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

3

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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, HNO₃ 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). 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 α -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 α -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 β -diversity
175 metrics (β 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 α -diversity in fungal community around*** 196 ***active tailings***

197 The α -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 α -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 α -diversity of fungal communities.
212 Positive linear regression between bacterial richness and α -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 α -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 β -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 β -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 α -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, α -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 β -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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453 **References**

454

- 455 Adiansyah, J.S., Rosano, M., Vink, S., Keir, G., 2015. A framework for a sustainable approach to mine
456 tailings management: disposal strategies. *J Clean Prod* 108, 1050-1062.
457 <https://doi.org/10.1016/j.jclepro.2015.07.139>.
- 458 Bardgett, R.D., van der Putten, W.H., 2014. Belowground biodiversity and ecosystem functioning.
459 *Nature* 515(7528), 505-511. <https://doi.org/10.1038/nature13855>.
- 460 Bastian, M., Heymann, S., Jacomy, M., 2009. Gephi: An Open Source Software for Exploring and
461 Manipulating Networks. *Proceedings of the International AAAI Conference on Web and Social Media*
462 3(1), 361-362. <https://doi.org/10.1609/icwsm.v3i1.13937>.
- 463 Bates, S.T., Berg-Lyons, D., Caporaso, J.G., Walters, W.A., Knight, R., Fierer, N., 2011. Examining
464 the global distribution of dominant archaeal populations in soil. *ISME J* 5(5), 908-917.
465 <https://doi.org/10.1038/ismej.2010.171>.
- 466 Beattie, R.E., Henke, W., Campa, M.F., Hazen, T.C., McAliley, L.R., Campbell, J.H., 2018. Variation
467 in microbial community structure correlates with heavy-metal contamination in soils decades after
468 mining ceased. *Soil Biol Biochem* 126, 57-63. <https://doi.org/10.1016/j.soilbio.2018.08.011>.
- 469 Becker, J., Eisenhauer, N., Scheu, S., Jousset, A., 2012. Increasing antagonistic interactions cause
470 bacterial communities to collapse at high diversity. *Ecol Lett* 15(5), 468-474.

471 <https://doi.org/10.1111/j.1461-0248.2012.01759.x>.

472 Bokulich, N.A., Mills, D.A., 2013. Improved selection of internal transcribed spacer-specific primers
473 enables quantitative, ultra-high-throughput profiling of fungal communities. *Appl Environ Microbiol*
474 79(8), 2519-2526. <https://doi.org/10.1128/AEM.03870-12>.

475 Camacho-Cristobal, J.J., Rexach, J., Gonzalez-Fontes, A., 2008. Boron in plants: deficiency and
476 toxicity. *J Integr Plant Biol* 50(10), 1247-1255. <https://doi.org/10.1111/j.1744-7909.2008.00742.x>.

477 Cavicchioli, R., Ripple, W.J., Timmis, K.N., Azam, F., Bakken, L.R., Baylis, M., Behrenfeld, M.J.,
478 Boetius, A., Boyd, P.W., Classen, A.T., Crowther, T.W., Danovaro, R., Foreman, C.M., Huisman, J.,
479 Hutchins, D.A., Jansson, J.K., Karl, D.M., Koskella, B., Mark Welch, D.B., Martiny, J.B.H., Moran,
480 M.A., Orphan, V.J., Reay, D.S., Remais, J.V., Rich, V.I., Singh, B.K., Stein, L.Y., Stewart, F.J.,
481 Sullivan, M.B., van Oppen, M.J.H., Weaver, S.C., Webb, E.A., Webster, N.S., 2019. Scientists'
482 warning to humanity: microorganisms and climate change. *Nat Rev Microbiol* 17(9), 569-586.
483 <https://doi.org/10.1038/s41579-019-0222-5>.

484 Chen, X.F., Wang, J.C., You, Y.M., Wang, R.Y., Chu, S.H., Chi, Y.W., Hayat, K., Hui, N., Liu, X.X.,
485 Zhang, D., Zhou, P., 2022. When nanoparticle and microbes meet: The effect of multi-walled carbon
486 nanotubes on microbial community and nutrient cycling in hyperaccumulator system. *Journal of*
487 *Hazardous Materials* 423. <https://doi.org/ARTN126947>
488 10.1016/j.jhazmat.2021.126947.

489 Chen, Y.C., Yin, S.W., Shao, Y., Zhang, K.R., 2022. Soil bacteria are more sensitive than fungi in
490 response to nitrogen and phosphorus enrichment. *Frontiers in Microbiology* 13. [https://doi.org/ARTN](https://doi.org/ARTN999385)
491 999385
492 10.3389/fmicb.2022.999385.

493 Cherabier, P., Ferriere, R., 2022. Eco-evolutionary responses of the microbial loop to surface ocean
494 warming and consequences for primary production. *ISME J* 16(4), 1130-1139.
495 <https://doi.org/10.1038/s41396-021-01166-8>.

496 Clarke, K.R., 1993. Non-parametric multivariate analyses of changes in community structure. *Austral*
497 *Ecology* 18(1), 117-143. <https://doi.org/10.1111/j.1442-9993.1993.tb00438.x>.

498 Cohen, P., Cohen, P., West, S.G., Aiken, L.S., 2014. *Applied Multiple Regression/Correlation Analysis*
499 *for the Behavioral Sciences*. Psychology Press, New York. <https://doi.org/10.4324/9781410606266>.

500 Deveau, A., Bonito, G., Uehling, J., Paoletti, M., Becker, M., Bindschedler, S., Hacquard, S., Herve, V.,
501 Labbe, J., Lastovetsky, O.A., Mieszkina, S., Millet, L.J., Vajna, B., Junier, P., Bonfante, P., Krom, B.P.,
502 Olsson, S., van Elsas, J.D., Wick, L.Y., 2018. Bacterial-fungal interactions: ecology, mechanisms and
503 challenges. *FEMS Microbiol Rev* 42(3), 335-352. <https://doi.org/10.1093/femsre/fuy008>.

504 Dold, B., 2014. Evolution of Acid Mine Drainage Formation in Sulphidic Mine Tailings. *Minerals-Basel*
505 4(3), 621-641. <https://doi.org/10.3390/min4030621>.

506 Flores-Torres, G., Solis-Hernandez, A.P., Vela-Correa, G., Rodriguez-Tovar, A.V., Cano-Flores, O.,
507 Castellanos-Moguel, J., Perez, N.O., Chimal-Hernandez, A., Moreno-Espindola, I.P., Salas-Luevano,
508 M.A., Chavez-Vergara, B.M., Rivera-Becerril, F., 2021. Pioneer plant species and fungal root
509 endophytes in metal-polluted tailings deposited near human populations and agricultural areas in
510 Northern Mexico. *Environ Sci Pollut Res Int* 28(39), 55072-55088.
511 <https://doi.org/10.1007/s11356-021-14716-6>.

