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1 **Bacterial diversity in typical abandoned multi-contaminated nonferrous**
2 **metal(loid) tailings during natural attenuation**

3
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36 **Abstract**

37 Abandoned nonferrous metal(loid) tailings sites are anthropogenic, and represent unique
38 and extreme ecological niches for microbial communities. Tailings contain elevated and toxic
39 content of metal(loid)s that had negative effects on local human health and regional
40 ecosystems. Microbial communities in these typical tailings undergoing natural attenuation
41 are often very poorly examined. The diversity and inferred functions of bacterial
42 communities were examined at seven nonferrous metal(loid) tailings sites in Guangxi (China),
43 which were abandoned between 3 and 31 years ago. The acidity of the tailings sites rose over
44 31 years of site inactivity. *Desulfurivibrio*, which were always coupled with sulfur/sulfide
45 oxidation to dissimilate the reduction of nitrate/nitrite, were specific in tailings with 3 years
46 abandonment. However, genus beneficial to plant growth (*Rhizobium*), and iron/sulfur-
47 oxidizing bacteria and metal(loid)-related genera (*Acidiferrobacter* and *Acidithiobacillus*)
48 were specific within tailings abandoned for 23 years or more. The increased abundance of
49 acid-generating iron/sulfur-oxidizing and metal(loid)-related bacteria and specific bacterial
50 communities during the natural attenuation could provide new insights for understanding
51 microbial ecosystem functioning in mine tailings. OTUs related to *Sulfuriferula*, *Bacillus*,
52 *Sulfurifustis*, *Gaiella*, and *Thiobacillus* genera were the main contributors differentiating the
53 bacterial communities between the different tailing sites. Multiple correlation analyses
54 between bacterial communities and geochemical parameters indicated that pH, TOC, TN, As,
55 Pb, and Cu were the main drivers influencing the bacterial community structures. PICRUSt
56 functional exploration revealed that the main functions were related to DNA repair and
57 recombination, important functions for bacterial adaptation to cope with the multi-
58 contamination of tailings. Such information provides new insights to guide future
59 metagenomic studies for the identification of key functions beyond metal-
60 transformation/resistance. As well, our results offers novel outlooks for the management of

61 bacterial communities during natural attenuation of multi-contaminated nonferrous metal(loid)
62 tailings sites.

63

64 **Keywords:** bacterial community succession; metal(loid)s; natural attenuation;
65 nonferrous metal(loid) tailings

66 **1. Introduction**

67 Mine tailings repositories are unwanted and uneconomic materials from the
68 mineral processing deposited exposure in the air. Tailings often contain elevated
69 concentrations of metal(loid)s, which are potentially toxic (Lecumberri-Sanchez et al.,
70 2014; Hudson-Edwards, 2016). Abandoned nonferrous metal(loid) tailings (i.e.,
71 facilities having no operator or successor) have received considerable attention around
72 the world because they represent a risk for the environment and human health
73 (Aleksandrovskii et al., 2015; COM, 2016). Guangxi (China) is one of the
74 predominant nonferrous mining areas in the world (Rademaekers et al., 2011). It is a
75 karst landform with many ecologically sensitive areas and is located upstream of the
76 Pearl River Basin (China's third longest river and second largest by volume) (Wang et
77 al., 2007). In Guangxi, different mining activities release waste that results in the
78 formation of tailings with heterogeneous composition containing high concentrations
79 of metal(loid)s and flotation reagents (Liu et al., 2018). Such level of multi-
80 component contamination is probably more serious than many other areas in the
81 world (Zhu et al., 2018).

82 Biotic and abiotic processes modify the speciation of metal(loid)s and physical-
83 chemical characteristics in tailings (Ye et al., 2017a; Ye et al., 2017b), which facilitate
84 metal(loid)s permeation into soil, surface runoff, and air transportation (Deng et al.,
85 2009; COM, 2016; Jiang et al., 2016; Yi et al., 2016; Yuan and Liu, 2016). Natural
86 attenuation occurs when natural processes (including pedogenesis) are managed to
87 recover an ecosystem to a point where the original fauna and flora are replicated
88 (Clewell, 2000). Natural attenuation is more economical for re-purposing tailings
89 compared to physical remediation, reclamation processes, or activated biochar
90 addition on remediation (Lima et al., 2016, Ye et al., 2019). However, natural

91 attenuation is a slow process that can take more than 100 years (Bradshaw, 1997;
92 Ciarkowska et al., 2016; Lima et al., 2016). With time, microbial colonization follows
93 the modification of physical-chemical parameters (Giloteaux et al., 2013) due to bio-
94 geochemical processes (Haferburg and Kothe, 2007). Knowledge of the colonization
95 of microbial communities during natural attenuation in mine tailings is limited
96 (Bruneel et al., 2008; Volant et al., 2014; Zhan and Sun, 2014; Chao et al., 2016),
97 particularly in the Guangxi area where only three tailings sites (Pb-Zn and Mn sites)
98 have been investigated (Jin et al., 2015; Liu et al., 2014; Li et al., 2015). Recent
99 results show that the distribution of bacterial communities in Guangxi nonferrous
100 metal(loid)s tailings was best correlated with the combination of pH, Cu, Pb, and Mn,
101 suggesting that these parameters influence the organization of bacterial communities
102 (Liu et al., 2018). However, the modification of bacterial communities during natural
103 attenuation in undisturbed nonferrous metal(loid) tailings is still uninvestigated.

104 To address this research gap, we examined nonferrous mine tailings sites with
105 different periods of abandonment (from 3 to 31 years) in the Guangxi mining area
106 (Fig. 1 and Table S1), which have different geochemical characteristics. We
107 hypothesize that temporal changes in biogeochemical factors, and bacterial diversity
108 and metabolic functions are part of the natural attenuation process occurring in these
109 tailings. The present study offers the possibility to examine the ecological changes,
110 such as primary succession of microbial communities during natural attenuation. The
111 objectives of this study were to: (1) investigate the structure of the microbial
112 community (by MiSeq sequencing of 16S rRNA genes) and predict the metabolic
113 functions in mine tailings, and (2) analyze the combined effects of geochemical
114 factors including pH, total organic carbon (TOC), total nitrogen (TN), total
115 phosphorus (TP), and metal(loid)s content on the bacterial community structure. This

116 study will provide a better understanding of microbial variations in nonferrous mine
117 tailings, and useful information for the management of bacterial resources during
118 natural attenuation of nonferrous metal(loid) tailings.

119

120 **2. Materials and methods**

121 **2.1 Site description and sampling**

122 Sampling was performed around Hechi City of Guangxi (China) (Fig. 1), which
123 has a subtropical monsoon climate (Bi et al., 2016). Seven tailings sites with different
124 composition and ages (ranging from three to 31 years old) were sampled to evaluate
125 changes in bacterial communities during the natural attenuation (Fig. 1). The
126 abandonment periods were determined using tailings pond records from the local
127 Environmental Protection Agency. The types of tail sand at these seven tailings sites
128 were mainly from Sb, Pb-Zn, and Sn mining and smelting industries (Table S1).
129 These tailings were not treated with amendments or any remediation technology.
130 There was no visible plant growth in all of the studied sites.

131 Surface samples (0-10 cm) with 3-10 subsamples for each site were collected in
132 June 2016, using a wooden shovel according to EU international guideline (Hansen et
133 al., 2007). All samples were directly placed into plastic pipes in cooler boxes (at 4°C)
134 and transported to laboratory at the University of Science and Technology Beijing
135 within 2 d of sampling. After thorough homogenization, the samples were split into
136 two parts. Approximately 500 g for each sample was then stored at -20°C until DNA
137 extraction. The remaining samples were used for geochemical analyses, and were
138 stored at 4°C.

139

< insert Fig. 1 >

140

141 2.2 Analysis of geochemical factors

142 Samples were air-dried and analyzed according to the technical specifications for
143 soil analysis for determination of pH, total organic carbon (TOC), total nitrogen (TN),
144 and total phosphorus (TP) as defined by the China National Agricultural Technology
145 Extension Center (2006). The operating conditions for the TOC solid sample module
146 (SSM-5000A, Shimadzu) were: the oven temperature, 680°C, and the gas flow of
147 high purity oxygen in TOC-V and SSM-5000A section, 150 and 500 mL/min,
148 respectively. Samples were extracted with a solution of nitric, hydrochloric, and
149 hydrofluoric acids (5:3:2, v/v/v) in a microwave unit to determine the total
150 metal(loid)s content (T-M). The acid soluble fraction of metal(loid)s (H-M) were
151 analyzed using the Chinese method HJ/T299-2007. This fraction of H-M represents
152 the leachable compartment that could be released into the environment. The leaching
153 solution was prepared by adding 0.09 mL of a solution of sulfuric and nitric acids (2:1,
154 v/v) and taken up to 1 L with ultra-pure water (Milli-Q Academic Lab Water System,
155 Millipore, USA). Certified reference materials of soil samples (GBW 07405 (GSS-5))
156 and polymetallic ore samples (GBW 07162 (GSO-1)) were used for quality control.
157 The limit of detection (LOD) for T-Ms was $> 0.10 \times 10^{-3}$ mg/kg (Liu et al., 2018) and
158 for H-Ms was > 0.10 mg/kg (Wang, 2018), according to the China Environmental
159 Monitoring Technical guideline (HJ 168-2010). The recoveries were between 85% -
160 110%. Samples were placed into 20 mL of leaching solution (pH 3.20 ± 0.05) and
161 shaken for about 20 h. Induced coupled plasma optical emission spectrometry (ICP-
162 OES) (iCAP 7000 SERIES, Thermo Scientific) was used to determine the
163 metal(loid)s concentrations. The operating conditions were: auxiliary gas flow, 0.5
164 L/min; plasma gas stable time, 10 min; ICP RF power, 1150 W; and pump rate, 45
165 rpm. All the samples were sieved at 100-mesh size (0.149 mm, US standard) to

166 determine the geochemical factors. The analyses were performed in duplicate to
167 evaluate precision.

168

169 **2.3 MiSeq sequencing and data processing**

170 Genomic DNA was extracted using the SoilGen DNA Kit (CWBio, Beijing,
171 China). DNA extraction kits allow to obtain high-quality DNA for PCR amplification
172 and sequencing (Bordenave et al., 2004; Bordenave et al., 2008). The potential
173 damage during DNA extraction are prevented by diluting metal(loid)s and eliminating
174 them in the first steps of the procedure allowing molecular analyses of highly
175 contaminated samples such as acid mine drainage (Giloteaux et al., 2010). The
176 universal primer set 338F/806R amplified the V3-V4 region of the bacterial 16S
177 rRNA gene, and an 8 bp-tag was used for the sample identification (Liu et al., 2016).
178 Polymerase chain reaction (PCR) amplification (20 μ L) was conducted in triplicate
179 and contained 10 ng DNA template, 4 μ L of 5 \times FastPfu Buffer, 2 μ L of 2.5 mM
180 dNTPs, 0.2 μ M of each primer, 0.4 μ L FastPfu Polymerase, 0.2 μ L bovine serum
181 albumin, and double-distilled water. PCR was started with an initial denaturation (3
182 min at 95°C), followed by 28 cycles of denaturation (30 s at 95°C), annealing (30 s at
183 55°C), and extension (45 s at 72°C), and a final extension (10 min at 72°C).
184 Sequencing using a MiSeq platform was performed at a commercial facility
185 (Shanghai Majorbio Bio-Pharm Technology Corporation, Shanghai, China).

