Title:

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

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Abstract:

Understanding the effects of land-use changes on arbuscular mycorrhizal fungal (AMF) communities may greatly benefit ecosystem conservation and restoration. However, how AMF communities respond to anthropogenetic land-use change (e.g., from natural ecosystems to farmland ecosystems) is still under debate. To enhance the preservation 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 composition and assembly processes in the ATF region (soil pH: 3.5-4.2) were investigated. Our results showed that AMF α-diversity indices in ATF were significantly higher than those in conventional tea plantations (CTP) and Forest. 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 addition, neutral processes dominated the AMF community assembly, and *Acaulospora* was the dominant genus of AMF indicator species in ATF. Moreover, land-use changes eliminated the neutral process of AMF community assembly in CTP and Forest by 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 addition, we found that high acidity soils may exert an ecological selection on the AMF community, as only species that adapt to strongly acidic soils persisted. Overall, our results indicated that mitigating soil acidification has potential as a method of improving the AMF community diversity and conserving and restoring ATF ecosystems in southwest China.

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

1. Introduction

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

In global terrestrial ecosystems, arbuscular mycorrhizal fungal (AMF) play an essential role in maintaining the growth of 80% of higher plants (Klironomos et al., 2011). Previous studies have proved that land-use changes can significantly impact AMF communities (House and Bever, 2018; Xiang et al., 2014; Xu et al., 2017). For instance, the increase in soil phosphorus (P) concentration and poor soil structure due 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 to agricultural ecosystems can lower AMF activity and subsequently reduce carbon (C) sequestration and deteriorate soil quality (Xu et al., 2017). The AMF communities in undisturbed grasslands reveal strong differentiation, whereas communities in disturbed grasslands exhibit more homogeneity (House and Bever, 2018). Overall, variation in environmental factors variations after land-use change contribute significantly to AMF community change. However, the specific mechanisms underpinning AMF community responses to environmental change is still unclear and needs to be addressed.

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

Ancient wild tea forests (ATF) have existed for thousands of years in southwest China (Zi et al., 2020). Interestingly, ATF stands under such long-term tea production use no, or limited, agronomic practices (e.g., fertilization, tillage, and trimming) compared to conventional tea plantations (CTP; that undergo annual fertilization, tillage, pesticide application, trimming, etc.). Therefore, AMF communities may play crucial roles in maintaining tea production in ATF (Singh et al., 2008; Yamato et al., 2008). Recent research has revealed that AMF effectively promoted tea plant nutrient acquisition (e.g., 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.

2. Materials and methods

2.1. Site description

Soil samples were collected from three different land-use types (i.e., ATF, CTP, 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 displayed in Table S1. The annual mean temperature and precipitation in this county were 19.2 °C and 1624.0 mm, respectively. Soils in this region are latosolic red soils. The ATF has existed for thousands of years in this area, and most wild tea trees live for 100-1000 years. The CTP in this region is planted with tea cultivar ("Yunkang 10") and is under standard agronomic management (i.e., annual trimming twice a year) and fertilization (600 kg compound fertilizer plus 300 kg urea per hectare)). Forest is typically mixed forest (i.e., mixed evergreen broad-leaved and deciduous broad-leaved forest).

2.2. Soil sampling, preparation and determination of soil properties

Six soil samples were randomly collected within a 20 m^2 square at each site. Litter and soil crusts were removed before the surface soil (0-20 cm) was collected. Fresh soils were then divided into three parts. Subsamples for soil pH, available P, available K, metal ions (i.e., Al^{3+}, Ca^{2+}, Mg^{2+}, Fe^{2+}, Mn^{2+}, Cu^{2+}, Zn^{2+}), total C, total N, total P, and total K were air-dried and passed through corresponding sieves of different sizes. Subsamples for the estimation of soil mineral N (NO_3^--N and NH_4^+-N), dissolved organic C and N (DOC and DON), microbial biomass C and N (MBC and MBN) were passed through a 2-mm sieve and stored at 4 ℃. Subsamples for DNA extraction were passed through a 2-mm sieve and stored at -80 ℃.

