Arbuscular Mycorrhizal Fungi Associated with Roots Reveal High Diversity Levels at Different Elevations in Tropical Montane Rainforests

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Abstract: (1) Background: Understanding the diversity of communities of arbuscular mycorrhizal fungi (AMF) is the basis for understanding the ecological functions of AMF. (2) Methods: The community diversity and distribution of AMF at different elevations in tropical montane rainforests of Mt. Jianfenfeng and Mt. Diaoluo were explored using high-throughput sequencing technology. (3) Results: A total of 283 AMF operational taxonomic units (OTUs) were identified from roots and the number of unique OTUs was 173, accounting for 61.13% of the total number discovered in these tropical montane rainforests. At different altitudes, high turnovers of AMF were observed, with the maximum proportion of unique OTUs between two altitudes being 45.16%, recorded between a.s.l. 250 m and 900 m on Mt. Diaoluo. The highest Sobs, Shannon and Pielou diversity indices appeared at 650 m on Mt. Diaoluo. For the two mountains, the soil properties of C, N and C/N have significant impacts on the genera Scutellospora, Paraglomus and unclassified in Archaeosporaceae, while the genera Glomus, Diversispora and Acaulospora are significantly affected by soil P and pH. It can be considered that altitude probably determines the presence of AMF communities by affecting edaphic properties. (4) Conclusions: There are abundant AMF associated with roots in the tropical montane rainforests of China. Furthermore, a high turnover of OTUs was found to exist between the mountains and at different altitudes, revealing diverse AMF community structures in tropical montane rainforests.

Keywords: arbuscular mycorrhizal fungi; communities; diversity; elevation; tropical montane rainforests

1. Introduction

As the main components of terrestrial vegetation, tropical forest ecosystems retain the highest levels of biodiversity, making great contributions to Earth’s total biodiversity. Notably, the role of soil microorganisms in regulating critical ecosystem processes cannot be ignored in the context of global climate change [1]. As the most widespread mutualists, arbuscular mycorrhizal fungi (AMF) form symbioses with 80% of terrestrial plant species and are ubiquitous in tropical forests [2,3]. Symbiotic associations can improve root mineral nutrient acquisition, especially low-mobility soil minerals. Studies have also proved that the presence of these fungi is even more important when soil nutrient contents are low, because AMF have acclimation mechanisms to cope with environmental stress, enhancing plant tolerance to abiotic stress [4–6]. What is more, interaction between soil microorganisms and plant roots is a key factor in shaping AMF communities, which is important for understanding biodiversity patterns and ecosystem functions [7]. Therefore, there is increasing evidence that the effect of mycorrhizal symbiosis on biodiversity and ecosystem functions is not to be ignored in various ecosystems [8–11].

AMF diversity is related to plant community diversity, structure and ecosystem function [5]. Exploring AMF diversity and community structure in various ecosystems may
play a role in predicting the evolution and succession of ecosystems [12]. On a global scale, Davison et al. found 350 to 1000 molecularly identified AMF taxa in root samples [13]; 90% of AMF species were found in more than one climatic zone and 93% of taxa were found on multiple continents, demonstrating a low level of endemism. However, at a regional scale, soil pH, soil nutrients and climate types are all important driving factors in the formation and turnover of AMF communities [14,15]. Therefore, against a background of globally low endemism of AMF, AMF taxa turnover and diversity along different environmental gradients at regional scales need to be further studied.

It is considered that AMF diversity is more prominent in tropical montane forests because of the relatively high rainfall, low annual temperature variations and high plant community diversity and heterogeneity levels [16]. Although tropical forests account for only 12% of the earth’s surface, they are the main reservoirs of biodiversity and play a vital role in regulating the earth’s climate and biogeochemical cycles [17,18]. What is more, tropical montane rainforests are unique climate-affected ecosystems, which makes them natural areas for studying biodiversity [19]. Elevation is a comprehensive factor in tropical montane ecosystems: elevation gradients lead to changes in environmental gradients. Elevation gradients are characterized by dramatic changes in climate and biotic turnover over short geographic distances, providing a unique chance to explore AMF diversity and turnover [20]. Research has shown that AMF communities are more phylogenetically clustered in high-altitude areas [21]. Zhang et al. showed that AMF diversity showed a cubic function trend with increasing altitude [22]. Vieira et al. suggested that with increasing altitude, AMF diversity showed a downward trend in temperate climates [23]. However, Shen et al. suggested that AMF diversity was not correlated with elevation [24]. These different results support the view that the main determinant of microbial community distribution and diversity is environmental filtration [21,25]. However, the diversity and turnover of AMF taxa in complex tropical mountain ecosystems need to be studied further. In addition, studying the distribution and diversity of AMF along different elevation gradients is key to predicting the biogeography of AMF, which helps to understand the generation and maintenance mechanisms of soil microbial diversity at regional scales and is the basis for predicting the directions of the evolution of terrestrial ecosystem functions [26,27].

