



**HAL**  
open science

## Assembly processes of bacterial and fungal communities in metal(loid)s smelter soil

Miaomiao Li, Jun Yao, Geoffrey Sunahara, Robert Duran, Bang Liu, Ying  
Cao, Hao Li, Wancheng Pang, Houquan Liu, Shun Jiang, et al.

► **To cite this version:**

Miaomiao Li, Jun Yao, Geoffrey Sunahara, Robert Duran, Bang Liu, et al.. Assembly processes of bacterial and fungal communities in metal(loid)s smelter soil. *Journal of Hazardous Materials*, 2023, 451, pp.131153. 10.1016/j.jhazmat.2023.131153 . hal-04036925

**HAL Id: hal-04036925**

**<https://univ-pau.hal.science/hal-04036925>**

Submitted on 20 Mar 2023

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution 4.0 International License



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 m<sup>2</sup> 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<sup>®</sup> 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<sup>™</sup>-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 ( $\beta$ NTI) and the Bray-Curtis-based  
209 Raup-Click ( $RC_{\text{bray}}$ ). 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 ( $\beta$ NTI > 2: variable selection; whereas  $\beta$ 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  $2301.93 \pm 897.79$  (mean  $\pm$  SD) (ranging from 3195.94 to 225.60), the Simpson  
263 index was  $0.02 \pm 0.02$  (from 0.005 to 0.084) and the Shannon index was  $5.32 \pm 0.92$  (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 *Biatriosporaceae*,  
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  $\beta$ NTI and  $RC_{\text{bray}}$  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  $\beta$ 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  $\beta$ 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.

## 612 **Acknowledgments**

613 This work has been supported partly by grants received from the Major National R & D  
614 Projects from the Chinese Ministry of Science and Technology (2019YFC1803500), the  
615 National Science Foundation of China (42230716, 41720104007), the 111 Project (B21017),  
616 and the 1000-Talents Plan project (WQ2017110423).

## 617 **Author contributions**

618 **Miaomiao Li:** Conceptualization, Methodology, Resources, Formal analysis, Writing -  
619 original draft, Writing – review & editing. **Jun Yao:** Writing – review & editing, Funding  
620 acquisition. **Geoffrey Sunahara, Robert Duran and Qinghua Zhang:** Conceptualization,  
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

626 **References**

627 Aponte, H., Herrera, W., Cameron, C., Black, H., Meier, S., Paolini, J., Tapia, Y., Cornejo, P.,  
628 2020. Alteration of enzyme activities and functional diversity of a soil contaminated  
629 with copper and arsenic. *Ecotoxicol Environ Saf.* 192, 110264.  
630 10.1016/j.ecoenv.2020.110264

631 Banerjee, S., van der Heijden, M.G.A., 2022. Soil microbiomes and one health. *Nat Rev*  
632 *Microbiol.* 10.1038/s41579-022-00779-w

633 Barberan, A., Bates, S.T., Casamayor, E.O., Fierer, N., 2012. Using network analysis to  
634 explore co-occurrence patterns in soil microbial communities. *ISME J.* 6(2), 343-51.  
635 10.1038/ismej.2011.119

636 Chen, G., Fan, J., Liu, R., Zeng, G., Chen, A., Zou, Z., 2012. Removal of Cd(II), Cu(II) and  
637 Zn(II) from aqueous solutions by live *Phanerochaete chrysosporium*. *Environ Technol.*  
638 33(22-24), 2653-9. 10.1080/09593330.2012.673015

639 Chen, J., He, F., Zhang, X., Sun, X., Zheng, J., Zheng, J., 2014. Heavy metal pollution  
640 decreases microbial abundance, diversity and activity within particle-size fractions of  
641 a paddy soil. *FEMS Microbiol Ecol.* 87(1), 164-81. 10.1111/1574-6941.12212

642 Chun, S.J., Kim, Y.J., Cui, Y., Nam, K.H., 2021. Ecological network analysis reveals  
643 distinctive microbial modules associated with heavy metal contamination of  
644 abandoned mine soils in Korea. *Environ Pollut.* 289, 117851.  
645 10.1016/j.envpol.2021.117851

646 Compton, J.E., Watrud, L.S., Arlene Porteous, L., DeGrood, S., 2004. Response of soil  
647 microbial biomass and community composition to chronic nitrogen additions at  
648 Harvard forest. *Forest Ecology and Management.* 196(1), 143-158.  
649 10.1016/j.foreco.2004.03.017

