

Article

Effects of Stand Types on Ectomycorrhizal Fungal Community Composition and Structure of *Pinus massoniana* in Subtropical Mountain Forest Ecosystems

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Abstract: Tree species composition in forest ecosystems is an important biological factor affecting the diversity of ectomycorrhizal fungi (EMF). However, little is known about the composition and diversity of EMF communities associated with *Pinus massoniana* in different *P. massoniana* association habitats (MpAHs) in subtropical mountains. This study investigated the EMF community characteristics of *P. massoniana* in different MpAHs using plant community surveys, soil property analyses, and mycorrhizal identification. A total of 56 operational taxonomic units (OTUs), belonging to 20 families and 22 genera, were identified. OTU richness of Basidiomycota (58.93%) was higher than that of Ascomycota (41.07%). Unclassified Helotiales, *Russula*, *Lactarius*, and *Tomentella* were the dominant groups. Different stand types significantly altered the EMF communities of *P. massoniana* ($p < 0.05$, for Shannon index) and the associations of *P. massoniana* + *Populus adenopoda* (Mp_Pa) had the highest diversity of EMF, while *P. massoniana* + *Cunninghamia lanceolata* (Mp_Cl) had the lowest diversity. The number of specific OTUs was higher than shared OTUs. Similarity index and principal coordinate analysis indicated that the EMF communities of *P. massoniana* varied significantly in different MpAHs ($R^2 = 0.21$, $p = 0.001$). The linear regression model showed that the EMF diversity of *P. massoniana* was positively related to tree species diversity, indicating that the EMF diversity of *P. massoniana* is influenced by tree species diversity. The findings provide a reasonable reference for tree species configuration in the process of mixed transformation or near-natural management of plantations.

Keywords: masson pine; *P. adenopoda*; tree species composition; EMF diversity; mixed forest



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1. Introduction

Ectomycorrhizal fungi (EMF) are important biological components of subtropical forest ecosystems that have a significant impact on plant diversity, productivity, and ecosystem functions [1,2]. The relationships between plant diversity and soil fungi (e.g., mycorrhiza) have long been a focus of research interest but also present certain challenges [3,4]. Typically, many different aspects of plant diversity can influence the composition and activities of EMF communities, e.g., host plant genus-level diversity, richness, and phylogenetic diversity [5–7]. Higher levels of plant diversity can increase the availability and heterogeneity of soil resources and produce more niches [8], which is usually related to the positive response of the EMF community composition and increased diversity [9]. Several studies have revealed that the EMF community diversity increases significantly with increasing tree species diversity and changes in tree species composition [10]. Other studies have shown that tree species richness has no significant effect on EMF community richness and composition [11], and no direct relationship exists between the structure of the plant community and EMF [12,13]. However, most of the current research focuses on host plants

and EMF. Plant–EMF interactions are significantly important in regulating the health and stability of ecosystems, but the results of different studies on the effects of (ectomycorrhizal and nonectomycorrhizal) tree species composition and diversity on EMF communities in forest ecosystems are inconsistent.

Given its excellent environmental resistance and ecological adaptability, *Pinus massoniana*, a typical EMF host plant in southern China, makes an important contribution to the regional forestry economy (e.g., timber sources) and ecological construction (e.g., water and soil conservation, carbon sequestration, and forest development) [14,15]. Studies on the EMF of *P. massoniana* conducted to date have mainly focused on the effects of different environmental conditions (e.g., heavy metal pollution and season) [16,17] and disturbance types (e.g., resin tapping) [18] on the EMF community structure and the physiological and biochemical characteristics of tree species [19,20]. Association is the basic unit of plant community classification in China. Usually, the species composition, the layer structure, and the dominant species (or marker species) of each layer for an association are basically the same. It can directly characterize the composition and structure of plant species, as well as the stand characteristics, and reflects the similarity of their habitats [21]. The association types of *P. massoniana* forests in southern China are diverse; they can be divided into 76 types [22], providing “natural experimental materials” for studying the effects of changes in tree species composition and forest community structure on the EMF community of the same host (*P. massoniana*).

In this study, we selected six typical *P. massoniana* associations in subtropical mountainous areas using both morphological identification and molecular biology methods to classify and identify EMF associated with *P. massoniana*. Our research objectives were to: (1) assess whether different *P. massoniana* association habitats (MpAHs) alter the composition and structure of EMF communities associated with *P. massoniana*; (2) determine whether MpAHs affect the diversity of EMF communities associated with *P. massoniana*; and (3) identify the factors that have a prominent influence on the EMF communities associated with *P. massoniana* in different MpAHs. This study aimed to provide a scientific basis for protecting the diversity of *P. massoniana* EMF resources and realizing the sustainable management of *P. massoniana* forests.

2. Materials and Methods

2.1. Study Area

The study area is located in Kaiyang County (26°48′–27°22′ N, 106°45′–107°17′ E), Guizhou Province, China, which is a mountainous plateau area with an altitude of 1000–1400 m. The geological structure is complex, showing a high trend in the southwest and a low trend in the northeast. The area predominantly has a humid subtropical monsoon climate with an average temperature of 13 °C and an average annual rainfall of approximately 1120 mm. Soil types are mainly yellow soil and yellow-brown soil. The zonal vegetation type is evergreen broad-leaved forest, and the tree species in the area mainly include Fagaceae, Betulaceae, Lauraceae, and Aquifoliaceae [23].