Soil pH was measured using a pH meter in a 1:2.5 suspension of soil: KCl solution (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, Netherlands). Soil available P was measured according to the Bray1 method. Soil available K and other metal ions were extracted using the Mehlich 3 method and then measured using inductively coupled plasma atomic emission spectroscopy (ICP-AES, Thermo Jarrell Ash Ltd). Soil total C and N were determined using a C/N elemental analyzer (Vario Max, Elementar, Germany). Soil total P and total K were measured using a digestion method. Briefly, 0.2 g samples were weighed, and then 5 mL HNO_3, 1 mL $HClO_4$, and 2 mL hydrogen fluoride were sequentially added. After digestion, the samples were diluted to 50 mL, and ICP-AES determined the total P and K content. For the soil MBC and MBN, the samples were fumigated with chloroform (with no ethyl alcohol) in an airtight and dark vessel and parallel samples were set but without fumigation. All the samples were extracted with 0.05 M K_2SO_4 and then measured using a total organic carbon analyzer (Multi N/C 2100/1, Analytic JENA ag., Jena, Germany). The results of non-fumigated samples were considered as soil DOC and DON, and results of fumigated minus non-fumigated were considered as soil MBC and MBN.

2.3. DNA extraction, purification, polymerase-chain-reaction (PCR), and high-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

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

PCR amplification of AM fungi was performed using the primer pairs AMV4.5NF (AAGCTCGTAGTTGAATTTCG) and AMDGR (5′-CCCAACTATCCCTATTAATCAT-3′) (Suzuki et al., 2020). The reaction conditions 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 for 7 min. The amplicons were purified using the Agencourt AMPure XP kit (Beckman Coulter, USA). The purified amplicons were sequenced using the Illumina MiSeq platform at Genesky Biotechnologies Inc. (Shanghai, China). The raw sequence data are available in the NCBI database with accession PRJNA701385.

2.4. Bioinformatics

USEARCH (v 11.0.667) was employed to process the raw high-throughput sequencing data (Edgar, 2013). In brief, paired raw sequences were merged and re-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 was used to denoise and identify all the biological real sequences and generate the table for representative sequences and amplicon sequence variants (ASVs). In this step, 4900 representative sequences were obtained. These sequences were then aligned to the MaarjAM database (http://maarjam.botany.ut.ee) (Öpik et al., 2010) with a threshold of 0.97 and 27 virtual taxa (VTX) were obtained; the sequence/ASV IDs of annotation results were used to create subset representative sequences only of AMF and ASVs table. Finally, 189225 sequences, from 116 AMF species, remained for further analysis.

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

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

2.5. Sloan neutral model analysis

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

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. Species below the 95% confidence interval (below prediction) were considered selected against by the host or having limited dispersal ability from the metacommunity.

2.6. Statistical analysis

In our study, soil environmental factors were characterized as physicochemical 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., Al^{3+}, Ca^{2+}, Mg^{2+}, Fe^{2+}, Mn^{2+}, Cu^{2+}, and 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) model was carried out to predict the relative importance of soil environmental factors contributing to the AMF community composition. The RF model was performed using the R packages "randomForest", "rfPermute", and "rfUtilities". In addition, variance decomposition was carried out to test the relative contribution of physicochemical, ionic, and biotic factors to the differentiation in AMF community composition. Variance decomposition analysis was performed using the function "rda" in the R package "vegan". Finally, a linear regression model was carried out to explore the 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.

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

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 3.50-4.21in 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 (NH_4^+-N and NO_3^--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., Ca^{2+}, Mg^{2+}, Zn^{2+}, Fe^{2+}, and Mn^{2+})
255 were elevated in CTP except for Cu^{2+}. ATF had the highest 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 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
265 (e.g., soil total N, NH_4^+-N, available P and K, DOC, DON, MBC, MBN, Ca^{2+}, Cu^{2+},
266 and Zn^{2+}) (Fig. S1).