186 All the 16S raw data were trimmed and filtered using Trimmomatic software
187 (Manual v0.32), by trimming the average base quality region below 20 bp (Trujillo et
188 al., 2014). The paired-end reads were merged using FLASH software. The sequences
189 assigned to chloroplasts, mitochondria or eukaryotes were removed in the
190 pretreatment of raw reads. Bacterial operational taxonomic units (OTUs) were

191 clustered with 97% similarity using Usearch version 7.0 (<http://drive5.com/uparse/>)
192 based on Silva Release128 (<http://www.arb-silva.de>). Taxonomy was assigned to
193 OTUs using Qiime (http://qiime.org/scripts/assign_taxonomy.html) and ribosomal
194 database project pipeline classification algorithm with a 70% confidence threshold
195 (Nakayama, 2010). Alpha diversity indices (ace, Shannon, Simpson evenness and
196 Boneh) and hierarchical clustering were calculated with Qiime. Circos-0.67-7 was
197 used to perform the bacterial composition of each sample, and the distribution ratio of
198 dominant bacteria in different samples. Functional prediction of bacterial
199 communities was determined using PICRUSt, a well-documented tool to assign
200 sequencing information based on 16S input data to reveal the functions encoded in
201 bacterial communities (Langille et al., 2013; Mchardy et al., 2013). Kyoto
202 Encyclopedia of Genes and Genomes (KEGG) databases (e-value cut-off 10^{-5}) were
203 used for functional annotation and metabolism analyses (Mchardy et al., 2013;
204 Kanehisa et al., 2014; Vrutika et al., 2016). The weighted nearest sequenced taxon
205 index (NSTI) was calculated to assess the accuracy of PICRUSt analysis (Langille et
206 al., 2013).

207

208 **2.4 Statistical analyses**

209 One-way ANOVA was applied to test the differences of geochemical factors; a
210 level of $p < 0.05$ was considered significant. The relationships between geochemical
211 factors and alpha-diversity indexes were analyzed using Spearman correlation (SPSS
212 v21). SIMPER analysis based on Bray-Curtis similarity measurement was used to test
213 microbial differences in the tailings. Non-metric multidimensional scaling (NMDS)
214 and distance-based redundancy analysis (db-RDA) analysis were conducted to test the
215 correlation between bacterial communities and geochemical factors of tailings sites

216 based on weighted normalized unifrac distance algorithm. The significance of
217 geochemical factor was tested with Monte Carlo permutations (permu = 999).
218 Correlations of each bacterial community and each geochemical factor were
219 calculated with p -values < 0.05 and plotted as a heatmap. Network analysis was used
220 to reflect the relationship between tailings sites and genera. After detection for genera
221 (Gephi software), each module was represented by network correlation shared values
222 of abundance profile by using modularity analysis. BIOENV analysis was used to
223 determine the combined effects of geochemical factors on the metabolic pathways of
224 bacterial communities. All the analyses were done using R software (v 3.4.1) unless
225 otherwise stated.

226

227 **3. Results and discussion**

228 **3.1 Geochemical parameters of nonferrous mine tailings samples**

229 The pH in the seven studied tailings (Table S1) decreased with the period of
230 abandonment, notably around pH 7.3 at the youngest sites (from T_3Y to T_15Y),
231 weakly acidic (pH 6.4) at site T_23Y, and extremely acidic (pH 2.6) at site T_31Y.
232 This is consistent with other reports showing that the pH decreased in mine tailings
233 undergoing more than 30 years of natural attenuation (Huang et al, 2011; Zhan and
234 Sun, 2014; Ciarkowska et al., 2016). The gradual acidification of tailings could be
235 caused by microbial mediated oxidative dissolution of pyrite (FeS_2) and other sulfide
236 minerals exposed to air and water during natural attenuation (Huang et al., 2016). The
237 low nutrient concentrations of C/N/P could nevertheless support the observed growth
238 of microorganisms (described below), at least during the early phases of natural
239 attenuation (Oudjehani, et al., 2002).

240 As expected at nonferrous metal(loid) tailings sites, the total metal(loid)s

241 contents (T-M) were higher compared with the reported tailing sites in other regions
242 around the world (Alakangas et al. 2010; Giloteaux et al., 2013; Bruneel et al., 2017).
243 The total arsenic content (T-As) was significantly correlated with total contents of Cd,
244 Cr, and Zn (Spearman rho = from -0.82 to 0.86, $p < 0.04$; Table 1). Similar
245 correlations between As, Zn, and Cd have been reported at mining- and alumina-
246 contaminated soils (Zacháry et al., 2015).

247 The acid soluble fraction of metal(loid)s (H-M), representing the leachable
248 fraction, was generally higher in tailing sites with 31 years abandonment (Table S1).
249 Significant differences were also observed among the tailings sites, particularly for H-
250 Cd, H-Cr, and H-Cu (ANOVA, $p < 0.05$, Table S1). Acid-soluble fraction in surface
251 tailings represents the elements releasable that can migrate laterally or downwards via
252 biotic and abiotic processes (Alakangas et al. 2010; Volant et al., 2014). The
253 increased concentration of H-Ms and decreased pH with the age of abandonment
254 suggested that a release of these metal(loid) was increasing over time, which was
255 reported for other tailings (Walder and Chavez, 1995; Shu et al., 2001).

256 < Insert Table 1 >

257

258 **3.2 Microbial community diversity and composition of nonferrous mine tailings**

259 To determine the bacterial dynamics in abandoned tailings, a total of 1,481
260 bacterial OTUs were identified of all quality 16S rRNA bacterial sequences (265,487
261 in total) after removing singletons and chimeric sequences (Tables S2 and S3). These
262 1,481 OTUs represent a high coverage (99.8 ± 0.1 %, Table S2), indicating that the
263 sequencing data could reflect the vast majority of microbial diversity in the real
264 environment. Furthermore, the Shannon diversity indexes were between 2.88 - 4.80,
265 which were similar to an earlier report of a Pb-Zn mining site (Chen et al., 2013).

266 Bacterial diversity showed a decreasing relationship with the age of abandonment
267 (Table S2 and Fig. S1). Nevertheless, the bacterial richness (Table S2) was up to eight
268 times higher than that reported in an abandoned Pb-Zn mine tailing site (Epelde et al.,
269 2015), but no significant trend could be observed with abandonment age. This could
270 be due to some more fundamental properties of tailings such as tailing matrix, mineral
271 phases, and chemical composition since the tailings are from different types of mining
272 and smelting industries (Fig. S1). In contrast, Chao et al. (2016) reported clear
273 differences, as well as a time-dependent increase, in bacterial richness among REE
274 (Rare Earth Elements) tailings sites that were abandoned for 3, 6, and 10 years. The
275 richness of bacterial communities had a significant correlation with PD (phylogenetic
276 diversity, $\rho = 0.972$, $p = 0.0002$; Table 1), which was statistically correlated with
277 TP ($\rho = 0.79$, $p = 0.036$; Table 1). Overall, our results indicated a high genetic
278 diversity in the Guangxi nonferrous mine tailings sites.

279 Over 98% of the OTUs could be assigned to a taxonomic phylum with 70%
280 confidence; while over 56% of sequences were generally identified as no-rank or
281 unclassified genera (Table S3), which was lower than that in a Sb-rich tailings dump
282 (Xiao et al., 2016). Coupled with the high coverage and sequencing depth (99.8 ± 0.1
283 %, Table S2), this low assigned rate suggested these tailings sites had vast
284 unidentified populations and microbial resources. These results were consistent with
285 an earlier report showing that 58% of the sequences in a vanadium- and 17% in a
286 gold- mine water from a South African mine, could not be assigned to a particular
287 phylum (Keshri et al., 2015). Specifically, the shared phyla of tailings sites were
288 Proteobacteria, Firmicutes, and Actinobacteria, accounting for 76% of total
289 microbial community (Fig. 2), which confirmed recent studies by Liu et al. (2018) at
290 abandoned nonferrous metal tailings sites, and by Chao et al (2016) at an abandoned

291 REE tailings facility. These studies reported the same or similar dominant bacterial
292 communities (at the phylum level), despite differences in pH and geochemical factors
293 of the tailings.

294 < insert Fig. 2 >

295 Among the total 507 genera identified from the seven tailings sites, 31 shared
296 genera (relative abundance > 1% of total sequences at least in one tailing site) had
297 different abundance among the seven tailings sites (Table S4). The differences
298 observed were mainly due to different abundances of *Sulfuriferula*, *Bacillus*,
299 *Sulfurifustis*, *Gaiella*, and *Thiobacillus* (Table 2). *Sulfurifustis* and *Thiobacillus* were
300 the most abundant genera shared by tailings sites that were abandoned for < 15 years
301 (Table S4), indicating that these two genera may have contributed to sulfur- and iron
302 oxidation at these sites. To date only three studies have detected *Sulfurifustis* that
303 could be involved in sulfur oxidation (Kojima, et al., 2015; Kojima, et al., 2016;
304 Umezawa, et al., 2016). *Thiobacillus* is capable of iron/sulfur-oxidization and
305 carbon/nitrogen fixation in the early stages of the acidification processes of tailings
306 (Yamanaka, 1996; Huang et al., 2016). *Ralstonia*, the most abundant genus in tailing
307 sites abandoned for up to 23 years (29%; Table S4), is a ubiquitous inhabitant of soil,
308 freshwater and even ultrapure water in industrial systems (Gan, et al., 2012). This
309 genus carries metal resistant genes, such as *czc* (resistance to cadmium, zinc, and
310 cobalt) and *ncc* (cobalt and cadmium) (Mergeay, et al., 2010). *Acidithiobacillus* was
311 most abundant at the extremely acidic tailing site (pH = 2.0, Table S1) abandoned for
312 31 years (accounting for 29% of total communities, Table S4). This genus was found
313 to be able of carbon/nitrogen fixation, iron/sulfur oxidation, and arsenic oxidation
314 (Huang et al., 2016). *Acidithiobacillus* may play an important role in the iron and
315 arsenic oxidation in late acidification of the present tailing sites as reported for other

316 tailings sites (Bruneel et al., 2005; Jorge et al., 2008; Huang et al., 2016).

