forests

MDPI

Article

Impact of Aspect on Arbuscular Mycorrhizal Fungal Diversity and Community Composition in a Natural *Toona ciliata* var. *pubescens* Forest in Subtropical China

Qiong Wang [1,2], Jianfeng Pan [1,2,3], Yunfang Ke [1,2], Suqin Yu [1,2], Philip J. Murray [4], Tianyu Luo [1,2], Lu Zhang [1,2,*] and Wei Liu [1,2,*]

1 Jiangxi Provincial Key Laboratory of Silviculture, Jiangxi Agricultural University, Nanchang 330045, China
2 College of Forestry, Jiangxi Agricultural University, Nanchang 330045, China
3 Nature Protected Area Construction Center of Jiangxi, Nanchang 330036, China
4 School of Agriculture, Food and Environment, Royal Agricultural University, Cirencester GL7 6JS, UK
* Correspondence: zhanglu856@mail.jxau.edu.cn (L.Z.); w_liu08@163.com (W.L.)

Abstract: The aspect can affect plant communities and soil physical and chemical properties through different microclimates. However, little is known about the effect of aspect on arbuscular mycorrhizal (AM) fungal diversity and community composition, although AM fungi are beneficial for plant nutrient absorption and natural restoration. In this study, AM fungal community and diversity distribution patterns in the rhizosphere soil and roots of seven widespread plants in a natural *Toona ciliata* var. *pubescens* (*Tc*) forest on the north-facing (NF) aspect and south-facing (SF) aspect were investigated using Illumina PE250 high-throughput sequencing in the Guanshan National Nature Reserve, Jiangxi Province, China. Our results exhibited that aspect did not affect AM fungal diversity but significantly affected AM fungal community structure and composition. Glomeraceae was the most common and abundant family in the *Tc* natural forest. The Glomeromycota sequence proportion of root AM fungal community was significantly larger on NF than on SF ($p < 0.05$). The relative abundance of Acaulosporaceae of root AM fungal community differed significantly with aspect, being greater on NF than on SF ($p < 0.05$). In addition, the number of Glomeromycota sequences was significantly larger on SF than on NF, while the number of OTUs and the relative abundance of unclassified fungi in rhizosphere soil in *Tc* showed the opposite trend ($p < 0.05$). The soil properties (organic matter, nitrogen, potassium, phosphorus, and pH) were significantly correlated with these changes. These findings indicate that the habitat of NF with low insolation, high soil moisture, and high nutrient content might promote the functional realization of AM fungi; the habitat of SF with high insolation, low soil moisture, and low soil nutrient content might be beneficial for the proliferation and preservation of AM fungal groups. This study provides important information on the ecological processes of AM fungal community construction and microbiological mechanisms in natural *Tc* forests.

Keywords: *Toona ciliata* var. *pubescens*; arbuscular mycorrhizal fungi; aspect; soil properties

Citation: Wang, Q.; Pan, J.; Ke, Y.; Yu, S.; Murray, P.J.; Luo, T.; Zhang, L.; Liu, W. Impact of Aspect on Arbuscular Mycorrhizal Fungal Diversity and Community Composition in a Natural *Toona ciliata* var. *pubescens* Forest in Subtropical China. *Forests* **2022**, *13*, 2100. https://doi.org/10.3390/f13122100

Academic Editor: Benjamin L. Turner

Received: 31 October 2022
Accepted: 7 December 2022
Published: 9 December 2022

1. Introduction

Biodiversity is an important feature of life, and species composition and community construction have long been core issues in ecological research [1,2]. Indeed, the biodiversity and structural composition and distribution of communities are closely related to ecosystem multifunctionality [2]. Topography is one of the most major natural factors that affects biodiversity. Topography belongs to biogeography and affects numerous ecological functions and processes, such as soil erosion and water storage [3], weathering and nutrient leaching [4], and migration and storage of soil organic matter [5].

Aspect (shady and sunny aspects) is a feature of natural geographical mountain landscapes [6]. Shady and sunny aspects are different habitats with climate and microen-

vironment differences that form a natural environmental gradient [7]. The difference in climate conditions between shady and sunny aspects leads to profound changes in vegetation, soil, landform, hydrology, and other factors [8,9]. The shady aspects are darker, wetter, and colder, with less light radiation, and the climate characteristics on sunny aspects are the opposite [10]. Changes in biodiversity distribution and community construction on shady and sunny aspect gradients, as well as the relationships between environmental factors and biological communities, can reflect the roles and contributions of various biological, physical, and chemical factors.

Most ecological studies on different aspects have predominantly focused on soil stoichiometry characteristics, vegetation distribution and succession, plant community diversity and structure, aboveground plant biomass changes, and plant functional traits [11–13]. Zhao et al. [14] focused on vegetation–microorganism–aspects complexes and concluded that soil microbial activity in mountain ecosystems was profoundly affected by these complexes. However, previous studies have not fully solved the problem of how aspects affect biodiversity directly or indirectly, and little is known about the coexistence of species and mechanisms of community construction. Therefore, it is necessary to study different aspects in typical mountain ecosystems and reveal the relationships among environmental factors (e.g., light, temperature, and soil's physical and chemical properties), biological factors (e.g., plant community composition, stoichiometric characteristics, and functional traits), and community composition and distribution rules of specific biotas.

Toona ciliata Roem. var. *pubescens* (Franch.) Hand.-Mazz. (*Tc*) is regarded as a national secondary protected plant in China. Moreover, *Tc* is an endangered species and a valuable woody plant of the *Toona* genus in the Meliaceae family [15]. Owing to a long period of exhaustive utilization and exploitation, habitats of *Tc* forest are affected by these effects and have disadvantageous factors for natural regeneration, meaning that the distribution area of natural *Tc* forests is reduced and fragmented [16]. Wild *Tc* is predominantly distributed on both sides of valleys and streams in virgin forests in subtropical China, breeding predominantly by seeds and clonal tillers [15]. Their special habitat preferences and breeding strategies may be the dominant cause of this endangerment. In recent years, scientists have addressed the problems of the natural regeneration of *Tc* forests [17], and found that transmission barriers [18] and microhabitat restrictions [19] might be major causes of failure of *Tc* regeneration. Sensitive habitat selection and lower forest regeneration capacity have caused difficulties in the protection and restoration of wild *Tc* forests [20]. Microhabitats involve many environmental factors, such as water, microorganisms, light, and soil. The growth of little trees is affected by these environmental factors, and there are large variations in the key factors of various plants. Soil microorganisms play a major role in seedling growth, improving soil quality, and promoting natural regeneration in *Tc* forests [20]. Huang et al. [21] found that soil pathogen infection was the dominant reason for seed rot and the natural regeneration failure of *Tc*. As an important soil microorganism, arbuscular mycorrhizal (AM) fungi has a symbiotic association with over 80% of terrestrial plants [22], and can effectively promote plant growth and natural regeneration of *Tc* [23,24]. The symbiotes formed by AM fungi and plants give rise to vital roles in plant nutrient absorption and resistance improvement to external adverse environments at the ecosystem or individual scale [22,25]. Wild *Tc* forests are largely distributed in habitats which have poor light and soil conditions, and *Tc* seedlings are often hindered by other plants during the initial growth stage [21]. Considering this, AM fungi may be important in wild *Tc* forests, as AM fungi can promote plant development and growth by increasing the chlorophyll content in plant leaves, improving photosynthesis, promoting C assimilation and metabolism of plants [26], and improving the competitiveness of plants in natural habitats.

Currently, the study of different aspect-related soil microbial community compositions and their distribution characteristics can only provide some sporadic information [27,28] and only a few studies have investigated the impact of aspect on AM fungal community structure. Chu et al. [28] reported the community composition of AM fungi on sunny and shady aspects in boreal forests of the Greater Khingan Mountains. Similarly, one study

reported the distribution of AM fungi on sunny and shady aspects in the arid ecosystem of Inner Mongolia [29]. However, the above studies covered only a few ecosystem types, and studies on particular species and habitats are lacking, limiting our insight into the community-building mechanisms associated with AM fungi. Shady and sunny aspects are comprehensive natural environmental gradients, and the AM diversity and relationship among AM fungi, environmental factors, and plant communities can reveal the comprehensive effects in natural *Tc* forests.

Considering the particularity of wild *Tc* forest distribution regions and the important role of AM fungi in natural regeneration and growth of wild *Tc* forests, this study aimed to reveal the community characteristics and biogeographic distribution rules (sunny and shady aspects) of AM fungi in a wild *Tc* forests. A gradient of shady and sunny aspects in the Guanshan National Nature Reserve in Jiangxi Province was selected as the research site. Field investigations of AM fungal community and diversity distribution patterns in the roots and rhizosphere soils of seven widespread plants in natural *Tc* forest were analyzed using the Illumina PE250 high-throughput sequencing method. The rich, continuous environmental (biotic and abiotic) gradients and distinct vegetation types in this region provide an excellent opportunity to discover the dynamics of AM fungi on different aspects in natural *Tc* forests. Our results will provide important information on the ecological processes of AM fungal community construction in natural *Tc* forests and a reference for discussing the microbiological mechanism of adaptation of Tc to microhabitats. The null hypothesis was that *Tc* in shady and sunny aspects would be associated with similar AM fungi diversity and composition, but that *Tc* recruited significantly different AM fungi compared to other plants. Changes in soil chemical properties and tree species may be responsible for these variations. The findings of this study will improve our ability to understand ecosystem dynamics and function in wild *Tc* forests and facilitate scientific protection and cultivation strategy decisions on subtropical mountain ecosystems in China.

2. Materials and Methods

2.1. Study Site

The Guanshan National Nature Reserve (28°30′–28°40′ N, 114°29′–114°45′ E) is located in the western part of the Jiuling Mountains in the northwest Jiangxi Province, China. It was one of the first national nature reserves in Jiangxi Province and belongs to the subtropical evergreen broad-leaved forest ecosystem. The mean annual temperature is 17.2 °C. The mean annual precipitation is 1680.2 mm. The total area is 11,500.5 ha, comprising 3621.1 ha in the core area, 1466.4 ha in the buffer zone, and 6413.0 ha in the experimental area. The soil type of this study area was typical red soil.

