COMMUNITY STRUCTURE AND DRIVING FACTORS FOR RHIZOSPHERE ECTOMYCORRHIZAL FUNGI OF *PINUS MASSONIANA* L. IN YUNTAI MOUNTAIN

WENQUAN ZHANG[1], DASHI XIE[1], HUAFANG YU[1], JIE DENG[2] AND QINGZHI YAO[1]

Inner Mongolia Agricultural University, Hohhot 010018, Inner Mongolia, China

Abstract

Pinus massoniana L. is an important andi typical ectomycorrhizal dependent tree species in Guizhou. In order to reveal the community structure and driving factors for ectomycorrhizal fungi (ECMF) of *P. massoniana* in Karst region, Illumina MiSeq high-throughput sequencing was used to study the diversities and community structure characteristics of ECMF in the rhizosphere soil of *P. massoniana* L. in the three sites of Xugongdian (SY1), Dishuiguanyin (SY2) and Yingtaowan (SY3) in Yuntai Mountain, Guizhou. The ectomycorrhizal fungi belonged to 2 phyla, 5 classes, 11 orders, and 38 genera. At the phylum level, ectomycorrhizal fungi mainly belonged to the Basidiomycota, accounting for 96.20% of all ectomycorrhizal fungi; the rest were Ascomycota fungi, accounting for only 3.8%. At the genus level, Russula, Cortinarius, Suillus, Inocybe, Tricholoma, Tomentella, Sebacina, Scleroderma, Cenococcum were the dominant ectomycorrhizal fungi of *P. massoniana* . The diversity and richness of ectomycorrhizal fungi communities in the three sites were significantly different. The ectomycorrhizal fungal community structures in SY1 and SY3 were similar, but were quite different from those in SY2. All soil factors explained 97.7% of the differences in the community composition of ECMF, with AP, TN, OM and AK being the main driving factors for the differences in the community composition of ectomycorrhizal fungi in the three sites.

Introduction

Soil microorganism is the core component of soil ecosystem, and its diversity and community composition largely determine the versatility of ecosystem (Tedersoo *et al.* 2014). Mycorrhizal fungi in soil microorganisms are one of the most important functional groups in soil ecosystem (Van Dam and Bouwmeester 2016). Ectomycorrhizal (ECM) fungi can promote the absorption of soil mineral nutrients and water by host plants and improve the ability of plants to resist early, salt and heavy metal stress (Garcia *et al.* 2016). They are widely distributed in forest ecosystem, and their community structure and diversity are affected by plant types, soil conditions, i climate conditions and other factors (Tedersoo *et al.* 2014, Ren *et al.* 2018, Rilig *et al.* 2019, Liu *et al.* 2020). Therefore, the diversity and community structure of mycorrhizal fungi can be used as one of the important indicators to evaluate the health and stability of their ecosystem (Delgado baquerizo *et al.* 2020).

Pinus massoniana is a pioneer native tree species for vegetation restoration in South China. It is of great significance for maintaining regional ecological balance. It is a typical Ectomycorrhizal fungi (ECM) tree species. Previous studies show that there are abundant ECM fungi resources with high diversity in *P. massoniana* forests in Guizhou., mainly Russulaceae, Cortinariaceae, Suillaceae, etc. (Li 2016, Luo 2013, Chen 2020). Yuntai Mountain of this study area is located in Shibing County, Guizhou Province. It is a typical dolomite karst area and has continuous karst

---

[1] Author for correspondence: <yaoqingzhi@imau.edu.cn>. [1]Loudi Vocational Technical College, Loudi, 417000, Hunan, China. [2]Kaili University, Kaili 556011, Guizhou, China.

natural forest land. There are significant differences with other areas in soil, climate and geography. These factors are important factors affecting the composition of ectomycorrhizal fungi community. The important question to solved is that is there a significant difference in the composition of ECM fungi community of *P. massoniana* under different ecological environment conditions, and what are the driving factors for the difference in the composition of ECM fungi community? Based on this, ECM fungi community structure was studied in the rhizosphere soil of *P. massoniana* in three typical distribution areas of Yuntai Mountain, Shibing, in order to explore the diversity and community structure characteristics of ECM fungi of *P. massoniana* in Dolomite Karst area, and the driving factors causing the difference of ECM fungi community composition were analyzed. Further the understanding of the composition of ECM fungi community was enhanced in Karst ecosystem.

Materials and Methods

The study site was located in Shibing County in the east of Guizhou Province (108° 01'E-108° 10'E, 27°13'N-27°04'N). Three sites with concentrated distribution of *Pinus massoniana* were selected as the sampling points: Xu Gongdian on the top of the mountain (SY1), Dishui Guanyin on the hillside (SY2) and Cherry bay at the foot of the mountain (SY3).

Four 25×25 m sample plots were randomly set at each point, with an interval of 20m. The sample plots were randomly arranged according to the S-type, and the rhizosphere soil of 10 healthy trees were collected. The surface litters were removed from the roots with a shovel and gently pull it to the root end of the tree, and then collect the rhizosphere soil at 20-50 cm depth. The collected soil samples are mixed evenly in each sample plot, stored in a low-temperature ice box and quickly brought back to the laboratory. The soil samples were screened through 2 mm soil sieve and divided into two parts, one for high-throughput sequencing (stored at -80°C) and the other for the detection of soil physical and chemical properties.

The physical and chemical properties of soil samples were tested in the laboratory according to the conventional determination method (Chen 2020).

The total DNA of soil genome was extracted by powerOil® DNA kit, and then PCR amplification and Operational Taxonomic Units (OTU) division were completed by sequencing company, using Illumina miseq sequencing platform. After high-throughput sequencing of fungi sequences, single databases were used for classification and annotation of ectomycorrhizal fungi (https://unite.ut.ee/index.php) database.

