UNIVERSITY of York

This is a repository copy of Land-use changes alter the arbuscular mycorrhizal fungal community composition and assembly in the ancient tea forest reserve.

White Rose Research Online URL for this paper: <u>https://eprints.whiterose.ac.uk/192546/</u>

Version: Accepted Version

Article:

Ji, Lingfei, Yang, Xiangde, Zhu, Chen et al. (8 more authors) (2022) Land-use changes alter the arbuscular mycorrhizal fungal community composition and assembly in the ancient tea forest reserve. Agriculture, Ecosystems & Environment. 108142. ISSN 0167-8809

https://doi.org/10.1016/j.agee.2022.108142

Reuse

This article is distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs (CC BY-NC-ND) licence. This licence only allows you to download this work and share it with others as long as you credit the authors, but you can't change the article in any way or use it commercially. More information and the full terms of the licence here: https://creativecommons.org/licenses/

Takedown

If you consider content in White Rose Research Online to be in breach of UK law, please notify us by emailing eprints@whiterose.ac.uk including the URL of the record and the reason for the withdrawal request.



eprints@whiterose.ac.uk https://eprints.whiterose.ac.uk/

1 **Title:**

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

4 Authors and affiliations:

- 5 Lingfei Ji^{1, 2}, Xiangde Yang¹, Chen Zhu², Lifeng Ma¹, Yupei Chen³, Ning Ling²,
 6 Zhongfan Zhou⁴, Kang Ni¹, Shiwei Guo², Thorunn Helgason⁵, Jianyun Ruan^{1, 6}
- 7

8 1. Tea Research Institute, Chinese Academy of Agriculture Sciences, Key Laboratory

9 of Tea Biology and Resource Utilization of Tea, the Ministry of Agriculture, Hangzhou
310008, China:

10 310008, China;

11 2. Jiangsu Provincial Key Lab of Solid Organic Waste Utilization, Jiangsu

12 Collaborative Innovation Center of Solid Organic Wastes, Educational Ministry

13 Engineering Center of Resource-saving fertilizers, Nanjing Agricultural University,

14 Nanjing 210095, Jiangsu, P.R. China;

15 3. Zhejiang Cultivated Land Quality and Fertilizer Management Station, Hangzhou

16 310020, China

17 4. The Communist Youth League of Lan'cang County, Lan'cang, 665600, China;

18 5. Department of Biology, University of York, York, YO10 5DD, UK;

19 6. Xihu National Agricultural Experimental Station for Soil Quality, Hangzhou 310008,

20 China.

21 Corresponding authors:

- 22 Shiwei Guo (sguo@niau.edu.cn); Tel: +86- 25-84396393, Fax: +86- 25-84396393
- 23 Jianyun Ruan (jruan@mail.tricaas.com); Tel: +86-571-86653938, Fax: +86-571-
- 24 86653938

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 changes influence the AMF community in acidic soils, the AMF community 31 32 composition and assembly processes in the ATF region (soil pH: 3.5-4.2) were 33 investigated. Our results showed that AMF α -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 decrease when ATF (12 VTXs) changed to Forest (8 VTXs) and CTP (3 VTXs). In 36 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, Mg^{2+} , and Cu^{2+} were important factors accounting for the AMF community change. In 41 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; Xiang et al., 2014). Additionally, native ecosystems (i.e., grassland, forest) converted 65 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 historically disturbed soils than adjacent undisturbed soils, whereas dispersal is more 84 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 acquisition (e.g., NH4⁺ and phosphorus) and increased growth (e.g., number of leaves, 97

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).

In this study, we collected 36 soil samples, from three land-use types, from the ATF reserve to test the following hypotheses: H1, land-use change from ATF to CTP and Forest will decrease AMF diversity and change the community composition; H2, different aboveground plant species after land-use change will significantly influence the AMF community; H3, neutral process dominate the AMF community assembly in 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 (100°10' E, 22°12' N). The detailed location information of each land use type is 127 128 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**

Six soil samples were randomly collected within a 20 m² square at each site. Litter 137 138 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 K, metal ions (i.e., Al³⁺, Ca²⁺, Mg²⁺, Fe²⁺, Mn²⁺, Cu²⁺, Zn²⁺), total C, total N, total P, 140 141 and total K were air-dried and passed through corresponding sieves of different sizes. 142 Subsamples for the estimation of soil mineral N (NO₃⁻-N and NH₄⁺-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 KCl and measured using a flow injection analyzer (SAN++, SKALAR Ltd., Breda, 148 149 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 HNO₃, 155 1 mL HClO₄, 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 K₂SO₄ 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