Davison’s study found that there was high diversity and low numbers of endemic species of AMF on the global scale [13]. Therefore, in the background of this conclusion, it can be inferred that there should be abundant AMF diversity and lower turnover of AMF taxa along different environmental gradients in tropical mountain rainforests. We try to reveal the reasons for the results obtained and hope to provide important information for the study of the biogeography of AMF in tropical rainforests in order to predict the service function of this type of ecosystem.

2. Materials and Methods

2.1. Study Region and Sample Collection

This study was conducted on Mt. Diaoluo (109°41′–110°40′ E, 18°38′–18°50′ N) and Mt. Jianfengling (108°44′–109°02′ E, 18°25′–18°52′ N) on Hainan Island, China. Mt. Diaoluo is one of the rarest and most precious primitive tropical rainforest areas in China. With increasing elevation, the vegetation types of Mt. Diaoluo proceed through tropical secondary forest, monsoon forest and evergreen broad-leaved forest, with a forest coverage rate of 96.26%. The annual average temperature of Mt. Diaoluo is 20.8 °C, and the climate is a tropical monsoon climate. Similar to Mt. Diaoluo, Mt. Jianfengling has a typical monsoon climate, with an average annual temperature of 19.7 °C and an average annual precipitation of 1300 to 3500 mm [26]. The vegetation types of Mt. Jianfengling are tropical semi-deciduous monsoon rainforest and tropical evergreen monsoon rainforest, with a forest coverage rate reaching 98%. Due to their special geographic locations and climates, the Mt. Diaoluo and Mt. Jianfengling areas are among the regions with the highest biodiversity levels in China.
Soil and root samples were collected from 4 elevations of 250, 450, 650, 900 m on Mt. Diaoluo and 6 elevations of 350 m, 600 m, 800 m, 1000 m, 1200 m and 1350 m on Mt. Jianfengling. At each target altitude, three 20 × 20 m independent quadrats were set up 50–100 m horizontally apart from each other. The root systems were excavated with adherent soil. Each quadrat was divided into 100 sub-quadrats with side lengths of 2 m. A total of 100 soil cores with diameters of 5 cm and depths of 0–30 cm were collected and then mixed to form one composite sample for each quadrat. Soil samples were used to determine soil nutrient concentrations at each altitude, and root samples were stored in a cooling bin containing dry ice to assess the diversity of AMF.

2.2. DNA Extraction and PCR Amplification

Microbial DNA was extracted from root samples using the FastDNA SPIN Kit (MP Biomedicals LLC, Santa Ana, CA, USA), according to the manufacturer’s protocols. Final DNA concentration and purification were determined with a NanoDrop 2000 UV–vis spectrophotometer at 260 nm (Thermo Fisher Scientific, Wilmington, NC, USA), and DNA quality was checked by 1% agarose gel electrophoresis. All extracted DNA samples were stored at −20 °C for further analysis. The extracted DNA was amplified according to a polymerase chain reaction (PCR) procedure using a Thermocycler PCR system (GeneAmp 9700, ABI, Carlsbad, CA, USA). The AMF region was amplified using the PCR amplifier of the ABI GeneAmp® 9700 device, with the universal primers AMV4-5NF (5′-AAGCTCGTAGTTGAA TTTCG-3′) and AMDGR (3′-CCCAACTATCCCTATTAATCAT-5′). The PCR reactions were conducted using the following program: 3 min of denaturation at 95 °C, 32 cycles of 30 s at 95 °C, 30 s for annealing at 55 °C, 45 s for elongation at 72 °C and a final extension for 10 min at 72 °C, dropping to 10 °C until halted by user. The second step PCR included: one cycle of 3 min at 95 °C, 30 cycles of 30 s at 95 °C, 30 s for annealing at 55 °C, 45 s for elongation at 72 °C and a final extension at 72 °C for 10 min, dropping to 10 °C until halted by user. PCR reactions were performed in triplicate using 20 µL mixture containing 4 µL of 5 × FastPfu Buffer, 2 µL of 2.5 mM dNTPs, 0.8 µL of forward and reverse primers (5 µM), 0.4 µL of FastPfu Polymerase, 0.2 µL of BSA and 10 ng of template DNA. The PCR products were separated by electrophoresis and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA), then quantified using QuantiFluor™-ST (Promega Biosciences, Madison, WI, USA), according to the manufacturer’s protocol.