2.2. Sample Collection and Processing

In July 2020, we selected six representative *P. massoniana* associations in Kaiyang County, Guizhou Province, which were designated on the basis of the dominant species at each association site as follows: *P. massoniana* + *Cunninghamia lanceolata* (Mp_Cl), *P. massoniana* + *Quercus fabri* (Mp_Qf), *P. massoniana* + *Populus adenopoda* (Mp_Pa), *P. massoniana* + *Liquidambar formosana* (Mp_Lf), *P. massoniana* middle-age plantation (Mp_MP), and *P. massoniana* old-age plantation (Mp_OP). At each site, we established a single 20 m × 20 m plant community survey plot, the site conditions and plant community information of which were recorded [23] (Table 1). In each of these plots, we randomly selected 10 healthy *P. massoniana* trees with relatively uniform diameters at breast height and they were separated by a minimum spatial distance of 3 m [24]. For each of these trees, using a root-cutting knife, we collected EMF root tips from within a 1–1.5 m radius of the base of the tree trunk [25].

Each sampled root system was separately placed in a plastic bag and maintained at 4 °C until further analysis. In addition, soil samples were excavated from the 0–20 cm topsoil layer at six points located along an “S” path drawn within each plot and thoroughly mixed into one composite sample. Six soil samples were collected (a mixed soil sample for each quadrat). For each sample, 500 g of soil was sieved through a 2 mm and 0.25 mm mesh and air-dried for physicochemical analyses [26].

Table 1. Vegetation and soil physicochemical features in different *Pinus massoniana* associations habitats.

Sites	<i>P. massoniana</i> Associations					
	Mp_Cl	Mp_Qf	Mp_Pa	Mp_Lf	Mp_MP	Mp_OP
Latitude/Longitude	106°59′27.39″ E, 27°5′30.25″ N	106°59′14.80″ E, 27°6′9.22″ N	106°59′7.78″ E, 27°6′9.22″ N	106°59′7.78″ E, 27°6′34.00″ N	106°59′15.28″ E, 27°16′34.98″ N	106°59′24.00″ E, 27°16′45.21″ N
Age (a)	30–40	30–40	30–40	20–30	20–30	150–200
Altitude (m)	1132	1162	1156	1080	977	943
Aspect (°)	175	23	48	229	128	136
Slope (°)	10	10	8	5	12	8
Canopy Coverage (%)	85	93	95	72	88	68
LAI	1.50	1.54	1.45	1.30	1.65	1.49
pH	4.76 ± 0.05 b	4.34 ± 0.02 e	4.66 ± 0.04 c	4.88 ± 0.04 a	4.65 ± 0.02 c	4.45 ± 0.01 d
SOM (g/kg)	49.34 ± 0.31 b	54.10 ± 2.38 a	53.04 ± 5.03 a	56.57 ± 1.06 a	27.26 ± 1.70 c	41.74 ± 5.22 b
TN (g/kg)	0.98 ± 0.04 a	1.18 ± 0.01 a	1.21 ± 0.01 a	1.38 ± 0.02 a	1.07 ± 0.02 a	0.98 ± 0.04 a
TP (g/kg)	0.40 ± 0.01 b	0.43 ± 0.01 b	0.54 ± 0.04 a b	0.68 ± 0.04 a	0.40 ± 0.01 b	0.40 ± 0.01 b
AN (mg/kg)	5.19 ± 0.63 b	6.08 ± 0.30 ab	3.92 ± 1.36 b	7.69 ± 0.65 a	5.25 ± 0.59 b	5.19 ± 0.63 b
AP (mg/kg)	17.81 ± 3.23 a	11.32 ± 1.72 ab	12.91 ± 3.44 ab	15.98 ± 2.68 ab	9.35 ± 1.14 b	10.43 ± 1.44 b
AK (mg/kg)	59.00 ± 9.54 b	39.00 ± 0.00 d	52.33 ± 1.15 b c	82.67 ± 0.58 a	44.33 ± 0.58 cd	60.00 ± 1.00 b
T_Shanon index	1.71	2.07	2.15	1.90	0.47	0.60
T_Simpson index	0.79	0.86	0.87	0.81	0.29	0.41
T_Pielou index	0.88	0.94	0.93	0.86	0.68	0.87

P. massoniana + *Cunninghamia lanceolata*, Mp_Cl; *P. massoniana* + *Quercus fabri*, Mp_Qf; *P. massoniana* + *Populus adenopoda*, Mp_Pa; *P. massoniana* + *Liquidambar formosana*, Mp_Lf; *P. massoniana* middle-age plantation, Mp_MP; *P. massoniana* old-age plantation, Mp_OP; leaf area index, LAI; soil organic matter, SOM; total nitrogen, TN; total phosphorus, TP; available nitrogen, AN; available phosphorus, AP; available potassium, AK; different lowercase letters for a variable indicate the significance of soil properties in different *P. massoniana* associations at the $p < 0.05$ level; ± indicates standard error.

2.3. Analysis of Soil Properties and Plant Diversity

Soil pH was determined by the potentiometric method (water/soil ratio was 2.5:1). Soil organic matter (SOM) was determined by the potassium dichromate oxidation–external heating method. Total nitrogen (TN) was determined using the semi-micro Kjeldahl method. Total phosphorus (TP) was determined by the molybdenum antimony anticolorimetric method. Available nitrogen (AN) was determined by the alkaline hydrolysis diffusion method. Available phosphorus (AP) was determined by the molybdenum blue method. Available potassium (AK) was determined by flame photometry [27]. A plant community survey of each association plot was conducted using the method described by Fang et al. [28]. The diversity indices (Shannon, Simpson, and Pielou) of tree species in the tree layers of each plot were calculated [23].