DNA was extracted from 0.25 g of fresh soil using a Powersoil DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA). Negative controls for extraction were included to ensure the kit reagents were not contaminated. The extracted raw DNA samples were purified to remove PCR inhibitors with a PowerClean® DNA Clean-Up Kit (MoBio 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 (AAGCTCGTAGTTGAATTTCG) AMDGR (5'and 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; 58 °C for 45 s for annealing, and 72 °C for 1 min; and a final elongation step at 72 °C 177 178 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 platform at Genesky Biotechnologies Inc. (Shanghai, China). The raw sequence data 180 181 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 expected errors > 0.5 and lengths < 250 bp were discarded. The UNOISE algorithm 186 187 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 (<u>http://maarjam.botany.ut.ee</u>) (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 a-diversity indices. Principal coordinates analysis (PCoA) was performed using BrayCurtis distance to evaluate the overall differences in AMF community 198 199 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 "adnois" 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 goodnessof-fit of the model was evaluated through R^2 , and the parameter m value represented 216 the migration rate. The occurrence frequency of the AMF species that fell within the 217 218 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,

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

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 P. available K, and total K), ionic factors (i.e., Al³⁺, Ca²⁺, Mg²⁺, Fe²⁺, Mn²⁺, Cu²⁺, and 226 227 Zn^{2+}), and biotic factors (i.e., MBC and MBN). One-way ANOVA was employed to test the significant difference among land-use types at P < 0.05. Random Forest (RF) 228 229 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.

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

239 **3. Results**

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

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

3.1. Changes in soil environmental factors, AMF diversity, and community 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 Forest (P < 0.05). Soil mineral N (NH₄⁺-N and NO₃⁻-N), available P, available K, DON, 251 252 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, concentrations of the majority divalent cations (i.e., Ca²⁺, Mg²⁺, Zn²⁺, Fe²⁺, and Mn²⁺) 254 were elevated in CTP except for Cu²⁺. ATF had the highest Cu²⁺ concentration 255 256 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 α -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 1.31-141 times 262 higher than Forest and CTP (Fig. 1). In addition, all α -diversity indices were slightly 263 higher in Forest than in CTP (Fig. 1). The Spearman correlation analysis revealed that 264 AMF α-diversity indices had significant correlations with soil environmental factors (e.g., soil total N, NH4⁺-N, available P and K, DOC, DON, MBC, MBN, Ca²⁺, Cu²⁺, 265 and Zn^{2+}) (Fig. S1). 266

267 AMF communities are significantly different for each land-use type. (PERMANOVA test: $R^2 = 0.36$, P = 0.001). in the distance-based PCoA, the first two 268 269 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). Indicator species VTX00024 and VTX00026 were dominant in ATF and 286 accounted for 25.60% and 13.59% of the total abundance, respectively (Fig. 2d). In 287

accounting for 18.82%, 6.73%, and 1.55% of the total abundance (Fig. 2d). In Forest,

CTP, the indicator species were VTX00030, VTX00420, and VTX00348, respectively,

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).

3.2. The fit of the neutral model for AMF community assembly across different 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 accounting for AMF community differentiation across ATF, CTP, and Forest. The RF 318 319 model (number of trees = 500) explained 79.41% of the AMF community differentiation ($R^2 = 0.79$, P < 0.001) and revealed that the soil Cu^{2+} (P < 0.05), NO_3^{-} -320 N (P < 0.05), Mg²⁺ (P < 0.05) and TK (P < 0.05) significantly impacted AMF 321 322 community differentiation (Fig. 4). According to the variance decomposition, soil physicochemical, ionic, and biotic properties explained 16.56% (P < 0.01), 22.06% (P323 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).