512 Freilich, S., Kreimer, A., Meilijson, I., Gophna, U., Sharan, R., Ruppin, E., 2010. The large-scale
513 organization of the bacterial network of ecological co-occurrence interactions. *Nucleic Acids Res*
514 38(12), 3857-3868. <https://doi.org/10.1093/nar/gkq118>.

515 Frey-Klett, P., Burlinson, P., Deveau, A., Barret, M., Tarkka, M., Sarniguet, A., 2011. Bacterial-Fungal
516 Interactions: Hyphens between Agricultural, Clinical, Environmental, and Food Microbiologists.
517 *Microbiol Mol Biol R* 75(4), 583-+. <https://doi.org/10.1128/Mmbr.00020-11>.

518 Gall, J.E., Boyd, R.S., Rajakaruna, N., 2015. Transfer of heavy metals through terrestrial food webs: a
519 review. *Environ Monit Assess* 187(4). <https://doi.org/ARTN> 201
520 10.1007/s10661-015-4436-3.

521 Gao, Q., Yang, Y.F., Feng, J.J., Tian, R.M., Guo, X., Ning, D.L., Hale, L.R., Wang, M.M., Cheng, J.M.,
522 Wu, L.W., Zhao, M.X., Zhao, J.S., Wu, L.Y., Qin, Y.J., Qi, Q., Liang, Y.T., Sun, B., Chu, H.Y., Zhou,
523 J.Z., 2019. The spatial scale dependence of diazotrophic and bacterial community assembly in paddy
524 soil. *Global Ecol Biogeogr* 28(8), 1093-1105. <https://doi.org/10.1111/geb.12917>.

525 Giller, K.E., Witter, E., McGrath, S.P., 1998. Toxicity of heavy metals to microorganisms and
526 microbial processes in agricultural soils: A review. *Soil Biol Biochem* 30(10-11), 1389-1414.
527 <https://doi.org/Doi> 10.1016/S0038-0717(97)00270-8.

528 Gravel, D., Guichard, F., Hochberg, M.E., 2011. Species coexistence in a variable world. *Ecol Lett*
529 14(8), 828-839. <https://doi.org/10.1111/j.1461-0248.2011.01643.x>.

530 Harris, J.A., 2003. Measurements of the soil microbial community for estimating the success of
531 restoration. *Eur J Soil Sci* 54(4), 801-808. <https://doi.org/10.1046/j.1351-0754.2003.0559.x>.

532 Jia, T., Wang, R.H., Fan, X.H., Chai, B.F., 2018. A Comparative Study of Fungal Community
533 Structure, Diversity and Richness between the Soil and the Phyllosphere of Native Grass Species in a
534 Copper Tailings Dam in Shanxi Province, China. *Appl Sci-Basel* 8(8). <https://doi.org/ARTN> 1297
535 10.3390/app8081297.

536 Jiao, P.P., Li, Z.W., Yang, L., He, J.J., Chang, X.F., Xiao, H.B., Nie, X.D., Tong, D., 2021. Bacteria
537 are more sensitive than fungi to moisture in eroded soil by natural grass vegetation restoration on the
538 Loess Plateau. *Science of the Total Environment* 756. <https://doi.org/ARTN> 143899
539 10.1016/j.scitotenv.2020.143899.

540 Jiao, S., Chen, W., Wei, G., 2022. Core microbiota drive functional stability of soil microbiome in
541 reforestation ecosystems. *Glob Chang Biol* 28(3), 1038-1047. <https://doi.org/10.1111/gcb.16024>.

542 Juge, C., Cossette, N., Jeanne, T., Hogue, R., 2021. Long-term revegetation on iron mine tailings in
543 northern Québec and Labrador and its effect on arbuscular mycorrhizal fungi. *Applied Soil Ecology*
544 168. <https://doi.org/10.1016/j.apsoil.2021.104145>.

545 Kandeler, E., Tschirko, D., Bruce, K.D., Stemmer, M., Hobbs, P.J., Bardgett, R.D., Amelung, W.,
546 2000. Structure and function of the soil microbial community in microhabitats of a heavy metal
547 polluted soil. *Biol Fert Soils* 32(5), 390-400. <https://doi.org/DOI> 10.1007/s003740000268.

548 Karaca, O., Cameselle, C., Reddy, K.R., 2018. Mine tailing disposal sites: contamination problems,
549 remedial options and phytocaps for sustainable remediation. *Rev Environ Sci Bio* 17(1), 205-228.
550 <https://doi.org/10.1007/s11157-017-9453-y>.

551 Li, D.J., Poisot, T., Waller, D.M., Baiser, B., 2018. Homogenization of species composition and
552 species association networks are decoupled. *Global Ecol Biogeogr* 27(12), 1481-1491.
553 <https://doi.org/10.1111/geb.12825>.

554 Li, S., Wu, J., Huo, Y., Zhao, X., Xue, L., 2021. Profiling multiple heavy metal contamination and
555 bacterial communities surrounding an iron tailing pond in Northwest China. *Sci Total Environ* 752,
556 141827. <https://doi.org/10.1016/j.scitotenv.2020.141827>.

557 Li, Y., Jia, Z., Sun, Q., Zhan, J., Yang, Y., Wang, D., 2016. Ecological restoration alters microbial
558 communities in mine tailings profiles. *Sci Rep* 6, 25193. <https://doi.org/10.1038/srep25193>.

559 Li, Z., Wu, S., Liu, Y., Yi, Q., Nguyen, T.A.H., Ma, Y., You, F., Hall, M., Chan, T.-S., Huang, Y.,
560 Huang, L., 2022. Plant biomass amendment regulates arbuscular mycorrhizal role in organic carbon
561 and nitrogen sequestration in eco-engineered iron ore tailings. *Geoderma* 428.
562 <https://doi.org/10.1016/j.geoderma.2022.116178>.

563 Lin, Y., Ye, Y., Hu, Y., Shi, H., 2019. The variation in microbial community structure under different
564 heavy metal contamination levels in paddy soils. *Ecotoxicol Environ Saf* 180, 557-564.
565 <https://doi.org/10.1016/j.ecoenv.2019.05.057>.

566 Liu, B., Yao, J., Chen, Z., Ma, B., Liu, J., Li, H., Zhu, X., Li, M., Cao, Y., Pang, W., Zhao, C., Mihucz,
567 V.G., Duran, R., 2022. Unraveling ecological risk of As/Sb and other metal(loid)s and fungal
568 community responses in As/Sb smelting-intensive zone: A typical case study of Southwest China. *J*
569 *Clean Prod* 338. <https://doi.org/10.1016/j.jclepro.2022.130525>.

