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1 **Assembly processes of bacterial and fungal communities in metal(loid)s**
2 **smelter soil**

3
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22 **Abstract**

23 The assembly processes of microbial communities governing the metal(loid)s
24 biogeochemical cycles at smelters are yet to be solved. Here, a systematic investigation
25 combines geochemical characterization, co-occurrence patterns, and assembly mechanisms of
26 bacterial and fungal communities inhabiting soil around an abandoned arsenic smelter.
27 *Acidobacteriota*, *Actinobacteriota*, *Chloroflexi*, and *Pseudomonadota* were dominant in the
28 bacterial communities, whereas *Ascomycota* and *Basidiomycota* dominated the fungal
29 communities. The random forest model indicated the bioavailable fractions of Fe were the
30 main positive factor driving the beta diversity of bacterial communities (9.58%) and the
31 negative impact of total N on fungal communities (8.09%). The microbial-contaminant
32 interactions demonstrate the positive impact of the bioavailable fractions of certain
33 metal(loid)s on bacteria (*Comamonadaceae* and *Rhodocyclaceae*) and fungi (*Meruliaceae*
34 and *Pleosporaceae*). The fungal co-occurrence networks exhibited higher connectivity and
35 complexity than the bacterial networks. Interestingly, positive links dominated all networks.
36 The keystone taxa were identified in bacterial (including the family *Diplorickettsiaceae*,
37 *norank_o__Candidatus_Woesebacteria*, *norank_o__norank_c__AT-s3-28*,
38 *norank_o__norank_c__bacteriap25*, and *Phycisphaeraceae*) and fungal (including
39 *Biatriosporaceae*, *Ganodermataceae*, *Peniophoraceae*, *Phaeosphaeriaceae*, *Polyporaceae*,
40 *Teichosporaceae*, *Trichomeriaceae*, *Wrightoporiaceae*, and *Xylariaceae*) communities.
41 Meanwhile, community assembly analysis revealed that deterministic processes (especially
42 variable selection) dominated the microbial community assemblies, which were highly
43 impacted by the pH, total N, and total and bioavailable metal(loid) content. This study

44 provides a deeper insight into the organization of microbial communities in response to
45 long-term metal(loid)s pollution and helpful information to develop bioremediation strategies
46 for the mitigation of metal(loid)s-polluted soils.

47

48 **Keywords:** Metal(loid)s, Bacterial and fungal community, Co-occurrence pattern, Assembly

49 mechanism

50

51 **1. Introduction**

52 More than 10 million sites present metal(loid)s polluted soils worldwide, being a major
53 concern for society and the scientific community (Fei et al., 2019; Zhang et al., 2020; Xu et
54 al., 2021a). Metal smelting activity is considered one of the main anthropogenic sources of
55 metal(loid)s pollution in soil (Yun et al., 2018). Smelting operations generate large amounts
56 of hazardous solid waste (e.g., smelting slag, combustion residues, and mine tailings) that are
57 deposited near the smelters (Xu et al., 2021a; Liu et al., 2022a). Thus, the dispersion of
58 smelter waste can affect the stability of surrounding ecosystems causing important ecological
59 disturbances (Zhang et al., 2022b). The presence of metal(loid)s can decrease the soil
60 microbial biomass, as well as disrupt the composition and function of soil microbial
61 communities (Park et al., 2021). To date, most studies have investigated the ecological
62 processes of microbial communities under metal(loid)s pressure, usually focusing on only
63 one microbial domain (Liu et al., 2021; Park et al., 2021; Liu et al., 2022a; Zhang et al.,
64 2022c). In addition to bacteria, fungi can play vital roles in soil, including biogeochemical
65 cycling of metal(loid) elements, decomposition of organic matter, and maintenance of
66 ecosystem stability and productivity (Wang et al., 2022b; Zhang et al., 2022c). However, the
67 simultaneous exploration of the responses of bacterial and fungal communities to long-term
68 metal(loid)s contamination in smelting environments has been little studied.

69 Co-occurrence network analysis has proved to be an efficient tool to explore the
70 responses of microbial communities to environmental stress (Price et al., 2021). Also, the
71 investigation of microbial interactions is critical to understand the complexity and diversity of
72 microbial communities (Zhou et al., 2020). The co-occurrence, competition, and antagonism

73 between different microbial compartments can be examined using network analyses based on
74 molecular data (Zhang et al., 2022c). Furthermore, the network topological features can
75 identify the keystone taxa. Although extensive studies have explored the complex microbial
76 interactions in metal(loid)s contaminated environments (Sun et al., 2020; Chun et al., 2021;
77 Liu et al., 2022c), only a few studies have investigated the differences between bacterial and
78 fungal communities from the perspective of co-occurrence patterns under metal(loid)s
79 contamination. Investigating the relative importance of assembly-regulating processes in
80 microbial communities under metal(loid)s exposure can help reveal the adaptive responses of
81 microbial communities to environmental stress (Zhang et al., 2022b), such as bacterial
82 communities living in mining and smelting sites (Liu et al., 2022a). However, there remains a
83 knowledge gap in the microbial community assembly processes, particularly when comparing
84 the ecological assembly process of bacterial and fungal communities under diverse
85 metal(loid)s exposure. Therefore, a holistic approach investigating all the components of the
86 microbial community (prokaryotes and eukaryotes) under long-term metal(loid)s
87 contamination is required for a better understanding of the assembly rules involved in the
88 microbial communities in response to metal(loid)s exposure.

89 We suspected that bacterial and fungal communities will have similar adaptive responses
90 to long-term metal(loid)s exposure. In the present study, soil samples were collected from the
91 Huilong nonferrous metal smelter, which produced mainly arsenic and arsenic trioxide, and
92 was closed for the past 20 years. Studies were conducted to reveal the differences in adaptive
93 responses between bacterial and fungal communities under metal(loid)s exposure. The
94 objectives of this study were to (1) investigate the effects of selected geochemical properties

95 on bacterial and fungal communities; (2) characterize the co-occurrence patterns of bacterial
96 and fungal communities, and (3) determine the ecological assembly processes of bacterial
97 and fungal under metal(loid)s exposure.

98 **2. Materials and methods**

99 *2.1 Study site description*

100 The study area (111°16'48.64"E, 24°27'48.41"N) has a total area of about 16,617 m² and
101 is located at Zhongshan City in Guangxi Province, southwestern China (Fig. 1). The study
102 region was divided into different areas, including site XY (belonging to the original roasting
103 reverberatory furnace production workshop, which was later used by another factory), site
104 YBS (the main production workshop, which was since demolished and leveled to the ground;
105 its topography was changed greatly), and site ZD (consisting mainly of large piles of
106 arsenic-containing waste slag and soot slag from the production plant). The surrounding areas
107 proximal to the Huilong factory were each identified numerically at site Y. The climate in this
108 region is subtropical monsoon with distinct wet and dry seasons. The mean annual
109 precipitation is 1576.7 mm, with 47% precipitation occurring between April and June. The
110 mean annual air temperature is 19.7°C, with 39.8°C maximal (in 2003 and 2007) and -3.7°C
111 minimal (in 1969) temperatures.

112 *2.2 Soil sampling*

113 A total of 18 topsoil samples (10-20 cm) were collected in October 2020 using a
114 sterilized shovel. Three soil samples were taken from the Y site, and five soil samples were
115 taken from XY, YBS, and ZD sites (Fig. 1). Each sample was a composite of five
116 sub-samples (each approximately 500 g from the four corners and the center point of a 4 m ×

117 4 m quadrat). Large-grain gravel and plant residues were removed during the sampling
118 process. Then these samples were stored in separate polyethylene bags placed on ice and
119 transported immediately to the laboratory. After a preliminary treatment (air-dried and pass
120 through a 10-mm mesh sieve), each composite sample was divided into two aliquots (each
121 approximately 50 g), one for the analysis of soil chemical properties at 4°C, and the other
122 was stored at -80°C for DNA extraction.

123 *2.3 Geochemical measurements*

124 Samples were air-dried at room temperature and passed through a 10-mm mesh sieve.
125 The moisture content (MC) was measured using a published procedure (Dettmann et al.,
126 2021). The soil pH and oxidation-reduction potential (ORP) were determined by suspending
127 soil in distilled water (1:3, solid to water, w/v), shaking for 10 min, and equilibrating for 30
128 min, before the supernatant was measured using a standard PE-28 pH meter (Mettler Toledo,
129 Shanghai). The electrical conductivity (EC) was determined by a Model DDS-11A
130 conductivity meter (Leici, Shanghai) using soil suspended in distilled water (1:5, w/v)
131 according to a previous study (Aponte et al., 2020). Soil total nitrogen (TN) contents were
132 measured by a Vario MACRO cube elemental analyzer (Elementar, Hanau, Germany). For
133 the total organic carbon (TOC) measurements, 5% HCl was added to digest the carbonates of
134 each soil sample and dried at 105°C, then the TOC was measured using a Shimadzu TOC-L
135 Analyzer (Japan). The sulfates were measured on an ICS-600 ion chromatograph (Thermo,
136 USA). The soil metal(loids)s were first digested with a mixture of aqua regia and HF (5:3, v/v)
137 and then the total metal concentrations (for Al, As, Cd, Cr, Cu, Fe, Mg, Mn, Ni, Pb, Sb, Ti, V,
138 and Zn) were determined by inductively coupled plasma optical emission spectrometry

139 (ICP-OES, Thermo iCAP 7000 SERIES, USA). In addition, the bioavailable fractions of
140 metals (Kim et al., 2014) were obtained using the modified BCR sequential extraction
141 method according to Zhu et al. (Zhu et al., 2018). Four metal(loid)s fractions were collected
142 and detailed information was consistent with a previous paper (Li et al., 2022b). Here the
143 bioavailable fractions are expressed as "element symbolmetal(loid)s_bio," such as As_bio
144 and Cd_bio, etc. Each sequential digested filtrate was rinsed with distilled water and filtered
145 before ICP-OES analysis. All samples were analyzed in triplicate for the quality of data.

