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Houquan Liu, Jun Yao, Bang Liu, Miaomiao Li, Jianli Liu, Shun Jiang,  
Wenjing Yu, Yuhui Zhao, Robert Duran

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1           **Active tailings disturb the surrounding vegetation soil fungal**  
2           **community: Diversity, assembly process and co-occurrence patterns**

3

4   Houquan Liu<sup>a</sup>, Jun Yao<sup>a\*</sup>, Bang Liu<sup>a</sup>, Miaomiao Li<sup>a</sup>, Jianli Liu<sup>a</sup>, Shun Jiang<sup>a</sup>, Wenjing Yu<sup>a</sup>,  
5    Yuhui Zhao<sup>a</sup>, Robert Duran<sup>a,b</sup>

6

7   <sup>a</sup> School of Water Resource and Environment, Research Center of Environmental Science and  
8   Engineering, MOE Key Laboratory of Groundwater Circulation and Environmental Evolution,  
9   China University of Geosciences (Beijing), 29 Xueyuan Road, Haidian District, Beijing,  
10   100083, P.R. China.

11   <sup>b</sup>Université de Pau et des Pays de l'Adour, E2S-UPPA, IPREM UMR CNRS 5254, BP 1155,  
12   64013, Pau Cedex, France.

13

14   **Keywords**

15   Fungal community diversity; biotic factor; community assembly; co-occurrence  
16   patterns; active tailings

17

18   **Highlights**

- 19   • Vegetation types and Cu together drive the fungal diversity.
- 20   • *Exophiala* has potential as a biomarker species indicative of restoration progress.
- 21   • Variable selection process dominates fungal community assembly.
- 22   • Fungal communities resist environmental stress by species coexistence.

23

## 24 **Abstract**

25        Soil fungi play an important role in the soil biogeochemical cycle and are  
26 important biological indicators for the ecological remediation of mine tailings  
27 contaminated sites, therefore understanding the characteristics of soil fungal  
28 communities is a key aspect of pollution remediation. However, the influence of  
29 biological factors on the characteristics of fungal community diversity; assembly  
30 mechanisms and co-occurrence patterns of fungal community along environmental  
31 gradients around tailings are not well understood. In this study, soil samples from  
32 forest, agriculture and grass around tailings were collected to reveal the assembly  
33 mechanisms and co-occurrence patterns of soil fungal community and to quantify the  
34 contribution of abiotic and biotic factors to fungal diversity. The results suggest that  
35 vegetation types and Cu concentration together drive the distribution of fungal  
36 diversity. We found that *Exophiala* has potential as a biomarker species indicative of  
37 restoration progress. Increased environmental stress accelerates the process of  
38 changing fungal community assemblages from stochastic to deterministic, while also  
39 allowing fungal communities tend to resist tailings-induced environmental stresses  
40 through species coexistence. Together, this study provides new insights into the  
41 influence of biological factors on fungal community diversity, as well as revealing  
42 mechanisms of fungal community assembly and co-occurrence patterns, which are  
43 important for understanding the maintenance mechanisms of fungal community  
44 diversity and ecological remediation of tailings-contaminated soils.

45

46

## 47 **1. Introduction**

48 Tailings are one of the main sources of heavy metal pollution in the mining  
49 industry (Adiansyah et al., 2015), generating acid mine drainage (Ramdani et al.)  
50 (Karaca et al., 2018). It is estimated that more than 10 billion tons of tailings are  
51 produced every year in the world, causing huge heavy metal pollution (Xie and van  
52 Zyl, 2020). Studies have demonstrated that heavy metals can severely disturb the  
53 evolutionary process of soil microorganisms, thereby limiting soil functions (Gall et  
54 al., 2015; Giller et al., 1998). In order to prevent continuing damage of the ecosystem  
55 by heavy metal pollution, it is necessary to restore the soil around the tailings. It is  
56 worth noting that irrespective of the adopted method for restoration, the  
57 autochthonous microbial community is involved during all process. As an important  
58 participant in the biogeochemical cycle, soil microbial community plays a key role in  
59 the process of restoration, which has received extensive attention (Cavicchioli et al.,  
60 2019; Jiao et al., 2022; Mohapatra et al., 2021).

61 Since Harris proposed that characterization of microbial communities could be  
62 used to measure the progress of the restoration process (Harris, 2003), the  
63 development of high-throughput sequencing technologies provides unprecedented  
64 tools for the characterization of microbial communities widely used to identify  
65 restoration biomarker (Mendez and Maier, 2008; Wang et al., 2012). In soil, both  
66 fungi and bacteria play a key role in geochemical cycling, but fungi have a lower

67 sensitivity in response to heavy metals than bacteria (Zhao, H.C. et al., 2021). This  
68 feature gives the soil fungal community the potential to become a biomarker  
69 indicative of ecological recovery of polluted soils. Disentangling the assembly  
70 mechanisms of soil fungal community is essential to better understand the  
71 maintenance and generation of fungal diversity. The structure and assembly processes  
72 of fungal communities have been demonstrated to be influenced by abiotic and biotic  
73 factors. During the phytoremediation of tailings, it has been shown that microbial  
74 succession occurred mainly in the surface tailings (0-20 cm) when comparing a 60 cm  
75 profile (Li et al., 2016). This result implies that the microbial community  
76 characteristics of the topsoil can better reflect the restoration process. In tailings,  
77 heavy metals are typical abiotic factors affecting the structure and assembly processes  
78 of fungal communities (Beattie et al., 2018; Kandeler et al., 2000). It has been  
79 confirmed that Cd, Pb, Cu, Zn and Cr were the main factors positively correlated with  
80 Ascomycota and Basidiomycota, the most abundant fungal phyla in Pb-Zn tailings in  
81 northwest China (Shen et al., 2022). It is important to note that the heavy metal  
82 contamination from tailings is depending on the type of tailings, the time, and the  
83 geographical environment (Liu et al., 2019). There are many studies demonstrating  
84 that fungal communities in tailings are often closely related to plants. In copper  
85 tailings, *Erythrobasidiales* sp. was found to be the key fungal species in the soil and  
86 the phyllosphere of four native grass species (Jia et al., 2018). It has been shown that  
87 the dark septate endophyte (DSE) fungi play a role in promoting plants for  
88 bioremediation of mine waste in a semi-arid climate (Flores-Torres et al., 2021). Thus,

89 vegetation is an important biotic factor in the study of fungal communities in  
90 tailings-contaminated soils. Bacteria and fungi can form a range of physical  
91 associations that depend on various modes of molecular communication for their  
92 development and functioning (Deveau et al., 2018; Frey-Klett et al., 2011). It is  
93 reasonable to speculate that bacteria have an important role in the process of fungal  
94 community construction and assembly. However, little attention has been paid on the  
95 influence of soil bacteria around tailings in the diversity patterns and assembly  
96 processes of fungal communities.

97 Here, we hypothesize that (i) mechanisms for maintaining the diversity of fungal  
98 communities are regulated by a combination of biotic and abiotic factors, and (ii)  
99 fungal communities in different ecological niches have different assembly processes  
100 and co-occurrence patterns. In the present study, we analyzed topsoil samples from  
101 agriculture, forest and grass around active lead-zinc tailings in Guizhou province,  
102 China, to gain a systematic and comprehensive understanding of the fungal  
103 community. Specifically, we explored the diversity, assembly processes and  
104 co-occurrence patterns of fungal communities in three vegetated soils around the  
105 tailings and quantified the contribution of abiotic and biotic factors. This study  
106 provides new insights for understanding the fungal diversity, assembly mechanisms  
107 and co-occurrence patterns of the fungal communities, and provides theoretical  
108 guidance for ecological remediation of polluted soils.