According to the RF model, soil Cu²⁺, Mg²⁺, NO₃⁻-N, and total K significantly 327 impacted the AMF community composition. A linear regression model was used to 328 329 explore the relationship between AMF species and these four environmental factors. ASV57 and ASV102 exhibited a significantly negative relationship with soil Cu^{2+} (Fig. 330 5a), while they displayed the reverse (positive) result for the soil NO₃⁻-N (Fig. 5c). Both 331 332 ASV57 and ASV102 belonged to VTX00024 (identified as Acaulospora sp.). On the other hand, soil Mg²⁺ showed a distinctly positive relationship with ASV38 and ASV86, 333 and these two species belonged to VTX00030 (also identified as Acaulospora sp.) (Fig. 334 335 5b). However, the linear regression result showed that soil total K did not correlate significantly with the AMF species. 336

337 4. Discussion

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

340 Studies have reported a negative correlation between land-use intensity and AMF biodiversity (Oehl et al., 2010; Schnoor et al., 2011). This was also verified in our study, 341 342 i.e., the AMF α -diversity significantly decreased in Forest and CTP because these two 343 land-use types are subject to more anthropogenetic influence than ATF (Fig. 1). Interestingly, the AMF α -diversity indices in CTP (Chao1 index: mean = 28.25; 344 345 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, grasslands, and forests (Chao1 index: 17.71-32.00; Shannon index: 1.58-2.21), while 347 348 ATF showed significantly higher AMF α -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 α -diversity because soil 356 available nutrients (e.g., available P and NH₄⁺-N) negatively affect AMF diversity 357 (Xiang et al., 2014; Zhu et al., 2018).

AMF community structure and composition significantly varied with the changes in land use (Fig. 2a). Several earlier published studies have proved that soil pH is a 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 long-term low soil pH condition results in a strong selection effect, and only AMF 363 364 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 to soil physicochemical, ionic, and biotic properties (Fig. 4), while Cu²⁺, NO₃⁻-N, Mg²⁺, 370 371 and total K significantly impact the community change (Fig. 4). The concentration of Mg^{2+} and Cu^{2+} has been reported to alter the fungal community. In particular, Mg^{2+} can 372 373 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 community change (Liu et al., 2012). In contrast, soil P content non-significantly 376 377 influenced the AMF community composition in the present study. This result was in line with the research showing that P did not affect AMF community composition under 378 379 55-year long-term fertilization (Williams et al., 2017).

The linear regression model showed that ASV57 and ASV102 had a significantly positive relationship with soil NO₃⁻-N and an antagonistic relationship with Cu²⁺. These ASVs were identified as VTX00024. VTX00024 is usually present in the roots of woody plants and has tolerance to various environments, including semi-arid and saline-alkaline conditions (Kaidzu et al., 2020). VTX00024 is also found in upland rice 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 benefit AMF colonization (Ibne Baki et al., 2021). Therefore, we suggest that 388 389 VTX00024 might have a tolerance to acidic stress. ASV38 and ASV86 were identified 390 as VTX00030 (Acaulospora spp) and showed significant positive relationships with soil Mg^{2+} content. Previous studies reported that *Acaulospora* spp. is more resistant to 391 biotic and abiotic stresses (Hart and Reader, 2002; Maherali and Klironomos, 2007). 392 Our results indicated that soil Mg²⁺ might promote the growth of Acaulospora spp., 393 394 such as VTX00030.

395 4.2. Host plant communities influence AMF community composition

Plant communities can significantly affect AMF diversity and community
composition (Faggioli et al., 2019; Xiang et al., 2014; Xu et al., 2017). A review of 111
published studies summarized that plant species distribution under global change can
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 Claroideoglomus was only detected in the ATF, whereas the indicator species 414 415 VTX00231 belonging to Archaeospora existed only in the Forest. Claroideoglomus 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 Claroideoglomus 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).

In addition to the host plant species, sampling time can also influence the AMF diversity because some VTX may exist in soils as active or dormant spores with seasonal variation (Dumbrell et al., 2011). On this basis, the sampling frequency of a year could affect the accuracy of determination of the AMF community diversity and composition (Hiiesalu et al., 2014). Thus, sampling only once in summer may have led 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 field433 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 suggests the difference in dispersal limitation. The relative importance of dispersal to 462 463 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 20

483 community composition. Our study revealed that the environmental factors, such as soil NO₃⁻-N, TK, Mg²⁺, and Cu²⁺, were relatively crucial for the dynamics of the AMF 484 community in the studied region. The soil pH was not considered the primary driver of 485 486 the AMF community change in extremely low soil pH conditions because strong ecological selection on the AMF community renders AMF species adapt to live in 487 488 strongly acidic soils. Cumulatively, the assembly of the AMF community was found to prefer a neutral process in the ATF reserve area. We suggest that mitigation of soil 489 490 acidification might be a potential means to improve the AMF community diversity, 491 which is beneficial for conserving and restoring ecosystems in southwest China.