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 components accounted for 53.38% of the change in AMF community structure (Fig. 2a). The AMF community composition consisted of seven genera (27 VTXs were included), including *Glomus*, *Claroideoglomus*, *Acaulospora*, *Archaeospora*, *Ambispora*, *Paraglomus*, and *Gigaspora*, and the dominant genus was *Glomus* (Fig. 2b). The pairwise comparison of AMF community composition abundance showed a significant difference among land-use types (Fig. 2b). *Archaeospora* and *Ambispora* showed the highest relative abundance in ATF. The relative abundance of *Acaulospora*, *Claroideoglomus*, and *Paraglomus* was the highest in CTP, while *Glomus* and *Gigaspora* had the highest relative abundance in the Forest. In addition, the VTXs within different genera also displayed significant differences among land-use types (Fig. 2b). Indicator species of AMF community in each land-use type were relatively enriched in their corresponding land-use type. The highest number of indicator species was present in ATF, while the lowest was in CTP (Fig. 2c and 2d). In addition, the average cumulative relative abundance of indicator species of ATF, CTP, and Forest accounted for 58.33%, 27.10%, and 38.93% of the total abundance (Fig. 2d). Furthermore, the number of indicator species (VTX) showed a remarkable decrease 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 accounted for 25.60% and 13.59% of the total abundance, respectively (Fig. 2d). In CTP, the indicator species were VTX00030, VTX00420, and VTX00348, respectively, accounting for 18.82%, 6.73%, and 1.55% of the total abundance (Fig. 2d). In Forest, VTX00328 and VTX00231 were the dominant indicator species and accounted for 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

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

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

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

The RF model was performed to identify the most important environmental factors accounting for AMF community differentiation across ATF, CTP, and Forest. The RF 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^--N ($P < 0.05$), Mg^{2+} ($P < 0.05$) and TK ($P < 0.05$) significantly impacted AMF community differentiation (Fig. 4). According to the variance decomposition, soil physicochemical, ionic, and biotic properties explained 16.56% ($P < 0.01$), 22.06% ($P < 0.001$), and 2.88% ($P > 0.05$) of the AMF community change, respectively, and all the environmental factors could cumulatively explain 87.65% of the AMF community change (Table 2).

According to the RF model, soil Cu^{2+}, Mg^{2+}, NO_3^--N, and total K significantly impacted the AMF community composition. A linear regression model was used to explore the relationship between AMF species and these four environmental factors. ASV57 and ASV102 exhibited a significantly negative relationship with soil Cu^{2+} (Fig. 5a), while they displayed the reverse (positive) result for the soil NO_3^--N (Fig. 5c). Both ASV57 and ASV102 belonged to VTX00024 (identified as *Acaulospora* sp.). On the other hand, soil Mg^{2+} showed a distinctly positive relationship with ASV38 and ASV86, and these two species belonged to VTX00030 (also identified as *Acaulospora* sp.) (Fig. 5b). However, the linear regression result showed that soil total K did not correlate significantly with the AMF species.

4. Discussion

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

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, i.e., the AMF α-diversity significantly decreased in Forest and CTP because these two 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; Shannon index: mean = 2.99) and the forest (Chao1 index: mean = 32.75; Shannon 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 ATF showed significantly higher AMF α-diversity indices (Chao1 index: mean = 59.33; Shannon index: mean = 2.99) (Verbruggen et al., 2012; Xiang et al., 2014; Xu et al., 2017). This phenomenon could be attributed to the passive development of the AMF species over a long timescale, whereas human disturbance could limit this development (García de León et al., 2016). Thus, the AMF species in ATF had more time to develop because of long-standing and relatively low disturbance compared to CTP and the forest in our study (Zi et al., 2020). Moreover, higher soil available nutrients in CTP and the forest might also be the reason for the decrease in AMF α-diversity because soil available nutrients (e.g., available P and NH_4^+-N) negatively affect AMF diversity (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

al., 2018). However, our results showed that pH was not a significant factor accounting 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 adapted to the low pH can survive. Therefore, soil pH is not the main factor affecting the AMF communities in the three land-use types. The AMF communities in our study were sensitive to other soil environmental factors rather than directly to soil pH. A recent study also found that soil nutrient availability had a more significant effect on AMF abundance and diversity than pH (Xiao et al., 2020).

Nevertheless, the RF model showed that the AMF community change was related to soil physicochemical, ionic, and biotic properties (Fig. 4), while Cu^{2+}, NO_3^--N, Mg^{2+}, 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 promote the colonization of AMF (Gryndler et al., 1992; Sutcliffe et al., 2018), which was consistent with results observed in our study and verified by the linear regression 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 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 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 NO_3^--N and an antagonistic relationship with Cu^{2+}. 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
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^{2+} 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^{2+} 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

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

4.3. Neutral process dominates AMF community assembly

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

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

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

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

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

5. Conclusion

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

community composition. Our study revealed that the environmental factors, such as soil NO_3^--N, TK, Mg^{2+}, and Cu^{2+}, were relatively crucial for the dynamics of the AMF community in the studied region. The soil pH was not considered the primary driver of 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 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 acidification might be a potential means to improve the AMF community diversity, which is beneficial for conserving and restoring ecosystems in southwest China.