570 Liu, B., Yao, J., Ma, B., Chen, Z., Zhu, X., Zhao, C., Li, M., Cao, Y., Pang, W., Li, H., Mihucz, V.G.,
571 Duran, R., 2022. Metal(loid)s diffusion pathway triggers distinct microbiota responses in key regions
572 of typical karst non-ferrous smelting assembly. *J Hazard Mater* 423(Pt B), 127164.
573 <https://doi.org/10.1016/j.jhazmat.2021.127164>.

574 Liu, J.L., Yao, J., Wang, F., Min, N., Gu, J.H., Li, Z.F., Sunahara, G., Duran, R., Solevic-Knudsen, T.,
575 Hudson-Edwards, K.A., Alakangas, L., 2019. Bacterial diversity in typical abandoned
576 multi-contaminated nonferrous metal(loid) tailings during natural attenuation. *Environ Pollut* 247,
577 98-107. <https://doi.org/10.1016/j.envpol.2018.12.045>.

578 Liu, Y.X., Qin, Y., Chen, T., Lu, M.P., Qian, X.B., Guo, X.X., Bai, Y., 2021. A practical guide to
579 amplicon and metagenomic analysis of microbiome data. *Protein Cell* 12(5), 315-330.
580 <https://doi.org/10.1007/s13238-020-00724-8>.

581 Liu, Z.C., Deng, Y.F., Ji, M.Z., Sun, W.Q., Fan, X.Y., 2020. Prophages domesticated by bacteria
582 promote the adaptability of bacterial cells. *Biocell* 44(2), 157-166.
583 <https://doi.org/10.32604/biocell.2020.09917>.

584 Macias-Perez, L.A., Levard, C., Barakat, M., Angeletti, B., Borschneck, D., Poizat, L., Achouak, W.,
585 Auffan, M., 2022. Contrasted microbial community colonization of a bauxite residue deposit marked
586 by a complex geochemical context. *J Hazard Mater* 424(Pt B), 127470.
587 <https://doi.org/10.1016/j.jhazmat.2021.127470>.

588 Mantel, N., 1966. The detection of disease clustering and a generalized
589 regression approach. *Cancer Res*.

590 Mendez, M.O., Maier, R.M., 2008. Phytostabilization of mine tailings in arid and semiarid
591 environments--an emerging remediation technology. *Environ Health Perspect* 116(3), 278-283.
592 <https://doi.org/10.1289/ehp.10608>.

593 Mohapatra, M., Yadav, R., Rajput, V., Dharne, M.S., Rastogi, G., 2021. Metagenomic analysis reveals
594 genetic insights on biogeochemical cycling, xenobiotic degradation, and stress resistance in mudflat
595 microbiome. *J Environ Manage* 292, 112738. <https://doi.org/10.1016/j.jenvman.2021.112738>.

596 O'Neill, M.A., Eberhard, S., Albersheim, P., Darvill, A.G., 2001. Requirement of borate cross-linking
597 of cell wall rhamnogalacturonan II for *Arabidopsis* growth. *Science* 294(5543), 846-849.
598 <https://doi.org/10.1126/science.1062319>.

599 Poudel, N., Baral, P., Neupane, M., Shrestha, S.M., Shrestha, A.K., Bhatta, S., 2022. Effect of Boron
600 on Growth and Yield Parameters of Cauliflower (*Brassica oleracea* var botrytis cv Snow Mystique) in
601 Terhathum, Nepal. *International Journal of Applied Sciences and Biotechnology* 10(1), 41-49.
602 <https://doi.org/10.3126/ijasbt.v10i1.44158>.

603 Ramdani, M., Flower, R.J., Elkhiaati, N., Kraïem, M.M., Fathi, A.A., Birks, H.H., Patrick, S.T., 2001.
604 North African wetland lakes: Characterization of nine sites included in the CASSARINA Project.
605 *Aquat Ecol* 35(3/4), 281-302. <https://doi.org/10.1023/a:1011957324901>.
606 Rodriguez, L., Ruiz, E., Alonso-Azcarate, J., Rincon, J., 2009. Heavy metal distribution and chemical
607 speciation in tailings and soils around a Pb-Zn mine in Spain. *J Environ Manage* 90(2), 1106-1116.
608 <https://doi.org/10.1016/j.jenvman.2008.04.007>.
609 Rooney, R.C., Azeria, E.T., Gillman, L.N., 2014. The strength of cross-taxon congruence in species
610 composition varies with the size of regional species pools and the intensity of human disturbance.
611 *Journal of Biogeography* 42(3), 439-451. <https://doi.org/10.1111/jbi.12400>.
612 Segata, N., Izard, J., Waldron, L., Gevers, D., Miropolsky, L., Garrett, W.S., Huttenhower, C., 2011.
613 Metagenomic biomarker discovery and explanation. *Genome Biol* 12(6), R60.
614 <https://doi.org/10.1186/gb-2011-12-6-r60>.
615 Shen, Y.Y., Li, H.J., Liu, Y., Gao, T.P., Li, G.W., Zuo, M.B., Ji, J., Li, C.M., Li, X.K., Chen, Y.L., Yin,
616 Z.X., Li, J., Zhang, W.L., 2022. Variations of fungal communities in lead-zinc tailings located in
617 Northwestern China. *Hum Ecol Risk Assess*. <https://doi.org/10.1080/10807039.2022.2098466>.
618 Shorrocks, V.M., 1997. The occurrence and correction of boron deficiency. *Plant and Soil* 193(2),
619 121-148. <https://doi.org/10.1023/a:1004216126069>.
620 Stegen, J.C., Lin, X.J., Fredrickson, J.K., Chen, X.Y., Kennedy, D.W., Murray, C.J., Rockhold, M.L.,
621 Konopka, A., 2013. Quantifying community assembly processes and identifying features that impose
622 them. *Isme Journal* 7(11), 2069-2079. <https://doi.org/10.1038/ismej.2013.93>.
623 Tanaka, M., Fujiwara, T., 2008. Physiological roles and transport mechanisms of boron: perspectives
624 from plants. *Pflügers Arch* 456(4), 671-677. <https://doi.org/10.1007/s00424-007-0370-8>.
625 Tedersoo, L., Sanchez-Ramirez, S., Koljalg, U., Bahram, M., Doring, M., Schigel, D., May, T., Ryberg,
626 M., Abarenkov, K., 2018. High-level classification of the Fungi and a tool for evolutionary ecological
627 analyses. *Fungal Divers* 90(1), 135-159. <https://doi.org/10.1007/s13225-018-0401-0>.
628 Tian, T., Chen, Z., Tian, Y., Gao, L., 2017. Microbial diversity in solar greenhouse soils in
629 Round-Bohai Bay-Region, China: The influence of cultivation year and environmental condition.
630 *Environ Sci Pollut Res Int* 24(29), 23236-23249. <https://doi.org/10.1007/s11356-017-9837-0>.
631 Wang, J., Zhang, T., Li, L., Li, J., Feng, Y., Lu, Q., 2017. The Patterns and Drivers of Bacterial and
632 Fungal beta-Diversity in a Typical Dryland Ecosystem of Northwest China. *Front Microbiol* 8, 2126.
633 <https://doi.org/10.3389/fmicb.2017.02126>.
634 Wang, Q., Wang, R.Q., Tian, C.Y., Yu, Y., Zhang, Y.L., Dai, J.L., 2012. Using microbial community
635 functioning as the complementary environmental condition indicator: A case study of an iron deposit
636 tailing area. *Eur J Soil Biol* 51, 22-29. <https://doi.org/10.1016/j.ejsobi.2012.03.004>.
637 Wu, K., Zhao, W., Li, M., Picazo, F., Soininen, J., Shen, J., Zhu, L., Cheng, X., Wang, J., 2020.
638 Taxonomic dependency of beta diversity components in benthic communities of bacteria, diatoms and
639 chironomids along a water-depth gradient. *Sci Total Environ* 741, 140462.
640 <https://doi.org/10.1016/j.scitotenv.2020.140462>.
641 Wu, Z., Zhang, L., Xia, T., Jia, X., Wang, S., 2020. Heavy metal pollution and human health risk
642 assessment at mercury smelting sites in Wanshan district of Guizhou Province, China. *RSC Adv* 10(39),
643 23066-23079. <https://doi.org/10.1039/d0ra01004a>.
644 Xiao, E., Ning, Z., Sun, W., Jiang, S., Fan, W., Ma, L., Xiao, T., 2021. Thallium shifts the bacterial and
645 fungal community structures in thallium mine waste rocks. *Environ Pollut* 268(Pt A), 115834.
646 <https://doi.org/10.1016/j.envpol.2020.115834>.

