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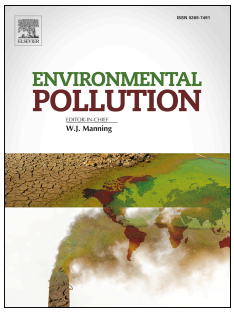

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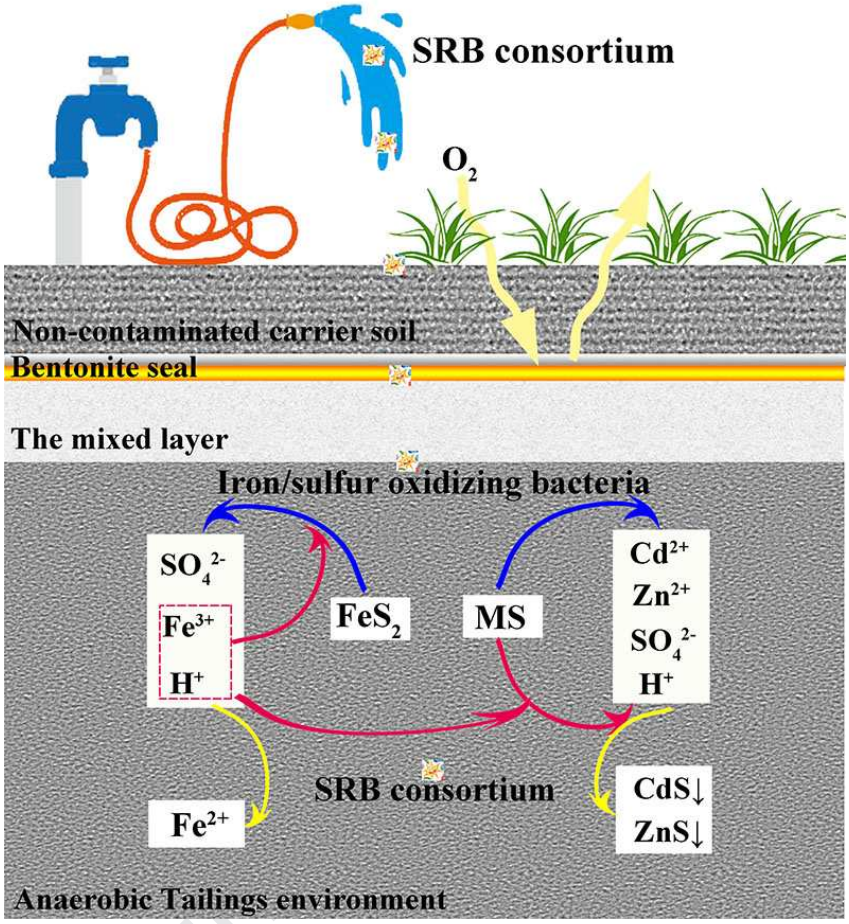

Bacterial shifts during in-situ mineralization bio-treatment to non-ferrous metal(loid) tailings

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Abstract

Nonferrous mine tailings have caused serious problems of co-contamination with metal(loid)s. It is still a global challenge to cost-effectively manage and mitigate the effect of the mining wastes. We conducted an in-situ bio-treatment of non-ferrous metal(loid) tailings using a microbial consortium of sulfate reducing bacteria (SRB). During the bio-treatment, the transformation of metal(loid)s (such as Cu, Fe, Mn, Pb, Sb, and Zn) into oxidizable and residual fractions in the subsurface tended to be higher than that observed in the surface. As well the mineral compositions changed becoming more complex, indicating that the sulfur reducing process of bio-treatment shaped the bio-transformation of metal(loid)s. The added SRB genera, especially *Desulfotomaculum* genus, colonized the tailings suggesting the coalescence of SRB consortia with indigenous communities of tailings. Such observation provides new insights for understanding the functional microbial community coalescence applied to bio-treatment. PICRUSt analysis revealed presence of genes involved in sulfate reduction, both assimilatory and dissimilatory. The potential for the utilization of both inorganic and organic sulfur compounds as S source, as well as the presence of sulfite oxidation genes indicated that SRB play an important role in the transformation of metal(loid)s. We advocate that the management of microorganisms involved in S-cycle is of paramount importance for the in situ bio-treatment of tailings, which provide new insights for the implementation of bio-treatments for mitigating the effect of tailings.

Keywords: microbial treatment; metal(loid) contamination; bacterial communities; sulfur metabolism

1 Introduction

Large quantities (~ 200,000 tons) of mine tailings are produced daily worldwide (Jakubick and Mckenna, 2003). In China, approximately 300 million hectares have been contaminated by mining tailings (Chen et al., 2016). Most tailings contain flotation reagents, used in the extraction procedures, which could form chelate compounds with metal(loid)s and lead to co-contamination. Such co-contamination results on a more aggravated contamination, which threaten the surroundings (Hudson-Edwards, 2016; Liu et al., 2018; Zhu et al., 2018). Therefore, management and treatment of mine tailings is a global issue requiring urgent solutions. However, conventional technologies for treatment of tailings, such as physical and chemical stabilization, are limited by their high costs (approximately US$1.50 - 450 m^{-3}) (Mendez and Maier, 2008). Similarly, the success of in-situ phyto-treatment is limited because acidification occurs in tailings during natural attenuation (Liu et al., 2019), which may hamper the survival of native plants (Mendez and Maier, 2008; Wang et al., 2017). Cost-effective microbial-treatment represents a promising alternative to overcome these limitations with potential application in tailing sites (Mkandawire, 2013; Gutiérrez et al., 2016).

Sulfate-reducing bacteria (SRB) have been detected in acid mine drainages (AMD), and mine tailings (Giloteaux et al., 2013; Volant et al., 2015). They participate to the formation of precipitates of metal(loid) as sulfur complexes (Le et al., 2017; Zhang et al., 2017). SRB have been considered as the most promising alternative for the decontamination of AMD (Martins et al., 2009; Zhang et al., 2017) involving several mechanisms such as bio-sorption, bio-chelation, bio-reduction, and bio-mineralization of metal(loid)s (Haferburg and Kothe, 2010; Mkandawire, 2013). In AMD, SRB are competing with acidophilic bacteria (Mkandawire, 2013), and therefore the success of a treatment involves that SRB outcompete acidophilic bacteria. It has been demonstrated that an amendment with organic carbon promotes SRB diversity in mine

tailings (Lindsay et al., 2011). Recently, we revealed that acidophilic bacteria were among the abundant bacterial populations at the abandoned nonferrous metal(loid) tailings of Guangxi (China) (Liu et al., 2018; Liu et al., 2019), which could limit SRB development. Bio-augmentation treatment, involving the addition of bacterial strains, has been demonstrated to be a green efficient approach for bio-treatment processes (Goñi-Urriza et al., 2013; Said et al., 2015). However, there were few practical application examples of SRB consortium to abandoned nonferrous metal(loid) tailings.

To address this gap, we applied a field treatment experiments to investigate the efficiency and impact of a bio-augmentation process to metal(loid)s, with a SRB consortium for the treatment of the nonferrous metal(loid) Guangxi's tailings, on the whole bacterial communities. We hypothesize that the bio-augmentation process shaped the temporal distribution of indigenous bacterial communities, and different metal(loid)s transformation and bacterial structure in surface and subsurface tailings sites. The bacterial communities were followed during the bio-augmentation treatment of nonferrous metal(loid) tailings in order to determine (1) the temporal variation of bacterial communities by high-throughput 16S rRNA gene sequencing technology, (2) the major geochemical drivers, and (3) the potential sulfur metabolism during bio-treatment. This study will provide a technical guidance on the pollution prevention, and a better understanding for the management and bio-treatment of nonferrous metal(loid) tailings by bacterial resources.

2 Materials and methods

2.1 Site description and sampling

The studied mine tailing site was located at Guangxi, (China; 107°38'12.69" E, 24°50'14.36" N; Fig. 1), a mountainous area. This nonferrous metal(loid) tailing site was

chosen because it is easy access for heavy equipment and vehicles. Field treatment experiments included a bio-augmentation by SRB (sulfate-reducing bacteria) consortium following an in-situ mineralization treatment method as previously proposed (Goñi-Urriza et al., 2013; Said et al., 2015). The SRB consortium were cultivated at 30 °C for 40 d as recommended by Zhang et al. (2017). The mixture material used for bio-treatment contained sugarcane sticks and poultry excrement (obtained from local farmland), desulphurized gypsum and steel slag powder (collected from the mining facilities), bentonite clay and non-contaminated carrier soil (bought from local facilities and farmland). An estimation of the cost for the implementation of the present bio-treatment was approximately US$1.45 m^{-3}, which were a little lower than traditional remediation techniques (range from US$1.50 - 450 m^{-3} for mine tailings (Mendez and Maier, 2008). The five-later of coverage technology patent number is 201710586719.1. Despite the facts that the plant growing on tailings may influence the microbial communities (Haferburg and Kothe, 2010; Ye et al., 2017), the bentonite clay and seal layer could provide anaerobic environment for the survival of SRB consortium, and prevent plants from growing the bottom of tailings in present bio-treatment system (Fig. 1). The SRB consortium was provided by Prof. Liu Xingyu from the National Engineering Laboratory of Biohydrometallurgy (Beijing, China). The initial SRB consortium was the Y3 obtain by Zhang (Zhang et al., 2017) containing mainly two SRB strains *Desulfosporosinus* and *Desulfotomaculum* with high abundance, and the acidophilic strain *Acidiphilium*. Based on previous laboratory-scale experiments (Zhang et al., 2017), the treatment was performed in 0.6 ha tailings. A mixture containing yeast extract (1.2 g L^{-1}) and the SRB consortium (5.0×10^8 cells L^{-1}) were sprayed during 10 d (approximately 1,000 t).

