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Indigenous cyanobacteria enhances remediation of arsenic-contaminated soils by regulating physicochemical properties, microbial community structure and function in soil microenvironment

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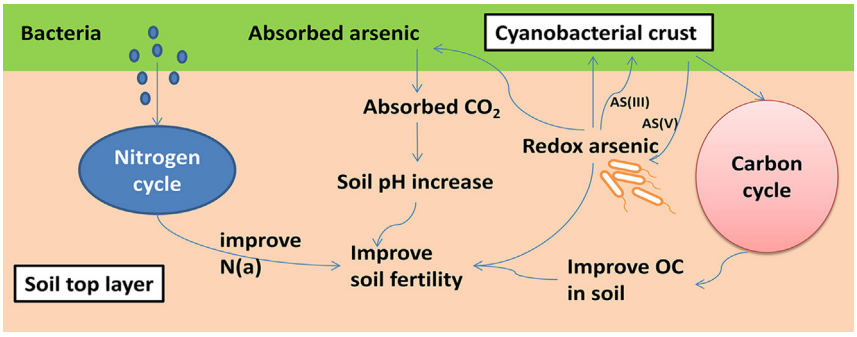
HIGHLIGHTS

- Cyanobacterial crust reduced the bioavailability of As in micro-scale soil layer.
- Cyanobacteria alter biotic and abiotic synergism to immobilize As.
- Non-EDTA-exchangeable As was immobilized on cyanobacterial crust.
- High abundance of *aioA* and *arsM* in mining soil with cyanobacteria inoculation
- Cyanobacteria inoculation improved the fertility of mining soil.

GRAPHICAL ABSTRACT



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ABSTRACT

Biocrust was widely used for the immobilization and removal of arsenic (As) in drainage systems of rice fields and mining areas. In this study, the role of an indigenous cyanobacteria (*Leptolyngbya* sp. XZMQ) was explored in the bioremediation of As-contaminated farmland and tailing soil. After 80 d of inoculation with cyanobacteria, total As (As(T)) accumulated in the cyanobacterial crust of farmland and tailing soil was 279.89 mg kg^{-1} and 269.57 mg kg^{-1}, respectively, and non-EDTA exchangeable fraction was the major fraction of it. The As(T) in farmland and tailing soil of micro-environment decreased by 10.76% and 12.73%, respectively. Meanwhile, the available As (As(a)) decreased by 21.25% and 27.65%, respectively. The XRD results showed that hematite and SiO_2 existed in cyanobacterial crust of farmland and tailing soil. FTIR spectra indicated that the adsorption of As in cyanobacterial crust was mediated by —OH and —CO. After inoculation of *Leptolyngbya* sp. XZMQ, in subcrust soil, As biotransformation gene *aioA* was the most abundant, followed by *arsM*. The dominant phyla of soil biota were Proteobacteria, Cyanobacteria, Actinobacteria, and Bacteroiota, which could play critical roles in shaping *aioA* and *arsM* harboring microbe communities in soil. Redundancy analysis (RDA) showed that soil organic carbon (OC), pH, and chlorophyll *a* (Chl *a*) were the most important environmental factors in altering soil bacterial communities. Correlation analysis showed the *Leptolyngbya* had a positive correlation with Chl *a*, effective nitrogen (N(a)), electrical conductivity (EC), OC, pH in the soil, respectively, while it had a significant negative correlation with As(a), As(III) and As(T). These results emphasized on the significance of cyanobacteria in the behavior of As in mine soils and offered a promising strategy for bioremediation of As-contaminated soil in the mining area.

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1. Introduction

Arsenic (As) as a high toxic carcinogen distributing extensively in the crust could potentially degrade soil quality. Increased mining and smelting activities have contributed to high As accumulation in soil (Francesconi et al., 2002). Low nutrient elements and high heavy metal content in the soil around mine tailings limit the capacity to restore ecosystem biodiversity. In addition, As and heavy metals in mining soil can directly raise the health concerns in surrounding population through rainwater infiltration and surface runoff. Over the past few decades, soil remediation technologies, including biological, chemical and physical remediation methods, for tailing areas have gained increasing attention (Sun et al., 2018). Bioremediation (e.g., phytoremediation and microbial remediation) is an economical, environment-friendly, and sustainable approach for ecosystem reclamation (Megharaj and Naidu, 2017). Phytoremediation potential is limited due to infertile soil and multiple metal pollutants in the mine tailings, while microbes can grow in a variety of environments and effectively improve soil properties, stabilize and degrade pollutants (Jin et al., 2018). Therefore, microbial remediation can be used for the restoration of mine areas.

Biological soil crusts (BSCs), composed of bacteria, algae, fungi, moss and soil particles (Chamizo et al., 2012), can influence soil characteristics, nutrients and hydrological processes. BSCs is one novel microbial remediation method. As ecosystem engineers in arid and semi-arid region (Rossi et al., 2017), BSCs primarily contain cyanobacteria that are essential in maintaining soil stability and fixing carbon and nitrogen. Some of these can also affect metal bioavailability in soil (Naveed et al., 2018). Cyanobacteria as photoautotrophic organisms are the earliest pioneer colonizers and capable of surviving in soils with extreme drought, pH, salinity, heat, cold (Mccutcheon et al., 2020), and heavy metal stress (Cabala et al., 2011). They can colonize rapidly and spread widely on soil substrates to form BSCs and further affect the physicochemical characteristics of the surface soil (Chamizo et al., 2012). Different from environmental factors, cyanobacteria, as the main participant of BSCs, may regulate the structure and function of BSCs. Therefore, they may participate in pollution remediation. Cyanobacteria can secrete extracellular polymers (EPS), which can react with Cd(II), Pb(II), and Cr(VI) via its functional groups to reduce the bioavailability of heavy metals (Cui et al., 2021). Cyanobacteria can combine heavy metals with metallothioneins (MTs) and phytochelatins (PCs) by intracellular absorption to reduce the toxicity of heavy metals (Turner and Robinson, 1995). In addition, filamentous cyanobacteria are able to entangle soil particles (Fattahi et al., 2020), which may prevent the further decomposition and release of mineral particles containing pollutants. Filamentous cyanobacteria can penetrate into minerals (Hu et al., 2003), making it difficult to release pollutants accumulated by algae, thus playing a role in the immobilization and storage of pollutants by minerals. In addition, cyanobacteria can fix nitrogen from the atmosphere and convert it into bioavailable forms through the action of nitrogenase (Qiu et al., 2022). Cyanobacteria are continuously decomposed by microorganisms such as secretions and residues to form humus (Yao et al., 2022), which may effectively promote the accumulation of soil organic matter and soil nutrients. Cyanobacteria are capable of stimulating the growth of other microorganisms (Acea et al., 2003). In addition, cyanobacteria can oxidize, reduce, enrich, and methylate As (Hussain et al., 2021), hence, reducing As toxicity up to a certain extent. Some cyanobacteria have high As bioaccumulation potential, which can be used as a tool for As bioremediation (Mitra et al., 2017). Algae can detoxify As by both extracellular enzymes and intracellular metabolism. As(V) crosses the plasma membrane via phosphate transporters, while As(III) enters algal cells through hexose infiltration and AQP (aquaglyceroporins) channels. As(V) is reduced to As(III) by *arsC* in algal cells, and then transformed into organic form of methyl As by *arsM* (Qin et al., 2009). Besides, As(III) can be oxidized to As(V) through extracellular carbonic anhydrase and extracellular phosphatase (Qin et al., 2009). Therefore, algae can reduce As toxicity in the environment through As metabolism. Although increasing attention has been paid on cyanobacteria, as a necessary tool for ecological restoration of degraded soils (Rocha et al., 2020), its application in soil restoration in As-contaminated mining areas still needs to be explored. The application of the cyanobacteria to construct the As contaminated soil bio-crust may stabilize As and improve soil fertility for plant growth.

Accordingly, this study aims to: (i) investigate the mobility and transformation of As in different substrates soil (mine tailing and farmland) in the presence of cyanobacterial crust, (ii) analyze the changes of crust biomass and physicochemical properties in different textured soils after cyanobacteria inoculation, and (iii) explore the effects of cyanobacteria inoculation on microbial community composition and As metabolism gene abundance in different matrix tailing soil for explaining the As bioremediation potential of *Leptolyngbya* sp. XZMQ. This work provides meaningful insights into the role of cyanobacteria in biogeochemical cycle and bioremediation of As especially in mining areas.

2. Materials and methods

2.1. Cyanobacteria culturing

The cyanobacteria strain, *Leptolyngbya* sp. XZMQ (Fig. S1) used in this study, was isolated from BSCs of Shimen realgar mine, China. *Leptolyngbya* sp. XZMQ was cultivated to obtain enough biomass in BG11 medium with constant sterile air at 26 °C under light: dark cycles (14: 10 h) at 4000 Lx. After the culture was filtered, cyanobacteria was washed twice with distilled water, and then resuspended in distilled water to obtain the inoculating concentration. The biomass was determined by dry weight after filtering and keeping in an oven at 80 °C for 24 h (Lam and Lee, 2012).

2.2. Experimental mesocosms

The experimental soil was collected from the 0–10 cm depth in farmland (29°40′7″N, 111°3′13″E) and tailing (29°38′11″N, 111°2′26″E) soil around the Shimen realgar mine, in Hunan Province. The physicochemical characteristics of the farmland and tailing soil in Table S1. The soil was naturally dried at room temperature and ground through a 2 mm sieve. The soil was divided into sterilized soil and non-sterilized soil. The sterilized soil was sterilized twice at 120 °C for an hour each time (Li et al., 2011). All cylindrical plexiglass microcosms (Φ150 mm, height 100 mm) contained 1.00 kg homogenized soil. The soil bulk density was 1.03 $g{\cdot}cm^{-3}$ and deionized water was added with 70% water holding capacity at constant temperature incubator aging 30 d backup. Treatments were set up as follows: (i) the non-sterilized control group consists of farmland soil (CK-N) and tailing soil (CK-Z) that represented the blank group, (ii) the non-sterilized treatment group consists of farmland soil (N) and tailing soil (Z) with cyanobacteria, (iii) the sterilized control group consists of sterilized farmland soil and tailing soil, named S-CK-N and S-CK-Z, respectively and (iv) the sterilized treatment group consists of sterilized farmland soil (S-N) and tailing soil (S-Z) with cyanobacteria. These treatments are summarized in Table S2. Each treatment was arranged in a completely random order of each microcosm, experiment was conducted in triplicates, as a whole, 24 experimental units were established, with 8 treatment groups. The inoculum was uniformly inoculated on the top of soil substrates with 80 g wet weight m^{-2} (about 6 g dry weight m^{-2}). Briefly, the algal cells collected by centrifugation were re-suspended in sterile water after cleaning with sterile water twice, and then the same volume of algal suspension were uniformly inoculated on the surface soil. After inoculation, all miniatures were incubated at 25 ± 1 °C for 80 d in a dark:light cycle of 10 h:14 h in the greenhouse. The equivalent amount of sterile deionized water was used in the control soil groups. The amount and frequency of water added to the soil was based on the average annual rainfall (1450 mm) of the study site while considering the duration of the experiments for 80 d (Du et al., 2013). The soil moisture was maintained by using a precise balance to weight and was supplemented with water every 3 d. In order to maintain the cyanobacterial growth, 3 mL of BG-11 medium was added to the inoculation groups, weekly.

2.3. Characterization and analysis of cyanobacterial crust

The crust biomass of *Leptolyngbya* sp. XZMQ, which was expressed by measuring the content of chlorophyll *a* (Chl *a*) (Rao et al., 2009). The Chl *a* of cyanobacterial crust was calculated based according to a previously defined method of Castle et al. (2011), using double ethanol extraction technique measuring absorbance at 665 nm by a UV–vis spectrophotometer (UV-1800PC, Shanghai Mapada Instruments Co., Ltd., China). Morphological features of cyanobacterial crust were observed by a scanning electron microscope (SEM, SU8010, Hitachi, Japan), after 80 d of cultivation. For this purpose, cyanobacterial crust was obtained per unit area, dehydrated using an alcohol gradient, freeze-dried for 48 h, and then tested with gold spraying. In addition, after the cyanobacterial crust was powdered, the crystal structures and compositions of cyanobacterial crust were analyzed by X-ray diffraction (XRD) (Bruker D8 Advance, Germany), which scanned between 5° and 90° 2θ at a step rate of 2.5° 2θ min^{-1}. The composition of functional groups in cyanobacterial crust was determined by FT-IR spectrometer (Nicolet iS50, Thermo Fisher Scientific, USA) at room temperature. The infrared spectra were recorded over the wavenumber ranging from 400 to 4000 cm^{-1}. X-ray photoelectron spectroscopy (XPS) (ESCALAB 250, Thermo Fisher Scientific) was used to analyze the elemental composition of cyanobacterial crust. The CasaXPS software was used to deconvolve XPS spectra using C 1s at a binding energy of 284.8 eV, as the energy standard for charge correction (version 2.3.13).

