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1 **Title:**

2 Land-use changes alter the arbuscular mycorrhizal fungal community composition and  
3 assembly in the ancient tea forest reserve

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25 **Abstract:**

26 Understanding the effects of land-use changes on arbuscular mycorrhizal fungal (AMF)  
27 communities may greatly benefit ecosystem conservation and restoration. However,  
28 how AMF communities respond to anthropogenetic land-use change (e.g., from natural  
29 ecosystems to farmland ecosystems) is still under debate. To enhance the preservation  
30 of vegetation diversity in ancient tea forest (ATF) regions and understand how land-use  
31 changes influence the AMF community in acidic soils, the AMF community  
32 composition and assembly processes in the ATF region (soil pH: 3.5-4.2) were  
33 investigated. Our results showed that AMF  $\alpha$ -diversity indices in ATF were  
34 significantly higher than those in conventional tea plantations (CTP) and Forest.  
35 Moreover, number of indicator species (as virtual taxa, VTX) showed a remarkable  
36 decrease when ATF (12 VTXs) changed to Forest (8 VTXs) and CTP (3 VTXs). In  
37 addition, neutral processes dominated the AMF community assembly, and *Acaulospora*  
38 was the dominant genus of AMF indicator species in ATF. Moreover, land-use changes  
39 eliminated the neutral process of AMF community assembly in CTP and Forest by  
40 enhancing the environmental filtering effects. The concentrations of soil nitrate, TK,  
41  $Mg^{2+}$ , and  $Cu^{2+}$  were important factors accounting for the AMF community change. In  
42 addition, we found that high acidity soils may exert an ecological selection on the AMF  
43 community, as only species that adapt to strongly acidic soils persisted. Overall, our  
44 results indicated that mitigating soil acidification has potential as a method of  
45 improving the AMF community diversity and conserving and restoring ATF  
46 ecosystems in southwest China.

47 **Keywords:** Land-use change; AMF diversity; Community composition; Community  
48 assembly; Acidic soils; Ancient tea forest

## 49 **1. Introduction**

50 Soil microbial communities are essential for ecosystem processes in terrestrial  
51 ecosystems, but they are threatened by changes in land-use types (Fierer, 2017; Osburn  
52 et al., 2021). Nearly 75% of the ice-free land area on earth is altered by the change in  
53 land-use types, which has significantly impacted microbial community structure and  
54 soil ecosystem functions (Gottshall et al., 2017; Jefwa et al., 2012; Pereira et al., 2014).  
55 Thus, understanding how land-use changes affect soil microbial communities remains  
56 a pressing need and would provide new insights into ecosystem conservation and  
57 restoration because soil microbial communities play vital roles in ecosystem functions  
58 and aboveground diversity.

59 In global terrestrial ecosystems, arbuscular mycorrhizal fungal (AMF) play an  
60 essential role in maintaining the growth of 80% of higher plants (Klironomos et al.,  
61 2011). Previous studies have proved that land-use changes can significantly impact  
62 AMF communities (House and Bever, 2018; Xiang et al., 2014; Xu et al., 2017). For  
63 instance, the increase in soil phosphorus (P) concentration and poor soil structure due  
64 to land-use changes have adverse effects on the AMF community (Bender et al., 2019;  
65 Xiang et al., 2014). Additionally, native ecosystems (i.e., grassland, forest) converted  
66 to agricultural ecosystems can lower AMF activity and subsequently reduce carbon (C)  
67 sequestration and deteriorate soil quality (Xu et al., 2017). The AMF communities in  
68 undisturbed grasslands reveal strong differentiation, whereas communities in disturbed  
69 grasslands exhibit more homogeneity (House and Bever, 2018). Overall, variation in  
70 environmental factors variations after land-use change contribute significantly to AMF  
71 community change. However, the specific mechanisms underpinning AMF community  
72 responses to environmental change is still unclear and needs to be addressed.

73 Changes in the soil microbial community occur in connection with different  
74 community assembly processes under diverse land-use types (Goss-Souza et al., 2017).  
75 Environmental factors (e.g., soil pH, moisture and nutrient level) are the primary drivers  
76 of these processes and show significant differences among land-use types (Jangid et al.,  
77 2011). For instance, differences in soil pH among land-use types can alter community  
78 assembly processes in structuring microbial communities through a strong filtering  
79 effect on soil microbes (Barnett et al., 2020). Additionally, land-use choice after  
80 deforestation could determine the patterns of microbial community assembly (Goss-  
81 Souza et al., 2017). For example, high homogenizing selection occurs when the  
82 Amazon rainforest converts into grassland communities (Rodrigues et al., 2013).  
83 Moreover, selection is more critical in microbial community assembly processes under  
84 historically disturbed soils than adjacent undisturbed soils, whereas dispersal is more  
85 critical in undisturbed soils than disturbed soils (Osburn et al., 2021). However, these  
86 studies primarily focus on the mechanisms of bacterial/ fungal community assembly,  
87 while the assembly processes of the AMF communities under land-use change still need  
88 to be examined. This could help us overcome the obstacles of artificially manipulating  
89 AMF communities to mitigate human impacts on land-use changes.

90 Ancient wild tea forests (ATF) have existed for thousands of years in southwest  
91 China (Zi et al., 2020). Interestingly, ATF stands under such long-term tea production  
92 use no, or limited, agronomic practices (e.g., fertilization, tillage, and trimming)  
93 compared to conventional tea plantations (CTP; that undergo annual fertilization,  
94 tillage, pesticide application, trimming, etc.). Therefore, AMF communities may play  
95 crucial roles in maintaining tea production in ATF (Singh et al., 2008; Yamato et al.,  
96 2008). Recent research has revealed that AMF effectively promoted tea plant nutrient  
97 acquisition (e.g.,  $\text{NH}_4^+$  and phosphorus) and increased growth (e.g., number of leaves,

98 leaf area, plant height, shoot length, root length, and so on) (Sharm and Kayang, 2017;  
99 Sun et al., 2020). Previous studies have revealed that intensive agronomic management  
100 can result in the loss of AMF diversity (House and Bever, 2018; Xiang et al., 2014; Xu  
101 et al., 2017). However, the AMF community in the ATF is still a black box. Whether  
102 the land-use changes in this area will decrease the AMF diversity and change the AMF  
103 community composition are needed to be verified. Furthermore, the ATF reserve has  
104 a relatively low soil pH (3.9-6.4) (Guo et al., 2010). Tea planting also results in natural  
105 soil acidification, and long-term tea planting leads to extremely low soil pH (mean pH  
106 value = 3.3) (Yang et al., 2018). It is likely that such low pH conditions will influence  
107 the AMF community diversity, composition and assembly need to be debated because  
108 pH is usually considered to be the main driver of microbial community change.