647 Xie, L., van Zyl, D., 2020. Distinguishing reclamation, revegetation and phytoremediation, and the
648 importance of geochemical processes in the reclamation of sulfidic mine tailings: A review.
649 *Chemosphere* 252, 126446. <https://doi.org/10.1016/j.chemosphere.2020.126446>.

650 Xu, M., Huang, Q., Xiong, Z., Liao, H., Lv, Z., Chen, W., Luo, X., Hao, X., 2021. Distinct Responses
651 of Rare and Abundant Microbial Taxa to In Situ Chemical Stabilization of Cadmium-Contaminated
652 Soil. *mSystems* 6(5), e0104021. <https://doi.org/10.1128/mSystems.01040-21>.

653 Xu, R., Li, T., Shen, M., Yang, Z.L., Zhao, Z.-W., 2020. Evidence for a Dark Septate Endophyte
654 (*Exophiala pisciphila*, H93) Enhancing Phosphorus Absorption by Maize Seedlings. *Plant and Soil*
655 452(1-2), 249-266. <https://doi.org/10.1007/s11104-020-04538-9>.

656 Yu, Y., Teng, Z., Mou, Z., Lv, Y., Li, T., Chen, S., Zhao, D., Zhao, Z., 2021. Melatonin confers heavy
657 metal-induced tolerance by alleviating oxidative stress and reducing the heavy metal accumulation in
658 *Exophiala pisciphila*, a dark septate endophyte (DSE). *BMC Microbiol* 21(1), 40.
659 <https://doi.org/10.1186/s12866-021-02098-1>.

660 Yuan, H., Zhang, W., Yin, H., Zhang, R., Wang, J., 2022. Taxonomic dependency of beta diversity for
661 bacteria, archaea, and fungi in a semi-arid lake. *Front Microbiol* 13, 998496.
662 <https://doi.org/10.3389/fmicb.2022.998496>.

663 Zelezniak, A., Andrejev, S., Ponomarova, O., Mende, D.R., Bork, P., Patil, K.R., 2015. Metabolic
664 dependencies drive species co-occurrence in diverse microbial communities. *Proc Natl Acad Sci U S A*
665 112(20), 6449-6454. <https://doi.org/10.1073/pnas.1421834112>.

666 Zeng, X.Y., Li, S.W., Leng, Y., Kang, X.H., 2020. Structural and functional responses of bacterial and
667 fungal communities to multiple heavy metal exposure in arid loess. *Sci Total Environ* 723, 138081.
668 <https://doi.org/10.1016/j.scitotenv.2020.138081>.

669 Zhao, H., Lin, J., Wang, X., Shi, J., Dahlgren, R.A., Xu, J., 2021. Dynamics of Soil Microbial
670 N-Cycling Strategies in Response to Cadmium Stress. *Environ Sci Technol* 55(20), 14305-14315.
671 <https://doi.org/10.1021/acs.est.1c04409>.

672 Zhao, H.C., Lin, J.H., Wang, X.H., Shi, J.C., Dahlgren, R.A., Xu, J.M., 2021. Dynamics of Soil
673 Microbial N-Cycling Strategies in Response to Cadmium Stress. *Environ Sci Technol* 55(20),
674 14305-14315. <https://doi.org/10.1021/acs.est.1c04409>.

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687 **Figure legends**

688

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) β -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 α -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 β 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 α -diversity of bacterial
739 communities. (B) β -diversities of bacterial communities based on non-metric
740 multidimensional scaling (NMDS) analysis. (C) The composition of bacterial
741 communities in three vegetation at phylum level. (D-F) The co-occurrence networks
742 of bacterial communities in Forest, Agriculture and Grass, respectively. A connection
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745 according to modularity classes.