109

## 110 **2. Materials and Methods**

## 111 ***2.1 Soil sampling***

112 Total 15 sites were selected, 5 in forest, 6 in agriculture and 4 in grass around  
113 tailings. The sampling sites extended from 26°25'40" N to 26°26'18"N, and  
114 105°38'30"E to 105°39'27"E around active lead-zinc tailings. Forest soil is the  
115 farthest from tailings, followed by Agriculture soil and then Grass soil. As the  
116 succession of microbial communities in topsoil is more representative, a total of  
117 approximately 50 g of soil was collected at 0-10 cm depth using a sterile spatula. The  
118 soil samples were placed in 50 mL conical tubes and stored at -80 °C until further  
119 analysis.

120

## 121 ***2.2 Determination of total metal(loid)s content***

122 To determine the contents of total metal(loid)s, 0.1 g of sieved samples was  
123 digested with a mixture of concentrated HCl, HNO<sub>3</sub> and HF (4:1:1, v/v) in a graphite  
124 heating block until solid particles disappeared completely (Liu, B. et al., 2022).  
125 Followed by measurements on an induced coupled plasma optical emission  
126 spectrometry (iCAP 7000 SERIES, Thermo Scientific, USA). Standard reference  
127 material (GSB 04-1767-2004, National Nonferrous Metal Analysis and Testing  
128 Center) was employed for the purpose of quality confirmation.

129

## 130 ***2.3 DNA extraction and PCR amplification***

131 Soil microbial communities were analyzed using high-throughput amplicon  
132 sequencing (Liu et al., 2021). Total genomic DNA was extracted from approximately

133 0.5 g of sample using FastDNA® SPIN Kit for soil (MP BIO, Qbiogene, USA)  
134 according to the manufacture's protocol. The microbial communities were profiled by  
135 targeting the V4 region of the 16S rRNA gene for bacteria, and the ITS1 region for  
136 fungi. The hyper-variable V4 region of 16S rRNA gene was amplified by the primer  
137 pairs ArBa515F (5'-GTGCCAGCMGCCGCG GTAA-3') and Arch806R  
138 (5'-GGACTACVSGGGTATCTAAT-3') (Bates et al., 2011). The ITS1 region (~300  
139 bp) was amplified using the specific primer sets of ITS1-F  
140 (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS2-R  
141 (5'-GCTGCGTTCTTCATCGAT GC-3') (Bokulich and Mills, 2013). The amplicon  
142 sequencing was performed on a high-throughput Illumina MiSeq platform at Majorbio  
143 Bio-pharm Technology Co., Ltd (Shanghai, China).

144

#### 145 ***2.4 Data processing and statistical analyses***

146 The raw data and bioinformatics data were analyzed on the online platform of  
147 Majorbio Cloud Platform ([www.majorbio.com](http://www.majorbio.com)). Briefly, the paired-end reads  
148 obtained from Miseq sequencing were first spliced according to the overlap  
149 relationship, while the sequence quality was quality-controlled and filtered to obtain  
150 an OTU table with 97% sequence identity. We calculated the Shannon and Chao1  
151 index of  $\alpha$ -diversity for bacteria and fungi, which were the most extensively used  
152 (Macias-Perez et al., 2022). Non-metric multidimensional scaling analysis (NMDS)  
153 were performed based on Bray-Curits distance of OTUs and analysis of similarities  
154 (ANOSIM) with 999 permutations was used to detect statistical significances between



155 microbial communities (both bacteria and fungi) in different groups (Clarke, 1993).  
156 The composition of the fungal and bacterial communities at the phylum level was  
157 calculated based on the OTU tables. An assessment of the high-dimensional microbial  
158 taxa among the three vegetated soils was performed using the linear discriminate  
159 analysis effect size (LEfSe) analysis with non-parametric factorial Kruskal-Wallis  
160 (KW) sum-rank test (Segata et al., 2011). The variance inflation factors (VIFs) were  
161 used for filtering soil geochemical variables for Canonical correlation analysis (CCA),  
162 in which VIFs greater than 10 were denied and the selected variables included pH, Fe,  
163 Al, Cu, Ni, Pb, Zn, Cr, B and biotic factors (Shannon and Chao1  $\alpha$ -diversity index for  
164 bacteria, named bio1 and bio2 respectively). Variance partitioning analysis (VPA)  
165 was used to quantify the relative explanations of metals and biotic factors on the  
166 variances of total fungal community (Cohen et al., 2014). Mantel tests were used to  
167 analyze the correlations between fungal communities and selected main  
168 environmental factors (Mantel, 1966). Null model analysis was carried out using the  
169 framework described by Stegen et al. (Stegen et al., 2013) to classify community pairs  
170 into underlying drivers of deterministic and stochastic processes. Considering the  
171 unreliable and less resolution of the phylogeny derived directly from ITS sequences,  
172 we compiled the phylogenetic trees by the taxonomy\_to\_tree.pl script using the OTU  
173 taxonomy matrix (Tedersoo et al., 2018). The variation in phylogenetic or taxonomic  
174 diversity was measured respectively using null-model-based phylogenetic  $\beta$ -diversity  
175 metrics ( $\beta$ NTI). Co-occurrence patterns of fungal and bacterial communities with key  
176 taxa were explored using "igraph" package with Spearman's correlation, and

177 visualized using the interactive platform Gephi (v0.9.2) (Bastian et al., 2009; Liu,  
178 Bang et al., 2022). Without otherwise statement elsewhere, R environment (v4.0.2)  
179 was used for data analyses and visualization throughout this study.

180

## 181 **3. Results**

### 182 ***3.1 The environmental stress induced by the active tailings***

183 Physical-chemical parameters in soil samples were determined, including heavy  
184 metal content (Pb, Zn, Cu, Cr, Ni), and other factors (Fe, Al, B, pH) (Fig. 1), to study  
185 the environmental stress caused by the continuous accumulation of active lead-zinc  
186 tailings. Only Cu exhibited a clear chemical concentration gradient (from 56.12  
187 mg/Kg to 1.69 mg/Kg) across the three vegetated soils, with the relative highest Cu  
188 concentration in the Grass soil, followed by Agriculture and Forest soils. The Pb, Zn  
189 and Ni contents were significantly similar in the three types of vegetated soils, while  
190 significant differences (Wilcoxon test,  $p < 0.001$ ) were observed for the Cr content.  
191 However, the content of these metals was largely below the soil background value in  
192 Guizhou Province, China (Wu, Z. et al., 2020), indicating that Cu was the main  
193 environmental factor in disturbing the surrounding soil of tailings.

194

### 195 ***3.2 Patterns and drivers of $\alpha$ -diversity in fungal community around*** 196 ***active tailings***

197 The  $\alpha$ -diversity analysis was performed on rarefied OTU tables by resampling to  
198 a minimum number of sequences obtained in a sample, corresponding to 28707 for

199 bacteria and 55981 for fungi. Although we observed that the Shannon index of Grass  
200 soil was lower than that of agriculture and forest soils, the difference was not  
201 statistically significant. Also, a distinct decrease of the fungal richness was observed  
202 (Fig. 2A). This suggests that the environmental stress caused by the tailings reduced  
203 the abundance and decreased the diversity of the fungal community.