109 In addition to variation in environmental factors caused by land-use change, the  
110 aboveground plant communities also influence on AMF communities (Liu et al., 2012).  
111 Plant community shift towards species that support fewer mycorrhizas may also reduce  
112 the AMF diversity. Recent research has reported that plant root exudates, which vary  
113 among plant species, can significantly influence the AMF growth. Root flavonoids have  
114 been shown to enhance AMF colonization of an invasive tree, while a high level of  
115 phenols and tannins in plant roots can result in a slower AMF colonization rate (Pei et  
116 al., 2020).

117 In this study, we collected 36 soil samples, from three land-use types, from the  
118 ATF reserve to test the following hypotheses: H1, land-use change from ATF to CTP  
119 and Forest will decrease AMF diversity and change the community composition; H2,  
120 different aboveground plant species after land-use change will significantly influence  
121 the AMF community; H3, neutral process dominate the AMF community assembly in  
122 ATF.

## 123 **2. Materials and methods**

### 124 **2.1. Site description**

125 Soil samples were collected from three different land-use types (i.e., ATF, CTP,  
126 and Forest) in the summer of 2019, Lan'cang county, Pu'er city, Yunnan province  
127 (100°10' E, 22°12' N). The detailed location information of each land use type is  
128 displayed in Table S1. The annual mean temperature and precipitation in this county  
129 were 19.2 °C and 1624.0 mm, respectively. Soils in this region are latosolic red soils.  
130 The ATF has existed for thousands of years in this area, and most wild tea trees live for  
131 100-1000 years. The CTP in this region is planted with tea cultivar ("Yunkang 10") and  
132 is under standard agronomic management (i.e., annual trimming twice a year) and  
133 fertilization (600 kg compound fertilizer plus 300 kg urea per hectare)). Forest is  
134 typically mixed forest (i.e., mixed evergreen broad-leaved and deciduous broad-leaved  
135 forest).

### 136 **2.2. Soil sampling, preparation and determination of soil properties**

137 Six soil samples were randomly collected within a 20 m<sup>2</sup> square at each site. Litter  
138 and soil crusts were removed before the surface soil (0-20 cm) was collected. Fresh  
139 soils were then divided into three parts. Subsamples for soil pH, available P, available  
140 K, metal ions (i.e., Al<sup>3+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup>, Fe<sup>2+</sup>, Mn<sup>2+</sup>, Cu<sup>2+</sup>, Zn<sup>2+</sup>), total C, total N, total P,  
141 and total K were air-dried and passed through corresponding sieves of different sizes.  
142 Subsamples for the estimation of soil mineral N (NO<sub>3</sub><sup>-</sup>-N and NH<sub>4</sub><sup>+</sup>-N), dissolved  
143 organic C and N (DOC and DON), microbial biomass C and N (MBC and MBN) were  
144 passed through a 2-mm sieve and stored at 4 °C. Subsamples for DNA extraction were  
145 passed through a 2-mm sieve and stored at -80 °C.

146 Soil pH was measured using a pH meter in a 1:2.5 suspension of soil: KCl solution  
147 (1 M) (Orion 3 Star, Thermo Ltd., USA). Soil mineral N was extracted using 2 M of  
148 KCl and measured using a flow injection analyzer (SAN++, SKALAR Ltd., Breda,  
149 Netherlands). Soil available P was measured according to the Bray1 method. Soil  
150 available K and other metal ions were extracted using the Mehlich 3 method and then  
151 measured using inductively coupled plasma atomic emission spectroscopy (ICP-AES,  
152 Thermo Jarrell Ash Ltd). Soil total C and N were determined using a C/N elemental  
153 analyzer (Vario Max, Elementar, Germany). Soil total P and total K were measured  
154 using a digestion method. Briefly, 0.2 g samples were weighed, and then 5 mL HNO<sub>3</sub>,  
155 1 mL HClO<sub>4</sub>, and 2 mL hydrogen fluoride were sequentially added. After digestion, the  
156 samples were diluted to 50 mL, and ICP-AES determined the total P and K content. For  
157 the soil MBC and MBN, the samples were fumigated with chloroform (with no ethyl  
158 alcohol) in an airtight and dark vessel and parallel samples were set but without  
159 fumigation. All the samples were extracted with 0.05 M K<sub>2</sub>SO<sub>4</sub> and then measured  
160 using a total organic carbon analyzer (Multi N/C 2100/1, Analytic JENA ag., Jena,  
161 Germany). The results of non-fumigated samples were considered as soil DOC and  
162 DON, and results of fumigated minus non-fumigated were considered as soil MBC and  
163 MBN.

### 164 **2.3. DNA extraction, purification, polymerase-chain-reaction (PCR), and high-** 165 **throughput sequencing**

166 DNA was extracted from 0.25 g of fresh soil using a Powersoil DNA Isolation Kit  
167 (MoBio Laboratories, Carlsbad, CA). Negative controls for extraction were included to  
168 ensure the kit reagents were not contaminated. The extracted raw DNA samples were  
169 purified to remove PCR inhibitors with a PowerClean® DNA Clean-Up Kit (MoBio  
170 Laboratories, Carlsbad, CA). The quality of the DNA was checked on the NanoDrop



171 2000C spectrophotometer. DNA concentrations were determined using a Quant-iT  
172 PicoGreen double-stranded DNA (dsDNA) assay kit (Invitrogen, USA).

173 PCR amplification of AM fungi was performed using the primer pairs AMV4.5NF  
174 (AAGCTCGTAGTTGAATTTTCG) and AMDGR (5'-  
175 CCCAACTATCCCTATTAATCAT-3') (Suzuki et al., 2020). The reaction conditions  
176 were as follows: initial denaturation at 95 °C for 5 min; 35 cycles of 95 °C for 45 s;  
177 58 °C for 45 s for annealing, and 72 °C for 1 min; and a final elongation step at 72 °C  
178 for 7 min. The amplicons were purified using the Agencourt AMPure XP kit (Beckman  
179 Coulter, USA). The purified amplicons were sequenced using the Illumina MiSeq  
180 platform at Genesky Biotechnologies Inc. (Shanghai, China). The raw sequence data  
181 are available in the NCBI database with accession PRJNA701385.

## 182 **2.4. Bioinformatics**

183 USEARCH (v 11.0.667) was employed to process the raw high-throughput  
184 sequencing data (Edgar, 2013). In brief, paired raw sequences were merged and re-  
185 oriented by comparing them to those in the SILVA database. Then sequences with  
186 expected errors > 0.5 and lengths < 250 bp were discarded. The UNOISE algorithm  
187 was used to denoise and identify all the biological real sequences and generate the table  
188 for representative sequences and amplicon sequence variants (ASVs). In this step, 4900  
189 representative sequences were obtained. These sequences were then aligned to the  
190 MaarjAM database (<http://maarjam.botany.ut.ee>) (Öpik et al., 2010) with a threshold  
191 of 0.97 and 27 virtual taxa (VTX) were obtained; the sequence/ASV IDs of annotation  
192 results were used to create subset representative sequences only of AMF and ASVs  
193 table. Finally, 189225 sequences, from 116 AMF species, remained for further analysis.