146 *2.4 DNA extraction, amplification, and sequencing*

147 DNA extraction was conducted using the MoBio PowerSoil[®] DNA isolation kit (MO
148 BIO Laboratories, Carlsbad, CA, USA) according to the manufacturer's instructions. The
149 quality of extracted DNA was evaluated by the NanoDrop 2.0 spectrophotometer (Thermo
150 Fisher Scientific, CA, USA). A Quant-IT Pico Green dsDNA Kit (Invitrogen Molecular
151 Probes Inc, Oregon, USA) was used to examine the final DNA concentration. Primer pairs
152 515F (GAGCCAGCMGCCGCGGTA) and 806R (GGACTACHVGGGTWTCTAAT) were
153 used to amplify the V4 region of the bacterial 16S rRNA gene. Primer pairs ITS1F
154 (CTTGGTCATTTAGAGGAAGTAA) and ITS2R (GCTGCGTTCTTCATCGATGC) were
155 used to amplify the ITS1 region of the fungal rRNA gene (Liu et al., 2022b). The products of
156 PCR amplification and tag-encoded high-throughput sequencing of the 16S and ITS1 region
157 were conducted by MajorBio (Shanghai, China) using the Illumina platform (Miseq, USA)
158 and quantified by QuantiFluor[™]-ST microfluorometry (Promega, USA).

159 The paired-end sequences were merged by the FLASH program based on the default
160 parameters of the equipment (Magoc and Salzberg, 2011). Then the merged sequences were

161 further filtered using QIIME 1.9 for quality, with a size greater than 20 as a threshold of the
162 average Phred quality score. The sequences of chimeric were removed using the USEARCH.
163 The sequences were classified into the operational taxonomic units (OTUs) at a similarity
164 level of 97%, and all singleton OTUs were removed. The representative OTUs of bacteria
165 were classified with the taxonomic information in the SILVA (v138.1) database and the
166 representative OTUs of fungi were classified with the taxonomic information in the UNITE
167 database. Finally, subsampling was performed to normalize the dataset to the sample with the
168 lowest number of reads. The alpha diversity indices (Shannon, Simpson, Chao) were
169 calculated from these samples to evaluate the microbial diversity and richness of bacteria and
170 fungi. The sequence reads of bacteria and fungi obtained here have been submitted to the
171 database of the (National Center of Biotechnology Information) NCBI (Accession No.
172 PRJNA853628 and PRJNA853717, respectively).

173 *2.5 Network construction*

174 The network was adopted to build the co-occurrence patterns of bacterial and fungal
175 communities, exploring the potential relationships among microbes. Initially, the OTU
176 taxonomy file, the relative abundance of OTUs, and the geochemical parameters were
177 assembled for future analysis. Only those genera with the sum of relative abundances higher
178 than 0.005 were kept and the OTUs were filtered that simultaneously appeared in five
179 samples. Subsequently, the Spearman's correlation coefficient analysis ($R > |0.6|$ and $P < 0.05$)
180 was performed to determine the interactions with each of the two genera (Barberan et al.,
181 2012). The correlation matrix was then transformed into the similarity matrix based on the
182 Spearman's correlation coefficient. The global network properties (the average path length,

183 connectivity, average clustering coefficient, and modularity) were calculated. Among these
184 properties, the average path length is the average distance between two nodes and the average
185 clustering coefficient represents the interconnection of nodes. Connectivity reflects the
186 connection strength between two nodes, and modularity is a measure to divide the network
187 into modules, which is identified as a group of nodes connected more densely than the nodes
188 outside the group (Olesen et al., 2007). The topological properties are commonly used to
189 describe the complex pattern of inter-relationships between OTUs (Wei et al., 2020). Network
190 hubs and connectors are considered the keystone nodes in a network, which play important
191 roles in the stability and resistance of microbial communities. Therefore, the OTUs related to
192 these nodes were defined as keystone species. To investigate the interactions between
193 environmental factors and microorganisms in the samples with different contaminant
194 concentrations, the networks between OTUs and the environmental factors were also
195 constructed. All the networks in this investigation were constructed and analyzed by Gephi
196 0.9.2 (<http://igraph.org>).

197 *2.6 Modeling microbial community assembly*

198 Microbial assembly processes are essential for establishing the co-occurrence patterns of
199 community composition within a given ecosystem (Feng et al., 2018). Two complementary
200 mechanisms regulate the assemblages of a microbial community, namely the deterministic
201 (niche-based theory) and stochastic (neutral theory) processes (Zhou and Ning, 2017). The
202 deterministic process emphasizes the dominant role of environmental selection (including
203 biological and non-biological factors) in the microbial assembly process, which largely
204 affects the abundance, composition, and distribution patterns of the microbial communities

205 (Zhang et al., 2022b). Whereas the stochastic process emphasizes the role of dispersal and
206 ecological drift (Zheng et al., 2021).

207 The deterministic and stochastic assembly processes of microbial communities observed
208 here were calculated based on the beta nearest taxon index (β NTI) and the Bray-Curtis-based
209 Raup-Click (RC_{bray}). These data were analyzed using the online tool of the Majorbio Cloud
210 Platform (<https://cloud.majorbio.com/page/tools/>). The value of $|\beta$ NTI| > 2 indicates that a
211 deterministic process (β NTI > 2: variable selection; whereas β NTI < -2: homogeneous
212 selection) dominates the community assembly process. Otherwise, it indicates the stochastic
213 process ($|\beta$ NTI| < 2) dominates the community assembly process (specifically, $|\beta$ NTI| < 2 and
214 $RC_{\text{bray}} < -0.95$: homogeneous dispersal; $|\beta$ NTI| < 2 and $RC_{\text{bray}} > 0.95$: dispersal limitation;
215 $|\beta$ NTI| < 2 and $|RC_{\text{bray}}| < 0.95$: undominated).

216 *2.7 Statistical analyses*

217 The alpha diversity (Shannon, Simpson, or Chao) was estimated using rarified libraries.
218 The Principal Component Analysis (PCoA, beta diversity) was determined by the Bray-Curtis
219 distance matrix to explain the difference of sample groups based on the R package ‘vegan’.
220 The Canonical Correspondence Analysis (De Agostini et al.) was also performed by R
221 package ‘vegan’ to analyze the relationships between geochemical parameters and the
222 microbial community in different groups. The Random Forest (Numberger, #24) prediction
223 was conducted in R using the ‘randomForest’ package. The comparison between different
224 groups was determined using one-way analysis of variance (Dell'Anno et al.) and SPSS
225 software (v8.0) and was considered significant at $P < 0.05$. All the statistical tests were
226 performed using R 4.1.0 unless otherwise stated.

227 **3. Results**

228 *3.1 Geochemical characteristics of soil*

229 The geochemical parameters, including selected soil properties and heavy metal(loid)
230 concentrations of 18 soil samples (from Y, XY, YBS, and ZD sites) are summarized in Fig. 2
231 and Fig. S1. The pH values of soil samples ranged from pH 3.88 to pH 8.37 and were not
232 significantly different (ANOVA, $P > 0.05$) among the four sites. The oxidation-reduction
233 potential (ORP) was significantly higher (ANOVA, $P < 0.05$) in Y and YBS sites than in XY
234 and ZD sites. The total nitrogen content in the Y site was significantly higher (ANOVA, $P <$
235 0.05) compared to the other sites. In addition, the total organic carbon (TOC) concentration in
236 the XY site was significantly lower ($P < 0.05$) than those in the Y and ZD sites. No
237 significant differences in moisture content (MC) or sulfate concentrations were observed
238 among the four sites.

239 The total concentrations of 14 heavy metal(loid)s, including Al, As, Cd, Cr, Cu, Fe, Mg,
240 Mn, Ni, Pb, Sb, Ti, V, and Zn were determined (Fig. 2). Specifically, the Cd, Cr, Fe, Mn, Ti, V,
241 and Zn concentrations in Y samples were significantly higher (ANOVA, $P < 0.05$) than those
242 in XY and ZD sites. There were no significant differences in concentrations of Al, As, Cu,
243 Mg, Ni, Pb, and Sb among the four sites. It is worth noting that the total concentrations of As,
244 Cr, and Sb in all samples greatly exceeded the background limits of the Chinese soil standard
245 (CSL, GB15618-2018), confirming that the Huilong plant (XY, YBS, and ZD sites) and its
246 nearby areas (Y site) were highly polluted by various heavy metal(loid)s; especially As, Cr,
247 and Sb being the main pollutants. BCR sequential extraction showed that the bioavailable
248 fraction of metal(loid)s in the ZD site was higher than those in the other three sites, except for

249 the As and Cd species (Fig. S2).