204 In order to find the factors driving the altered  $\alpha$ -diversity of fungal communities  
205 in three vegetation soils, we performed correlation analysis of environmental factors  
206 (Fig. 2C) as well as linear regression analysis (Fig. 2D). Across all abiotic factors  
207 (caused by tailings) and biotic factors, there was a significant negative correlation  
208 between Cu and fungal community alpha-diversity. This result was confirmed by a  
209 negative linear regression between Cu and Chao1 ( $R^2 = 0.4$ ,  $p = 0.011$ ) and Shannon  
210 ( $R^2 = 0.31$ ,  $p = 0.031$ ) index of fungal community. It is noteworthy that there is a  
211 positive correlation between bacterial richness and  $\alpha$ -diversity of fungal communities.  
212 Positive linear regression between bacterial richness and  $\alpha$ -diversity of fungal  
213 community (Chao1,  $R^2 = 0.35$ ,  $p = 0.019$ ; Shannon,  $R^2 = 0.33$ ,  $p = 0.024$ ) was  
214 confirmed this observation. We observed a positive correlation between B and Chao1  
215 index of fungal community. The B is essential for plants (Camacho-Cristobal et al.,  
216 2008). Its availability in soil and irrigation water is an important determinant of  
217 agricultural production (Tanaka and Fujiwara, 2008). Many studies have  
218 demonstrated that the content of B is positively correlated with the growth of  
219 vegetation (O'Neill et al., 2001; Poudel et al., 2022; Shorrocks, 1997). Therefore, we  
220 can use the content of B to indicate the growth of vegetation. It is thus likely that the

221 relationship between plant and fungal communities is mutually beneficial rather than  
222 competitive, which is consistent with other studies (Juge et al., 2021; Li et al., 2022).  
223 These results illustrate that vegetation and Cu together drive the distribution pattern of  
224 fungal community  $\alpha$ -diversity in soils around tailings.

225

### 226 ***3.3 Exophiala has potential as a biomarker species indicative of*** 227 ***restoration progress***

228 We compared the  $\beta$ -diversity of fungal communities in three vegetated soils to  
229 explore the fungal species that could be used as potential biomarkers in the soil  
230 around the tailings. The NMDS ordination based on Bray-Curtis matrix showed that  
231 the fungal communities in the three vegetation soils had distinct segregation  
232 characteristics (Fig. 2B, ANOSIM, stress: 0.141, R = 0.296, p = 0.005). This result  
233 suggests that the disturbance of the surrounding soil by the tailings caused changes in  
234 the structure of the fungal community. This observation was similar to the studies  
235 reporting that plant species and heavy metals can significantly alter the  $\beta$ -diversity of  
236 soil fungal communities (Li et al., 2021). For comprehensively understand the  
237 differences in fungal community structure, we first profiled the soil fungal community  
238 composition around tailings at different taxonomic levels (Fig. 3). We observed that  
239 most of the sequences belonged to the phyla Ascomycota, Basidiomycota,  
240 Mortierellomycota and so forth, which showed different cross-vegetation distributions  
241 (Fig. 3A). Ascomycota were more abundant in Grass soil, while the relative  
242 abundance of Basidiomycota was higher in Forest soil. The maximum OTU number

243 (2266) was observed in Forest soil (Fig. 3C). The number of unique OTUs decreased  
244 as follow Forest > Agriculture > Grass, consistent with the observation of  $\alpha$ -diversity.  
245 The fungal composition at the phylum and class levels (Fig. 3B, 3D) shows the fungal  
246 community was dominated by Ascomycota in all soils, suggesting that members of  
247 this class exhibit a broad range of life modes (Chen, X.F. et al., 2022). Particularly,  
248 Ascomycota dominates the soil fungal community around tailings with heavy metal  
249 contamination, which is consistent with the previous observations (Xiao et al., 2021;  
250 Zeng et al., 2020). Similarly, Basidiomycota was present in all sites but its relative  
251 abundance decreased as follow Forest > Agriculture > Grass, which may be explained  
252 by stronger selection pressure closer to the tailings. Thus, Basidiomycota is sensitive  
253 to the presence of metals indicating that it likely plays a role in environment with  
254 relatively low pollution level, which is consistent with previous research (Lin et al.,  
255 2019; Tian et al., 2017). The relative abundance of Chytridiomycota was higher in  
256 Grass soil suggesting that Chytridiomycota can adapt to the presence of heavy metals.

257 We explored fungal species that differed significantly in relative abundance  
258 among the three vegetation soils. The relative abundance distribution of these species  
259 showed that nearly 40% of the species had a relative abundance of less than 1% (Fig.  
260 4A), which can be considered as rare microorganisms. This finding suggests that rare  
261 microbes are more sensitive to environmental changes, which is consistent with Xu's  
262 observations (Xu et al., 2021). *Paraphoma* and *Exophiala* ranked top 2 in the linear  
263 discriminant analysis (LDA) (Fig. 4B), being significantly more abundant in Forest  
264 and Agricultural soils respectively. It is likely that they play an important role in the

265 composition of the fungal community around the tailings. *Exophiala* has also been  
266 shown to be present in the roots of Bengal asparagus from a mine smelter in Yunnan  
267 Province, China, being highly resistant to heavy metals while promoting phosphorus  
268 uptake by plants (Xu et al., 2020; Yu et al., 2021). In our study, the abundance of  
269 *Exophiala* in the Agricultural soil reached a non-negligible 5.5%, indicating that it can  
270 also accumulate in soil. These evidences, combined with the LDA results, suggest that  
271 *Exophiala* has potential as a biomarker species indicative of restoration progress. We  
272 also observed that the number of key species (LDA>2.5) in the fungal community of  
273 Grass soil was lower than that of Agriculture and Forest soils, which may indicate that  
274 aggregation processes occur in fungal communities in the Grass soil.

275

### 276 ***3.4 Factors responsible for the variance in fungal communities***

277 Canonical correspondence analysis (CCA) and partition analysis of variance  
278 (VPA) were performed to demonstrate the influence of environmental factors and  
279 quantify their contributions (Fig. 5). The SF factors (B, Al, Fe, pH) were the main  
280 factors responsible for the variance in fungal communities, followed by biotic factors  
281 (Shannon and Chao1 index of bacteria communities) and heavy metals (Cu, Pb, Zn,  
282 Ni, Cr) (Fig. 5B). Notably,  $\alpha$ -diversity of bacterial communities (bio1 and bio2)  
283 explained 21.11% of the variance. It shows that the interaction between the bacterial  
284 community and the fungal community plays a critical role on shaping the fungal  
285 community structure. However, this critical interaction has always been overlooked  
286 during microbial community construction in tailings environments. Unsurprisingly,

287 heavy metals contributed the least to fungal community changes (10.67% of variance  
288 explained independently). This result also confirmed that, in this case, heavy metals  
289 significantly changed the abundance of the fungal community but not the diversity.

290

### 291 ***3.5 Assembly mechanisms of fungal community in the three vegetated*** 292 ***soils around tailings***

293 We used the null model to investigate the assembly process of fungal  
294 communities in three vegetation soils. The null model revealed that stochastic  
295 processes ( $|\beta\text{NTI}| < 2$ ) dominated the fungal community assembly in agriculture and  
296 forest, while deterministic processes ( $|\beta\text{NTI}| > 2$ ) dominated the fungal community  
297 assembly in the Grass soil (Fig. 6A). Based on the null model results, the variable  
298 selection process increased from agriculture to grass soils (Fig. 6B). The deterministic  
299 process of fungal community assembly was significantly increased in all three  
300 vegetation types, indicating that the fungal communities were disturbed by the tailings.  
301 Therefore, we infer that the continuous accumulation of tailings leads to the  
302 accumulation of heavy metals in the surrounding soil, which leads to changes in the  
303 assembly processes driving the spatial distribution of fungal communities. And this  
304 change is mainly dominated by the variable selection in the deterministic process,  
305 which gradually increases with the environmental pressure.

306

### 307 ***3.6 Co-occurrence networks of fungal community in three vegetation*** 308 ***soils around tailings***

309 The properties of co-occurrence networks for fungal community differed among  
310 the vegetation types (Fig. 7A). The network node number of fungal community in the  
311 Grass soil was lower than in the Agricultural and Forest soils, indicating that the  
312 community networks became less complex with an increase in environmental stress.  
313 While, the fungal community of the Grass soil had the highest average degree and  
314 average clustering coefficient among the three vegetated soils. This indicates a higher  
315 degree of aggregation of fungal communities in The Grass soil. The co-occurrence  
316 network of the three fungal communities showed clear modularity of taxa, but the  
317 degree of modularity did not differ significantly. These results suggest that as  
318 environmental stresses rise, fungal community network complexity decreases and taxa  
319 cluster towards dominant species. The co-occurrence networks of fungal communities  
320 in three vegetation soils confirmed this point (Fig. 7B).