194 Soil AMF phylogenetic diversity was calculated using the R package "picante"

195 (Kembel et al., 2010), and the Chao1 and Shannon indices were calculated using the R  
196 package "vegan". Kruskal-Wallis's rank-sum test was conducted to test the significance  
197 of AMF  $\alpha$ -diversity indices. Principal coordinates analysis (PCoA) was performed  
198 using BrayCurtis distance to evaluate the overall differences in AMF community  
199 structure, where the input matrix was the ASV table with a percentage transformation.  
200 One-way permutational analysis of variance (PERMANOVA) was used to analyze the  
201 effects of land-use types on the community structure of AM fungi by using the function  
202 "adonis" in the R package "vegan". The AMF community composition difference was  
203 tested using the "heat\_tree" function in the R package "Metacoder" (Foster et al., 2017).  
204 Then the composition differences between land-use types were determined using the  
205 Kruskal-Wallis rank-sum test followed by Benjamini-Hochberg (false discovery rate)  
206 correction for multiple comparisons. The indicator species of each land-use type were  
207 calculated using the function "indval" in the R package "labdsv".

## 208 **2.5. Sloan neutral model analysis**

209 The Sloan neutral model was used to infer the contribution of neutral processes  
210 (i.e., dispersal and ecological drift) to AMF community assembly (Burns et al., 2016).  
211 This neutral model predicts the relationship between the occurrence frequency of  
212 operational taxonomic units in the local community and their abundance in the  
213 metacommunity (Burns et al., 2016; Sloan et al., 2006; Wang et al., 2020). Herein,  
214 AMF species from the same land-use type were considered the local community, and  
215 species from all three land-use types were considered metacommunity. The goodness-  
216 of-fit of the model was evaluated through  $R^2$ , and the parameter  $m$  value represented  
217 the migration rate. The occurrence frequency of the AMF species that fell within the  
218 95% confidence intervals of the neutral model's best fit was considered neutrally

219 distributed. Species distributed above the model's 95% confidence intervals (henceforth,  
220 above prediction) are positively selected by the host or have a strong dispersal ability.  
221 Species below the 95% confidence interval (below prediction) were considered selected  
222 against by the host or having limited dispersal ability from the metacommunity.

## 223 **2.6. Statistical analysis**

224 In our study, soil environmental factors were characterized as physicochemical  
225 factors (i.e., mineral N, pH, total C, total N, C: N ratio, DOC, DON, available P, total  
226 P, available K, and total K), ionic factors (i.e.,  $\text{Al}^{3+}$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Fe}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Cu}^{2+}$ , and  
227  $\text{Zn}^{2+}$ ), and biotic factors (i.e., MBC and MBN). One-way ANOVA was employed to  
228 test the significant difference among land-use types at  $P < 0.05$ . Random Forest (RF)  
229 model was carried out to predict the relative importance of soil environmental factors  
230 contributing to the AMF community composition. The RF model was performed using  
231 the R packages "randomForest", "rfPermute", and "rfUtilities". In addition, variance  
232 decomposition was carried out to test the relative contribution of physicochemical,  
233 ionic, and biotic factors to the differentiation in AMF community composition.  
234 Variance decomposition analysis was performed using the function "rda" in the R  
235 package "vegan". Finally, a linear regression model was carried out to explore the  
236 response of AMF species to changes in the environmental factor.

237 The visualizations of analysis results in this study were performed by using R  
238 packages "ggplot2", "vcd", and "VennDiagram" in R, version 3.6.1.

## 239 **3. Results**

240 We recovered 9123778 reads from 36 samples, ranging from 142312 to 414818  
241 reads per sample (minimum length: 25 bp, mean length: 258 bp, maximum length: 286

242 bp). Due to the specificity of primers, sequence reads that do not belong to  
243 Glomeromycota were abandoned. 12.41% (189225 reads) of our sequence reads  
244 remained and successfully mapped against the MaarjAM database with a threshold of  
245 0.97. These remained reads were aligned to 27 AM VTX in seven genera.

### 246 **3.1. Changes in soil environmental factors, AMF diversity, and community** 247 **composition across different land-use types**

248 The soil pH ranged from 3.50-4.21 in the study areas, but there was no significant  
249 change in soil pH across different land-use types ( $P > 0.05$ ) (Table 1). In addition, soil  
250 total N, total C, total K, and DOC significantly decreased in CTP compared to ATF and  
251 Forest ( $P < 0.05$ ). Soil mineral N ( $\text{NH}_4^+\text{-N}$  and  $\text{NO}_3^-\text{-N}$ ), available P, available K, DON,  
252 and total P displayed the highest value in CTP, and these soil environmental factors  
253 were significantly higher than in ATF and Forest ( $P < 0.05$ ) (Table 1). In addition,  
254 concentrations of the majority divalent cations (i.e.,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Fe}^{2+}$ , and  $\text{Mn}^{2+}$ )  
255 were elevated in CTP except for  $\text{Cu}^{2+}$ . ATF had the highest  $\text{Cu}^{2+}$  concentration  
256 compared to Forest and CTP ( $P < 0.05$ ). Soil biotic factors, MBC and MBN, revealed  
257 remarkable differences among land-use types and demonstrated the same variation  
258 tendency, i.e., ATF > Forest > CTP ( $P < 0.05$ ) (Table 1).

259 The AMF  $\alpha$ -diversity indices (including phylogenetic diversity (PD), richness, and  
260 Shannon indices) showed similar variation across land-use types. PD, richness and  
261 Shannon indices in ATF were 1.81-2.10 times, 1.29-1.40 times and 1.31-1.41 times  
262 higher than Forest and CTP (Fig. 1). In addition, all  $\alpha$ -diversity indices were slightly  
263 higher in Forest than in CTP (Fig. 1). The Spearman correlation analysis revealed that  
264 AMF  $\alpha$ -diversity indices had significant correlations with soil environmental factors  
265 (e.g., soil total N,  $\text{NH}_4^+\text{-N}$ , available P and K, DOC, DON, MBC, MBN,  $\text{Ca}^{2+}$ ,  $\text{Cu}^{2+}$ ,  
266 and  $\text{Zn}^{2+}$ ) (Fig. S1).

