity in the quantitative assays was evaluated by melt-curve analysis, which showed a single dominant peak for each target gene. No-template controls were not routinely included in the quantitative assays. Each sample was analyzed in technical triplicate.

Gene copy numbers were calculated from the corresponding standard curves and corrected for DNA elution volume and sample mass. Results were expressed as copies g^{−1} sample. Technical replicates were used only to assess analytical repeatability.

2.7. Data Analysis

Alpha-diversity indices, bacterial community composition, and functional gene abundances were evaluated descriptively. One composite sample was analyzed for each soil treatment. No inferential statistical tests were applied to the molecular data.

3. Results and Discussion

3.1. Periphyton Biofilm Development and Growth Characteristics

The cultivation sequence in Figure 1 demonstrates that field-derived periphyton could be successfully maintained and expanded in BG11 medium, providing a stable culture for subsequent interface experiments. SEM observations revealed that the cultivated periphyton formed a heterogeneous three-dimensional biofilm rather than a uniform algal layer (Figure 2). Fibrous structures formed interconnected networks around pores and

microbial aggregates, while filamentous, rod-shaped, and particulate components were embedded within an extracellular matrix-like material. This structural organization is consistent with previous observations of paddy-field biofilms, in which microorganisms and EPSs form closely associated aggregates at the soil–water interface [20,21]. The resulting heterogeneous architecture provides diverse attachment sites and internal microsites that can facilitate the retention and exchange of dissolved and particulate substances. Such extracellular matrices may contribute to As retention through reactive functional groups and binding sites, consistent with previous evidence for enhanced As adsorption and accumulation in biofilms [24]. These observations indicate that the cultivated periphyton constitutes a structurally complex and potentially reactive biofilm interface, rather than a simple surface algal layer, providing a physical and ecological basis for subsequent examination of its development and interactions with the underlying paddy soil.

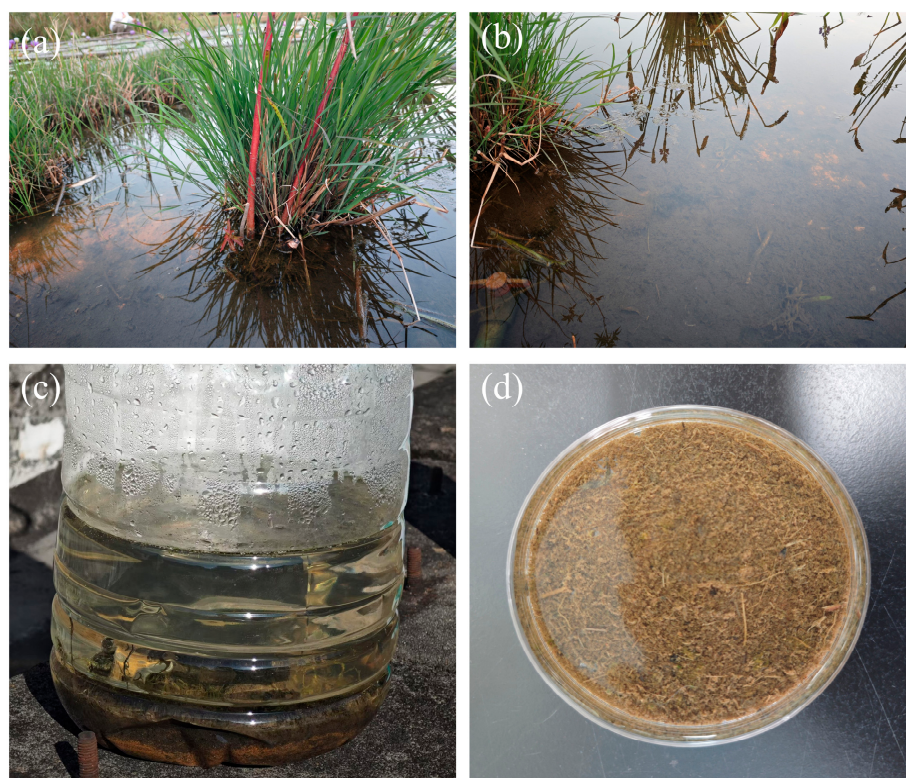


Figure 1. Sampling, cultivation, and preparation of paddy periphyton. (a,b) Paddy field at sampling point; (c) laboratory enrichment culture of field-collected periphyton; and (d) representative periphyton samples used for subsequent experiments.