2.4. Classification and Identification of EMF

The mycorrhizal samples were taken out and placed in a Petri dish, washed repeatedly with tap water, and then observed under a stereomicroscope (Motic China Group Co., Ltd., SMZ-171, Xiamen, China) for examination at 2.25–200× magnification. Preliminary classification of the mycorrhizae was performed based on characteristics such as morphology, branching, color, and presence or absence of mycelium [29], and two to three mycorrhizal root tips of each morphology were picked for DNA analysis to identify EMF species [30]. The primers ITS1F (5′-CTTGGTCATTTAGAGGAAGTAA-3′) and ITS4 (5′-TCCTCCGCTTATTGATATATATGC-3′) were used for PCR amplification. The PCR system volume was 25 µL [31]. The PCR reaction conditions were as follows: predenaturation at

94 °C for 3 min; denaturation at 94 °C for 30 s; annealing at 55 °C for 30 s; and extension at 72 °C for 60 s, followed by 34 cycles and extension at 72 °C for 10 min at the end of the cycle. After PCR products were verified by 1% agarose gel electrophoresis in 2 µL volumes, the qualified samples were sent to Sangon Bioengineering (Shanghai, China) Co., Ltd. for sequencing. DNA sequences from this study were submitted to GenBank.

2.5. Statistical Analysis

DNA sequences were aligned using the National Center for Biotechnology Information (NCBI) (<https://www.ncbi.nlm.nih.gov/> accessed on 13 May 2023) Basic Local Alignment Search Tool (BLAST) with 97% sequence similarity for operational taxonomic unit (OTU) classification. SPSS 22.0 was used for the analysis of variance (ANOVA), and Tukey's post hoc test was used to test the significance of the soil factors in different associations ($p < 0.05$). We used an UpSet diagram to characterize the quantitative characteristics of the EMF community species associated with *P. massoniana* in different MpAHs and used Gephi (version 0.9.3) to create the OTU coexistence network diagram. Principal coordinate analysis (PcoA) was used to analyze and visualize the composition of EMF associated with *P. massoniana* in different MpAHs. A Pearson correlation analysis heat map was used to evaluate the correlation between soil factors and the EMF community of *P. massoniana*. Simple linear regression analysis of tree species diversity and EMF diversity of *P. massoniana* in the natural secondary *P. massoniana* mixed forests was performed using the ggTrendline.

3. Results

3.1. EMF Community Composition of *P. massoniana*

In total, 210 effective sequences were obtained from ectomycorrhizal roots. After the OTUs were aligned ($\geq 97\%$), using UNITE and NCBI to remove the duplicate sequences, 56 OTUs belonging to 20 families and 22 genera were identified (Table 2). The dilution curves of the six MpAHs differed significantly (Figure 1a). Most of the curves continued to rise, indicating that the sample size should be increased in future studies. Only one OTU was observed in all the MpAHs, and the specific OTUs were Mp_Pa (9) > Mp_OP (7) = Mp_MP (7) > Mp_Qf (5) = Mp_Cl (5) > Mp_Lf (4) (Figure 1b). The ratio of OTU richness of Basidiomycota to that of Ascomycota differed distinctly in most MpAHs (Figure 2a), mainly Mp_Lf (8:3), Mp_Pa (2:1), Mp_MP (11:7), Mp_OP (1:1), Mp_Cl (1:1), and Mp_Qf (7:8). At the genus level, *Tomentella*, unclassified Helotiales, and *Russula* were the dominant genera of the EMF community in Mp_Cl; an unclassified Helotiales was in Mp_Qf; *Lactarius* and *Russula* were in Mp_Pa; *Cenococcum*, *Russula*, and *Lactarius* were in Mp_Lf; *Tomentella* and *Lactarius* were the dominant genera of Mp_MP; and unclassified Helotiales and *Lactarius* were in Mp_OP (Figure 2b).

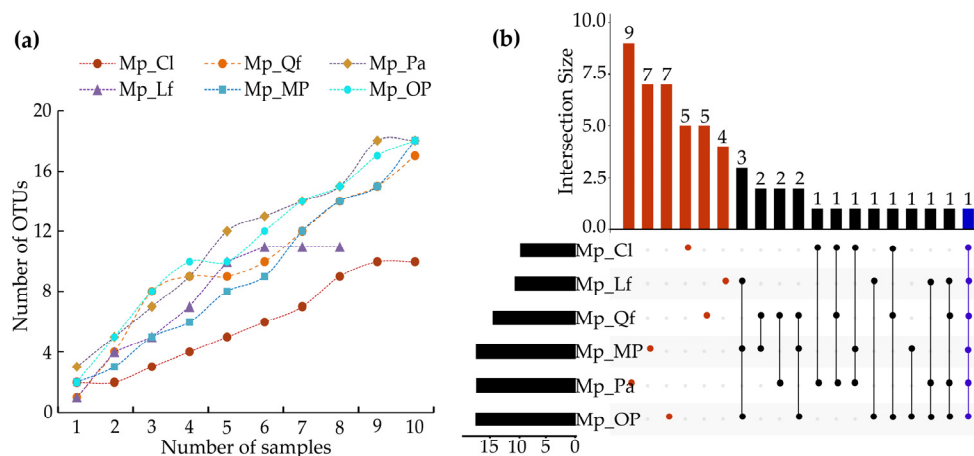


Figure 1. Dilution curves (a) and UpSet Venn diagram (b) of *Pinus massoniana* ectomycorrhizal fungi in different *P. massoniana* association habitats.