Purified amplicons were pooled in equimolar and paired-end sequences (2 × 300) on an Illumina MiSeq platform (Illumina, San Diego, CA, USA), according to the standard protocols of Majorbio BioPharm Technology Co. Ltd. (Shanghai, China). Operational taxonomic units (OTUs) were clustered with 97% similarity cutoff using UPARSE (UPARSE OTU clustering. Available online: http://drive5.com/uparse/; accessed on 20 May 2022).

2.3. Soil Properties Analysis

Soil C and N concentrations were determined with an elemental analyzer. Soil pH was determined with a 1:2.5 suspension of soil in water using a precision pH meter (PHs-3C). The concentration of soil total phosphorus was determined using the Olsen and Sommers method [29].

2.4. Statistical Data Analysis

AMF taxa found on Mt. Diaoluo and Mt. Jiangfengling at different elevations were analyzed based on OTU levels, which were presented in Venn diagrams using R software (version 3.3.1). AMF turnover was expressed as the percentage of the number of unique OTUs relative to the total number of OTUs between two altitudes and was analyzed using Excel 2019. AMF relative richness and occurrence frequency were analyzed based on genus level using Excel 2019. Sobs, Shannon and Pielou alpha diversity indices were determined and represented AMF community richness, diversity, and evenness, respectively. The diversity indices were represented by the average values for each altitude. One-way ANOVA
was conducted at \( p < 0.05 \) for different altitudes, and Duncan’s multiple comparison method was used to test the significance of the differences in SPSS 25.0. A redundancy analysis (RDA) was used to explore the relationships between the environment factors and AMF community structure. Quantification of the effects of environmental factors on the fungal communities was performed with R software (version 3.3.1) using the “vegan” package.

3. Results

3.1. AMF Community Composition and Distribution

A total of 283 unique OTUs were identified from two tropical montane forests (Figure 1A). Among them, 208 and 185 OTUs were identified on Mt. Diaoluo and Mt. Jianfengling, respectively. Ninety-eight unique OTUs were isolated on Mt. Diaoluo, accounting for 47.12% of the total number of OTUs on the mountain. The number of unique OTUs was 75 on Mt. Jianfengling, accounting for 40.54% of the total number of OTUs. In total, 173 unique OTUs were isolated, accounting for 61.13% of the total number of OTUs in these tropical forests, with 110 OTUs being shared between the two mountains, accounting for 38.89% of the total number of OTUs in these forests.

![Figure 1](image1.png)

**Figure 1.** Venn diagrams showing the shared AMF OTUs between the two mountains (A) and at different altitudes on Mt. Diaoluo (B) and Mt. Jianfengling (C). Different colors represent different altitudes.

On Mt. Diaoluo, the numbers of OTUs ranged from 59 to 127 at different altitudes. The numbers of unique OTUs ranged from 17 to 64. The highest number of unique OTUs occurred at an altitude of 250 m, accounting for 50.39% of the total number of OTUs at this altitude. Ten OTUs were shared by the four altitudes on Mt. Diaoluo. On Mt. Jianfengling, the number of AMF OTUs changed from 37 to 103 at different altitudes, and the largest number of OTUs occurred at 1350 m. Consistently, the highest number of unique OTUs was 47 at the altitude of 1350 m, accounting for 45.63% of the total number of OTUs at this altitude. In addition, 8 OTUs was shared by these six elevations.

The turnover of AMF taxa was expressed as the percentage of the number of unique OTUs relative to the total number of OTUs between two altitudes. On Mt. Diaoluo, AMF OTU turnover ranged from 20.25 to 45.16% between pairs of altitudes (Figure 2A). The highest turnover occurred between 250 m and 900 m, with 45.16%. At the same time, the lowest turnover occurred between 450 m and 650 m. The number of unique OTUs changed from 5 to 47 on Mt. Jianfengling. A turnover of OTUs greater than 20% was recorded. The highest turnover of OTUs was 38.61%, which occurred between 600 and 1350 m (Figure 2B).
Figure 2. Counts of total OTUs and unique OTUs (in brackets) found at each altitude and the percentages of unique OTUs at different altitudes on Mt. Diaoluo (A) and Mt. Jianfengling (B).