3.2. Macroscopic Periphyton Development

Macroscopic observations revealed progressive periphyton development over the 14-d incubation period (Figure 3). At day 7, only limited biofilm growth was visible across treatments, whereas by day 14, distinct green biofilm patches had developed under illumination. In contrast, little visible green biofilm developed under light exclusion. The enhanced biofilm development under illumination is consistent with the phototrophic nature of paddy periphyton, which commonly develops at the soil–water interface of flooded paddy fields [19,20]. Collectively, these observations indicate that illumination was associated with visible periphyton development. Accordingly, periphyton developed under illumination was selected for subsequent microscopic, elemental, microbial community, and functional gene analyses.

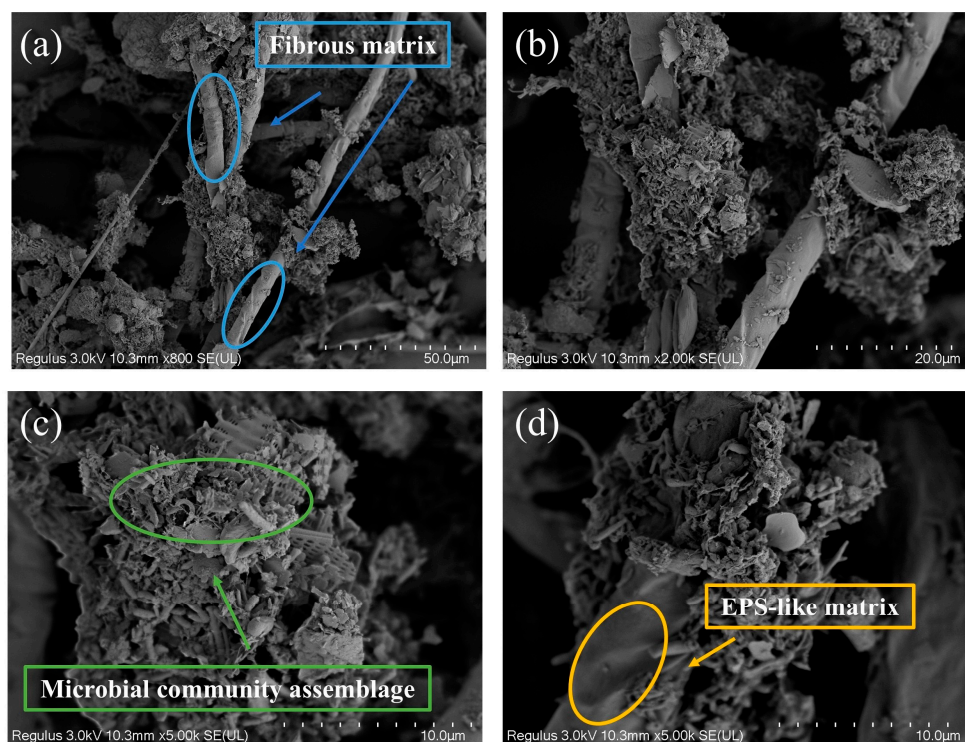


Figure 2. Morphological characteristics of cultivated periphyton. (a,b) Representative SEM images at 800 $\times$ and 3000 $\times$ magnification, respectively; (c,d) representative SEM images at 5000 $\times$ magnification. EPS-like matrix denotes putative extracellular matrix material identified based on SEM morphology.