Table 2. Ectomycorrhizal fungi colonizing *Pinus massoniana* root from different *P. massoniana* association habitats were identified based on morphotyping and sequencing of the internal transcribed spacer (ITS) rDNA.

Number	OTUs	Sequence Length (bp)	The Alignment Number	Identity (%)	Genbank ID
1	Agaricales sp.	789	FJ266729	97.36	OR467492
2	<i>Aleurina imaii</i>	672	MG871292	98.15	OR469906
3	<i>Amphinema</i> sp. 1	592	LC013707	98.37	OR482662
4	<i>Amphinema</i> sp. 2	606	JN943925	99.15	OR482663
5	<i>Archaeorhizomyces borealis</i>	499	NR_126144	98.8	OR482664
6	<i>Astrosporina</i> sp.	533	JQ991646	99.06	OR483811
7	<i>Cenococcum</i> sp. 1	592	LC095124	94.90	OR482665
8	<i>Cenococcum</i> sp. 2	545	AB769888	98.45	OR482666
9	<i>Cladophialophora</i> sp.	636	LC229676	98.66	OR482667
10	<i>Clavulina amethystina</i>	695	MK422194	99.23	OR482668
11	<i>Clavulina</i> sp.	695	ON794325	94.14	OR482669
12	<i>Clavulina thindii</i>	463	MG892054	98.15	OR482670
13	Helotiales sp. 1	587	KP866121	98.96	OR482671
14	Helotiales sp. 2	641	KP866122	96.54	OR482672
15	Helotiales sp. 3	512	KP866123	99.45	OR482673
16	Helotiales sp. 4	566	KX440153	98.92	OR482674
17	Helotiales sp. 5	461	MG670433	98.81	OR483812
18	Helotiales sp. 6	460	AB636433	99.22	OR483813
19	<i>Hyaloscypha aff.hepaticicola</i>	562	AB847066	99.42	OR482675
20	<i>Hyaloscypha</i> sp. 1	583	OQ430740	99.79	OR482676
21	<i>Hyaloscypha</i> sp. 2	586	MT522552	99.26	OR482677
22	<i>Hyaloscypha</i> sp. 3	904	OQ207649	99.77	OR482678
23	Hyaloscyphaceae sp.	571	KU141214	99.30	OR482679
24	<i>Hymenoscyphus</i> sp.	550	KF679808	98.29	OR482680
25	<i>Inocybe</i> sp. 1	584	MT237516	91.55	OR482681
26	<i>Inocybe</i> sp. 2	584	LC175093	98.28	OR482682
27	<i>Lactarius hatsudake</i>	709	EF685076	99.57	OR482683
28	<i>Lactarius kesiya</i>	732	KR025614	99.17	OR482684
29	<i>Lactarius parallelus</i>	743	MH984997	98.84	OR482685
30	<i>Oidiiodendron</i> sp.	568	EU888629	99.46	OR482686
31	<i>Phialocephala fortinii</i>	901	KX440179	99.20	OR482687
32	<i>Russula cascadenis</i>	653	MT522568	99.67	OR482688
33	<i>Russula indocatillus</i>	684	MN581483	99.51	OR482689
34	<i>Russula rosea</i>	693	MZ221554	99.23	OR482690
35	<i>Russula</i> sp. 1	690	MK770275	98.77	OR482691
36	<i>Russula</i> sp. 2	663	OQ421796	99.75	OR482692
37	<i>Russula</i> sp. 3	671	OQ430675	98.65	OR482693
38	<i>Russula</i> sp. 4	690	LC367779	98.12	OR482694
39	<i>Russula</i> sp. 5	702	KU205301	93.64	OR482695
40	<i>Russula vesca</i>	676	HM189953	97.64	OR482696
41	Russulaceae sp.	683	FJ454965	97.39	OR482697
42	<i>Sebacina</i> sp. 1	623	OM236634	97.81	OR482698
43	<i>Sebacina</i> sp. 2	642	KP013014	91.72	OR482699
44	<i>Sebacina</i> sp. 3	646	KF000417	94.51	OR482700
45	<i>Sphaerospora</i> sp.	602	MW476527	98.00	OR482701
46	Thelephoraceae sp.	660	AB634273	99.07	OR482702
47	<i>Tomentella</i> sp. 1	687	MN970734	98.88	OR482703
48	<i>Tomentella</i> sp. 2	666	KP866136	99.39	OR482704
49	<i>Tomentella</i> sp. 3	662	JX630406	96.91	OR482705
50	<i>Tomentella stuposa</i>	669	MK602778	97.30	OR482706
51	<i>Tomentella sublilacina</i>	660	OQ430790	99.70	OR482707
52	Trechisporales sp.	645	LC436083	99.81	OR482708
53	<i>Trichoderma</i> sp.	609	MK870953	99.67	OR482709
54	<i>Tylospora</i> sp. 1	605	AB456677	98.66	OR482710
55	<i>Tylospora</i> sp. 2	598	KF007260	99.50	OR482711
56	<i>Venturia</i> sp.	562	MT522585	99.61	OR482712