<Insert Fig. 1>

Tailing samples were collected in June 2016 (before the 10 d spraying procedure). More

detailed information of the nonferrous metal(loid) tailings site has been provided previously (Liu et al., 2018). The sampling method followed a random sampling strategy according to the technical specifications for soil environmental monitoring of State Environmental Protection Administration (HJ/T 166-2004). In order to highlight the temporal distribution of bacterial communities during bio-treatment, samples before bio-treatment were used as references. Samples during bio-treatment were collected on 10 July 2016 (one month after spraying), October (2016), February (2017), and June (2017), respectively. The temperatures for each sampling month were 26.0, 25.9, 17.4, 11.1, and 23.6 °C, respectively. The rainfalls for each sampling month were 70.8, 24.8, 2.80, 16.7, and 185 mm. The rainfall data were obtained from China Meteorological Administration. It has been determined that pH and water content of tailings sites could directly mediate the metabolic rates and biochemical processes of adapted microbial communities (Liu et al., 2019). As well, soil water content showed strong correlation with soil pH, which could change availability of plant nutrient (Wu et al., 2019). Therefore, the acidity of the studied tailings sites with bio-treatment (average pH = 7.5, Table S1) and water content also correlate with the variation of microbial communities. The samples codes are explained in the supplemental materials' Table S1. Additionally, to determine whether sulfate reduction process could shape the different variations to metal(loid) transformation and bacterial compositions at different tailings sites layers, samples were collected at two different depths, including the surface (0 - 10 cm) and subsurface (40 - 50 cm). The non-contaminated carrier soil was removed when collecting the surface samples during bio-treatment. In order to take into account the heterogeneity of tailing impoundment, which could lead to the undetectable effects of environmental factor on microbial diversity (Zhou *et al.*, 2016), composite samples by five-point sampling within a site accounted for the environmental heterogeneity. The duplicates sampling design was implemented, and five

147 sub-samples were collected for both surface and subsurface layers. Then these five
148 sub-samples of each duplicate were mixed into one composite sample. The 24 composite
149 tailings samples (500 g each) were kept in cool box (4 °C) and transported to the lab within 2 d
150 of sampling for subsequent analyses as previously described (Liu et al., 2018).

151

152 ***2.2 Geochemical characterization***

153 For the determination of geochemical parameters, samples were air dried and sieved at
154 100-mesh size (0.149 mm, US standard). The electrical conductivity (EC) and pH were
155 determined in deionized water (1:5.0, w/v) using EC electrodes (DDS 307, LEICI) and a
156 soil-water mixture (1:2.5, w/v) using a pH meter (Starter 3C, OHAUS). EC reflected the
157 salinity of samples (Chen et al., 2015). Total organic carbon (TOC) and total nitrogen (TN)
158 contents were determined using a Total Organic Carbon Analyzer (TOC-VCPH) and Total
159 Nitrogen Module (TNM-1, Shimadzu, Japan), respectively. The total phosphorus (TP) was
160 analyzed according to the Chinese standard method for determination of soil total phosphorus
161 (GB 9837-88). Total sulfur (TS) was determined by infrared absorption carbon-sulfur analyzer
162 (CS-8620, Wuxi, China). The total content of metal(loid)s (T-M; As, Cd, Cr, Cu, Fe, Mn, Pb,
163 Sb, and Zn) was determined by inductively coupled plasma optical emission spectrometry
164 (ICP-OES; iCAP 7000 SERIES, Thermo Scientific,USA) after digested with HNO_3:HCl:HF
165 (5:3:2, v/v/v, GR) as described earlier (Liu et al., 2018). The limit of detection for metal(loid)s
166 was from 0.10×10^{-3} (Zn) to 4.22×10^{-3} (Sb) mg kg^{-1}, and the recovery of the spiked standard
167 was 99.0% - 103% (Table S1).

168 To investigate the mobility and migration of metal(loid)s during treatment, the
169 fractionation of metal(loid)s was determined by the modified BCR sequential extraction
170 method (Liang et al., 2017). Four distinct fractions were considered: (1) exchangeable fraction

(BCR-1), linked to the mobility of metal(loid)s under mild acid conditions; (2) reducible fraction (BCR-2), associated to Fe and Mn oxy/hydroxides; (3) oxidizable fraction (BCR-3), changed with oxidizing conditions; and (4) residual fraction (BCR-4), which is difficult to extract under the above conditions and exist in the lattice of silicates and minerals (Ning et al., 2016; Zhu et al., 2018). All chemical reagents, including ethylic acid (0.11 M HAc), hydroxylamine hydrochloride (0.5 M $NH_2OH \cdot HCl$), and ammonium acetate (1.0 M NH_4OAC) were of guaranteed reagent grade (JONK, Wuhan, China), and used for BCR extraction experiment, respectively. Between each step of BCR sequential extraction, samples were rinsed with deionized water. Each fraction of metal(loid)s were tested by ICP-OES. A previous study showed that the transformation rate of metal speciation from the labile fractions toward stable fractions could reflect the rate of metal mobility factor (Zheng and Zhang, 2011). The transformation rate was calculated following the relationship:

$$\text{transformation rate} = (BCR1 + BCR2) / (BCR1 + BCR2 + BCR3 + BCR4) \quad (1)$$

where BCR1, BCR2, BCR3, and BCR4 represent the proportion of corresponding metal(loid) speciation. The larger the value of changing rate, the greater the hazard and instability.

The mineralogy of samples was characterized by X-ray diffraction (XRD) (Yikexue Company, China), and XRD data were analyzed with Jade 6.5 software. All the data for geochemical parameters were determined in technical triplicates.

2.3 MiSeq sequencing and bioinformatic analysis

Genomic DNA was extracted from each sample using the SoilGen DNA kit (CWBio, Beijing, China) according to the manufacturer's protocol for sequencing. The universal primer set 341F/518R was used for PCR amplification of the V3 - V4 region of 16S rRNA bacterial gene as described previously (Liu et al., 2018). Each amplification was done in triplicate

pooled. Sequencing was performed at the Illumina MiSeq platform (Shanghai Majorbio Bio-pharm Technology Co., Ltd., Shanghai, China).

The fast length adjustment of short reads (FLASH) software was used for merging the paired-end reads of 16S rRNA gene raw sequences. Differences between sequences for each sample were distinguished based on unique barcodes. The parameters for denoising the unqualified sequences were set up as previous described (Liu et al., 2018). The chimeras were eliminated and sequences related to chloroplasts, mitochondria, and eukaryote were removed. Then the sequences were used to affiliate the operational taxonomic units (OTUs) with a threshold of 97% similarity using the Silva (Release128 http://www.arb-silva.de) database. Qiime (http://qiime.org/scripts/assign_taxonomy.html) and ribosomal database project classifier (version 2.2 http://sourceforge.net/projects/rdp-classifier/) were used for OTUs and taxonomic annotate analysis with 70% confidence threshold. Alpha-diversity indices were calculated using Mothur software (v.1.30.1) based on the OTUs at 97% similarity level. Phylogenetic investigation of communities by reconstruction of unobserved states (PICRUSt) analysis was used to predict the potential Kyoto Encyclopedia of Genes and Genomes (KEGG) metabolic pathways of bacterial communities. The nearest sequenced taxon index (NSTI) of phylogenetic distances to the nearest reference genome was used to test the accuracy of PICRUSt prediction (Langille et al., 2013). The NSTI values were 0.10 and 0.13 for surface and subsurface tailings samples respectively (Table S4), indicating that the PICRUSt analysis was robust. The sequence read archive accession number for the 16S rRNA sequences is PRJNA552653.

2.4 Statistical analyses

All statistical analyses were performed using IBM SPSS statistics 21.0 and RStudio free

219 software. Differences of geochemical parameters, alpha-diversity indices, bacterial relative
220 abundance, and KEGG metabolic pathways were statistically tested by one-way ANOVA and
221 student's t test, and $p < 0.05$ was considered significant. Correlations among these indices were
222 analyzed by Spearman correlation analysis, linear univariate model, distance-based
223 redundancy analysis (db-RDA), and canonical correlation analysis (CCA). Modularity analysis
224 of co-occurrence network analysis reflected the correlation of bacterial communities among
225 different samples using Gephi-0.9.2 software. To illustrate the clustering of bacterial
226 community structures, a non-metric multidimensional scaling (NMDS) ordination was based
227 on the unweighted-unifrac similarity distance. Analysis of similarity (ANOSIM) based on
228 Bray-Curtis distance and weighted normalized unifrac distance were conducted using 999
229 permutations to test the variations of bacterial structures. Two-tailed Wilcoxon rank-sum test
230 was used for further emphasizing statistically significant differences among tailings samples
231 with different bio-treatment phase at both surface and subsurface levels, based on Welch's
232 inverted calculation method ($p < 0.05$).
233

234 **3 Results and Discussion**

235 ***3.1 Tailing characteristics***

236 The geochemical and mineralogical characteristics were determined at the surface (0 - 10
237 cm) and subsurface (50 cm) layers. The tailings became neutral and slightly alkali environment
238 (pH = 7.59) showing significant differences after starting the treatment ($p < 0.05$; Table S1).
239 Such observation indicated that the treatment and the added bacterial consortium inhibited the
240 acidification of tailings. The tailings before bio-treatment were highly contaminated by
241 metal(loid)s (Table S1), which concentrations were similar to those found in other tailings in
242 China (Chen et al., 2013) and UK (Bleuven and Landry, 2016). After bio-treatment setup, a

243 dilution effect was observed. Indeed, the concentration of As, Cd, Cu, Fe, Pb, and Zn
244 decreased obviously, which were significantly different during bio-treatment ($p < 0.05$; Table
245 S1). Consistent with the fact that sample collected before dilution contained higher amount of
246 metal(loid)s than that collected during other bio-treatment periods, it is likely that the mixing
247 with uncontaminated soil and other materials used for the treatment diluted the metal(loid)s
248 content.

249 It has been demonstrated that microbial activities promote the transformation of
250 metal(loid)s into the oxidizable and residual fractions (Tokalıoğlu and Kartal, 2005; Zhu et al.,
251 2018). It is likely that these oxidizable fractions have a strong tendency to bind to activated
252 groups of organic and sulphide compounds, which were hard to release at neutral or alkaline
253 environments as previously shown (Tokalıoğlu and Kartal, 2005). In order to determine the
254 changes in the proportion of the different BCR sequential extraction fractions during the
255 bio-treatment, the proportion of the BCR fractions between the first and the 12^{th} months of the
256 bio-treatment were compared. The BCR sequential extraction showed that the oxidizable and
257 residual fractions of metal(loid)s, such as Cu, Fe, Mn, Pb, Sb, and Zn, showed an increased
258 trend in the subsurface, which were much higher than that in the surface during bio-treatment
259 (Fig. 2). Similar results were obtained for the bio-treatment of metals in acid tailings by mixed
260 microbial mats (Phillips and Bender, 1998). It has been demonstrated that SRB plays a key
261 role in metal rich ecosystems (Giloteaux et al., 2010). Previous studies on the metal(loid)s
262 bio-treatment process using SRB in Cd contaminated sediments and Mn/Pb contaminated acid
263 mine drainage showed that SRB removed 93% Mn^{2+}, 90% Pb^{2+}, and 78 - 96% Cd (Miao et al.,
264 2018; Peng et al., 2018). Despite residual fraction of As, Cd, and Cr metal(loid)s were reduced
265 with bio-treatment, but exchangeable and reducible fractions remains low, indicating that there
266 is a kind of “metal extraction” during bio-treatment (Fig. 2). It has been demonstrated that

Arsenate reducing bacteria was able to transfer As^{3+} into a lower valence condition (Dia et al., 2015). Based on the solubility products of metallic sulfides, different transformation rates of As and Cd in different bio-treatment systems may be affected by the sulfur deficiency in the post bio-treatment (Zhang et al., 2017). The metal(loid)s transformation was probably due to different processes including mineralization by microorganisms, or mobilization by runoff and rain lixiviation as previously reported (Haferburg and Kothe, 2010).