- 650 Coyte, K.Z., Schluter, J., Foster, K.R., 2015. The ecology of the microbiome: networks,  
651 competition, and stability. *Science*. 350, 663-666.
- 652 De Agostini, A., Caltagirone, C., Caredda, A., Cicatelli, A., Cogoni, A., Farci, D., Guarino, F.,  
653 Garau, A., Labra, M., Lussu, M., Piano, D., Sanna, C., Tommasi, N., Vacca, A., Cortis,  
654 P., 2020. Heavy metal tolerance of orchid populations growing on abandoned mine  
655 tailings: A case study in Sardinia Island (Italy). *Ecotoxicol Environ Saf.* 189, 110018.  
656 10.1016/j.ecoenv.2019.110018
- 657 Dell'Anno, A., Beolchini, F., Rocchetti, L., Luna, G.M., Danovaro, R., 2012. High bacterial  
658 biodiversity increases degradation performance of hydrocarbons during  
659 bioremediation of contaminated harbor marine sediments. *Environmental Pollution*.  
660 167, 85-92. 10.1016/j.envpol.2012.03.043
- 661 Dell'Anno, F., Rastelli, E., Tangherlini, M., Corinaldesi, C., Sansone, C., Brunet, C., Balzano,  
662 S., Ianora, A., Musco, L., Montereali, M.R., Dell'Anno, A., 2021. Highly  
663 Contaminated Marine Sediments Can Host Rare Bacterial Taxa Potentially Useful for  
664 Bioremediation. *Front Microbiol.* 12, 584850. 10.3389/fmicb.2021.584850
- 665 Deng, Y., Zhang, P., Qin, Y., Tu, Q., Yang, Y., He, Z., Schadt, C.W., Zhou, J., 2016. Network  
666 succession reveals the importance of competition in response to emulsified vegetable  
667 oil amendment for uranium bioremediation. *Environ Microbiol.* 18(1), 205-18.  
668 10.1111/1462-2920.12981
- 669 Dettmann, U., Kraft, N.N., Rech, R., Heidkamp, A., Tiemeyer, B., 2021. Analysis of peat soil  
670 organic carbon, total nitrogen, soil water content and basal respiration: Is there a 'best'  
671 drying temperature? *Geoderma*. 403. 10.1016/j.geoderma.2021.115231
- 672 Fashola, M.O., Ngole-Jeme, V.M., Babalola, O.O., 2016. Heavy Metal Pollution from Gold  
673 Mines: Environmental Effects and Bacterial Strategies for Resistance. *Int J Environ*  
674 *Res Public Health*. 13(11). 10.3390/ijerph13111047
- 675 Faust, K., Raes, J., 2012. Microbial interactions: from networks to models. *Nat Rev*  
676 *Microbiol.* 10(8), 538-50. 10.1038/nrmicro2832
- 677 Fei, X., Christakos, G., Xiao, R., Ren, Z., Liu, Y., Lv, X., 2019. Improved heavy metal  
678 mapping and pollution source apportionment in Shanghai City soils using auxiliary  
679 information. *Sci Total Environ.* 661, 168-177. 10.1016/j.scitotenv.2019.01.149



- 680 Feng, M., Adams, J.M., Fan, K., Shi, Y., Sun, R., Wang, D., Guo, X., Chu, H., 2018.  
681 Long-term fertilization influences community assembly processes of soil diazotrophs.  
682 Soil Biology and Biochemistry. 126, 151-158. 10.1016/j.soilbio.2018.08.021
- 683 Flieder, M., Buongiorno, J., Herbold, C.W., Hausmann, B., Rattei, T., Lloyd, K.G., Loy, A.,  
684 Wasmund, K., 2020. Novel taxa of Acidobacteriota involved in seafloor sulfur cycling.  
685 bioRxiv. 10.1101/2020.10.01.322446
- 686 Gao, W., Liu, P., Ye, Z., Zhou, J., Wang, X., Huang, X., Deng, X., Ma, L., 2022. Divergent  
687 prokaryotic microbial assembly, co-existence patterns and functions in surrounding  
688 river sediments of a Cu-polymetallic deposit in Tibet. Sci Total Environ. 851(Pt 1),  
689 158192. 10.1016/j.scitotenv.2022.158192
- 690 Ghoul, M., Mitri, S., 2016. The Ecology and Evolution of Microbial Competition. Trends  
691 Microbiol. 24(10), 833-845. 10.1016/j.tim.2016.06.011
- 692 Hoek, T.A., Axelrod, K., Biancalani, T., Yurtsev, E.A., Liu, J., Gore, J., 2016. Resource  
693 Availability Modulates the Cooperative and Competitive Nature of a Microbial  
694 Cross-Feeding Mutualism. PLoS Biol. 14(8), e1002540.  
695 10.1371/journal.pbio.1002540
- 696 Jia, S., Tian, Y., Li, J., Chu, X., Zheng, G., Liu, Y., Zhao, W., 2022. Field study on the  
697 characteristics of scales in damaged multi-material water supply pipelines: Insights  
698 into heavy metal and biological stability. J Hazard Mater. 424(Pt A), 127324.  
699 10.1016/j.jhazmat.2021.127324
- 700 Jia, T., Guo, T.Y., Wang, R.H., Chai, B.F., 2020. Effects of heavy metal contents on  
701 phyllosphere and rhizosphere fungal communities for bothriochloa ischaemum in  
702 copper tailings area. Environmental Science. 41(11), 5193-5200.
- 703 Jiang, Y., Liang, Y., Li, C., Wang, F., Sui, Y., Suvannang, N., Zhou, J., Sun, B., 2016. Crop  
704 rotations alter bacterial and fungal diversity in paddy soils across East Asia. Soil  
705 Biology and Biochemistry. 95, 250-261. 10.1016/j.soilbio.2016.01.007
- 706 Jiao, S., Liu, Z., Lin, Y., Yang, J., Chen, W., Wei, G., 2016. Bacterial communities in oil  
707 contaminated soils: Biogeography and co-occurrence patterns. Soil. Biol. Biochem.  
708 98, 64-73. 10.1016/j.soilbio.2016.04.005
- 709 Keshri, J., Mankazana, B.B., Momba, M.N., 2015. Profile of bacterial communities in South

710 African mine-water samples using Illumina next-generation sequencing platform.  
711 *Appl Microbiol Biotechnol.* 99(7), 3233-42. 10.1007/s00253-014-6213-6

712 Kim, E.J., Yoo, J.C., Baek, K., 2014. Arsenic speciation and bioaccessibility in  
713 arsenic-contaminated soils: sequential extraction and mineralogical investigation.  
714 *Environ Pollut.* 186, 29-35. 10.1016/j.envpol.2013.11.032

715 Li, H., Yao, J., Min, N., Chen, Z., Li, M., Pang, W., Liu, B., Cao, Y., Men, D., Duran, R.,  
716 2022a. Comprehensive evaluation of metal(loid)s pollution risk and microbial activity  
717 characteristics in non-ferrous metal smelting contaminated site. *Journal of Cleaner*  
718 *Production.* 344. 10.1016/j.jclepro.2022.130999