250 *3.2 Diversity and taxonomic characteristics of microbial communities*

251 The sequences of 16S rRNA and ITS1 genes were amplified by Illumina Miseq
252 sequencing to analyze the bacterial and fungal compositions. After quality filtration, chimeras
253 were removed and sequences were assembled, a total of 1,211,767 high-quality reads were
254 obtained from the 18 samples for bacteria, and 1,003,712 high-quality reads were obtained
255 from the 17 samples for fungi (sample YBS5 did not successfully amplify fungi, maybe
256 because the fungal biomass was too low). The bacterial reads ranged from 37,345 to 74,602,
257 whereas the reads of fungi ranged from 39,272 to 74,314. Based on the 97% sequence
258 similarity, a total of 9,704 and 2,638 OTUs were identified with valid reads for the bacterial
259 and fungal communities, respectively. All coverage indices were > 0.98 , indicating that the
260 reads libraries were reliable for the subsequent study and that the sequences typified the
261 actual bacterial and fungal communities (Fig. S3). For the bacterial community, the Chao
262 index was 2301.93 ± 897.79 (mean \pm SD) (ranging from 3195.94 to 225.60), the Simpson
263 index was 0.02 ± 0.02 (from 0.005 to 0.084) and the Shannon index was 5.32 ± 0.92 (from
264 6.31 to 3.50). Although slight biases were observed in these indices, there were no significant
265 differences (ANOVA, $P > 0.05$) in bacterial community diversity and richness between these
266 four sites. Fungal community diversity showed that the Chao index (ranging from 1061.85 to
267 35.50) and Shannon index (from 4.74 to 1.81) in the XY site were significantly higher than
268 those in the Y site (ANOVA, $P < 0.05$). No significant differences in the Simpson index
269 (ranging from 0.35 to 0.03) were observed among the four sites (ANOVA, $P > 0.05$). These
270 results indicated that the alpha diversity of bacterial communities in different regions was not

271 significantly different, whereas a slight difference was observed in fungal communities.

272 PCoA analysis was calculated based on the sequences to determine the differences in the
273 microbial communities among different sites (Fig. S4). PCoA1 and PCoA2 explained 15.81%
274 and 13.22% of the variation of the bacterial community, respectively. Similarly, PCoA1 and
275 PCoA2 explained 13.06% and 9.36% of the variation of fungal community, respectively. It
276 was worth noting that the Y samples clustered separately, compared to the other three site
277 samples (XY, YBS, and ZD sites) that were closely clustered. These results demonstrated that
278 microbial communities were clustered by their corresponding origin. Taxonomic assignment
279 analysis showed that a total of 65 bacterial and 14 fungal phyla were identified in the OTUs
280 dataset. Among these, 14 bacterial phyla comprised > 90% of the whole raw reads (Fig. S6A),
281 which included four bacterial dominant phyla (the relative abundance > 5%) of
282 *Pseudomonadota* (24.43%-48.84%), *Chloroflexi* (8.92%-19.94%), *Actinobacteriota*
283 (7.05%-15.74%), and *Acidobacteriota* (5.96%-10.38%). The dominant phyla
284 *Pseudomonadota* were composed of *Alphaproteobacteria* and *Gammaproteobacteria* classes,
285 which contributed to 20.57% and 21.48% of the bacterial community, respectively. And the
286 dominant phyla *Actinobacteriota* in the Y site had higher relative abundances than the ZD site
287 ($P < 0.05$) (Fig. S7A). Also, the relative abundances of some bacteria (such as the *Bacillota*
288 phylum) in the YBS site were significantly higher ($P < 0.05$) than those at the XY and ZD
289 sites (Fig. S7A). In addition, other phyla with low relative abundances were also identified
290 among samples (Fig. S7A), such as *Bacteroidota*, *Desulfobacterota*, *Bacillota*,
291 *Gemmatimonadota*, *Methylomirabilota*, *Patescibacteria*, *Planctomycetota*, etc.

292 For the fungal community, *Ascomycota* (21.40%-57.32%) and *Basidiomycota*

293 (3.42%-17.13%) were the dominant fungal phyla among four sites (except for the Y site) (Fig.
294 S6B). The dominant phylum *Ascomycota* was significantly lower in the Y site than in the
295 other three sites ($P < 0.05$). Also, the relative abundance of fungi-like *Basidiomycota* phylum
296 in the Y site was significantly lower ($P < 0.05$) than that in the ZD site. In contrast, the
297 relative abundances of some fungi (such as *Monoblepharomycota* and *unclassified_k__Fungi*
298 phyla) in the Y site were significantly higher ($P < 0.05$) than those at the YBS and ZD sites
299 (Fig. S7B). Additionally, the phyla *unclassified_k__Fungi* accounted for the fungal sequences
300 with a relative abundance of 59.66% in the Y site, 32.38% in the XY site, 14.74% in the YBS
301 site, and 18.92% in the ZD site, indicating that many unknown species existed in the fungal
302 community. The above results indicated that there were significant differences in the
303 distribution of bacterial and fungal communities in different smelting areas.

304 3.3 The importance of geochemical properties on microbial communities

305 RDA (Redundancy analysis) analysis (Fig. S4 and Fig. S5) and the RF (Random Forest)
306 model (Fig. 3 and Fig. S8) were used to determine the role of geochemical properties on
307 bacterial and fungal communities, respectively. According to the RDA analysis, the
308 geochemical variables (RDA1 39.5%, RDA2 25.12% for the edaphic variables; RDA1
309 24.34%, RDA2 17.04% for the total metal(loid)s; RDA1 25.5%, RDA2 20.16% for the
310 bioavailable metal(loid)s) explained the variations of bacterial communities (Fig. S4c,e and
311 Fig. S5A), whereas the geochemical variables (RDA1 40.83%, RDA2 19.89% for the edaphic
312 variables; RDA1 26.49%, RDA2 11.49% for the total metal(loid)s; RDA1 24.23%, RDA2
313 18.03% for the bioavailable metal(loid)s) explained the variations of the fungal communities
314 (Fig. S4d,f and Fig. S5B). The relative abundance of the geochemical variables that affected

315 the beta diversity (NMDS1) was also predicted by the RF model (Fig. 3 and Fig. S8), which
316 explained 50.69% and 31.74% of the total variations of bacterial and fungal communities,
317 respectively. For bacteria, the Fe_bio and the TOC were the most important geochemical
318 variables on the beta diversity, which explained more than 15% of the variations, followed by
319 Cr_bio, Al_bio, Ni_bio, Zn_bio, and other environmental factors (Fig. 3). Many bioavailable
320 metal(loid)s showed positive effects on the beta diversity, such as Fe_bio, Ni_bio, Zn_bio,
321 V_bio, Mn_bio, As_bio, Sb_bio, and Ti_bio, whereas edaphic properties such as TN showed
322 negative impacts on the beta diversity of bacterial communities. The effects of other
323 geochemical variables on beta diversity were not clear and will not be further discussed. For
324 fungi, the dominant environmental factors were TN and As_bio, which explained more than
325 10% of the variations. Sulfate, as well as the Pb_bio, Cu_bio, and Cr_bio also influenced the
326 beta diversity of fungal communities (the relative abundance was more than 2%). The As_bio,
327 Ni_bio, and Pb_bio were positively related to the beta diversity, whereas the TN and Cd_bio
328 were negatively related to the beta diversity (Fig. S8).

329 The interactions between geochemical variables and microbial taxa (the top 500
330 abundant OTUs of bacterial (Table S1) and fungal (Table S2) communities) were further
331 analyzed using co-occurrence networks, with Spearman's correlations ($|R| > 0.6$ and $P < 0.05$)
332 (Fig. 4). For the bacterial community, Fe_bio had the most important impact on 25 OTUs,
333 which agreed well with the results of the Random Forest model. Cr_bio also had important
334 impacts on the bacterial community (13 OTUs), followed by Ni_bio (12 OTUs), the moisture
335 content (MC) (12 OTUs), and Fe_tot (10 OTUs) (Fig. 4A). A total of 88 OTUs displayed
336 significant correlations with geochemical variables, and the network was divided into ten

337 modules. The microbial taxa of *Burkholderiaceae* (2 OTUs), *Chitinophagaceae* (2 OTUs),
338 *Comamonadaceae* (4 OTUs), *Hyphomonadaceae* (1 OTUs), *Nocardiaceae* (3 OTUs),
339 *norank_o__IMCC26256* (1 OTUs), *norank_o__norank_c__P2-11E* (1 OTUs),
340 *Oxalobacteraceae* (2 OTUs), *Rhodocyclaceae* (3 OTUs), *UBA12409* (1 OTUs), and
341 *unclassified_c__Acidimicrobiia* (1 OTUs) families displayed the highest correlations with
342 geochemical variables (Table S2). Some groups, such as *Chitinophagaceae*,
343 *Comamonadaceae*, *Nocardiaceae*, *Oxalobacteraceae*, *Rhodocyclaceae*, and *UBA12409*,
344 showed positive correlations with the bioavailable fractions of metals (Table S3). For the
345 fungal community, a total of 135 OTUs displayed strong correlations with the geochemical
346 variables, and the network was divided into eight modules. Mn_tot had the most important
347 impact on 85 OTUs, followed by Cr_tot (79 OTUs), Ni_tot (62 OTUs), TOC (57 OTUs),
348 V_tot (43 OTUs), and MC (30 OTUs) (Fig. 4B). The OTUs affiliated with the
349 *Didymosphaeriaceae* (5 OTUs), *Fomitopsidaceae* (3 OTUs), *Lophiostomataceae* (2 OTUs),
350 *Lycoperdaceae* (3 OTUs), *Meruliaceae* (12 OTUs), *Phaeosphaeriaceae* (5 OTUs),
351 *Pleosporaceae* (4 OTUs), *Steccherinaceae* (3 OTUs), and *Xenasmataceae* (2 OTUs) families
352 were strongly influenced by multiple environmental parameters. These families (except for
353 *Lophiostomataceae* and *Xenasmataceae*) showed positive correlations with the bioavailable
354 fractions of metal(loid)s, whereas negative correlations were found with the total metal(loid)
355 content and the other environmental parameters (Table S4).