2.4. Sample analysis

For soil As speciation, 1 M ortho-phosphoric acid (H_3PO_4) and 0.5 M ascorbic acid ($C_6H_8O_6$) were used as extractants according to the previous study (Giral et al., 2010). The sequential extraction method was used to extract different combined forms of As in soils, and ammonium dihydrogen phosphate ($NH_4H_2PO_4$, 0.05 M) was used to extract bioavailable As (As(a)) in soils (Wenzel et al., 2001; Khan et al., 2010). To determine the total As (As(T)) in soils and cyanobacterial crust, 0.1 g of soil and cyanobacterial crust samples were processed under microwave digestion with HNO_3, HCL, and HF mixtures (volume ratio = 6:3:1). Hydride generation atomic fluorescence spectrometer (HG-AFS; AFS-830, Beijing Jitian Instrument Co., Ltd., China) was used to determine the concentrations of As(III), As (V), and As(T).

To acquire the fine earth fraction, soil samples were air-dried and sieved to 2 mm. Aliquots of these samples were taken and mashed in an agate mortar to obtain the 0.5 mm particle size necessary for the determination of organic C (OC). Electrical conductivity (EC) and pH were measured by an electrical conductivity meter and pH meter in a 1:5 soil-water suspension (Zhan and Sun, 2012). The H_2SO_4-$K_2Cr_2O_7$ oxidation method (Hu et al., 2015) was used to measure OC. The soil effective nitrogen (N(a)) was determined according to the method described by Gao et al. (2014).

2.5. DNA extraction and Illumina Miseq sequencing

At the end of the incubation, DNA was extracted from soil samples (0.5 g fresh weight) using the DNeasy PowerSoil Pro Kit for Soil (M.P. Biomedicals, USA) according to the manufacturer's instructions. After checking the quality and concentrations of extracted DNA by agarose gel electrophoresis, DNA amplification was performed by polymerase chain reaction (PCR). The universal primers 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) were used to amplify the V3–V4 region of the 16 S rRNA gene (Xu et al., 2016). After that, the obtained products were prepared for high-throughput sequencing via an Illumina MiSeq platform (Illumina, San Diego, CA, USA) at Majorbio BioPharm Technology Co., Ltd. (Shanghai, China).

2.6. Real-time PCR detection of 16S rDNA and As metabolism genes

As metabolism genes in soil mainly included As(III) oxidase gene (*aioA*), As(V) detoxification reductase gene (*arsC*), As(V) respiratory reductase gene (*arrA*), and As(III) methyltransferase gene (*arsM*). In order to further obtain the abundance of As metabolism genes, *aioA*, *arrA*, *arsC*, and *arsM*, 16S rRNA gene amplification was performed by PCR using primers aioA-F2/aioA-R2, AS1F/AS1R, amlt-42F/amlt-376R, arsMF1/arsMR2 and 1369F/1492R, respectively (Table S3). The standard curve was generated for each gene for the absolute quantification of *aioA*, *arrA*, *arsC*, and *arsM* gene and 16S rDNA gene (details of the procedures provided in supplementary material). The amplification efficiency was ranged from 90.04% to 93.88% (Table S4), and the standard curves' correlation coefficients (R^2) were >0.99.

2.7. Statistical analyzes

The data of As content between different treatments were processed by SPSS version 20.0 (SPSS, Inc., Chicago, IL, United States), and statistical analysis was performed using Independent-Samples T Test. A one-way analysis of variation (ANOVA) were used to assess the significant difference among different stages of treatment. $P < 0.05$ was considered statistically significant. The redundancy analysis (RDA) was performed to explore the relationships between bacterial community structure and soil environmental parameters using R with the vegan package. Pearson's correlation analysis by SPSS version 20.0 was also performed to determine the links between the bacterial community and soil environmental parameters. All data were presented as the mean ± standard error (n = 3).

3. Results

3.1. Characteristics and composition of cyanobacterial crust

According to SEM, the filamentous cyanobacteria were the main organisms in the crust (Fig. 1a), tangled with clay particles and mineral particles (Fig. 1b and d). Therefore, it was identified as cyanobacterial crust. The SEM images revealed that the morphology of cyanobacterial crust was uneven, rough and porous. In addition, a large number of EPS was found in the cyanobacterial crust (Fig. 1c). The cyanobacterial crust contained a range of mineral particles, including quartz, illite, albite, hematite and chlorite (Fig. S2). SiO_2 was found to be the dominant crystalline mineral in the cyanobacterial crust followed by illite, pyrite, chlorite and albite. Hematite existed in the cyanobacterial crust (12.69–14.69 wt%), as a prominent mineral (Table S5). Moreover, XPS results indicated that the Fe 2p spectra was divided into two peaks at binding energies of 709.88 eV and 711.55 eV

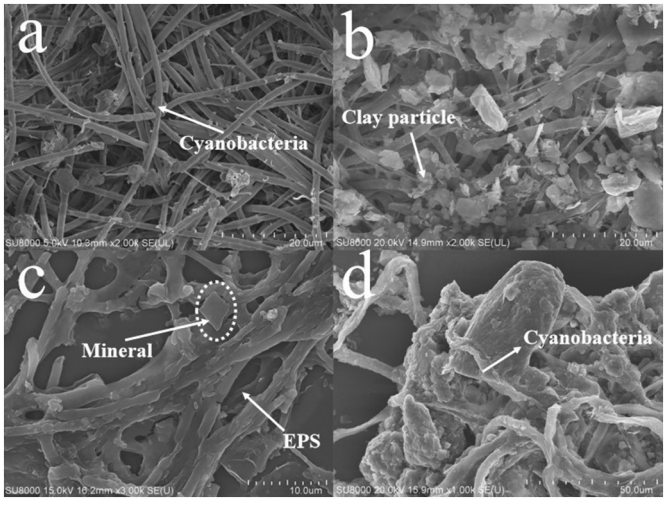

Fig. 1. SEM images of morphological characteristics and composition of cyanobacterial crust. (a, b) cyanobacteria and clay particles in cyanobacterial crust; (c) EPS and mineral particles in cyanobacterial crust; (d) the entanglement of filamentous cyanobacteria on soil mineral particles in cyanobacterial crust.

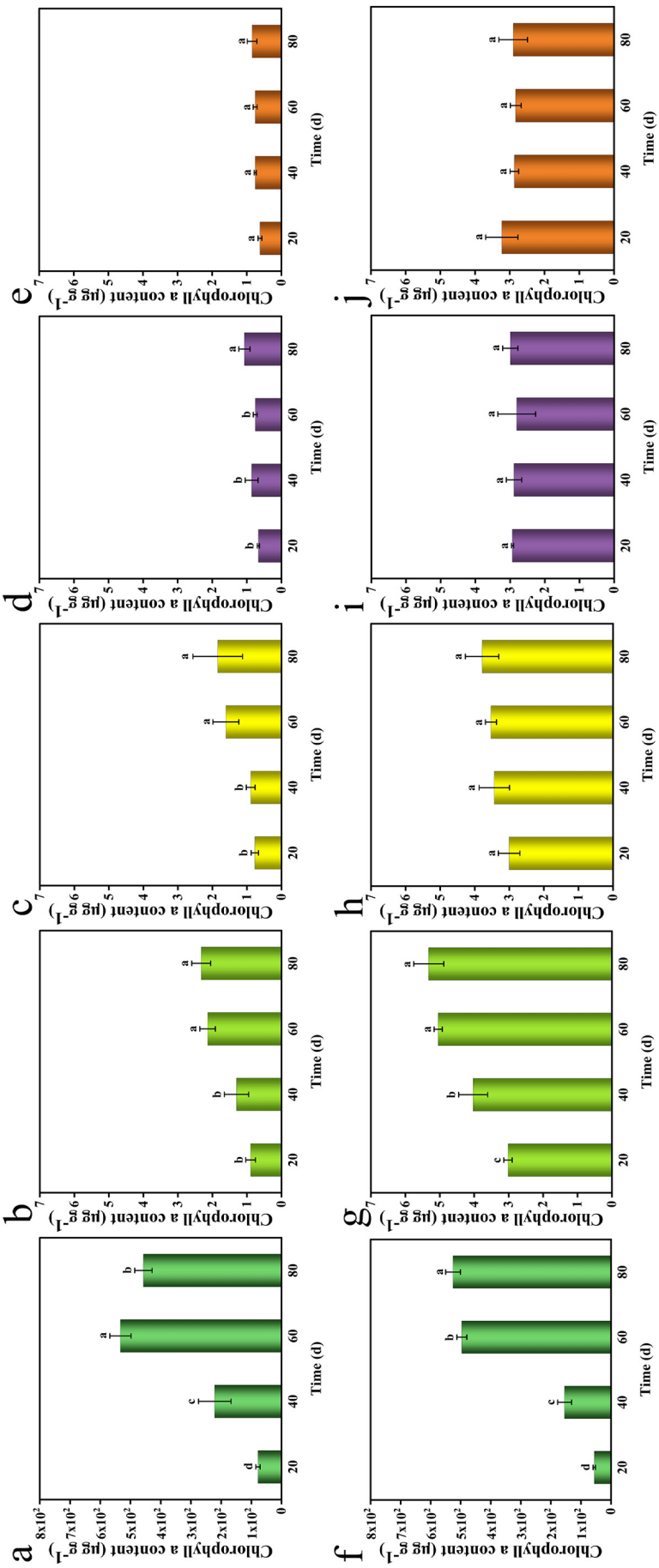

Fig. 2. Chl *a* content in cyanobacterial crust and soil at different depths in farmland soil and tailing soil inoculated with *Leptolyngbya* sp. XZMQ (a, f is content of Chl *a* in cyanobacterial crust of farmland soil and tailing soil, respectively; b, c, d, e is the Chl *a* content of farmland soil at 0–0.5, 0.5–1.5, 1.5–3.5, 3.5–5.5 cm depth, respectively; g, h, i, j is the Chl *a* content of tailing soil at 0–0.5, 0.5–1.5, 1.5–3.5, 3.5–5.5 cm depth, respectively). Error bars show mean ± standard deviation. Different letters above bars indicate significant differences at $P < 0.05$.

(Figs. S3–S4), which represented the Fe_2O_3 and FeOOH (Biesinger et al., 2011). Accordingly, the percentages of Fe_2O_3 and FeOOH in the cyanobacterial crust of farmland soil were 39.91% and 60.09%, respectively. Likewise, for the cyanobacterial crust of tailing soil, the percentages of Fe_2O_3 and FeOOH were 41.32% and 58.68%, respectively. These results indicated that iron oxides and FeOOH existed in the cyanobacterial crust, which could coprecipitate with As by the cyanobacterial crust. In addition, Fig. S5 showed the FTIR spectra of cyanobacterial crust of farmland soil and tailing soil. These results also suggested that functional groups such as —OH and —CO participated in the adsorption of As by cyanobacterial crust.

3.2. Growth of cyanobacteria in crust and soil

The Chl *a* content was calculated to assess the algal biomass during the 80 d of culture and was shown in Fig. 2. The Chl *a* content of cyanobacterial crust showed an increasing trend before 60 d of cultivation, and the highest content of cyanobacterial crust in farmland and tailing soil were 533.28 and 525.40 μg g^{-1}, after 80 d of cultivation, respectively (Fig. 2a, f). In addition, compared with tailing soil, the cyanobacterial crust of farmland soil grew faster in the first 40 d. Nevertheless, the tailing soil grew faster from 40 to 60 d and its growth was reduced after 60 d. The content of Chl *a* showed a gradual decrease with the increase of soil depth. After 80 d of culture, the Chl *a* content in 0–0.5 cm, 0.5–1.5 cm, 1.5–3.5 cm and 3.5–5.5 cm depth of farmland soil were 2.33, 1.85, 1.07, 0.85 μg g^{-1}, respectively (Fig. 2b–e). However, the content in tailing soil ranged between 5.32 and 2.90 μg g^{-1} (Fig. 2g–j). In addition, the Chl *a* content in soil under cyanobacterial crust was significantly lower than that of cyanobacterial crust, and the changes trend of Chl *a* was consistent.