719 Li, M., Yao, J., Sunahara, G., Hawari, J., Duran, R., Liu, J., Liu, B., Cao, Y., Pang, W., Li, H.,  
720 Li, Y., Ruan, Z., 2022b. Novel microbial consortia facilitate metalliferous  
721 immobilization in non-ferrous metal(loid)s contaminated smelter soil: Efficiency and  
722 mechanisms. *Environ Pollut.* 313, 120042. 10.1016/j.envpol.2022.120042

723 Lin, Y., Ye, Y., Hu, Y., Shi, H., 2019. The variation in microbial community structure under  
724 different heavy metal contamination levels in paddy soils. *Ecotoxicol Environ Saf.*  
725 180, 557-564. 10.1016/j.ecoenv.2019.05.057

726 Liu, B., Yao, J., Chen, Z., Ma, B., Li, H., Wancheng, P., Liu, J., Wang, D., Duran, R., 2022a.  
727 Biogeography, assembly processes and species coexistence patterns of microbial  
728 communities in metalloids-laden soils around mining and smelting sites. *J Hazard*  
729 *Mater.* 425, 127945. 10.1016/j.jhazmat.2021.127945

730 Liu, B., Yao, J., Chen, Z., Ma, B., Liu, J., Li, H., Zhu, X., Li, M., Cao, Y., Pang, W., Zhao, C.,  
731 Mihucz, V.G., Duran, R., 2022b. Unraveling ecological risk of As/Sb and other  
732 metal(loid)s and fungal community responses in As/Sb smelting-intensive zone: A  
733 typical case study of Southwest China. *Journal of Cleaner Production.* 338.  
734 10.1016/j.jclepro.2022.130525

735 Liu, B., Yao, J., Ma, B., Chen, Z., Zhao, C., Zhu, X., Li, M., Cao, Y., Pang, W., Li, H., Feng,  
736 L., Mihucz, V.G., Duran, R., 2021. Microbial community profiles in soils adjacent to  
737 mining and smelting areas: Contrasting potentially toxic metals and co-occurrence  
738 patterns. *Chemosphere.* 282, 130992. 10.1016/j.chemosphere.2021.130992

739 Liu, B., Yao, J., Ma, B., Chen, Z., Zhu, X., Zhao, C., Li, M., Cao, Y., Pang, W., Li, H.,

740 Mihucz, V.G., Duran, R., 2022c. Metal(loid)s diffusion pathway triggers distinct  
741 microbiota responses in key regions of typical karst non-ferrous smelting assembly. *J.*  
742 *Hazard. Mater.* 423(Pt B), 127164. 10.1016/j.jhazmat.2021.127164

743 Liu, J., Bao, Z., Wang, C., Wei, J., Wei, Y., Chen, M., 2022d. Understanding of mercury and  
744 methylmercury transformation in sludge composting by metagenomic analysis. *Water*  
745 *Res.* 226, 119204. 10.1016/j.watres.2022.119204

746 Liu, Z.B., Zhang, J.L., Papp, V., Dai, Y.C., 2022e. Taxonomy and Phylogeny of Meruliaceae  
747 with Descriptions of Two New Species from China. *J Fungi (Basel)*. 8(5).  
748 10.3390/jof8050501

749 Magoc, T., Salzberg, S.L., 2011. FLASH: fast length adjustment of short reads to improve  
750 genome assemblies. *Bioinformatics*. 27(21), 2957-63. 10.1093/bioinformatics/btr507

751 Marghoob, M.U., Rodriguez-Sanchez, A., Imran, A., Mubeen, F., Hoagland, L., 2022.  
752 Diversity and functional traits of indigenous soil microbial flora associated with  
753 salinity and heavy metal concentrations in agricultural fields within the Indus Basin  
754 region, Pakistan. *Front Microbiol.* 13, 1020175. 10.3389/fmicb.2022.1020175

755 Moberly, J., D'Imperio, S., Parker, A., Peyton, B., 2016. Microbial community signature in  
756 Lake Coeur d'Alene: Association of environmental variables and toxic heavy metal  
757 phases. *Applied Geochemistry*. 66, 174-183. 10.1016/j.apgeochem.2015.12.013

758 Mori, A.S., Isbell, F., Seidl, R., 2018. beta-Diversity, Community Assembly, and Ecosystem  
759 Functioning. *Trends Ecol Evol.* 33(7), 549-564. 10.1016/j.tree.2018.04.012

760 Olesen, J.M., Bascompte, J., Dupont, Y.L., Jordano, P., 2007. The modularity of pollination  
761 networks. *PNAS*. 104 (50) 19891-19896.

762 Park, S.C., Boyanov, M.I., Kemner, K.M., O'Loughlin, E.J., Kwon, M.J., 2021. Distribution  
763 and speciation of Sb and toxic metal(loid)s near an antimony refinery and their effects  
764 on indigenous microorganisms. *J Hazard Mater.* 403, 123625.  
765 10.1016/j.jhazmat.2020.123625

766 Pereira, L.B., Vicentini, R., Ottoboni, L.M., 2014. Changes in the bacterial community of soil  
767 from a neutral mine drainage channel. *PLoS One.* 9(5), e96605.  
768 10.1371/journal.pone.0096605

769 Price, G.W., Langille, M.G.I., Yurgel, S.N., 2021. Microbial co-occurrence network analysis

770 of soils receiving short- and long-term applications of alkaline treated biosolids. *Sci*  
771 *Total Environ.* 751, 141687. 10.1016/j.scitotenv.2020.141687