356 *3.4 Co-occurrence patterns and keystone taxa analysis of microbial communities*

357 In the present study, the co-occurrence patterns of bacterial and fungal communities
358 were individually constructed by strong correlations (Spearman $R > |0.6|$) and significant ($P <$

359 0.05) among the top 500 abundant OTUs in the Huilong smelter regions. For bacteria, the
360 networks of the Huilong smelter regions contained 473 nodes (OTUs) and 5120 edges
361 (connection between OTUs). Whereas for fungi, the networks of the Huilong smelter regions
362 contained 490 nodes and 16559 edges. Analogously, the average degree, clustering
363 coefficient, and density in fungal communities were higher than those in bacterial
364 communities. It was worth noting that, the proportions of positive edges tended to be higher
365 than negative edges in the networks of bacterial and fungal communities (Table 1). Moreover,
366 the nodes were assigned by phylum (Fig. 5A and C) and modularity class (Fig. 5B and D).

367 A total of 11 dominant taxa (at the phylum level) were identified from the co-occurrence
368 patterns of bacterial communities, which accounted for 86.26% of total nodes, and the
369 remaining nodes (65 nodes) were classified as others (13.74%). *Pseudomonadota* was the
370 most dominant phylum, which accounted for 32.98% of total nodes, followed by
371 *Actinobacteriota* (10.99%), *Chloroflexi* (7.82%), *Acidobacteriota* (5.92%), *Planctomycetota*
372 (5.5%), and *Bacteroidota* (5.5%) in the bacterial networks (Fig. 5A). All nodes were divided
373 into 11 modules, including 5 major modules (Fig. 5B). Additionally, the OTUs with nodes
374 degree > 30 and betweenness centrality < 100 were typically identified as the keystone
375 phylotypes in the present study. Here a total of 5 nodes were identified as the keystone taxa,
376 which are mainly distributed in Module III and Module IV. The keystone OTUs were
377 affiliated to *Diplorickettsiaceae*, *norank_o__Candidatus_Woesebacteria*,
378 *norank_o__norank_c__AT-s3-28*, *norank_o__norank_c__bacteriap25*, and
379 *Phycisphaeraceae* families.

380 Accordingly, for fungi, a total of 11 phyla (Fig. 5C), including *Ascomycota* (51.84%),

381 *Basidiomycota* (41.63%), *Glomeromycota* (2.24%), *Chytridiomycota* (2.04%), *Rozellomycota*
382 (0.61%), *Mucoromycota* (0.41%), *Olpidiomycota* (0.41%), *Mortierellomycota* (0.2%),
383 *Kickxellomycota* (0.2%), *Calcarisporiellomycota* (0.2%) and *Monoblepharomycota* (0.2%),
384 were identified from the co-occurrence patterns of fungal communities. All nodes were
385 divided into 9 modules, including 6 major modules (Fig. 5D). Here a total of 9 nodes were
386 identified as the keystone OTUs, which were affiliated to *Biatrisporaceae*,
387 *Ganodermataceae*, *Peniophoraceae*, *Phaeosphaeriaceae*, *Polyporaceae*, *Teichosporaceae*,
388 *Trichomeriaceae*, *Wrightoporiaceae*, *Xylariaceae* families, mainly distributing in Module I,
389 Module III and Module IV.

390 *3.5 Ecological assembly processes of microbial communities*

391 The calculated β NTI and RC_{bray} were compared between bacterial and fungal
392 communities, and the assembly processes were determined. There were slight differences in
393 the scale of the assembly process at each site. The assembly process was judged according to
394 the criteria described in the above method. For the whole bacterial communities, the majority
395 of the β NTI in the whole microbial community (91.5%) was greater than 2, indicating that the
396 deterministic process controlled the bacterial community assembly (Fig. 6A). The Y and YBS
397 sites had similar assembly processes, with the variable selection accounting for 94.5% and
398 undominated process accounting for 5.5%. Similarly, the variable selection process (90.1%
399 and 87.7%) also governed the bacterial community assembly of XY and ZD sites, whereas
400 the dispersal limitation (4.4% and 2.3%) and undominated (5.5% and 10%) incurred
401 variations in bacterial communities of the XY and ZD sites, respectively. For the whole
402 fungal communities, the relative contribution of the deterministic process (90.2%) was higher

403 than that of the stochastic process (9.8%) (Fig. 6B). Within fungal communities of each site,
404 variable selection accounted for a high percentage of community assembly, including Y
405 (82.3%), XY (92.9%), YBS (91.2%), and ZD sites (90.5%). The stochastic process contained
406 dispersal limitation (7.9%), homogenizing dispersal (3.9%), and undominated (5.9%) in the Y
407 site, whereas only undominated processes (7.1%, 8.8%, and 9.5%) were present in the XY,
408 YBS, and ZD sites, respectively. These results indicated that deterministic processes
409 dominated the assembly of microbial communities regardless of bacterial or fungal
410 communities.

411 A correlation matrix between edaphic variables and the β NTI was assembled to
412 investigate the factors influencing the ecological assembly process of microbial communities.
413 The assembly process of bacterial communities was more affected by the tested geochemical
414 properties than the fungal communities (Table S1). In particular, the pH, TN, total
415 metal(loid)s, and the bioavailable fractions of metal(loid)s (except for As, Cd, and Ti) had
416 significant impacts on either the bacterial or fungal assembly processes in this study (Table
417 S1-1).

418 **4. Discussion**

419 Metal(loid)s pollution of soil is a high-priority research topic in which microbial
420 strategies for stabilizing metal(loid)s are recognized as a promising alternative relative to
421 traditional remediation technologies. Microbial communities are identified as potential
422 indicators of soil function and health in contaminated and surrounding environments
423 (Banerjee and van der Heijden, 2022). Most recent studies of metal(loid)s contamination
424 focus on only one microbial domain at a time (Park et al., 2021; Liu et al., 2022a; Liu et al.,

425 2022c; Zhang et al., 2022c). However, the co-occurrence patterns and ecological assembly
426 processes of both bacterial and fungal communities under diverse metal(loid)s exposures
427 have not been investigated so far. Therefore, it is necessary to comprehensively compare the
428 responses of indigenous bacterial and fungal communities to metal(loid)s pollution.

429 *4.1 Geochemical characterizations*

430 In the Huilong smelter area, the concentrations of total As, Cr, and Sb exceeded the
431 Chinese *Soil Environmental Quality Construction Land Soil Pollution Risk Management and*
432 *Control Standard (Trial)* (GB36600-2018), which is the second land use standard for
433 screening purposes. Although no significant differences in these total metal(loid)s
434 concentrations (except for total Cr) were found among the four studied sites. In contrast,
435 significant differences were observed in the bioavailable fraction of these metal(loid)s among
436 the four contaminated areas. For example, the bioavailable fraction proportion of As (As_bio)
437 in three sites (XY, YBS, and ZD) was higher than that in the Y site (Fig. S2). Recent studies
438 of the evaluation of metal(loid)s pollution risk at other sites of the Huilong smelter (Li et al.,
439 2022a) showed that the high-risk metal elements were As and Sb. These results suggested
440 that smelting activities released these metal(loid)s to the local community, despite being shut
441 down more than two decades ago. Additionally, both TN and TOC were significantly higher
442 in the Y site than in the other three sites. It was likely that the Y site was located near
443 farmland, as the levels of TN and TOC in agricultural soils tend to be higher than in other soil
444 types (Compton et al., 2004). Therefore, the above results indicated that significant
445 differences were observed in metal(loid)s and environmental factors between the Huilong
446 smelter area (XY, YBS, and ZD sites) and the surrounding area (Y site), which might be

447 related to the artificial smelting activities.