2.2. Experimental Design and Sampling

The natural *Tc* forest community is predominantly distributed in a subtropical evergreen broad-leaved forest (Figure 1). The *Tc* is dominantly distributed sporadically on both sides of valleys and streams and on both sides of the aspects with different orientations and illumination and hydrothermal conditions. The photophilous tree species is mainly distributed in the arborous layer. A field survey was started at 2017, and eight experimental sample plots of 20 × 20 m were set up in a natural *Tc* forest (north-facing [NF] aspect—4, and south-facing [SF] aspect—4). In the sample plot, we selected seven dominant plants that were distributed in NF and SF, as follows: *Toona ciliates* (*Tc*), *Padus buergeriana* (*Pb*), *Maesa japonica* (*Mj*), *Melliodendron xylocarpum* (*Mx*), *Ilex chinensis* (*Ic*), *Alniphyllum fortunei* (*Af*), and *Mallotus japonicas* (*Mj2*). At least one individual sample from each plant was selected in each plot. Five individual root samples and five individual rhizosphere soil samples were randomly selected from each plant, respectively. Fine fibrous roots in the 0–20 cm soil depth were collected from the root samples, and 1–2 kg of soil around the root was collected with a clean small steel shovel to extract AM fungal spores. Finally, 70 root samples and 70 rhizosphere soil samples per plot (2 aspects × 7 plants × 5 individuals) were collected. The collected fine roots were rinsed with water and stored at −80 °C for

DNA extraction. The collected soil samples were passed through a 2 mm sieve to eliminate stones, roots, and litter for DNA extraction (−80 °C) and soil experiments (air-dried soil, 0.149 mm mesh).

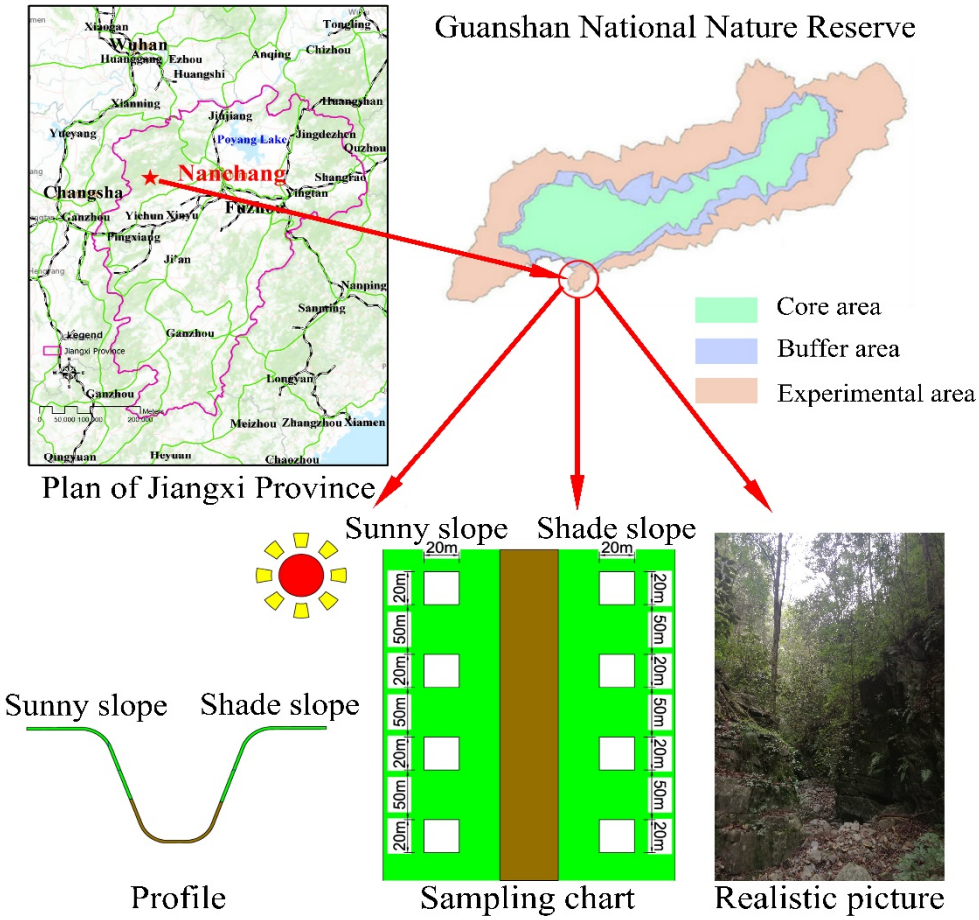

Figure 1. Location of Guanshan National Nature and distribution of study sites.

2.3. Soil Chemical Property Determination

The soil pH was measured using a glass electrode at 1:2.5 (*w*/*v*). The soil organic matter (SOM, $g \cdot kg^{-1}$) content was measured using the $K_2Cr_2O_7$ heating method. Soil available phosphorus (AP) content was determined after extraction with 0.5 $mol \cdot L^{-1}$ $NaHCO_3$. Total potassium (TK) content was extracted using a 1 M NH_4OAc solution (pH 7.0) and measured by flame photometry. Total phosphorus (TP) content was determined using the H_2SO_4-$HClO_4$ heating digestion method, and the total nitrogen (TN) content was measured using the Kjeldahl method. The detailed steps can be found in SD Bao [30].

2.4. DNA Extraction Method

Soil DNA was extracted using a Fast DNA SPIN Kit (MP Biomedicals LLC, Santa Ana, CA, USA) according to the instructions and using 0.5 g fresh soil samples. Root DNA was extracted using a Fast Plant Kit (Beijing Tiangen) according to the instructions and using 0.05 g frozen root samples. The total concentration of the extracted soil/root DNA in each soil or root sample was determined using a NanoDrop ND-8000 spectrophotometer (NanoDrop, Wilmington, DE, USA). The DNA quality was checked by 1% agarose gel electrophoresis, diluted to 10–20 $ng \cdot \mu L^{-1}$ with ultrapure water, and preserved at −20 °C in a refrigerator for molecular biological analysis.

2.5. PCR Reaction Method

To guarantee the reliability and accuracy of the further data analysis, the PCR reaction needs to meet the following two requirements: (1) use a small number of amplification cycles and (2) ensure that the number of cycles amplified by each sample is consistent. The SSU rDNA of AM fungi in soil and plant roots was amplified using AM FUNGI-specific primers AMV4.5NF-F (5′-AAGCTCGTAGTTGAATTTCG-3′) and AMDGR-R (5′-CCCAACTATCCCTATTAATCAT-3′). Then, PCR was performed using TransStart Fastpfu DNA Polymerase, with a 20 µL reaction system. Each sample was repeated three times. The reaction system is shown in Table 1.

Table 1. PCR amplification system used in Illumina PE250 high-throughput sequencing.

PCR Amplification System (20 µL)	
5 × FastPfu Buffer	4 µL
2.5 mM dNTPs	2 µL
Forward Primer (5 µM)	0.8 µL
Reverse Primer (5 µM)	0.8 µL
FastPfu Polymerase	0.4 µL
Template DNA	10 µL
Add ddH_2O	2 µL

The PCR reaction system was mixed mildly and put into the PCR instrument (ABI GeneAmp® 9700 type), and the reaction conditions were as follows: (1) 5 min of incipient denaturation at 95 °C; (2) 30 s of denaturation at 95 °C; (3) 30 s of annealing at 55 °C; (4) 45 s of elongation at 72 °C; (5) 27 cycles from step 4 to step 2; and (6) incubation at 72 °C for 10 min. Each sample was subjected to three replicates, and the PCR products of the same sample were blended and detected using 2% agarose gel electrophoresis.

2.6. Illumina High-Throughput Sequencing and Bioinformatics

The PCR products were subjected to 1% agarose gel electrophoresis in 1 × TAE buffer (added with Biotake producing a green as blue nucleic acid dye). After electrophoresis at 125 V for 30 min, the PCR results of the gel were observed under UV light. Then, the target band for recovering the PCR products was cut off using a AxyPrepDNA gel recovery kit (AXYGEN), which was eluted with Tris-HCl and detected by 2% agarose electrophoresis. The PCR products were observed and identified using 2% agarose gel, while the electrophoresis condition was 120 V for 40 min. The target bands were separated by electrophoresis, cut, and purified (Aidlab Biotechnologies Co., Ltd., Tianjin, China). The purified product was quantified, homogenized using a TBS-380 fluorescence spectrometer, and sequenced using the Illumina PE250 high-throughput sequencing platform (Shanghai BIOZERON Co., Ltd., Shanghai, China) referring to standard methods. A 97% identity threshold was used to group sequences into operational taxonomic units (OTUs) [31]. The largest ample sequence from each OTU was regarded as the typical sequence for the OTU. The Usearch (version 7.1 http://drive5.com/uparse/, accessed on 25 December 2017) software was used to cluster sequences. Taxonomy was allotted to the fungal OTUs against a subset of the Silva 104 database (http://www.arb-silva.de/download/archive/qiime/, accessed on 28 December 2017). The GenBank (http://www.ncbi.nlm.nih.gov/, accessed on 31 December 2017) for the OTUs was used to confirm the typical sequences that could not be recognized at the family or class level in the fungal database. The original sequences described here can be accessed through GenBank with SRP277481 and accession numbers SAMN15815414 to SAMN15815425 and SAMN15815492 to SAMN15815503.

2.7. Calculation Methods of Diversity Correlation Indexes Data Analysis

We used the Chao index to calculate the total number of species in an ecosystem [32]. Here, Chao 1 was used to calculate the number of OTUs, as follows:

$$S_{chao1} = S_{obs} + \frac{n_1(n_1 - 1)}{2(n_2 + 1)}$$

where S_{chao1} is the number of OTUs, S_{obs} is the number of observed OTUs, n_1 is the number of OTUs containing only one sequence (singletons), and n_2 is the number of OTUs containing only two sequences (doubletons).

The Simpson index was used to quantitatively calculate the microbial diversity [33]. The larger the Simpson index, the lower the community diversity, as follows:

$$D_{simpson} = \frac{\sum_{i=1}^{S_{obs}} n_i(n_i - 1)}{N(N - 1)}$$

where S_{obs} is the number of observed OTUs, n_i is the sequence number of OTU-i, and N is the total number of sequences.