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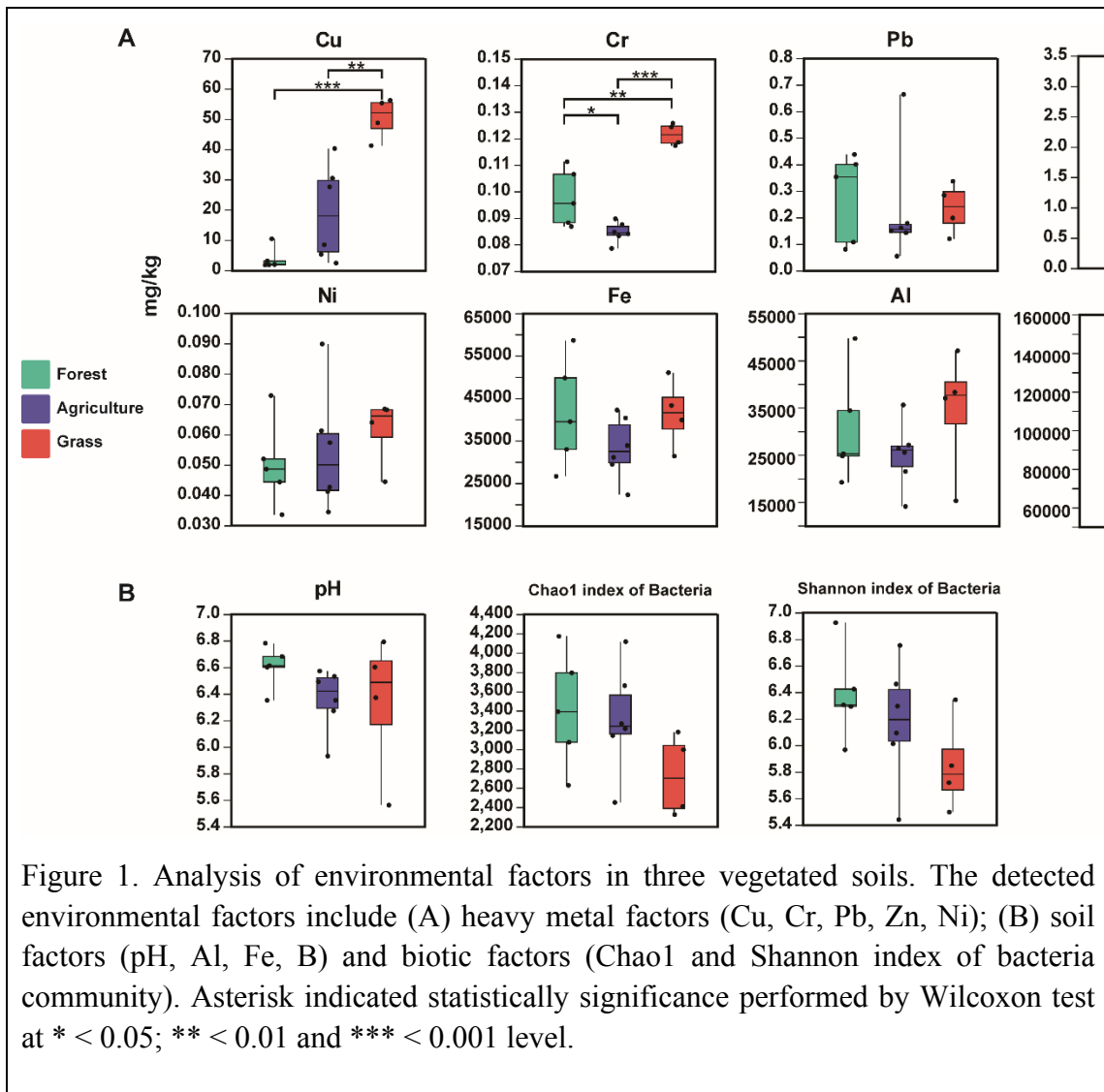


Figure 1. Analysis of environmental factors in three vegetated soils. The detected environmental factors include (A) heavy metal factors (Cu, Cr, Pb, Zn, Ni); (B) soil factors (pH, Al, Fe, B) and biotic factors (Chao1 and Shannon index of bacteria community). Asterisk indicated statistically significance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