493 Acknowledgements:

- 494 This work was financially supported by Yunnan Province Science and Technology
- 495 Department (202102AE090038), National Science Foundation of China (32172634),
- 496 Ministry of Agriculture and Rural Affairs of China (CARS 19, CAAS-ASTIP-
- 497 TRICAAS,1610212022008,1610212022015).

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.

503 **Reference**

- Barnett, S.E., Youngblut, N.D., Buckley, D.H., 2020. Soil characteristics and land-use
 drive bacterial community assembly patterns. FEMS Microbiol. Ecol. 96.
 https://doi.org/10.1093/femsec/fiz194
- 507 Bender, S.F., Schlaeppi, K., Held, A., Van der Heijden, M.G.A., 2019. Establishment 508 success and crop growth effects of an arbuscular mycorrhizal fungus inoculated 509 fields. Ecosyst. Environ. into Swiss corn Agric. 273. 13–24. 510 https://doi.org/10.1016/j.agee.2018.12.003
- 511Brundrett, M.C., Jasper, D.A., Ashwath, N., 1999. Glomalean mycorrhizal fungi from512tropicalAustralia.Mycorrhiza8,315–321.513https://doi.org/10.1007/s005720050252
- Burns, A.R., Stephens, W.Z., Stagaman, K., Wong, S., Rawls, J.F., Guillemin, K.,
 Bohannan, B.J., 2016. Contribution of neutral processes to the assembly of gut
 microbial communities in the zebrafish over host development. ISME J. 10, 655–
 664. https://doi.org/10.1038/ismej.2015.142
- Caruso, T., Hempel, S., Powell, J.R., Barto, E.K., Rillig, M.C., 2012. Compositional
 divergence and convergence in arbuscular mycorrhizal fungal communities.
 Ecology 93, 1115–1124. <u>https://doi.org/10.1890/11-1030.1</u>
- 521 Chaudhary, V.B., Nolimal, S., Sosa-Hernández, M.A., Egan, C., Kastens, J., 2020.
 522 Trait-based aerial dispersal of arbuscular mycorrhizal fungi. New Phytologist

523 228, 238-252. https://doi.org/10.1111/nph.16667 524 Davison, J., Moora, M., Öpik, M., Adholeya, A., Ainsaar, L., Bâ, A., Burla, S., Diedhiou, A.G., Hiiesalu, I., Jairus, T., Johnson, N.C., Kane, A., Koorem, K., 525 526 Kochar, M., Ndiaye, C., Pärtel, M., Reier, Ü., Saks, Ü., Singh, R., Vasar, M., Zobel, 527 M., 2015. Global assessment of arbuscular mycorrhizal fungus diversity reveals very low endemism. Science. https://doi.org/10.1126/science.aab1161 528 529 Dumbrell, A.J., Ashton, P.D., Aziz, N., Feng, G., Nelson, M., Dytham, C., Fitter, A.H., 530 Helgason, T., 2011. Distinct seasonal assemblages of arbuscular mycorrhizal fungi 531 revealed by massively parallel pyrosequencing. New Phytol. 190, 794-804. 532 https://doi.org/10.1111/j.1469-8137.2010.03636.x 533 Dumbrell, A.J., Nelson, M., Helgason, T., Dytham, C., Fitter, A.H., 2010. Relative roles 534 of niche and neutral processes in structuring a soil microbial community. ISME J. 535 4, 337–345. https://doi.org/10.1038/ismej.2009.122 536 Edgar, R.C., 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nat. Methods 10, 996–998. https://doi.org/10.1038/nmeth.2604 537 538 Faggioli, V.S., Cabello, M.N., Grilli, G., Vasar, M., Covacevich, F., Öpik, M., 2019. 539 Root colonizing and soil borne communities of arbuscular mycorrhizal fungi 540 differ among soybean fields with contrasting historical land use. Agric. Ecosyst. 541 Environ. 269, 174–182. https://doi.org/10.1016/j.agee.2018.10.002 542 Fierer, N., 2017. Embracing the unknown: disentangling the complexities of the soil 543 microbiome. Nat. Rev. Microbiol. 15, 579-590. 544 https://doi.org/10.1038/nrmicro.2017.87 545 Foster, Z.S.L., Sharpton, T.J., Grünwald, N.J., 2017. Metacoder: An R package for 546 visualization and manipulation of community taxonomic diversity data. PLOS Comput. Biol. 13, e1005404. https://doi.org/10.1371/journal.pcbi.1005404 547 548 García de León, D., Moora, M., Öpik, M., Jairus, T., Neuenkamp, L., Vasar, M., Bueno, C.G., Gerz, M., Davison, J., Zobel, M., 2016. Dispersal of arbuscular mycorrhizal 549 550 fungi and plants during succession. Acta Oecologica 77, 128-135. 551 https://doi.org/10.1016/j.actao.2016.10.006 552 Gosling, P., Mead, A., Proctor, M., Hammond, J.P., Bending, G.D., 2013. Contrasting 553 arbuscular mycorrhizal communities colonizing different host plants show a 554 similar response to a soil phosphorus concentration gradient. New Phytol. 198, 555 546-556. https://doi.org/10.1111/nph.12169 556 Goss-Souza, D., Mendes, L.W., Borges, C.D., Baretta, D., Tsai, S.M., Rodrigues, 557 J.L.M., 2017. Soil microbial community dynamics and assembly under long-term 558 land use change. FEMS Microbiol. Ecol. 93. 559 https://doi.org/10.1093/femsec/fix109 560 Gottshall, C.B., Cooper, M., Emery, S.M., 2017. Activity, diversity and function of 561 arbuscular mycorrhizae vary with changes in agricultural management 562 intensity. Agric. Ecosyst. Environ. 241, 142-149. https://doi.org/10.1016/j.agee.2017.03.011 563 564 Gryndler, M., Vejsadová, H., Vančura, V., 1992. The effect of magnesium ions on the vesicular-arbuscular mycorrhizal infection of maize roots. New Phytol. 122, 565 566 455-460. https://doi.org/10.1111/j.1469-8137.1992.tb00073.x Guo, J.H., Liu, X.J., Zhang, Y., Shen, J.L., Han, W.X., Zhang, W.F., Christie, P., 567 Goulding, K.W.T., Vitousek, P.M., Zhang, F.S., 2010. Significant Acidification in 568 569 Major Chinese Croplands. Science. https://doi.org/10.1126/science.1182570 570 Hart, M.M., Reader, R.J., 2002. Taxonomic basis for variation in the colonization 571 strategy of arbuscular mycorrhizal fungi. New Phytol. 153, 335-344. https://doi.org/10.1046/j.0028-646X.2001.00312.x 572