The Shannon index (i.e., the Shannon–Weiner index) was also applied to quantitatively calculate the microbial diversity. The larger the Shannon index, the greater the community diversity, as follows:

$$H_{shannon} = -\sum_{i=1}^{S_{obs}} \frac{n_i}{N} \ln \frac{n_i}{N}$$

where S_{obs} is the number of observed OTUs, n_i is the sequence number of OTU-i, and N is the total number of sequences.

Subsequently, the coverage index was calculated as follows:

$$C = 1 - \frac{n_1}{N}$$

where C is the coverage of OTUs, n_1 is the number of OTUs containing only one sequence (singletons), and N is the total sequence.

2.8. Data Analysis

Firstly, the normal distribution test is carried out on all the data used in the analysis. If the data does not conform to the normal distribution, the natural logarithm or trigonometric function is used for data conversion. Secondly, the Duncan's multiple comparative analysis (version 26.0; IBM, Armonk, NY, USA) was conducted to analyze the variations in soil chemical properties (pH, SOM, AP, TK, TP, and TN), soil and root AM fungi community diversity (the Chao, Shannon, Simpson, and coverage indices), and sequence parameters (the total sequence number, the number of Glomeromycota sequences, the proportion of Glomeromycota sequences, the number of OTUs, and the relative abundance of the below-ground AM fungi community sequence) in *Tc* natural forest in different aspects. Pairwise comparative analysis was used to compare the difference of the corresponding value between the north and south aspects. Thirdly, there were, respectively, 18 response factors for AM fungal community characteristics in soil and root. Through factor analysis, 6 types of comprehensive factors (which mainly reflected the information of 11 key factors) were used to reflect the information of 18 response factors, so as to reduce the dimension, and the key response factors were determined for further analysis. Therefore, the redundancy analysis (RDA) was applied to analyze the relationships between soil environmental variables and the key characteristics of the AM fungal communities by using the standardized data (Canoco 5.0, Biometrics Wageningen, The Netherlands).

3. Results

3.1. Soil Chemical Properties

In general, soil TN, TP, and AP differed significantly with aspect, being greater on NF than on SF ($p < 0.05$, Table 2); moreover, the significant effects mainly showed in soils of *Tc*, *Pb*, *Ic*, and *Mj* (Figure 2). However, there was no significant effect of species on soil TN and TP ($p > 0.05$, Table 2). Although soil TK showed no obvious difference with aspect ($p > 0.05$), significant species effect and interaction were found in Table 2 and Figure 2 ($p < 0.05$). For example, the soil TK of *MJ2* was significantly higher than other trees on SF. Additionally, regarding soil pH and SOM, there was no significant difference between SF and NF ($p > 0.05$, Table 2 and Figure 2).

Table 2. Results of variance analysis for the effects of aspect and species on the variables.

Variables	Aspect		Species		Aspect × Species	
	F	*p*	F	*p*	F	*p*
TN	4.519	0.045	0.363	NS	1.074	NS
TK	0.117	NS	2.876	0.023	2.555	0.039
pH	0.694	NS	2.291	NS	1.147	NS
SOM	0.208	NS	1.107	NS	0.099	NS
TP	3.701	0.044	1.063	NS	0.449	NS
AP	9.150	0.005	4.102	0.004	2.689	0.031
S-Sequence	3.332	NS	0.634	NS	0.846	NS
S-Glomeromycota sequence	0.465	NS	1.092	NS	2.373	0.049
S-Glomeromycota sequence proportion	0.577	NS	0.872	NS	1.314	NS
S-OTUs	0.203	NS	5.683	0.000	1.033	NS
S-Chao	2.716	NS	1.758	NS	0.184	NS
S-Shannon	2.576	NS	1.964	NS	0.122	NS
S-Simpson	0.672	NS	2.097	NS	0.226	NS
S-Coverage	0.065	NS	2.764	0.026	0.551	NS
R-Sequence	6.472	0.015	1.969	NS	0.792	NS
R-Glomeromycota sequence	3.986	NS	0.676	NS	0.800	NS
R-Glomeromycota sequence proportion	10.116	0.003	1.664	NS	1.109	NS
R-OTUs	3.725	NS	1.536	NS	0.171	NS
R-Chao	0.462	NS	5.100	0.001	1.134	NS
R-Shannon	0.426	NS	1.961	NS	0.989	NS
R-Simpson	0.179	NS	0.839	NS	0.744	NS
R-Coverage	3.323	0.031	5.003	0.009	0.720	NS

Abbreviations are as follows: S, soil; R, root; NS means that there is no significant difference ($p > 0.05$).

3.2. Sequence Characteristics of AM Fungi

The basic sequence information of soil AM fungal communities showed no significant difference with aspect ($p > 0.05$) (Tables 2 and 3). Due to the Glomeromycota sequences making up the largest proportion of the total sequences, we conducted the related analysis (Table 3). Although the number of Glomeromycota sequences showed no obvious difference with aspect ($p > 0.05$), a significant interaction between aspect and species was found in Tables 2 and 3 ($p < 0.05$). The number of Glomeromycota sequences of *Tc* and *Mj2* was significantly higher on SF than on NF ($p < 0.05$, Table 3).

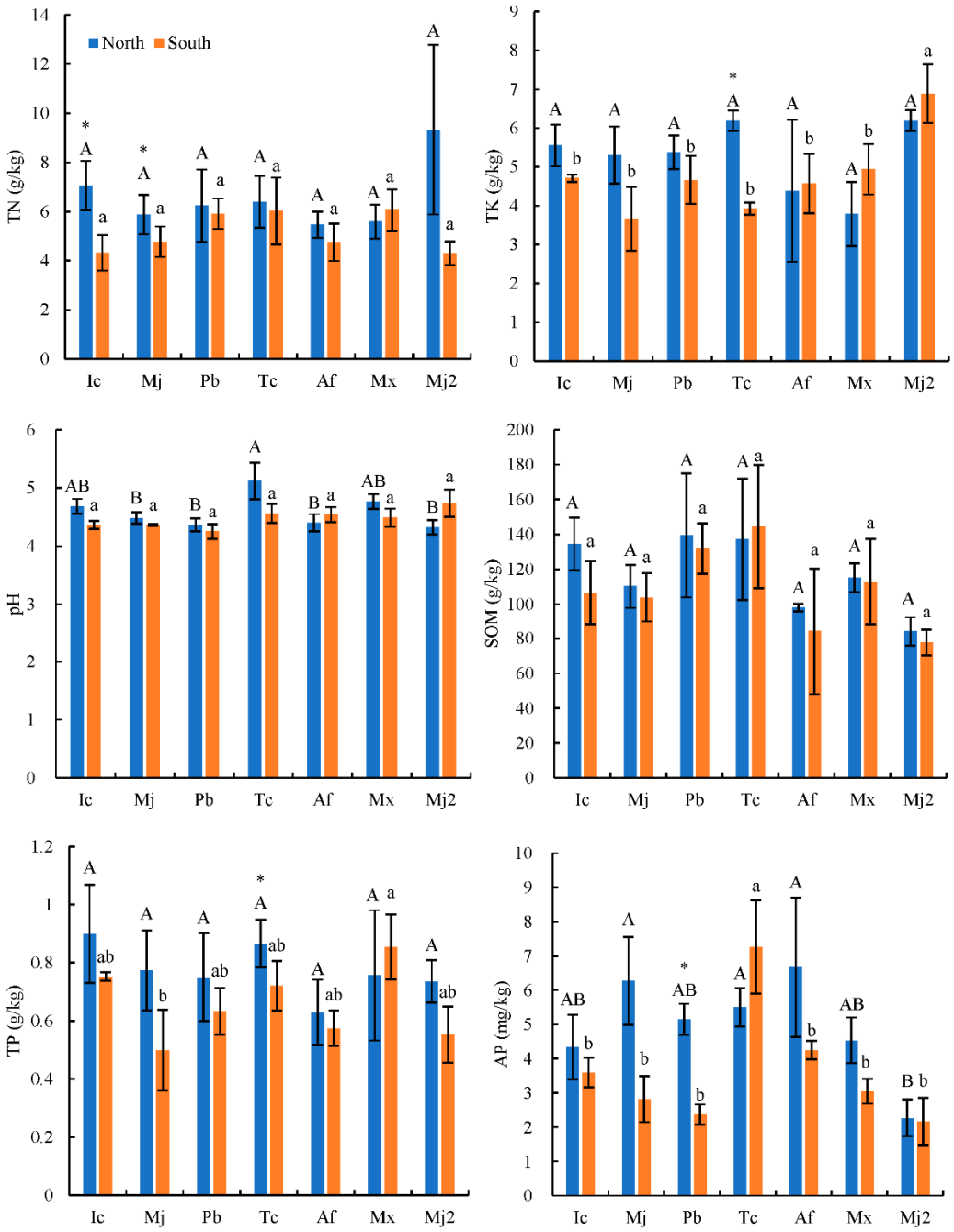

Figure 2. Soil chemical properties (AP, TK, SOM, pH, TP, and TN) in *Tc* natural forest in different aspects. The error line is the standard error. Data with different capital letters means significant difference in NF at $p < 0.05$. Data with different lowercase letters means significant difference in SF at $p < 0.05$; * means that there is significant difference between SF and NF. Abbreviations are as follows: Tc, *Toona ciliates*; Pb, *Padus buergeriana*; Mj, *Maesa japonica*; Mx, *Melliodendron xylocarpum*; Ic, *Ilex chinensis*; Af, *Alniphyllum fortunei*; Mj2, *Mallotus japonicas*.

The sequence information of the root AM fungal communities in *Tc* natural forest at different aspects is shown in Table 4. On the whole, the number of sequence differed significantly with aspect ($p < 0.05$, Tables 2 and 4), being greater on SF than on NF. However, there was no significant effect of species on the number of sequence ($p > 0.05$, Table 4). The Glomeromycota sequence proportion showed significant differences between SF and NF ($p < 0.05$, Tables 2 and 4). Moreover, the significant effect of species on the Glomeromycota sequence proportion was found in *Mx* ($p < 0.05$, Table 4).