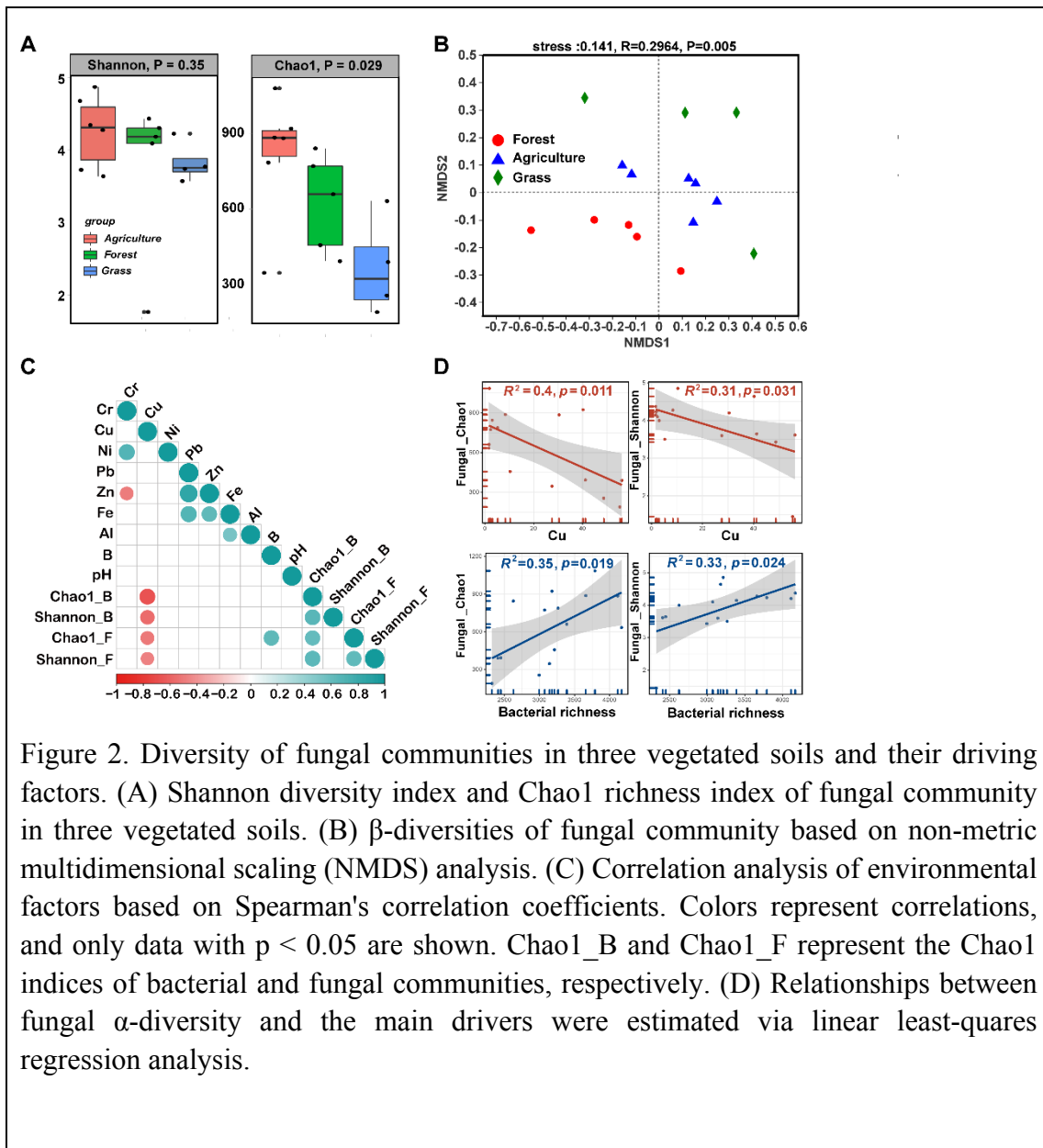


Figure 2. Diversity of fungal communities in three vegetated soils and their driving factors. (A) Shannon diversity index and Chao1 richness index of fungal community in three vegetated soils. (B) β -diversities of fungal community based on non-metric multidimensional scaling (NMDS) analysis. (C) Correlation analysis of environmental factors based on Spearman's correlation coefficients. Colors represent correlations, and only data with $p < 0.05$ are shown. Chao1_B and Chao1_F represent the Chao1 indices of bacterial and fungal communities, respectively. (D) Relationships between fungal α -diversity and the main drivers were estimated via linear least-squares regression analysis.

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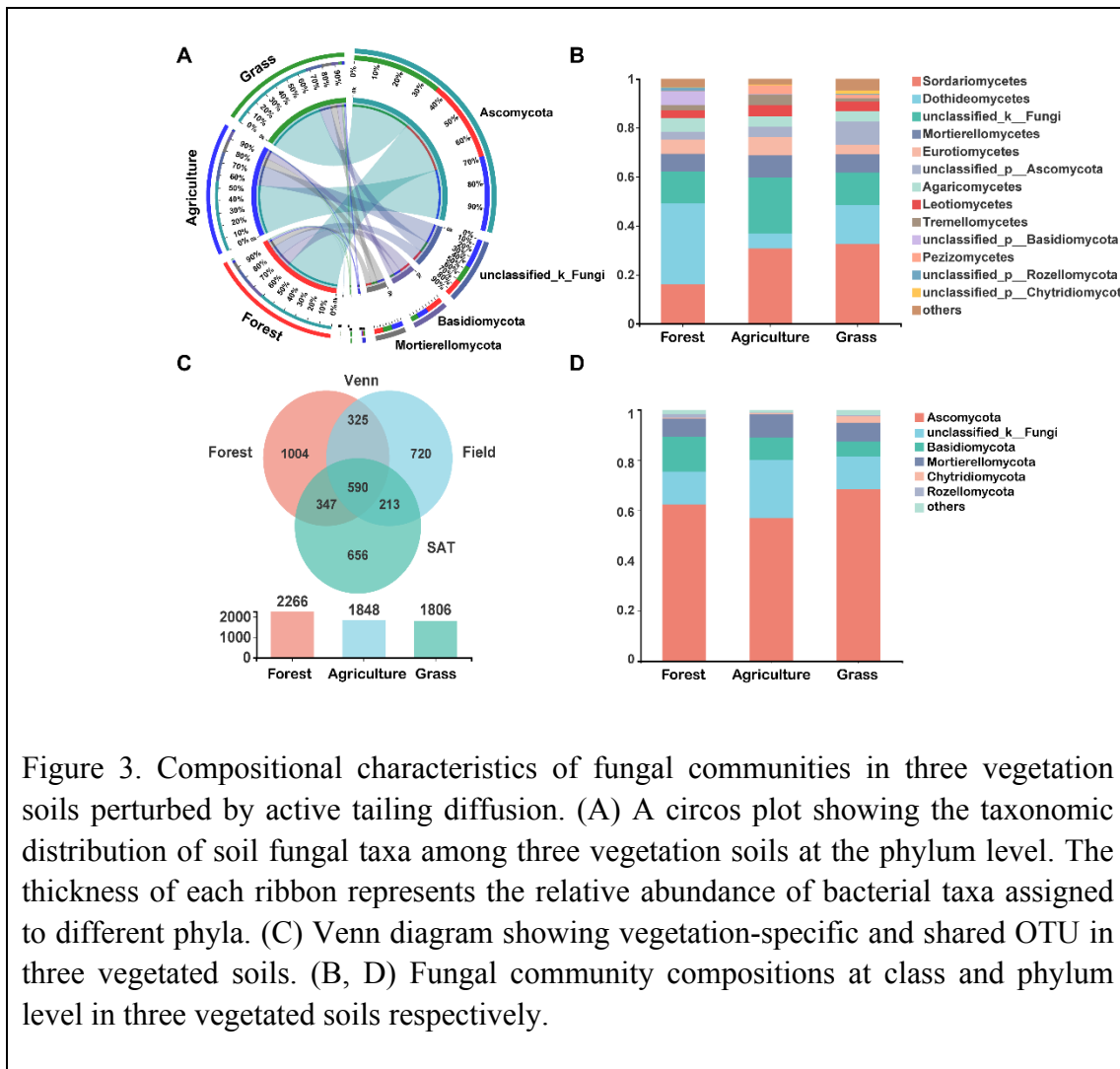


Figure 3. Compositional characteristics of fungal communities in three vegetation soils perturbed by active tailing diffusion. (A) A circos plot showing the taxonomic distribution of soil fungal taxa among three vegetation soils at the phylum level. The thickness of each ribbon represents the relative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