<Insert Fig. 2>

In addition, correlation analysis between BCR fractions of metal(loid)s and geochemical parameters showed that the oxidation fraction of Pb had significant positive correlation with TOC (r = 0.69, p < 0.05; Table S2). Such observation indicated that peritization process in presence of natural organic matter might make metal(loid)s hard to be released even under oxidizing condition. It has been shown that trace metals may be bound to various forms of organic matter, including living organisms, detritus and coated on mineral particles (Charlesworth et al., 2004). However, a strong negative correlations between BCR sequential extraction fractions of Sb and TOC content (r = -0.745 to -0.830, p < 0.01; Table S2), indicating that TOC did not influenced the distribution and concentration of Sb elements during tailing bio-treatment. The BCR sequential extraction fractions of metal(loid)s, except for Sb, appeared to be fluctuant and unstable at the fourth month of bio-treatment, which may probably be due to the mineralization or transformation by microorganisms. It has been demonstrated that siderophore-producing bacteria (SPB) enhanced the bio-available concentration of Cr and Pb via forming stable complexes, which were diverted from the iron (essential elements) channel (Ye et al., 2017). Despite the decreased content of residual fraction of metal(loid)s during bio-treatment (Fig. 2), the transformation of metal(loid)s into the more oxidizable fractions is beneficial to the bio-treatment system at the present neutral or

alkaline tailings sites.

XRD analysis showed more complex profiles indicating the formation of metal(loid)s precipitates such as Zinc sulfide and quintinite-3T (Fig. 3). It is likely that bacterial communities transformed metal(loid)s into precipitates probably throughout H_2S biologically produced by SRB as proposed earlier (Zhang et al., 2017). Before bio-treatment, S, As, and Fe were present in tailing samples, and mainly existed in the forms of FeS_2 and FeAsS, which were observed in the third and fifth micro-structural analysis of EDS (Fig. S1). While for the tailings samples during bio-treatment, the EDS spectra analysis showed the presence of FeAsS (Fig. S1). Meanwhile, the percentage of oxide mineral showed great decreased trend, and oxygen element were mainly present in quartz and calcite during bio-treatment (Fig. S1). The low content of metallic oxides indicated that the added bacteria consortium had the capacity to transform metal(loid)s either into more oxidizable forms or make precipitates as reported previously (Haferburg and Kothe, 2010). At the same time, the microorganisms decomposed large minerals particles (20 - 50 μm) into small particles (10 μm) during bio-treatment as shown by the EDS analysis (Fig. S1). But the presence of these small particles will reduce the permeability of tailings, reducing thus the leaching rate and the vertical permeability as previously demonstrated by XRF and XRD analyses (Sasaki et al., 2002). Consistent with the XRD analysis, EDS analysis may explain increase/decrease alternation observed in the BCR profiles for each metal(loid). From the perspective of bio-treatment, the small particles and precipitation under the activity of microorganisms, is conducive for preventing the possibility of metal(loid)s penetrating into the deep underground of tailings. Overall, our results showed that the main trend was the removal of metal(loid)s during bio-treatment.

<Insert Fig. 3>

3.2 Bacterial diversity and composition

The bacterial diversity was determined by MiSeq Illumina 16S rRNA gene sequencing. A total of 746,341 sequences (average of 37,137 ± 4,440 per sample) were obtained. After normalization 30,007 sequences were retained that were assigned to 2,787 OTUs with a threshold identity 97% (Table S3). The coverage (> 98.5%) and rarefaction curves indicated that the sequencing depth was enough to estimate the bacterial diversity (Table S3; Fig. S2). The alpha-diversity indices (species richness, Shannon, Simpson, ace, chao1, and phylogenetic diversity (PD)) increased after treatment of tailings in both surface and subsurface layers (Table S3), specially after the first month of bio-treatment, which was consistent with the dilution effect observed for metal(loid)s (Table S1). However, in Cd contaminated sediment environment (0 - 25 mg kg^{-1}), it was observed that the alpha-diversity indices (except Simpson index) first decreased and then increased between 32 and 166 d of bio-treatment (Peng et al., 2018). The dilution effect was also observed in the bacterial composition that was modified after the first month of bio-treatment. Before the treatment setup, the bacterial communities were dominated by *Proteobacteria*, *Firmicutes*, and *Deinococcus-Thermus* at the surface; while *Firmicutes* dominated largely the subsurface (Fig. 4a). During all the bio-treatment, the bacterial communities were dominated by *Proteobacteria* (Fig. 4a). At the genus level, ecological succession was observed, bacterial communities showing different compositions at each time for both surface and subsurface layers (Fig. 4b). At the 12^{th} month, bacterial community of the surface layer showed similar compositions to that observed at the subsurface layer, which was also similar to that found at the first month ($p > 0.05$; Fig. S3a). This observation suggested the resilience of bacterial communities. Microbial communities are often resilient after a disturbance returning to their initial structure (Allison and Martiny, 2008). Such resilient capacity has been reported after oil spill (Bordenave et al., 2006), and metal

339 contamination (Azarbad et al., 2016).

340 During the ecological succession, we also observed the succession of genera related to
341 sulfur metabolism. The main sulfate-reducing bacteria (SRB) genus was *Desulfotomaculum*
342 (Fig. 4c), which was related to the SRB genus added for the bio-treatment. It showed a high
343 abundance at twelfth month of bio-treatment (12%; Fig. 4c). Increased abundance of
344 *Desulfotomaculum* genus suggested that it was able to colonize the tailings during
345 bio-treatment. It has been determined that *Desulfotomaculum* was able to precipitate the
346 arsenic trisulfide (As_2S_3) playing an important role in the biogeochemical cycle of arsenic
347 (Newman et al., 1997). In contrast, the abundance of the added *Desulfosporosinus* genus
348 showed a decreased trend, being detected at very low abundance mostly at the twelfth month
349 of bio-treatment (< 0.01%; Fig. 4c). *Desulfosporosinus* has been demonstrated to promote
350 metal sulfide precipitation via specific bio-mineralization precipitation pathway (Sitte et al.,
351 2013). It also showed the capacity to degrade toluene and reduce Fe^{3+} (Pester et al., 2012). It is
352 likely that the added *Desulfosporosinus* SRB genus was not able to compete with autochthous
353 bacteria. Such observation has been reported for bio-augmentation treatments where the added
354 bacteria could not colonize the environment (Li et al., 2018). In addition, the abundance of
355 indigenous SRB genera, particularly *Desulfurispora* and *Desulfuromonas*, increased (Fig. 4c).
356 These observations suggested that both members of the added SRB consortium and indigenous
357 SRB populations played a key role in metal(loid)s precipitation. The main sulfur-oxidizing
358 bacteria (SOB) were *Sulfurifustis*, *Thiobacillus*, and *Sulfuricurvum* genera, and the relative
359 proportion of SOB decreased by 20% during bio-treatment process (Fig. 4d). As well, the
360 *Sulfuricurvum* genus showed significant differences in the subsurface tailings sites during the
361 bio-treatment ($p < 0.001$; Fig. S3b). Such results indicated that the conditions prevailing in the
362 tailings environment were more favorable for SRB survival.

<Insert Fig. 4>

The bacterial communities of the surface layer were compared with those of the subsurface layer during the bio-treatment by Venn diagram analyses (Fig. S6). The shared OTUs increased during the bio-treatment (Fig. S6), notably after the first month of the bio-treatment, corresponding to the observed “dilution effect” that was the consequence of mixing bacterial communities from soil to the tailings. It is likely that the bio-treatment activated the autochthous bacteria increasing the shared OTUs between the different horizons, which was benefic for the mineralization of metal(loid)s. The number of specific OTUs, for both the surface and the subsurface, increased slightly during the bio-treatment. But the surface bacterial communities contained more specific OTUs than that of the subsurface (Fig. S6). The specific OTUs attested the different conditions prevailing in each horizon depending on air (oxygen) availability, water and other perturbations including the release of contaminants (Blume et al., 2002). Similar to previous study of bioremediation to contaminated marine sediments (Fonti et al., 2015), the SRB bio-tretament determined a strong changes in both bacterial diversity and composition, including the indigenous and added SRB bacterial consortium. But compared with previous study, which enhanced metal(loid)s mobility with potential detrimental consequences during bio-treatment (Fonti et al., 2015), the metal(loid)s were changed to more stable fraction (such as oxidation, reduction, and residual fraction) due to the changes of bacterial communities.