772 Qiao, L., Liu, X., Zhang, S., Zhang, L., Li, X., Hu, X., Zhao, Q., Wang, Q., Yu, C., 2021.  
773 Distribution of the microbial community and antibiotic resistance genes in farmland  
774 surrounding gold tailings: A metagenomics approach. *Sci Total Environ.* 779, 146502.  
775 10.1016/j.scitotenv.2021.146502

776 Sheik, C.S., Mitchell, T.W., Rizvi, F.Z., Rehman, Y., Faisal, M., Hasnain, S., McInerney, M.J.,  
777 Krumholz, L.R., 2012. Exposure of soil microbial communities to chromium and  
778 arsenic alters their diversity and structure. *PLoS One.* 7(6), e40059.  
779 10.1371/journal.pone.0040059

780 Sun, X., Kong, T., Xu, R., Li, B., Sun, W., 2020. Comparative characterization of microbial  
781 communities that inhabit arsenic-rich and antimony-rich contaminated sites:  
782 Responses to two different contamination conditions. *Environ Pollut.* 260, 114052.  
783 10.1016/j.envpol.2020.114052

784 Tian, Z., Li, G., Tang, W., Zhu, Q., Li, X., Du, C., Li, C., Li, J., Zhao, C., Zhang, L., 2022.  
785 Role of *Sedum alfredii* and soil microbes in the remediation of ultra-high content  
786 heavy metals contaminated soil. *Agriculture, Ecosystems & Environment.* 339.  
787 10.1016/j.agee.2022.108090

788 Wan, Y., Devereux, R., George, S.E., Chen, J., Gao, B., Noerpel, M., Scheckel, K., 2022.  
789 Interactive effects of biochar amendment and lead toxicity on soil microbial  
790 community. *J Hazard Mater.* 425, 127921. 10.1016/j.jhazmat.2021.127921

791 Wang, C., Jia, Y., Wang, Q., Yan, F., Wu, M., Li, X., Fang, W., Xu, F., Liu, H., Qiu, Z., 2022a.  
792 Responsive change of crop-specific soil bacterial community to cadmium in  
793 farmlands surrounding mine area of Southeast China. *Environ Res.* 214(Pt 1), 113748.  
794 10.1016/j.envres.2022.113748

795 Wang, C., Liu, S., Wang, P., Chen, J., Wang, X., Yuan, Q., Ma, J., 2021. How sediment  
796 bacterial community shifts along the urban river located in mining city. *Environ Sci*  
797 *Pollut Res Int.* 28(31), 42300-42312. 10.1007/s11356-020-12031-0

798 Wang, N., Zhang, S., He, M., 2018. Bacterial community profile of contaminated soils in a  
799 typical antimony mining site. *Environ Sci Pollut Res Int.* 25(1), 141-152.

800 10.1007/s11356-016-8159-y

801 Wang, W., Wang, H., Cheng, X., Wu, M., Song, Y., Liu, X., Loni, P.C., Tuovinen, O.H.,  
802 2022b. Different responses of bacteria and fungi to environmental variables and  
803 corresponding community assembly in Sb-contaminated soil. *Environmental Pollution*.  
804 298. 10.1016/j.envpol.2022.118812

805 Wei, G., Li, M., Shi, W., Tian, R., Chang, C., Wang, Z., Wang, N., Zhao, G., Gao, Z., 2020.  
806 Similar drivers but different effects lead to distinct ecological patterns of soil bacterial  
807 and archaeal communities. *Soil Biology and Biochemistry*. 144.  
808 10.1016/j.soilbio.2020.107759

809 Xie, Y., Bu, H., Feng, Q., Wassie, M., Ameer, M., Jiang, Y., Bi, Y., Hu, L., Chen, L., 2021.  
810 Identification of Cd-resistant microorganisms from heavy metal-contaminated soil  
811 and its potential in promoting the growth and Cd accumulation of bermudagrass.  
812 *Environ Res.* 200, 111730. 10.1016/j.envres.2021.111730

813 Xu, D.M., Fu, R.B., Liu, H.Q., 2021a. Current knowledge from heavy metal pollution in  
814 Chinese smelter contaminated soils, health risk implications and associated  
815 remediation progress in recent decades: A critical review. *J. Clean. Prod.* 286.  
816 10.1016/j.jclepro.2020.124989

817 Xu, M., Huang, Q., Xiong, Z., Liao, H., Lv, Z., Chen, W., Luo, X., Hao, X., 2021b. Distinct  
818 Responses of Rare and Abundant Microbial Taxa to In Situ Chemical Stabilization of  
819 Cadmium-Contaminated Soil. *mSystems*. 6(5), e0104021.  
820 10.1128/mSystems.01040-21

821 Yan, C., Wang, F., Geng, H., Liu, H., Pu, S., Tian, Z., Chen, H., Zhou, B., Yuan, R., Yao, J.,  
822 2020. Integrating high-throughput sequencing and metagenome analysis to reveal the  
823 characteristic and resistance mechanism of microbial community in metal  
824 contaminated sediments. *Sci Total Environ.* 707, 136116.  
825 10.1016/j.scitotenv.2019.136116

826 Yun, S.W., Baveye, P.C., Kim, D.H., Kang, D.H., Lee, S.Y., Kong, M.J., Park, C.G., Kim,  
827 H.D., Son, J., Yu, C., 2018. Analysis of metal(loid)s contamination and their  
828 continuous input in soils around a zinc smelter: development of methodology and a  
829 case study in South Korea. *Environ Pollut.* 238, 140-149.