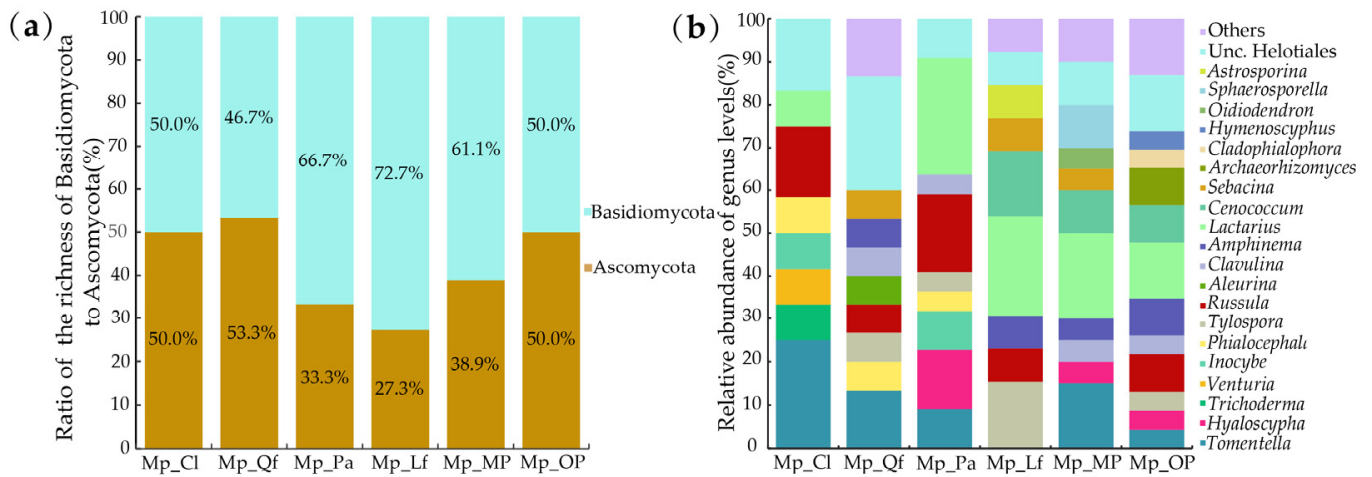


Figure 2. Ratios of Ascomycota to Basidiomycota fungal phyla (a) and relative abundance at the ectomycorrhizal fungal genus level (b) in different *Pinus massoniana* association habitats.

P. massoniana EMF communities showed significant differences in different MpAHs, mainly in terms of their species composition. The number of specific OTUs was significantly higher than that of common species (Figure 3). The common OTU in the six associations was *Helotiales* sp. 4. The common OTU among the four associations was *Tylospora* sp. 2. The OTUs of the three associations were *Tomentella* sp. 2, *Helotiales* sp. 1, *Phialocephala fortinii*, *Cenococcum* sp. 1, *Cenococcum* sp. 2, *Lactarius kesiyae*, *Russula indocatillus*, *Amphinema* sp. 2, and *Lactarius parallelus*.

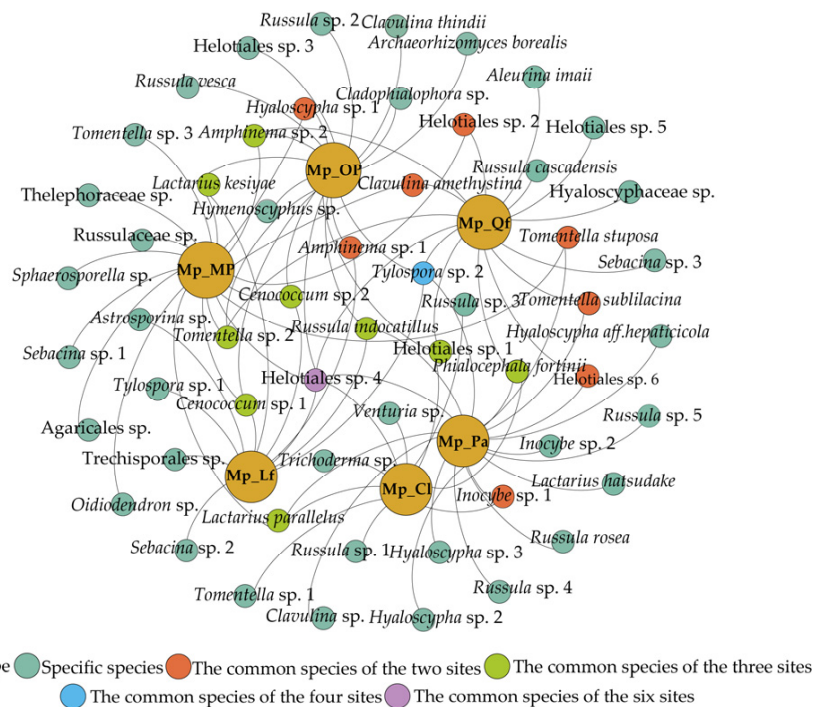


Figure 3. Co-occurrence network of *Pinus massoniana* ectomycorrhizal fungal communities in different *P. massoniana* association habitats.

3.2. EMF Community Diversity of *P. massoniana*

The EMF diversity indices of *P. massoniana* differed significantly among different MpAHs (Tables 3 and 4). In general, the diversity and evenness indices of the EMF communities in the pure forest habitats of *P. massoniana* were higher than those in the mixed forest habitats of *P. massoniana*. The EMF community diversity and richness index (Chao1) of *P. massoniana* in Mp_Pa was highest in the mixed forest habitats of *P. massoniana*. The Sorensen index was less than 0.5 and the Jaccard index was less than 0.2 among EMF communities. The results showed that different MpAHs altered the composition of the *P. massoniana* EMF community.