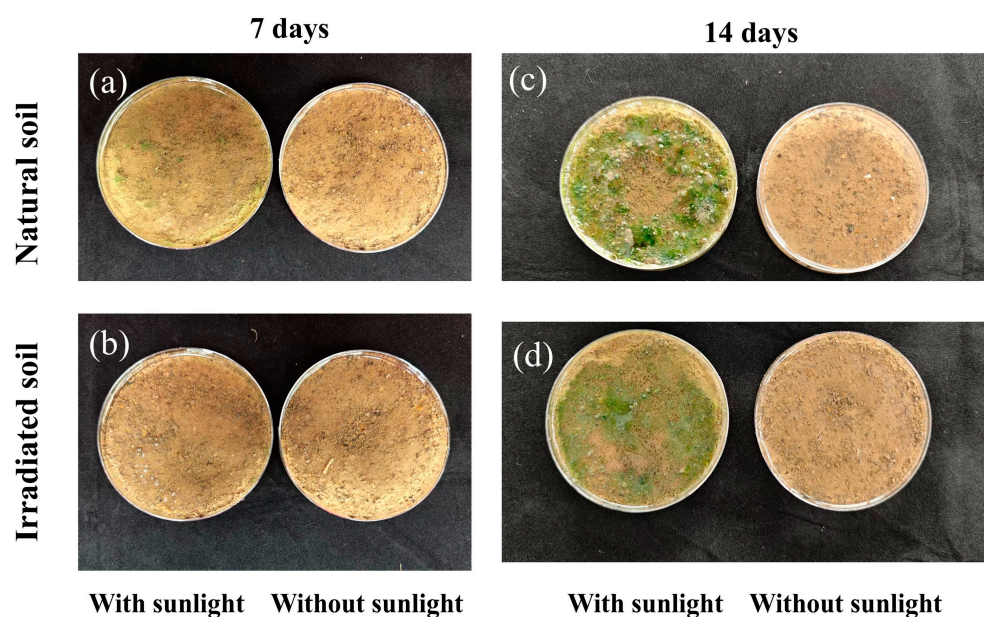


Figure 3. Macroscopic development of periphyton on natural and Co-60 γ -irradiated As-contaminated paddy soils under illuminated and light-excluded conditions. (a,b) Periphyton development after 7 d on natural and Co-60 γ -irradiated soils, respectively; (c,d) periphyton development after 14 d on natural and Co-60 γ -irradiated soils, respectively. In each panel, the left dish was incubated under illumination and the right dish under light exclusion. Each 90-mm Petri dish contained 20 g of soil and 40 mL of ultrapure water. Three replicate dishes were prepared for each treatment, and the total incubation period was 15 d.

3.3. Microstructure and Elemental Composition of Periphyton

Figure 4 further reveals the microstructural organization of periphyton developed on the two soil backgrounds. On natural As-contaminated soil, fibrous structures formed an interconnected network at 1000 $\times$ and 2000 $\times$ magnification, with dense microbial aggregates and matrix-associated materials becoming increasingly evident at higher magnification. Periphyton developed on Co-60 γ -irradiated soil exhibited a broadly similar architecture, characterized by fibrous structures and microbial aggregates, although relatively larger void spaces were observed in some regions. These observations indicate that periphyton formed a heterogeneous three-dimensional matrix comprising microbial aggregates, extracellular matrix materials, and soil-derived particles. At the end of incubation, periphyton-associated As was 1.16 and 2.37 mg kg⁻¹, while dissolved As in the overlying water averaged 5.85 and 2.06 $\mu\text{g L}^{-1}$ in the natural and irradiated-soil systems, respectively (Table S3). The two As pools showed contrasting patterns between the soil backgrounds, with higher periphyton-associated As in the irradiated-soil sample but higher dissolved As in the overlying water of the natural-soil system. Because these measurements were obtained from single composite samples, this pattern is interpreted descriptively. Initial As speciation was not determined, which further limits mechanistic interpretation of these differences.