3.2. AMF Genus Relative Abundances and Occurrence Frequencies at Different Elevations at OTU Level

The relative abundances of genera at OTU level were obtained and it was found that *Glomus* was the dominant genus, with the highest relative abundances of 78.31% and 83.45% on Mt. Diaoluo and Mt. Jianfengling, respectively (Figure 3A). Regarding altitude gradients, the relative abundances of *Glomus* were the highest at all elevations (Figure 3B,C). The highest relative abundances of *Glomus* occurred at 650 m and 350 m—93.39% and 99.99%, respectively—on Mt. Diaoluo and Mt. Jianfengling. In addition to *Glomus*, the genus *unclassified in Glomeromycetes* was the second dominant on Mt. Diaoluo, and the highest relative abundance was 30.81% at the altitude of 250 m. In addition, the relative abundances of *Acaulospora* at the altitudes of 450 and 650 m were 5.26% and 6.55%.

For Mt. Jianfengling, the relative abundances of *Glomus* showed a trend of decreasing with increasing altitude. The highest and lowest relative abundances of *Glomus* occurred at 350 m and 1200 m, these being 99.99% and 61.70%, respectively. At the altitude of 1200 m, the relative abundance of *unclassified in Archaeosporaceae* was higher, with 23.09%. The relative abundance of *Paraglomus* was also higher at the altitude of 1350 m, with 27.95%.

The dominant genus *Glomus* appeared at every altitude, and the occurrence frequency was 100% in the tropical forest of Mt. Diaoluo and Mt. Jianfeng (Table 1). In addition, the genus of *Acaulospora* reached the highest occurrence frequency at 650 m, with 100% on Mt. Diaoluo. The genus of *Diversispora* only occurred at 900 m on Mt. Diaoluo, with an occurrence frequency of 33.33%. A similar observation was made for the *Diversispora* genus of *Archaeospora*, which only appeared at 600 m on Mt. Jianfeng, with an occurrence frequency of 33.33%. On the whole, the genus with the highest occurrence frequency was *Glomus*.

Table 1. AMF genus occurrence frequencies at different elevations in tropical forests of China.

| Genus                  | Occurrence Frequency (%) | Total |
|------------------------|--------------------------|-------|
|                        | DL          | JF      |       |
| 250 m | 450 m | 650 m | 900 m | 350 m | 600 m | 800 m | 1000 m | 1200 m | 1350 m |       |
| Acaulospora            | 66.67       | 25      | 100    | 33.33 | 0      | 0      | 0      | 66.67  | 33.33  | 66.67  | 40    |
| Archaeospora           | 0           | 0       | 0      | 0     | 0      | 0      | 0      | 66.67  | 33.33  | 66.67  | 33.33 |
| Diversispora           | 0           | 0       | 0      | 33.33 | 0      | 0      | 0      | 0      | 66.67  | 33.33  | 33.33 |
| Glomus                 | 100         | 100     | 100    | 100   | 100    | 100    | 100    | 100    | 100    | 100    | 100   |
| Norank                 | 0           | 0       | 66.67  | 0     | 0      | 0      | 0      | 0      | 0      | 66.67  |
| Paraglomus             | 33.33       | 25      | 0      | 50    | 0      | 0      | 0      | 33.33  | 33.33  | 33.33  | 20    |
| Scutellomycetis        | 0           | 0       | 33.33  | 0     | 0      | 33.33  | 66.67  | 33.33  | 33.33  | 66.67  | 26.67 |
| Unclassified in Glomeromycetes | 33.33    | 50      | 33.33  | 66.67 | 0      | 66.67  | 33.33  | 0      | 33.33  | 33.33  | 40    |
| Unclassified in Archaeosporaceae | 33.33     | 0       | 0      | 0     | 0      | 0      | 33.33  | 33.33  | 33.33  | 33.33  | 13.33 |
| Unclassified in Gigasporaceae | 33.33   | 25      | 66.67  | 33.33 | 0      | 0      | 66.67  | 100    | 33.33  | 66.67  | 43.33 |
3.3. AMF Diversity at Different Elevations

The Sobs index ranged from 20.25 to 50.00, with an average of 33.69 on Mt. Diaoluo (Figure 4A). As with the Sobs index, the highest and lowest Shannon and Pielou indices also occurred at 650 m and 450 m (Figure 4B,C). Elevation had no significant influence on AMF diversity index, including Shannon and Pielou evenness, on Mt. Diaoluo.