Table 3. Diversity indices of ectomycorrhizal fungal community of *Pinus massoniana* in different *P. massoniana* association habitats.

Diversity Index	Mp_Cl	Mp_Qf	Mp_Pa	Mp_Lf	Mp_MP	Mp_OP
Shannon	2.062	2.385	2.651	2.216	2.728	2.815
Simpson	0.833	0.864	0.908	0.870	0.923	0.934
Pielou	0.896	0.881	0.917	0.924	0.944	0.974
Chao1	13.750	33.333	63.500	39.000	34.500	53.000

P. massoniana + *Cunninghamia lanceolata*, Mp_Cl; *P. massoniana* + *Quercus fabri*, Mp_Qf; *P. massoniana* + *Populus adenopoda*, Mp_Pa; *P. massoniana* + *Liquidambar formosana*, Mp_Lf; *P. massoniana* middle-age plantation, Mp_MP; *P. massoniana* old-age plantation, Mp_OP.

Table 4. Sorensen similarity index (lower left) and Jaccard similarity index (upper right) of ectomycorrhizal fungal community of *Pinus massoniana* in different *P. massoniana* association habitats.

Sites	Similarity Index					
	Mp_Cl	Mp_Qf	Mp_Pa	Mp_Lf	Mp_MP	Mp_OP
Mp_Cl		0.107	0.125	0.045	0.069	0.065
Mp_Qf	0.222		0.132	0.071	0.135	0.128
Mp_Pa	0.286	0.303		0.094	0.125	0.098
Mp_Lf	0.095	0.154	0.207		0.125	0.189
Mp_MP	0.148	0.313	0.286	0.286		0.163
Mp_OP	0.211	0.294	0.162	0.467	0.211	

P. massoniana + *Cunninghamia lanceolata*, Mp_Cl; *P. massoniana* + *Quercus fabri*, Mp_Qf; *P. massoniana* + *Populus adenopoda*, Mp_Pa; *P. massoniana* + *Liquidambar formosana*, Mp_Lf; *P. massoniana* middle-age plantation, Mp_MP; *P. massoniana* old-age plantation, Mp_OP.

3.3. The Relationship between EMF Community of *P. massoniana*, Tree Species Diversity, and Soil Properties

The PCoA clustering map showed an apparent dissimilarity significantly in the EMF communities of different MpAHs ($R^2 = 0.21$, $p = 0.001$) (Figure 4a). The correlation heatmap indicated that *P. massoniana*'s EMF community composition was significantly correlated with soil properties (Figure 4b). *Tylospora* was significantly positively correlated with TP ($p < 0.05$); *Astrosporina* was significantly and positively correlated with TP and AK ($p < 0.05$); unclassified Helotiales was significantly and negatively correlated with pH ($p < 0.01$); *Clavulina* was significantly and negatively correlated with AP ($p < 0.01$); and *Sphaerospora* and *Oidiodendron* were significantly and negatively correlated with SOM ($p < 0.05$). Linear regression analysis showed that the diversity indices of *P. massoniana* EMF had a significant positive relationship with the diversity indices of tree species ($p < 0.05$, for Shannon; $p > 0.05$, for Simson) but a negative relationship with the evenness index of tree species ($p > 0.05$, for Pielou) in natural secondary MpAHs (Figure 5).

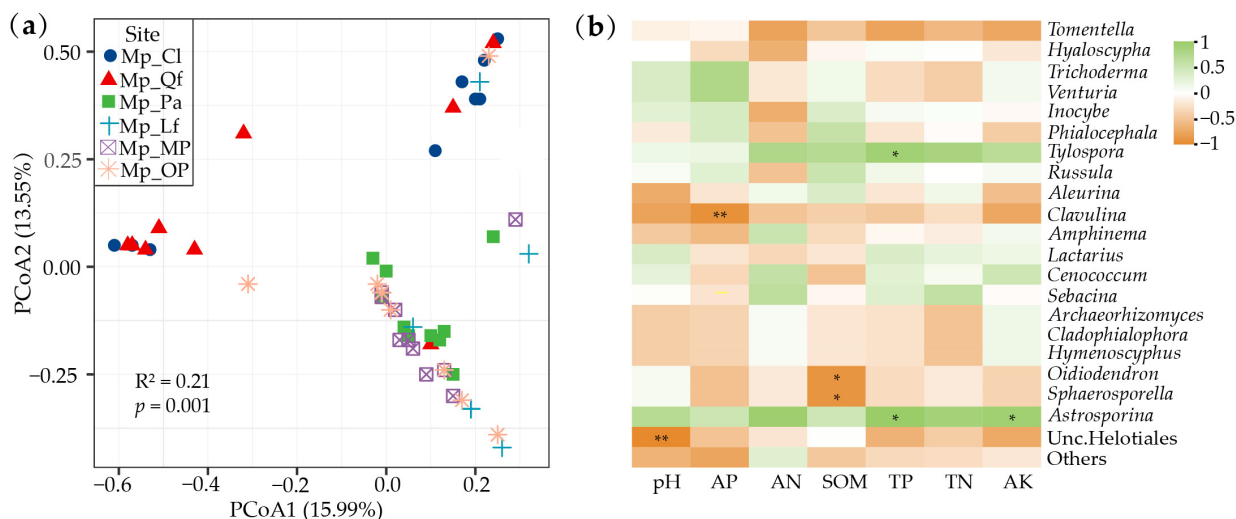


Figure 4. Principal coordinates analysis (PCoA) (a) and Pearson correlation heat map (b) of *Pinus massoniana* ectomycorrhizal fungal community composition and soil properties. Green is positive, red is negative; the darker color means the correlation is stronger. *: significant at $p < 0.05$; **: significant at $p < 0.01$.