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Data availability

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

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Figure legends

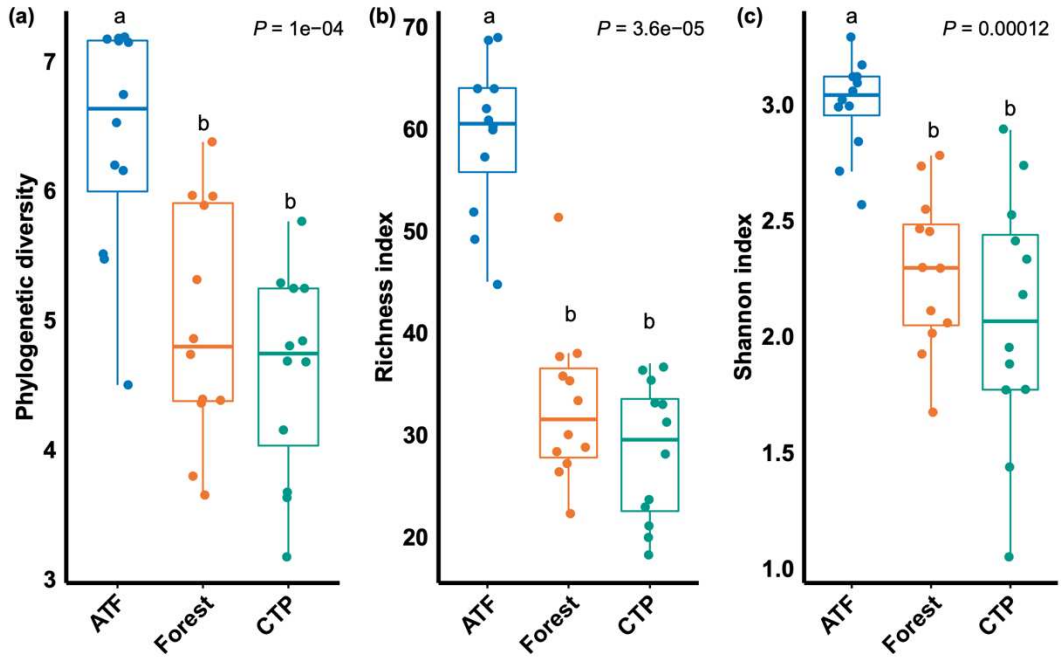


Fig. 1 AMF community α-diversity. (a) Phylogenic diversity, (b) Richness index, (c) Shannon index. The different letters above every figure mean significant differences between land-use types tested by the Kruskal-Wallis method at $P = 0.05$. The dotted line represents the mean value of all samples.