- Hiiesalu, I., Pärtel, M., Davison, J., Gerhold, P., Metsis, M., Moora, M., Öpik, M.,
 Vasar, M., Zobel, M., Wilson, S.D., 2014. Species richness of arbuscular
 mycorrhizal fungi: associations with grassland plant richness and biomass. New
 Phytol. 203, 233–244. https://doi.org/10.1111/nph.12765
- House, G.L., Bever, J.D., 2018. Disturbance reduces the differentiation of mycorrhizal
 fungal communities in grasslands along a precipitation gradient. Ecol. Appl. 28,
 736–748. https://doi.org/10.1002/eap.1681
- Ibne Baki, M.Z., Suzuki, K., Takahashi, K., Chowdhury, S.A., Asiloglu, R., Harada, N.,
 2021. Molecular genetic characterization of arbuscular mycorrhizal fungi
 associated with upland rice in Bangladesh. Rhizosphere 18, 100357.
 https://doi.org/10.1016/j.rhisph.2021.100357
- Jangid, K., Williams, M.A., Franzluebbers, A.J., Schmidt, T.M., Coleman, D.C.,
 Whitman, W.B., 2011. Land-use history has a stronger impact on soil microbial
 community composition than aboveground vegetation and soil properties. Soil
 Biol. Biochem. 43, 2184–2193. https://doi.org/10.1016/j.soilbio.2011.06.022
- 588 Jefwa, J.M., Okoth, S., Wachira, P., Karanja, N., Kahindi, J., Njuguini, S., Ichami, S., Mung'atu, J., Okoth, P., Huising, J., 2012. Impact of land use types and farming 589 590 practices on occurrence of arbuscular mycorrhizal fungi (AMF) Taita-Taveta 591 Ecosyst. Environ. 157. district in Kenva. Agric. 32-39. 592 https://doi.org/10.1016/j.agee.2012.04.009
- 593 Kaidzu, T., Suzuki, K., Sugiyama, H., Akca, M.O., Ergül, A., Turgay, O.C., Nonaka, 594 M., Harada, N., 2020. The composition characteristics of arbuscular mycorrhizal 595 fungal communities associated with barley in saline-alkaline soils in Central 596 Anatolia. Soil Plant Nutr. 268-274. Sci. 66. 597 https://doi.org/10.1080/00380768.2019.1706432
- Kembel, S.W., Cowan, P.D., Helmus, M.R., Cornwell, W.K., Morlon, H., Ackerly,
 D.D., Blomberg, S.P., Webb, C.O., 2010. Picante: R tools for integrating
 phylogenies and ecology. Bioinformatics 26, 1463–1464.
 https://doi.org/10.1093/bioinformatics/btq166
- Kivlin, S.N., Hawkes, C.V., Treseder, K.K., 2011. Global diversity and distribution of
 arbuscular mycorrhizal fungi. Soil Biol. Biochem. 43, 2294–2303.
 https://doi.org/10.1016/j.soilbio.2011.07.012
- Klironomos, J., Zobel, M., Tibbett, M., Stock, W.D., Rillig, M.C., Parrent, J.L., Moora,
 M., Koch, A.M., Facelli, J.M., Facelli, E., Dickie, I.A., Bever, J.D., 2011. Forces
 that structure plant communities: quantifying the importance of the mycorrhizal
 symbiosis. New Phytol. 189, 366–370. https://doi.org/10.1111/j.14698137.2010.03550.x
- Liu, Y., Shi, G., Mao, L., Cheng, G., Jiang, S., Ma, X., An, L., Du, G., Collins Johnson,
 N., Feng, H., 2012. Direct and indirect influences of 8 yr of nitrogen and
 phosphorus fertilization on Glomeromycota in an alpine meadow ecosystem. New
 Phytol. 194, 523–535. https://doi.org/10.1111/j.1469-8137.2012.04050.x
- Maherali, H., Klironomos, J.N., 2007. Influence of Phylogeny on Fungal Community
 Assembly and Ecosystem Functioning. Science.
 https://doi.org/10.1126/science.1143082
- Monkai, J., Goldberg, S.D., Hyde, K.D., Harrison, R.D., Mortimer, P.E., Xu, J., 2018.
 Natural forests maintain a greater soil microbial diversity than that in rubber
 plantations in Southwest China. Agric. Ecosyst. Environ. 265, 190–197.
 https://doi.org/10.1016/j.agee.2018.06.009
- 621 Oehl, F., Laczko, E., Bogenrieder, A., Stahr, K., Bösch, R., van der Heijden, M.,
 622 Sieverding, E., 2010. Soil type and land use intensity determine the composition