3.3. Geochemical drivers of bacterial communities

In order to reveal the main geochemical drivers of the bacterial community structure during the ecological succession, a correlation analysis between bacterial composition and chemical data (BCR metal fractionation and geochemical parameters) was performed. The

correlation analysis was performed compiling surface and subsurface data for more robustness. The analysis allowed us to point out four main groups (Fig. 5). The first group showing positive correlation with the fraction BCR4 of most metal(loid)s (Pb, Mn, Cd, As, Cu, Fe, Zn, and Cr), was composed by *Bacillus*, *Enterococcus*, and *Lactococcus* (Fig. 5). These three genera encode specific metal resistant genes such as *cadC*, *cop*, and *mntH* conferring the Cd/Cu resistance or proton-coupled Mn^{2+}/Fe^{2+} transport homolog (Turner et al., 2007; Pereira et al., 2015; Jung et al., 2016). It has been reported that these genera also produce flocculants to precipitate the carbonate minerals (Yao et al., 2013). They probably play a major role in transforming and solidification of metals in tailings. The second group showing positive correlation with the fractions BCR1/2/3 of most metal(loid)s (Mn, As, Cu, Zn, Cd, Pb, and Fe) included *Aquicella*, *Bradyrhizobium*, *Gaiella*, *Meiothermus*, *Pseudolabrys*, *Sphingomonas*, *Sulfurifustis*, and three unclassified genera (Fig. 5). It has been reported that *Bradyrhizobium* strains were tolerant to Cd, Cr, Pb, Zn and Cu, which were important for the plants and formerly included 'slow growing' strains of the genus *Rhizobium* (Wani and Khan, 2014). *Sphingomonas* genus has been shown resistant to different type of metal(loid)s, such as Al, Li, Ba, and Ni. It includes some species of photo-organotrophic microorganisms with the ability to form aggregates or attach goethite (<alpha>-FeOOH) in their cells (Csotonyi et al., 2010; Ozer et al., 2013). *Meiothermus* genus has been demonstrated to reduce Cr^{5+} (Ozer et al., 2013). Thus, it is likely that these three genera also play an important role in metal transformation. Only few information is available for the other members of this group, which were not found in contaminated environments: *Pseudolabrys* use organic acids as substrate (Kampfer et al., 2006), *Gaiella* is a strictly aerobic *Actinobacteria* (Albuquerque et al., 2011), *Aquicella* was isolated from a spa, and *Sulfurifustis* is a sulfur-oxidizing bacteria (Albuquerque et al., 2011).

The third group, which was negatively correlated with the fractions BCR1/2/3 of most

411 metal(loid)s (Mn, As, Cu, Zn, Cd, Pb, and Fe), included *Sulfuricella*, and two unclassified
412 genera (Fig. 5). *Sulfuricella*, described as sulfur-oxidizing and denitrification bacteria, might
413 play an important role in metal detoxification and in the nitrogen cycle as previously described
414 (Watanabe et al., 2014). The presence of *Sulfuricella* genus resulted on decreasing fractions
415 BCR1/2/3, participating thus to the detoxification of metal(loid)s as the main BCR fraction
416 was the residual fraction (Fig. 2). In addition, metal(loid)s could react with H_2S to form
417 precipitates which has been determined by previous studies (Zhang et al., 2017; Zhu et al.,
418 2018). Finally, the fourth group, showing negative correlation with the fraction BCR4 of most
419 metal(loid)s (Pb, Mn, Cd, As, Cu, Fe, Zn, and Cr), included *Thiobacter* and five unclassified
420 genera (Fig. 5). *Thiobacter*, sulfur/thiosulfate-oxidizing genera (Hirayama et al., 2005) has
421 been found able to solubilize Cu, As, Hg and other metal(loid)s from minerals (Valdés et al.,
422 2008). Although the *Thiobacter* genus (SOB) showed a low abundance in tailings (Fig. 4d), its
423 presence in the tailings suggested that *Thiobacter* plays an important role in the
424 bio-availability of metal(loid)s.

425 <Insert Fig. 5>

426

427 ***3.4 Predicted functional variation and sulfur metabolism***

428 The bacterial functional capabilities were predicted by PICRUSt analysis. The obtained
429 NSTI value was low (0.06 - 0.18; Table S3) indicating that the prediction was accurate as
430 previously shown (Langille et al., 2013). Consistent with the biodiversity indexes, the
431 correspondence analysis, based on KEGG pathways, clearly separated the samples before
432 bio-treatment from those after setting the bio-treatment (Fig. 5), confirming the dilution effect.
433 The analysis also showed that the surface layers at 12^{th} month of bio-treatment clustered with
434 functions involved in “Transport” and “DNA repair and recombination” (Fig. 6). These

functions might have an important role in the detoxification of metals in tailings as several metal-efflux pumps (Kerr, 2004; Zhou et al., 2009), and in maintaining the genomic stability (Barber et al., 2008). This observation suggested that the bacterial communities of the surface layer were adapted to the conditions at the end of experiment. The subsurface layers during bio-treatment (except at 4^{th} month) were associated with electron transfer carriers and organic degradation functions (Fig. 6), indicating that the possible input of organic matter/compounds came from medium used in the treatment and carbon fixed by microbiota. Functions of "electron transfer carriers" ensure the transfer of electrons from electron donors to electron acceptors via redox reactions (Murray et al., 2003). Correlation analysis with geochemical parameters showed that the KEGG pathways of transcription related proteins, isoflavonoid biosynthesis were positively correlated with TOC, total concentration of As and Fe (r = 0.80 - 0.85, $p < 0.01$; Fig. 6).

<Insert Fig. 5>

The sulfur cycle plays an important role in mine tailings (Zhang et al., 2017). Sulfur has various chemical species that are cycled by a suite of coupled biogeochemical processes catalyzed by various microbe-encoded genes (Cao et al., 2014). To deepen our understanding of bio-treatment ecosystems, it is crucial to know which S cycling potential pathways are operating and how they respond to the bio-treatment. Among the 6,907 inferred genes, 29 genes were associated with sulfur metabolism, corresponding mainly to assimilatory and dissimilatory sulfate reduction (Fig. 7a). Noteworthy, the genes K17725, K17229, and K17230 involved in the production of sulfur from sulfite or sulfide were not revealed by PICRUSt. Such observation suggested that the S-cycle was incomplete. The most abundant genes included the genes encoding the phosphoadenosine phosphosulfate reductase (K00390, 4.8% - 10.2%), the ATP sulfurylase (K00958, 5.6% - 11.2%) and the thiosulfate/3-mercaptopyruvate

sulfurtransferase (K01011, 15.2% - 31.2%) (Fig. 7b). The ATP sulfurylase (K00958) catalyze the reaction producing APS or PAPS from sulfate, corresponding to the first reaction of both assimilatory and dissimilatory sulfate reduction (Cao et al., 2014). Thus, the bacterial communities own sulfidogenesis capacity, a key mechanism in metals/metalloid immobilization, involved in some treatment and metal recovery processes from acid drainages (Sorokin et al., 2010). This metal immobilization does not exclude the possibility of metals be carried by rain (runoff). The second reaction of the sulfate reduction involves the phosphoadenosine phosphosulfate reductase (CysH; K00390), adenylylsulfate reductase (AprAB; K00394 and K00395) (Cao et al., 2014). Noteworthy, CysH (K00390) was found high abundant at the surface (4.8% - 9.2%) and subsurface (5.2% - 10.2%) during bio-treatment of tailings (Fig. 6b). The relative abundances of AprAB (K00394 and K00395) increased during the bio-treatment in both the surface and the subsurface (Fig. 7b).

In the third reaction, sulfite is transformed either in sulfate or in sulfide. The transformation to sulfate involves the sulfite oxidase/dehydrogenase (SUOX; K00387 and K05301). Although the SUOX (K00387 and K05301) were found at lower abundances (0.2% - 3.6%), it is likely that these functions ensure the recycling of sulfate from sulfite in tailings maintaining thus the substrate for sulfate-reducers. The transformation of sulfite to sulfide involves K00380, K00381, K00385, K00392, K11180, and K11181 genes (Zhang et al., 2017). PICRUST also revealed genes involved in the metabolism of thiosulfate and sulfur-organic compounds. For example, the sulfurtransferases (TST; K01011) catalyze the transfer of sulfur from 3-mercaptopyruvate to cyanide or other thiol compounds (Zhang et al., 2017). Its presence suggested that bacterial assemblages in the bio-treatment of tailings have the capacity to use organic sulfur as S source. The fact that the genes involved in sulfur metabolism were inferred by PICRUSt suggested that the bacterial activity participate to the mineralization and

transformation of metal(loid)s in tailings during the bio-treatment, which was consistent with a previous study demonstrating that the mineral formation and stability in extreme depositional environment was affected by sulfur cycle activities (Johnson et al., 2015).

<Insert Fig. 7>

4 Conclusions

The bio-treatment resulted in a more complex mineral composition for most of the metal(loid)s including Cd, Cr, Cu, Fe, and Mn. The oxidizable and residual fractions transformation of metal(loid)s in the subsurface showed a higher rate than that in the surface during bio-treatment. As well, a dilution effect of metal content and resilience of bacterial communities inhabiting the tailings were observed that subsequently affected the bacterial composition and organization during the bio-treatment. But, we observed bacterial community coalescence as the added SRB consortium and indigenous bacterial communities were mixed during the bio-treatment. The added SRB, *Desulfotomaculum*, colonized the tailings during the bio-treatment process. Additionally, PICRUSt analysis revealed that the bacterial communities had the potential to ensure assimilatory and dissimilatory sulfate reduction during the bio-treatment. The presence of the genes involved in the elimination of the toxic sulfite, producing sulfate, maintain the substrate for sulfate reducers. Such observation indicated that the conditions for active sulfate reduction were present in the tailings. Thus, our results suggested that persistent periodic spraying of the SRB consortium is necessary in order to ensure the success of the bio-augmentation for a better mineralization and transformation of metal(loid)s. However, more detailed information is still required to fully understand the microbial mechanism involved in the transformation of metal(loid)s, such as As, Cd, Sb, and Zn. Such information is of paramount importance for a better management of nonferrous

metal(loid) tailings, which will provide significant advance for the implementation of cost-effective bio-treatment of nonferrous metal(loid) tailings.

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References

Albuquerque, L., França, L., Rainey, F.A., et al., 2011. *Gaiella occulta* gen. Nov., Sp. Nov., a novel representative of a deep branching phylogenetic lineage within the class *Actinobacteria* and proposal of *Gaiellaceae* fam. Nov. and *Gaiellales* ord. Nov. Syst. Appl. Microbiol. 34, 595-599.

Allison, S.D., Martiny, J.B.H., 2008. Resistance, resilience, and redundancy in microbial communities. Proc Natl Acad Sci USA 105, 11512-11519.

Azarbad, H., Gestel, C.A.M.V., Niklińska, M., et al., 2016. Resilience of soil microbial communities to metals and additional stressors: DNA-based approaches for assessing "Stress-on-Stress" responses. Int. J. Mol. Sci. 17, 933-953.

Barber, L.J., Youds, J.L., Ward, J.D., et al., 2008. RTEL1 maintains genomic stability by suppressing homologous recombination. Cell 135, 261-271.

Bleuven, C., Landry, C.R., 2016. Molecular and cellular bases of adaptation to a changing environment in microorganisms. Proc. R. Soc. B. 283, 1458-1467.