830 10.1016/j.envpol.2018.03.020

831 Zhang, B., Zhang, J., Liu, Y., Shi, P., Wei, G., 2018. Co-occurrence patterns of soybean  
832 rhizosphere microbiome at a continental scale. *Soil Biology and Biochemistry*. 118,  
833 178-186. 10.1016/j.soilbio.2017.12.011

834 Zhang, K., Li, K., Tong, M., Xia, Y., Cui, Y., Liu, Z., Chen, Q., Li, Q., Hu, F., Yang, F., 2022a.  
835 Distribution Pattern and Influencing Factors of Heavy Metal Resistance Genes in the  
836 Yellow River Sediments of Henan Section. *Int J Environ Res Public Health*. 19(17).  
837 10.3390/ijerph191710724

838 Zhang, M., Ye, J., He, J.S., Zhang, F., Ping, J., Qian, C., Wu, J., 2020. Visual detection for  
839 nucleic acid-based techniques as potential on-site detection methods. A review. *Anal*  
840 *Chim Acta*. 1099, 1-15. 10.1016/j.aca.2019.11.056

841 Zhang, M., Zhang, T., Zhou, L., Lou, W., Zeng, W., Liu, T., Yin, H., Liu, H., Liu, X.,  
842 Mathivanan, K., Praburaman, L., Meng, D., 2022b. Soil microbial community  
843 assembly model in response to heavy metal pollution. *Environ Res*. 213, 113576.  
844 10.1016/j.envres.2022.113576

845 Zhang, X., Chen, B., Yin, R., Xing, S., Fu, W., Wu, H., Hao, Z., Ma, Y., Zhang, X., 2022c.  
846 Long-term nickel contamination increased soil fungal diversity and altered fungal  
847 community structure and co-occurrence patterns in agricultural soils. *J Hazard Mater*.  
848 436, 129113. 10.1016/j.jhazmat.2022.129113

849 Zhang, Z.F., Pan, J., Pan, Y.P., Li, M., 2021. Biogeography, Assembly Patterns, Driving  
850 Factors, and Interactions of Archaeal Community in Mangrove Sediments. *mSystems*.  
851 6(3), e0138120. 10.1128/mSystems.01381-20

852 Zheng, W., Zhao, Z., Gong, Q., Zhai, B., Li, Z., 2018. Responses of fungal–bacterial  
853 community and network to organic inputs vary among different spatial habitats in soil.  
854 *Soil Biology and Biochemistry*. 125, 54-63. 10.1016/j.soilbio.2018.06.029

855 Zheng, W., Zhao, Z., Lv, F., Wang, R., Wang, Z., Zhao, Z., Li, Z., Zhai, B., 2021. Assembly  
856 of abundant and rare bacterial and fungal sub-communities in different soil aggregate  
857 sizes in an apple orchard treated with cover crop and fertilizer. *Soil Biology and*  
858 *Biochemistry*. 156. 10.1016/j.soilbio.2021.108222

859 Zhou, H., Gao, Y., Jia, X., Wang, M., Ding, J., Cheng, L., Bao, F., Wu, B., 2020. Network

860 analysis reveals the strengthening of microbial interaction in biological soil crust  
861 development in the Mu Us Sandy Land, northwestern China. *Soil Biology and*  
862 *Biochemistry*. 144. 10.1016/j.soilbio.2020.107782

863 Zhou, J.Z., Ning, D.L., 2017. Stochastic community assembly: does it matter in microbial  
864 ecology. *Microbiol. Mol. Biol. Rev.* 10.1128/MMBR

865 Zhou, L., Zhao, Z., Shao, L., Fang, S., Li, T., Gan, L., Guo, C., 2022. Predicting the  
866 abundance of metal resistance genes in subtropical estuaries using amplicon  
867 sequencing and machine learning. *Ecotoxicol Environ Saf.* 241, 113844.  
868 10.1016/j.ecoenv.2022.113844

869 Zhu, X., Yao, J., Wang, F., Yuan, Z., Liu, J., Jordan, G., Knudsen, T.S., Avdalovic, J., 2018.  
870 Combined effects of antimony and sodium diethyldithiocarbamate on soil microbial  
871 activity and speciation change of heavy metals. Implications for contaminated lands  
872 hazardous material pollution in nonferrous metal mining areas. *J Hazard Mater.* 349,  
873 160-167. 10.1016/j.jhazmat.2018.01.044

874 Zou, H.Y., He, L.Y., Gao, F.Z., Zhang, M., Chen, S., Wu, D.L., Liu, Y.S., He, L.X., Bai, H.,  
875 Ying, G.G., 2021. Antibiotic resistance genes in surface water and groundwater from  
876 mining affected environments. *Sci Total Environ.* 772, 145516.  
877 10.1016/j.scitotenv.2021.145516

878

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.

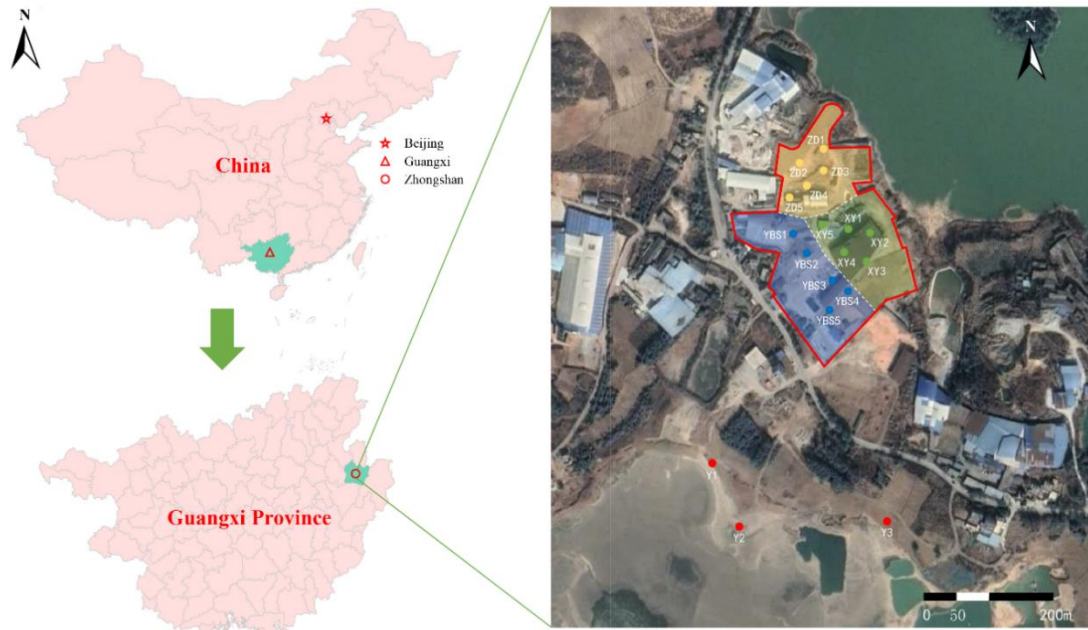
891 **Fig. 2.** Boxplots showing the soil total metal(loid)s concentrations in the non-ferrous  
892 metal(loid)s smelter and surrounding sites. Different lowercase letters in the  
893 boxplots indicate significant differences ( $P < 0.05$ ) among the tested sites.