Table 3. Basic sequence information of soil AM fungal communities in *Tc* natural forest in different aspects.

Species	Aspects	The Number of Sequence	The Number of Glomeromycota Sequence	Glomeromycota Sequence Proportion (%)
Toona ciliates	South	50,890 (±2465) a	15,143 (±241) a	29.86 (±1.08) a
	North	40,187 (±3655) A	12,062 (±2219) A *	30.63 (±6.19) A
Padus buergeriana	South	40,673 (±2257) a	12,076 (±1251) b	29.46 (±1.78) a
	North	37,203 (±2745) A	14,939 (±387) A	40.93 (±3.59) A *
Maesa japonica	South	44,598 (±4338) a	14,748 (±345) a	33.66 (±3.08) a
	North	39,575 (±7654) A	15,104 (±200) A	39.75 (±8.19) A
Melliodendron xylocarpum	South	49,182 (±5272) a	14,960 (±224) a	31.13 (±3.39) a
	North	35,266 (±7225) A	15,320 (±96) A	51.71 (±13.96) A
Ilex chinensis	South	49,826 (±3474) a	14,698 (±302) a	30.00 (±2.45) a
	North	40,715 (±5318) A	15,044 (±418) A	37.91 (±3.75) A
Alniphyllum fortunei	South	42,443 (±5429) a	14,584 (±402) a	36.43 (±5.48) a
	North	48,785 (±5089) A	13,194 (±1122) A	28.59 (±5.07) A
Mallotus japonicus	South	39,661 (±12,721) a	15,486 (±65) a	53.09 (±22.60) a
	North	38,330 (±3760) A	13,652 (±405) A *	36.42 (±2.97) A

Note: Data are exhibited as the mean ± standard error. Data with different capital letters means significant difference in NF at $p < 0.05$. Data with different lowercase letters means significant differences in SF at $p < 0.05$; * means that there is significant difference between SF and NF.

Table 4. Basic sequence information of root AM fungal communities in *Tc* natural forest in different aspects.

Species	Aspects	The Number of Sequence	The Number of Glomeromycota Sequence	Glomeromycota Sequence Proportion (%)
Toona ciliates	South	49,755 (±4673) a	30,186 (±70) a	62.28 (±5.72) a
	North	50,058 (±3202) AB	30,068 (±122) A	60.88 (±4.28) AB
Padus buergeriana	South	50,257 (±2626) a	29,920 (±157) a	60.06 (±3.35) a
	North	52,982 (±2217) A	30,126 (±89) A	57.16 (±2.40) B
Maesa japonica	South	53,405 (±655) a	29,985 (±136) a	56.18 (±0.91) a
	North	45,872 (±5774) AB	30,119 (±97) A	68.95 (±8.83) AB
Melliodendron xylocarpum	South	48,572 (±4766) a	28,840 (±1099) a	61.66 (±7.98) a
	North	42,850 (±5793) AB	30,185 (±30) A	74.13 (±9.20) AB *
Ilex chinensis	South	53,724 (±1835) a	29,754 (±69) a	55.59 (±2.00) a
	North	45,667 (±3392) AB	30,200 (±83) A *	67.20 (±4.82) AB
Alniphyllum fortunei	South	48,351 (±1445) a	28,635 (±1425) a	59.30 (±3.02) a
	North	39,375 (±3677) AB	30,124 (±118) A	78.25 (±6.26) AB
Mallotus japonicus	South	45,732 (±1246) a	29,803 (±302) a	65.31 (±1.88) a
	North	38,492 (±4235) B	30,015 (±166) A	80.64 (±8.23) A

Note: Data are exhibited as the mean ± standard error. Data with different capital letters means significant difference in NF at $p < 0.05$. Data with different lowercase letters means significant differences in SF at $p < 0.05$; * means that there is significant difference between SF and NF.

3.3. OTUs Analysis of AM Fungi

Although the relative OTU abundance of AM fungal communities of soil and root showed no difference between SF and NF ($p > 0.05$), the significant effect of species was found in soil ($p < 0.05$) (Tables 2 and 5). Moreover, we found that the relative OTU abundance in rhizosphere soil of *Tc* was significantly higher for NF than for SF ($p < 0.05$, Table 5).

3.4. Analysis of AM Fungal Community Composition

At the family level, the relative abundance of each composition changed obviously in the soil AM fungal community in the *Tc* natural forest at different aspects (Figure 3). Glomeraceae was the most common and plentiful family in the *Tc* natural forest soil, but there was no significant difference between SF and NF ($p > 0.05$, Tables A1 and A2). The

relative abundance of unclassified fungi showed a significant interaction between aspect and species ($p < 0.05$, Table A2).

Table 5. Relative OTU abundance of AM fungal communities on different aspects in *Tc* natural forest.

Species	South		North	
	Root	Soil	Root	Soil
Toona ciliata	15.1% a	13.7% bc	11.3% B	16.4% AB *
Maesa japonica	21.7% a	17.9% a	23.0% A	18.6% A
Padus buergeriana	12.3% a	16.0% ab	14.3% AB	16.2% ABC
Melliodendron xylocarpum	14.0% a	10.6% c	16.6% AB	12.5% BC
Alniphyllum fortunei	12.0% a	13.9% bc	13.6% B	11.0% C
Ilex chinensis	9.8% a	13.1% bc	8.3% B	12.5% BC
Mallotus japonicus	15.1% a	14.8% ab	12.9% B	12.8% BC

Note: Data with different capital letters means significant difference in NF at $p < 0.05$. Data with different lowercase letters means significant difference in SF at $p < 0.05$; * means that there is significant difference between SF and NF.

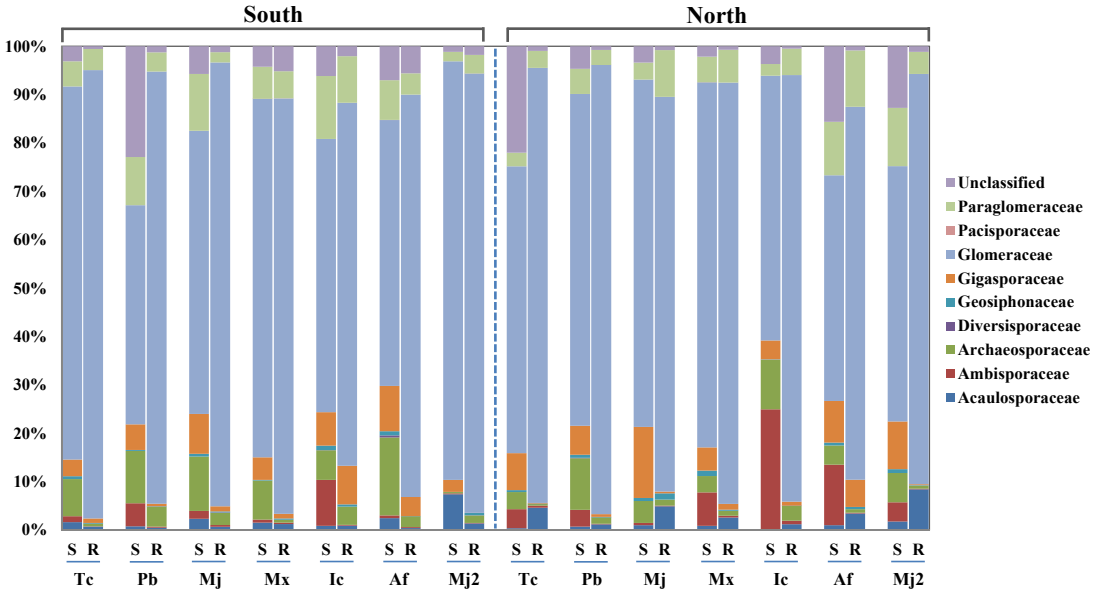

Figure 3. The AM fungal community composition in *Tc* natural forest (at the family level) in different aspects. Tc represents *Toona ciliates*; Pb represents *Padus buergeriana*; Mx represents *Melliodendron xylocarpum*; Mj represents *Maesa japonica*; Ic represents *Ilex chinensis*; Af represents *Alniphyllum fortunei*; Mj2 represents *Mallotus japonicas*.

Similarly, Glomeraceae was the most common and abundant AM fungi group in the *Tc* natural forest root (Figure 3). The relative abundance of Glomeraceae in the *Tc* root was 92.68% in SF, and it was 90.10% in NF. Generally, the relative abundance of Acaulosporaceae differed significantly with aspect, being greater on NF than on SF ($p < 0.05$, Tables A1 and A2, Figure 3). However, there was no significant effect of species on the relative abundance of Acaulosporaceae ($p > 0.05$, Table A2). In addition, the relative abundance of Archaeosporaceae showed significant differences among different species ($p < 0.05$, Tables A1 and A2, Figure 3).

3.5. Diversity of AM Fungi Community

The diversity of the soil and root AM fungal communities in the *Tc* natural forest at different aspects is shown in Tables 6 and 7. The coverage index of the root AM fungal communities differed significantly with aspect ($p < 0.05$, Tables 2 and 7), being greater on SF than on NF. Moreover, there was significant effect of species on the coverage index of the soil and root AM fungal communities ($p < 0.05$, Tables 2, 6 and 7). Although the Chao index

of the root AM fungal communities showed no obvious difference with aspect ($p > 0.05$, Table 2), a significant species effect was found in Tables 2 and 7 ($p < 0.05$). In addition, the Shannon and Simpson indices of the soil and root AM fungal community found no significant difference between SF and NF ($p > 0.05$).

Table 6. The diversity of soil AM fungal communities in *Tc* natural forest in different aspects.