Blume, E., Bischoff, M., Reichert, J.M., et al., 2002. Surface and subsurface microbial biomass, community structure and metabolic activity as a function of soil depth and season. Appl.

534 Soil Ecol. 20, 171-181.
535 Bordenave, S., Jézéquel, R., Fourçans, B., et al., 2006. Degradation of the “Erika” oil. Aquat.
536 Living Resour. 17, 261-267.
537 Cao, H., Wang, Y., Lee, O., et al., 2014. Microbial sulfur cycle in two hydrothermal chimneys on
538 the southwest Indian ridge. Mbio. 5, 913-980.
539 Charlesworth, S., Everett, M., Mccarthy, R., et al., 2004. A comparative study of heavy metal
540 concentration and distribution in deposited street dusts in a large and a small urban area:
541 Birmingham and Coventry, West Midlands, UK. Environ. Int. 29, 563-573.
542 Chen, F., Yao, Q., Tian, J., 2016. Review of ecological restoration technology for mine tailings
543 in China. Eng. Rev. 36, 115-121.
544 Chen, L., Li, J., Chen, Y., et al., 2013. Shifts in microbial community composition and function
545 in the acidification of a lead/zinc mine tailings. Environ. Microbiol. 15, 2431-2444.
546 Chen, Q., Zhou, Y., An, Y., 2015. Study on resource utilization of tailings. Geol. Rev. 61,
547 979-980.
548 Csotonyi, J.T., Swiderski, J., Stackebrandt, E., et al., 2010. A new environment for aerobic
549 anoxygenic phototrophic bacteria: biological soil crusts. Environ. Microbiol. R. 2, 651-656.
550 Dia, A., Lauga, B., Davranche, M., et al., 2015. Bacteria-mediated reduction of As(V)-doped
551 lepidocrocite in a flooded soil sample. Chem. Geol. 406, 34-44.
552 Giloteaux, L., Duran, R., Casiot, C., et al., 2013. Three-year survey of sulfate-reducing bacteria
553 community structure in Carnoulès acid mine drainage (France), highly contaminated by
554 arsenic. FEMS Microbiol. Ecol. 83, 724-737.
555 Giloteaux, L., Goñi-Urriza, M., Duran, R., 2010. Nested PCR and new primers for analysis of
556 sulfate-reducing bacteria in low-cell-biomass environments. Appl. Environ. Microbiol. 76,
557 2856-2865.
558 Goñi-Urriza, M.S., Cravo-Laureau, C., Duran, R., 2013. Microbial Bioremediation of Aquatic
559 Environments. in: Férard, J., Blaise, C. (Eds.), Encyclopedia of Aquatic Ecotoxicology.
560 Springer Netherlands, Dordrecht, pp. 709-720.
561 Gutiérrez, M., Mickus, K., Camacho, L.M., 2016. Abandoned Pb-Zn mining wastes and their
562 mobility as proxy to toxicity: a review. Sci. Total Environ. 565, 392-400.
563 Fonti, V., Beolchini, F., Rocchetti, L., et al. Bioremediation of contaminated marine sediments
564 can enhance metal mobility due to changes of bacterial diversity. Water Res. 2015, 68,
565 637-650.

566 Haferburg, G., Kothe, E., 2010. Microbes and metals: interactions in the environment. J. Basic
567 Microb. 47, 453-467.
568 Hirayama, H., Takai, K., Inagaki, F., et al., 2005. *Thiobacter subterraneus* gen. Nov., Sp nov., an
569 obligately chemolithoautotrophic, thermophilic, sulfur-oxidizing bacterium from a
570 subsurface hot aquifer. Int. J. Syst. Evol. Micr. 55, 467-472.
571 Hudson-Edwards, K., 2016. Tackling mine wastes. Science, 352, 288-290.
572 Jakubick, T., Mckenna, G., 2003. Stabilisation of tailings deposits: international experience.
573 Mining and the Environment III, Sudbury, Ontario, Canada, 25-28 May, 2003, 1-9
574 Johnson, S.S., Chevrette, M.G., Ehlmann, B.L., et al., 2015. Insights from the metagenome of an
575 acid salt lake: the role of biology in an extreme depositional environment. PLoS One 10,
576 e0122869-e0122888.
577 Jung, J., Jeong, H., Kim, H.J., et al., 2016. Complete genome sequence of *Bacillus*
578 *oceanisediminis* 2691, a reservoir of heavy-metal resistance genes. Mar. Genom. 30, 73-76.
579 Kampfer, Peter, Young, C., Arun, A.,et al., 2006. *Pseudolabrys taiwanensis* gen. Nov., Sp. Nov.,
580 an alphaproteobacterium isolated from soil. Int. J. Syst. Evol. Microbiol. 56, 2469-2472.
581 Kerr, I.D., 2004. Sequence analysis of twin ATP binding cassette proteins involved in
582 translational control, antibiotic resistance, and ribonuclease L inhibition. Biochem. Bioph.
583 Res. Co. 315, 166-173.
584 Langille, M.G., Zaneveld, J., Caporaso, J.G., et al., 2013. Predictive functional profiling of
585 microbial communities using 16S rRNA marker gene sequences. Nat. Biotechnol. 31,
586 814-821.
587 Le, P.P., Battaglia-Brunet, F., Parmentier, M., et al., 2017. Complete removal of arsenic and zinc
588 from a heavily contaminated acid mine drainage via an indigenous SRB consortium. J.
589 Hazard. Mater. 321, 764-772.
590 Li, J., Xue, S., He, C., et al., 2018. Effect of exogenous inoculants on enhancing oil recovery and
591 indigenous bacterial community dynamics in long-term field pilot of low permeability
592 reservoir. World J. Microb. Biot. 34, 53-63.
593 Liang, J., Yang, Z., Tang, L., et al., 2017. Changes in heavy metal mobility and availability from
594 contaminated wetland soil remediated with combined biochar-compost. Chemosphere, 181,
595 281-288.
596 Lindsay, M., Wakeman, K.D., Rowe, O.F., et al., 2011. Microbiology and geochemistry of mine
597 tailings amended with organic carbon for passive treatment of pore water. Geomicrobiol. J.

28, 229-241.

Liu, J., Yao, J., Wang, F., et al., 2018. China's most typical nonferrous organic-metal facilities own specific microbial communities. Sci. Rep-UK 8, 12570-12580.

Liu, J., Yao, J., Wang, F., et al., 2019. Bacterial diversity in typical abandoned multi-contaminated nonferrous metal(loid) tailings during natural attenuation. Environ. Pollut. 247, 98-107.

Martins, M., Faleiro, M.L., Barros, R.J., et al., 2009. Characterization and activity studies of highly heavy metal resistant sulphate-reducing bacteria to be used in acid mine drainage decontamination. J. Hazard. Mater. 166, 706-713.

Mendez, M.O., Maier, R.M., 2008. Phytostabilization of mine tailings in arid and semiarid environments: an emerging remediation technology. Environ. Health Persp. 116, 278-283.

Miao, Z.Y., He, H., Tan, T., et al., 2018. Biotreatment of Mn^{2+} and Pb^{2+} with sulfate-reducing bacterium *Desulfuromonas alkenivorans* S-7. J. Environ. Eng. 144, 04017116

Mkandawire, M., 2013. Biogeochemical behaviour and bioremediation of uranium in waters of abandoned mines. Environ. Sci. Pollut. R. 20, 7740-7767.

Murray, R.K., Granner, D.K., Mayer, P.A., et al., 2003. Harper`s Illustrated Biochemistry. New York, NY: Lange Medical Books/ MgGraw Hill.

Newman, D.K., Beveridge, T.J., Morel, F.M.M., 1997. Precipitation of arsenic trisulfide by *Desulfotomaculum auripigmentum*. Appl. Environ. Microbiol. 63, 2022-2028.

Ning, D., Liang, Y., Song, A., et al., 2016. In situ stabilization of heavy metals in multiple-metal contaminated paddy soil using different steel slag-based silicon fertilizer. Environ. Sci. Pollut. R. 23, 1-10.

Ozer, G., Ergene, A., Icgen, B., 2013. Biochemical and molecular characterization of strontium-resistant environmental isolates of *Pseudomonas fluorescens* and *Sphingomonas paucimobilis*. Geomicrobiol. J. 30, 381-390.

Peng, W., Li, X., Liu, T., et al., 2018. Biostabilization of cadmium contaminated sediments using indigenous sulfate reducing bacteria: efficiency and process. Chemosphere 201, 697-707.

Pereira, L.B., Vicentini, R., Ottoboni, L.M.M., 2015. Characterization of the core microbiota of the drainage and surrounding soil of a Brazilian copper mine. Genet. Mol. Biol. 38, 484-489.

Pester, M., Brambilla, E., Alazard, D., et al., 2012. Complete genome sequences of

Desulfosporosinus orientis DSM765(T), *Desulfosporosinus youngiae* DSM17734(T), *Desulfosporosinus meridiei* DSM13257(T), and *Desulfosporosinus acidiphilus* DSM22704(T). J. Bacteriol. 194, 6300-6301.

Phillips, P., Bender, J., 1998. Bioremediation of Metals in Acid Tailings by Mixed Microbial Mats. In: Geller W., Klapper H., Salomons W. (eds) Acidic Mining Lakes. Environ. Sci. Springer, Berlin, Heidelberg.

Said, O., Louati, H., Soltani, A., et al., 2015. Changes of benthic bacteria and meiofauna assemblages during bio-treatments of anthracene-contaminated sediments from Bizerta lagoon (Tunisia). Environ. Sci. Pollut. R. 22, 15319-15331.

Sasaki, K., Haga, T., Hirajima, T., et al., 2002. Distribution and transition of heavy metals in mine tailing dumps. Mater. Trans. 43:2778-2783.

Sitte, J., Pollok, K., Langenhorst, F., et al., 2013. Nanocrystalline nickel and cobalt sulfides formed by a heavy metal-tolerant, sulfate-reducing enrichment culture. Geomicrobiol. J. 30, 36-47.

Sorokin, D.Y., Rusanov, I.I., Pimenov, N.V., et al., 2010. Sulfidogenesis under extremely haloalkaline conditions in soda lakes of Kulunda Steppe (Altai, Russia). FEMS Microbiol. Eco. 73, 1444-1453.

Tokalıoğlu, Ş., Kartal, Ş., 2005. Comparison of metal fractionation results obtained from single and BCR sequential extractions. B. Environ. Contam. Tox. 75, 180-188.