321 It has been reported that species interactions can determine the functional  
322 properties or ecological niche occupancy of microbial communities (Becker et al.,  
323 2012; Freilich et al., 2010), and play an important role in stimulating ecosystem  
324 processes (Bardgett and van der Putten, 2014). The relatively high number of nodes  
325 detected in fungal communities in the Agricultural soil indicates a wider distribution  
326 of interactions within the community (more nodes and lower clustering coefficients)  
327 and a greater occupancy of ecological niches. We also found that fungal communities  
328 in the Grass soil are dominated by a few core species and the taxa are decreasing in  
329 association (lower node number and higher average degree). This observation  
330 suggests that persistently increasing environmental stress triggers species coexistence



331 within fungal communities, leading to microbial aggregation, which is consistent with  
332 (Zelezniak et al., 2015). While species coexistence is generally viewed as one  
333 potential outcome between two competing species, numerous community members  
334 may be involved in this process (Gravel et al., 2011). It has been reported that species  
335 coexistence, which is typically used in ecology and biogeography as a proxy for  
336 species interaction in the community (Li et al., 2018), has a significant impact on  
337 microbial community  $\beta$ -diversity (Gao et al., 2019). Therefore, we hypothesize that  
338 species coexistence is also a non-negligible biological factor influencing the structure  
339 of fungal communities.

340

### 341 ***3.7 Diversity and co-occurrence networks of bacterial community in*** 342 ***three vegetation soils around tailings***

343 Soil bacterial communities were also investigated in order to gain a  
344 comprehensive understanding of the effects of vegetation and Cu on soil  
345 microorganisms. The results show that the alpha diversity of bacterial community in  
346 three vegetation decreased in the following order (high to low): Forest > Agriculture >  
347 Grass (Fig. 8A). In the grassland soil samples, fungal communities and bacteria  
348 showed the same decreasing trend. The bacterial community structures have obvious  
349 similarity ( $p>0.05$ ) (Fig. 8B). We also found that Actinobacteriota, Proteobacteria,  
350 Acidobacteriota and Chloroflexi dominated three bacterial communities, which  
351 indicated that the composition of bacterial communities had no significant difference  
352 in three vegetation (Fig. 8C). Although the networks of three bacterial communities

353 show the similarly patterns (Fig. 8D-F), the attenuation of modularity degree was  
354 observed in the network of bacterial community in Grass (Fig. 8F). Therefore, the  
355 structure and co-occurrence patterns of the bacterial communities did not change  
356 significantly. Compared to the results of the fungal community response, we suggest  
357 that vegetation type exerted strong effects on fungal but not bacterial communities.

358

## 359 **4. Discussion**

### 360 *4.1 Ecological restoration of active tailings needs to be carried out as* 361 *soon as possible*

362 In the study here, we detected heavy metal concentrations in soil samples and  
363 found that copper was the main environmental factor disturbing the soil around the  
364 tailings. However, this result is not entirely consistent with findings from other  
365 lead-zinc mine contaminated sites. Heavy metal contamination from a Pb-Zn tailings  
366 pond in Spain that had been closed for nearly 20 years has been reported to be mainly  
367 Pb, Zn, Cr and Cu (Rodriguez et al., 2009). We note that the pH range the soils  
368 studied here was 5.56-6.79, while the pH range of the Spanish Pb-Zn mine soils was  
369 3.02-8.20. Some studies have reported that under natural conditions, the tailings will  
370 gradually complete acidification, forming acidic mine wastewater as well as leaching  
371 of large amounts of heavy metals (Dold, 2014; Liu et al., 2019). Environmental  
372 factors have been reported to influence the evolution and biogeochemical function of  
373 microbial communities (Cherabier and Ferriere, 2022; Liu et al., 2020). Without any  
374 treatment of the tailings, the microorganisms inhabiting soil around the tailings will

375 be subject to continuous environmental stress, eventually impacting the evolutionary  
376 direction and biogeochemical function of the microbial community. Therefore, we  
377 speculate that the acidification process at the active Pb-Zn tailings in Guizhou, China,  
378 has not yet allowed the migration of heavy metals from the tailings into the  
379 surrounding soil. This also indicates that the remediation of contaminated soil around  
380 the tailings is urgently needed.

381

#### 382 ***4.2 Alpha diversity in three vegetation soils is taxonomically dependent*** 383 ***among fungi and bacteria.***

384 We found a significant correlation of alpha diversity between fungal community  
385 and bacterial community through linear regression analysis (Fig. 2D). Understanding  
386 the correlation between fungi and bacteria is important for revealing the maintenance  
387 mechanism of soil microbial diversity around tailings. In study here, this strong  
388 correlation can be further explained as taxonomic dependency. Such cross-taxon  
389 congruence can occur if different organisms covary in space regarding alpha or beta  
390 diversity (Rooney et al., 2014). Wu observed that beta diversity in lake ecosystems  
391 was taxonomically dependent across bacteria, diatoms and chironomids (Wu, K. et al.,  
392 2020). This taxonomic dependence also widely exists between bacteria and fungi. For  
393 example, studies have shown that the taxonomically dependent among bacteria and  
394 archaea was observed in semi-arid lake ecosystems (Yuan et al., 2022). Soil bacteria  
395 shows a high correlation with fungi in an arid inland river basin of northwest China  
396 (Wang et al., 2017). Our results also support this conclusion, showing that taxonomic

397 dependence between fungi and bacteria persists under tailings disturbance. This  
398 suggests that we should consider the effects of cross-classification when undertaking  
399 ecological remediation of tailings.

400

#### 401 ***4.3 The response of fungal community to the disturbance of active*** 402 ***tailings is more significant***

403 The finding of a strong positive correlation between bacterial abundance and  
404 alpha-diversity of fungal communities prompted further research into the structure  
405 and coexistence patterns of bacterial communities disturbed by active tailings. The  
406 results show that the alpha diversity of bacterial community in three vegetation  
407 decreased in the following order (high to low): Forest > Agriculture > Grass (Fig. 8A).  
408 In the grassland soil samples, fungal communities and bacteria showed the same  
409 decreasing trend. The bacterial community structures have obvious similarity ( $p>0.05$ )  
410 (Fig. 8B). This means that the bacterial community structure did not show significant  
411 changes in the three vegetated soils. Previous studies have shown that bacteria are  
412 more sensitive to environmental changes than fungi. The results of Jiao et al. showed  
413 that bacteria were more sensitive than fungi to changes in soil moisture (Jiao et al.,  
414 2021). The results of Chen et al. suggest that soil bacteria are more sensitive than  
415 fungi in response to N and P enrichment (Chen, Y.C. et al., 2022). Zhao et al. reported  
416 a higher tolerance of fungi than bacteria when faced with Cd stress (Zhao, H. et al.,  
417 2021). However, we obtained the opposite result. This may be the result of a balance  
418 between the inhibitory effect on the bacterial community of risk factors released by

419 the active tailings and the promoting effect of vegetation and soil factors on the  
420 bacterial community. Although the networks of three bacterial communities show the  
421 similarly patterns (Fig. 8D-F), the attenuation of modularity degree was observed in  
422 the network of bacterial community in Grass (Fig. 8F). Under the same conditions, the  
423 structure of the fungal communities in the three vegetation has changed significantly  
424 (Fig. 2B), while the bacterial communities has a similar structure (Fig. 8B). The  
425 results of co-occurrence networks also showed that the change of fungal community  
426 could better reflect the impact of tailings on soil (Fig. 8D-F, Fig. 7). Therefore, fungal  
427 communities have a more obvious response than bacterial communities in the face of  
428 tailings disturbance and are better suited as biomarkers for the ecological recovery of  
429 contaminated soils.