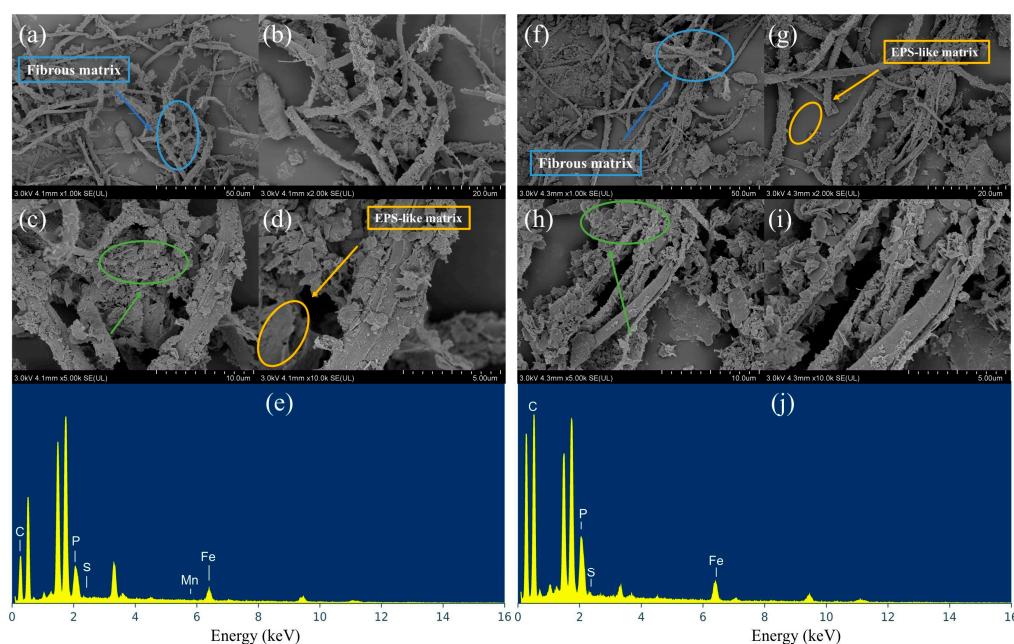


Figure 4. Microscopic morphology and elemental composition of periphyton developed on natural and Co-60 γ -irradiated As-contaminated soils. (a–d) Representative SEM images of periphyton developed on natural As-contaminated soil at 1000 $\times$, 2000 $\times$, 5000 $\times$, and 10,000 $\times$ magnification, respectively; (e) corresponding EDS spectrum. (f–i) Representative SEM images of periphyton developed on Co-60 γ -irradiated As-contaminated soil at 1000 $\times$, 2000 $\times$, 5000 $\times$, and 10,000 $\times$ magnification, respectively; (j) corresponding EDS spectrum.

EDS analysis of selected periphyton regions detected C, P, S, and Fe in both soil backgrounds, while Mn was detected in one selected region of the natural-soil sample. These results indicate heterogeneous local elemental compositions within the periphyton matrix. Semi-quantitative EDS results are provided in Table S2. The values represent local elemental compositions of selected regions rather than bulk concentrations of the periphyton. Fe- and Mn-bearing phases are known to influence As retention and mobilization at paddy soil–water interfaces [36]. Periphytic biofilms have also been reported to facilitate Fe accumulation through interactions with their matrix components [25], while EPSs can

provide abundant binding sites for As adsorption and accumulation within biofilms [24]. Collectively, the observed heterogeneous architecture and elemental composition characterize periphyton as a structurally complex interface containing both microbial and mineral components.

3.4. Bacterial Community Diversity and Composition

Sequencing coverage was high in both periphyton samples, with Good's coverage values above 0.99 (Table 1). The natural-soil composite contained 2615 observed ASVs and had a Chao1 value of 2681.3. The corresponding values for the irradiated-soil composite were 1124 and 1124.35. Shannon diversity was also higher in the natural soil sample. Pielou's evenness showed the same pattern, while Simpson diversity changed little. Overall, the natural-soil composite showed greater bacterial richness and higher community diversity.