Species	Aspects	Chao	Shannon	Simpson	Coverage
Toona ciliates	South	421 (±156) a	3.67 (±0.67) ab	0.07 (±0.05) a	0.9986 (±0.0005) ab
	North	236 (±73) B	3.03 (±0.36) A	0.10 (±0.03) A	0.9990 (±0.0004) AB
Padus buergeriana	South	356 (±139) a	3.81 (±0.36) ab	0.05 (±0.02) a	0.9985 (±0.0006) ab
	North	312 (±65) AB	3.28 (±0.46) A	0.11 (±0.05) A	0.9985 (±0.0004) AB
Maesa japonica	South	630 (±86) a	4.35 (±0.10) a	0.03 (±0.00) a	0.9972 (±0.0000) b
	North	505 (±98) A	3.91 (±0.07) A	0.04 (±0.01) A	0.9971 (±0.0014) B
Melliodendron xylocarpum	South	367 (±86) a	3.85 (±0.10) ab	0.04 (±0.01) a	0.9989 (±0.0003) a
	North	353 (±93) AB	3.56 (±0.31) A	0.06 (±0.01) A	0.9977 (±0.0010) AB
Ilex chinensis	South	272 (±170) a	2.80 (±0.75) b	0.18 (±0.10) a	0.9992 (±0.0005) a
	North	161 (±26) B	2.72 (±0.48) A	0.15 (±0.06) A	0.9995 (±0.0001) A
Alniphyllum fortunei	South	337 (±72) a	3.70 (±0.18) ab	0.04 (±0.01) a	0.9987 (±0.0003) a
	North	301 (±37) AB	3.41 (±0.14) A	0.08 (±0.02) A	0.9989 (±0.0001) AB
Mallotus japonicus	South	382 (±83) a	3.85 (±0.10) ab	0.04 (±0.01) a	0.9985 (±0.0002) ab
	North	277 (±45) B	3.68 (±0.25) A	0.05 (±0.01) A	0.9985 (±0.0003) AB

Note: Data are exhibited as mean ± standard error. Data with different capital letters means significant difference in NF at $p < 0.05$. Data with different lowercase letters means significant difference in SF at $p < 0.05$.

Table 7. The diversity of root AM fungal communities in *Tc* natural forest in different aspects.

Species	Aspects	Chao	Shannon	Simpson	Coverage
Toona ciliates	South	409 (±52) bc	3.52 (±0.29) a	0.08 (±0.02) a	0.9988 (±0.0002) ab
	North	466 (±63) AB	3.93 (±0.05) A	0.05 (±0.01) A	0.9983 (±0.0002) A
Padus buergeriana	South	471 (±18) ab	3.88 (±0.08) a	0.05 (±0.01) a	0.9985 (±0.0002) ab
	North	463 (±40) AB	3.80 (±0.18) A	0.05 (±0.01) A	0.9984 (±0.0002) A
Maesa japonica	South	534 (±34) a	4.11 (±0.17) a	0.04 (±0.01) a	0.9983 (±0.0001) b
	North	517 (±59) A	3.91 (±0.15) A	0.05 (±0.01) A	0.9980 (±0.0002) A
Melliodendron xylocarpum	South	308 (±31) c	3.70 (±0.20) a	0.05 (±0.01) a	0.9990 (±0.0002) a
	North	358 (±30) B	3.79 (±0.28) A	0.07 (±0.04) A	0.9986 (±0.0002) A
Ilex chinensis	South	387 (±31) bc	3.63 (±0.13) a	0.07 (±0.02) a	0.9988 (±0.0002) ab
	North	374 (±25) B	3.47 (±0.15) A	0.07 (±0.01) A	0.9985 (±0.0001) A
Alniphyllum fortunei	South	448 (±32) ab	3.57 (±0.11) a	0.06 (±0.01) a	0.9983 (±0.0002) b
	North	335 (±35) B	3.44 (±0.17) A	0.05 (±0.01) A	0.9983 (±0.0002) A
Mallotus japonicus	South	455 (±37) ab	3.83 (±0.18) a	0.05 (±0.01) a	0.9983 (±0.0002) b
	North	399 (±35) AB	3.47 (±0.09) A	0.07 (±0.01) A	0.9979 (±0.0001) A *

Note: Data are exhibited as mean ± standard error. Data with different capital letters means significant difference in NF at $p < 0.05$. Data with different lowercase letters means significant difference in SF at $p < 0.05$. * means that there is significant difference between SF and NF.

3.6. Redundancy Analysis in Different Aspects

Through the factor analysis, we extracted the key response factors for RDA analysis. The environmental variables (aspects and soil physicochemical properties) and response variables (characteristics of AM fungal communities of dominant tree species in *Tc* natural forest) were analyzed using RDA (Figure 4). The results showed that soil AM fungi communities had a higher total sequence numbers, Glomeromycota sequence numbers, Chao index, Shannon index, Archaeosporaceae abundance, and Acaulosporaceae abundance in SF, while the opposite trend was shown in NF (Figure 4A). Furthermore, SOM, TK, TN, TP, and AP were negatively correlated with total sequence numbers, Glomeromycota sequence numbers, Chao index, Shannon index and Archaeosporaceae abundance, and negatively correlated with the relative abundance of unclassified fungi (Figure 4A). The SOM and TK were positively correlated with OTUs (Figure 4A).

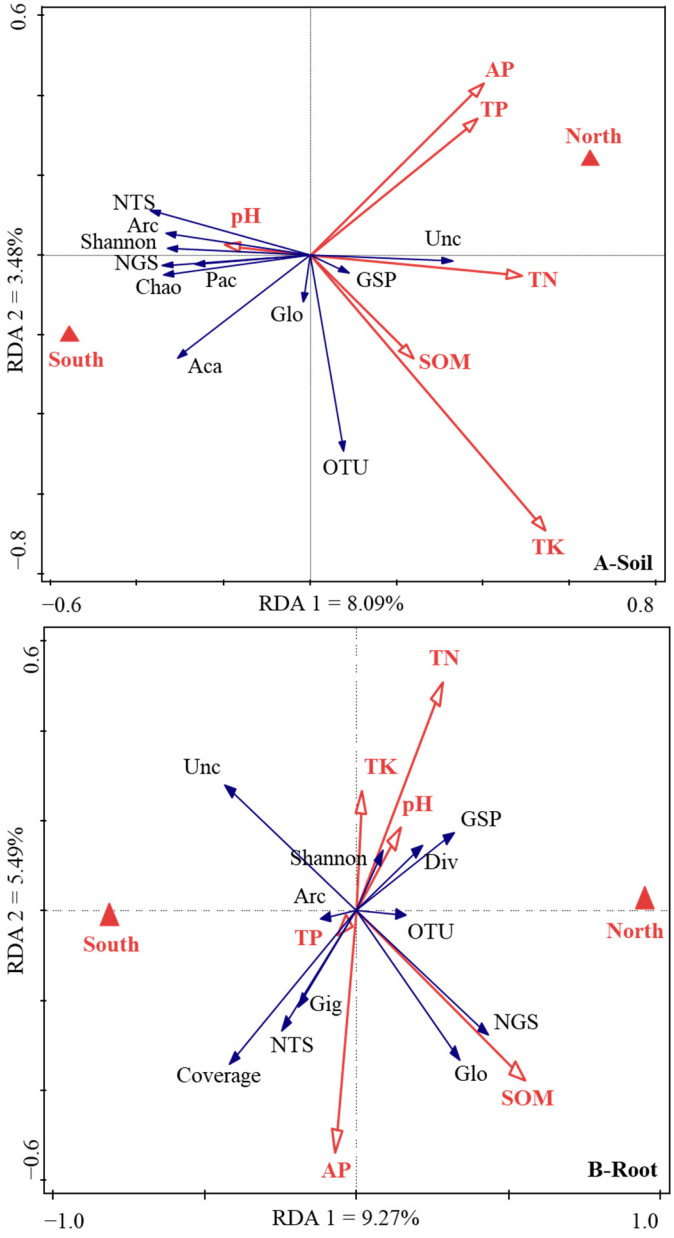

Figure 4. Redundancy ordination (RDA) results (**A**, soil; **B**, root) of the characteristics of the AM fungi community and aspects and soil physicochemical properties in *Tc* natural forest. Abbreviations are as follows: GSP, the proportion of Glomeromycota sequences; NTS, the number of total sequences; NGS, the number of Glomeromycota sequences; Div, Diversisporaceae; Gig, Gigasporaceae; Arc, Archaeosporaceae; Unc, unclassified fungi; Glo, Glomeraceae; Pac, Pacisporaceae; Aca, Acaulosporaceae.

The RDA results (Figure 4B) showed that the proportion of Glomeromycota sequences, Glomeromycota sequence numbers, Diversisporaceae abundance, and Shannon index of the root AM fungi community were relatively high in NF, while total sequence numbers, coverage index, Gigasporaceae abundance, and unclassified fungi abundance of the root AM fungi community were relatively high on SF. The SOM was positively correlated with Glomeromycota sequence numbers and Glomeraceae abundance (Figure 4B). The AP was positively correlated with coverage index, total sequence numbers, and Gigasporaceae abundance, while it was negatively correlated with unclassified fungi abundance, the proportion of Glomeromycota sequences, Diversisporaceae abundance, and Shannon index (Figure 4B). Moreover, TN, TK, and pH showed the opposite trend with AP.

4. Discussion

4.1. Soil AM Fungal Community Diversity and Composition in Tc Forest at Different Aspects

An Illumina PE250 high-throughput sequencing method was applied to explore the features of the AM fungi community in a natural *Tc* forest at different aspects and to analyze the AM fungal community characteristics and diversity changes. The AM fungal community structure refers to the composition and quantitative characteristics of the fungal community in each habitat, and is mainly characterized by abundance, density, frequency, dominance, importance value, and community coefficient [34]. Stability is an important factor in achieving ecological balance. Therefore, studying the structural changes in AM fungal communities in specific ecosystems and their influencing factors can provide a basis for optimizing the natural *Tc* forest ecosystem structure and regulating ecological functions. Indeed, AM fungal species diversity is an important component of soil microbial diversity and has a vital ecological and physiological role in providing plant nutrient uptake, enhancing soil structure stability, and changing the global C and N cycles [35]. Many factors influence the diversity of AM fungal communities, including nutrient inputs [36], anthropogenic disturbances [37], transmission limitations [38], plant communities [39], and other environmental factors [40].