267 AMF communities are significantly different for each land-use type.  
268 (PERMANOVA test:  $R^2 = 0.36$ ,  $P = 0.001$ ). in the distance-based PCoA, the first two  
269 components accounted for 53.38% of the change in AMF community structure (Fig.  
270 2a). The AMF community composition consisted of seven genera (27 VTXs were  
271 included), including *Glomus*, *Claroideoglomus*, *Acaulospora*, *Archaeospora*,  
272 *Ambispora*, *Paraglomus*, and *Gigaspora*, and the dominant genus was *Glomus* (Fig.  
273 2b). The pairwise comparison of AMF community composition abundance showed a  
274 significant difference among land-use types (Fig. 2b). *Archaeospora* and *Ambispora*  
275 showed the highest relative abundance in ATF. The relative abundance of *Acaulospora*,  
276 *Claroideoglomus*, and *Paraglomus* was the highest in CTP, while *Glomus* and  
277 *Gigaspora* had the highest relative abundance in the Forest. In addition, the VTXs  
278 within different genera also displayed significant differences among land-use types (Fig.  
279 2b). Indicator species of AMF community in each land-use type were relatively  
280 enriched in their corresponding land-use type. The highest number of indicator species  
281 was present in ATF, while the lowest was in CTP (Fig. 2c and 2d). In addition, the  
282 average cumulative relative abundance of indicator species of ATF, CTP, and Forest  
283 accounted for 58.33%, 27.10%, and 38.93% of the total abundance (Fig. 2d).  
284 Furthermore, the number of indicator species (VTX) showed a remarkable decrease  
285 when ATF (12 VTXs) change to Forest (8 VTXs) and CTP (3 VTXs) (Fig. 2d).

286 Indicator species VTX00024 and VTX00026 were dominant in ATF and  
287 accounted for 25.60% and 13.59% of the total abundance, respectively (Fig. 2d). In  
288 CTP, the indicator species were VTX00030, VTX00420, and VTX00348, respectively,  
289 accounting for 18.82%, 6.73%, and 1.55% of the total abundance (Fig. 2d). In Forest,  
290 VTX00328 and VTX00231 were the dominant indicator species and accounted for  
291 19.45% and 7.99%, respectively, of the total abundance (Fig. 2d).

292 **3.2. The fit of the neutral model for AMF community assembly across different**  
293 **land-use types**

294 ATF, CTP and Forest fitted the neutral model well ( $R^2 = 0.30-0.49$ ) (Fig. 3a). ATF  
295 ( $m = 0.00138$ ) had the highest migration rate, which was significantly higher than those  
296 of CTP ( $m = 0.00039$ ) and Forest ( $m = 0.00042$ ) (Fig. 3a). The cumulative relative  
297 abundance of the neutrally distributed AMF species was 71.07%, 87.73%, and 86.26%  
298 in ATF, CTP, and Forest, respectively (Fig. 3b). The sum of the relative abundance of  
299 above prediction species in ATF (26.73%) was higher than that in CTP (10.50%) and  
300 Forest (8.30%). In comparison, the sum of the relative abundance of the below  
301 prediction was in the order of Forest (5.44%) > ATF (2.19%) > CTP (1.73%) (Fig. 3b).

302 In addition, the composition of neutrally distributed, above prediction, and below  
303 prediction species were distinctly different. The above prediction species in ATF (18  
304 species), CTP (6 species), and Forest (9 species) consisted of 7, 4, and 6 different VTXs  
305 respectively (Fig. 3b). The below prediction species displayed a relatively low species  
306 diversity; only six VTXs were identified, with 3, 2, and 1 VTXs from ATF, Forest and  
307 CTP, respectively. The composition of neutrally distributed species was distinctly  
308 different across three land-use types. VTX00024 was a dominant species of neutrally  
309 distributed species and accounted for 29.32%, 44.97%, and 46.54% of the relative  
310 abundance of the neutrally distributed species in the ATF, CTP, and Forest. Our results  
311 also revealed that ATF had a more diverse VTX pattern ( $n=12$ ; relative abundance >  
312 1%) than that of CTP and Forest ( $n=7$ ) (Fig. S2). Overall, *Acaulospora* sp. was the  
313 dominant genus of neutrally distributed species in ATF, CTP, and Forest and accounted  
314 for 58.08%, 76.04%, and 77.29% of the relative abundance, respectively (Fig. 3c).

### 315 3.3. Relationships between environmental factors and arbuscular mycorrhizal 316 fungal community composition

317 The RF model was performed to identify the most important environmental factors  
318 accounting for AMF community differentiation across ATF, CTP, and Forest. The RF  
319 model (number of trees = 500) explained 79.41%% of the AMF community  
320 differentiation ( $R^2 = 0.79$ ,  $P < 0.001$ ) and revealed that the soil  $\text{Cu}^{2+}$  ( $P < 0.05$ ),  $\text{NO}_3^-$ -  
321 N ( $P < 0.05$ ),  $\text{Mg}^{2+}$  ( $P < 0.05$ ) and TK ( $P < 0.05$ ) significantly impacted AMF  
322 community differentiation (Fig. 4). According to the variance decomposition, soil  
323 physicochemical, ionic, and biotic properties explained 16.56% ( $P < 0.01$ ), 22.06% ( $P$   
324  $< 0.001$ ), and 2.88% ( $P > 0.05$ ) of the AMF community change, respectively, and all  
325 the environmental factors could cumulatively explain 87.65% of the AMF community  
326 change (Table 2).

327 According to the RF model, soil  $\text{Cu}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{NO}_3^-$ -N, and total K significantly  
328 impacted the AMF community composition. A linear regression model was used to  
329 explore the relationship between AMF species and these four environmental factors.  
330 ASV57 and ASV102 exhibited a significantly negative relationship with soil  $\text{Cu}^{2+}$  (Fig.  
331 5a), while they displayed the reverse (positive) result for the soil  $\text{NO}_3^-$ -N (Fig. 5c). Both  
332 ASV57 and ASV102 belonged to VTX00024 (identified as *Acaulospora* sp.). On the  
333 other hand, soil  $\text{Mg}^{2+}$  showed a distinctly positive relationship with ASV38 and ASV86,  
334 and these two species belonged to VTX00030 (also identified as *Acaulospora* sp.) (Fig.  
335 5b). However, the linear regression result showed that soil total K did not correlate  
336 significantly with the AMF species.