The two composite samples showed different phylum-level community profiles (Figure 5). Proteobacteria and Cyanobacteria were the dominant phyla in both communities, but their relative abundances differed between soil backgrounds. In periphyton developed on natural soil, Cyanobacteria accounted for approximately 41% of the community, followed by Proteobacteria at approximately 30%. In the irradiated-soil composite, Proteobacteria accounted for approximately 51% and Cyanobacteria for approximately 37%. Other relatively abundant phyla included Gemmatimonadota, Acidobacteriota, Chloroflexi, Actinobacteriota, and Bacteroidota. The dominance of Proteobacteria and Cyanobacteria is consistent with previous observations of paddy periphyton communities [21,37]. Cyanobacteria represent key phototrophic members of periphytic biofilms and can provide organic carbon and metabolites through photosynthesis, potentially supporting metabolic interactions with heterotrophic microorganisms. Such trophic interactions have recently been implicated in shaping As-methylating populations and methylated As production in paddy periphyton [23]. Therefore, the contrasting community profiles between the two soil backgrounds provide an ecological context for interpreting the differences in As-related functional genes discussed below.

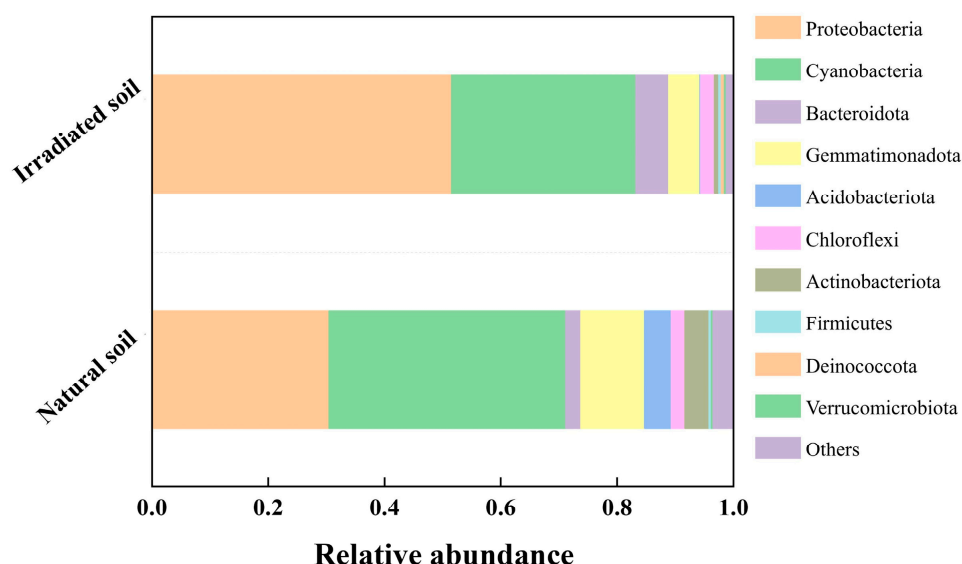


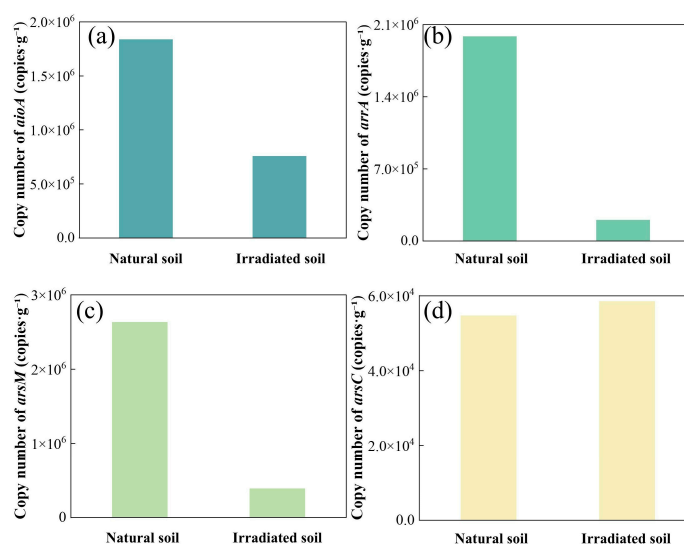
Figure 5. Phylum-level bacterial community composition of composite periphyton samples developed on natural and Co-60 γ -irradiated As-contaminated soils. Relative abundances of the dominant bacterial phyla are shown, with less abundant taxa grouped as Others.

Table 1. Alpha diversity and sequencing coverage of the two composite periphyton samples developed on natural and irradiated As-contaminated soils.