Aspect, one of the most common environmental impact factors of natural mountain forests, influences the diversity of AM fungal communities in plant soils [41]. Soil microorganisms are seen as the driving force of productivity diversity in terrestrial ecosystems [42]. In this study, species richness and diversity indices were used to describe the diversity characteristics of AM fungal communities in different aspects of *Tc* natural forests, including Chao, Shannon, and Simpson. In terms of species richness, the number of OTUs of AM fungal communities in the inter-rhizosphere soil of *Tc* was significantly higher on NF (16.4%) than on SF (13.7%). In terms of diversity indices, the Chao, Shannon, and Simpson indices of AM fungal communities in the rhizosphere soil and root of *Tc* were not significantly different between NF and SF. This phenomenon may be because the composition of the AM fungi community in the roots and rhizosphere soil primarily consisted of Glomeraceae, reaching 45.07%–86.39% and 74.82%–93.08%, respectively. Some scholars have studied the AM fungal diversity and community structure of farmland, wetland, forest, and grassland, and found that the AM fungal community composition is mainly determined by Glomeraceae [43,44], which is the same as the result of this study. The reason is that the Glomeraceae has a unique reproductive mode and can tolerate different environmental changes [45]. However, the rhizosphere soil contained more rare AM fungal taxa with low relative abundance, which lowered the community diversity index (Figure 3; Table 5). We found that there were a large number of rare AM fungal taxa with very few sequences in the *Tc* natural forest, and these rare taxa may be the main reason for the functional redundancy in the *Tc* natural forest, which should be investigated in a follow-up study. The results suggest that the effects of various environmental factors on AM fungal communities in natural ecosystems, especially in complex forest ecosystems, should not be evaluated simply in terms of diversity or species richness but should be considered comprehensively to obtain more accurate and objective analysis results.

4.2. Effects of Aspects and Other Factors on the Characteristics of AM Fungal Community

The AM fungi are mainly distributed in the soil around the roots; therefore, they are closely related to the soil environment. Soil type, depth, water status, fertility, pH, and other factors have important effects on the AM fungal community [46]. In a past study, ectomycorrhizal fungal diversity was higher on sunny aspects than on shady aspects, probably due to differences in the distribution of plants in different aspect orientations caused by differences in insolation, water, and heat conditions [47]. This study found that soil nutrient characteristics were relatively better on NF than on SF. The differences may be due to the variations of space and climate which were observed in the selected *Tc* community distribution area.

This is similar to the results of other studies, which found that the taxonomy and spectral composition of AM fungal communities significantly differed between sunny and shady aspects through NMDS sorting and PERMANOVA analysis, with sunny aspects having higher light intensity, soil temperature, and soil pH, and shady aspects having higher soil moisture and soil fertility index [48]. Community genealogy analysis also showed that AM fungal communities were spectrally aggregated on sunny aspects and spectrally random on shady aspects, indicating that the primary ecological process of AM fungal community construction is a shift from ecological niche-dominated filtering to a synthesis of competitive exclusion and ecological niche filtering [49]. The diversity of mycorrhizal fungal communities (evenness index, Simpson index, Shannon–Wiener index, and richness) in different areas differed according to aspect, altitude, soil moisture, and human disturbance [50].

4.3. Effects of Unique Tree Species Characteristics of Tc on AM Fungal Community Characteristics

The characteristics of *Tc* and its symbiotic relationship with AM fungi jointly affected the soil AM fungal community. Symbiotics play a vital role in plant nutrient uptake, improving ecosystem or individual resistance to adverse environments [22,25], which can help break the regeneration dilemma caused by transmission barriers [21] and microhabitat restrictions in *Tc* forests [51], and are conducive to the protection and restoration of wild *Tc* forests [52]. The *Tc* is the main tree species in the *Tc* natural forest, but its seeds are susceptible to soil pathogens, which affects its natural forest regeneration ability. Therefore, it does not have an absolute advantage in community competition [53]. In different aspects in the natural *Tc* forest, the diversity and relative abundance of AM fungal communities in the roots and rhizosphere soil of *Tc* were only at the medium level compared to those of the other six species. This is because there is a certain selectivity between different mycorrhizal fungi and host plants, which affects the binding ability of plants to mycorrhizal fungi [54], and different plants have different adaptabilities to different AM fugal community [55] and aspect habitats. In addition, the unclassified fungi were found less in roots (0%–1%) than in rhizosphere soil (6%–17%). Published research recognized more new species of Glomeromycota in the rhizospheres of endemic plants [56]. Therefore, *Tc* still has a certain development scope for AM fungal infections. It can enhance its competition for environmental resources by increasing AM fungal infection, which may be of great value for the natural regeneration of *Tc* and protection of the *Tc* natural community.

4.4. AM Fungal Library in SF and 'Functional' AM Fungi in NF

The AM fungi in soil are often considered a library, and their significance is to preserve and maintain the diversity of AM fungal resources. The AM fungi in roots are often considered functional AM fungi, and their presence helps realize some plant functions. In this study, the number of OTUs (16.4%) of AM fungal communities in rhizosphere soil on NF of *Tc* was significantly larger than that on SF (13.7%), whereas the number of OTUs (11.3%) of AM fungal communities in roots on NF of *Tc* was lower than on SF (15.1%). This difference is precisely why taxonomic and functional diversities are not synchronized [57] with aspect. Moreover, abiotic factors, such as soil physicochemical properties, had an important influence on AM fungal community composition [58]. In this study, the low insolation, high moisture, and high nutrient content (TP, TK, and TN) conditions on NF might be beneficial for the functional realization of AM fungi. In contrast, the high insolation, low soil moisture, and low soil nutrient content conditions (TP, TK, and TN) on SF might be conducive to the proliferation and preservation of AM fungal groups. For example, one study showed that when SOM content was high, the associated microorganisms of AM fungi promote mass-production due to more nutrients, thus, inhibiting the infiltration of roots of AM fungi [59]. The changes in vegetation and environment factors did not lead to the extinction of a large number of soil AM fungi, but only caused the change in their relative proportion. Therefore, the results of this study found that the soil AM fungal pool might be relatively stable in the *Tc* natural forest.

4.5. Implications

The rhizosphere is the main medium for the interaction between plant and soil. A complex feedback process occurs at the interface between plant and soil. Soil fungi form a dense underground mycelium bridge network in soil through coupling with plant roots, thus, promoting water and nutrient absorption by the plant [60]. Most terrestrial plants can form mycorrhizal symbionts with AM fungi, and the fungi cannot grow and complete their life cycle without host plants [22]. In this study, Illumina high-throughput sequencing technology was used to study the composition and functional characteristics of the AM fungal communities in the natural forest of *Tc*. The results of this study can provide a theoretical basis for better understanding of the natural regeneration law of the community of *Tc*. We provide a reference for ecological restoration, protection, and utilization of *Tc* from the perspective of underground microbial diversity, as well as for the protection of rare and endangered plants, sustainable use of forests, maintenance of ecosystem balance and protection of biodiversity.

5. Conclusions

We analyzed the diversity and composition of AM fungal communities in a natural *Tc* forest using Illumina high-throughput sequencing of rhizosphere soil and roots from north-facing and south-facing aspects. The number of Glomeromycota sequences was significantly larger on SF than on NF, while the number of OTUs on NF was significantly higher than on SF in *Tc* soil. The composition of the AM fungi community in roots and rhizosphere soil was primarily consisted of *Glomeraceae*, reaching 45.07%–86.39% and 74.82%–93.08%, respectively. Moreover, the relative abundance of unclassified fungi in rhizosphere soil of *Tc* on NF was significantly higher than that on SF. However, the Chao, Shannon, and Simpson indices of the soil and root AM fungal community found no significant difference between SF and NF. Furthermore, our study revealed that the low insolation, high soil moisture, and high nutrient content conditions on NF might be beneficial for the functional realization of AM fungi. In contrast, the high insolation, low soil moisture, and low soil nutrient content conditions on SF might be beneficial for the proliferation and preservation of AM fungal groups. In the future, how topography and various environmental factors to regulate AM fungal function should be given attention due to the fact that AM symbiosis plays non-negligible roles in ecosystems.

Author Contributions: Q.W., J.P., W.L. and L.Z. conceptualized the study, and J.P. identified, processed, and collected samples with help from S.Y. and T.L.; Q.W. and Y.K. primarily interpreted the data with help from P.J.M. and L.Z.; Q.W., J.P., L.Z. and W.L. wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the National Natural Science Foundation of China (31860202) and the Forestry Science and Technology Innovation Special Project of Jiangxi Provincial Department of Forestry (Innovation Special Project [2019] No. 25).

Data Availability Statement: Not applicable.

Acknowledgments: We would especially like to thank the Administration of Guanshan National Natural Reserve for assisting in the investigation.

Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

Table A1. Analysis of relative abundance of belowground AM fungal communities' sequence in dominant tree species in *T. ciliate* natural forest in different aspects.

Species	Aspects	Unclassified	Paraglomeraceae	Pacisporaceae	Glomeraceae	Gigasporaceae	Geosiphonaceae	Diversisporaceae	Archaeosporaceae	Ambisporaceae	Acaulosporaceae
Soil											
Toona ciliates	South	b	a	a	ab	a	a	a	ab	a	a
	North	A *	B	A	A	A	A	A	A	A	A
Padus buergeriana	South	a	a	a	c	a	a	a	ab	a	a
	North	A	AB	A	A	A	A	A	A	A	A
Maesa japonica	South	b	a	a	abc	a	a	a	ab	a	a
	North	A	AB	A	A	A	A	A	A	A	A
Melliodendron xylocarpum	South	b	a	a	abc	a	a	a	ab	a	a
	North	A	AB	A	A	A	A	A	A	A	A
Ilex chinensis	South	b	a	a	bc	a	a	a	ab	a	a
	North	A	B	A	A	A	A	A	A	A	A
Alniphyllum fortunei	South	b	a	a	bc	a	a	a	a	a	a
	North	A	AB	A	A	A	A	A	A	A	A
Mallotus japonicus	South	b	a	a	a	a	a	a	b	a	a
	North	A *	A *	A	A *	A	A	A	A	A	A
Root											
Toona ciliates	South	a	a	a	a	a	a	a	b	a	a
	North	A	A	A	A	B	B	B	A	AB	A
Padus buergeriana	South	a	a	a	a	a	a	a	a	a	a
	North	A	A	A	A	B	B	B	A	B	A
Maesa japonica	South	a	a	a	a	a	a	a	ab	a	a
	North	A	A	A	A	B	A	B	A	B	A
Melliodendron xylocarpum	South	a	a	a	a	a	a	a	b	a	a
	North	A	A	A	A	B	B	A	A	AB	A
Ilex chinensis	South	A	A	a	a	a	a	a	ab	a	a
	North	A *	A	A	A	B	B	B	A	A	A
Alniphyllum fortunei	South	a	a	a	a	a	a	a	ab	a	a
	North	A	A	A	A	A	AB	B	A *	B	A *
Mallotus japonicus	South	a	a	a	a	a	a	a	ab	a	a
	North	A	A	A	A	B	B	B	A	B	A

Note: Data with different capital letters means significant difference in NS at $p < 0.05$. Data with different lowercase letters means significant difference in SS at $p < 0.05$; * means that there is significant difference between SS and NS in *Tc*.