Turner, M., Tan, Y., Giffard, P., 2007. Inactivation of an iron transporter in *Lactococcus lactis* results in resistance to tellurite and oxidative stress. Appl Environ. Microbiol. 73, 6144-6149.

Valdés, J., Pedroso, I., Quatrini, R., et al., 2008. *Acidithiobacillus ferrooxidans* metabolism: from genome sequence to industrial applications. BMC Genomics 9, 597-597.

Volant, A., Bruneel, O., Desoeuvre, A., et al., 2015. Diversity and spatiotemporal dynamics of bacterial communities: physicochemical and other drivers along an acid mine drainage. FEMS Microbiol. Ecol. 90, 247-263.

Wang, L., Ji, B., Hu, Y., et al., 2017. A review on in situ phytoremediation of mine tailings. Chemosphere, 184, 594-600.

Wani, P.A., Khan, M.S., 2014. Screening of multiple metal and antibiotic resistant isolates and their plant growth promoting activity. Pak. J. Biol. Sci. 17, 206-212.

Watanabe, T., Kojima, H., Fukui, M., 2014. Complete genomes of freshwater sulfur oxidizers

662 *Sulfuricella denitrificans* skB26 and *Sulfuritalea hydrogenivorans* sk43H: genetic insights
663 into the sulfur oxidation pathway of *Betaproteobacteria*. Syst. Appl. Microbiol. 37,
664 387-395.
665 Wu, R., Cheng, X., Han, H., 2019. The effect of forest thinning on soil microbial community
666 structure and function. Forests 10, 352-368.
667 Yao, M., Lian, B., Dong, H., et al., 2013. Iron and lead ion adsorption by microbial flocculants in
668 synthetic wastewater and their related carbonate formation. Acta. Sci. Circum. 25,
669 2422-2428.
670 Ye, S., Zeng, G., Wu, H., et al., 2017. Biological technologies for the remediation of
671 co-contaminated soil. Crit. Rev. Biotechnol. 37, 1-15.
672 Zhang, M., Liu, X., Li, Y., et al., 2017. Microbial community and metabolic pathway succession
673 driven by changed nutrient inputs in tailings: effects of different nutrients on tailing
674 remediation. Sci. Rep-UK 7, 474-480.
675 Zheng, S., Zhang, M., 2011. Effect of moisture regime on the redistribution of heavy metals in
676 paddy soil. J. Environ. Sci. 23, 434-443.
677 Zhou, J., He, W., Wang, W., et al., 2009. Molecular cloning and characterization of an
678 ATP-binding cassette (ABC) transmembrane transporter from the white shrimp Litopenaeus
679 vannamei. Comp. Biochem. Physiol. Part C, 150, 450-458.
680 Zhou, J., Ye, D., Shen, L., et al., 2016. Temperature mediates continental-scale diversity of
681 microbes in forest soils. Nat. Commun. 7, 12083-12093.
682 Zhu, X., Yao, J., Wang, F., et al., 2018. Combined effects of antimony and sodium
683 diethyldithiocarbamate on soil microbial activity and speciation change of heavy metals.
684 Implications for contaminated lands hazardous material pollution in nonferrous metal
685 mining areas. J. Hazard. Mater. 349,160-167.

686 **Figure legends**

687 **Fig. 1 Map showing the sampling site and field photographs of bio-treatment.** Samples
688 were collected during four different months representing different seasons of the year.
689 At each sampling site (●), nine cores (●, the black doted box in the lower left) were
690 collected and mixed for geochemical parameters and bacterial community analysis.
691 Beijing city is denoted by red star (★). Samples were collected at four different seasons.
692 a, b, c, d, and e represented the tailings samples before bio-treatment, and at the first
693 month, fourth month, eighth month, and twelfth month, respectively.

694 **Fig. 2 Distribution of metal(loid)s (such as As, Cd, Sb, and Zn) among the BCR**
695 **fractions during the bio-treatment.** BCR1, exchangeable fraction; BCR2, fraction of
696 reducible fraction; BCR3, oxidizable fraction; BCR4, residual fraction.

697 **Fig. 3 Identification of mineral products by XRD during bio-treatment.**

698 **Fig. 4 Bacterial composition at the phylum (a) and the genus (b) levels.** Only phyla with
699 relative abundance > 1% in at least one tailing sample are shown. Relative abundance
700 of sulfate reducing bacteria (SRB, c) and sulfur oxidizing bacteria (SOB, d) in both
701 surface and subsurface layers.

702 **Fig. 5 Spearman correlations between bacterial OTUs and geochemical parameters.** The
703 analysis includes the OTUs (genus level) of the top 50 most abundant genera
704 considering surface and subsurface layers. Positive correlations are in red, negative
705 correlations are in blue. Non-significant correlations are shown in white. * $0.01 < p \leq 0.05$; ** $0.001 < p \leq 0.01$; *** $p \leq 0.001$.

707 **Fig. 6 Correlation of KEGG pathways with treatment samples (a) and with geochemical**

708 **parameters (b).** a) Detrended correspondence analysis (DCA), based on KEGG
709 pathways of bacteria communities in tailing during bio-treatment. Red triangles
710 correspond to the samples, and diamonds to KEGG pathways. The ellipse shows the
711 subsurface samples that cluster together, discussed in the text. Particularly, the black
712 diamonds inside the ellipse of left panel represent the KEGG pathways associated with
713 "electron transfer carriers" and "organic degradation functions". As well, the black
714 diamonds outside the ellipse of left panel represent the KEGG pathways of "transport"
715 and "DNA repair and recombination". b) Canonical correlation analysis (CCA) of
716 KEGG pathways (triangles) and selected geochemical factors (arrows) in tailings sites.
717 The black triangles represent the KEGG pathways of transcription related proteins.

718 **Fig. 7 PICRUSt of sulfur KEGG pathway prediction.** (a) A simplified scheme of the sulfur
719 cycle based on the KEGG sulfur metabolism pathway. (b) Abundance of sulfur KEGG
720 pathway genes in the different tailing samples. The percentage of associated gene reads
721 were predicted using PICRUSt based on KEGG database.

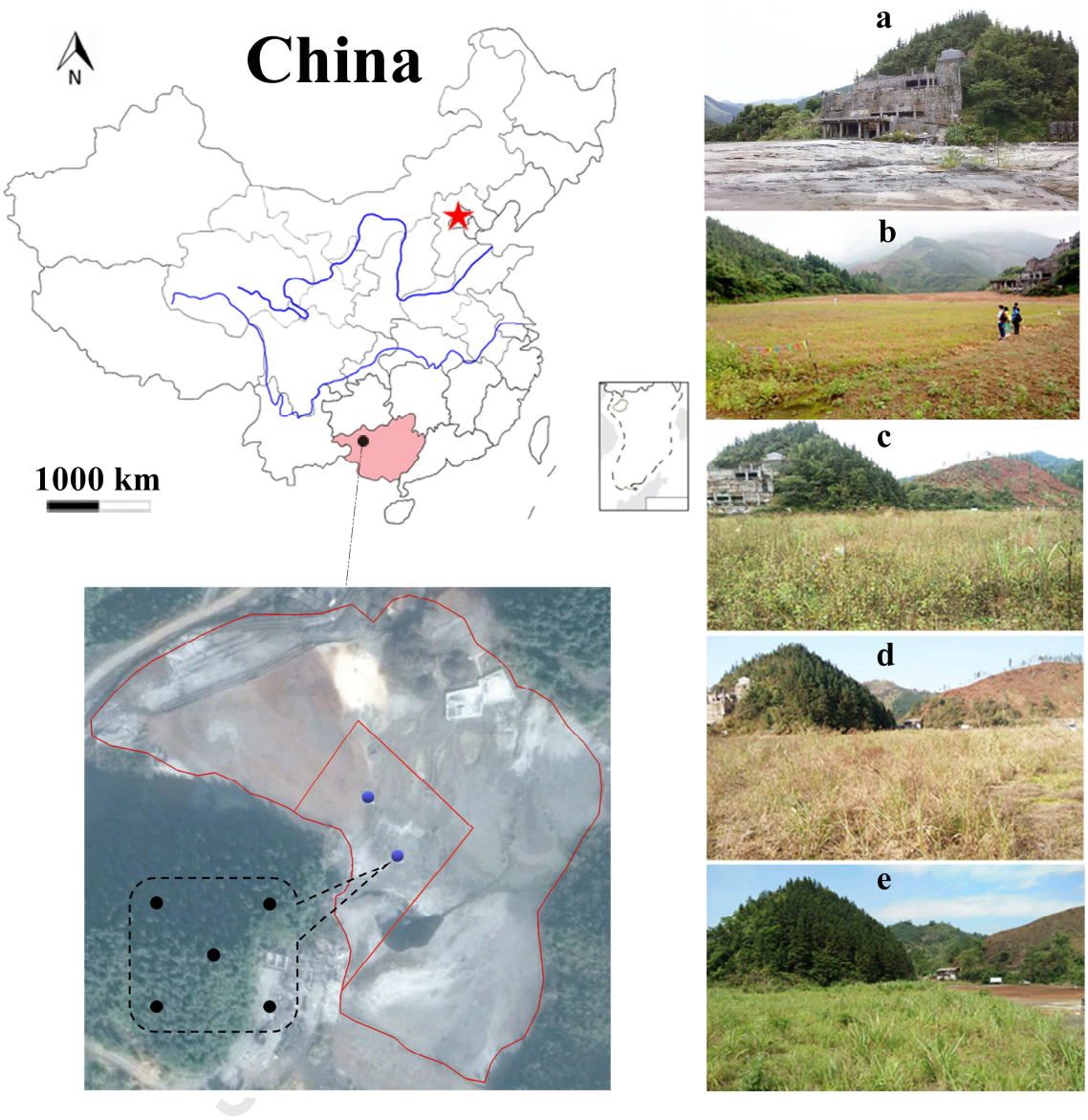
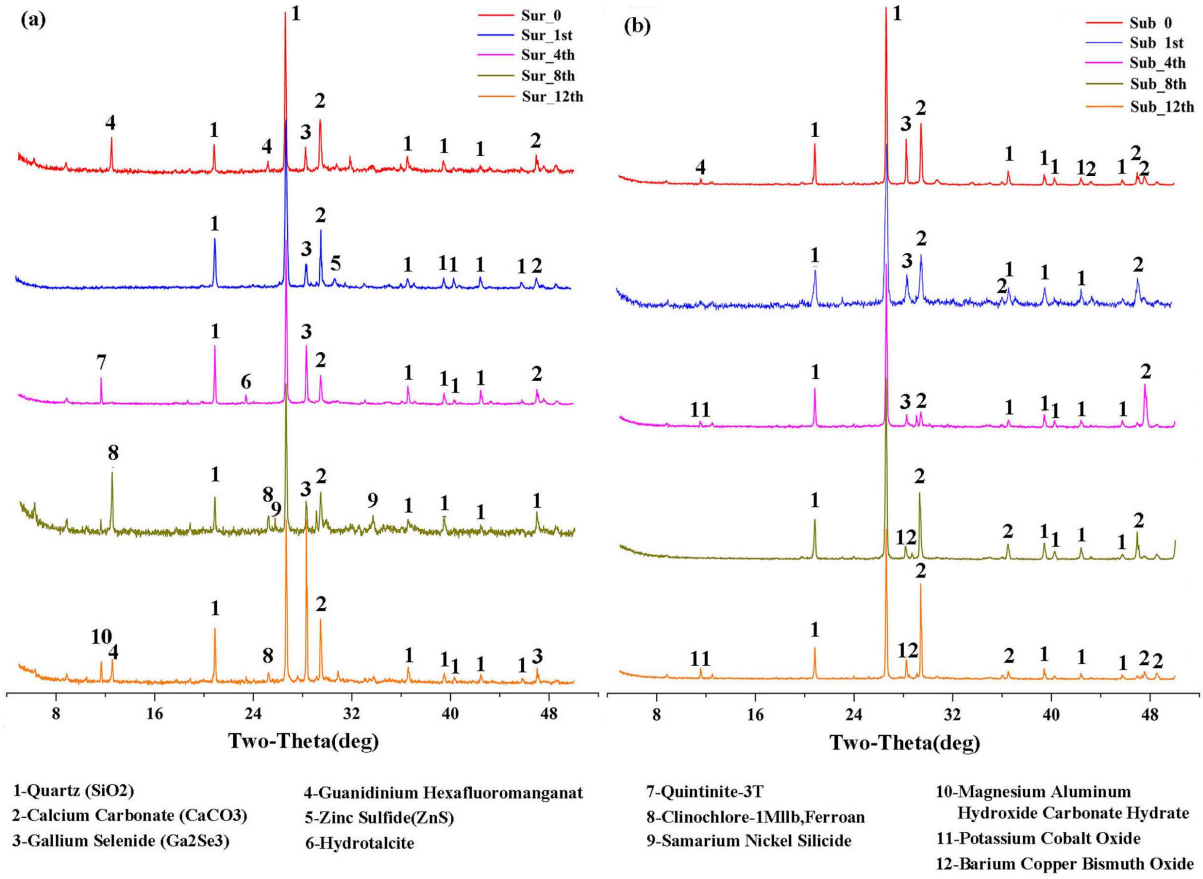