430

## 431 **5. Conclusion**

432 Our results reveal the distribution of fungal and bacterial diversity in the  
433 surrounding soils of tailings. We provide evidence on the processes involved in  
434 community assembly, highlighting the critical contribution of biological factors, the  
435 vegetation and the Cu content together being the main drive of the fungal community  
436 changes. We have also observed taxonomic dependence between fungi and bacteria  
437 under tailings stress. We found that vegetation type exerted strong effects on fungal  
438 but not bacterial communities. In response to increasing environmental stress, the  
439 fungal community assembly processes are dominated by variable selection in  
440 deterministic processes. It is likely that the fungal community around tailings tend to

441 react to environmental stresses with a co-occurrence pattern of species coexistence.  
442 This study provides new insights into understanding the diversity, assembly  
443 mechanisms and co-occurrence patterns of the fungal community in tailings. Our  
444 results fill a crucial gap in our understanding of fungal and bacterial interactions  
445 within tailings-affected areas.

446

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## 687 **Figure legends**

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689 Figure 1. Analysis of environmental factors in three vegetated soils. The detected  
690 environmental factors include (A) heavy metal factors (Cu, Cr, Pb, Zn, Ni); (B) soil  
691 factors (pH, Al, Fe, B) and biotic factors (Chao1 and Shannon index of bacteria  
692 community). Asterisk indicated statistically significance performed by Wilcoxon test  
693 at \* < 0.05; \*\* < 0.01 and \*\*\* < 0.001 level.

694

695 Figure 2. Diversity of fungal communities in three vegetated soils and their driving  
696 factors. (A) Shannon diversity index and Chao1 richness index of fungal community  
697 in three vegetated soils. (B)  $\beta$ -diversities of fungal community based on non-metric  
698 multidimensional scaling (NMDS) analysis. (C) Correlation analysis of environmental  
699 factors based on Spearman's correlation coefficients. Colors represent correlations,  
700 and only data with  $p < 0.05$  are shown. Chao1\_B and Chao1\_F represent the Chao1  
701 indices of bacterial and fungal communities, respectively. (D) Relationships between  
702 fungal  $\alpha$ -diversity and the main drivers were estimated via linear least-quares  
703 regression analysis.

704

705 Figure 3. Compositional characteristics of fungal communities in three vegetation  
706 soils perturbed by active tailing diffusion. (A) A circo plot showing the taxonomic  
707 distribution of soil fungal taxa among three vegetation soils at the phylum level. The  
708 thickness of each ribbon represents the relative abundance of bacterial taxa assigned  
709 to different phyla. (C) Venn diagram showing vegetation-specific and shared OTU in  
710 three vegetated soils. (B, D) Fungal community compositions at class and phylum  
711 level in three vegetated soils respectively.

712

713 Figure 4. (A) Differential species analysis and (B) linear discriminant analysis of  
714 fungal communities in three vegetated soils. Data only show species with LDA > 2.5.

715

716 Figure 5. (A) Canonical Correspondence Analysis (CCA) plots showing the impact of

717 metal and other factors on the indigenous fungal communities. Bio1 and bio2  
718 represent the Shannon and Chao1 indices of bacterial communities, respectively. The  
719 length of arrows denotes the relative contribution of the variables on the fungal  
720 community. (B) Variance Partitioning Analysis (VPA) showing the relative  
721 explanations of HM (Cu, Pb, Zn, Ni, Cr), SF (Al, Fe, B, pH) and Biotic factors (bio1  
722 and bio2) on the total variance of fungal community.

723

724 Figure 6. Deterministic and stochastic assembly processes in soil fungal community  
725 formation. (A) Distribution pattern of  $\beta$ NTI in different fungal communities. (B) Null  
726 model analysis of the fungal community assembly process.

727

728 Figure 7. Network analysis shows the co-occurrence patterns among three vegetated  
729 soils. (A) The inner relationship of the co-occurrence networks among three  
730 vegetation soils. The node average degree, modularity, average clustering coefficient  
731 and max degree of fungal networks are shown in the figure. (B) The co-occurrence  
732 networks of fungal community in three vegetation soils. A connection represents a  
733 strong ( $r > 0.8$ ) and significant ( $P < 0.01$ ) correlation. The size of each node is  
734 proportional to the number of connections (degree). Nodes are colored according to  
735 modularity classes. The top 200 nodes are shown.

736

737 Figure 8. Characteristics of bacterial communities in three vegetation under the  
738 tailings disturbance. (A) The Shannon and Chao1 index of  $\alpha$ -diversity of bacterial  
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740 multidimensional scaling (NMDS) analysis. (C) The composition of bacterial  
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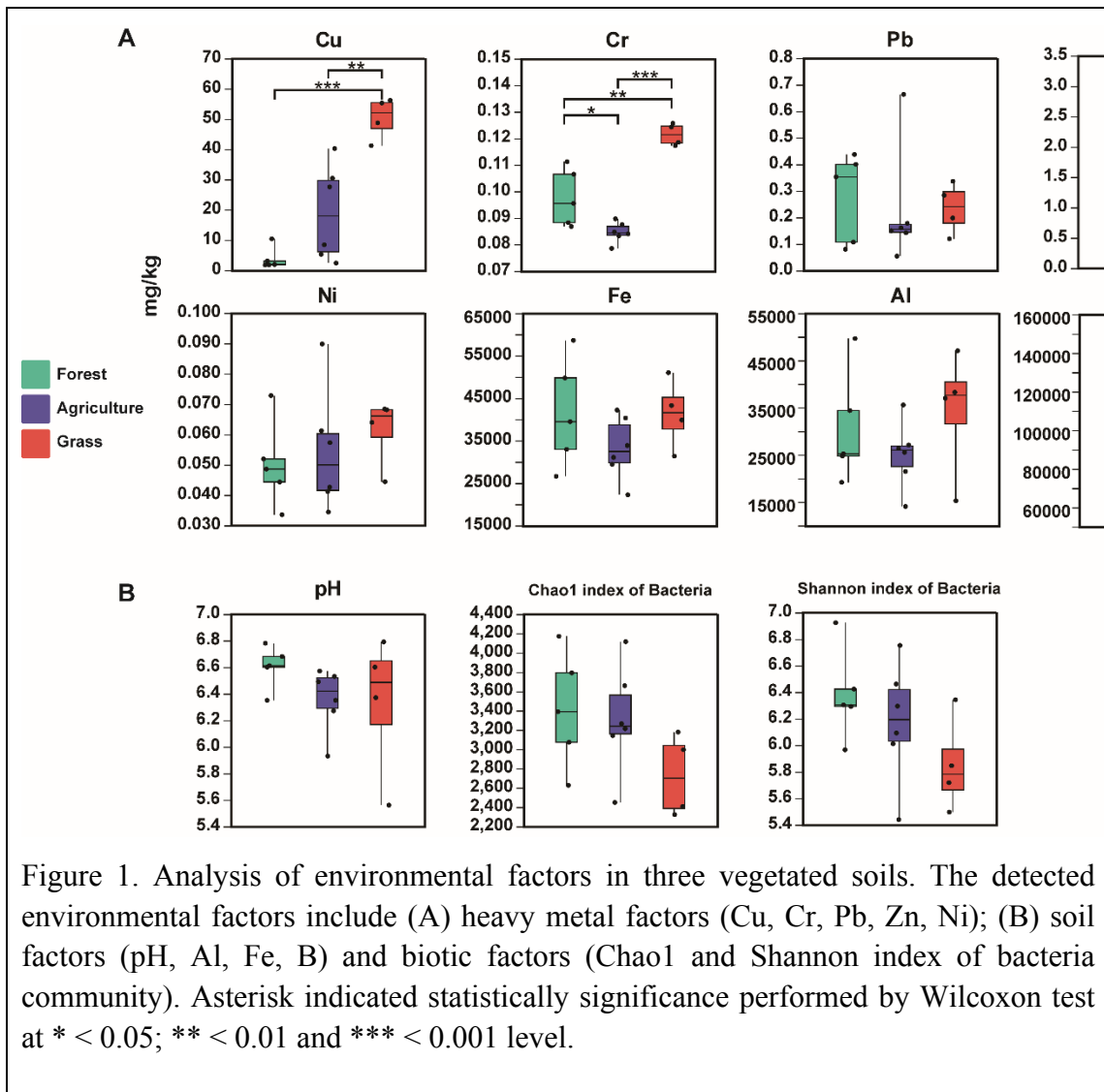