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.  
917  
918



919

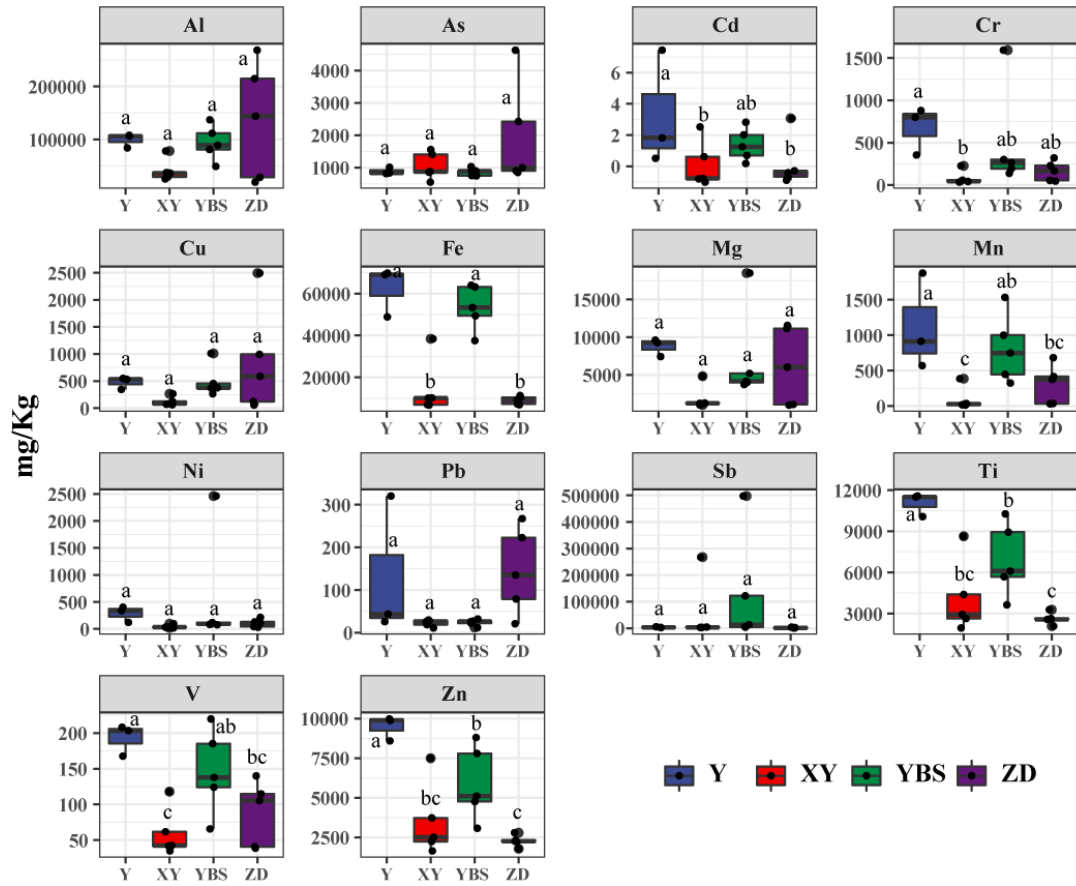
920 **Fig. 1.** Location of the abandoned non-ferrous metal(loid)s Huilong smelters (left)

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.