Fig. 1 Map showing the sampling site and field photographs of bio-treatment. Samples were collected during four different months representing different seasons of the year. At each sampling site (●), nine cores (●, the black doted box in the lower left) were collected and mixed for geochemical parameters and bacterial community analysis. Beijing city is denoted by red star (★). Samples were collected at four different seasons. a, b, c, d, and e represented the tailings samples before bio-treatment, and at the first month, fourth month, eighth month, and twelfth month, respectively.

Fig. 2 Distribution of metal(loid)s (such as As, Cd, Sb, and Zn) among the BCR fractions during the bio-treatment. BCR1, exchangeable fraction; BCR2, fraction of reducible fraction; BCR3, oxidizable fraction; BCR4, residual fraction.



733

734 **Fig. 3** Identification of mineral products by XRD during bio-treatment.

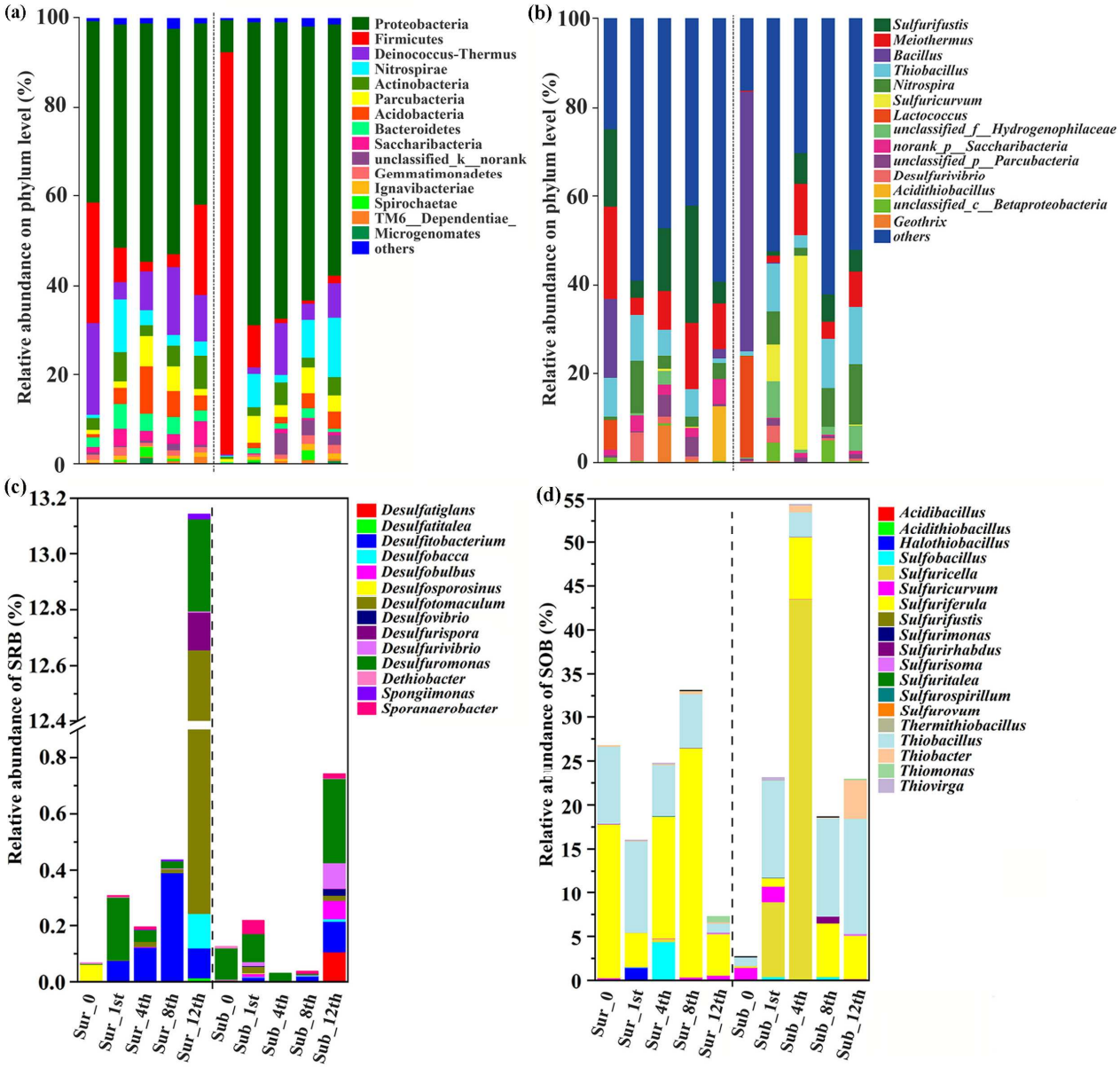
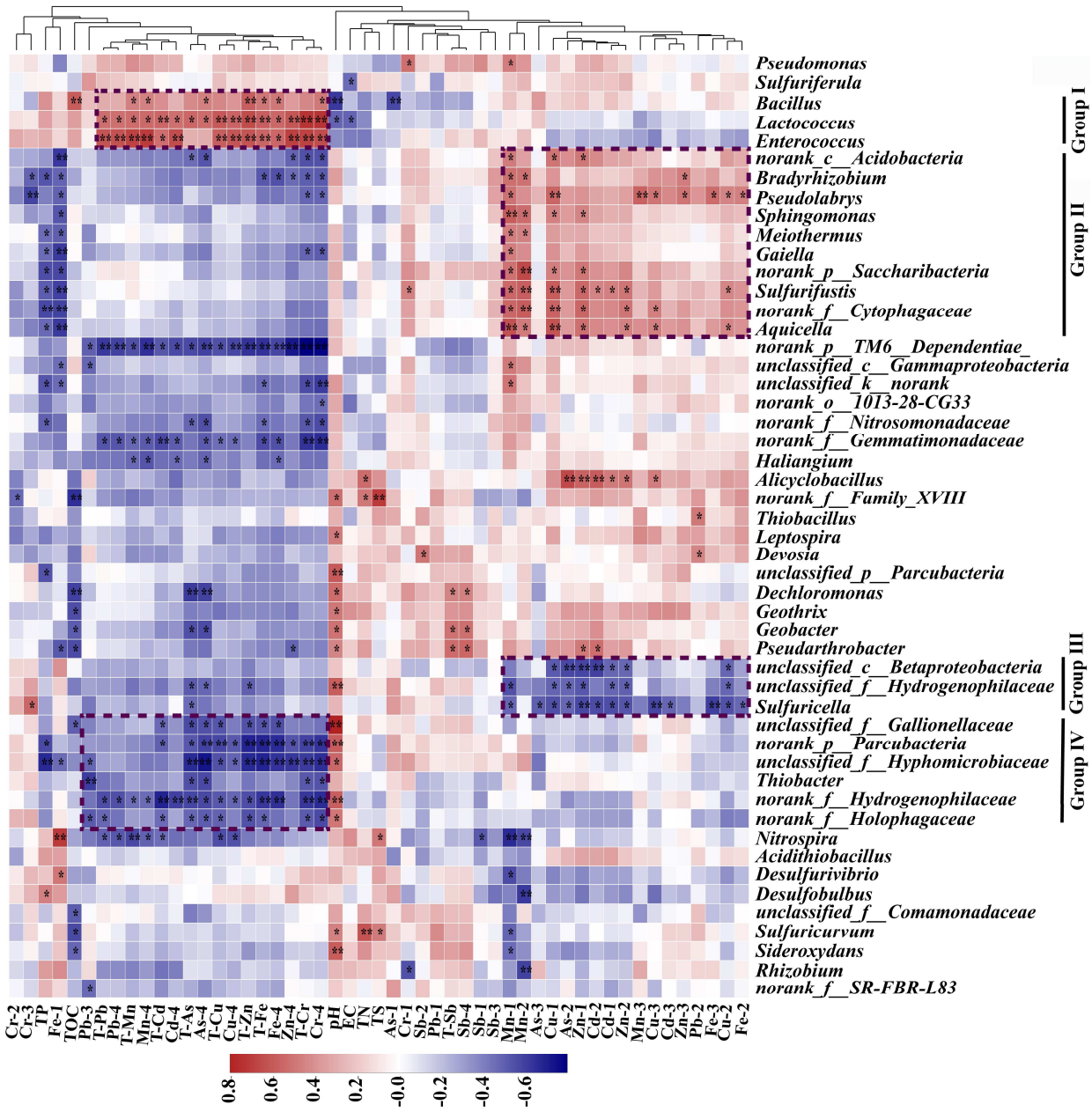
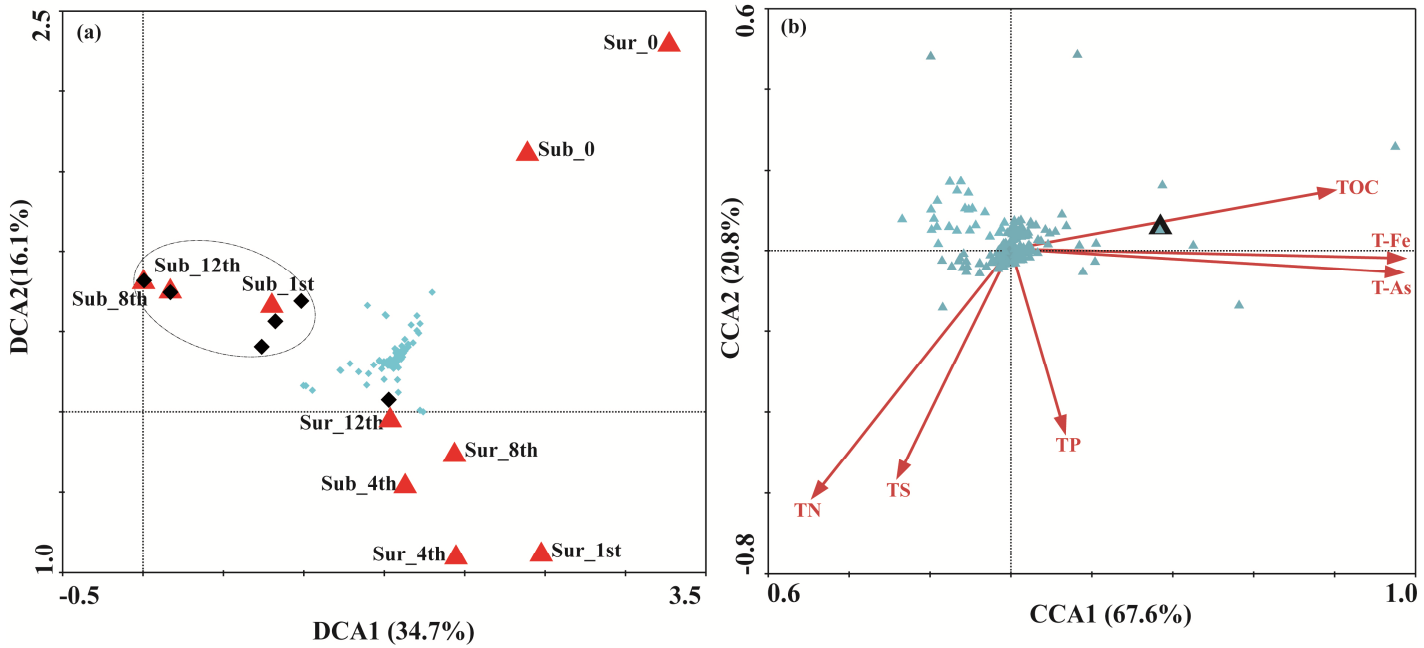