- of arbuscular mycorrhizal fungal communities. Soil Biol. Biochem. 42, 724–738.
 https://doi.org/10.1016/j.soilbio.2010.01.006
- Öpik, M., Vanatoa, A., Vanatoa, E., Moora, M., Davison, J., Kalwij, J.M., Reier, Ü.,
 Zobel, M., 2010. The online database MaarjAM reveals global and ecosystemic
 distribution patterns in arbuscular mycorrhizal fungi (Glomeromycota). New
 Phytol. 188, 223–241. https://doi.org/10.1111/j.1469-8137.2010.03334.x
- Osburn, E.D., Aylward, F.O., Barrett, J.E., 2021. Historical land use has long-term
 effects on microbial community assembly processes in forest soils. ISME
 Commun. 1, 1–4. https://doi.org/10.1038/s43705-021-00051-x
- Pei, Y., Siemann, E., Tian, B., Ding, J., 2020. Root flavonoids are related to enhanced
 AMF colonization of an invasive tree. AoB PLANTS 12, plaa002.
 https://doi.org/10.1093/aobpla/plaa002
- Pereira, C.M.R., Silva, D.K.A. da, Ferreira, A.C. de A., Goto, B.T., Maia, L.C., 2014.
 Diversity of arbuscular mycorrhizal fungi in Atlantic forest areas under different
 land uses. Agric. Ecosyst. Environ. 185, 245–252.
 https://doi.org/10.1016/j.agee.2014.01.005
- Rodrigues, J.L.M., Pellizari, V.H., Mueller, R., Baek, K., Jesus, E. da C., Paula, F.S.,
 Mirza, B., Hamaoui, G.S., Tsai, S.M., Feigl, B., Tiedje, J.M., Bohannan, B.J.M.,
 Nüsslein, K., 2013. Conversion of the Amazon rainforest to agriculture results in
 biotic homogenization of soil bacterial communities. Proc. Natl. Acad. Sci. 110,
 988–993. https://doi.org/10.1073/pnas.1220608110
- 644 Schnoor, T.K., Lekberg, Y., Rosendahl, S., Olsson, P.A., 2011. Mechanical soil
 645 disturbance as a determinant of arbuscular mycorrhizal fungal communities in
 646 semi-natural grassland. Mycorrhiza 21, 211–220. https://doi.org/10.1007/s00572647 010-0325-3
- 648 Sharma, D., Kayang, H., 2017. Effects of arbuscular mycorrhizal fungi (amf) on
 649 Camellia sinensis (L.) o. kuntze under greenhouse conditions. J. Exp. Biol. Agric.
 650 Sci. 5, 235–241.
- Singh, S., Pandey, A., Chaurasia, B., Palni, L.M.S., 2008. Diversity of arbuscular
 mycorrhizal fungi associated with the rhizosphere of tea growing in 'natural' and
 'cultivated' ecosites. Biol. Fertil. Soils 44, 491–500.
 https://doi.org/10.1007/s00374-007-0231-9
- Sloan, W.T., Lunn, M., Woodcock, S., Head, I.M., Nee, S., Curtis, T.P., 2006.
 Quantifying the roles of immigration and chance in shaping prokaryote
 community structure. Environ. Microbiol. 8, 732–740.
 https://doi.org/10.1111/j.1462-2920.2005.00956.x
- Sommermann, L., Geistlinger, J., Wibberg, D., Deubel, A., Zwanzig, J., Babin, D.,
 Schlüter, A., Schellenberg, I., 2018. Fungal community profiles in agricultural
 soils of a long-term field trial under different tillage, fertilization and crop rotation
 conditions analyzed by high-throughput ITS-amplicon sequencing. PLOS ONE 13,
 e0195345. https://doi.org/10.1371/journal.pone.0195345
- Sun, M., Yuan, D., Hu, X., Zhang, D., Li, Y., 2020. Effects of mycorrhizal fungi on
 plant growth, nutrient absorption and phytohormones levels in tea under shading
 condition. Not. Bot. Horti Agrobot. Cluj-Napoca 48, 2006–2020.
 https://doi.org/10.15835/nbha48412082
- Sutcliffe, B., Chariton, A.A., Harford, A.J., Hose, G.C., Greenfield, P., Midgley, D.J.,
 Paulsen, I.T., 2018. Diverse fungal lineages in subtropical ponds are altered by
 sediment-bound copper. Fungal Ecol. 34, 28–42.
 https://doi.org/10.1016/j.funeco.2018.03.003
- 672 Suzuki, K., Takahashi, K., Harada, N., 2020. Evaluation of primer pairs for studying