317 < insert Table 2 >

318

319 **3.3 Structure of tailings microbial communities**

320 Although the tailings sites shared bacterial populations, the whole bacterial
321 structures were different (Fig. 3). Correlation analysis of geochemical factors and
322 bacterial structure of tailings revealed four cluster groups: i) OTUs in T_4Y, T_8Y,
323 and T_15Y correlated with TOC, ii) OTUs in T_6Y correlated with TN, T-As, and H-
324 Pb, iii) OTUs in T_23Y correlated with pH and H-Sb, and iv) OTUs in T_31Y
325 correlated with pH and TP (Fig. 3). These findings were consistent with earlier studies
326 indicating that pH, total metal(loid)s, and acid leachable metal(loid)s were correlated
327 with microbial communities in tailings sites (Bruneel et al., 2017; Gupta et al., 2017;
328 Hao et al., 2017). The acid leachable or bio-accessible fractions of metal(loid)s (such
329 as H-Pb) can easily migrate with applications of acid rain or during natural
330 acidification, and this process can be accelerated by the metabolic processes of
331 adapted microbial communities (Haferburg and Kothe, 2007). For example, to survive
332 in aquatic and soil environments with Pb²⁺ contamination, some microbes have
333 developed Pb²⁺ resistance, involved extracellular binding, intracellular sequestration,
334 active transport, and exclusion by forming a permeable barrier (Pan et al., 2017).

335 < insert Fig. 3 >

336 Specific genera in each tailing site were also observed (Fig. 4A), suggesting that
337 these tailings sites represent unique ecological niches during tailing colonization and
338 natural attenuation. The distribution of these genera correlated with a combination of
339 pH, TOC, H-Pb, and T-As ($r_M = 0.80$, $p = 0.01$; Fig. 4B; Table 3), indicating that
340 these four geochemical factors may play a key role in the distribution of microbial

341 communities. In tailing sites with 3 years abandonment, *Desulfurivibrio* were specific
342 (Fig. 4A), which always grow chemolithotrophically by sulfur/sulfide oxidation and
343 dissimilate the reduction of nitrate/nitrite in slight alkali environments (Sorokin et al.,
344 2008; Thorup et al., 2017). Although no plants were observed at the tailings sites,
345 specific *Rhizobium* genus, beneficial for plant growth (Sujkowska-Rybkowska and
346 Ważny, 2018), were observed in site T_23Y. This observation could be explained by
347 aerial seeding by plants from the surrounding areas. The distribution of bacterial
348 communities in T_23Y was correlated with pH and H-Sb content (Fig. 3B). In the
349 extremely acidic T_31Y tailing site, most of the specific genera, such as
350 *Acidithiobacillus* and *Acidiferrobacter*, were related to sulfur/iron oxidation (Fig. 4A).
351 These genera had significant and negative correlations with pH, and significant
352 positive correlations with H-As, H-Cr, and H-Cu contents ($p < 0.001$, Fig. 5). This
353 observation would be expected because *Acidiferrobacter* and *Acidithiobacillus*
354 species participate in the metabolism of iron, sulfur, arsenic, and organic matter (Fan
355 et al., 2016; Bruneel et al., 2017). In addition, acidophilic *Acidithiobacillus*-related
356 sequences can generate AMD waters, and oxidize the ferrous sulfate to immobilize
357 As^{5+} in arsenic-contaminated soil (Huang et al., 2016; Yang et al., 2017), suggesting
358 that this species may have an important ecological role for increasing metal sulfide
359 dissolution and controlling AMD production. The frequently encountered distribution
360 and numerous dominance of iron/sulfur-oxidizing and metal-related genera in acidic
361 environments during the long process of natural attenuation reflects their potential
362 role in the natural attenuation of metal(loid)s and generating AMD at tailings sites
363 (Chen et al., 2013; Huang et al., 2016).

364 < insert Fig. 4 >

365 < insert Fig. 5 >

366 < insert Table 3 >

367 Chao et al. (2016) showed that soil microbiota can vary significantly at different
368 abandoned REE tailing sites, by the co-development of microbial and plant
369 communities during natural attenuation. These studies showed that site-specific
370 factors induced microbial changes within subgroups of abandoned sites, which is
371 consistent with our findings. However, the tailing samples in the Chao et al. (2016)
372 contained vegetal material compared to the present study. Therefore, it is not known if
373 the microbial changes described in the Chao et al. study are related to site factors or
374 plant development, or both. Ridl et al. (2016) demonstrated that plants, and not the
375 use of fertilizers, were the drivers of microbial community structure in contaminated
376 soil, with the magnitude of effect depending on the type of plant species. Based on
377 our study, in which plants were not observed, it is likely that microbial changes were
378 caused by geochemical factors and the extremely unfavorable growing conditions
379 (such as low C/N/P contents and high metal(loid)s concentrations).

380

381 **3.4 Potential functional metabolism of bacterial communities**

382 For successful survival and adaptation to a multi-contaminated environment,
383 which constitutes an evolutionary challenge for organisms, sophisticated resistance
384 strategies and mechanisms are required for microbial succession (Guan et al., 2017).
385 PICRUSt analysis was used for exploring the possible metabolism pathways
386 associated with the detoxification of metal(loid)s and transport of geochemical
387 elements in tailings undergoing natural attenuation. The NSTI (nearest sequenced
388 taxon index) values in the present study were less than 0.18 (except at site T_3Y)
389 indicating that the PICRUSt prediction analysis was accurate (Table S2). The
390 relationship between the KEGG pathways and bacterial community structures

391 revealed that each tailing site had its specific functional pathways (Fig. 6). KEGG
392 pathways related to DNA replication and repair, and recombination proteins were
393 mainly clustered close to tailing sites with 31 years natural attenuation (Fig. 6). As
394 well, the distribution of these predicted functional metabolic pathways was strongly
395 correlated with pH, TOC, TP, T-As, T-Zn, and H-Cr ($r = 0.98$, Table S7). It is known
396 that environmental stresses (such as pH, As, and Pb) can directly or indirectly damage
397 the structure of DNA, which results in the mismatch of nucleic acids, and DNA
398 degradation, thus affecting the diversity and structure of microorganisms (Amaral-
399 Zettler et al., 2011; Bruneel et al., 2017; Guan et al., 2017; Hao et al., 2017). These in
400 turn could ultimately lead to microbial cell injury, protein degradation, and gene
401 mutation (Dai et al., 2013; Guan et al., 2017). It is possible that the DNA repair
402 system participated in the sensitive targets of microbial metal(loid)s toxicity observed
403 in our study, resulting in the adaptation of bacterial communities to the extreme
404 tailings environments.

405 SIMPER analysis using the KEGG database indicated that the metabolic
406 pathways directly related to ATP, methane, nitrogen, and energy generation (such as
407 ABC transporters) also contributed to the differences of bacterial community
408 structures in the seven tailings sites (Table S5 and S6). ABC transporters constitute
409 large amounts of membrane proteins and could transport many diverse substrates,
410 such as metal(loid)s and secondary metabolites (Theodoulou and Kerr, 2015). As
411 discussed above, metal(loid) oxidation-related genus of *Acidithiobacillus* could also
412 encode the ABC transporter genes involved with zinc ion transport (Hou et al., 2012).
413 Previous studies also confirmed that genetic expression of iron/sulfur-oxidizing and
414 metal(loid) tolerance may propagate through horizontal gene transfer (Sandoval et al.,
415 2004; Bouzat and Hoostal, 2013), which enables bacterial communities to acquire a

416 gene (or genes) favoring the adaptation of bacterial communities to extreme
417 environments during natural attenuation. To better understand the mechanisms of
418 bacterial communities undergoing natural attenuation in nonferrous metal tailings,
419 further analyses combining geochemical parameters (such as inorganic C,
420 sulfides/sulfates/iron contents, and the neutralization capacity) with
421 metatranscriptomic and metagenomic analyses will provide useful information.

422

423 **Conclusions**

424 Our study provides greater insight into the temporal dynamics of bacterial
425 communities during natural attenuation. Each tailing site was identified as a unique
426 ecological niche. Tailings abandoned for ≤ 15 years were in a pre-acidification phase
427 and undergoing acidification. Tailings ≥ 23 years abandonment had higher acid
428 soluble As concentrations and the metal(loid)s that may represent a risk for human
429 health and the environment (COM, 2016; Hudson-Edwards, 2016). A gradual
430 succession of bacterial genera in the tailings sites was observed suggesting that the
431 bacterial communities become more acidophilic and metal-resistant. Functional
432 metabolic pathways of DNA repair and recombination may be the main potential
433 mechanisms for the microbes to cope with oligotrophic and extreme tailings habitats.
434 The present study suggests that although natural attenuation may be a key strategy
435 towards sustainability, careful monitoring of abandoned tailings sites should be
436 considered as early as possible, to enable the timely management of any potential
437 environmental risks present at these sites.

438

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450

451 **Appendix A. Supporting information**

452 Supplementary data related to this article can be found at the website of
453 *Environmental Pollution*.

454

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673 **Figure legends**

674 **Fig. 1.** Sample location map (top left) and aerial photograph (top right) of the seven
675 tailings sites (T_3Y to T_31Y) close to Hechi city (● shown on map), Guangxi
676 (China), where the surface samples (● on aerial photo) were collected for the present
677 study. Field photographs of tailing sample sites are shown in the bottom panels (T_3Y
678 to T_31Y). As an example, T_3Y corresponds to the tailing sample code. T_3Y
679 means the tailing samples were taken from a three years old abandoned site (not-used).

680

681 **Fig. 2.** Relative abundances of bacterial phyla in the seven abandoned tailings sites.
682 The relative abundances of Alpha-, Beta-, Gamma-, Delta- *Proteobacteria*, and
683 *Actinobacteria* classes are shown in the insert diagram.

684

685 **Fig. 3.** (A) Non-metric multidimensional scaling (NMDS) analysis of bacterial
686 communities in seven tailings sites at genus level. (B) Distance-based redundancy
687 analysis (db-RDA) of genus and selected geochemical factors in seven tailings sites.
688 Both NMDS and db-RDA analysis were based on the weighted normalized unfrac
689 distance algorithms. Direction and magnitude of arrows indicate the correlation of
690 geochemical factors and genera.