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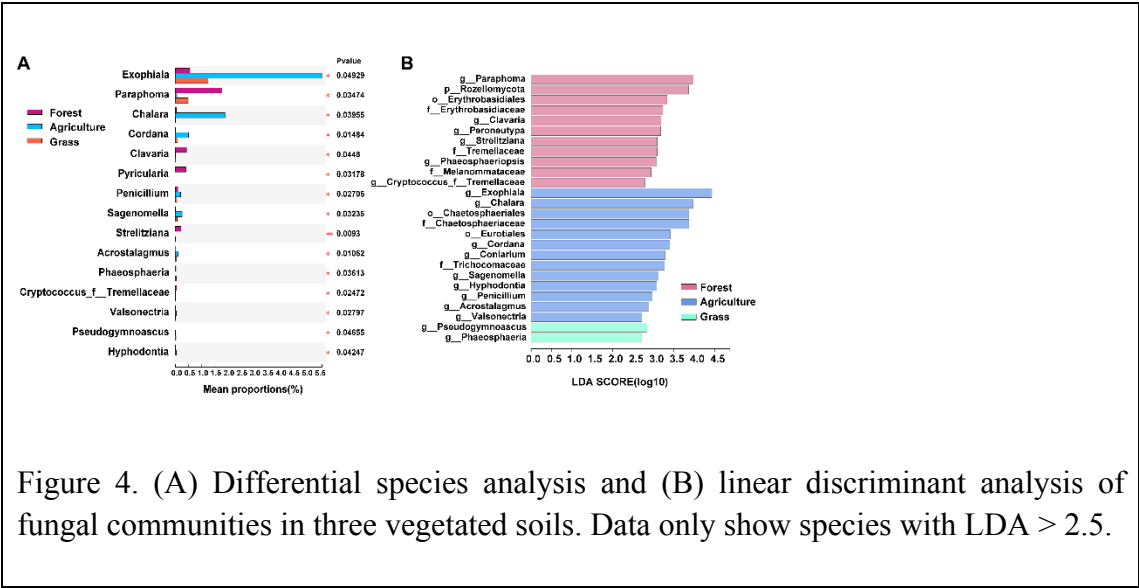
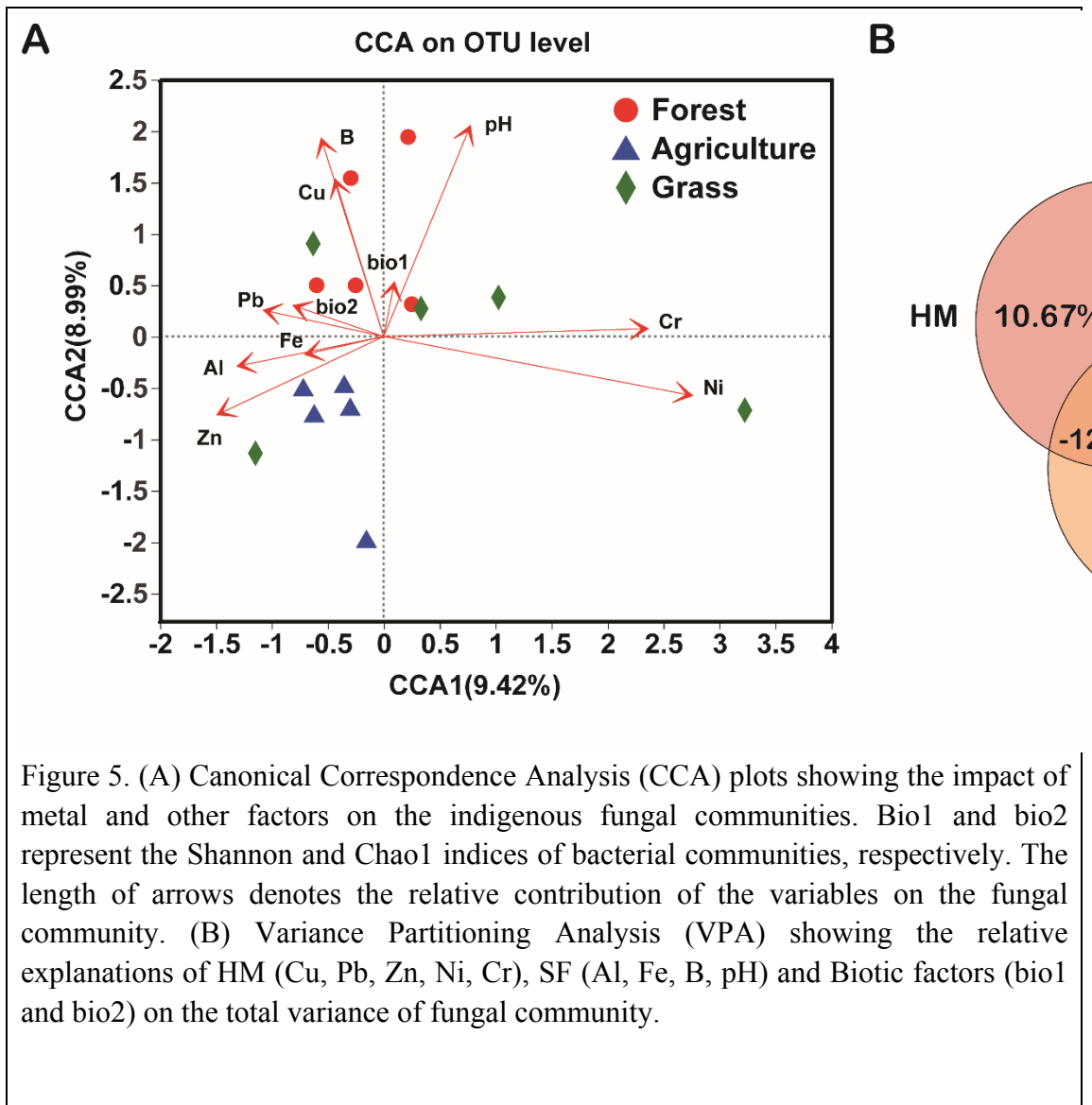