448 *4.2 Effects of geochemical characteristics on bacterial and fungal communities*

449 There were no significant differences in bacterial diversity and richness (Fig. S3A).
450 Such observations demonstrate that the alpha diversity of bacterial communities was not
451 significantly affected by the variations of metal(loid)s and environmental factors, which is
452 similar to previous studies of metal(loid)s contaminated environments near mining and
453 smelting sites (Pereira et al., 2014; Chun et al., 2021; Liu et al., 2021). In contrast, significant
454 differences in alpha diversity (except the Simpson indices) and richness were observed for
455 fungi. From the perspective of microbial diversity, the toxic effects of metal(loid)s can often
456 reduce microbial diversity during the initial phase of metal(loid)s exposure (Liu et al., 2022a).
457 However, the microbes could then develop an adaptive response mechanism to metal(loid)s
458 contamination, as evidenced by an increase in their diversity and richness (Yan et al., 2020).
459 In particular, the diversity and richness of fungal communities in the XY site (in Huilong
460 smelter area) were significantly higher than in the Y site (in the surrounding area), which
461 demonstrated that smelting activities maybe increase the fungal diversity and richness.

462 Notably, beta-diversity patterns were distinguished in both bacterial and fungal
463 communities, with significant differences among the four sites as revealed by PCoA (Fig. S4)
464 and RDA analyses (Fig. S4 and S5). Such observations may be due to the combined effect of
465 metal(loid)s and other geochemical parameters. The RF model prediction showed that the
466 bioavailability of certain metal(loid)s (Al, As, Cr, Fe, Ni, Pb, Sb, Ti, and Zn) had positive
467 effects on the beta-diversity of bacterial communities (Fig. 3). Similarly, bioavailable As, Ni,
468 and Pb had positive effects on the beta diversity of fungal communities (Fig. S8). These

469 results suggest that the bioavailable fractions of metal(loid)s have substantial impacts on the
470 beta diversities of the bacterial and fungal communities (Wang et al., 2018), although the
471 dominant factors differed for the bacterial and fungal communities. As previously reported,
472 bioavailable Al, As, Fe, and Sb have strong negative effects on microbial diversity (Liu et al.,
473 2022c) and significant decreases in community richness in As-and Cr-contaminated soils
474 (Sheik et al., 2012). In contrast, the bioavailable fractions of certain metal(loid)s presented
475 here generally showed positive effects on the beta diversity of microbial communities, as
476 shown in the RF model, probably due to sensitive indigenous microorganisms that were
477 stimulated by the metal(loid)s.

478 The microbial-contaminant interactions demonstrated that some bacteria and fungi were
479 responsive to some but not all bioavailable metals in soil. For example, three OTUs
480 associated with *Comamonadaceae* showed positive relationships with the bioavailable Fe, Cr,
481 and Ni. Members of *Comamonadaceae* have been identified as Fe(III)-reducing bacteria,
482 which could adapt to the detrimental environment by changing their metabolic ways (Wang et
483 al., 2021; Zhang et al., 2022a). Consistently, two OTUs associated with *Rhodocyclaceae*
484 showed positive relationships with the bioavailable Fe and Cr. It also has been reported that
485 Pb exhibited a strong correlation with the Fe(III)-reducing bacterial lineages *Rhodocyclaceae*
486 (Moberly et al., 2016; Jia et al., 2022). Apart from these bacteria, the fungi, e.g., *Meruliaceae*
487 and *Pleosporaceae*, showed similar trends. Members of *Meruliaceae* were the most
488 influenced by multiple environmental parameters, three OTUs associated with *Meruliaceae*
489 showed positive relationships with the bioavailable Fe, Ni, and V. Although *Meruliaceae*
490 often grows on rotten gymnosperm wood (Liu et al., 2022e), the relationship of *Meruliaceae*

491 with metal(loid)s has not been studied. Additionally, two OTUs associated with
492 *Pleosporaceae* showed positive relationships with the bioavailable Fe and V. It was consistent
493 with the observation of rhizosphere fungal communities in a Cu tailings area (Jia et al., 2020).
494 The observations presented here demonstrate the adaptation of the promising candidates (e.g.,
495 bacteria, *Comamonadaceae*, *Rhodocyclaceae*, and fungi, *Meruliaceae*, *Pleosporaceae*) to the
496 conditions prevailing at Huilong nonferrous metal smelter.

497 *4.3 The co-occurrence patterns of bacterial and fungal communities*

498 Soil microbes do not thrive well in isolated environments; instead, they prefer complex
499 networks of interactions. In the present study, the co-occurrence patterns of bacterial and
500 fungal communities were established using networks based on the random matrix theory to
501 reveal the interactions between microbes (Faust and Raes, 2012). The topological
502 characteristics of networks generally represent the levels of interactions and connectedness
503 (Zheng et al., 2018). Compared to bacterial networks, the fungal networks had more links
504 (Table 1 and Fig. 5), which indicated that inter-species relationships among different adaptive
505 fungal species would be strengthened and stabilized in metal(loid)s contaminated areas. On
506 the other hand, the bacterial communities were less adapted and were weaker as evidenced by
507 their few inter-species relationships. These data suggest that fungal communities were less
508 sensitive and more resistant to HM toxicity than the bacterial communities residing at this
509 abandoned smelter site. These results indicated that the frequency of connections for fungal
510 communities was higher than those in bacterial communities. Generally, there were similar
511 trends in network topological properties for both bacterial and fungal communities.

512 The keystone species inhabiting the heavily polluted areas may indicate that they have

513 special ecological roles in harsh environments. In the present study, the OTU with nodes
514 degree > 30 and betweenness centrality < 100 were typically identified as the keystone
515 phylotypes. In the bacterial networks, the members of the family *Diplorickettsiaceae*,
516 *norank_o__Candidatus_Woesebacteria*, *norank_o__norank_c__AT-s3-28*,
517 *norank_o__norank_c__bacteriap25*, and *Phycisphaeraceae* were considered as the keystone
518 taxa in the bacterial communities. Within these keystone taxa, members of
519 *Diplorickettsiaceae*, belonging to the phylum of *Pseudomonadota*, have been identified as
520 Pb-sensitive taxa in the soil microbial community (Wan et al., 2022). Consistently, several
521 previous studies have shown that the members of *Pseudomonadota* possess a large number of
522 metal-related operons, which are responsible for the detoxification of metal(loid)s (Qiao et al.,
523 2021; Zou et al., 2021). The *Pseudomonadota* was abundant in the Huilong area, suggesting
524 that this phylum mitigates metal(loid)s toxicity through a special adaptive mechanism (Keshri
525 et al., 2015; Fashola et al., 2016). Moreover, members of
526 *norank_o__Candidatus_Woesebacteria* belong to the phylum of *Patescibacteria*, which phyla
527 has As, Fe, and Ag resistance genes (Zhou et al., 2022) and can transport various heavy
528 metals from massive soils to the rhizosphere (Tian et al., 2022). Consistent with previous
529 studies (Flieder et al., 2020; Liu et al., 2022c), the members of
530 *norank_o__norank_c__AT-s3-28* family, associated with the phylum *Acidobacteriota*, play a
531 key role in bacterial communities in areas such as Huilong, which exhibited a resistance
532 capacity to metal(loid)s pollution (Wang et al., 2022a). It is not surprising that the family
533 *norank_o__norank_c__bacteriap25* (*Myxococcota*) and *Phycisphaeraceae* (*Planctomycetota*)
534 were identified as keystone taxa in the Huilong site, owing to their ability to tolerate and

535 resist metals ions using the complexation and adsorption mechanisms (Dell'Anno et al., 2021;
536 Liu et al., 2022d; Marghoob et al., 2022; Gao et al., 2022). These findings could help develop
537 remediation strategies for Huilong contaminated areas, for example, by purposefully
538 screening key species for biological materials.

539 The fungi with a strong resistance to metal(loid)s pollution can play a key role in the
540 restoration of metal(loid)s polluted ecosystems. In the fungal networks, OTUs affiliated to the
541 *Biatriosporaceae* (*Ascomycota*), *Ganodermataceae* (*Basidiomycota*), *Peniophoraceae*
542 (*Basidiomycota*), *Phaeosphaeriaceae* (*Ascomycota*), *Polyporaceae* (*Basidiomycota*),
543 *Teichosporaceae* (*Ascomycota*), *Trichomeriaceae* (*Ascomycota*), *Wrightoporiaceae*
544 (*Basidiomycota*), *Xylariaceae* (*Ascomycota*) families were considered as the keystone taxa.
545 These OTUs mainly belonged to the phylum of *Ascomycota* and *Basidiomycota*. *Ascomycota*,
546 the most abundant and widely existing soil fungi in the world, was the keystone phylum in
547 the fungal networks of Huilong sites, consistent with the previous studies (Lin et al., 2019;
548 De Agostini et al., 2020; Xie et al., 2021). This phylum has a strong tolerance to metal(loid)s;
549 in fact, even its abundance was increased with metal(loid)s concentrations (Lin et al., 2019).
550 Furthermore, *Basidiomycota* was also a dominant phylum in the networks of fungal
551 communities. *Basidiomycota* (filamentous fungi) can be used to remove metal(loid)s (Chen et
552 al., 2012). Therefore, *Basidiomycetes* could be associated with their adaptation and function
553 in metal(loid)s contaminated environments. These results agree with a previous report (Chen
554 et al., 2014) that *Ascomycota* and *Basidiomycota* were the most important components in
555 metal(loid)s polluted soils.