Group	Observed Species	Chao1	Good's Coverage	Simpson	Shannon	Pielou
Natural soil	2615	2681.3	0.9926	0.972	8.63	0.760
Irradiated soil	1124	1124.4	0.9997	0.975	7.22	0.712

3.5. Arsenic-Transforming Functional Potential

Figure 6 shows the copy numbers of four As-related functional genes in the two composite samples. In the natural-soil composite, *arsM* was the most abundant gene (2.63×10^6 copies g^{-1} sample), followed by *arrA* (1.99×10^6 copies g^{-1}) and *aioA* (1.84×10^6 copies g^{-1}). The *arsC* copy number was 5.47×10^4 copies g^{-1} . In the irradiated-soil composite, *aioA*, *arrA*, *arsM*, and *arsC* were 7.57×10^5 , 2.02×10^5 , 3.64×10^5 , and 5.85×10^4 copies g^{-1} sample, respectively. The natural-soil composite showed 2.4-, 9.8-, and 7.2-fold higher *aioA*, *arrA*, and *arsM* copy numbers, whereas *arsC* was similar between the two samples. These values represent descriptive comparisons between the two composite samples.

**Figure 6.** Copy numbers of As-related functional genes in composite periphyton samples developed on natural and Co-60 γ -irradiated soils. Panels show (a) *aioA*, (b) *arrA*, (c) *arsM*, and (d) *arsC*. Gene copy numbers are expressed as copies g^{-1} sample.

The coexistence of *aioA*, *arrA*, *arsC*, and *arsM* suggests that periphyton harbors a broad genetic potential for multiple As transformation pathways. Specifically, *aioA* is associated with As(III) oxidation, *arrA* with respiratory As(V) reduction, *arsC* with intracellular As(V) reduction for detoxification, and *arsM* with As methylation. The higher *arsM* copy number in the natural-soil composite is consistent with previous observations of abundant As methylation genes in paddy periphyton [15,16], while recent evidence indicates that interactions between phototrophic and heterotrophic microorganisms can further shape *arsM*-harboring populations within periphyton [23]. Thus, the contrasting gene patterns were associated with differences between the two soil backgrounds. Together with the community profiles above, these gene patterns provide a descriptive comparison of the two soil backgrounds. The simultaneous occurrence of genes involved in As oxidation, reduction, detoxification, and methylation further highlights the potential for multiple and potentially competing As transformation pathways within the periphytic biofilm. However, the different responses of *aioA*, *arrA*, *arsM*, and *arsC* cannot be mechanistically resolved from

gene abundance alone. Their relative activity and regulation require direct measurements of gene expression and As transformation under defined microenvironmental conditions. These findings provide a functional basis for integrating microbial community structure and As transformation potential into the conceptual model of periphyton-mediated As cycling at the paddy soil–water interface [6].

3.6. Conceptual Microbial Pathways of Arsenic Transformation

Collectively, the morphological, elemental, microbial, and functional gene profiles characterize periphyton as a structurally and microbiologically complex interface at the paddy soil–water boundary. Its heterogeneous biofilm matrix, comprising microbial aggregates and mineral particles, provides reactive surfaces and microsites that may support diverse microbial processes. The coexistence of *aioA*, *arrA*, *arsC*, and *arsM* further indicates the potential for multiple, and potentially opposing, As transformation pathways, including As(III) oxidation, As(V) reduction, cellular detoxification, and methylation (Figure 7). These opposing potentials may be spatially separated within the biofilm. Photosynthetic O₂ production can maintain more oxic conditions near the illuminated surface, whereas respiration and diffusion limitation may create O₂-depleted zones in deeper layers. Such microscale gradients could support the coexistence of *aioA*-associated oxidation and *arrA*-associated respiratory reduction within the same matrix. However, these microenvironmental conditions were not measured directly in this study. Differences in bacterial community composition and functional gene abundance between soil backgrounds further suggest that the surrounding microbial context may shape the transformation potential of periphyton [6,7,16]. Moreover, interactions between phototrophic and heterotrophic microorganisms, supported by carbon fixation and metabolite exchange, may further regulate As-transforming populations and methylated As production [23]. Thus, the observed biofilm structure, microbial community, and As-related functional genes provide a conceptual basis for considering periphyton as a potential site of multiple As-related microbial processes at the paddy soil–water interface. Irradiation may have altered both microbial and physicochemical properties of the soil. Thus, the observed molecular differences cannot be attributed solely to indigenous soil microorganisms.