691

692 **Fig. 4.** (A) Network analysis for the detected bacterial communities (genus level) in
693 different tailings sites. Color was coded by tailings sites. Each node indicates one
694 genus. Colors of node represent the different major phyla. The size the species-node
695 denotes abundance of species. Black nodes represented the no_rank/un-classified
696 genera which were shared by tailings sites. Light green nodes represented the
697 no_rank/un-classified genera, which were specific in different tailings sites. The

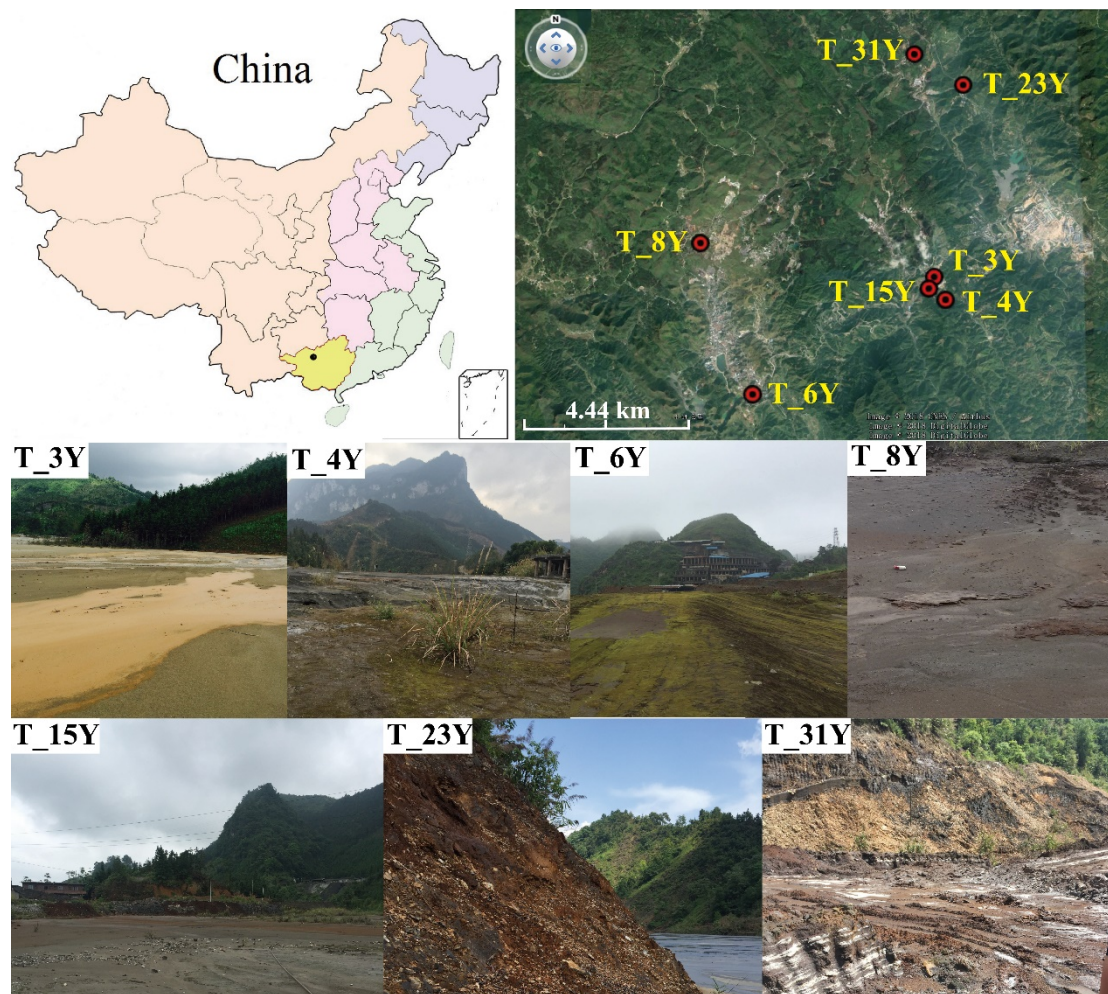
698 degree of node was assessed by the numbers of nodes connected directly to that node.
699 The more lines on the node denotes the higher degree of correlation between the sites
700 and other genera. (B) The sub-network analysis for modularity of genera. Colors of
701 node represent the different module. Node size is proportional to the modularity class.
702 The nodes without labels represented the no_rank/un-classified genera. B. P.,
703 *Burkholderia-Paraburkholderia*

704

705 **Fig. 5.** Correlation analysis based on the Pearson test showing the relation between
706 the geochemical factors and the relative abundance of bacterial communities at the
707 phylum (A) and genus (B) levels. Only the top 30 bacterial communities are shown in
708 this figure. Color key for the correlation values is shown on the right panel inset;
709 positive correlations are in red text, negative correlations are in green, non-significant
710 correlations are shown in white. * $0.01 < p \leq 0.05$, ** $0.001 < p \leq 0.01$, *** $p \leq 0.001$

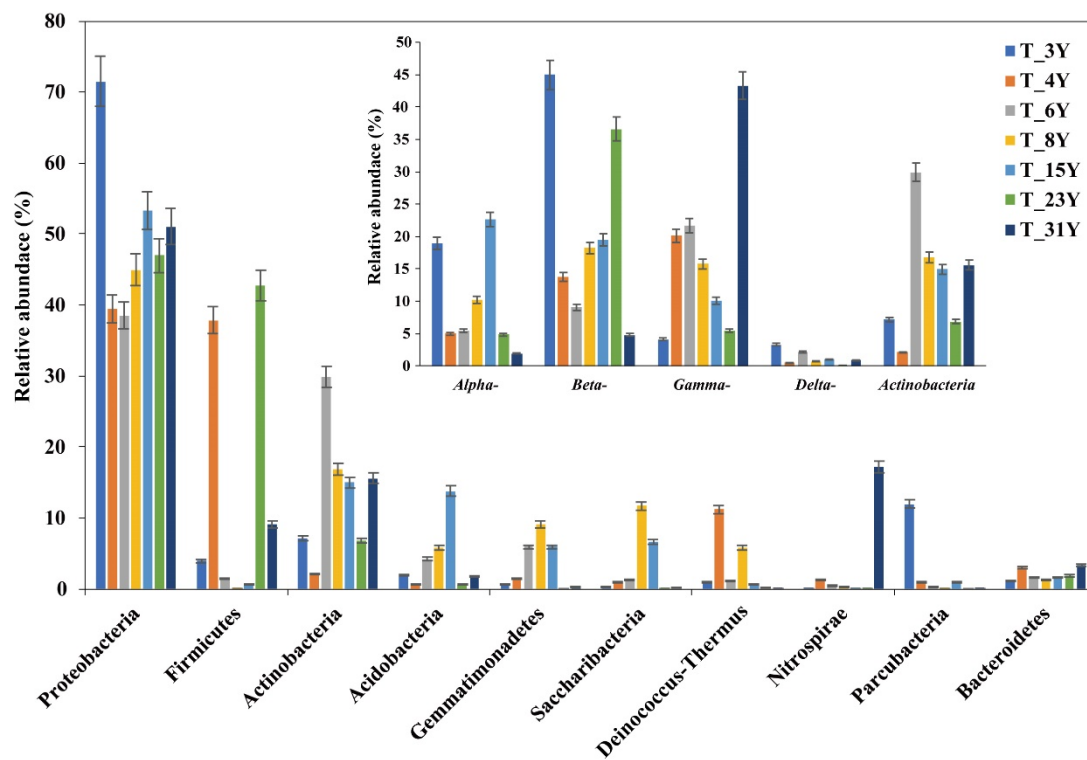
711

712 **Fig. 6** Principal Components Analysis (PCA) for bacterial community structure and
713 KEGG metabolic functional pathways based on 16S rRNA sequencing reads.



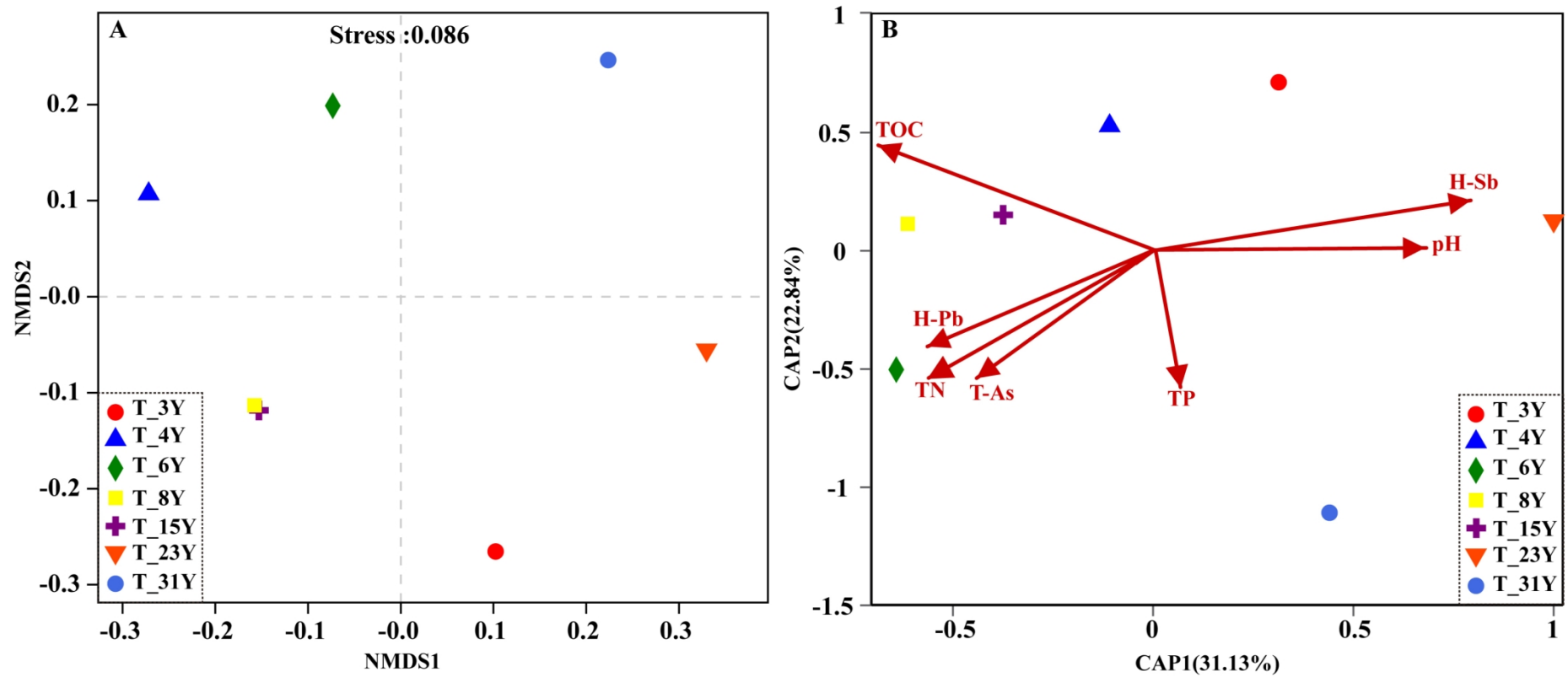
714

715 **Fig. 1.** Sample location map (top left) and aerial photograph (top right) of the seven
 716 tailings sites (T_3Y to T_31Y) close to Hechi city (● shown on map), Guangxi
 717 (China), where the surface samples (● on aerial photo) were collected for the present
 718 study. Field photographs of tailing sample sites are shown in the bottom panels (T_3Y
 719 to T_31Y). As an example, T_3Y corresponds to the tailing sample code. T_3Y
 720 means the tailing samples were taken from a three years old abandoned site (not-used).