3.3. Effects of cyanobacterial crust on physicochemical properties of soil with different substrates

3.3.1. Effects of cyanobacterial crust on As fractions and species in soil

The content of As(T) and bound As in the groups of S-CK-N and S-N at different depths are shown in Fig. 3. After 80 d of inoculation, compared with the S-CK-N, the As(T) decreased most obviously in the S-N at 0–0.5 cm soil depth under cyanobacterial crust ($P < 0.05$) (Fig. 3a). The content of amorphous iron aluminum oxide bound As (F3) in 0–0.5 cm soil layer was decreased by 11.39% in the S-N. Moreover, the contents of non-specifically bound As (F1) and specifically bound As (F2) also decreased by 72.16% and 25.40%, respectively. However, the binding of the crystalline iron/aluminum oxides and residual As did not significantly change over the experiment. Besides, under the cyanobacterial crust, the changes of total and bound As in 0.5–1.5 cm, 1.5–3.5 cm, and 3.5–5.5 cm soil layer were not obvious ($P > 0.5$) (Fig. 3b–d).

Similarly, Fig. 3e–h shows the changes in the binding forms of As in the groups of S-CK-Z and S-Z at different depths. The main binding forms of As in the S-Z group at 0–0.5 cm soil depth was amorphous iron aluminum oxide bound As, which decreased by 9.36%, compared with the group of S-CK-Z (Fig. 3e). Furthermore, the contents of non-specifically bound As (F1) and specifically bound As (F2) also decreased by 56.40% and 22.83%, respectively. These results indicated that As in soil under cyanobacterial crust changed from amorphous bound As to bioavailable and adsorbed As. Nevertheless, there was no significant change in As (T) ($P > 0.5$) in the 0.5–5.5 cm soil layer under the crust, indicating that cyanobacteria had limited effect on As in deep soil (Fig. 3f–h).

Fig. 4 shows the amounts of As(a), As(V) and As(III) in farmland soil and tailing soil under cyanobacterial crust after 80 d of incubation at 0–0.5 cm depth. Cyanobacteria inoculation exhibited the obvious effect on reducing the As(a) in the soil, which decreased significantly ($P < 0.05$) by 21.25% compared to in the S-CK-N. Furthermore, the As(a) in the S-Z decreased by 27.65% after 80 d compared to the S-CK-Z. These results indicated that cyanobacteria inoculation to the As-contaminated soil significantly reduced As(a) in soil at 0–0.5 cm depth under the cyanobacterial crust.

3.3.2. Effects of cyanobacterial crust for organic C, available N, pH, and EC in the soil

The inoculation of *Leptolyngbya* sp. XZMQ significantly affected the changes of OC, N(a), pH and EC in soil layers 0–0.5 cm (Fig. 5). For farmland soil, the OC and N(a) contents could reach up to 30.09 g kg^{-1} and 268.25 mg kg^{-1}, after 80 d of culture, respectively (Fig. 5a, c). Nevertheless, for tailing soil, the contents of OC and N(a) were 43.64 g kg^{-1} and 253.25 mg kg^{-1} (Fig. 5b, d). The OC and N(a) contents were increased with incubation time in sub-crust soil, while they were stable in deeper soil layers. It indicated that with the cyanobacterial crust developed, the contents of carbon and nitrogen in soil under the cyanobacterial crust were higher than in deeper soil layers. Fig. 5e, f shows changes in pH of the treatments and controls in each soil layer during the experiment. The pH of farmland soil increased 0.84 in 0–0.5 cm soil layer, and tailing soil increased 1.08. Likewise, the EC values of farmland soil and tailing soil increased 0.98, 0.55, respectively, but the values in deeper layers were not significantly changed ($p > 0.05$) (Fig. 5g, h). These results showed that cyanobacterial crust could regulate soil physicochemical properties, but their depth of action was limited.

3.4. As distribution and speciation in cyanobacterial crust

The distribution of As in cyanobacterial crust involves EPS-bound, sorbed and non-EDTA-exchangeable fraction, reflecting various biotic and abiotic As immobilization mechanisms. As shown in Fig. 6, the sorbed fraction, EPS-bound fraction, and non-EDTA exchangeable fraction in the cyanobacterial crust of farmland soil, accounted for 13.65%, 25.13%, and 61.22% for As after 40 d, respectively. Moreover, in the cyanobacterial crust of tailing soil, the EPS-bound fraction was 7.59%. Remarkably, 80.86% of As was found in the non-EDTA-exchangeable fraction in the cyanobacterial crust, while 11.55% of As was found in the sorbed fraction. After 80 d of culture, the highest content of As was found to be as high as 279.89 mg kg^{-1} and 269.57 mg kg^{-1} in the cyanobacterial crust of farmland soil and tailing soil, respectively. SEM morphology image and EDS spectrum of cyanobacterial crust are shown in Fig. 7 and Fig. S6. EDS analysis results showed that the main elements on cyanobacterial crust surface were non-metallic elements (e.g., Si, C, O, S), followed by the metal(loid)s (e.g., Al, Fe, Ca, Mn, As). In the cyanobacterial crust of S-N, the weight percentages of Al, Fe, Ca, Mn and As are 2.09, 1.48, 0.28, 0.30 and 0.64, respectively (Fig. S6a). However, in the cyanobacterial crust of S-Z, the weight percentages are 1.76, 1.32, 0.10, 0.08 and 0.86, respectively (Fig. S6b). The color of C and O was deep and concentrated, which represented the high EPS or cyanobacteria in the region. In addition, cyanobacteria pass through the soil gap and form soil aggregates with soil particles and mineral particles. Through SEM-EDS, it can be clearly seen that the distribution of As on the surface of cyanobacterial crust is attached with Al, Si, Fe, Ca and Mn elements. These results indicated that non-biological co-precipitation and adsorption participated in immobilization of As.

3.5. Effects of cyanobacterial inoculation in different soil substrates bacterial richness and diversity

At the phylum level, in the N group was mainly composed of Proteobacteria with an average relative abundance of 35.72%, followed by Cyanobacteria (32.10%) and Actinobacteria (17.33%). Similarly, Proteobacteria, Cyanobacteria, and Actinobacteria were the main prokaryotic communities in the Z group, having an average relative abundance of 29.00%, 34.01% and 12.16%, respectively (Fig. 8a). However, the average abundance of cyanobacteria in CK-N and CK-Z groups were just 14.82% and 11.63%, respectively. At the class level, the Cyanobacteria (20.10%), Alphaproteobacteria (22.07%), Actinobacteria (15.63%), Gammaproteobacteria (10.20%) and Bacteroides (4.06%) were the highly abundant in N treatment (Fig. 8b). The tailing soil's biota contained Cyanobacteriia (34.01%), Alphaproteobacteria (20.00%), Actinobacteria (10.04%) and Gammaproteobacteria (8.99%). Among

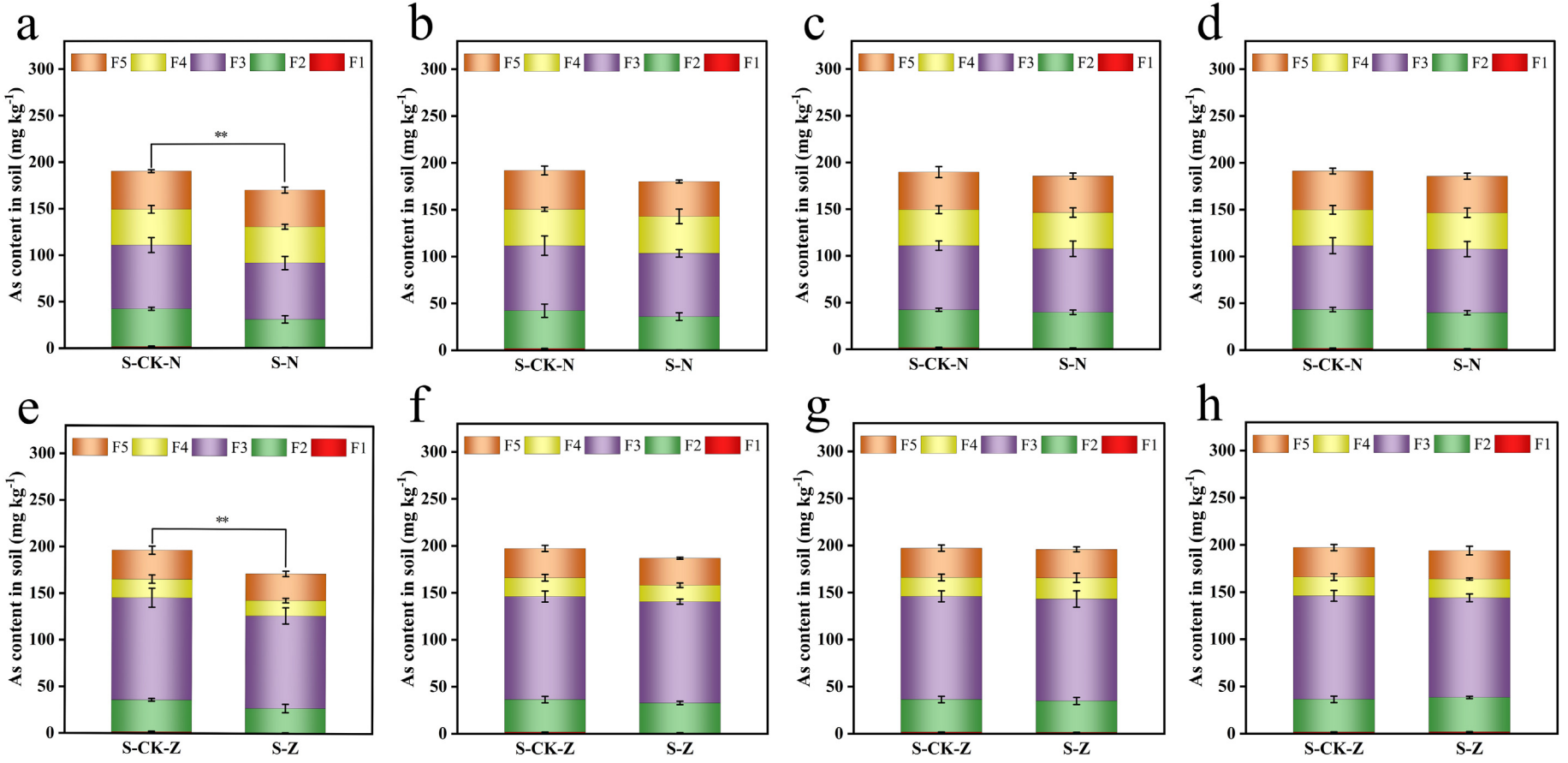

Fig. 3. As content in soil layers of different depths in the groups of S-CK-N, S-N, S-CK-Z, and S-Z after 80 d of inoculation (a, b, c, d is the As content of farmland soil at 0–0.5, 0.5–1.5, 1.5–3.5, 3.5–5.5 cm depth, respectively; e, f, g, h is the As content of tailing soil at 0–0.5, 0.5–1.5, 1.5–3.5, 3.5–5.5 cm depth, respectively; F1 non-specific adsorption state, F2 specific adsorption state, F3 amorphous iron aluminum oxide bound arsenic, F4 crystalline iron aluminum oxide bound arsenic, F5 residual state). * indicates significant difference at 0.05 in As(T) between the treatment groups (with cyanobacteria) and the control groups (without cyanobacteria). As(T) is the sum of the As content of F1, F2, F3, F4 and F5.