## 337 **4. Discussion**

### 338 **4.1. Land-use change from natural ecosystem to cultivation decreases AMF** 339 **community diversity and changes the community composition**

340 Studies have reported a negative correlation between land-use intensity and AMF  
341 biodiversity (Oehl et al., 2010; Schnoor et al., 2011). This was also verified in our study,  
342 i.e., the AMF  $\alpha$ -diversity significantly decreased in Forest and CTP because these two  
343 land-use types are subject to more anthropogenetic influence than ATF (Fig. 1).  
344 Interestingly, the AMF  $\alpha$ -diversity indices in CTP (Chao1 index: mean = 28.25;  
345 Shannon index: mean = 2.99) and the forest (Chao1 index: mean = 32.75; Shannon  
346 index: mean = 2.28) were similar to those reported in previous studies in farmlands,  
347 grasslands, and forests (Chao1 index: 17.71-32.00; Shannon index: 1.58-2.21), while  
348 ATF showed significantly higher AMF  $\alpha$ -diversity indices (Chao1 index: mean = 59.33;  
349 Shannon index: mean = 2.99) (Verbruggen et al., 2012; Xiang et al., 2014; Xu et al.,  
350 2017). This phenomenon could be attributed to the passive development of the AMF  
351 species over a long timescale, whereas human disturbance could limit this development  
352 (García de León et al., 2016). Thus, the AMF species in ATF had more time to develop  
353 because of long-standing and relatively low disturbance compared to CTP and the forest  
354 in our study (Zi et al., 2020). Moreover, higher soil available nutrients in CTP and the  
355 forest might also be the reason for the decrease in AMF  $\alpha$ -diversity because soil  
356 available nutrients (e.g., available P and  $\text{NH}_4^+$ -N) negatively affect AMF diversity  
357 (Xiang et al., 2014; Zhu et al., 2018).

358 AMF community structure and composition significantly varied with the changes  
359 in land use (Fig. 2a). Several earlier published studies have proved that soil pH is a  
360 major driver of the changes in the AMF community (Dumbrell et al., 2010; Monkai et



361 al., 2018). However, our results showed that pH was not a significant factor accounting  
362 for the AMF community composition changes (Fig. 4). Instead, we suggest that the  
363 long-term low soil pH condition results in a strong selection effect, and only AMF  
364 adapted to the low pH can survive. Therefore, soil pH is not the main factor affecting  
365 the AMF communities in the three land-use types. The AMF communities in our study  
366 were sensitive to other soil environmental factors rather than directly to soil pH. A  
367 recent study also found that soil nutrient availability had a more significant effect on  
368 AMF abundance and diversity than pH (Xiao et al., 2020).

369 Nevertheless, the RF model showed that the AMF community change was related  
370 to soil physicochemical, ionic, and biotic properties (Fig. 4), while  $\text{Cu}^{2+}$ ,  $\text{NO}_3^-$ -N,  $\text{Mg}^{2+}$ ,  
371 and total K significantly impact the community change (Fig. 4). The concentration of  
372  $\text{Mg}^{2+}$  and  $\text{Cu}^{2+}$  has been reported to alter the fungal community. In particular,  $\text{Mg}^{2+}$  can  
373 promote the colonization of AMF (Gryndler et al., 1992; Sutcliffe et al., 2018), which  
374 was consistent with results observed in our study and verified by the linear regression  
375 model (Fig. 5). Soil N and P availability were reported as crucial factors for the AMF  
376 community change (Liu et al., 2012). In contrast, soil P content non-significantly  
377 influenced the AMF community composition in the present study. This result was in  
378 line with the research showing that P did not affect AMF community composition under  
379 55-year long-term fertilization (Williams et al., 2017).

380 The linear regression model showed that ASV57 and ASV102 had a significantly  
381 positive relationship with soil  $\text{NO}_3^-$ -N and an antagonistic relationship with  $\text{Cu}^{2+}$ . These  
382 ASVs were identified as VTX00024. VTX00024 is usually present in the roots of  
383 woody plants and has tolerance to various environments, including semi-arid and  
384 saline-alkaline conditions (Kaidzu et al., 2020). VTX00024 is also found in upland rice  
385 and is supposed to adapt to low nutrient conditions (Ibne Baki et al., 2021). However,

386 in previous studies (Verbruggen et al., 2012; Xiang et al., 2014; Xu et al., 2017), the  
387 soil pH ranged from 4.58 to 8.40, higher than that in our sites. Slightly acidic conditions  
388 benefit AMF colonization (Ibne Baki et al., 2021). Therefore, we suggest that  
389 VTX00024 might have a tolerance to acidic stress. ASV38 and ASV86 were identified  
390 as VTX00030 (*Acaulospora* spp) and showed significant positive relationships with  
391 soil Mg<sup>2+</sup> content. Previous studies reported that *Acaulospora* spp. is more resistant to  
392 biotic and abiotic stresses (Hart and Reader, 2002; Maherali and Klironomos, 2007).  
393 Our results indicated that soil Mg<sup>2+</sup> might promote the growth of *Acaulospora* spp.,  
394 such as VTX00030.

#### 395 **4.2. Host plant communities influence AMF community composition**

396 Plant communities can significantly affect AMF diversity and community  
397 composition (Faggioli et al., 2019; Xiang et al., 2014; Xu et al., 2017). A review of 111  
398 published studies summarized that plant species distribution under global change can  
399 alter the AMF community composition (Kivlin et al., 2011).

400 In the present study, *Acaulospora* and *Glomus* were the dominant genera and were  
401 relatively enriched in Forest compared to the ATF and CTP. The growth of  
402 *Acaulospora* and *Glomus* showed a strong dependency on host plant roots, indicating  
403 less effective colonization of the AMF in the tea plants than in the forest (Fig. 2b and  
404 Fig. S2). This may also be due to the high polyphenol content in the roots of tea plants,  
405 as a high level of phenols and tannins in plant roots can result in a slower AMF  
406 colonization rate (Pei et al., 2020). However, analysis of indicator species showed a  
407 more diverse pattern of VTXs in ATF than in Forest and CTP, and most of these VTXs  
408 belonged to *Acaulospora* (Fig. 2). *Acaulospora* seem to follow an intermediate trend,  
409 colonizing from propagule fractions, colonized roots (Klironomos and Hart, 2002), and

410 spores (Brundrett et al., 1999). We suggested indicator species under the ATF  
411 vegetation type, i.e., VTX00024, VTX00026, VTX00028, VTX00030, VTX00328, and  
412 VTX00378, were more likely present as spores because of the high level of phenols and  
413 tannins (Pei et al., 2020). The indicator species VTX00193 belonging to  
414 *Claroideoglossum* was only detected in the ATF, whereas the indicator species  
415 VTX00231 belonging to *Archaeospora* existed only in the Forest. *Claroideoglossum*  
416 were more abundant in the spores and extraradical mycelium (ERM) fractions than in  
417 roots. *Archaeospora* were detected mainly in roots and spores and were almost absent  
418 from the ERM (Varela-Cervero et al., 2015). Therefore, compared to CTP and Forest,  
419 ATF could be predicted to have more ERM. However, this study did not identify if  
420 *Claroideoglossum* can form ERM or spores. In addition, CTP had the least number of  
421 VTXs. However, the host species tested by Varela-Cervero et al. (2015) were from a  
422 Mediterranean biome, very different to the tea habitats studied here. We suggest that  
423 annual fertilization and tillage are more likely factors and may reduce AMF diversity  
424 (Sommermann et al., 2018).