Table A2. Results of variance analysis for the effects of aspect and species on the relative abundance of belowground AM fungal communities' sequence.

Factors	Aspect		Species		Aspect × Species	
	F	*p*	F	*p*	F	*p*
S-Acaulosporaceae	2.939	NS	1.299	NS	0.606	NS
S-Ambisporaceae	2.171	NS	1.326	NS	0.443	NS
S-Archaeosporaceae	1.503	NS	0.938	NS	1.292	NS
S-Diversisporaceae	0.468	NS	0.755	NS	0.865	NS
S-Geosiphonaceae	0.274	NS	0.381	NS	1.170	NS
S-Gigasporaceae	1.338	NS	0.757	NS	0.425	NS
S-Glomeraceae	0.419	NS	1.478	NS	1.789	NS
S-Pacisporaceae	1.284	NS	0.754	NS	0.754	NS
S-Paraglomeraceae	1.582	NS	0.416	NS	1.975	NS
S-Unclassified fungi	0.465	NS	1.092	NS	2.373	0.049
R-Acaulosporaceae	7.598	0.009	0.903	NS	0.705	NS
R-Ambisporaceae	1.934	NS	1.779	NS	2.057	NS
R-Archaeosporaceae	2.516	NS	3.996	0.036	0.640	NS
R-Diversisporaceae	0.743	NS	0.811	NS	1.175	NS
R-Geosiphonaceae	0.276	NS	1.331	NS	1.866	NS
R-Gigasporaceae	0.535	NS	1.299	NS	0.652	NS
R-Glomeraceae	0.112	NS	1.319	NS	1.042	NS
R-Pacisporaceae	1.274	NS	1.318	NS	1.336	NS
R-Paraglomeraceae	1.337	NS	1.028	NS	1.427	NS
R-Unclassified fungi	3.986	NS	0.676	NS	0.800	NS

Abbreviations are as follows: S, soil; R, root; NS means that there is no significant difference ($p > 0.05$).

References

1. Cardinale, B.J.; Duffy, J.E.; Gonzalez, A.; Hooper, D.U.; Perrings, C.; Venail, P.; Narwani, A.; Mace, G.M.; Tilman, D.; Wardle, D.A.; et al. Biodiversity loss and its impact on humanity. *Nature* **2012**, *486*, 59–67. [CrossRef] [PubMed]
2. Jing, X.; Sanders, N.J.; Shi, Y.; Chu, H.; Classen, A.T.; Zhao, K.; Chen, L.; Shi, Y.; Jiang, Y.; He, J.S. The links between ecosystem multifunctionality and above- and belowground biodiversity are mediated by climate. *Nat. Commun.* **2015**, *6*, 8159. [CrossRef] [PubMed]
3. Mvondo Owono, F.; Ntamak-Nida, M.-J.; Dauteuil, O.; Guillocheau, F.; Njom, B. Morphology and long-term landscape evolution of the South African plateau in South Namibia. *Catena* **2016**, *142*, 47–65. [CrossRef]
4. Bennie, J.; Huntley, B.; Wiltshire, A.; Hill, M.O.; Baxter, R. Slope, aspect and climate: Spatially explicit and implicit models of topographic microclimate in chalk grassland. *Ecol. Model.* **2008**, *216*, 47–59. [CrossRef]
5. Liu, S.L.; An, N.N.; Yang, J.J.; Dong, S.K.; Wang, C.; Yin, Y.J. Prediction of soil organic matter variability associated with different land use types in mountainous landscape in southwestern Yunnan province, China. *Catena* **2015**, *133*, 137–144. [CrossRef]
6. Zhang, Z.W.; Yin, H.Y.; Chang, J.J.; Xue, J. Spatial variability of surface soil water content and its influencing factors on shady and sunny slopes of an alpine meadow on the Qinghai–Tibetan Plateau. *Glob. Ecol. Conserv.* **2022**, *34*, e02035. [CrossRef]
7. Wei, T.F.; Shangguan, D.H.; Tang, X.L.; Qin, Y. The role of glacial gravel in community development of vascular plants on the glacier forelands of the Third Pole. *J. Arid Land* **2022**, *14*, 1022–1037. [CrossRef]
8. Conforti, M.; Lucà, F.; Scarciglia, F.; Matteucci, G.; Buttafuoco, G. Soil carbon stock in relation to soil properties and landscape position in a forest ecosystem of southern Italy (Calabria region). *Catena* **2016**, *144*, 23–33. [CrossRef]
9. Huang, Y.M.; Liu, D.; An, S.S. Effects of slope aspect on soil nitrogen and microbial properties in the Chinese Loess region. *Catena* **2015**, *125*, 135–145. [CrossRef]
10. Small, C.J.; McCarthy, B.C. Spatial and temporal variability of herbaceous vegetation in an eastern deciduous forest. *Plant Ecol.* **2003**, *164*, 37–48. [CrossRef]
11. Måren, I.E.; Karki, S.; Prajapati, C.; Yadav, R.K.; Shrestha, B.B. Facing north or south: Does slope aspect impact forest stand characteristics and soil properties in a semiarid trans-Himalayan valley? *J. Arid Environ.* **2015**, *121*, 112–123. [CrossRef]
12. Pereira, P.; Cerda, A.; López, A.J.; Zavala, L.M.; Mataix-Solera, J.; Arcenegui, V.; Misiūnė, I.; Keesstra, S.D.; Novara, A.J.L.D. Short-Term Vegetation Recovery after a Grassland Fire in Lithuania: The Effects of Fire Severity, Slope Position and Aspect. *Land Degrad. Dev.* **2016**, *27*, 1523–1534. [CrossRef]
13. Wang, Y.Y.; Yu, J.H.; Xiao, L.; Zhong, Z.L.; Wang, Q.; Wang, W.J. Dominant species abundance, vertical structure and plant diversity response to nature forest protection in Northeastern China: Conservation effects and implications. *Forests* **2020**, *11*, 295. [CrossRef]