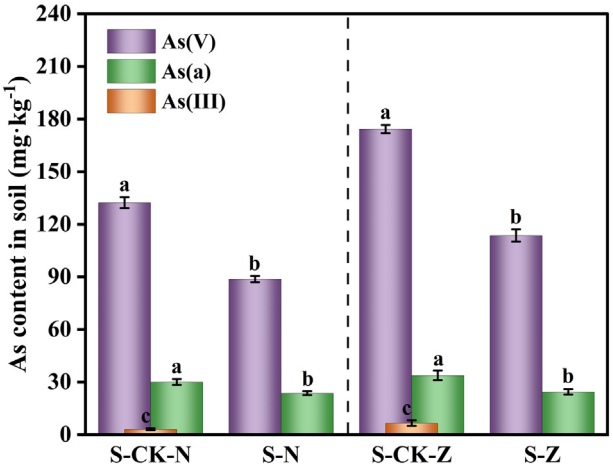

Fig. 4. Content of As(III), As(V), As(a) in soil after inoculation of *Leptolyngbya* sp. XZMQ 80 d at 0–0.5 cm depth in farmland soil and tailing soil. Different letters indicate significant differences between treatments. The results with $P < 0.05$ were considered significant.

these genera, *Leptolyngbya* (20.75%), *Micromonospora* (10.14%), *Chloroplast* (8.03%), *Actinophytocola* (4.95%), *Nordella* (4.77%), *Microcoleus* (4.33%), and *Steroidobacter* (4.07%) were the dominant in N treatment, whereas *Leptolyngbya* (17.50%), *Chloroplast* (14.58%), *Actinophytocola* (7.08%), *Tumebacillus* (6.42%), *Nordella* (4.66%), *Microcoleus* (3.26%), and *Niastella* (2.98%) relatively dominated in the Z treatment (Fig. 8c). After inoculation with cyanobacteria, the average abundance of *Leptolyngbya* in the N and Z groups increased by 12.85% and 8.48%, respectively, compared with their control groups. However, the relative average abundances of *Micromonospora* and *Tumebacillus* decreased slightly by 2% and 3%, respectively.

3.6. Effects of cyanobacterial inoculation on the abundance and diversity of As metabolism genes in different soil substrates

After cyanobacteria inoculation, *aioA* and *arsM* were the main As metabolism genes in N and Z treatments (Fig. 9). In the N treatment, the average relative abundance of *aioA* and *arsM* were 2.99×10^{-3} and 5.73×10^{-4}, respectively. Secondly, the average relative abundance of *arsC* was 6.7×10^{-7}, and the minimum *arrA* was 2.83×10^{-7}. Similar trends were also observed in Z treatment. The average relative abundances of *aioA*, *arsM*, *arsC* and *arrA* were 7.08×10^{-3}, 1.43×10^{-3}, 1.29×10^{-6}, 2.25×10^{-6}, respectively. In addition, the results also revealed that the average relative abundance of As metabolism genes in the Z treatment was higher than that in the N treatment.

3.7. Effects of cyanobacterial inoculation on the relationships of soil physicochemical characteristics and microbial communities

RDA ordination diagram of bacterial community and soil parameters are shown in Fig. 10. Our results showed that OC, Chl *a* and pH, were the most essential factors regulating the soil bacterial community composition. Axis 1 and 2 could explain 74.1% and 3.8%, 78.8% and 4.1%, 70.1% and 6.4% of the total variation of bacterial phylum, class and genus, respectively. As indicated by the RDA analysis of relative abundance at genus level, the As(a) ($r^2 = 0.9132$, $p = 0.001$), As(III) ($r^2 = 0.8693$, $p = 0.001$), OC ($r^2 = 0.9529$, $p = 0.001$), pH ($r^2 = 0.9115$, $p = 0.001$), N (a) ($r^2 = 0.9498$, $p = 0.001$), EC ($r^2 = 0.9578$, $p = 0.001$), Chl *a* ($r^2 = 0.9829$, $p = 0.002$) and As(T) ($r^2 = 0.9214$, $p = 0.002$) significantly correlated with the composition of the bacterial communities (Fig. 10c). Pearson correlation analysis revealed that *Leptolyngbya*, *Luteimonas*, and *Chloroplast* had a significantly positive correlation with Chl *a*, OC, N(a), and EC ($P < 0.05$), while they were significantly negatively correlated with As(a), As(III), and As(T) ($P < 0.01$) (Table S6(c)).

4. Discussion

4.1. Characterization of cyanobacterial crust

Characteristics of biological soil crust are vulnerable to be influenced by the environmental conditions and components (Ngosong et al., 2020). In this study, the characteristic of cyanobacterial crust was uneven and had gaps, which could make it to have a larger specific surface area and provide convenience for capturing soluble As in soil. Because high specific surface area of cyanobacterial crust had the potential to provide more adsorption sites for As. Similar benefits of biocrust have also been observed in the care of mine drainage, as porous surface of the biocrust can act as habitat for microorganisms such as oxygen-producing cyanobacteria and diatoms that may bio-accumulate metal(loid) (Wang et al., 2021). Thus, it can adsorb soluble As and has a positive role in reducing soluble As in soil once formed this characteristic cyanobacterial crust on the topsoil. Additionally, the most obvious characteristic of cyanobacterial crust is the presence of a large number of filamentous cyanobacteria (Fig. 1), which can have the ability to oxidize As(III) and accumulate As to reduce the toxicity and content of As in soil (Mao et al., 2022; Zhu et al., 2020). Moreover, another characteristic is the presence of EPS in cyanobacterial crust (Fig. 1c), which can not only adsorb metal ions through complexation and electrostatic interactions but also adsorb As (Naveed et al., 2019). Furthermore, some As-tolerant bacteria (e.g., Proteobacteria and Bacteroidetes) have been found in soil under cyanobacterial crust, which are involved in the transformation and accumulation of As by cyanobacterial crust (Yan et al., 2021).

However, the cyanobacterial crust not only included the presence of organisms but also other non-biological forms. Among of them, soil clay particles and mineral particles were wrapped or adsorbed in the network of the cyanobacterial crust (Fig. S2 and Table S5), which might increase the roughness of cyanobacterial crust surface and provide more adsorption sites (Cheng et al., 2022). In addition, metal oxides, hydroxides, and clay minerals can adsorb various As species (Xie and Cheng, 2021), and their presence in cyanobacterial crust facilitates the adsorption of more As, thereby reducing the content of As in the environment. As a whole, cyanobacterial crust has all the attributes that are required to support As bioremediation.

4.2. Influence of cyanobacterial crust on physical and chemical properties of soil

Soil cyanobacteria as an important part of soil microorganisms and could induce the rapid formation of biological crust and directly or indirectly affect the changes in soil physical and chemical properties (Kheirfam and Roohi, 2020; Nisha et al., 2007).

In our study, a large number of cyanobacteria survived in the soil under the cyanobacterial crust (Fig. 2). These cyanobacteria could fill the gaps between soil particles and produce EPS (Issa et al., 2009), which contributed to soil aggregation, increasing soil stability (Tiwari et al., 2019), and further preventing As from being released into the environment. Inoculation of cyanobacteria slightly increased the pH of the soil microenvironment (Fig. 5e, f), which was conducive to the conversion of As(III) to As(V) in the soil, thereby reducing the toxicity of As (Zhou et al., 2018). On the contrary, Kheirfam and Roohi (2020) found that soil pH decreased after inoculation with cyanobacteria. This might be due to the fact that the inoculated cyanobacteria could secrete more small molecular organic acids and there were a large number of acid-producing bacteria in the soil. Although high pH was not beneficial for the adsorption of As in soil (Goh and Lim, 2004), it increased the adsorption of some co-existing metal cations in soil, and the co-precipitation effect of metal ions on As might be much higher than that of pH.

Furthermore, due to As pollution, soil microbial activity was inhibited and soil fertility decreased (Das et al., 2013). The remediation of As-

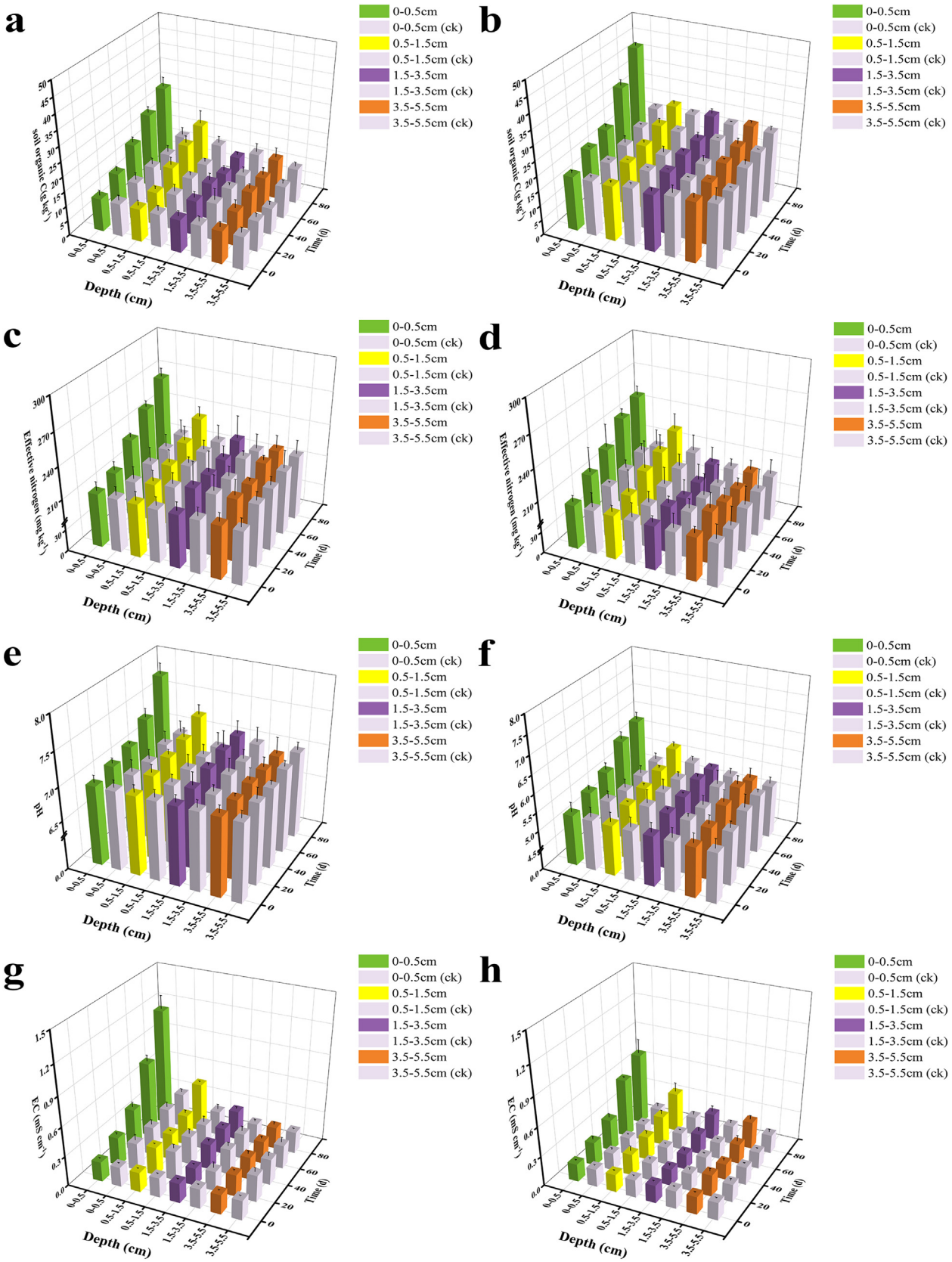

Fig. 5. Changes of Organic C, available N, pH, and EC in the soil at different depths of inoculation with and without *Leptolyngbya* sp. XZMQ (a, c, e, g is farmland soil; b, d, f, h is tailing soil).