425 In addition to the host plant species, sampling time can also influence the AMF  
426 diversity because some VTX may exist in soils as active or dormant spores with  
427 seasonal variation (Dumbrell et al., 2011). On this basis, the sampling frequency of a  
428 year could affect the accuracy of determination of the AMF community diversity and  
429 composition (Hiiesalu et al., 2014). Thus, sampling only once in summer may have led  
430 to an underestimation of the AMF diversity in the region.

### 431 **4.3. Neutral process dominates AMF community assembly**

432 The neutral model effectively evaluates the AMF community assembly on a field  
433 scale (Davison et al., 2015). The three land-use types in this study fitted the neutral

434 model well (Fig. 3a). Previous studies have revealed that the regional AMF species pool,  
435 dispersal and chance, and environmental and host filters can significantly affect the  
436 assembly of the AMF community (Davison et al., 2015; Verbruggen et al., 2012). In  
437 the region studied here, AMF species in ATF showed the best model fit and migration  
438 rate compared to CTP and Forest (Fig. 3a), which indicated that dispersal processes  
439 dominated the assembly of the AMF community of ATF but were limited in CTP and  
440 Forest vegetation types. We propose that fewer disturbances in ATF, e.g., no  
441 fertilization and tillage and only natural litterfall, lead to this outcome because  
442 environmental heterogeneity or anthropogenic disturbance can mask neutral processes,  
443 especially for dispersal (Caruso et al., 2012). In addition, annual fertilization, tillage,  
444 and trimming in the CTP may cause the lowest model fit. Annual agronomic  
445 management showed a strong environmental filter, thus habitat filtering, or dispersal  
446 limitation may be the primary driver of AMF community assembly (Kivlin et al., 2011).

447 The above prediction AMF species in ATF were more diverse than in CTP and the  
448 forest (Fig. 3b). We suggest that a long period of no disturbance in the ATF has led to  
449 a continuous succession of the AMF community, which suggests high dispersal ability  
450 of more AMF species in the ATF land-use type (Wang et al., 2020). Moreover,  
451 VTX00024 (*Acaulospora*, 15.67%) and VTX00245 (*Archaeospora*, 5.73%),  
452 VTX00370 (*Glomus*, 8.71%) and VTX00348 (*Paraglomus*, 1.2%), and VTX00370  
453 (*Glomus*, 5.18%) and VTX00328 (*Acaulospora*, 3.92%), respectively, were dominant  
454 among the above predicted AMF species in ATF, CTP, and Forest (Fig 3b and 3c). The  
455 discrepancies in relative abundance also suggest differences in dispersal ability among  
456 land-use types. Since *Acaulospora*, *Archaeospora*, and *Glomus* are present in soils as  
457 spores (Varela-Cervero et al., 2015), and tend to have traits that favour dispersal  
458 (Chaudhary et al., 2020), they are more likely to disperse. This is consistent with our

459 suggestion that AMF species in ATF show higher dispersal ability than the other two  
460 land-use types, thus maintaining the higher AMF diversity in ATF. Furthermore, the  
461 differences in the below prediction AMF species among three land-use types also  
462 suggests the difference in dispersal limitation. The relative importance of dispersal to  
463 environmental filtering is scale-dependent and varies, and soil physicochemical  
464 properties (e.g., soil pH, C: N ratio, and soil temperature) can influence this relative  
465 importance (Dumbrell et al., 2010; Kivlin et al., 2011). The results were further verified  
466 by the differences in soil environmental factors (Table 1). For example, soil available  
467 nutrients (i.e., mineral N, available K, and available P) in CTP were significantly higher  
468 than in ATF and Forest. At the same time, total C and total N contents showed contrary  
469 results. In addition, ATF showed the highest MBC and MBN, followed by the Forest  
470 and CTP. Thus, ATF vegetation type placed only a minor limitation on dispersal  
471 compared to CTP.

472 Overall, our results show that neutral processes dominate AMF community  
473 assembly. However, dispersal and neutral processes in CTP and the Forest were partly  
474 masked by the heterogeneity of the environment due to the anthropogenic disturbance.  
475 The effects of the host plants on AMF community assembly were not considered in the  
476 present study. Host plants play a primary role in AMF community assembly because  
477 different plant species or the same plant species at different growth stages can form  
478 various AMF symbionts despite growing in the same soils (Gosling et al., 2013;  
479 Sýkorová et al., 2007). Thus, we suggest that the effect of host plants should be  
480 considered when assessing the soil AMF community assembly in future studies.

## 481 **5. Conclusion**

482 In summary, land-use changes significantly altered the AMF diversity and

483 community composition. Our study revealed that the environmental factors, such as soil  
484  $\text{NO}_3^-$ -N, TK,  $\text{Mg}^{2+}$ , and  $\text{Cu}^{2+}$ , were relatively crucial for the dynamics of the AMF  
485 community in the studied region. The soil pH was not considered the primary driver of  
486 the AMF community change in extremely low soil pH conditions because strong  
487 ecological selection on the AMF community renders AMF species adapt to live in  
488 strongly acidic soils. Cumulatively, the assembly of the AMF community was found to  
489 prefer a neutral process in the ATF reserve area. We suggest that mitigation of soil  
490 acidification might be a potential means to improve the AMF community diversity,  
491 which is beneficial for conserving and restoring ecosystems in southwest China.  
492

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## 498 **Data availability**

499 Raw sequence data were available in the NCBI database with accession  
500 PRJNA701385 (<https://www.ncbi.nlm.nih.gov/sra/PRJNA701385>). The data that  
501 support the findings of this study are available from the corresponding author upon  
502 reasonable request.