The highest and lowest Sobs indices appeared at 800 m and 350 m, with the highest being 42.67 and the lowest being 18.8 (Figure 4D), on Mt. Jianfengling. The Sobs index values were significantly lower at 350 m and 600 m than that at the high altitudes of 800 m and 1350 m. The Shannon and Pielou indices ranged from 1.21 to 2.01 and 0.38 to 0.62, with means of 1.73 and 0.51 (Figure 4E,F). At the same time, elevation had no significant effect on AMF Shannon and Pielou evenness indices.
Figure 4. Differences in fungal diversity indices, including Sobs, Shannon and Pielou indices, on Mt. Diaoluo (A–C) and Mt. Jianfengling (D–F). Deviation bars represent standard errors. Different lowercase letters (a, b) above each column indicate significant differences ($p < 0.05$).

3.4. Effects of Soil Factors on AMF Diversity

The concentrations of soil N and C ranged from 0.12% to 0.23% and 1.46% to 3.19% on Mt. Diaoluo (Table 2). The maximum concentrations of C, N and C/N all appeared at 900 m and were significantly higher than the concentrations recorded at other altitudes. Similarly, the maximum concentration of P also occurred at 900 m, with 0.49 g/kg. In addition, elevation had no significant effect on soil P concentration. The range of soil pH was from 4.61 to 5.81, and the highest soil pH occurred at 650 m on Mt. Diaoluo. On Mt. Jianfengling, the highest concentrations of soil C and N both occurred at 1350 m, which were significantly higher than at other altitudes. The concentrations of soil P ranged from 0.17 to 0.36 g/kg, and elevation had no significant effect on these levels. The highest and lowest soil pHs occurred at 600 m and 800 m—5.88 and 4.63, respectively.

Table 2. The concentrations of soil nutrients at different altitudes.

| Mountain | Elevation (m) | N (%) | C (%) | C/N | P (g/kg) | pH |
|----------|---------------|-------|-------|------|----------|----|
| DL       | 250           | 0.20 ± 0.05 ab | 2.23 ± 0.43 ab | 11.81 ± 0.89 ab | 0.26 ± 0.05 a | 4.86 ± 0.27 bc |
|          | 450           | 0.19 ± 0.03 ab | 2.12 ± 0.45 ab | 10.90 ± 0.74 ab | 0.46 ± 0.03 a | 5.57 ± 0.15 ab |
|          | 650           | 0.12 ± 0.01 b  | 1.46 ± 0.14 b  | 12.13 ± 0.26 b  | 0.36 ± 0.01 a | 5.81 ± 0.13 a  |
|          | 900           | 0.23 ± 0.01 a  | 3.19 ± 0.21 a  | 13.65 ± 0.53 a  | 0.49 ± 0.01 a | 4.61 ± 0.43 c  |
| JF       | 350           | 0.23 ± 0.05 b  | 3.32 ± 0.60 b  | 14.78 ± 0.24 bc | 0.36 ± 0.05 a | 5.63 ± 0.21 ab |
|          | 600           | 0.19 ± 0.03 b  | 3.09 ± 0.72 b  | 16.13 ± 1.72 ab | 0.21 ± 0.10 a | 5.88 ± 0.49 a  |
|          | 800           | 0.17 ± 0.01 b  | 2.89 ± 0.30 b  | 16.39 ± 0.39 ab | 0.36 ± 0.06 a | 4.63 ± 0.14 b  |
|          | 1000          | 0.12 ± 0.01 b  | 1.54 ± 0.22 b  | 13.20 ± 0.35 c  | 0.17 ± 0.03 a | 5.13 ± 0.45 ab |
|          | 1200          | 0.29 ± 0.06 b  | 4.80 ± 1.21 b  | 16.69 ± 0.29 ab | 0.31 ± 0.03 a | 4.83 ± 0.08 ab |
|          | 1350          | 0.62 ± 0.16 a  | 11.13 ± 2.64 a | 17.97 ± 0.69 a  | 0.31 ± 0.15 a | 4.91 ± 0.24 ab |