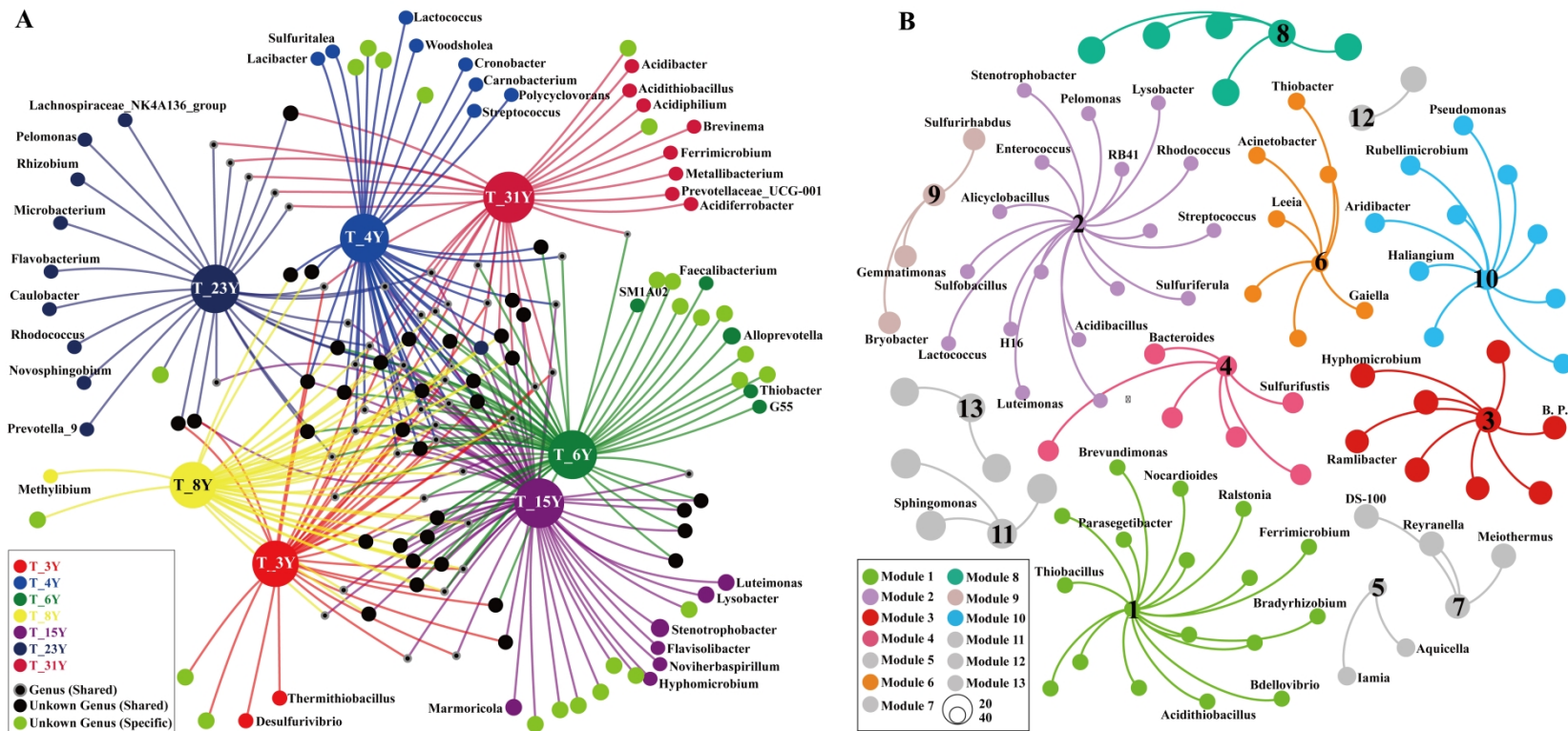
721

722 **Fig. 2.** Relative abundance of bacterial communities at phylum level in the seven
 723 abandoned tailings sites. Relative abundance of Alpha-, Beta-, Gamma-, Delta-
 724 *Proteobacteria*-related and *Actinobacteria* classes are shown in the map inset.



725

726 **Fig. 3.** (A) Non-metric multidimensional scaling (NMDS) analysis of bacterial communities in seven tailings sites at genus level. (B) Distance-
 727 based redundancy analysis (db-RDA) of genus and selected geochemical factors in seven tailings sites. Both NMDS and db-RDA analysis were
 728 based on the weighted normalized unifracs distance algorithms. Direction and magnitude of arrows indicate the correlation of geochemical factors
 729 and genera.



730

731 **Fig. 4.** (A) Network analysis for the detected bacterial communities (genus level) in different tailings sites. Color was coded by tailings sites.

732 Each node indicates one genus. Colors of node represent the different major phyla. The size the species-node denotes abundance of species.

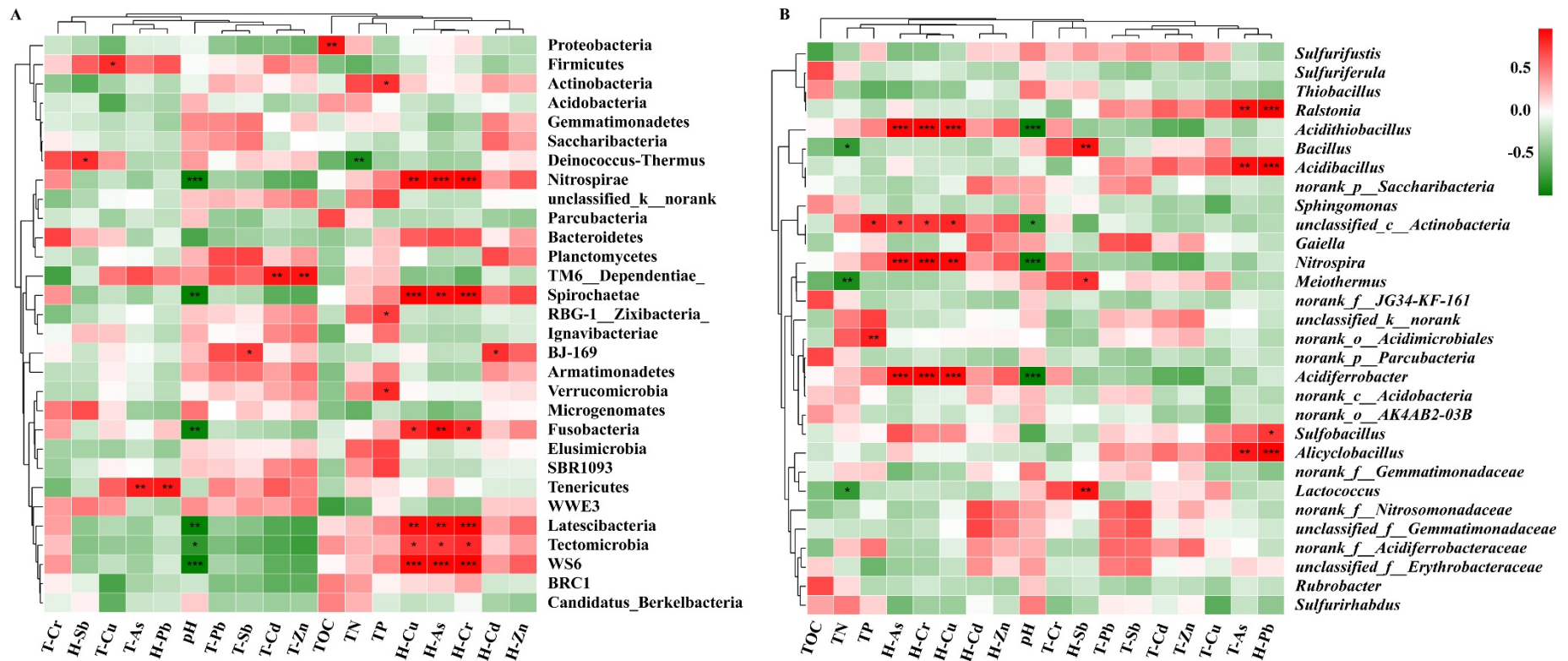
733 Black nodes represented the no_rank/un-classified genera which were shared by tailings sites. Light green nodes represented the no_rank/un-

734 classified genera, which were specific in different tailings sites. The degree of node was assessed by the numbers of nodes connected directly to

735 that node. The more lines on the node denotes the higher degree of correlation between the sites and other genera. (B) The sub-network analysis

736 for modularity of genera. Colors of node represent the different module. Node size is proportional to the modularity class. The nodes without

737 labels represented the no_rank/un-classified genera. B. P., *Burkholderia-Paraburkholderia*



738

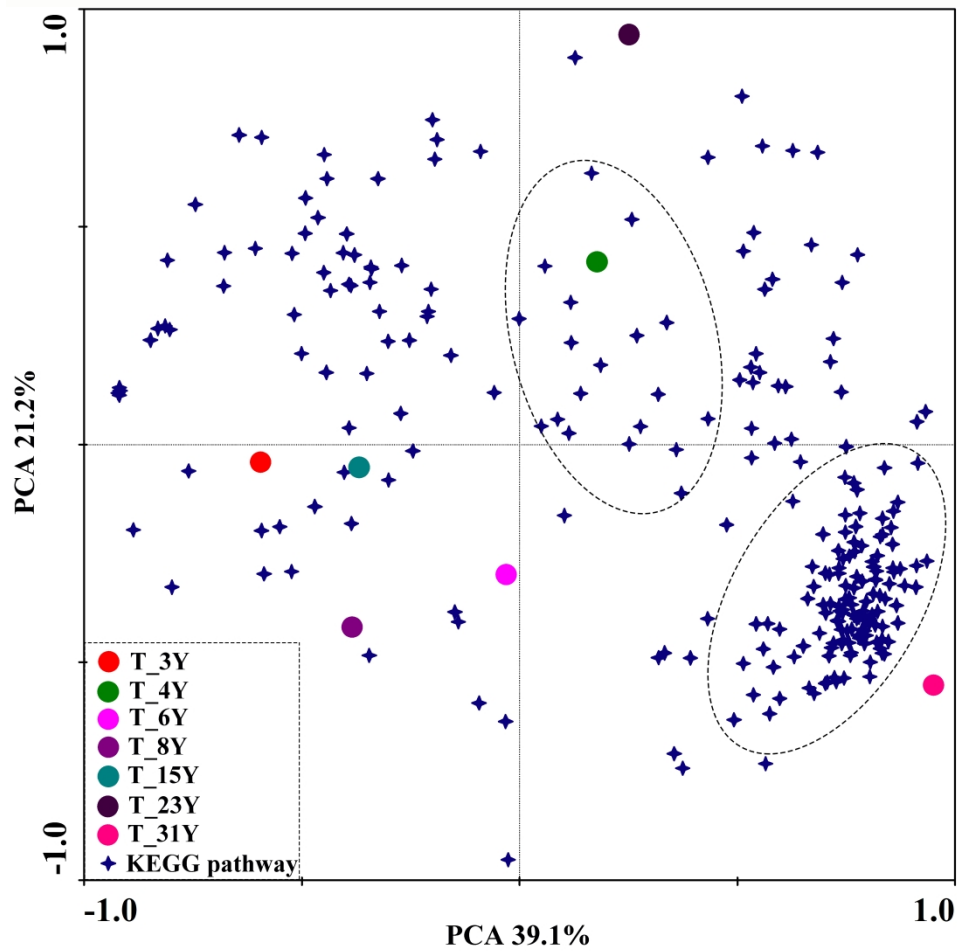
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Fig. 5. Correlation analysis based on the Pearson test showing the relation between the geochemical factors and the relative abundance of bacterial communities at the phylum (A) and genus (B) levels. Only the top 30 bacterial communities are shown in this figure. Color key for the correlation values is shown on the right panel inset; positive correlations are in red text, negative correlations are in green, non-significant correlations are shown in white. * $0.01 < p \leq 0.05$, ** $0.001 < p \leq 0.01$, *** $p \leq 0.001$



743

744 **Fig. 6** Principal Components Analysis (PCA) for bacterial community structure and
 745 KEGG metabolic functional pathways based on 16S rRNA sequencing reads.