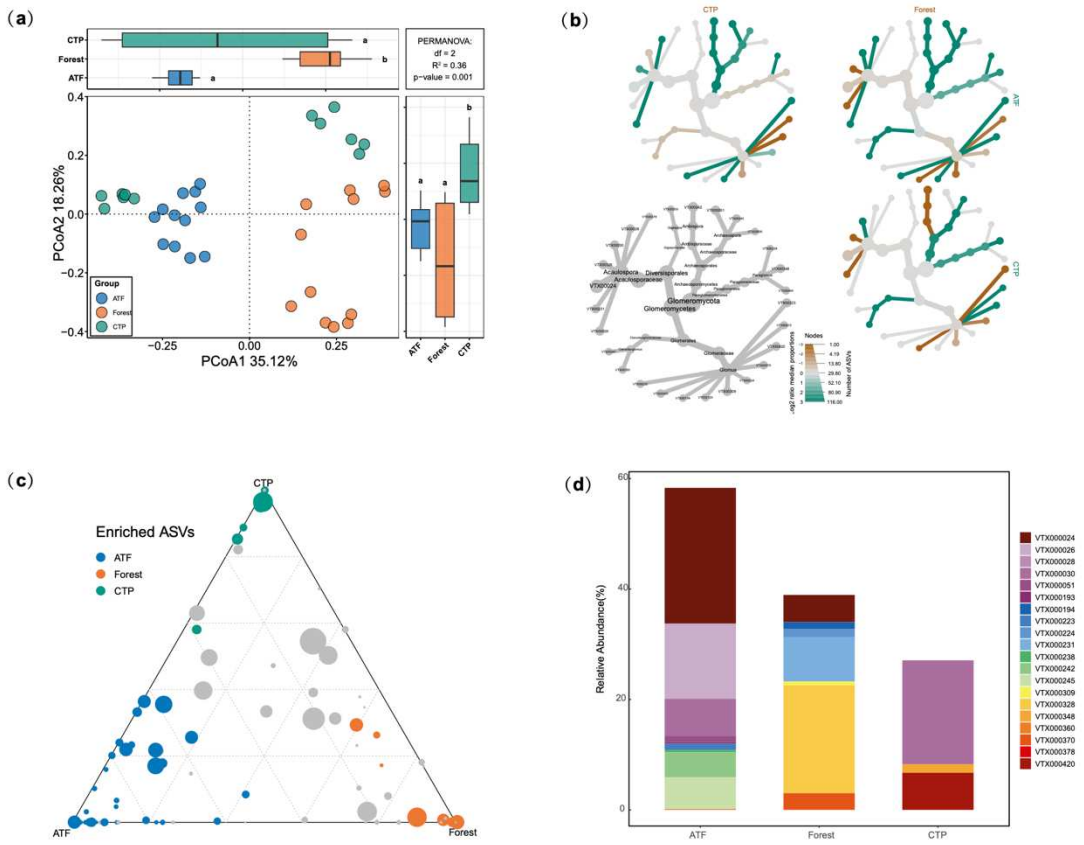
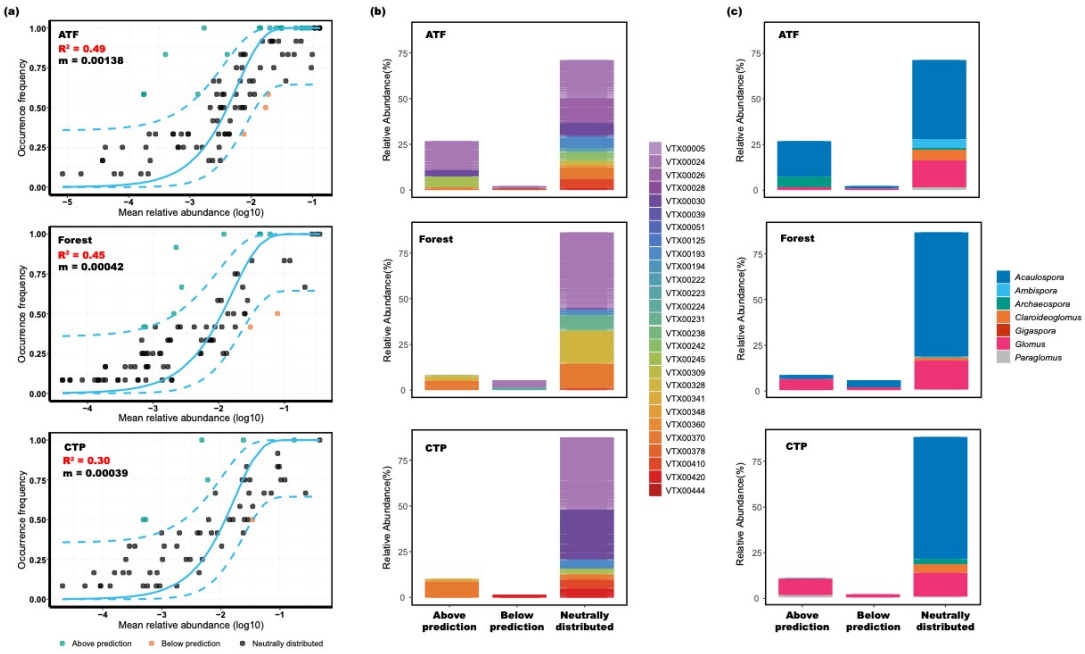


Fig. 2 AMF community β-diversity and composition difference. (a), Principal Coordinates Analysis (PCoA) of AM fungal community based on species matrices from three land-use types. One-way PERMANOVA was used to analyze the effects of land-use type on the community structure of AMF. (b), Heat tree of AMF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