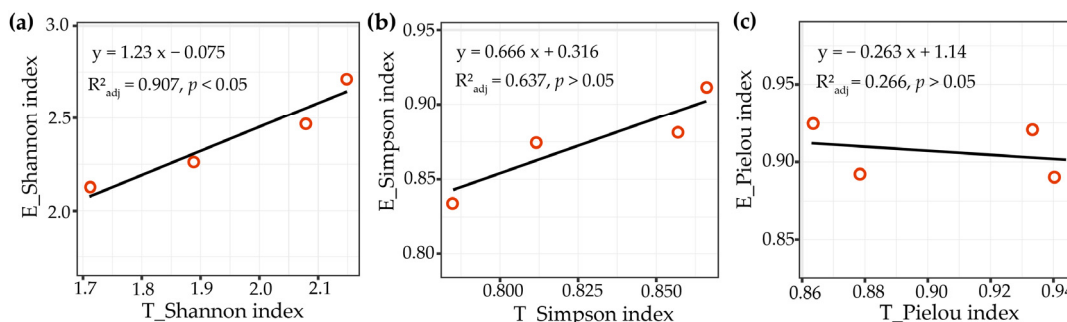


Figure 5. Linear regression analysis of ectomycorrhizal fungi of *Pinus massoniana* diversity indices against tree species diversity indices in different mixed forest stands.

4. Discussion

4.1. Composition and Structure of *P. massoniana* EMF Community

P. massoniana forests are widely distributed in Southwest China, where EMF species are abundant. A total of 56 EMF OTUs belonging to 20 families and 22 genera were identified. The OTU richness of Basidiomycota was significantly higher than that of Ascomycota, which is consistent with previous studies on other Pinaceae species [32,33], reflecting the dominant position of Basidiomycota EMF in the pine forest ecosystem. Unclassified Helotiales, *Russula*, *Lactarius*, and *Tomentella* were the dominant groups in the *P. massoniana* EMF community in the MpAHs. Unclassified Helotiales EMF are members of Ascomycota and comprise the main EMF group (particularly in the Mp_Qf) that may be closely associated with the density of the plant community and habitat conditions at the succession stage [34]. It is speculated that this type of EMF is more adapted to the mid-stages of forest succession (with density competition characteristics), which is similar to the regional forest vegetation succession stage of *P. massoniana* associations. *Russulaceae* is the dominant family of EMF in southern China [35]. *Russula* and *Lactarius*, which have high economic and ecological values, are important EMF in *Russulaceae* [36] and the dominant genera of EMF communities in other forest ecosystems [37–39]. *Lactarius* can enhance the aluminum (Al) toxicity tolerance of plants. *L. deliciosus* can enhance Al tolerance in *P. massoniana* seedlings used for forest plantation and ecosystem restoration in acidic soils, particularly in Southwest China [40]. *Tomentella* species are a typically dominant group in EMF communities of coniferous and deciduous forests [41], the members of which play a broad range of saprophytic roles and

can readily adapt to environmental change by altering their survival strategies [42]. We found that the number of specific OTUs in the EMF community of *P. massoniana* was higher than the shared common OTUs and the composition of the EMF community of *P. massoniana* was significantly different in different MpAHs. The differences in stand structures of different MpAHs greatly changed the characteristics of the habitat, and competition for resources among different tree species affects the growth of the neighborhood [43], importantly impacting the composition of the EMF community. In addition, the differences in the biological characteristics of different EMF species are also a vital reason for the formation of specific EMF communities in different MpAHs.

4.2. Diversity of *P. massoniana* EMF Community

The tree species composition in forest ecosystems is a vital biological factor that determines the diversity of EMF [10]. The survey plots in this study were differentiated by the origin of the *P. massoniana* stands, and with the exception of the Mp_MP and Mp_OP, which were *P. massoniana* plantations, the remainder were natural secondary *P. massoniana* mixed forest stands. The diversity of *P. massoniana* EMF communities in different MpAHs showed obvious differences. These influencing factors can be due to two reasons: first, in terms of the direct effect on plants, different forest composition structures can change soil microhabitats through different litter compositions and root exudates, thereby improving the effectiveness of the niche and causing changes in EMF diversity [8] and the competition for niche between different EMF species [44], which can importantly impact the distribution of EMF communities. We found that the diversity of *P. massoniana*-associated EMF in the Mp_Pa was higher than that of others in all natural secondary *P. massoniana* mixed forest types, which could be attributable to the fact that *P. massoniana* and *P. adenopoda* are tree species with regional vegetation succession, and both are typical EMF hosts, while *P. adenopoda* (deciduous tree species) has little competitive advantage over *P. massoniana* (evergreen coniferous species) in the community. The dominant trees in Mp_Cl and Mp_Lf were *L. formosana* and *C. lanceolata*, both of which are host plants for arbuscular mycorrhizal fungi [45,46] and can exert synergistic or antagonistic effects on EMF in the process of nutrient absorption and mineralization that may affect the EMF community composition of *P. massoniana* [47,48]. The EMF diversity of *P. massoniana* in Mp_OP was higher than that in Mp_MP, indicating that the EMF diversity of *P. massoniana* increased with increasing tree age and that *P. massoniana* planted in Mp_OP was approximately 100 years old and grew well. Its high plant productivity levels can provide more restrictive resources to soil fungi, increasing its scale and diversity [49]. Thus, the diversity of EMF may also be affected by the level of nutrition provided by the plants. In conclusion, the EMF community diversity of *P. massoniana* may be significantly affected by the composition and structure of (host and nonhost) tree species in MpAHs.