Note: Different lowercase letters (a, b, c) indicate significant differences at different altitudes on Mt. Diaoluo and Mt. Jianfengling ($p < 0.05$).
Redundancy analysis (RDA) indicated that AMF diversity at the level of genus was attributable to both soil conditions and altitude (Figure 5). The total explanation of canonical variables was 39.17%, while the main component 1 explained 22.84% and the main component 2 explained 16.33% on Mt. Diaoluo. The resulting ordination diagram indicated that the occurrence of Glomus was highly correlated with soil carbon and pH. For the study of Mt. Jianfengling, the total explanation of canonical variables was 20.23%, while the main component 1 explained 11.36% and the main component 2 explained 8.87%. The occurrences of unclassified in Archaeosporaceae and unclassified in Glomeromycetes were significantly affected by soil pH. For the two mountains, the soil properties of C, N and C/N had significant impacts on the genera Scutellospora, unclassified in Archaeosporaceae and Paraglomus, while the genera Glomus, Diversispora and Acaulospora were significantly correlated with soil P and pH.

**Figure 5.** Graphical representations of the redundancy analysis (RDA) for AMF genera and soil properties of C, N, P, pH and C/N on Mt. Diaoluo (A), Mt. Jianfengling (B) and the two mountains (C). Genera: Aca (Acaulospora), Arc (Archaeospora), Div (Diversispora), Glo (Glomus), norank, Par (Paraglomus), Scu (Scutellospora), unclassified in Glo (unclassified in Glomeromycetes), unclassified in Arc (unclassified in Archaeosporaceae), unclassified in Gig (unclassified in Gigasporaceae).
4. Discussion

Among terrestrial ecosystems, tropical montane rainforests are areas rich in microbes [30]. Understanding the responses of soil microbial communities to changes in environmental gradients is of great significance for understanding the functions of ecosystems. The role that microorganisms play in shaping ecosystem responses to global change drivers is especially important. Therefore, this study explored community diversity and turnover of AMF taxa in order to explore the role of AMF in tropical mountain ecosystems.

A total of 283 OTUs were identified in roots in tropical forests of Mt. Jianfengling and Mt. Diaoluo. The number of unique OTUs was 173, accounting for 61.13% of the total number for the two tropical montane rainforests. This showed a higher turnover of OTUs and more diverse AMF communities in tropical montane rainforests. In addition, the highest OTU turnover occurred between the highest and lowest altitudes, on Mt. Diaoluo and on Mt. Jianfengling. These results indicate that AMF community structure was affected by both distance and altitude. This is consistent with AMF community richness in individual plots varying with environmental gradients and spatial distances, as described by Davison et al. [13]. This may be due to dispersal limitation, with geographic distance influencing AMF biogeographic patterns [26]. Furthermore, environmental conditions and spatial configurations at different altitudes may also account for the different AMF communities. In addition, studies have shown that AMF communities vary in their environmental preferences and their responses to environmental gradients [31–33], hence the high turnover of AMF observed across altitude gradients in this study.

Glomus was found to be the dominant genus in the two tropical montane rainforests. Similar to this result, Susana et al. suggested that the genus Glomus accounted for 73% of total virtual taxa and was the dominant genus in tropical Africa [14]. Apart from tropical ecosystems, studies of temperate mountain ecosystems have also shown that Glomus was the dominant genus in both morphological investigations and molecular identifications [22,34]. Zhao et al. also indicated that Glomus dominates in all soil types [35]. What is more, Zhao et al. confirmed this result and indicated that Glomus was the dominant genus in different seasons and locations in desert ecosystems [36]. However, different to Glomus, the genus of Archaeospora only occurred at 600 m on Mt. Jianfengling and the genus of Diversispora only appeared at the altitude of 900 m on Mt. Diaoluo (Table 1). This result showed the differences in environmental preferences of AMF. This difference also confirmed the general assumption in microbial biogeography that “Everything is everywhere, but the environment selects” [37]. Moreover, Sousa et al. demonstrated that many AMF taxa are physiologically and ecologically adaptable to specific environments [38], which also indicates that the distribution mechanism of microorganisms is shaped by geography and environment.