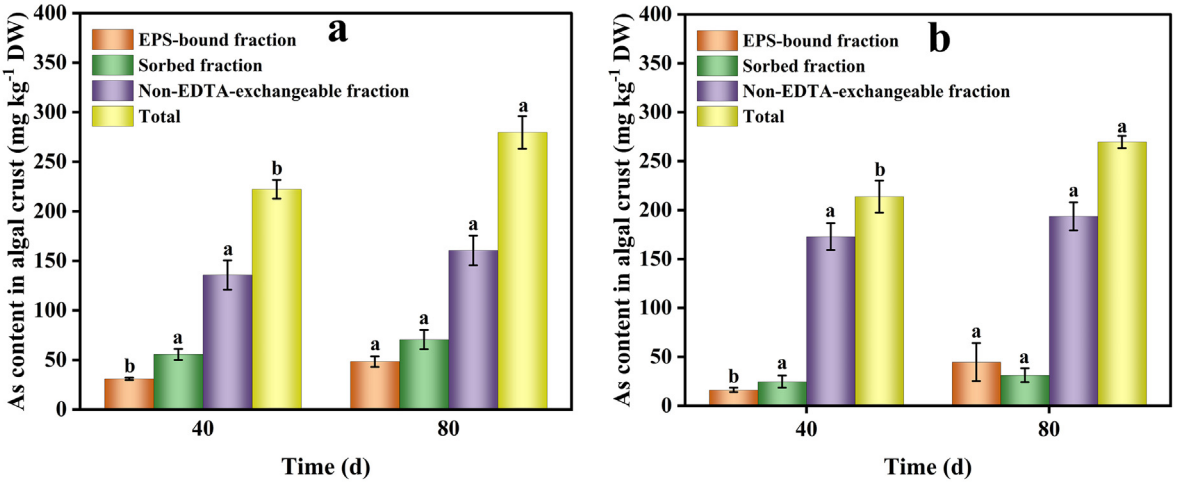

Fig. 6. Distribution of As in cyanobacterial crust of (a) S-N, and (b) S-Z. Different letters indicate significant differences at the 0.05 level in As content between the 40th and 80th days.

contaminated soil in mining area not only needs to reduce the As content and toxicity, but also needs to improve soil fertility. We found that after 80 days of culture, cyanobacterial crust significantly increased the fertility of two different types of As-contaminated soil. Two reasons may account for it as follows: on the one hand, with the growth of cyanobacterial crust, cyanobacteria were able to secrete more EPS to increase the content of OC in soil (Renuka et al., 2018). On the other hand, cyanobacteria could use sunlight to absorb carbon dioxide from the atmosphere and convert it into oxygen and hydrocarbons, thereby increasing soil OC content (Rossi et al., 2015). Muñoz-Rojas et al. (2018) also found that inoculation of cyanobacteria enhanced carbon sequestration in dryland soil matrix, thereby increasing soil fertility, which further strengthened our view.

Moreover, cyanobacterial crust also improved soil fertility by increasing soil N(a) content to enhance the remediation of As contaminated soil. Because CnfR (cyanobacterial nitrogen fixation regulator) of *Leptolyngbya boryana* could regulate nitrogenase activity for nitrogen fixation under microaerobic conditions, which was different from cyanobacteria (e.g., *Nostoc* and *Anabaena*) using heterocysts for nitrogen fixation (Tsujimoto et al., 2014). In addition, we found that the abundance of *Luteimonas* increased in the soil after inoculation of cyanobacteria (Fig. 8c), and *Luteimonas* as a key genus in nitrogen transformation, can affect the content of nitrogen in soil (Sun et al., 2019). Generally, cyanobacterial crust enhanced the content of OC and N(a) in soil, and there was a significant positive correlation between OC and N(a) ($P < 0.05$) (Table S6c). Hence, increased OC and N(a) under the cyanobacterial crust further increased soil fertility.

4.3. Influence of cyanobacterial crust on soil microbial structure, fertility and As bioremediation

The biocrust can influence the composition and diversity of soil microorganisms (Miralles et al., 2020), and some of microorganisms might affect the content and toxicity of As in soil through their own functions. This is also correlated with the increased soil organic carbon and fertility as

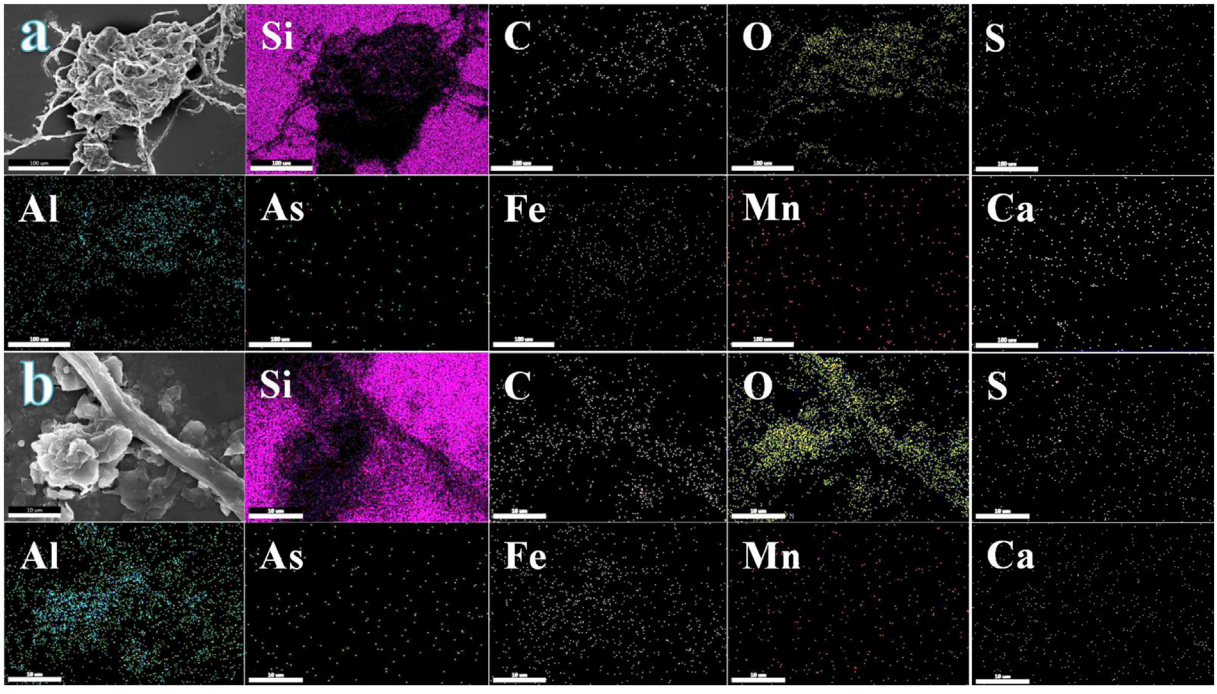

Fig. 7. SEM-based mapping of elements (Si, C, O, S, Al, As, Fe, Mn, Ca) in cyanobacterial crust of (a) S-N, and (b) S-Z.

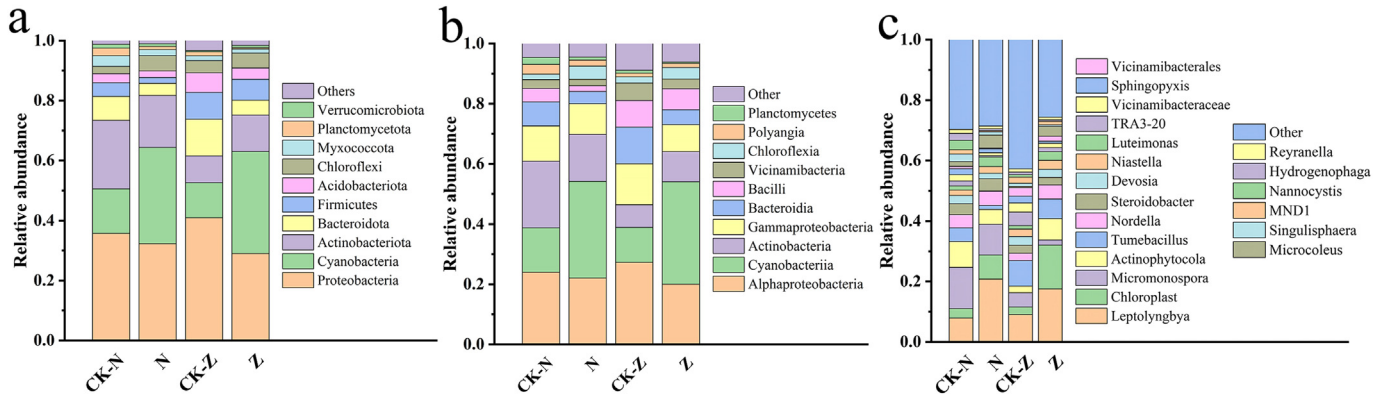

Fig. 8. Changes in the composition and relative abundances of bacterial phyla (a), classes (b), and genera (c) in different treatment groups.
Note: (a) Relative abundances of top 10 phyla. (b) Relative abundances of top 10 classes. (c) Relative abundances of top 20 genera. CK-N: farmland soil; N: farmland soil inoculated with cyanobacteria; CK-Z: tailing soil; Z: tailing soil inoculated with cyanobacteria.

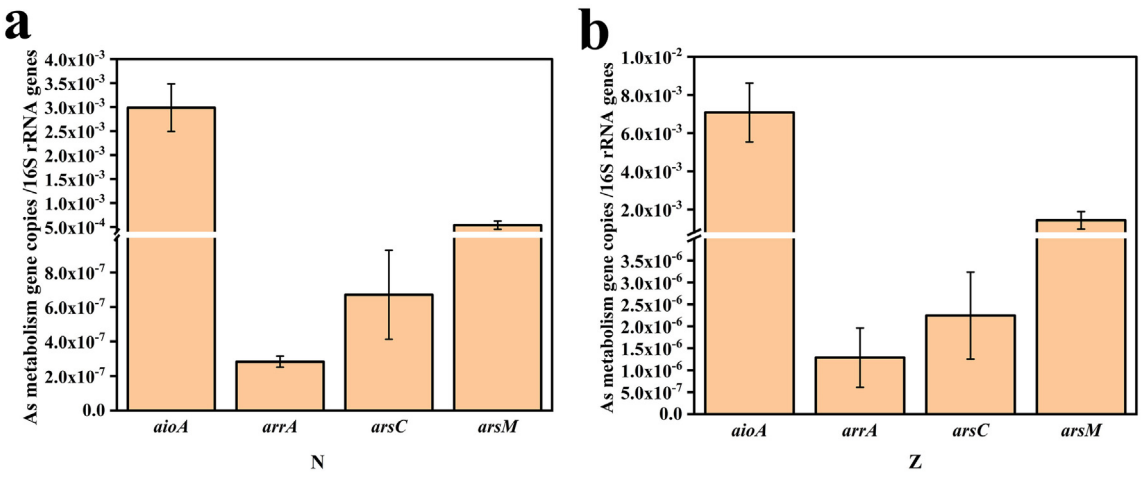

Fig. 9. The relative abundances of As metabolism genes in the treatment groups of (a) N and (b) Z.

mentioned in Section 4.2. According to our results, Proteobacteria, Cyanobacteria, Actinobacteria, and Bacteroidetes were the main bacterial phyla in the mining area of the soil (Fig. 8a), which have been identified to involve in the As methylation process and have the ability to volatilize As (Zhai et al., 2017; Dong et al., 2020). After inoculation of cyanobacteria, we also found that *Leptolyngbya* and *Luteimonas* had a significant negative correlation with As(a), As(T), and As(III) in soil (Table S6(c)), which might be due to their ability of As oxidation and methylation to reduce As toxicity and content in soil (Zhu et al., 2020; Mu et al., 2016). Similarly, these bacteria also could reduce the toxicity of heavy metals in soil and

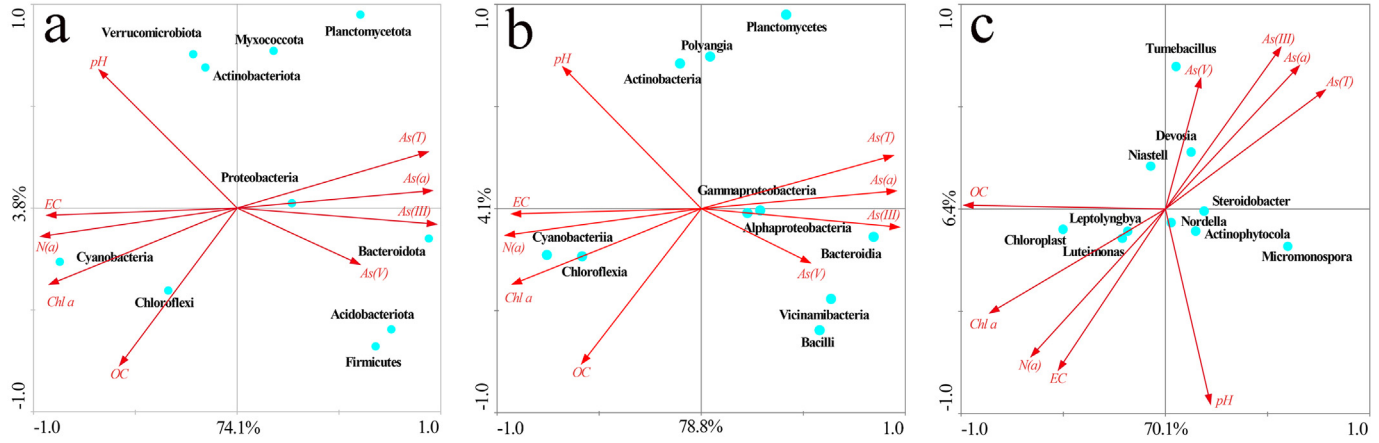

Fig. 10. RDA ordination diagram of bacterial community and soil environmental variables.
Note: (a), (b), (c) were based on the relative abundance at phylum (relative abundances of top 10 phyla), classes (relative abundances of top 10 classes and soil environmental parameters), genera (relative abundances of top 20 genera) level and soil environmental parameters.

remove heavy metals by using EPS and intracellular enzymes (Gongi et al., 2022; Wang et al., 2020). Notably, under cyanobacterial crust, the abundance of Cyanobacteria and Chloroflexi in the soil increased, while the Proteobacteria and Bacteroides, which are responsible for As methylation (Guo et al., 2021), decreased after cyanobacteria inoculation (Fig. 8a), so, Cyanobacteria and Chloroflexi played a more critical role in As-contaminated soil remediation under through oxidization of As(III) (Zhu et al., 2020; Summers et al., 2013). Interestingly, we also found higher *aioA* and *arsM* in the soil under the cyanobacterial crust (Fig. 9), further indicating that these microorganisms were involved in the process of As(III) oxidation and methylation, which was regarded as an effective method for As detoxification and removal (Kruger et al., 2013).