757

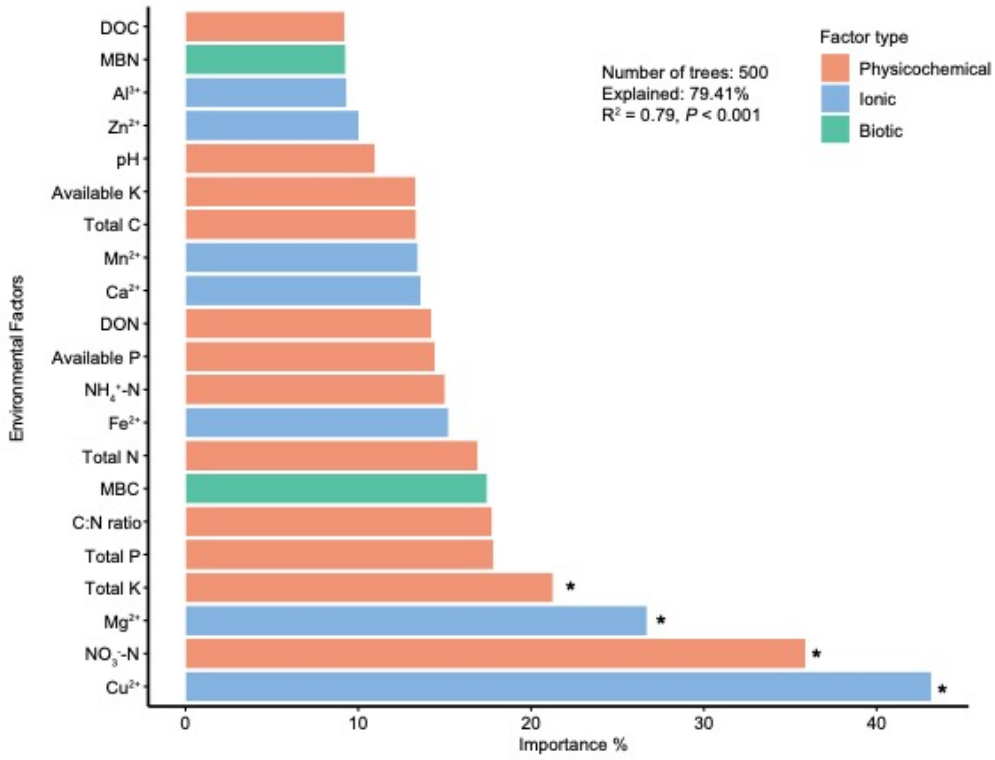
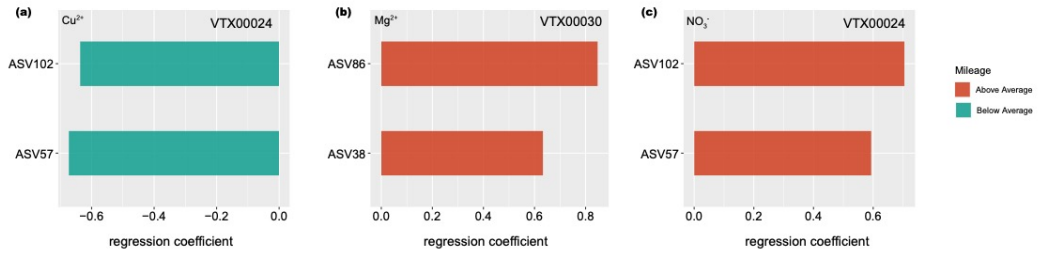


Fig. 4 Environmental factors contribute to the AMF community differentiation. (a), The relative importance of different environmental factors contribute to the differentiation of AMF communities under different vegetation based on Random Forest (RF) analysis. * means the level of significance. Orange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



768

769 **Fig. 5** Results of regression analysis between environmental factors and AMF species,
770 environmental factors were Cu^{2+}, Mg^{2+} and NO_3^- 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

Supplementary figure legends

Fig. S1 Spearman correlation analysis between AMF α-diversity indices and environmental factors. "*" represents significantly correlated, "*" and "**" means P < 0.05 and *P* < 0.0,1 respectively.

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

Table S1 Detailed location information of sample sites