- arbuscular mycorrhizal fungal community compositions using a MiSeq platform.
 Biol. Fertil. Soils 56, 853–858. https://doi.org/10.1007/s00374-020-01431-6
- Sýkorová, Z., Ineichen, K., Wiemken, A., Redecker, D., 2007. The cultivation bias:
 different communities of arbuscular mycorrhizal fungi detected in roots from the
 field, from bait plants transplanted to the field, and from a greenhouse trap
 experiment. Mycorrhiza 18, 1–14. https://doi.org/10.1007/s00572-007-0147-0
- 679 Varela-Cervero, S., Vasar, M., Davison, J., Barea, J.M., Öpik, M., Azcón-Aguilar, C., 680 2015. The composition of arbuscular mycorrhizal fungal communities differs among the roots, spores and extraradical mycelia associated with five 681 682 Mediterranean plant species. Environ. Microbiol. 17, 2882-2895. 683 https://doi.org/10.1111/1462-2920.12810
- Verbruggen, E., Van Der HEIJDEN, M.G.A., WEEDON, J.T., KOWALCHUK, G.A.,
 RÖLING, W.F.M., 2012. Community assembly, species richness and nestedness
 of arbuscular mycorrhizal fungi in agricultural soils. Mol. Ecol. 21, 2341–2353.
 https://doi.org/10.1111/j.1365-294X.2012.05534.x
- Williams, A., Manoharan, L., Rosenstock, N.P., Olsson, P.A., Hedlund, K., 2017.
 Long-term agricultural fertilization alters arbuscular mycorrhizal fungal
 community composition and barley (Hordeum vulgare) mycorrhizal carbon and
 phosphorus exchange. New Phytologist 213, 874–885.
 https://doi.org/10.1111/nph.14196
- Wang, Y., Wang, K., Huang, L., Dong, P., Wang, S., Chen, H., Lu, Z., Hou, D., Zhang,
 D., 2020. Fine-scale succession patterns and assembly mechanisms of bacterial
 community of Litopenaeus vannamei larvae across the developmental cycle.
 Microbiome 8, 106. https://doi.org/10.1186/s40168-020-00879-w
- Kiang, D., Verbruggen, E., Hu, Y., Veresoglou, S.D., Rillig, M.C., Zhou, W., Xu, T.,
 Li, H., Hao, Z., Chen, Y., Chen, B., 2014. Land use influences arbuscular
 mycorrhizal fungal communities in the farming–pastoral ecotone of northern
 China. New Phytol. 204, 968–978. https://doi.org/10.1111/nph.12961
- Xiao, D., Che, R., Liu, X., Tan, Y., Yang, R., Zhang, W., He, X., Xu, Z., Wang, K.,
 2019. Arbuscular mycorrhizal fungi abundance was sensitive to nitrogen addition
 but diversity was sensitive to phosphorus addition in karst ecosystems. Biol Fertil
 Soils 55, 457–469. https://doi.org/10.1007/s00374-019-01362-x
- 705 Xu, M., Li, Xiaoliang, Cai, X., Li, Xiaolin, Christie, P., Zhang, J., 2017. Land use alters 706 arbuscular mycorrhizal fungal communities and their potential role in carbon 707 sequestration Plateau. on the Tibetan Sci. Rep. 7, 3067. https://doi.org/10.1038/s41598-017-03248-0 708
- Yamato, M., Ikeda, S., Iwase, K., 2008. Community of arbuscular mycorrhizal fungi in a coastal vegetation on Okinawa island and effect of the isolated fungi on growth of sorghum under salt-treated conditions. Mycorrhiza 18, 241–249. https://doi.org/10.1007/s00572-008-0177-2
- 713 Yang, X., Ni, K., Shi, Y., Yi, X., Zhang, Q., Fang, L., Ma, L., Ruan, J., 2018. Effects 714 of long-term nitrogen application on soil acidification and solution chemistry of a 715 plantation in China. Agric. Ecosyst. Environ. 252, 74-82. tea 716 https://doi.org/10.1016/j.agee.2017.10.004
- Zhu, C., Tian, G., Luo, G., Kong, Y., Guo, J., Wang, M., Guo, S., Ling, N., Shen, Q.,
 2018. N-fertilizer-driven association between the arbuscular mycorrhizal fungal
 community and diazotrophic community impacts wheat yield. Agric. Ecosyst.
 Environ. 254, 191–201. https://doi.org/10.1016/j.agee.2017.11.029
- Zi, H., Jiang, Y., Cheng, X., Li, W., Huang, X., 2020. Change of rhizospheric bacterial
 community of the ancient wild tea along elevational gradients in Ailao mountain,