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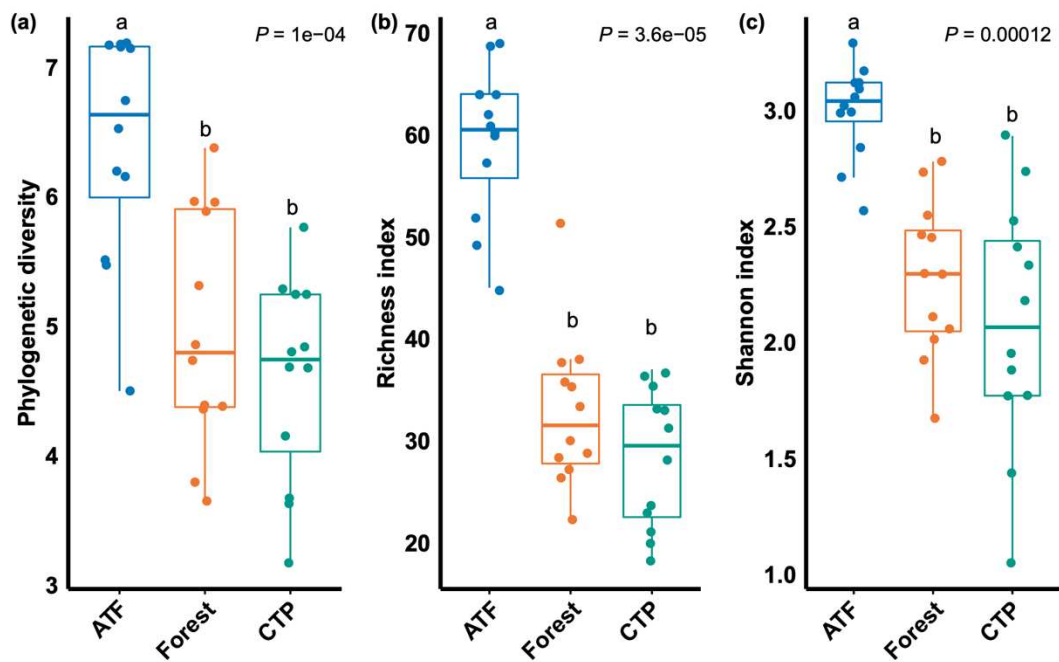
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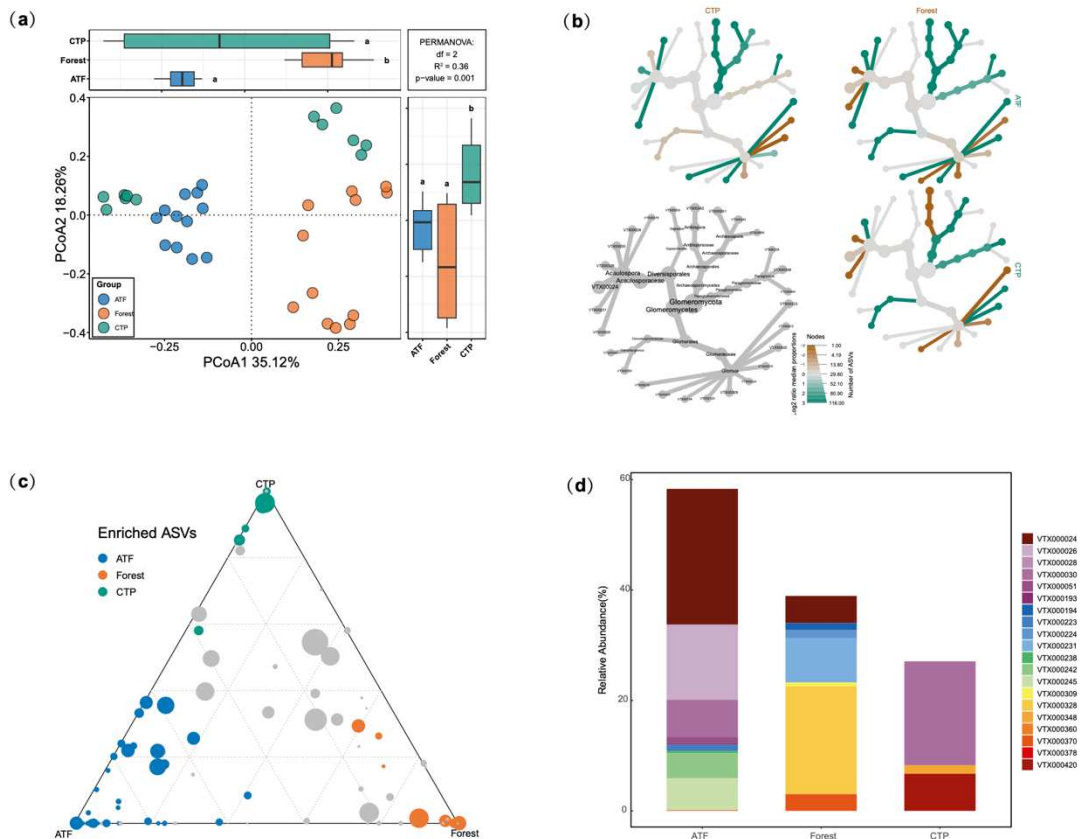
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725 **Figure legends**

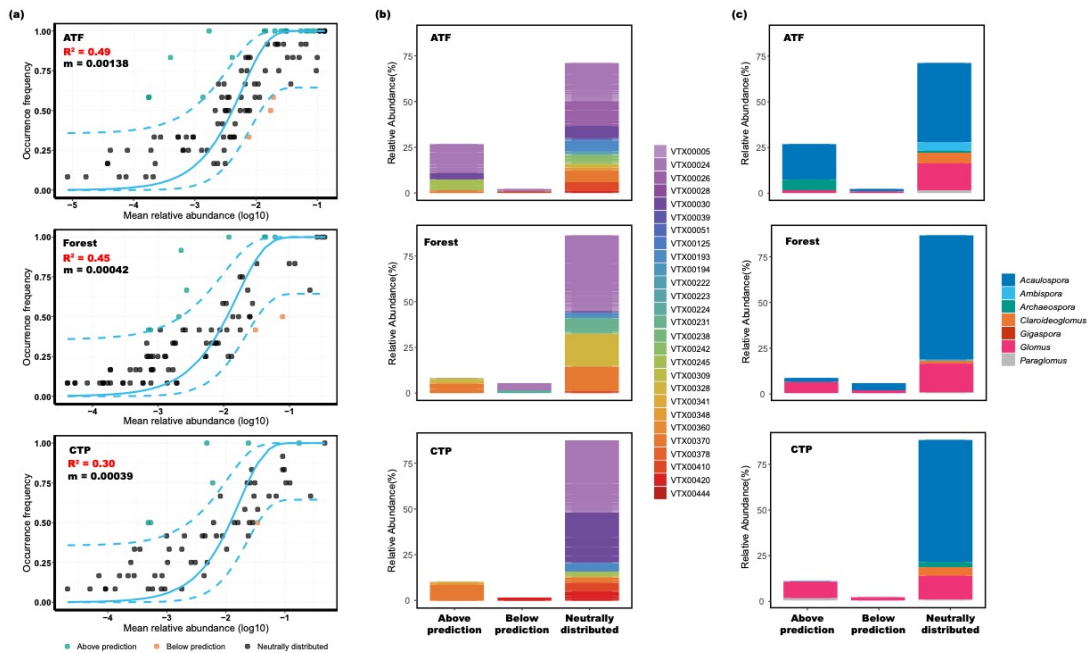


726 **Fig. 1** AMF community  $\alpha$ -diversity. (a) Phylogenetic diversity, (b) Richness index, (c)  
727 Shannon index. The different letters above every figure mean significant differences  
728 between land-use types tested by the Kruskal-Wallis method at  $P = 0.05$ . The dotted  
729 line represents the mean value of all samples.