Fig. 4 Bacterial composition at the phylum (a) and the genus (b) levels. Only phyla with relative abundance > 1% in at least one tailing sample are shown. Relative abundance of sulfate reducing bacteria (SRB, c) and sulfur oxidizing bacteria (SOB, d) in both surface and subsurface layers.



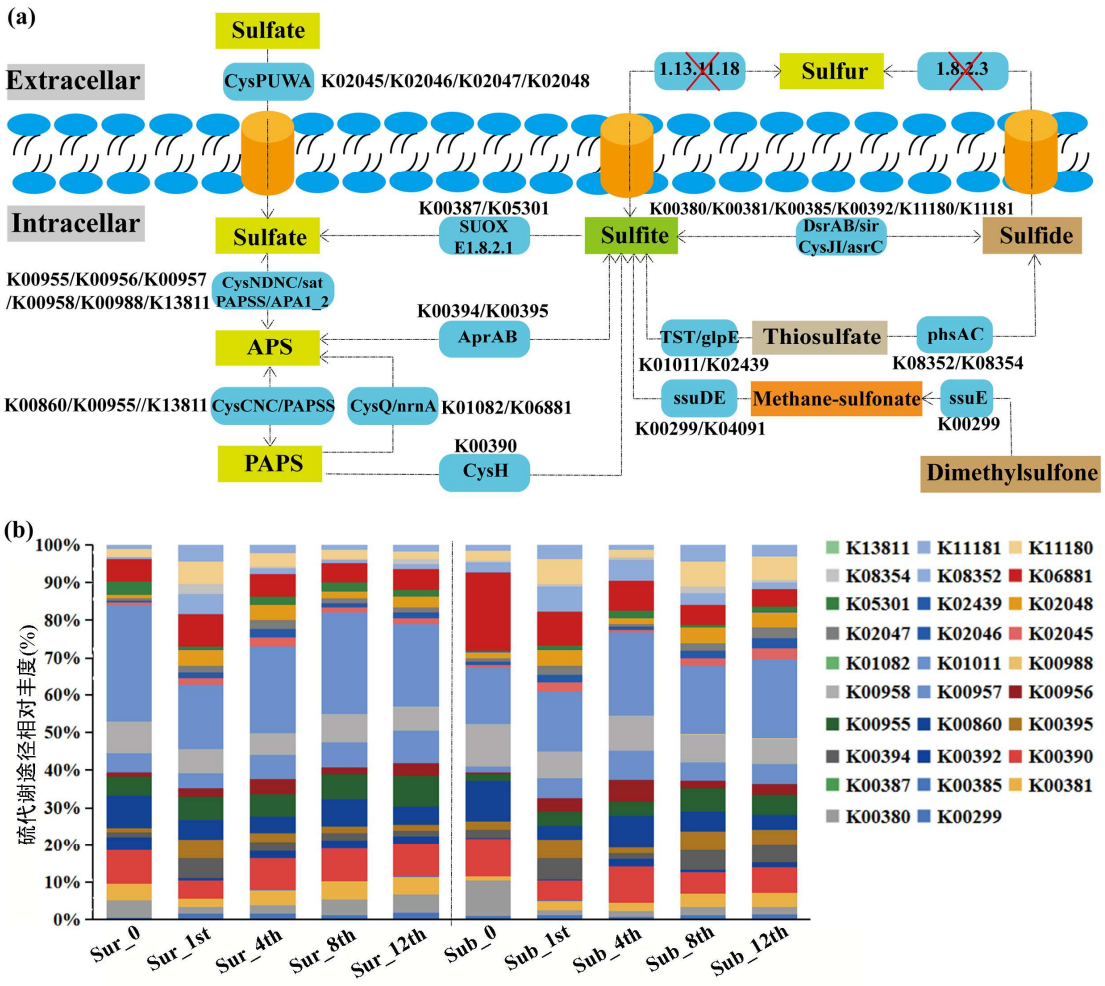
740

741 **Fig. 5 Spearman correlations between bacterial OTUs and geochemical parameters.** The
742 analysis includes the OTUs (genus level) of the top 50 most abundant genera
743 considering surface and subsurface layers. Positive correlations are in red, negative
744 correlations are in blue. Non-significant correlations are shown in white. * $0.01 < p$
745 ≤ 0.05; ** $0.001 < p \leq 0.01$; *** $p \leq 0.001$



746

747 **Fig. 6 Correlation of KEGG pathways with treatment samples (a) and with geochemical parameters (b).** a) Detrended correspondence
748 analysis (DCA), based on KEGG pathways of bacteria communities in tailing during bio-treatment. Red triangles correspond to the
749 samples, and diamonds to KEGG pathways. The ellipse shows the subsurface samples that cluster together, discussed in the text.
750 Particularly, the black diamonds inside the ellipse of left panel represent the KEGG pathways associated with electron transfer carriers and
751 organic degradation functions. As well, the black diamonds outside the ellipse of left panel represent the KEGG pathways of Transport and
752 DNA repair and recombination. b) Canonical correlation analysis (CCA) of KEGG pathways (triangles) and selected geochemical factors
753 (arrows) in tailings sites. The black triangles represent the KEGG pathways of transcription related proteins.



754

755 **Fig. 7 PICRUSt of sulfur KEGG pathway prediction.** (a) A simplified scheme of
756 the sulfur cycle based on the KEGG sulfur metabolism pathway. (b) Abundance
757 of sulfur KEGG pathway genes in the different tailing samples. The percentage
758 of associated gene reads were predicted using PICRUSt based on KEGG
759 database.

Highlight

- The in-situ bio-treatment of nonferrous metal(loid) tailings was effective
- The treatment contribute to the transformation of metal(loid)s
- The added consortium coalesce with indigenous communities and colonize the tailings
- Bacterial communities were enhanced during bio-treatment
- PICRUSt predicted genes for using inorganic and organic sulfur compounds as S sources

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Dear Editor:

We wish to submit the attached manuscript entitled:

"**Bacterial shifts during in-situ mineralization bio-treatment to non-ferrous metal(loid) tailings**"

by Liu *et al.*

for consideration in *Environmental Pollution*.

On behalf of my co-authors, I declare that the described work is original research that has not been published previously, and is not under consideration for publication elsewhere (in whole or in part). No conflict of interest exists in the submission of this manuscript. All the authors listed have approved the attached manuscript.

We appreciate your consideration of our manuscript, and we look forward to a favorable response. If you have any queries, please contact me. Thank you and with best regards.

Yours sincerely,

Jun Yao