723 China. Sci. Rep. 10, 9203. https://doi.org/10.1038/s41598-020-66173-9



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



Fig. 2 AMF community β -diversity and composition difference. (a), Principal 731 732 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.



746 Fig. 3 Fit of the neutral models for different vegetation AMF communities. (a), The ASVs that occurred more frequently than predicted by the model are shown in green, 747 748 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 within the confidence intervals considered neutrally distributed. R² values present the 750 goodness of fit of the neutral model, ranging from 0 (no fit) to 1 (perfect fit), m value 751 752 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.





759 Fig. 4 Environmental factors contribute to the AMF community differentiation. (a), The relative importance of different environmental factors contribute to the differentiation 760 761 of AMF communities under different vegetation based on Random Forest (RF) analysis. * means the level of significance. Orange color means physicochemical factors, 762 including mineral N, pH, Total C, Total N, C: N ratio, DOC, DON, Available P, Total 763 P, Available K, Total K. Blue color represents ionic factors, including the Al³⁺, Ca²⁺, 764 Mg²⁺, Fe²⁺, Mn²⁺, Cu²⁺, Zn²⁺. Green color means biotic factors, including MBC and 765 766 MBN. 767



Fig. 5 Results of regression analysis between environmental factors and AMF species, environmental factors were Cu^{2+} , Mg^{2+} and NO_3^{-} for (a), (b) and (c) respectively. In the RF analysis, environmental factors have significant effects on AMF community composition were selected for the regression analysis. Above and below average represent the positive and negative correlation coefficient, respectively. Only significant results were shown in the diagram.

776 Supplementary figure legends

777 Fig. S1 Spearman correlation analysis between AMF α -diversity indices and

- environmental factors. "*" represents significantly correlated, "*" and "**" means P < 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