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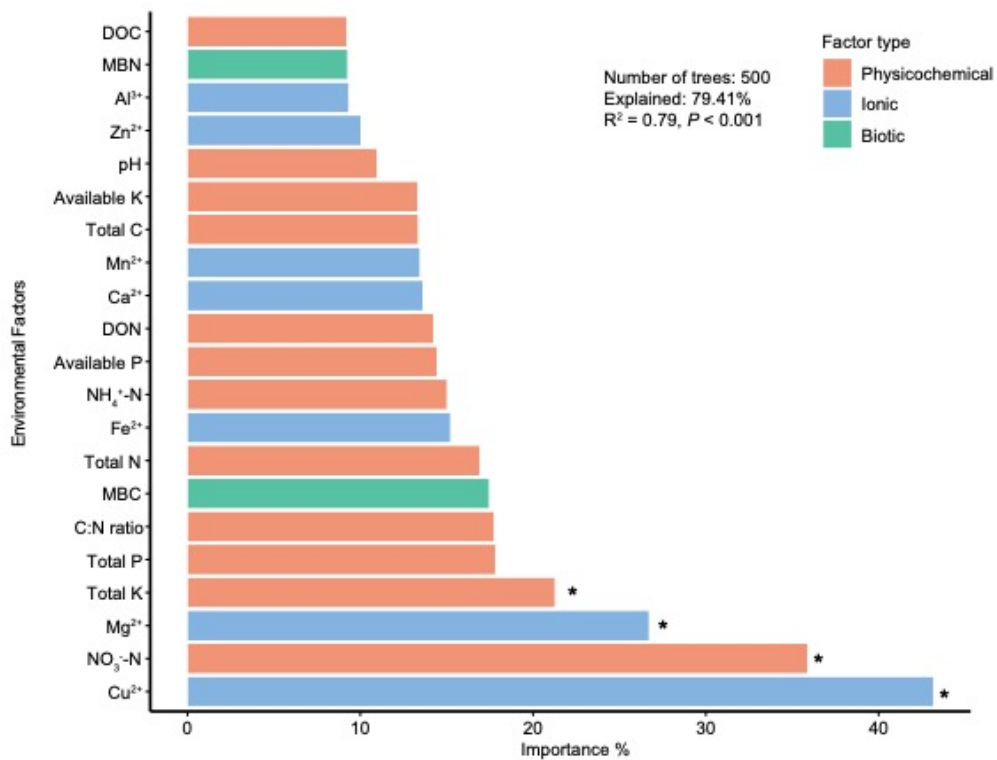


731 **Fig. 2** AMF community  $\beta$ -diversity and composition difference. (a), Principal  
 732 Coordinates Analysis (PCoA) of AM fungal community based on species matrices from  
 733 three land-use types. One-way PERMANOVA was used to analyze the effects of land-  
 734 use type on the community structure of AMF. (b), Heat tree of AMF community  
 735 composition. The gray tree on the lower left functions as a key for the smaller unlabeled  
 736 trees. The color of each taxon represents the log-2 ratio of median proportions of reads  
 737 observed at each land-use type. Only significant differences are colored, determined  
 738 using a Wilcox rank-sum test followed by a Benjamini-Hochberg (FDR) correction for  
 739 multiple comparisons. Taxa colored green are enriched in the part of the land-use shown  
 740 in the row, and those colored brown are enriched in the part of the land-use shown in  
 741 the column. (c), Ternary diagram of AMF community. The enriched ASVs were the  
 742 indicators species in each vegetation and colored with its corresponding color. (d),  
 743 Relative abundance of indicator species under each land-use type.  
 744



746 **Fig. 3** Fit of the neutral models for different vegetation AMF communities. (a), The  
 747 ASVs that occurred more frequently than predicted by the model are shown in green,  
 748 while those occurred less frequently than predicted are shown in orange. Blue dashed  
 749 lines represent 95% confidence intervals around the model prediction and the ASVs fall  
 750 within the confidence intervals considered neutrally distributed.  $R^2$  values present the  
 751 goodness of fit of the neutral model, ranging from 0 (no fit) to 1 (perfect fit),  $m$  value  
 752 means the migration rate. (b), VTX shows the taxonomic distribution of three categories  
 753 of ASVs (above prediction, below prediction, neutrally distributed) in different AMF  
 754 communities. (c), Taxonomic distribution of three categories of ASVs (above  
 755 prediction, below prediction, neutrally distributed) in different AMF communities are  
 756 shown by genus.

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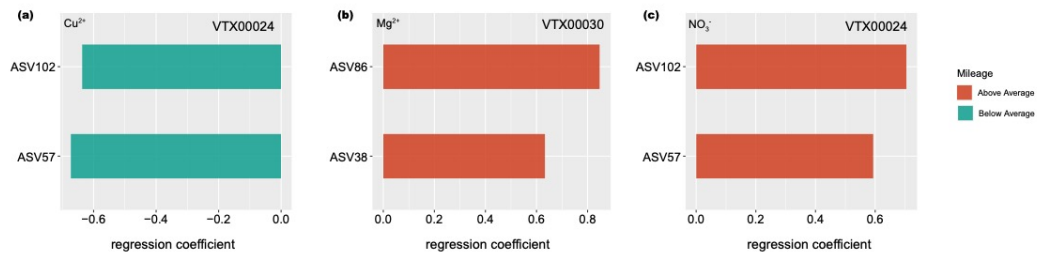


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759 **Fig. 4** Environmental factors contribute to the AMF community differentiation. (a), The  
 760 relative importance of different environmental factors contribute to the differentiation  
 761 of AMF communities under different vegetation based on Random Forest (RF) analysis.  
 762 \* means the level of significance. Orange color means physicochemical factors,  
 763 including mineral N, pH, Total C, Total N, C: N ratio, DOC, DON, Available P, Total  
 764 P, Available K, Total K. Blue color represents ionic factors, including the Al<sup>3+</sup>, Ca<sup>2+</sup>,  
 765 Mg<sup>2+</sup>, Fe<sup>2+</sup>, Mn<sup>2+</sup>, Cu<sup>2+</sup>, Zn<sup>2+</sup>. Green color means biotic factors, including MBC and  
 766 MBN.

767





768

769 **Fig. 5** Results of regression analysis between environmental factors and AMF species,  
 770 environmental factors were Cu<sup>2+</sup>, Mg<sup>2+</sup> and NO<sub>3</sub><sup>-</sup> for (a), (b) and (c) respectively. In the  
 771 RF analysis, environmental factors have significant effects on AMF community  
 772 composition were selected for the regression analysis. Above and below average  
 773 represent the positive and negative correlation coefficient, respectively. Only  
 774 significant results were shown in the diagram.

775

776 **Supplementary figure legends**

777 **Fig. S1** Spearman correlation analysis between AMF  $\alpha$ -diversity indices and  
778 environmental factors. “\*” represents significantly correlated, “\*\*” and “\*\*\*” means  $P <$   
779  $0.05$  and  $P < 0.0,1$  respectively.

780 **Fig. S2** Relative Abundance of Neutrally distributed AMF species colored by VTX.

781 **Table S1** Detailed location information of sample sites

782

783