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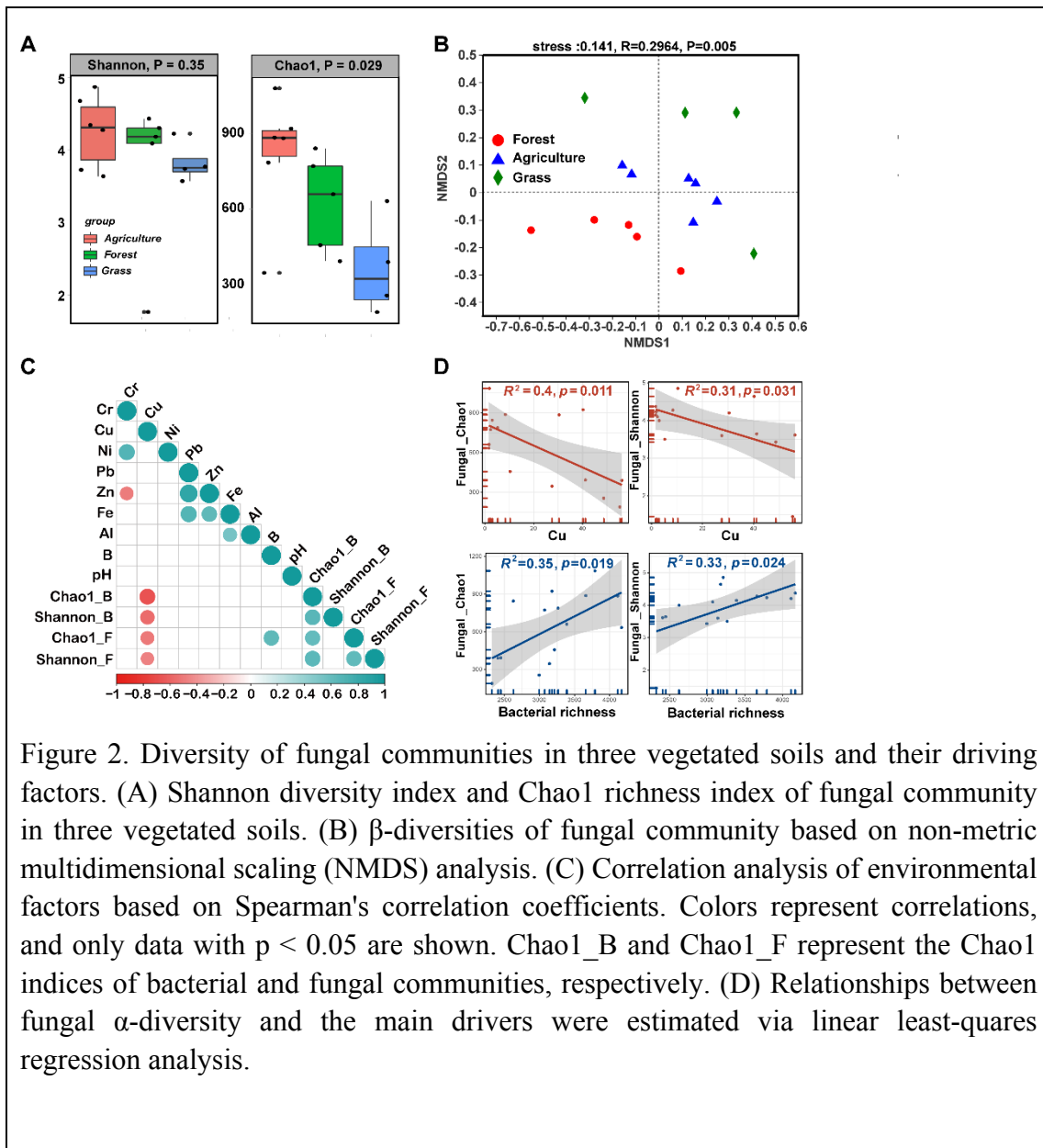


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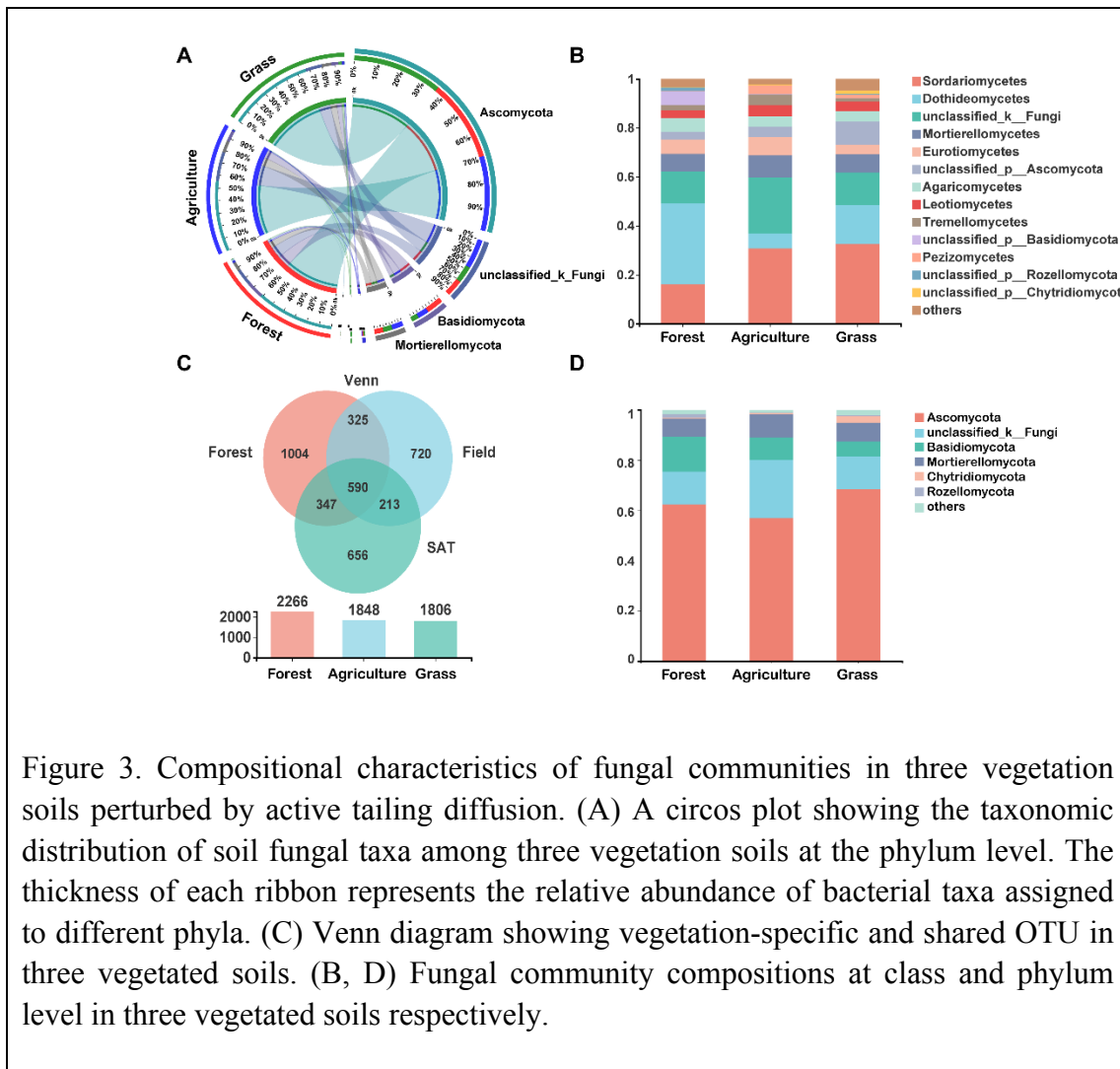