556 In addition, positive and negative links (representing positive and negative correlations)

557 may reflect cooperation (or niche overlap) and competition (or niche separation) among
558 species (Deng et al., 2016; Ghoul and Mitri, 2016; Zhang et al., 2018). Here there were more
559 positive links than negative links in both bacterial and fungal networks (Table 1). This
560 reflects the importance of microbial synergy, where most microbial species potentially
561 cooperate to withstand metal(loid)s pollution (Hoek et al., 2016). It is worth noting that the
562 proportion of positive connections is significantly higher in fungal networks than in bacterial
563 networks. This indicates that environmental pollution at the studied Huilong factories, as well
564 as other anthropogenic sources, increased the competition of species among the fungal
565 community (Zhou et al., 2020). Competition can stabilize the co-oscillation of the community
566 and promote the stability of the network (Coyte et al., 2015).

567 *4.4 Ecological assembly processes of bacterial and fungal communities*

568 Revealing the assembly process of the microbial community in ecological niches can
569 uncover the responses of a microbial community to perturbations by metal(loid) exposures
570 (Jiao et al., 2016; Zhang et al., 2021; Liu et al., 2022a). In the current study, variable selection
571 accounted for the highest contribution rate (82.3-94.5%) in the deterministic process among
572 bacterial and fungal communities (Fig. 6). However, the stochastic processes contribute
573 differently to the assemblies of fungal and bacterial communities. For example, the fungal
574 community showed a stronger dispersal limitation than the bacterial community in the
575 influenced area (Y site), which was consistent with previous studies (Jiang et al., 2016). That
576 it is because the body size of fungi hindered their spread or spatial aggregation, and limited
577 long distance dispersal compared with the smaller bacteria (Xu et al., 2021b). An opposite
578 trend occurred in Huilong factories area (XY, YBS, and ZD sites), in which high levels of

579 dispersal limitation were found in bacterial communities, possibly due to the differences of
580 metal(loid) concentrations and geochemical properties between the influenced area (Y site)
581 and Huilong factories area (XY, YBS, and ZD sites). Above observations agreed with several
582 previous studies (Zhou and Ning, 2017; Mori et al., 2018; Zheng et al., 2021) reporting that
583 the deterministic and stochastic processes could jointly regulate the natural community
584 assembly, and their relative importance varies with the environmental context. Furthermore,
585 the present study showed that the pH, TN, total metal(loid) concentrations, and the
586 bioavailable fractions of metal(loid)s (except for the As, Cd, and Ti) had significant impacts
587 on the microbial assembly process. These results confirm the combined effect of metal(loid)
588 exposures and geochemical properties on the microbial community. However, ecological
589 deterministic and stochastic processes exist that can jointly contribute to the soil microbial
590 community assembly in response to metal(loid)s exposure. Their detailed contributions can
591 vary in bacterial and fungal communities depending on the environmental context.

592 **5. Conclusion**

593 The response of bacterial and fungal communities to different geochemical variables, as
594 well as their ecological assembly processes, were investigated in the present study. The
595 smelting activities had indeed brought metal(loid)s pollution, especially As and Sb, to the
596 locality, although Huilong smelter has been shut down for decades. Both metal(loid)s and
597 edaphic variables drive the microbial communities, but very different responses of innate
598 microbiota were observed. The bioavailable Fe and TOC had considerable impacts on the
599 beta diversity of bacterial communities, whereas TN and bioavailable As mainly influenced
600 the fungal communities. The inter-species interactions of fungal co-occurrence networks were

601 more complex than the bacterial networks, suggesting inter-species relationships among
602 different fungal species were more stable than the bacterial species in this metal(loid)s
603 contaminated area. Moreover, the relatively higher positive interaction of microbiota
604 indicated the importance of microbial synergy among species with stronger resistance to
605 metal(loid)s polluted environments. The keystone taxa were investigated and revealed their
606 adaptive superiority to metal(loid)s stress. Additionally, the deterministic process (variable
607 selection) dominated all bacterial and fungal assemblies, which was highly impacted by the
608 pH, TN, total metal(loid)s, and the bioavailable fractions of certain metal(loid)s. These
609 findings enhance our understanding of the microbial responses to anthropogenically induced
610 smelting activities and provide a vital guideline for developing the bioremediation practice of
611 metal(loid)s contaminated sites.

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617 **Author contributions**

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621 Data Curation, Visualization, Writing – review & editing. **Bang Liu, Ying Cao, Hao Li,**

622 **Wancheng Pang, Houquan Liu, Shun Jiang, Junjie Zhu:** Writing – review & editing.

623 **Conflicts of interest**

624 The authors declare that there are no conflicts of interest.

625

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879 **Highlights**

- 880 ● The interaction between microbes, metal(loid)s, and soil properties was clarified.
- 881 ● The links of fungal co-occurrence networks were higher than the bacterial
882 networks.
- 883 ● The keystone taxa were investigated in bacterial and fungal communities.
- 884 ● Deterministic assemblies played a vital role in shaping the microbial community.

885 **Figure captions**

886 **Fig. 1.** Location of the abandoned non-ferrous metal(loid)s Huilong smelters (left)
887 and distribution of sampling points (right), located in Zhongshan, Guangxi
888 province, southwest China. The study area is delineated in red and different
889 background colors represent different sites. The red dots represent the impact
890 zone south of the smelter.

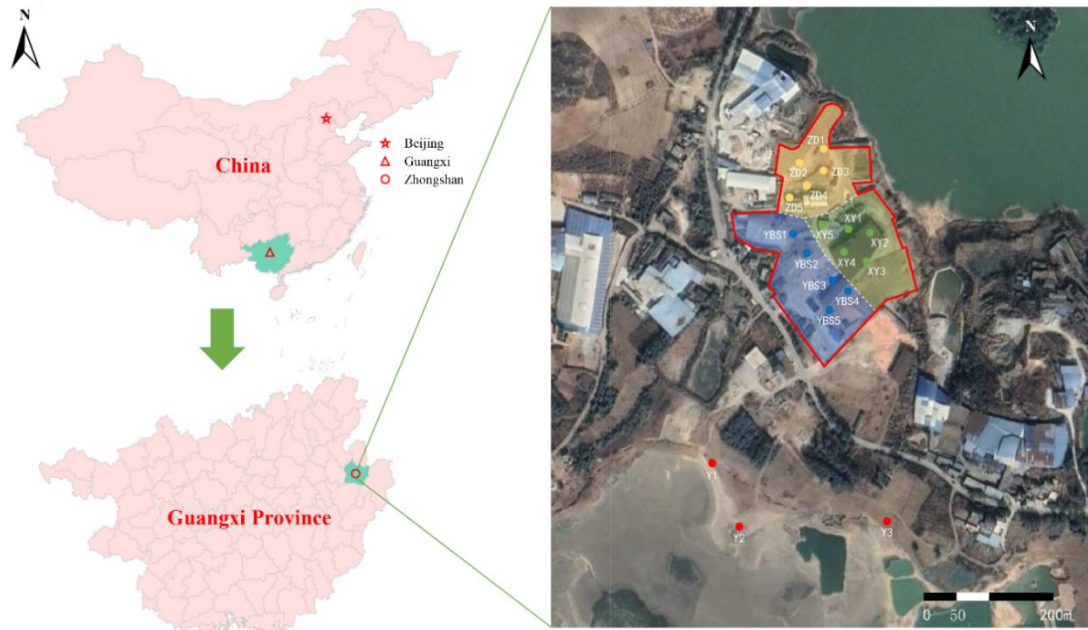
891 **Fig. 2.** Boxplots showing the soil total metal(loid)s concentrations in the non-ferrous
892 metal(loid)s smelter and surrounding sites. Different lowercase letters in the
893 boxplots indicate significant differences ($P < 0.05$) among the tested sites.

894 **Fig. 3.** Contributions of bioavailable metal(loid)s and edaphic parameters on bacterial
895 beta diversity (NMDS1) in Huilong smelter and surrounding sites as
896 predicted by the random forest model (A), and partial dependence of the
897 predicted model (B), the black points and blue lines are the partial
898 dependence data and trend, the red lines are the local polynomial regression
899 fitting trends, with the 95% confidence limits (the gray band).

900 **Fig. 4.** Networks analysis between environmental variables and bacterial (A) and
901 fungal (B) species among the top 500 abundant OTUs in the Huilong smelter
902 regions. Edges are shown by strong correlations (Spearman $R > |0.6|$) and
903 significant ($P < 0.05$). The nodes were colored by modularity class. The size
904 of the node corresponds to the number of connections. The thickness of the
905 edges corresponds to the strength of the correlation. Pink lines indicate
906 positive interactions and green lines indicate negative interactions.

907 **Fig. 5.** Co-occurrence patterns of bacterial (A and B) and fungal (C and D)
908 communities among the top 500 abundant OTUs in the Huilong regions.
909 Edges are shown by strong correlations (Spearman $R > |0.6|$) and significant
910 ($P < 0.05$). The nodes were colored by phylum (A and C) and modularity
911 class (B and D). The thickness of the edges corresponds to the strength of the
912 correlation. Light purple lines indicate positive interactions and green lines
913 indicate negative interactions.