4.3. Factors Influencing of *P. massoniana* EMF Community

The differences in host habitat conditions affect the changes in root-related microbial composition [50], including soil factors and vegetation communities (e.g., tree species composition and plant diversity). Soil pH is a key factor affecting the species composition of soil fungi, which can indirectly change the distribution of fungal communities by changing the availability of soil nutrients [51]. Most EMF prefer to grow in weakly acidic soils; however, the mycelium of mycorrhizal fungi can secrete high-concentration and low-molecular-weight organic acids to reduce pH [52]. In this study, the soil pH ranged from 4.50 to 5.00, which can affect the EMF community. SOM provides the main metabolites and energy for mycorrhizal fungi, and EMF are thought to have a key role in mobilizing organic nitrogen that is trapped in SOM [53,54]. N and P are important factors affecting the symbiotic relationship between EMF and host plants [55]. High N content can decrease the biodiversity of soil fungal communities, and mycorrhizal fungi are sensitive to changes in soil N content [56]. Soil P was limited, and the growth and biomass of EMF hyphae decreased [57]. K is one of the large nutrient elements required by vegetation, and no K⁺

or high K^+ inhibits the growth of EMF [58]. Changes in the forest vegetation type can lead to changes in AN, AP, and other soil factors [59]. In our study, these soil properties showed significant differences in different MpAHs (Figure 4b). The K^+ content of Mp_Lf was significantly higher than that of the other associations, which may be the reason for the lowest EMF diversity in other mixed forests. Thus, the EMF community structure of *P. massoniana* varied significantly with the soil properties of the different MpAHs. Notably, these soil variables were not correlated to the abundance of several EMF, which may be related to the preferences of different EMF for soil nutrients. Some studies show that dominant trees differentially modify soil properties [60], and fungal communities, both in litter and soil, are strongly affected by dominant vegetation [61]; the effect of plant diversity on fungal richness and community composition may override that of abiotic variables [62]. Therefore, except for nutrient preferences, the possibility then exists that other tree species in different mixed habitats have a greater impact on the EMF community diversity of *P. massoniana* than these soil factors, especially in codominant species.

In the four natural secondary *P. massoniana* mixed forests assessed in this study, we detected a positive correlation between the EMF diversity of *P. massoniana* and tree species diversity, indicating that tree species diversity influences the diversity of the EMF associated with *P. massoniana*, which is consistent with the findings of previous studies on the relationship between EMF diversity and tree species diversity in the tree layer [10]. The effects of tree characteristics and their dominance on ecosystem function are often stronger than species diversity in temperate forests with low tree richness [63]. The effect of tree species composition on EMF communities is often related to the characteristics of the hosts and their surrounding tree species [11]. Tree species resources in mixed forests are separate and often show different intensities of competition [64]. Competition between adjacent trees often inhibits the growth of the target tree species [65]. *C. lanceolata* and *P. massoniana* have similar ecological habits [66] and usually occupy similar niches, resulting in fierce competition that may lead to the inhibition of EMF growth. *P. massoniana* and *L. formosana* (broad-leaved tree species) are strong, positive, fast-growing tree species. During the course of plant community succession, the rapid growth of *L. formosana* in the tree layer intensifies the competition for light resources, which may influence the growth of *P. massoniana* [67]. *Q. fabri* is a further EMF host plant that is characterized by a long generation time and slow rates of growth and evolution [68,69]. We found *Q. fabri* seedlings were rich in Mp_Qf association, which may have a strong competitive effect in the later stages of succession, allowing it to gradually replace pioneer tree species (*P. massoniana*). Other naturally regenerated tree species also compete with *P. massoniana*, but the number of trees is small and competition is weak. Thus, we speculate that the differences in competitive advantage between *P. massoniana* and its codominant species (Mp_Pa > Mp_Qf > Mp_Lf > Mp_Cl) in different MpAHs may explain the differences in the EMF communities of *P. massoniana*. However, given that the effects of tree species composition and diversity on the local EMF communities of *P. massoniana* may be influenced by multiple factors, these should be considered in future studies, including the effects of sampling and other potential abiotic and biotic variables.

5. Conclusions

This study provides new information on the effects of plant diversity and soil factors on the ectomycorrhizal diversity of *P. massoniana* in different associations. It indicated that different stand types in different mountainous areas could significantly affect the EMF community composition and diversity of *P. massoniana*. These results provide a better understanding of the effects of different tree species and mixed *P. massoniana* stands on the soil EMF community and will aid the sustainable and ecological management of *P. massoniana* plantations. Additional research is also necessary to further explore appropriate plot areas and sample sizes and quantify the effects of variables such as climate, soil factors, and plant composition on EMF diversity in different *P. massoniana* stands.

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