Additionally, we found that the inoculation of cyanobacteria could stimulate the activity of some microorganisms, thereby increasing soil fertility (Fig. 5a–d). As mentioned in Section 4.2, biocrusts affected the physical and chemical properties of soil, thus affecting the changes of microbial community structure (Miralles et al., 2020). In turn, changes in microbial structure and function also affect soil properties. After inoculation with cyanobacteria, the abundance of *Steroidobacter*, *Luteimonas* and *Niastella* in the soil under cyanobacterial crust increased (Fig. 8c). The *Steroidobacter* could participate in the utilization of carbon in the rhizosphere of maize (Jeewani et al., 2020), which meant that the bacteria might also promote the growth of cyanobacterial crust to strengthen the remediation of As-contaminated soil. Furthermore, *Luteimonas* was identified to be involved in the nitrogen cycle to produce N(a), which could not only be absorbed by other microorganisms to maintain their normal growth, but also improve the fertility of As-contaminated soil (Sun et al., 2019). Meanwhile, *Niastella* can perform N_2-generating denitrification (Nishizawa et al., 2014). Although these processes adversely affect soil nitrogen fixation, the abundance of these bacteria (<3%) is much lower than that of *Leptolyngbya*, so their effects may be lower. Similarly our results found that N(a) was strongly and positively correlated with the abundance of *Leptolyngbya*, *Chloroplast* and *Luteimonas*, while negatively correlated with the abundance of *Tumebacillus* (Table S6c). This might indicate that the presence of *Tumebacillus* was not conducive to the accumulation of N (a) in soil. Previous study has shown that the *Tumebacillus* has strong the root colonization ability and nitrogen metabolism's ability (Li et al., 2022), and can use N(a) in soil for growth and enrichment. Therefore, the decreased *Tumebacillus* abundance was beneficial to reduce the consumption of N(a) and improve the soil fertility of microenvironment. Furthermore, some soil microorganisms have the ability to oxidize and methylate As, so they can be used as tools for the remediation of As-contaminated soil.

Besides, soil physicochemical properties also can regulate the toxic effects of As (Singh et al., 2022); thereby affecting the role of microorganisms. High pH may promote microbial growth by enhancing metal complexation to reduce the bioavailability of heavy metals (Xiao et al., 2019). Therefore, metal ions might also reduce the bioavailability of As by co-precipitation to reduce toxic effects. This study found that soil pH was significantly positively correlated with the abundance of *Nordella*, indicating that *Nordella* had the effect of regulating soil pH, which might reduce the toxic effects of metal ions and As in soil. Ma et al. (2020) observed that *Nordella* showed strong positive correlations with pH in the soil of Sb mine. Hence, the inoculation of indigenous cyanobacteria on As-contaminated mining soils formed cyanobacterial crust on the soil surface, which regulated the soil microbial structure, maintained soil pH, inspired the functions of As oxidation and methylation microorganisms, and increased the abundance of nitrogen metabolism functional bacteria.

4.4. As accumulation in cyanobacterial crust

The soil around the abandoned Shimen Realgar Mine contained high levels of soluble As, which has a high risk of emigration (Chen et al., 2020). However, our study found that the total As accumulated in cyanobacterial crust of farmland soil and tailing soil was as high as 279.89 mg kg^{-1} and 269.57 mg kg^{-1}, respectively (Fig. 6). BSCs can prevent As migration and reduce As content in soil by accumulating As in biotic and abiotic parts, including EPS-bounding, sorption, and non-EDTA-exchangeable parts, thereby enhancing the remediation of As-contaminated soil. The peaks shift at 1637 cm^{-1} and 3440 cm^{-1} indicated the presence of —CO (Nuhoglu and Malkoc, 2009) and —OH (Lian et al., 2019) functional groups in cyanobacterial crust (Fig. S5), which were mainly present in EPS produced by algae, showed that EPS could have bounded As and enhanced the adsorption of As in the cyanobacterial crust (Tuzen et al., 2009). Our findings were further supported by Zhu et al. (2018) who found these functional groups could effectively participate in the biosorption of As by periphytic biofilm to remove As from wastewater. In addition, EPS might indirectly adsorb As by adsorbing other soluble metal ions (e.g., iron, aluminum, manganese, and calcium) from the soil, because these metal ions had a strong affinity for As (Cheng et al., 2009).

Another mechanism of cyanobacterial crust remediation of soil As pollution was that the cyanobacterial crust could immobilize As in the form of non-EDTA-exchangeable, reducing the content of soluble As in soil and making the binding form of As in soil more stable. Our study found that non-EDTA-exchangeable fraction was the main part of As enrichment in cyanobacterial crust (Fig. 6), indicating that more As was easily absorbed inside the cyanobacterial cell or adsorbed in the inorganic solid phases. Because previous studies have shown that once As entered cells, it could bind to phytochelatins (PCs) and store them in vacuoles, or bind to glutathione to reduce As toxicity (Pawlik-Skowrońska et al., 2004). The existence of FeOOH and Fe_2O_3 in the cyanobacterial crust could have co-precipitate with As (Figs. S3–S4), and tended to have good stability, i.e., not easily re-released into the environment (Muedi et al., 2021). Moreover, high mobility and higher bioavailability make As seem to more easily bioaccumulate in the liquid phase. In the soil environment, cyanobacterial crust tended to accumulate As by adsorbing more colloidal substances and mineral particles (Yan et al., 2021). Consequently, it was very beneficial to reduce As in soil and prevent the migration of soluble As. In addition, cyanobacteria, as an oxygen-producing microorganism, might have induced the formation of iron minerals, e.g., pyrite (Wang et al., 2021). Filamentous cyanobacteria (*Leptolyngbya*) have sheath to absorb iron ions from wastewater and soil to form iron membranes (Zada et al., 2021; Mao et al., 2022), so these iron membranes can be involved in As adsorption by *Leptolyngbya* sp. XZMQ. Hence, iron mineral and EPS of cyanobacterial crust played a key role in the As bioremediation from tailing and farmland soil.

4.5. Mechanism of soil As bioremediation by indigenous cyanobacteria *Leptolyngbya sp. XZMQ*

There was a higher As content in farmland soil and tailing soil around the mining area (Table S1). In both soils, *Leptolyngbya* sp. XZMQ was able to form cyanobacterial crust (Fig. 1), which increased soil organic matter by EPS production that absorbed As through functional groups (e.g., —CO and —OH) and reduced As stress from the soil (Fig. S5), hence, soil stability, organic content and microbial diversity were improved. This enhanced microbial diversity that not only promoted further As bioremediation but also supported the growth of *Leptolyngbya* sp. XZMQ in symbiotic association. Some key microbial communities in soil are; *Leptolyngbya* (biosorbed and bio-accumulated As) (Mao et al., 2022), *Chloroplast*, *Luteimonas* (regulated nitrogen) (Sun et al., 2019), *Steroidobacter* (improved soil OC) (Jeewani et al., 2020), *Niastella* (regulated soil pH) (Ma et al., 2020), Chloroflexi (oxidized As(III) to As(V)) (Summers et al., 2013), Proteobacteria, Cyanobacteria, Actinobacteria, and Bacteroidetes (caused As methylation) (Guo et al., 2021; Zhai et al., 2017; Dong et al., 2020). During its growth, *Leptolyngbya* sp. XZMQ used sunlight and CO_2 to form hydrocarbons (Rossi et al., 2015), which increased soil pH and supported the oxidation of As(III) to As(V) (Fig. 5e–f and Fig. 4), which was precipitated by other cations effects (Fig. 7). Moreover, After 80 d of inoculation, cyanobacterial crust induced by *Leptolyngbya* sp. XZMQ could accumulate 279.89 mg kg^{-1} and 269.57 mg kg^{-1} of As, from farmland and tailing soil, respectively (Fig. 6). So, As

bio-remediation took place by EPS binding, methylation, oxidation, precipitation and bioaccumulation by *Leptolyngbya* sp. XZMQ and as an additional effect soil fertility was also improved through increased microbial activity, soil organic and nitrogen content.

Leptolyngbya sp. XZMQ is proposed to effectively colonize the soil surface by screening indigenous cyanobacteria in As-contaminated soil of Shimen mining area, and improve its status through cyanobacteria metabolism, so as to achieve ecological restoration of As-contaminated soil in mining area. This can not only effectively prevent secondary pollution caused by soil erosion, but also improve soil fertility and reduce As pollution. It is a cost effective solution to As pollution in soil ecosystem of Shimen mining area. Inoculation of indigenous cyanobacteria to form biological crusts on the surface of As-contaminated soil is more suitable and environmentally friendly because most As can be converted into less toxic organic forms. In addition, it can also promote soil fertility, provide suitable environmental conditions for soil microorganisms and higher plants, and promote soil ecological restoration in tailing areas. However, there are concerns about As release from As-enriched cyanobacterial crust after their death. Using cyanobacteria, functional microorganisms and hyperaccumulation plants to improve the efficiency of remediation of As-contaminated soil is our future work.

5. Conclusions

In this study, the native cyanobacteria (*Leptolyngbya* sp. XZMQ) was used to construct cyanobacterial crust on As-contaminated soil of the Shimen mining area. After 80 d of cultivation, the accumulation of As by cyanobacterial crust of farmland soil and mine soil reached up to 279.89 mg kg^{-1} and 269.57 mg kg^{-1}, respectively. Non-EDTA exchangeable fraction is the major fraction of As(T) accumulated in cyanobacterial crust. The functional groups (e.g., —OH, —CO) are involved by adsorption of As in the cyanobacterial crust. Furthermore, iron oxides and clay particles exist in cyanobacterial crust. These results indicated cyanobacteria altered biotic and abiotic synergism to immobilize As. Moreover, the inoculation of *Leptolyngbya* sp. XZMQ significantly reduced the content of As(T) and As(a) in 0–0.5 cm soil depth, while increased the soil pH, OC, N(a) and EC. The changes of these characteristics are the behavior of As toxicity reduction and soil fertility improvement in the mining area. Besides, As metabolism genes, including *aioA*, *arrA*, *arsC*, and *arsM* are identified in the soil, and the abundance of *aioA* and *arsM* is higher in the soil. In particular, the Alphaproteobacteria and Cyanobacteria classes may have *aioA* and *arsM* gene, which suggests the participation of these microbes in As detoxification. Overall, the findings strongly demonstrates that cyanobacterial crust constructed by the inoculation of indigenous cyanobacteria on As-contaminated soil in mining areas can not only reduce As toxicity, but also improve soil fertility. Hence, *Leptolyngbya* sp. XZMQ inoculation is a promising method for bioremediation of soil in the As mining area. These results are helpful to the development of BSCs based tailings bioremediation technology.