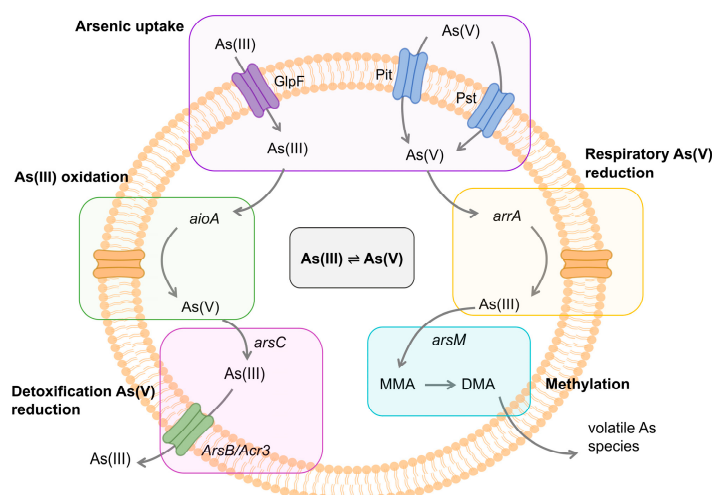


Figure 7. Conceptual model of potential microbial As transformation pathways in periphyton at the paddy soil–water interface. The functional genes *aioA*, *arrA*, *arsC*, and *arsM* represent the potential for As(III) oxidation, respiratory As(V) reduction, intracellular As(V) reduction and As(III) efflux, and As methylation, respectively. As uptake through aquaglyceroporins and phosphate transport systems is also illustrated. The model represents genetic potential only and does not indicate measured gene expression or actual in situ As transformation rates.

4. Conclusions

This study characterizes periphyton as a structurally and microbiologically complex interface at the paddy soil–water boundary. The heterogeneous biofilm matrix, composed of microbial aggregates, extracellular matrix materials, and mineral particles, provides a spatial framework in which microbial and mineral components coexist. The natural-soil composite showed higher bacterial richness and Shannon diversity than the irradiated-soil composite, with 2615 versus 1124 observed ASVs and Shannon indices of 8.63 versus 7.22. The *aioA*, *arrA*, and *arsM* copy numbers were 2.4-, 9.8-, and 7.2-fold higher in the natural-soil composite, whereas *arsC* showed similar copy numbers in the two samples. These molecular patterns are descriptive because one composite sample was analyzed for each soil treatment. The coexistence of *aioA*, *arrA*, *arsC*, and *arsM* indicates the genetic potential for multiple As-related pathways, including oxidation, reduction, detoxification, and methylation. Collectively, these findings characterize periphyton as a microbial interface in which biofilm architecture, community composition, and As-related functional potential coexist. These features provide a basis for incorporating periphyton into conceptual frameworks of As cycling in flooded paddy ecosystems. This study was conducted in controlled microcosms and therefore does not fully capture the spatial and temporal complexity of field paddy systems. Accordingly, the present findings should be extrapolated cautiously to field-scale As mobilization and As accumulation risks in rice. Future studies should quantify in situ transformation rates and gene expression dynamics to resolve how competing As transformation pathways are regulated across spatial and temporal gradients within the periphyton–soil interface. Integrating multi-omics approaches with microscale geochemical measurements and rice uptake experiments will further clarify whether periphyton ultimately enhances or constrains As mobility and transfer to rice under field-relevant conditions.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/w18192394/s1>. Table S1. Primers and qPCR performance for arsenic-related functional genes [32–35]. Table S2. Semi-quantitative elemental composition of selected EDS regions in periphyton developed on natural and Co-60 γ -irradiated soils. Table S3. Arsenic concentrations in the experimental soil, overlying water, and periphyton samples.

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