746 **Table 1**

747 Spearman correlation analysis for geochemical factor variables and a-diversity index

748 ($p < 0.05$).

| Variables used in analysis | Correlated variables $ rho > 0.75$ | <i>rho</i> | <i>p</i> -value |
|-------------------------------|--|------------|-----------------|
| TOC | H-Cr | 0.786* | 0.036 |
| T-As | T-Cd | 0.786* | 0.036 |
| | T-Cr | -0.821* | 0.023 |
| | T-Zn | 0.857* | 0.014 |
| T-Cd | T-Cu | 0.786* | 0.036 |
| | T-Zn | 0.929** | 0.003 |
| | H-Cr | -0.893** | 0.007 |
| T-Pb | H-Cd | 0.821* | 0.023 |
| | H-Pb | 0.821* | 0.023 |
| T-Zn | H-Cr | -0.893** | 0.007 |
| H-Cd | H-Cu | 0.929** | 0.003 |
| | H-Zn | 0.929** | 0.003 |
| H-Cu | H-Zn | 0.929** | 0.003 |
| ace | PD | 0.964** | 0.0004 |
| PD | TP | -0.786* | 0.036 |

749 TOC, total organic carbon; TN, total nitrogen; TP, total phosphorus; T-(metal), total content of

750 metal(loid)s; H-(metal), the acid extraction of metal(loid)s; ace, microbial richness; PD,

751 phylogenetic diversity; *rho*, Spearman coefficient of product-moment correlation

752 **Table 2**

753 Main genus contributed to the differences between different bacterial communities of
 754 tailings sites with different abandoned time. The relative abundances of genera ≥ 1 at
 755 least at one tailing site are shown.

| Genus | Contrib (%) | | | | | | | | |
|---------------------------|-------------|-------|-------|-------|-------|-------|-------|-------|-------|
| | a & b | a & c | a & d | a & e | b & c | b & d | b & e | c & d | c & e |
| Dissi | 68.3 | 68.4 | 59.2 | 73.5 | 52.7 | 56.9 | 83.2 | 49.6 | 78.5 |
| <i>Acidibacillus</i> | - | - | - | 3.65 | - | - | 3.15 | - | 2.98 |
| <i>Acidiferrobacter</i> | - | - | - | 2.52 | - | - | 2.17 | - | 2.06 |
| <i>Acidithiobacillus</i> | - | - | - | 3.78 | - | - | 3.27 | - | 3.09 |
| <i>Acinetobacter</i> | 0.93 | 0.69 | 0.75 | 0.97 | - | 0.70 | 1.56 | 0.73 | 1.37 |
| <i>Alicyclobacillus</i> | - | - | - | 2.81 | - | - | 2.43 | - | 2.30 |
| <i>Bacillus</i> | 7.34 | - | 0.67 | 0.40 | 8.17 | 7.89 | 5.36 | 0.71 | - |
| <i>Bdellovibrio</i> | 2.37 | 1.81 | 2.18 | 2.47 | - | - | - | - | - |
| <i>Burkholderia-</i> | - | - | - | 1.63 | - | - | 1.59 | - | 1.28 |
| <i>DS-100</i> | - | 1.59 | 1.34 | - | 2.04 | 1.38 | - | 1.04 | 1.37 |
| <i>Enterococcus</i> | 1.51 | 0.96 | - | - | - | 1.87 | 1.14 | 1.43 | 0.76 |
| <i>Erysipelothrix</i> | 3.04 | 2.70 | 3.33 | 1.96 | - | - | 0.68 | - | - |
| <i>Gaiella</i> | - | 4.48 | 5.01 | - | 4.59 | 4.01 | 0.78 | 1.79 | 3.73 |
| <i>Gemmatimonas</i> | 0.58 | - | 1.12 | 0.90 | 1.22 | 1.79 | - | 0.60 | 1.11 |
| <i>Iamia</i> | 0.82 | 2.75 | 0.67 | - | 2.56 | - | 0.47 | 2.88 | 2.12 |
| <i>Lactococcus</i> | 4.89 | - | - | - | 2.68 | 5.45 | 3.75 | - | - |
| <i>Meiothermus</i> | 3.54 | 0.59 | 2.09 | 1.57 | 3.23 | 2.39 | 4.11 | 2.13 | 1.78 |
| <i>Nitrospira</i> | 1.18 | 0.98 | - | 2.77 | - | 0.75 | 2.40 | 0.68 | 2.27 |
| <i>Ralstonia</i> | 0.81 | - | - | - | - | - | 3.68 | - | 3.11 |
| <i>Rhodococcus</i> | - | - | - | 1.35 | - | - | 1.29 | - | 1.22 |
| <i>Rubellimicrobium</i> | - | - | 1.09 | 0.44 | - | - | - | - | - |
| <i>Rubrobacter</i> | 3.91 | 2.66 | 3.16 | 3.54 | 1.02 | 1.13 | - | - | 0.68 |
| <i>Sphingomonas</i> | - | 0.65 | 3.91 | 1.42 | 1.18 | - | 0.98 | - | 1.70 |
| <i>Sulfobacillus</i> | - | - | - | 3.27 | - | - | 2.82 | - | 2.67 |
| <i>Sulfuriferula</i> | 8.46 | 8.20 | 10.3 | 7.57 | - | 0.96 | 0.91 | - | 0.86 |
| <i>Sulfurifustis</i> | 5.39 | 7.09 | 5.18 | 0.44 | 2.93 | 1.92 | 4.57 | 3.74 | 6.25 |
| <i>Sulfurirhabdus</i> | 1.69 | 1.12 | 1.11 | 1.53 | - | 3.01 | - | - | - |
| <i>Thermithiobacillus</i> | 2.84 | 2.52 | 3.11 | 2.56 | - | - | - | - | - |
| <i>Thiobacillus</i> | 1.97 | 3.53 | 0.65 | 5.07 | 2.27 | 1.53 | 2.84 | 3.87 | 1.20 |
| <i>Thiobacter</i> | - | 2.40 | - | - | 2.46 | - | 0.41 | 2.59 | 2.00 |

756 Contrib, the contribution of each genus to the differences of bacterial communities of tailings sites;
 757 a, T_3Y; b, T_4Y; c, T_6Y; d, T_8Y and T_15Y; e, T_23Y and T_31Y; “-”, the contribution data
 758 < 0.4.

759 **Table 3**

760 Correlation analysis of modules eigengenes in the bacterial community network (Fig.
761 4) and selected geochemical factors by BIOENV analysis and Monte-Carlo test.

| Combination of geochemical factors | | Module 1 | Module 2 | Module 3 |
|---|----------|-----------------|-----------------|-----------------|
| T-As | rM | 0.51 | 0.29 | 0.54 |
| | <i>p</i> | 0.07 | 0.28 | 0.10 |
| TOC + H-Pb | rM | 0.32 | 0.58 | 0.59 |
| | <i>p</i> | 0.20 | 0.03 | 0.07 |
| pH + TOC + H-Pb | rM | 0.84 | 0.33 | 0.26 |
| | <i>p</i> | 0.01 | 0.11 | 0.19 |
| TOC + H-Pb + T-As | rM | 0.37 | 0.53 | 0.62 |
| | <i>p</i> | 0.16 | 0.06 | 0.08 |
| pH + TOC + H-Pb + T-As | rM | 0.80 | 0.37 | 0.48 |
| | <i>p</i> | 0.01 | 0.10 | 0.13 |
| TOC + H-Pb + T-As + H-Sb | rM | 0.27 | 0.69 | 0.51 |
| | <i>p</i> | 0.23 | 0.08 | 0.13 |

762

Supporting Information

Bacterial diversity in typical abandoned multi-contaminated nonferrous metal(loid) tailings during natural attenuation

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Supplementary Tables: 7

Supplementary Figures: 1

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Table S1.

Types of tail sand for mining and smelting industries and geochemical factors of the studied tailings sites.

| | T_3Y | T_4Y | T_6Y | T_8Y | T_15Y | T_23Y | T_31Y |
|-----------------|------------------|------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Mining activity | Sb | Pb-Zn | Sn | Pb-Zn | Sb | Pb-Zn | Sn |
| pH | 7.40 ± 0.05a | 7.55 ± 0.30a | 7.39 ± 0.05a | 7.45 ± 0.35ab | 7.44 ± 0.09a | 6.44 ± 2.69ab | 2.59 ± 0.07b |
| TOC | 973 ± 108ab | 358 ± 139ab | 449 ± 233ab | 487 ± 378ab | 864 ± 29.3ab | 511 ± 209ab | 629 ± 104ab |
| TN | 57.1 ± 15.8ab | 18.1 ± 6.46b | 74.1 ± 22.6a | 37.6 ± 13.3ab | 64.6 ± 23.4ab | 48.5 ± 6.46ab | 62.2 ± 23.5ab |
| TP | 169 ± 5.08c | 180 ± 61.6c | 1470 ± 677a | 205 ± 10.7c | 343 ± 111bc | 145 ± 80.1c | 1119 ± 333ab |
| T-As | 17300 ± 1060ab | 11500 ± 6950ab | 22700 ± 4010ab | 20700 ± 4010ab | 14600 ± 2820ab | 55700 ± 39400ab | 8313 ± 864ab |
| T-Cd | 26.0 ± 1.67ab | 36.2 ± 23.0ab | 46.8 ± 0.53ab | 32.0 ± 17.3ab | 19.9 ± 2.41ab | 53.7 ± 21.0ab | 11.9 ± 7.78ab |
| T-Cr | 12.7 ± 0.58ab | 30.6 ± 11.2ab | 7.82 ± 0.81ab | 19.4 ± 12.0ab | 15.1 ± 1.60ab | 8.12 ± 3.91ab | 24.8 ± 16.8ab |
| T-Cu | 134 ± 35.2ab | 290 ± 272ab | 203 ± 25.2ab | 225 ± 46.3ab | 76.0 ± 6.92ab | 343 ± 191ab | 155 ± 110ab |
| T-Pb | 407 ± 71.3ab | 384 ± 254ab | 4270 ± 358ab | 7100 ± 4600ab | 266 ± 23.2ab | 5940 ± 920ab | 435 ± 225ab |
| T-Sb | 378 ± 33.1ab | 1830 ± 625ab | 3550 ± 192ab | 6980 ± 450ab | 775 ± 142ab | 4920 ± 758ab | 412 ± 248ab |
| T-Zn | 2280 ± 60.2ab | 3770 ± 2270ab | 5370 ± 149ab | 3890 ± 1860ab | 1840 ± 215ab | 5180 ± 2691ab | 630 ± 62.8ab |
| H-As | 0.115 ± 0.08ab | 0.40 ± 0.21ab | 0.303 ± 0.05ab | 0.963 ± 0.70ab | 0.540 ± 0.01ab | 7.31 ± 1.21ab | 29.3 ± 3.25ab |
| H-Cd | 0.009 ± 0.007b | 0.011 ± 0.01b | 0.041 ± 0.004b | 0.141 ± 0.06a | 0.007 ± 0.005b | 0.021 ± 0.02ab | 0.092 ± 0.08ab |
| H-Cr | 0.004 ± 0.003ab | 0.002 ± 0.001b | 0.002 ± 0.0004b | 0.004 ± 0.003ab | 0.009 ± 0.006ab | 0.003 ± 0.002ab | 0.05 ± 0.04a |
| H-Cu | 0.008 ± 0.006b | 0.011 ± 0.01b | 0.08 ± 0.008b | 0.28 ± 0.20b | 0.010 ± 0.009b | 0.053 ± 0.009b | 1.00 ± 0.516a |
| H-Pb | 0.006 ± 0.0003ab | 0.0004 ± 0.002ab | 0.023 ± 0.002ab | 0.084 ± 0.08ab | 0.01 ± 0.009ab | 0.893 ± 0.154ab | 0.035 ± 0.014ab |
| H-Sb | 0.41 ± 0.35ab | 1.85 ± 1.19ab | 0.23 ± 0.10ab | 0.407 ± 0.32ab | 0.70 ± 0.18ab | 0.613 ± 0.41ab | 0.084 ± 0.05ab |
| H-Zn | 0.71 ± 0.58ab | 2.32 ± 1.68ab | 2.78 ± 0.66ab | 7.63 ± 5.61ab | 0.45 ± 0.21ab | 1.31 ± 0.16ab | 7.70 ± 0.84ab |