In addition to different distributions, the Sobs, Shannon and Pielou diversity indices were also different at each altitude, though altitude had no significant effect on them. Different from this result, Zhang et al. studied AMF diversity in a temperate region of Mt. Taibai and found that altitude had a significant impact on AMF diversity indices [22]. Different results relating to AMF diversity and altitude in tropical and temperate zones may be due to the relatively small temperature change ranges in tropical ecosystems, since altitude gradients affect microbial communities mainly by affecting temperature and precipitation [39]. Therefore, altitude played a negligible role in AMF diversity in tropical mountains in this study. AMF OTU richness ranged from 18.5 to 50 in this study, which was higher than the OTU richness levels recorded for the alpine plateau climate of the Qinghai–Tibet Plateau [40] and for Mt. Segrila [21]. It may be geographic location and climate that have greater influences on AMF richness in tropical montane rainforests. Furthermore, it also may be that tropical montane rainforests tend to have higher diversities of plant species [41]. AMF Shannon diversity in this study was higher than the result of Bonfim’s study in a Brazilian tropical forest [42]. However, it was lower than the results obtained for the temperate mountain forest of Mt. Taibai [22,43]. Pepin et al. have shown that the temperature change ranges of high-altitude mountains are larger than those of
low-altitude mountains [44]. Temperature is considered to be an important predictor of microbial diversity along the entire altitude gradient [45]. Mt. Taibai is more than 3500 m high and its temperature variation range is larger than that of this study; therefore, this may be the reason for its high diversity. Temperature will thus be an important factor in studying the diversity of AMF communities in the next phase of our research.

The interactions between soil microorganisms and plant roots were also found to be key factors shaping AMF communities, so these are important for understanding biodiversity patterns and ecosystem functions [46]. With increasing altitude, temperature and precipitation continued to drop, which led to changes in soil factors. Therefore, altitude probably determined AMF communities by affecting edaphic properties. In this study, the soil concentrations of C and N at the highest altitudes of 900 m and 1350 m were significantly higher than at other altitudes. Tashi et al. used a meta-analysis of global data to establish concentrations of C and N and found that total soil C and N contents significantly increased with altitude [47]. However, studies have shown that nutrient addition caused decreases in AMF diversity and abundance [27,48,49]. Corresponding changes in soil factors have different degrees of impact on AMF community and diversity [49–51]. Differences in concentrations of nutritional factors may lead to the loss of some fungal species and promote the dominance of certain AMF species [52]. In this study, the genus of Diversispora only occurred at the altitude of 900 m on Mt. Diaolou. Moreover, RDA analysis showed that there was a significant correlation between soil P concentration and the occurrence of Diversispora. Gao’s study also showed that different phosphorus concentrations had significant impacts on AMF community structure [53]. Some studies have found that root systems can regulate symbioses with AMF according to their own nutritional statuses, which is called “self-regulation” [54,55]. When phosphorus supply is sufficient, roots directly absorb phosphorus from the soil. When the supply of phosphorus is insufficient, roots can undergo symbioses with AMF and thus facilitate hyphae to absorb phosphorus [56–58]. Therefore, this also confirms that soil phosphorus concentration plays an important role in AMF community structure.

In this study, the genera of Glomus, Archaeosporaceae, norank and Acaulospora were significantly affected by soil pH. In the process of AMF community formation, the importance of pH may be due to its direct impact on plant species with different pH tolerance ranges [59]. Different plant species have different affinities for AMF genera, which explains why some genera have a high correlation with soil pH, while the occurrence of some genera is not affected by soil pH. Xu et al. and Rozek et al. also suggested that soil pH was the strongest predictor of AMF richness and that it provides important information for AMF community changes [26,60]. Xu et al. have shown that different soil pH levels may lead to changes in AMF community structure, and this conclusion was consistent with the results of this study [31]. Therefore, soil pH may be the main selective factor influencing AMF community composition [61,62]. Studies have also suggested that AMF taxa differ in their habitat preferences and responses to local edaphic properties [15,63,64]. Similar results were obtained in our study, showing that AMF taxa have different environmental preferences and high turnover in tropical montane rainforests.

5. Conclusions

There is abundant AMF diversity in tropical montane rainforests, and the number of unique OTUs was shown to be high between the two mountains included in this study and at different altitudes, revealing a high turnover of AMF OTUs and diverse AMF communities in these tropical montane rainforests. Altitude probably determined AMF communities by affecting edaphic properties, since different AMF genera have different correlations with soil factors. Further, the environmental preferences of AMF resulted in their limited dispersal abilities in these tropical montane rainforests.
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