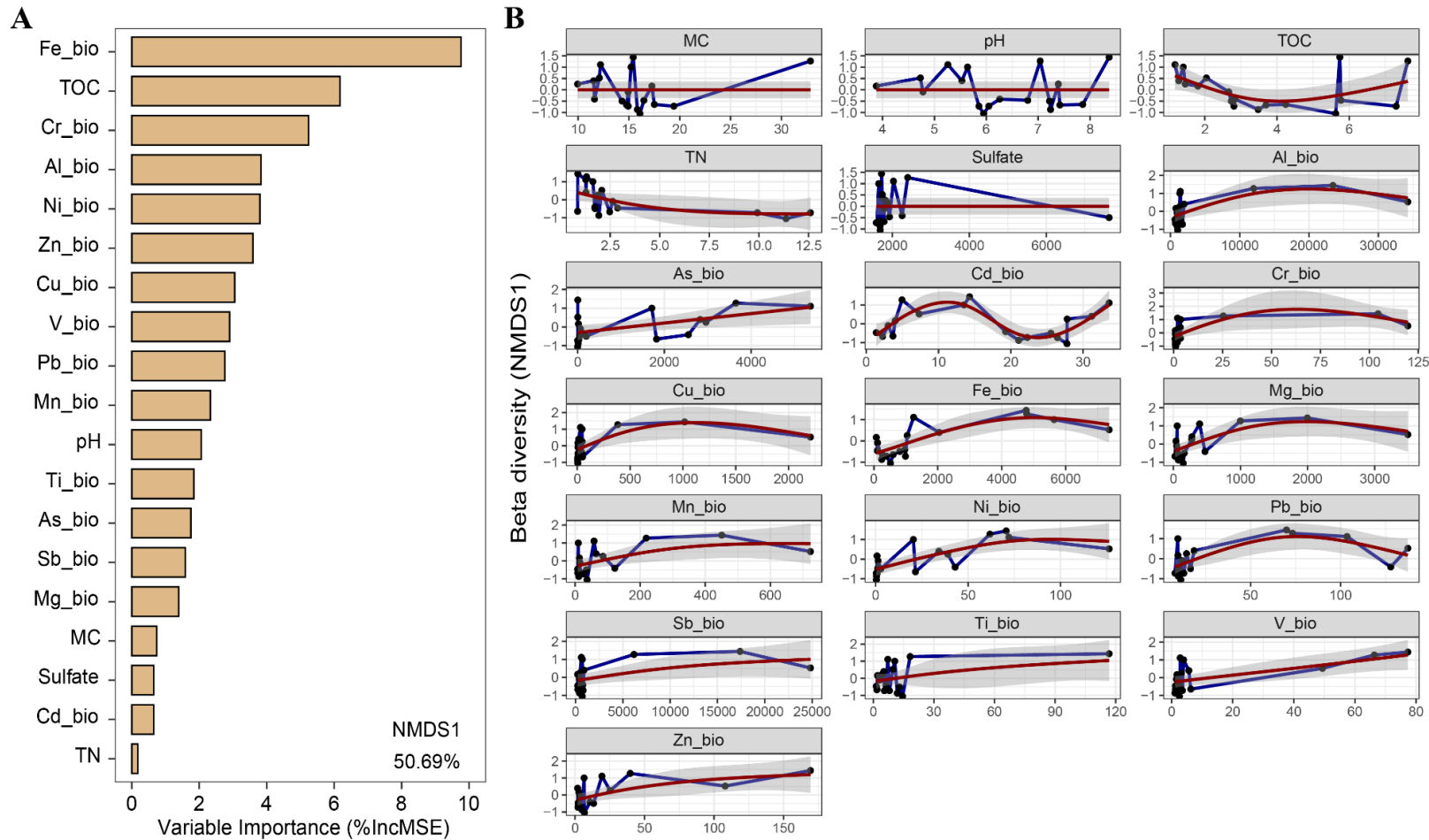
924



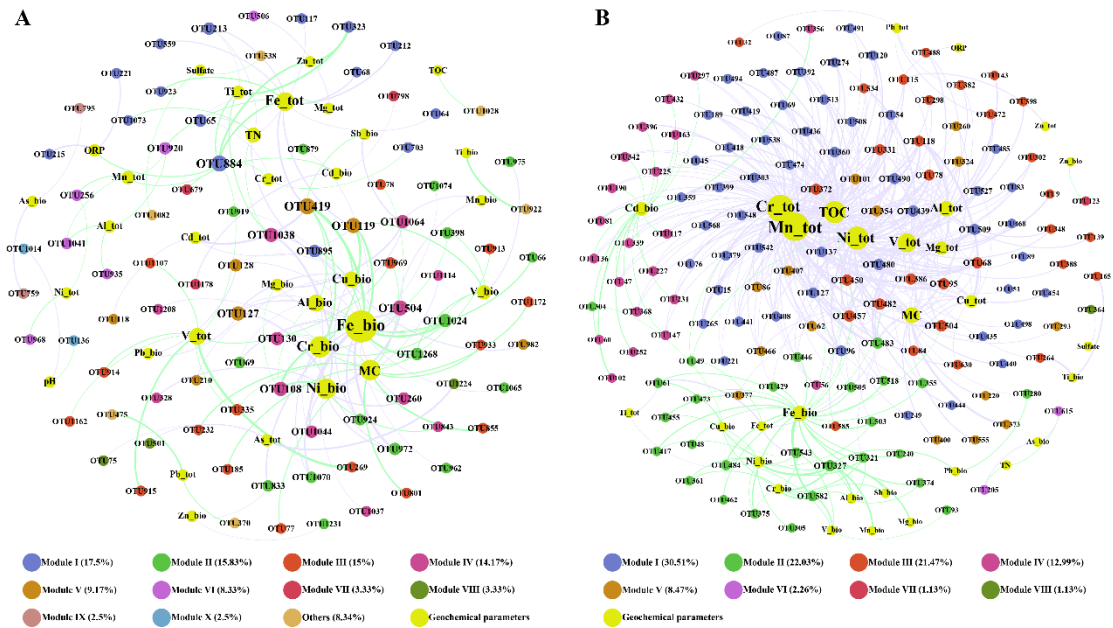
925

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

929



**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).

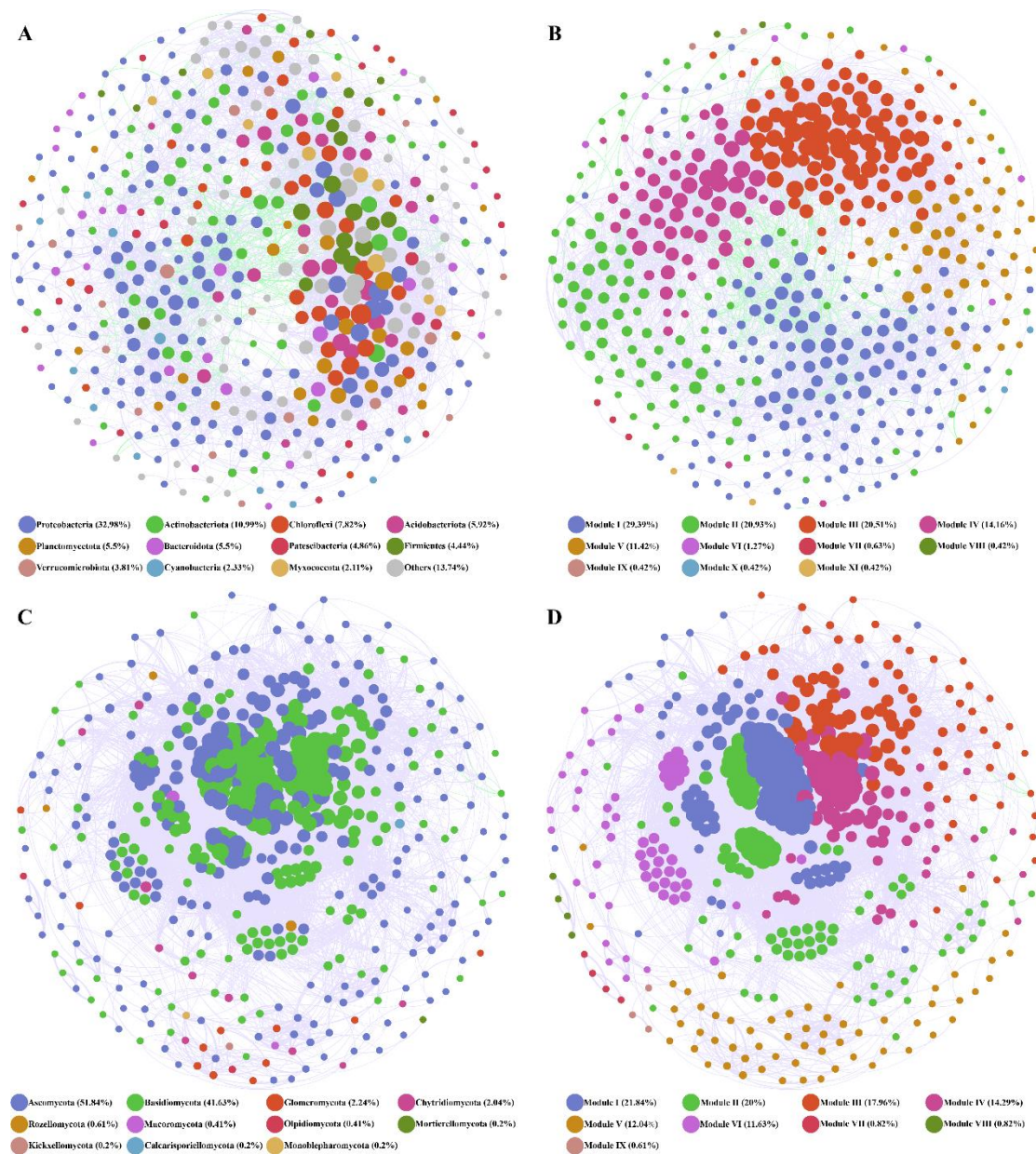


931

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.





939

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

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