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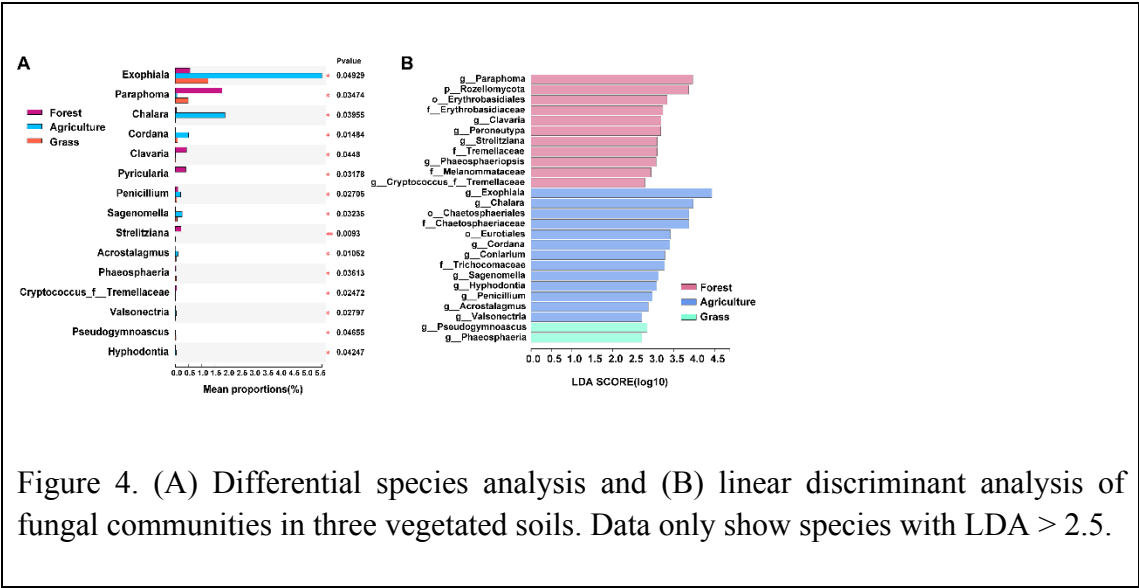
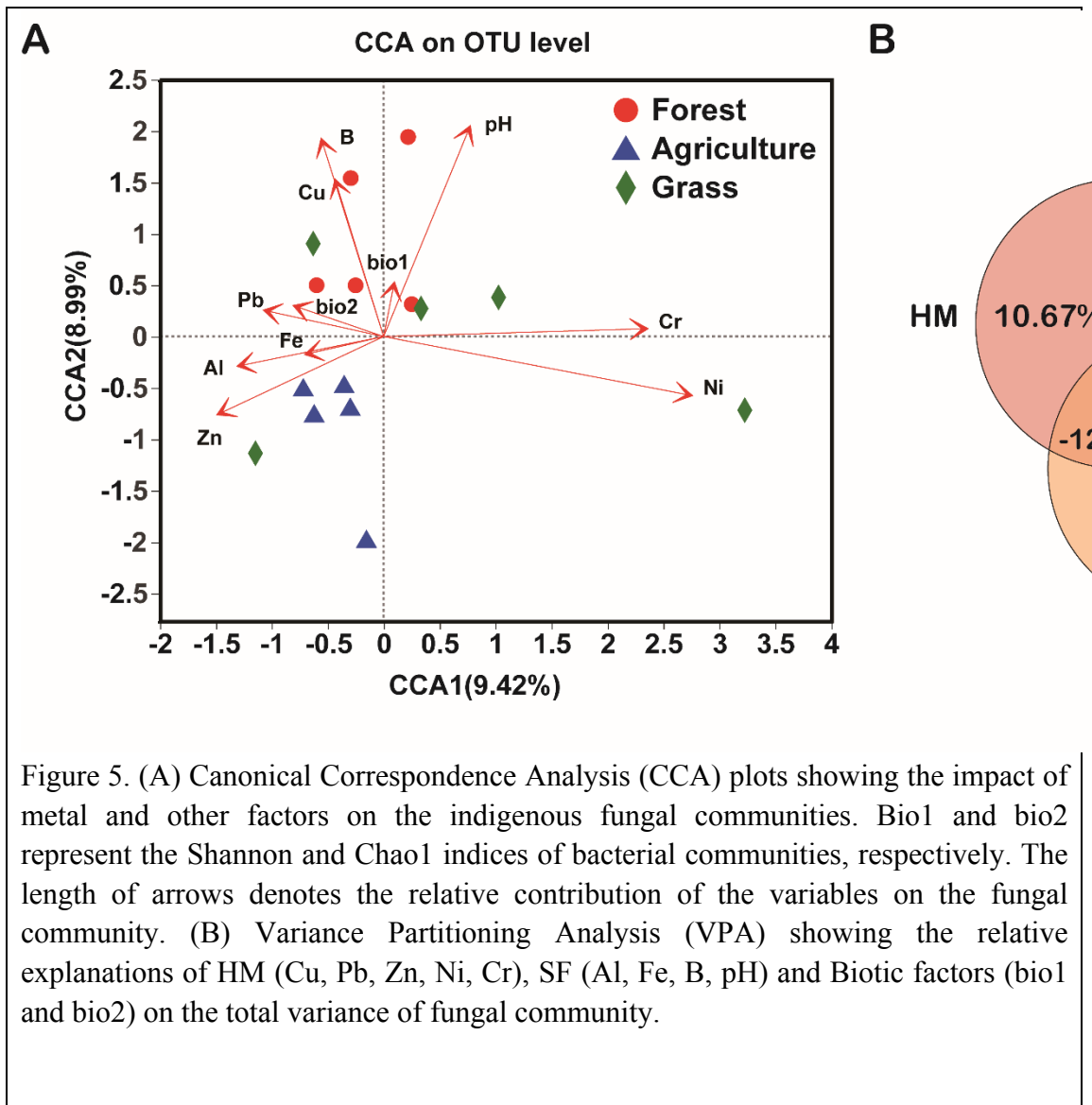