14. Zhao, N.N.; Guggenberger, G.; Shibistova, O.; Thao, D.T.; Shi, W.J.; Li, X.G. Aspect-vegetation complex effects on biochemical characteristics and decomposability of soil organic carbon on the eastern Qinghai-Tibetan Plateau. *Plant Soil* **2014**, *384*, 289–301. [CrossRef]
15. Liu, J.; Jiang, J.; Chen, Y. Genetic diversity of central and peripheral populations of Toona ciliata var. pubescens, an endangered tree species endemic to China. *Genet. Mol. Res. GMR* **2014**, *13*, 4579–4590. [CrossRef]
16. Zhan, X.; Li, P.; Hui, W.K.; Deng, Y.W.; Gan, S.M.; Sun, Y.; Zhao, X.H.; Chen, X.Y.; Deng, X.M. Genetic diversity and population structure of Toona ciliata revealed by simple sequence repeat markers. *Biotechnol. Biotechnol. Equip.* **2019**, *33*, 214–222. [CrossRef]
17. Huang, H.L.; Zhang, L.; Jia, L.M.; Liang, Y.L.; Cai, J.H. Fitness of sexual reproduction of Toona ciliata var. pubescens natural populations and their sexual reproduction and regeneration. *J. Appl. Ecol.* **2018**, *29*, 1098–1106. [CrossRef]
18. Münzbergová, Z.; Herben, T. Seed, dispersal, microsite, habitat and recruitment limitation: Identification of terms and concepts in studies of limitations. *Oecologia* **2005**, *145*, 1–8. [CrossRef]
19. Marques, M.C.M.; Burslem, D.F.R.P. Multiple stage recruitment limitation and density dependence effects in two tropical forests. *Plant Ecol.* **2015**, *216*, 1243–1255. [CrossRef]
20. Guo, X.Y.; Fu, L.; Zhang, L.; Su, H.; Liang, Y.L. Effects of forest soil and soil-borne fungi on seed germination and seedling survival of Toona ciliata var. pubescens. *For. Res.* **2017**, *30*, 871–877.
21. Huang, H.L.; Zhang, L.; Liao, C.K. Seed rain, soil seed bank, and natural regeneration of natural Toona ciliata var. pubescens forest. *Chin. J. Appl. Ecol.* **2012**, *23*, 972–978.
22. Smith, S.E.; Read, D.J. *Mycorrhizal Symbiosis*; Academic Press: Cambridge, MA, USA, 2008.
23. Mridha, M.A.U.; Dhar, P.P. Biodiversity of arbuscular mycorrhizal colonization and spore population in different agroforestry trees and crop species growing in Dinajpur, Bangladesh. *J. Res.* **2007**, *18*, 91–96. [CrossRef]
24. Pan, J.; Wang, Q.; Guo, X.; Jiang, X.; Cheng, Q.; Fu, L.; Liu, W.; Zhang, L. Local patterns of arbuscular mycorrhizal fungal diversity and community structure in a natural Toona ciliata var. pubescens forest in South Central China. *PeerJ* **2021**, *9*, e11331. [CrossRef]
25. Hoeksema, J.D.; Chaudhary, V.B.; Gehring, C.A.; Johnson, N.C.; Karst, J.; Koide, R.T.; Pringle, A.; Zabinski, C.; Bever, J.D.; Moore, J.C.; et al. A meta-analysis of context-dependency in plant response to inoculation with mycorrhizal fungi. *Ecol. Lett.* **2010**, *13*, 394–407. [CrossRef] [PubMed]
26. Liu, R.J.; Li, X.L. *Arbuscular Mycorrhiza and Its Application*; Science Press: Beijing, China, 2000.
27. Ehrlich, R.; Schulz, S.; Schloter, M.; Steinberger, Y. Effect of slope orientation on microbial community composition in different particle size fractions from soils obtained from desert ecosystems. *Biol. Fertil. Soils* **2015**, *51*, 507–510. [CrossRef]
28. Chu, H.Y.; Xiang, X.J.; Yang, J.; Adams, J.M.; Zhang, K.P.; Li, Y.T.; Shi, Y. Effects of slope aspects on soil bacterial and arbuscular fungal communities in a Boreal forest in China. *Pedosphere* **2016**, *26*, 226–234. [CrossRef]
29. Liu, M.; Zheng, R.; Bai, S.; Bai, Y.; Wang, J. Slope aspect influences arbuscular mycorrhizal fungus communities in arid ecosystems of the Daqingshan Mountains, Inner Mongolia, North China. *Mycorrhiza* **2017**, *27*, 189–200. [CrossRef]
30. Bao, S.D. *Soil and Agricultural Chemistry Analysis*; China Agriculture Press: Beijing, China, 2000.
31. Mahmoudi, N.; Cruz, C.; Mahdhi, M.; Mars, M.; Caeiro, M.F. Arbuscular mycorrhizal fungi in soil, roots and rhizosphere of Medicago truncatula: Diversity and heterogeneity under semi-arid conditions. *PeerJ* **2019**, *7*, e6401. [CrossRef]
32. Chao, A. Nonparametric-estimation of the number of classes in a population. *Scand. J. Stat.* **1984**, *11*, 265–270.
33. Simpson, E.H. Measurement of Diversity. *Nature* **1949**, *163*, 688. [CrossRef]
34. Liu, M.; Zheng, R.; Bai, S.L.; Wang, J.G.; Li, L.; Duan, G.Z. Advances of species diversity of arbuscular mycorrhizal fungi. *Microbiol. China* **2016**, *43*, 1836–1843.
35. Yang, H.S.; Xiong, Y.Q.; Wang, Q.; Guo, Y.; Dai, Y.J.; Xu, M.M. Arbuscular mycorrhizal fungal species diversity: Ecological functioning, determinants and assembling mechanisms. *Acta Ecol. Sin.* **2016**, *36*, 2826–2832.
36. Veresoglou, S.D.; Chen, B.; Rillig, M.C. Arbuscular mycorrhiza and soil nitrogen cycling. *Soil Biol. Biochem.* **2012**, *46*, 53–62. [CrossRef]
37. Kabir, Z. Tillage or no-tillage: Impact on mycorrhizae. *Can. J. Plant Sci.* **2005**, *85*, 23–29. [CrossRef]
38. Dumbrell, A.J.; Nelson, M.; Helgason, T.; Dytham, C.; Fitter, A.H. Idiosyncrasy and overdominance in the structure of natural communities of arbuscular mycorrhizal fungi: Is there a role for stochastic processes. *J. Ecol.* **2010**, *98*, 419–428. [CrossRef]
39. Řezáčová, V.; Řezáč, M.; Gryndler, M.; Hršelová, H.; Gryndlerová, H.; Michalová, T. Plant invasion alters community structure and decreases diversity of arbuscular mycorrhizal fungal communities. *Appl. Soil Ecol.* **2021**, *167*, 104039. [CrossRef]
40. Hawkes, C.V.; Kivlin, S.N.; Rocca, J.D.; Huguet, V.; Thomsen, M.; Suttle, K.B. Fungal community responses to precipitation. *Glob. Chang. Biol.* **2011**, *17*, 1637–1645. [CrossRef]
41. Guo, Y.N.; Zhang, H.D.; Bao, Y.Y.; Tan, H.Z.; Liu, X.H.; ur Rahman, Z. Distribution characteristics of soil AM fungi community in soft sandstone area. *J. Environ. Manag.* **2022**, *316*, 115193. [CrossRef]
42. van der Heijden, M.G.; Bardgett, R.D.; van Straalen, N.M. The unseen majority: Soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecol. Lett.* **2008**, *11*, 296–310. [CrossRef]
43. Jeffries, P.; Gianinazzi, S.; Perotto, S.; Turnau, K.; Barea, J.-M. The contribution of arbuscular mycorrhizal fungi in sustainable maintenance of plant health and soil fertility. *Biol. Fertil. Soils* **2003**, *37*, 1–16. [CrossRef]
44. Scheublin, T.R.; Ridgway, K.P.; Young, J.P.; van der Heijden, M.G. Nonlegumes, legumes, and root nodules harbor different arbuscular mycorrhizal fungal communities. *Appl. Environ. Microbiol.* **2004**, *70*, 6240–6246. [CrossRef] [PubMed]

45. Brito, I.; Goss, M.J.; de Carvalho, M.; Chatagnier, O.; van Tuinen, D. Impact of tillage system on arbuscular mycorrhiza fungal communities in the soil under Mediterranean conditions. *Soil Till. Res.* **2012**, *121*, 63–67. [CrossRef]
46. Canini, F.; Zucconi, L.; Pacelli, C.; Selbmann, L.; Onofri, S.; Geml, J. Vegetation, pH and water content as main factors for shaping fungal richness, community composition and functional guilds distribution in soils of Western Greenland. *Front. Microbiol.* **2019**, *10*, 2348. [CrossRef] [PubMed]
47. Voortman, B.R.; Fujita, Y.; Bartholomeus, R.P.; Aggenbach, C.; Witte, J.-P.M. How the evaporation of dry dune grasslands evolves during the concerted succession of soil and vegetation. *Ecohydrology* **2017**, *10*, e1848. [CrossRef]
48. Chai, Y.X. Diversity and Community Structure of Arbuscular Mycorrhizal Fungi in Yinyang Slope of Qilian Mountains. Ph.D. Thesis, Lanzhou University, Lanzhou, China, 2018.
49. Li, R.; Liu, M.X.; Zhang, C.; Zhao, R.D.; Shao, P. Distribution characteristics of soil microbial communities along different slope direction of Gannan sub-alpine meadows. *Ecol. Environ. Sci.* **2017**, *26*, 1884–1891.
50. Sun, X.F.; Su, Y.Y.; Zhang, Y.; Wu, M.Y.; Zhang, Z.; Pei, K.Q.; Sun, L.F.; Wan, S.Q.; Liang, Y. Diversity of arbuscular mycorrhizal fungal spore communities and its relations to plants under increased temperature and precipitation in a natural grassland. *ChSBu* **2013**, *58*, 4109–4119. [CrossRef]
51. Guo, Y.; He, Y.J.; Wu, P.; Wu, B.L.; Lin, Y.; He, M.H.; Han, X.; Xia, T.T.; Shen, K.P.; Kang, L.L.; et al. The interspecific competition presents greater nutrient facilitation compared with intraspecific competition through AM fungi interacting with litter for two host plants in karst soil. *J. Plant Ecol.* **2021**, *15*, 399–412. [CrossRef]
52. Liang, Y.M.; He, X.Y.; Chen, C.Y.; Feng, S.Z.; Liu, L.; Chen, X.B.; Zhao, Z.W.; Su, Y.R. Influence of plant communities and soil properties during natural vegetation restoration on arbuscular mycorrhizal fungal communities in a karst region. *Ecol. Eng.* **2015**, *82*, 57–65. [CrossRef]
53. Huang, H.L.; Zhang, L.; Guo, X.Y.; Liang, Y.L.; Liu, Z.G. Fruiting characteristics and sexual fecundity of Toona ciliata var. pubescens populations in Jiulianshan national nature reserve. *Sci. Silvae Sin.* **2013**, *49*, 170–174.
54. Helgason, T.; Merryweather, J.W.; Denison, J.; Wilson, P.; Young, J.P.W.; Fitter, A.H. Selectivity and functional diversity in arbuscular mycorrhizas of co-occuring fungi and plants from a temperate deciduous woodland. *J. Ecol.* **2002**, *90*, 371–384. [CrossRef]
55. Lovelock, C.E.; Andersen, K.; Morton, J.B. Arbuscular mycorrhizal communities in tropical forests are affected by host tree species and environment. *Oecologia* **2003**, *135*, 268–279. [CrossRef] [PubMed]
56. López-García, Á.; Varela-Cervero, S.; Vasar, M.; Öpik, M.; Barea, J.M.; Azcón-Aguilar, C. Plant traits determine the phylogenetic structure of arbuscular mycorrhizal fungal communities. *Mol. Ecol.* **2017**, *26*, 6948–6959. [CrossRef] [PubMed]
57. Van Diepen, L.; Lilleskov, E.; Pregitzer, K. Simulated nitrogen deposition affects community structure of arbuscular mycorrhizal fungi in northern hardwood forests. *Mol. Ecol.* **2011**, *20*, 799–811. [CrossRef] [PubMed]
58. Jamiołkowska, A.; Księżniak, A.; Gałązka, A.; Hetman, B.; Kopacki, M.; Skwaryło-Bednarz, B. Impact of abiotic factors on development of the community of arbuscular mycorrhizal fungi in the soil: A Review. *Int. Agrophys.* **2018**, *32*, 133–140. [CrossRef]
59. Wang, Q.; Wang, J.; Li, Y.; Chen, D.; Ao, J.; Zhou, W.; Shen, D.; Li, Q.; Huang, Z.; Jiang, Y. Influence of nitrogen and phosphorus additions on N(2)-fixation activity, abundance, and composition of diazotrophic communities in a Chinese fir plantation. *Sci. Total Environ.* **2018**, *619–620*, 1530–1537. [CrossRef] [PubMed]
60. Corrales, A.; Ovrebo, C.L. Fungi of the Fortuna Forest Reserve: Taxonomy and ecology with emphasis on ectomycorrhizal communities. *Ecol. Environ. Conserv.* **2020**, *4*, 1–29.