Mining activity, the types of these tail sand; TOC, total organic carbon; TN, total nitrogen; TP, total phosphorus; T-(metal), total content of metal(loid)s; H-(metal), the acid leachable metal(loid)s. The unit for TOC, TN, TP, and content of metal(loid)s is mg/kg. Different letters in the same row denote significant differences between tailings sites at $p < 0.05$ level. “-” = no data.

40 **Table S2.**41 **The average sequences data for 16S rRNA in each tailing site.**

| Sample ID | Seq_num | Mean_lengt | Shannon | ace | coverage | PD | NSTI |
|-----------|---------|------------|---------|-----|----------|------|------|
| T_3Y | 36309 | 440 | 2.88 | 601 | 0.998 | 45.5 | 0.34 |
| T_4Y | 41493 | 444 | 3.67 | 621 | 0.999 | 51.2 | 0.12 |
| T_6Y | 44161 | 438 | 4.67 | 678 | 0.999 | 58.6 | 0.18 |
| T_8Y | 37562 | 439 | 4.43 | 571 | 0.997 | 43.7 | 0.17 |
| T_15Y | 37001 | 438 | 4.80 | 598 | 0.999 | 47.7 | 0.17 |
| T_23Y | 33465 | 445 | 3.12 | 255 | 0.997 | 25.9 | 0.07 |
| T_31Y | 35496 | 445 | 3.37 | 794 | 0.998 | 60.8 | 0.18 |

42 Seq_num, the sequences number for each tailing site; Mean_length, the average length of
43 sequences; NSTI, nearest sequenced taxon index. Four α -diversity indices (Shannon, ace,
44 PD, and coverage) are shown to estimate bacterial community diversity, richness,
45 phylogenetic diversity, and community coverage. The analysis for bacterial communities in
46 seven tailings sites at OTU level were based on 97% similarity. Data points for T_3Y,
47 T_4Y, T_6Y, T_8Y, T_15Y, T_23Y, and T_31Y represent tailings sites having 3, 4, 6, 8,
48 15, 23, and 31 years of abandonment, respectively.

49 **Table S3.**

50 **Average OTU distribution for 16S rDNA sequences in each tailing site.** T_3Y, T_4Y,

51 T_6Y, T_8Y, T_15Y, T_23Y and T_31Y represent different tailing sites with different ages

52 of abandonment. The table has been listed as a separate worksheet file.

53 **Table S4.**
 54 **Relative abundance (%) of genera in the seven studied tailing sites.** Only genera with
 55 relative abundance > 1% in at least one tailing samples are shown.

| Genus | T_3Y | T_4Y | T_6Y | T_8Y | T_15Y | T_23Y | T_31Y |
|--------------------------------------|-------------|-------------|-------------|-------------|--------------|--------------|--------------|
| <i>Acidibacillus</i> | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 23.9 | 0.40 |
| <i>Acidiferrobacter</i> | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 12.5 |
| <i>Acidithiobacillus</i> | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 28.3 |
| <i>Acinetobacter</i> | 0.40 | 0.00 | 0.00 | 0.00 | 0.40 | 0.34 | 1.10 |
| <i>Alicyclobacillus</i> | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 9.10 | 1.30 |
| <i>Bacillus</i> | 0.10 | 25.0 | 0.10 | 0.00 | 0.50 | 0.80 | 0.20 |
| <i>Bdellovibrio</i> | 2.70 | 0.10 | 0.10 | 0.10 | 0.30 | 0.00 | 0.00 |
| <i>Brevundimonas</i> | 0.10 | 0.00 | 0.00 | 0.10 | 0.40 | 1.30 | 0.00 |
| <i>Burkholderia-Paraburkholderia</i> | 0.00 | 0.00 | 0.00 | 0.00 | 0.10 | 5.10 | 0.30 |
| <i>DS-100</i> | 0.00 | 0.00 | 0.80 | 0.10 | 1.20 | 0.00 | 0.00 |
| <i>Enterococcus</i> | 0.00 | 1.10 | 0.30 | 0.00 | 0.00 | 0.00 | 0.00 |
| <i>Erysipelothrix</i> | 3.10 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.20 |
| <i>Gaiella</i> | 0.10 | 0.90 | 6.30 | 10.5 | 2.50 | 0.10 | 0.10 |
| <i>Gemmatimonas</i> | 0.30 | 0.10 | 0.50 | 1.30 | 0.70 | 0.00 | 0.00 |
| <i>Iamia</i> | 0.00 | 0.30 | 2.00 | 0.10 | 0.10 | 0.00 | 0.00 |
| <i>Lactococcus</i> | 0.00 | 9.60 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| <i>Luteimonas</i> | 0.00 | 0.00 | 0.00 | 0.00 | 3.10 | 0.00 | 0.00 |
| <i>Meiothermus</i> | 1.00 | 11.2 | 1.20 | 5.70 | 0.30 | 0.00 | 0.00 |
| <i>Nitrospira</i> | 0.10 | 1.20 | 0.60 | 0.40 | 0.10 | 0.10 | 17.2 |
| <i>Ralstonia</i> | 0.20 | 0.00 | 0.10 | 0.30 | 0.20 | 28.8 | 1.10 |
| <i>Rhodococcus</i> | 0.10 | 0.00 | 0.00 | 0.00 | 0.00 | 4.70 | 0.00 |
| <i>Rubellimicrobium</i> | 0.10 | 0.10 | 0.10 | 0.20 | 1.10 | 0.00 | 0.00 |
| <i>Rubrobacter</i> | 5.60 | 0.00 | 0.20 | 0.30 | 0.30 | 0.00 | 0.00 |
| <i>Sphingomonas</i> | 1.40 | 1.20 | 1.60 | 3.50 | 13.1 | 0.00 | 0.20 |
| <i>Sulfobacillus</i> | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 6.70 | 4.80 |
| <i>Sulfuriferula</i> | 31.0 | 0.50 | 0.00 | 0.00 | 0.00 | 0.00 | 2.20 |
| <i>Sulfurifustis</i> | 0.10 | 13.9 | 14.7 | 11.5 | 2.40 | 0.00 | 0.00 |
| <i>Sulfurirhabdus</i> | 1.00 | 0.00 | 1.80 | 1.30 | 2.20 | 0.00 | 0.00 |
| <i>Thermithiobacillus</i> | 2.70 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| <i>Thiobacillus</i> | 11.6 | 6.10 | 0.70 | 8.10 | 4.90 | 0.10 | 0.00 |
| <i>Thiobacter</i> | 0.00 | 0.10 | 1.50 | 0.10 | 0.00 | 0.00 | 0.00 |

| | | | | | | | | |
|----------------------|---|------|------|------|------|------|------|------|
| | | | | | | | | |
| | | | | | | | | — |
| | Oxidative phosphorylation | 0.01 | 0.02 | 0.02 | 0.02 | 0.02 | 0.01 | 0.02 |
| | Peptidases | 0.01 | 0.02 | 0.01 | 0.01 | 0.02 | 0.02 | 0.02 |
| | Pyrimidine metabolism | 0.01 | 0.02 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |
| | Amino acid related enzymes | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |
| | Arginine and proline metabolism | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |
| | Pyruvate metabolism | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |
| | Methane metabolism | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |
| | Butanoate metabolism | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |
| | Carbon fixation pathways in prokaryotes | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |
| | Glycolysis / Gluconeogenesis | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |
| | Propanoate metabolism | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |
| | Amino sugar and nucleotide sugar metabolism | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |
| | Porphyrin and chlorophyll metabolism | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |
| | Alanine, aspartate and glutamate metabolism | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |
| | Valine, leucine and isoleucine degradation | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.00 |
| | Citrate cycle (TCA cycle) | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |
| | Lipid biosynthesis proteins | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |
| | Glycine, serine and threonine metabolism | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |
| | Energy metabolism | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |
| | Fatty acid metabolism | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.00 |
| | Glyoxylate and dicarboxylate metabolism | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |
| | Cysteine and methionine metabolism | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |
| | Valine, leucine and isoleucine biosynthesis | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |
| | Nitrogen metabolism | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |
| | Phenylalanine, tyrosine and tryptophan biosynthesis | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |
| | Peptidoglycan biosynthesis | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |
| Poorly characterized | General function prediction only | 0.02 | 0.04 | 0.04 | 0.04 | 0.03 | 0.03 | 0.03 |
| | Function unknown | 0.01 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 |
| Others | | 0.56 | 0.35 | 0.36 | 0.36 | 0.37 | 0.37 | 0.36 |

61

Table S6.

62

Main KEGG pathways contributed to the differences between different metabolic pathways of tailings sites. The top fifty metabolic pathways of KEGG subsystems (at level 3) are shown.