914 **Fig. 6.** Relative contributions of deterministic and stochastic processes for bacterial
915 (A) and fungal (B) communities in the Huilong smelter and surrounding
916 regions were determined using the null model framework.
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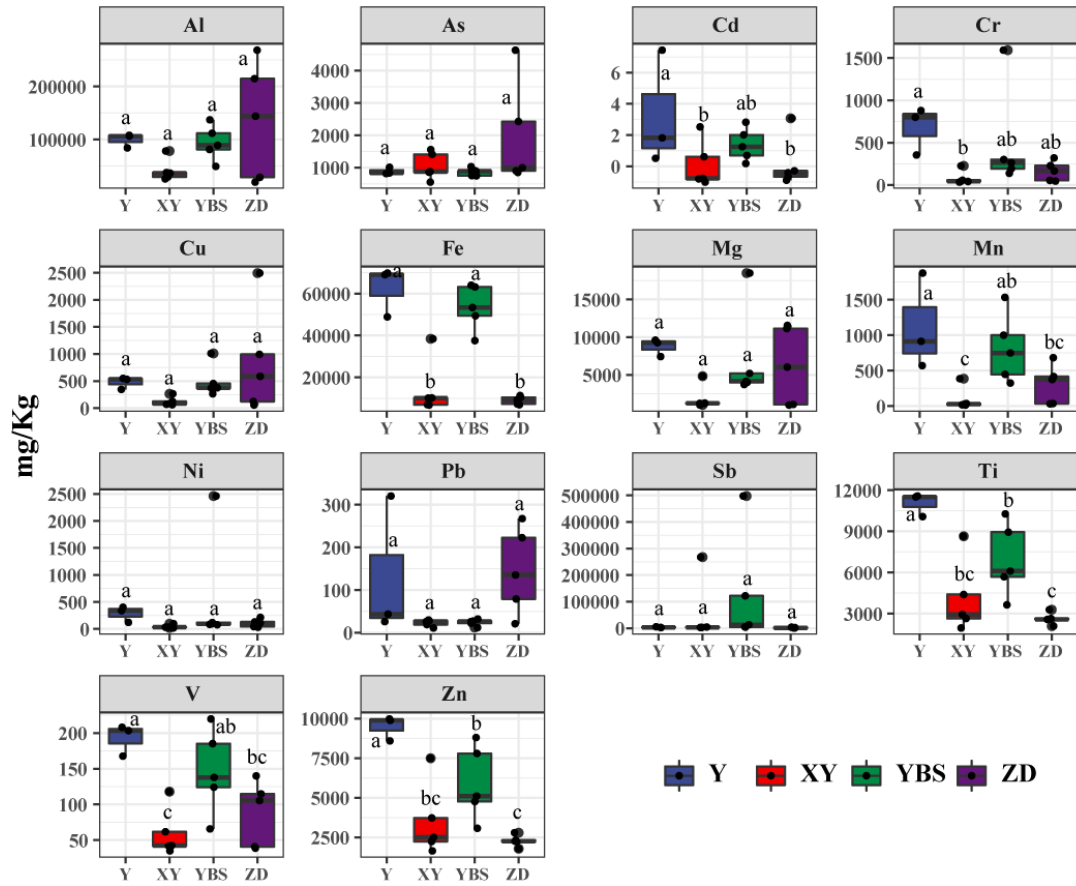
920 **Fig. 1.** Location of the abandoned non-ferrous metal(loid)s Huilong smelters (left)

921 and distribution of sampling points (right), located in Zhongshan, Guangxi province,

922 southwest China. The study area is delineated in red and different background colors

923 represent different sites. The red dots represent the impact zone south of the smelter.

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Fig. 2. Boxplots showing the soil total metal(loid)s concentrations in the non-ferrous metal(loid)s smelter and surrounding sites. Different lowercase letters in the boxplots indicate significant differences ($P < 0.05$) among the tested sites.

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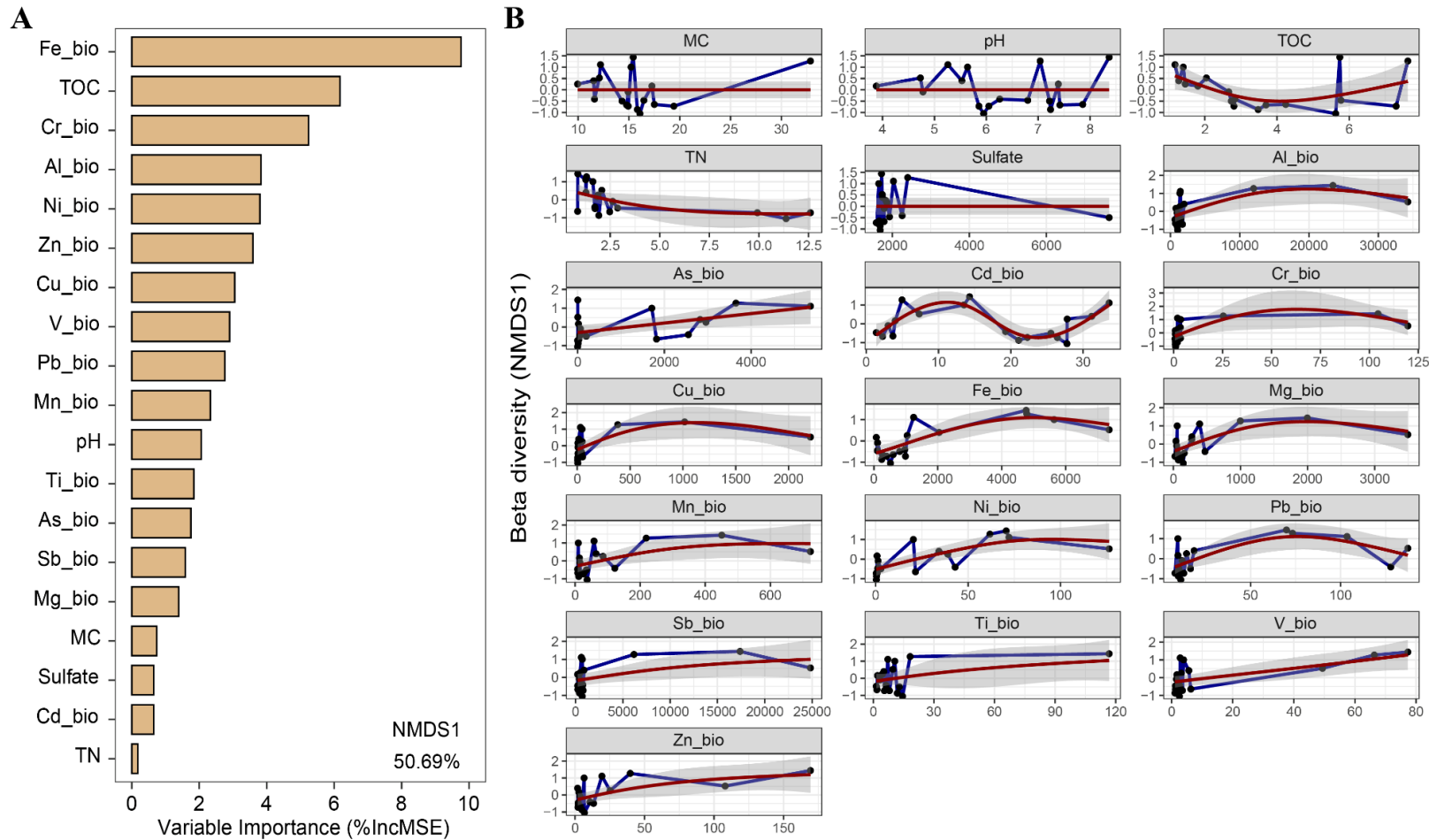
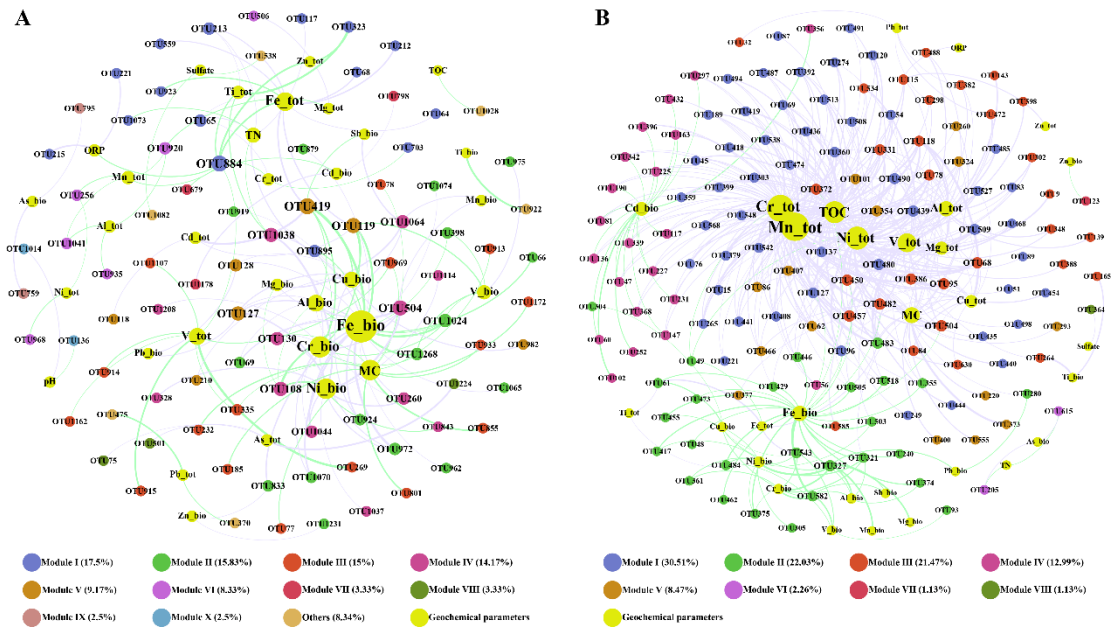