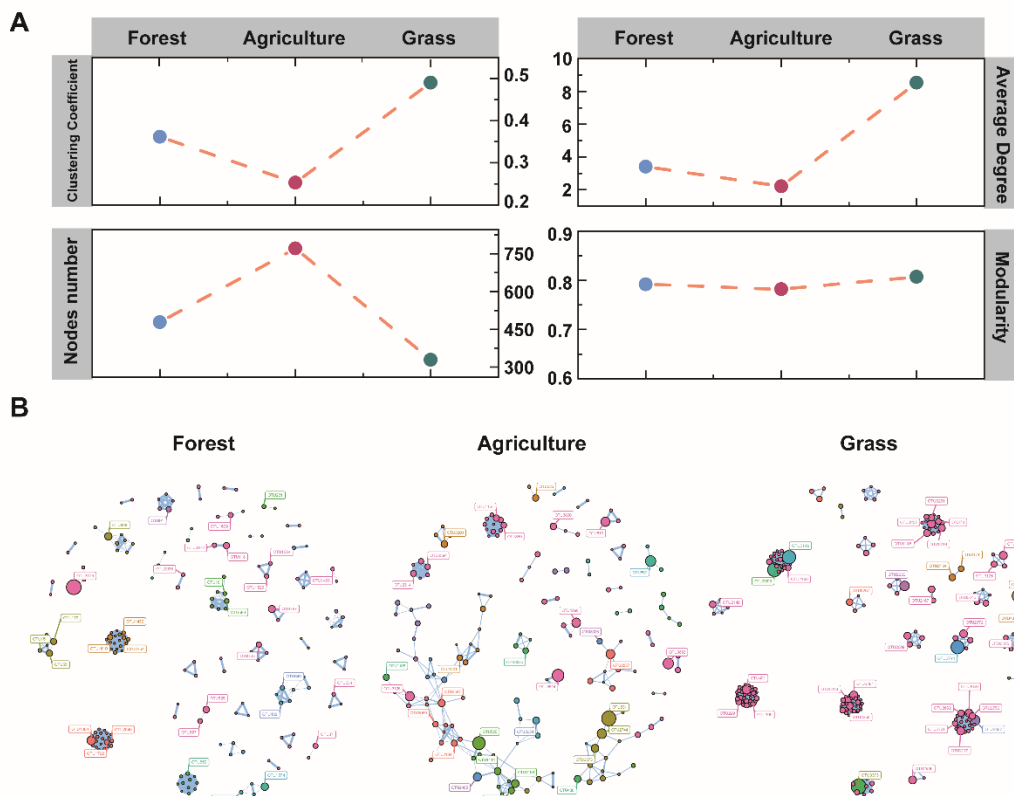
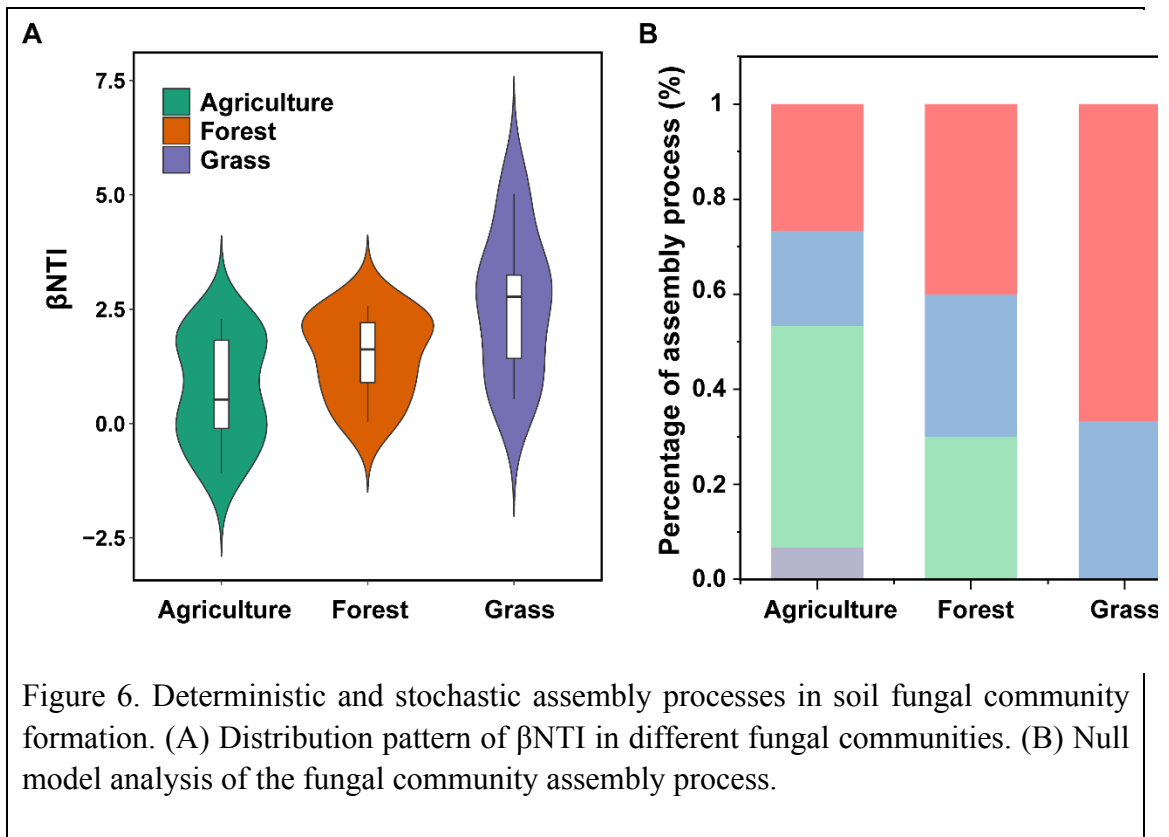
Figure 4. (A) Differential species analysis and (B) linear discriminant analysis of fungal communities in three vegetated soils. Data only show species with LDA > 2.5.

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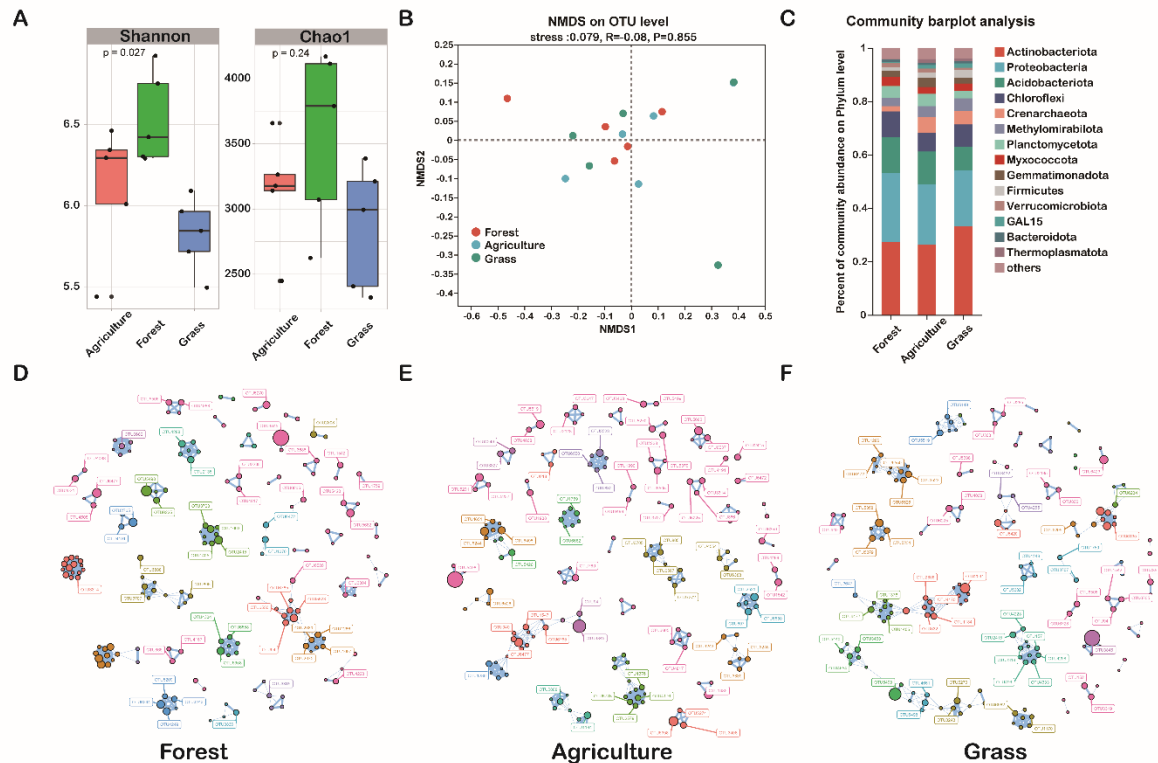
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