Statement of informed consent, human/animal rights

No conflicts, informed consent, human or animal rights applicable.

CRediT authorship contribution statement

Qing Mao: Conceptualization, Methodology, Software, Investigation, Writing-original draft. Zuoming Xie: Writing-review & editing, Supervision, Funding acquisition. Sana Irshad and Sakinatu Issaka: Formal analysis. Fuwen Pei and Gilbert randrianarison: Investigation. All authors reviewed the manuscript.

Data availability

No data was used for the research described in the article.

Declaration of competing interest

Authors declare no financial and non-financial conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2022.160543.

References

Acea, M.J., Prieto-Fernández, A., Diz-Cid, N., 2003. Cyanobacterial inoculation of heated soils: effect on microorganisms of C and N cycles and on chemical composition in soil surface. Soil Biol. Biochem. 35, 513–524. https://doi.org/10.1016/S0038-0717(03)00005-1.

Biesinger, M.C., Payne, B.P., Grosvenor, A.P., Lau, L.W., Gerson, A.R., Smart, R.S.C., 2011. Resolving surface chemical states in XPS analysis of first row transition metals, oxides and hydroxides: Cr, Mn, Fe,Co and Ni. Appl. Surf. Sci. 257 (7), 2717–2730. https://doi.org/10.1016/j.apsusc.2010.10.051.

Cabala, J., Rahmonov, O., Jablonska, M., Teper, E., 2011. Soil algal colonization and its ecological role in an environment polluted by past Zn-Pb mining and smelting activity. Water Air Soil Pollut. 215 (1–4), 339–348. https://doi.org/10.1007/s11270-010-0482-1.

Castle, S.C., Morrison, C.D., Barger, N.N., 2011. Extraction of chlorophyll a from biological soil crusts: a comparison of solvents for spectrophotometric determination. Soil Biol. Biochem. 43 (4), 853–856. https://doi.org/10.1016/j.soilbio.2010.11.025.

Chamizo, S., Cantón, Y., Miralles, I., Domingo, F., 2012. Biological soil crust development affects physicochemical characteristics of soil surface in semiarid ecosystems. Soil Biol. Biochem. 49, 96–105. https://doi.org/10.1016/j.soilbio.2012.02.017.

Chen, X., Zeng, X.C., Kawa, Y.K., Wu, W., Zhu, X., Ullah, Z., Wang, Y., 2020. Microbial reactions and environmental factors affecting the dissolution and release of arsenic in the severely contaminated soils under anaerobic or aerobic conditions. Ecotoxicol. Environ. Saf. 189, 109946. https://doi.org/10.1016/j.ecoenv.2019.109946.

Cheng, H., Hu, Y., Luo, J., Xu, B., Zhao, J., 2009. Geochemical processes controlling fate and transport of arsenic in acid mine drainage (AMD) and natural systems. J. Hazard. Mater. 165 (1–3), 13–26. https://doi.org/10.1016/j.jhazmat.2008.10.070.

Cheng, L., Yue, Y., Zhou, H., Fei, B., Xiu, X., Sun, Y., Jia, X., 2022. Biological soil crusts enhance the role of non-rainfall water in the water input in alpine sandy land ecosystems. J. Hydrol. 127966. https://doi.org/10.1016/j.jhydrol.2022.127966.

Cui, L., Fan, L., Li, Z., Wang, J., Chen, R., Zhang, Y., Shen, L., 2021. Characterization of extracellular polymeric substances from Synechocystis sp. PCC6803 under Cd (II), Pb (II) and Cr (VI) stress. J. Environ. Chem. Eng. 9 (4), 105347. https://doi.org/10.1016/j.jece.2021.105347.

Das, S., Jean, J.S., Kar, S., Chakraborty, S., 2013. Effect of arsenic contamination on bacterial and fungal biomass and enzyme activities in tropical arsenic-contaminated soils. Biol. Fertil. Soils 49 (6), 757–765. https://doi.org/10.1007/s00374-012-0769-z.

Dong, Y., Gao, M., Liu, X., Qiu, W., Song, Z., 2020. The mechanism of polystyrene microplastics to affect arsenic volatilization in arsenic-contaminated paddy soils. J. Hazard. Mater. 398, 122896. https://doi.org/10.1016/j.jhazmat.2020.122896.

Du, J., Fang, J., Xu, W., Shi, P., 2013. Analysis of dry/wet conditions using the standardized precipitation index and its potential usefulness for drought/flood monitoring in Hunan Province, China. Stoch. Env. Res. Risk A. 27 (2), 377–387. https://doi.org/10.1007/s00477-012-0589-6.

Fattahi, S.M., Soroush, A., Huang, N., 2020. Wind erosion control using inoculation of aeolian sand with cyanobacteria. Land Degrad. Dev. 31 (15), 2104–2116. https://doi.org/10.1002/ldr.3590.

Francesconi, K., Visoottiviseth, P., Sridokchan, W., Goessler, W., 2002. Arsenic species in an arsenic hyperaccumulating fern, Pityrogramma calomelanos: a potential phytoremediator of arsenic-contaminated soils. Sci. Total Environ. 284 (1–3), 27–35. https://doi.org/10.1016/S0048-9697(01)00854-3.

Gao, G.L., Ding, G.D., Zhao, Y.Y., Wu, B., Zhang, Y.Q., Qin, S.G., Liu, Y.D., 2014. Fractal approach to estimating changes in soil properties following the establishment of Caragana korshinskii shelterbelts in Ningxia, NW China. Ecol. Indic. 43, 236–243. https://doi.org/10.1016/j.ecolind.2014.03.001.

Giral, M., Zagury, G.J., Deschênes, L., Blouin, J.P., 2010. Comparison of four extraction procedures to assess arsenate and arsenite species in contaminated soils. Environ. Pollut. 158 (5), 1890–1898. https://doi.org/10.1016/j.envpol.2009.10.041.

Goh, K.H., Lim, T.T., 2004. Geochemistry of inorganic arsenic and selenium in a tropical soil: effect of reaction time, pH, and competitive anions on arsenic and selenium adsorption. Chemosphere 55 (6), 849–859. https://doi.org/10.1016/j.chemosphere.2003.11.041.

Gongi, W., Cordeiro, N., Pinchetti, J.L.G., Ouada, H.B., 2022. Functional, rheological, and antioxidant properties of extracellular polymeric substances produced by a thermophilic