63

| Metabolism pathway on level 1 | Metabolism pathway on level 3 | b&c | b& | b&e | b&f | b& | d&e | d&f | d& | d&c | e&f | e&a | e&c | f&a | f&c | a&c |
|--------------------------------------|--|------|------|------|------|------|-----|-----|------|-----|-----|------|-----|------|-----|------|
| Contrib (%) | | | | | | | | | | | | | | | | |
| Cellular Processes | Bacterial motility proteins | - | - | - | - | - | - | - | - | - | - | 0.6 | - | - | - | 0.49 |
| | Flagellar assembly | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Environmental Information Processing | Transporters | 1.21 | 2.00 | 1.00 | 1.05 | 0.60 | - | - | - | - | - | - | - | - | - | - |
| | ABC transporters | 1.26 | 1.7 | 1.2 | 1.12 | - | - | - | - | - | - | - | - | - | - | - |
| | Two-component system | - | - | - | - | 0.60 | - | - | 0.58 | - | - | - | - | - | - | - |
| | Secretion system | - | - | - | - | - | - | - | 0.61 | - | - | 0.72 | - | 0.50 | - | 0.48 |
| | Other ion-coupled transporters | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | Bacterial secretion system | - | - | - | - | 0.50 | - | - | 0.63 | - | - | 0.71 | - | 0.50 | - | 0.51 |
| Genetic Information Processing | DNA repair and recombination proteins | - | - | - | - | 0.60 | - | - | 0.52 | - | - | 0.61 | - | 0.50 | - | 0.52 |
| | Ribosome | - | - | - | - | - | - | - | - | - | - | - | - | 0.50 | - | - |
| | Chromosome | - | - | - | - | 0.60 | - | - | - | - | - | 0.68 | - | 0.50 | - | - |
| | Transcription factors | 1.14 | 0.80 | - | - | 0.50 | - | - | 0.59 | - | - | 0.58 | - | 0.50 | - | - |
| | Ribosome Biogenesis | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | Aminoacyl-tRNA biosynthesis | - | - | - | - | - | - | - | 0.52 | - | - | 0.62 | - | - | - | - |
| | Chaperones and folding catalysts | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | Transcription machinery | - | - | 0.50 | 0.71 | 0.60 | - | - | 0.57 | - | - | - | - | - | - | - |
| | DNA replication proteins | - | - | - | - | - | - | - | 0.54 | - | - | 0.68 | - | 0.60 | - | 0.55 |
| | Protein folding and associated processing | - | - | - | - | - | - | - | - | - | - | - | - | 0.60 | - | 0.54 |
| | Translation proteins | - | 0.60 | - | - | 0.60 | - | - | - | - | - | 0.60 | - | - | - | - |
| | Homologous recombination | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | Replication, recombination and repair proteins | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |

64

| | | | | | | | | | | | | | | | | |
|----------------------|---|------|------|---|------|------|---|------|------|------|------|------|---|------|---|------|
| Mtabolism | Purine metabolism | - | - | - | - | 0.70 | - | - | - | - | - | - | - | - | - | - |
| | Oxidative phosphorylation | - | - | - | - | 0.60 | - | - | 0.62 | - | - | - | - | 0.50 | - | - |
| | Peptidases | - | - | - | - | 0.70 | - | - | - | - | - | - | - | - | - | - |
| | Pyrimidine metabolism | - | 0.50 | - | - | 0.70 | - | - | - | - | - | - | - | - | - | - |
| | Amino acid related enzymes | - | - | - | - | 0.70 | - | - | - | - | - | 0.62 | - | - | - | - |
| | Arginine and proline metabolism | - | 0.70 | - | 0.88 | - | - | - | - | - | - | - | - | - | - | - |
| | Pyruvate metabolism | - | - | - | - | 0.70 | - | - | - | - | - | - | - | - | - | - |
| | Methane metabolism | - | - | - | - | 0.60 | - | - | 0.62 | - | - | 0.57 | - | 0.60 | - | 0.55 |
| | Butanoate metabolism | 1.22 | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | Carbon fixation pathways in prokaryotes | - | - | - | - | 0.70 | - | - | - | - | - | - | - | - | - | - |
| | Glycolysis / Gluconeogenesis | - | - | - | - | 0.70 | - | - | - | - | - | 0.58 | - | - | - | - |
| | Propanoate metabolism | - | - | - | 0.89 | - | - | - | - | - | - | - | - | - | - | - |
| | Amino sugar and nucleotide sugar metabolism | - | - | - | - | - | - | - | 0.48 | - | - | 0.54 | - | 0.40 | - | - |
| | Porphyrin and chlorophyll metabolism | - | - | - | 0.38 | - | - | - | - | - | - | - | - | - | - | - |
| | Alanine, aspartate and glutamate metabolism | - | 0.60 | - | 0.72 | - | - | - | - | - | - | - | - | - | - | - |
| | Valine, leucine and isoleucine degradation | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | Citrate cycle (TCA cycle) | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | Lipid biosynthesis proteins | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | Glycine, serine and threonine metabolism | 1.01 | - | - | 0.74 | - | - | - | - | - | - | - | - | - | - | - |
| | Energy metabolism | - | - | - | 0.88 | - | - | - | - | - | - | - | - | - | - | - |
| | Fatty acid metabolism | 1.37 | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | Glyoxylate and dicarboxylate metabolism | - | - | - | - | 0.60 | - | - | - | - | - | 0.61 | - | 0.50 | - | - |
| | Cysteine and methionine metabolism | - | - | - | - | 0.60 | - | - | - | - | - | - | - | - | - | - |
| | Valine, leucine and isoleucine biosynthesis | 0.99 | - | - | 0.59 | - | - | 0.74 | - | 1.24 | - | - | - | - | - | 0.48 |
| | Nitrogen metabolism | - | - | - | - | 0.60 | - | - | 0.60 | - | - | 0.66 | - | 0.50 | - | 0.48 |
| | Phenylalanine, tyrosine and tryptophan | - | - | - | - | 0.60 | - | - | 0.60 | - | - | - | - | - | - | - |
| | Peptidoglycan biosynthesis | - | - | - | - | - | - | - | 0.57 | - | - | - | - | - | - | 0.54 |
| Poorly Characterized | General function prediction only | - | - | - | - | - | - | - | - | - | 0.63 | - | - | - | - | |
| | Function unknown | - | 0.60 | - | 0.60 | 0.70 | - | - | - | - | - | - | - | - | - | |

65 Contrib, the contribution of each genus to the differences of bacterial communities of tailings sites. "-", there was no contribution to the the differences between different
66 metabolic pathways of tailings sites.

67

Table S7.

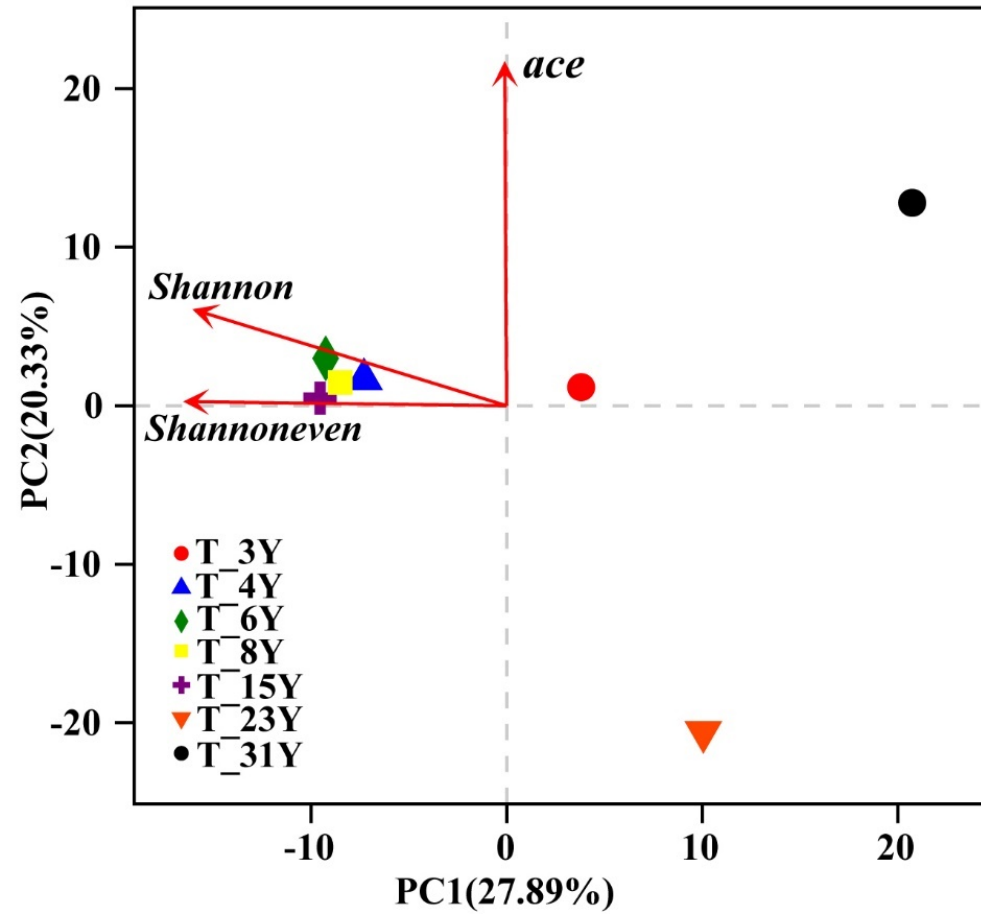
68

BIOENV analysis for the relationships between geochemical variables and functional prediction of KEGG pathways.

| Combination of geochemical factors | <i>r</i> |
|--|-----------------|
| H-Cr | 0.796 |
| T-Zn + H-Cr | 0.846 |
| pH + TP + T-Zn | 0.900 |
| pH + TOC + TP + T-Cd | 0.943 |
| pH + TOC + TP + T-As + H-Cr | 0.957 |
| pH + TOC + TP + T-As + T-Zn + H-Cr | 0.979 |
| pH + TOC + TP + T-As + T-Zn + H-Cr + H-Cu | 0.975 |
| pH + TOC + TP + T-As + T-Zn + H-As + H-Cr + H-Cu | 0.964 |
| pH + TOC + TN + TP + T-Cd + T-Zn + H-Cr + H-Cu + H-Pb | 0.946 |
| pH + TOC + TN + TP + T-Cd + T-Zn + H-As + H-Cr + H-Cu + H-Pb | 0.943 |
| pH + TOC + TP + T-Cd + T-Cr + T-Cu + T-Zn + H-As + H-Cr + H-Cu + H-Pb | 0.946 |
| pH + TOC + TN + TP + T-As + T-Cd + T-Sb + T-Zn + H-As + H-Cr + H-Cu + H-Pb | 0.921 |
| pH + TOC + TN + TP + T-As + T-Cd + T-Cu + T-Zn + H-As + H-Cr + H-Cu + H-Pb + H-Zn | 0.900 |
| pH + TOC + TN + TP + T-As + T-Cd + T-Cr + T-Cu + T-Pb + T-Zn + H-As + H-Cr + H-Cu + H-Pb | 0.875 |
| pH + TOC + TN + TP + T-As + T-Cd + T-Cr + T-Cu + T-Sb + T-Zn + H-As + H-Cr + H-Cu + H-Pb + H-Sb | 0.846 |
| pH + TOC + TN + TP + T-As + T-Cd + T-Cr + T-Cu + T-Pb + T-Zn + H-As + H-Cr + H-Cu + H-Pb + H-Sb + H-Zn | 0.800 |
| pH + TOC + TN + TP + T-As + T-Cd + T-Cr + T-Cu + T-Pb + T-Sb + T-Zn + H-As + H-Cu + H-Cr + H-Pb + H-Sb + H-Zn | 0.750 |
| pH + TOC + TN + TP + T-As + T-Cd + T-Cr + T-Cu + T-Pb + T-Sb + T-Zn + H-As + H-Cd + H-Cr + H-Cu + H-Sb + H-Zn + H-Pb | 0.646 |

69

Resulting values were weighted Spearman rank correlation coefficients (*r*).



70

71 **Figure S1. Principal component analysis based on relative genus abundance showing major differences in different seven tailings sites with different**
72 **abandoned times.**