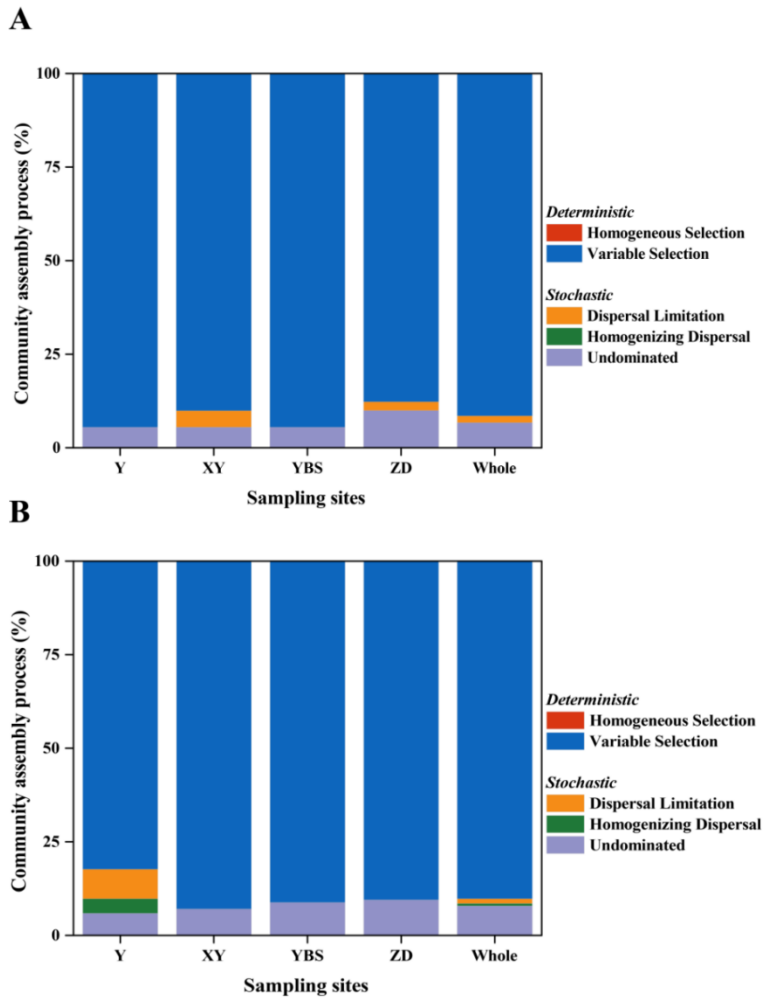
942 shown by strong correlations (Spearman  $R > |0.6|$ ) and significant ( $P < 0.05$ ). The

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

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

945 indicate positive interactions and green lines indicate negative interactions.

946



948

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.