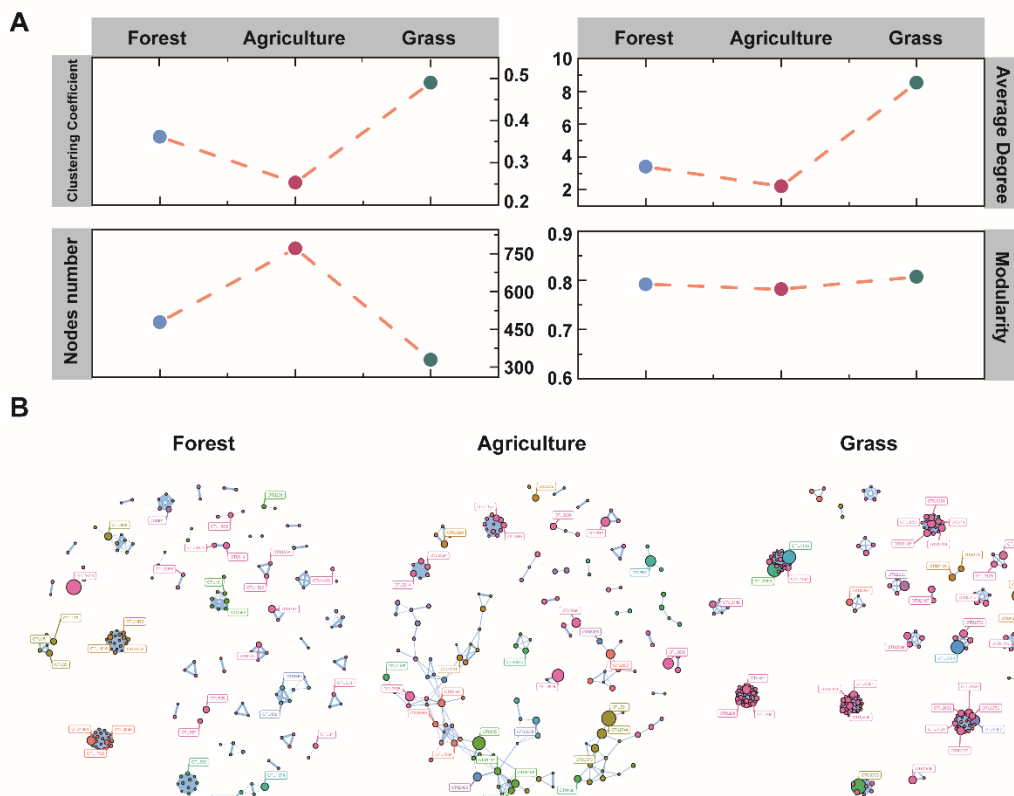
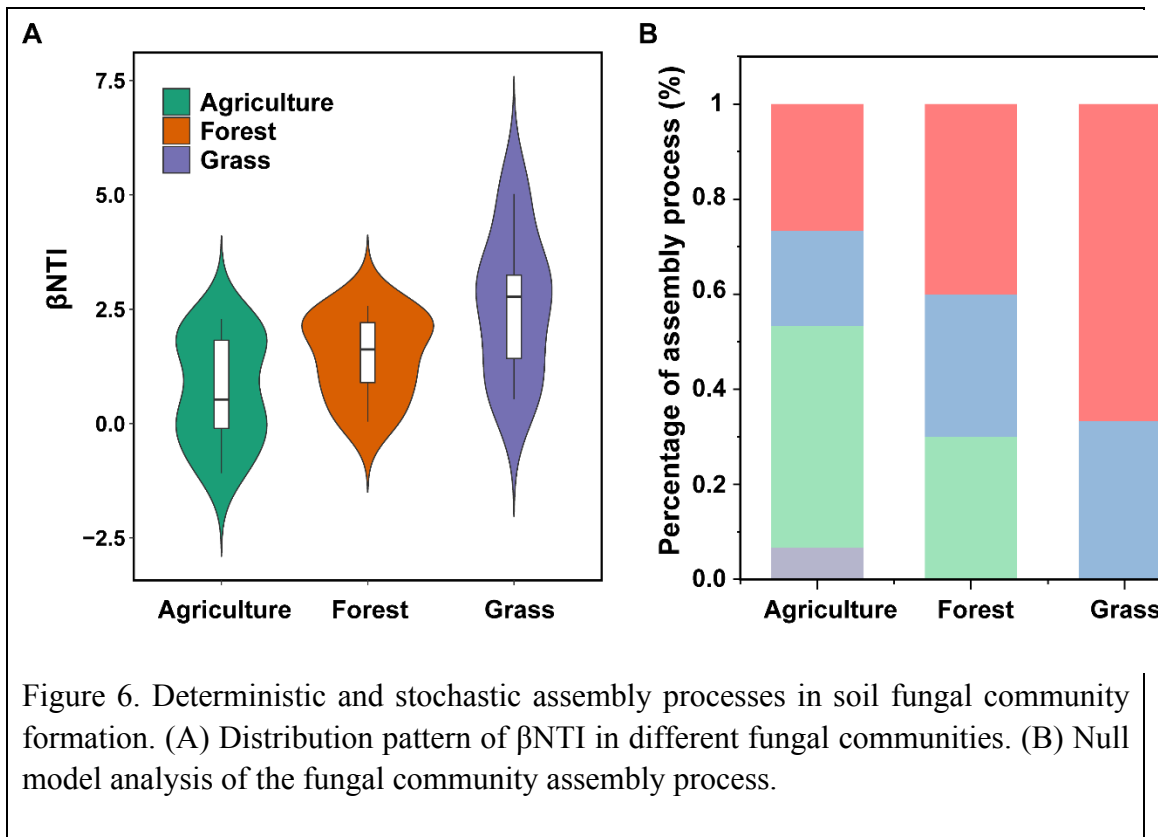
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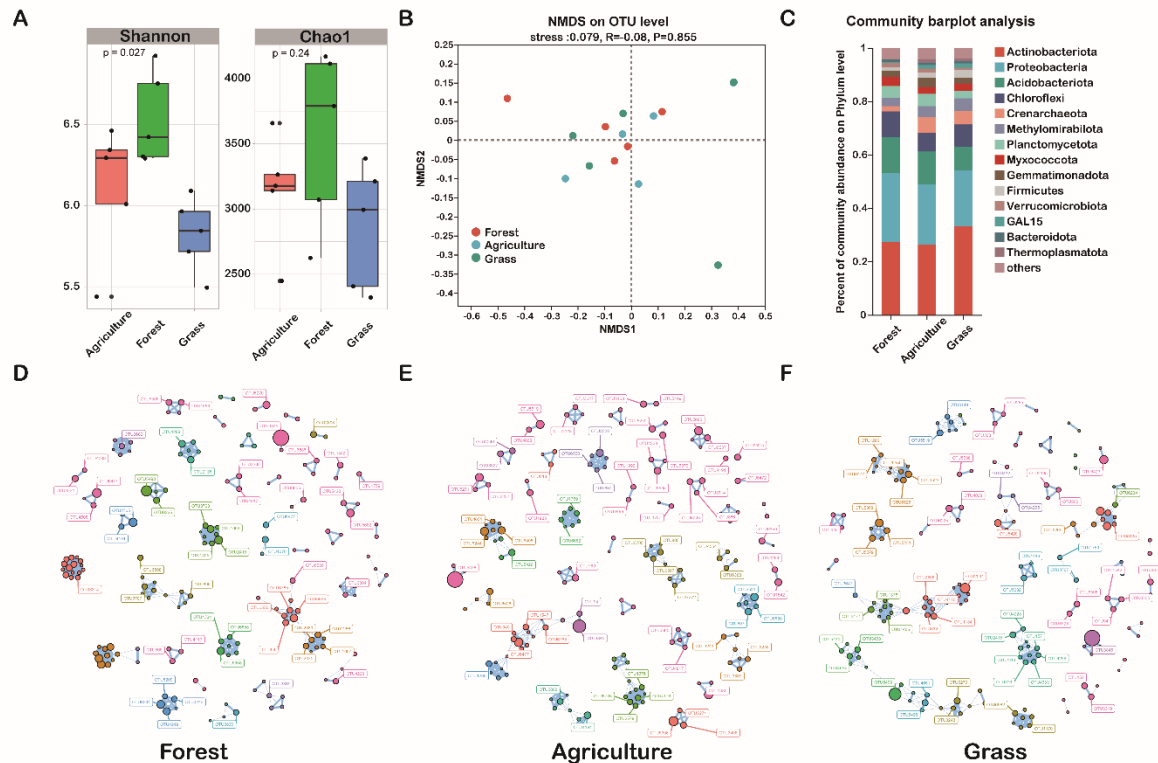
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