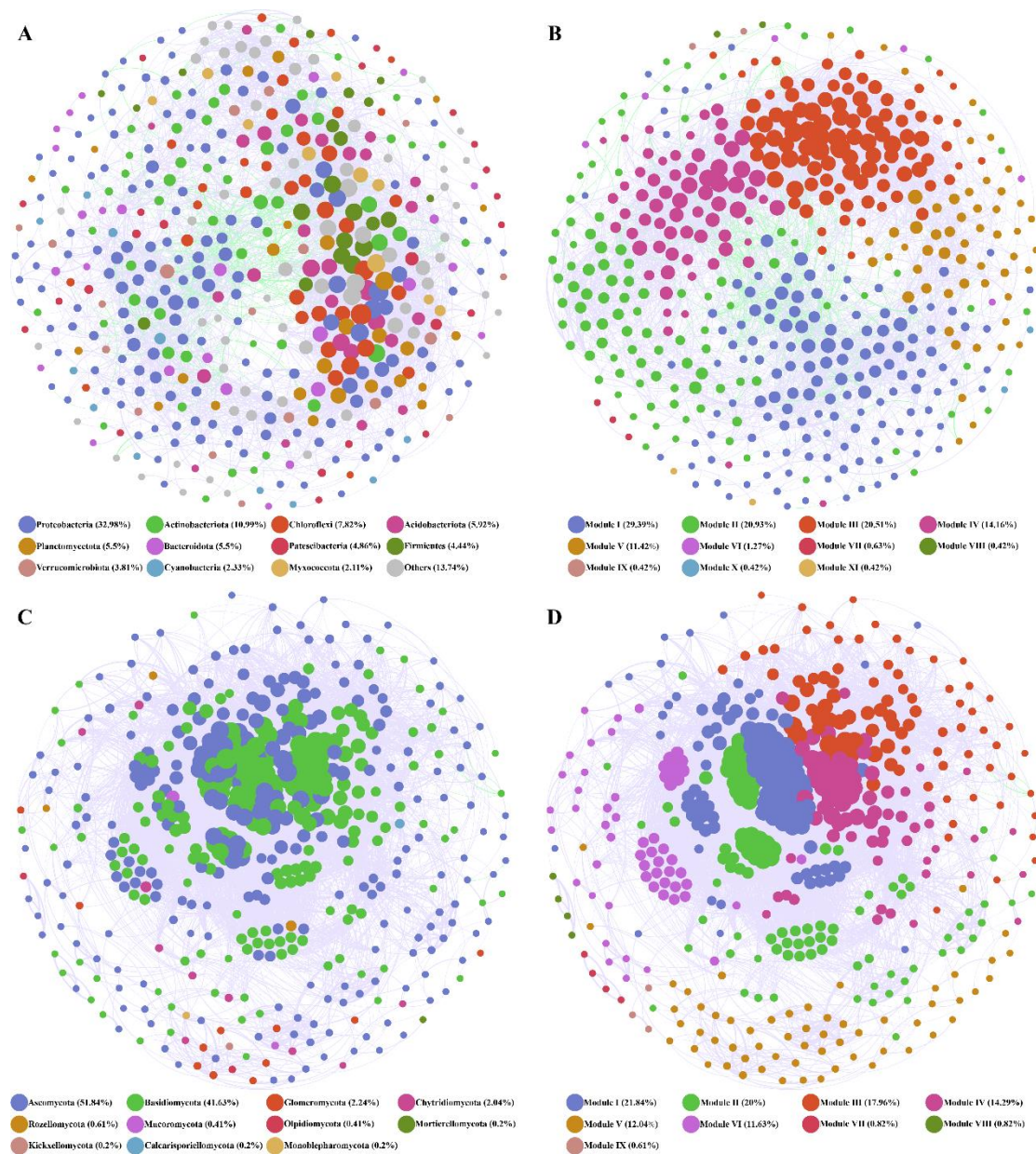
Fig. 3. Contributions of bioavailable metal(loid)s and edaphic parameters on bacterial beta diversity (NMDS1) in Huilong smelter and surrounding sites as predicted by the random forest model (A), and partial dependence of the predicted model (B), the black points and blue lines are the partial dependence data and trend, the red lines are the local polynomial regression fitting trends, with the 95% confidence limits (the gray band).



931

932 **Fig. 4.** Networks analysis between environmental variables and bacterial (A) and
 933 fungal (B) species among the top 500 abundant OTUs in the Huilong smelter regions.

934 Edges are shown by strong correlations (Spearman $R > |0.6|$) and significant ($P <$
 935 0.05). The nodes were colored by modularity class. The size of the node corresponds
 936 to the number of connections. The thickness of the edges corresponds to the strength
 937 of the correlation. Pink lines indicate positive interactions and green lines indicate
 938 negative interactions.



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Fig. 5. Co-occurrence patterns of bacterial (A and B) and fungal (C and D)

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communities among the top 500 abundant OTUs in the Huilong regions. Edges are

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shown by strong correlations (Spearman $R > |0.6|$) and significant ($P < 0.05$). The

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nodes were colored by phylum (A and C) and modularity class (B and D). The

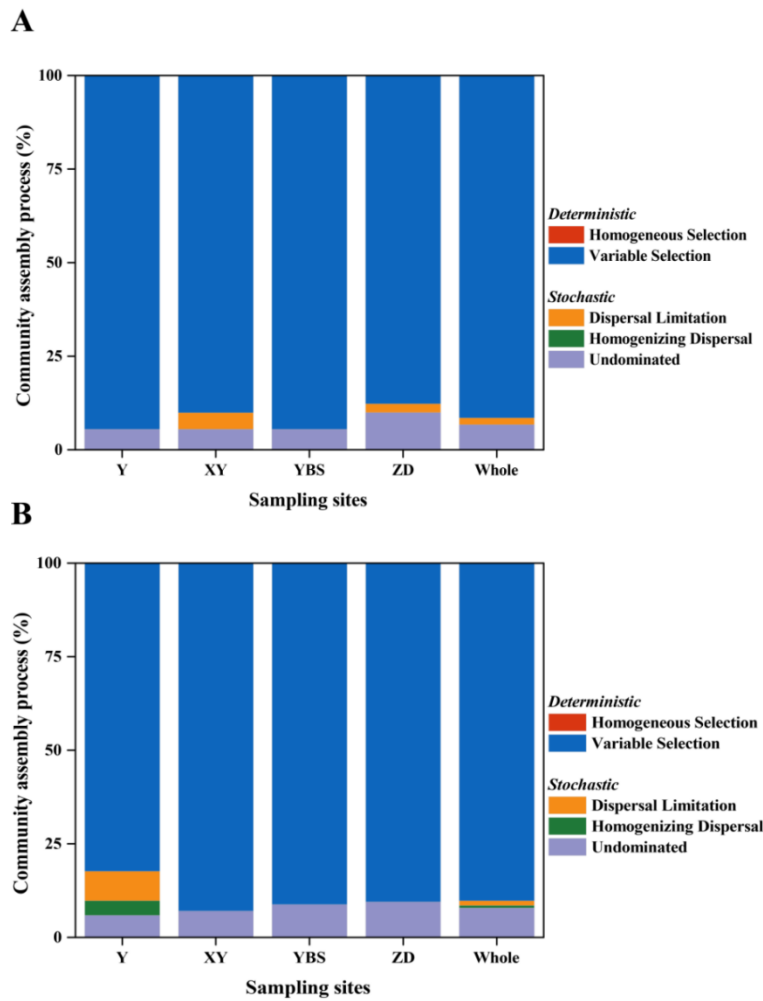
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thickness of the edges corresponds to the strength of the correlation. Light purple lines

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indicate positive interactions and green lines indicate negative interactions.

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949 **Fig. 6.** Relative contributions of deterministic and stochastic processes for bacterial
 950 (A) and fungal (B) communities in the Huilong smelter and surrounding regions were
 951 determined using the null model framework.

952

953 **Table 1.** Topological characteristics of bacterial and fungal networks in Y, XY, YBS,
 954 and ZD sites.

Topological properties	Bacterial networks		Fungal networks	
	1*	2*	1*	2*
Nodes	473	120	490	177
Total links	5120	159	16559	520
Positive links	4706	82	16553	89
Negative links	414	77	6	431
Average degree	21.649	2.65	67.588	5.876
Clustering coefficient	0.433	0	0.584	0
Path distance	3.219	4.229	2.656	3.181
Density	0.046	0.022	0.138	0.033
Modularity	0.508	0.63	0.29	0.341

955 * abbreviations: 1, nodes of co-occurrence patterns were colored by phylum and
 956 modularity class in the bacterial or fungal communities network analysis; 2, networks
 957 analysis between environmental variables and bacterial or fungal species.