cyanobacterium Leptolyngbya sp. J. Appl. Phycol. 34 (3), 1423–1434. https://doi.org/10.1007/s10811-022-02695-1.
Guo, T., Gustave, W., Lu, H., He, Y., Tang, X., Buchwalter, D.B., Xu, J., 2021. Periphyton enhances arsenic release and methylation at the soil-water interface of paddy soils. J. Hazard. Mater. 409, 124946. https://doi.org/10.1016/j.jhazmat.2020.124946.
Hu, C., Zhang, D., Huang, Z., Liu, Y., 2003. The vertical microdistribution of cyanobacteria and green algae within desert crusts and the development of the algal crusts. Plant Soil 257 (1), 97–111. https://doi.org/10.1023/A:1026253307432.
Hu, R., Wang, X.-P., Pan, Y.-X., Zhang, Y.-F., Zhang, H., Chen, N.J.C., 2015. Seasonal variation of net N mineralization under different biological soil crusts in Tengger Desert, North China. Catena 127, 9–16. https://doi.org/10.1016/j.catena.2014.12.012.
Hussain, M.M., Bibi, I., Niazi, N.K., Wang, J., Shahid, M., 2021. Arsenic speciation and biotransformation pathways in the aquatic ecosystem: the significance of algae. J. Hazard. Mater. 403, 124027. https://doi.org/10.1016/j.jhazmat.2020.124027.
Issa, O.M., Défarge, C., Trichet, J., Valentin, C., Rajot, J.L., 2009. Microbiotic soil crusts in the Sahel of Western Niger and their influence on soil porosity and water dynamics. Catena 77 (1), 48–55. https://doi.org/10.1016/j.catena.2008.12.013.
Jeewani, P.H., Gunina, A., Tao, L., Zhu, Z., Kuzyakov, Y., Van Zwieten, L., Xu, J., 2020. Rusty sink of rhizodeposits and associated keystone microbiomes. Soil Biol. Biochem. 147, 107840. https://doi.org/10.1016/j.soilbio.2020.107840.
Jin, Y., Luan, Y., Ning, Y., Wang, L., 2018. Effects and mechanisms of microbial remediation of heavy metals in soil: a critical review. Appl. Sci. 8 (8), 1336. https://doi.org/10.3390/app8081336.
Khan, M.A., Stroud, J.L., Zhu, Y.G., McGrath, S.P., Zhao, F.J., 2010. Arsenic bioavailability to rice is elevated in Bangladeshi paddy soils. Environ. Sci. Technol. 44 (22), 8515–8521. https://doi.org/10.1021/es101952f.
Kheirfam, H., Roohi, M., 2020. Accelerating the formation of biological soil crusts in the newly dried-up lakebeds using the inoculation-based technique. Sci. Total Environ. 706, 136036. https://doi.org/10.1016/j.scitotenv.2019.136036.
Kruger, M.C., Bertin, P.N., Heipieper, H.J., Arsène-Ploetze, F., 2013. Bacterial metabolism of environmental arsenic–mechanisms and biotechnological applications. Appl. Microbiol. Biotechnol. 97 (9), 3827–3841. https://doi.org/10.1007/s00253-013-4838-5.
Lam, M.K., Lee, K.T., 2012. Immobilization as a feasible method to simplify the separation of microalgae from water for biodiesel production. Chem. Eng. J. 191, 263–268. https://doi.org/10.1016/j.cej.2012.03.013.
Li, H., Ye, Z., Chan, W., Chen, X., Wu, F., Wu, S., Wong, M., 2011. Can arbuscular mycorrhizal fungi improve grain yield, as uptake and tolerance of rice grown under aerobic conditions? Environ. Pollut. 159 (10), 2537–2545. https://doi.org/10.1016/j.envpol.2011.06.017.
Li, Z., Zheng, Y., Li, Y., Cheng, X., Huang, S., Yang, X., Qin, Y., 2022. Genotype-specific recruitment of rhizosphere bacteria from sandy loam soil for growth promotion of Cucumis sativus var. hardwickii. Front. Microbiol. 13, 910644. https://doi.org/10.3389/fmicb.2022.910644.
Lian, F., Yu, W., Wang, Z., Xing, B., 2019. New insights into black carbon nanoparticle-lnduced dispersibility of goethite colloids and configuration-dependent sorption for phenanthrene. Environ. Sci. Technol. 53 (2), 661–670. https://doi.org/10.1021/acs.est.8b05066.
Ma, Y., Wang, Y., Chen, Q., Li, Y., Guo, D., Nie, X., Peng, X., 2020. Assessment of heavy metal pollution and the effect on bacterial community in acidic and neutral soils. Ecol. Indic. 117, 106626. https://doi.org/10.1016/j.ecolind.2020.106626.
Mao, Q., Xie, Z., Irshad, S., Zhong, Z., Liu, T., Pei, F., Li, L., 2022. Effect of arsenic accumulation on growth and antioxidant defense system of Chlorella thermophila SM01 and Leptolyngbya sp.XZMQ. Algal Res. 66, 102762. https://doi.org/10.1016/j.algal.2022.102762.
Mccutcheon, J., Mcquaid, J., Benning, L., Stockdale, A., 2020. Mineral phosphorus drives glacier algal blooms on the Greenland Ice Sheet. Nat. Commun. 12, 570. https://doi.org/10.1038/s41467-020-20627-w.
Megharaj, M., Naidu, R., 2017. Soil and brownfield bioremediation. Microb. Biotechnol. 10 (5), 1244–1249. https://doi.org/10.1111/1751-7915.12840.
Miralles, I., Lázaro, R., Sánchez-Marañón, M., Soriano, M., Ortega, R., 2020. Biocrust cover and successional stages influence soil bacterial composition and diversity in semiarid ecosystems. Sci. Total Environ. 709, 134654. https://doi.org/10.1016/j.scitotenv.2019.134654.
Mitra, A., Chatterjee, S., Gupta, D.K., 2017. Uptake, transport, and remediation of arsenic by algae and higher plants. In: Gupta, D.K., Chatterjee, S. (Eds.), Arsenic Contamination in the Environment: The Issues and Solutions. Springer International Publishing, Cham, pp. 145–169 https://doi.org/10.1007/978-3-319-54356-7_7.
Mu, Y., Pan, Y., Shi, W., Liu, L., Jiang, Z., Luo, X., Li, W.J., 2016. Luteimonas arsenica sp. nov., an arsenic-tolerant bacterium isolated from arsenic-contaminated soil. Int. J. Syst. Evol. Microbiol. 66 (6), 2291–2296. https://doi.org/10.1099/ijsem.0.001024.
Muedi, K.L., Brink, H.G., Masindi, V., Maree, J.P., 2021. Effective removal of arsenate from wastewater using aluminium enriched ferric oxide-hydroxide recovered from authentic acid mine drainage. J. Hazard. Mater. 414, 125491. https://doi.org/10.1016/j.jhazmat.2021.125491.
Muñoz-Rojas, M., Román, J., Roncero-Ramos, B., Erickson, T., Merritt, D., Aguila-Carricondo, P., Cantón, Y., 2018. Cyanobacteria inoculation enhances carbon sequestration in soil substrates used in dryland restoration. Sci. Total Environ. 636, 1149–1154. https://doi.org/10.1016/j.scitotenv.2018.04.265.
Naveed, S., Dong, B., Zhang, C., Ge, Y., 2018. Microalgae and their effects on metal bioavailability in paddy fields. J. Soils Sediments 18, 936–945. https://doi.org/10.1007/s11368-017-1881-3.
Naveed, S., Li, C., Lu, X., Chen, S., Yin, B., Zhang, C., Ge, Y., 2019. Microalgal extracellular polymeric substances and their interactions with metal(loid)s: a review. Crit. Rev. Environ. Sci. Technol. 49 (19), 1769–1802. https://doi.org/10.1080/10643389.2019.1583052.
Ngosong, C., Buse, T., Ewald, M., Richter, A., Glaser, K., Schöning, I., Ruess, L., 2020. Influence of management intensity and environmental conditions on microbiota in biological soil crust and crust-free soil habitats of temperate forests. Soil Biol. Biochem. 144, 107761. https://doi.org/10.1016/j.soilbio.2020.107761.
Nisha, R., Kaushik, A., Kaushik, C.P., 2007. Effect of indigenous cyanobacterial application on structural stability and productivity of an organically poor semi-arid soil. Geoderma 138 (1–2), 49–56. https://doi.org/10.1016/j.geoderma.2006.10.007.
Nishizawa, T., Quan, A., Kai, A., Tago, K., Ishii, S., Shen, W., Senoo, K., 2014. Inoculation with N2-generating denitrifier strains mitigates N2O emission from agricultural soil fertilized with poultry manure. Biol. Fertil. Soils 50 (6), 1001–1007. https://doi.org/10.1007/s00374-014-0918-7.
Nuhoglu, Y., Malkoc, E., 2009. Thermodynamic and kinetic studies for environmentaly friendly Ni(II) biosorption using waste pomace of olive oil factory. Bioresour. Technol. 99 (8), 2375–2380. https://doi.org/10.1016/j.biortech.2008.11.016.
Pawlik-Skowrońska, B., Pirszel, J., Kalinowska, R., Skowroński, T., 2004. Arsenic availability, toxicity and direct role of GSH and phytochelatins in As detoxification in the green alga Stichococcus bacillaris. Aquat. Toxicol. 70 (3), 201–212. https://doi.org/10.1016/j.aquatox.2004.09.003.
Qin, J., Lehr, C.R., Yuan, C., Le, X.C., McDermott, T.R., Rosen, B.P., 2009. Biotransformation of arsenic by a Yellowstone thermoacidophilic eukaryotic alga. PNAS 106 (13), 5213–5217. https://doi.org/10.1073/pnas.0900238106.
Qiu, G.W., Koedooder, C., Qiu, B.S., Shaked, Y., Keren, N., 2022. Iron transport in cyanobacteria–from molecules to communities. Trends Microbiol. 30 (3), 229–240. https://doi.org/10.1016/j.tim.2021.06.001.
Rao, B., Liu, Y., Wang, W., Hu, C., Dunhai, L., Lan, S., 2009. Influence of dew on biomass and photosystem II activity of cyanobacterial crusts in the Hopq Desert, northwest China. Soil Biol. Biochem. 41 (12), 2387–2393. https://doi.org/10.1016/j.soilbio.2009.06.005.
Renuka, N., Guldhe, A., Prasanna, R., Singh, P., Bux, F., 2018. Microalgae as multi-functional options in modern agriculture: current trends, prospects and challenges. Biotechnol. Adv. 36 (4), 1255–1273. https://doi.org/10.1016/j.biotechadv.2018.04.004.
Rocha, F., Esteban Lucas-Borja, M., Pereira, P., Muñoz-Rojas, M., 2020. Cyanobacteria as a nature-based biotechnological tool for restoring salt-affected soils. Agronomy 10 (9), 1321. https://doi.org/10.3390/agronomy10091321.
Rossi, F., Olguín, E.J., Diels, L., De Philippis, R., 2015. Microbial fixation of CO2 in water bodies and in drylands to combat climate change, soil loss and desertification. New Biotechnol. 32 (1), 109–120. https://doi.org/10.1016/j.nbt.2013.12.002.
Rossi, F., Li, H., Liu, Y., De Philippis, R., 2017. Cyanobacterial inoculation (cyanobacterisation): perspectives for the development of a standardized multifunctional technology for soil fertilization and desertification reversal. Earth-Sci. Rev. 171, 28–43. https://doi.org/10.1016/j.earscirev.2017.05.006.
Singh, S.B., Naseem, M., Raghuvanshi, R., Srivastava, P.K., 2022. Application of selected nutrient amendments to regulate soil properties for reducing arsenic accumulation in rice. Soil Sediment Contam. Int. J. 1–17. https://doi.org/10.1080/15320383.2022.2061417.
Summers, E.A., Johnson, L.R., Porter, M.L., 2013. Arsenite oxidase gene diversity among chloroflexi and proteobacteria from EI Tatio Geyser Field, Chile. FEMS Microbiol. Ecol. 3, 745–756. https://doi.org/10.1111/1574-6941.12030.
Sun, W., Ji, B., Khoso, S.A., Tang, H., Liu, R., Wang, L., Hu, Y., 2018. An extensive review on restoration technologies for mining tailings. Environ. Sci. Pollut. Res. 25 (34), 33911–33925. https://doi.org/10.1007/s11356-018-3423-y.
Sun, Y., Men, M., Xu, B., Meng, Q., Bello, A., Xu, X., Huang, X., 2019. Assessing key microbial communities determining nitrogen transformation in composting of cow manure using illumina high-throughput sequencing. Waste Manag. 92, 59–67. https://doi.org/10.1016/j.wasman.2019.05.007.
Tiwari, O.N., Bhunia, B., Mondal, A., Gopikrishna, K., Indrama, T., 2019. System metabolic engineering of exopolysaccharide-producing cyanobacteria in soil rehabilitation by inducing the formation of biological soil crusts: a review. J. Clean. Prod. 211, 70–82. https://doi.org/10.1016/j.jclepro.2018.11.188.
Tsujimoto, R., Kamiya, N., Fujita, Y., 2014. Transcriptional regulators ChlR and CnfR are essential for diazotrophic growth in nonheterocystous cyanobacteria. PNAS 111 (18), 6762–6767. https://doi.org/10.1073/pnas.1323570111.
Turner, J.S., Robinson, N.J., 1995. Cyanobacterial metallothioneins: biochemistry and molecular genetics. J. Ind. Microbiol. 14 (2), 119–125. https://doi.org/10.1007/BF01569893.
Tuzen, M., Sarı, A., Mendil, D., Uluozlu, O.D., Soylak, M., Dogan, M., 2009. Characterization of biosorption process of As(III) on green algae Ulothrix cylindricum. J. Hazard. Mater. 165 (1–3), 566–572. https://doi.org/10.1016/j.jhazmat.2008.10.020.
Wang, L., Wang, L.A., Zhan, X., Huang, Y., Wang, J., Wang, X., 2020. Response mechanism of microbial community to the environmental stress caused by the different mercury concentration in soils. Ecotoxicol. Environ. Saf. 188, 109906. https://doi.org/10.1016/j.ecoenv.2019.109906.
Wang, G., Yuan, Y., Morel, J.L., Feng, Z., Qiu, R., 2021. Biological aqua crust mitigates metal (loid) pollution and the underlying immobilization mechanisms. Water Res. 190 (1), 116736. https://doi.org/10.1016/j.watres.2020.116736.
Wenzel, W.W., Kirchbaumer, N., Prohaska, T., Stingeder, G., Adriano, D.C., 2001. Arsenic fractionation in soils using an improved sequential extraction procedure. Anal. Chim. Acta 436 (2), 309–323. https://doi.org/10.1016/S0003-2670(01)00924-2.
Xiao, E., Ning, Z., Xiao, T., Sun, W., Qiu, Y., Zhang, Y., Chen, Y., 2019. Variation in rhizosphere microbiota correlates with edaphic factor in an abandoned antimony tailing dump. Environ. Pollut. 253, 141–151. https://doi.org/10.1016/j.envpol.2019.06.097.
Xie, X., Cheng, H., 2021. Adsorption and desorption of phenylarsonic acid compounds on metal oxide and hydroxide, and clay minerals. Sci. Total Environ. 757, 143765. https://doi.org/10.1016/j.scitotenv.2020.143765.
Xu, N., Tan, G., Wang, H., 2016. Effect of bihar additions to soil on nitrogen leaching, microbial biomass and bacterial community structure. Eur. J. Soil Biol. 74, 1–8. https://doi.org/10.1016/j.ejsobi.2016.02.004.
Yan, S., Yang, J., Zhou, S., Yan, Y., Tang, X., Ma, Y., Hu, H., A, W.Y., 2021. Biological soil crusts alleviate the stress of arsenic on rice germination and the underlying immobilization mechanisms. Ecotoxicol. Environ. Saf. 227, 112839. https://doi.org/10.1016/j.ecoenv.2021.112839.

Yao, Y., Li, D., Chen, Y., Han, X., Wang, G., Han, R., 2022. High-resolution characteristics and mechanisms of endogenous phosphorus migration and transformation impacted by algal blooms decomposition. Sci. Total Environ. 820, 152907. https://doi.org/10.1016/j.scitotenv.2021.152907.

Zada, S., Lu, H., Khan, S., Iqbal, A., Ahmad, A., Ahmad, A., Zhang, X., 2021. Biosorption of iron ions through microalgae from wastewater and soil: optimization and comparative study. Chemosphere 265, 129172. https://doi.org/10.1016/j.chemosphere.2020.129172.

Zhai, W., Wong, M.T., Luo, F., Hashmi, M.Z., Liu, X., Edwards, E.A., Xu, J., 2017. Arsenic methylation and its relationship to abundance and diversity of arsM genes in composting manure. Sci. Rep. 7 (1), 1–11. https://doi.org/10.1038/srep42198.

Zhan, J., Sun, Q., 2012. Diversity of free-living nitrogen-fixing microorganisms in the rhizosphere and non-rhizosphere of pioneer plants growing on wastelands of copper mine tailings. Microbiol. Res. 167 (3), 157–165. https://doi.org/10.1016/j.micres.2011.05.006.

Zhou, Y., Niu, L., Liu, K., Yin, S., Liu, W., 2018. Arsenic in agricultural soils across China: distribution pattern, accumulation trend, influencing factors, and risk assessment. Sci. Total Environ. 616, 156–163. https://doi.org/10.1016/j.scitotenv.2017.10.232.

Zhu, N., Zhang, J., Tang, J., Zhu, Y., Wu, Y., 2018. Arsenic removal by periphytic biofilm and its application combined with biochar. Bioresour. Technol. 248, 49–55. https://doi.org/10.1016/j.biortech.2017.07.026.

Zhu, F., Yang, M., Luo, Z.X., Yu, R.L., Hu, G.R., Yan, Y., 2020. Bioaccumulation and biotransformation of arsenic in Leptolyngbya boryana. Environ. Sci. Pollut. Res. 27, 29993–30000. https://doi.org/10.1007/s11356-020-09294-y.