The diversity indexes of ectomycorrhizal fungi community were analyzed by language R, QIIME software and Mothur software. SPSS 22.0 statistical software was used to test the significant differences in the physical and chemical properties and ectomycorrhizal fungi diversity index of different sites through one-way ANOVA and multiple comparison ($p < 0.05$). Pearson correlation analysis was made between the relative abundance of dominant genera of ectomycorrhizal fungi and soil physical and chemical factors: using Canoco 5.0 software, RDA and PCA were carried out between environmental factors and ectomycorrhizal fungi community; Least Significant Difference were made for the mean values of different treatments at the 5% significance level.

Results and Discussion

The physical and chemical properties of the rhizosphere soil of *P. massoniana* at the three sites of Shibing Yuntai Mountain were shown in Table 1. The results showed that the total nitrogen content, available potassium content, available phosphorus content, organic matter content and water content of the rhizosphere soil at the three sites were significantly different. Among them,

the total nitrogen content, available potassium content and organic matter content at Xugongdian were the highest, and the water content at Dishui Guanyin was significantly higher than that in other sites. The available phosphorus content in Cherry bay was significantly higher than that in Xugongdian and Dishui Guanyin. The contents of hydrolyzed nitrogen and organic matter in the rhizosphere soil of *P. massoniana* in Xugongdian were significantly higher than those in the other two sites. It can be seen that there were significant differences in soil properties in the three sites.

Table 1. Physical and chemical properties of rhizosphere soil of *Pinus massoniana*.

Sampling site	Total N (g/kg)	Total P (g/kg)	Total K (g/kg)	Hydrolysis N (mg/kg)
SY1	$2.21 \pm 0.36a$	$0.24 \pm 0.01ab$	$32.02 \pm 5.44a$	$206.9 \pm 19.00a$
SY2	$1.06 \pm 0.95b$	$0.18 \pm 0.002ab$	$14.77 \pm 0.11b$	$129.00 \pm 0.00b$
SY3	$1.21 \pm 0.07ab$	$0.21 \pm 0.016ab$	$27.80 \pm 2.10a$	$170.9 \pm 9.00bc$

Table 1. Physical and chemical properties of rhizosphere soil of *P. massoniana* continued

Available P (mg/kg)	Available K (mg/kg)	Organic matter (g/kg)	WC (%)	pH
$3.30 \pm 1.20a$	$45.00 \pm 5.00a$	$57.20 \pm 7.45a$	$14.67 \pm 1.53c$	$5.32 \pm 0.46ab$
$1.34 \pm 0.15bc$	$33.00 \pm 1.00bc$	$29.57 \pm 0.32c$	$29.00 \pm 1.00a$	$5.12 \pm 0.31ac$
$2.44 \pm 0.95b$	$38.00 \pm 9.00bc$	$43.24 \pm 3.48b$	$22.67 \pm 1.53b$	$5.04 \pm 0.19ac$

Under the similarity level of 97%, the number of ECM fungi OTUs at each site was obtained, and the Venn diagram was used to show the number of common OTUs and unique OTUs of different sites (Fig. 1). A total of 128 OTUs sequences of ECM fungi were detected in the three sites, and the OTUs of SY1, SY2 and SY3 were 98, 67 and 92 respectively. There are 42 ECM fungi OTUs, 17 SY1 specific OTUs, 10 SY2 specific OTUs and 15 SY3 specific OTUs in the three sites, indicating that there are some differences in the composition of ECM fungi OTUs rhizosphere soil of *P. massoniana* in different sites.

The ECM fungi identified in all samples were divided into two phyla: Ascomycota and Basidiomycota, consisting 5 classes, 11 orders, 25 families and 38 genera. Basidiomycota accounts for 96.20%, while Ascomycota accounts for 3.80%. There are only Agaricomycetes in Basidiomycota, while Ascomycota includes Dothideomycetes, Pezizomycetes, Leotiomycetes and Eurotiomycetes, accounting for 46.76%, 36.30%, 16.24% and 0.70% of this phylum respectively. Agaricomycetes mainly includes 11 families, Russulaceae (20.87%), Cortinariaceae (17.92%), Inocybaceae (15.81%), Suillaceae (11.97%), Thelephoraceae (7.48%), Tricholomataceae (6.51%), Sebacinaceae (4.89%), Sclerodermataceae (2.92%), Hygrophoraceae (2.37%), Clavulinaceae (1.67%), Atheliaceae (1.35%).
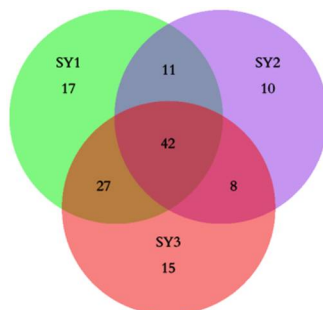

Fig. 1. Number of ectomycorrhizal fungal Operational Taxonomic Units (OTUs).

The analysis of the dominant genera of ECM fungi in the rhizosphere soil of *P. massoniana* showed that the dominant genera of ECM fungi in the three sites were *Russula* (20.33%), *Cortinarius* (17.59%), *Inocybe* (15.81%), *Suillus* (11.97%), *Tomentella* (7.43%), *Tricholoma* (6.51%), *Sebacina* (4.89%), *Scleroderma* (2.92%), *Hygrophorus* (2.37%), and *Clavulina* (1.67%), These 11 genera account for 93.25% of all ECM fungal OTUs. Ascomycetes include *Cenococcum, Melinomyces, Wilcoxina, Tuber* and other genera, but none of them belong to the dominant genus.

The distribution of dominant OTUs of ECM fungi in different sites was shown in Fig. 2. The dominant OTUs of ECM fungi in SY1 accounts for 50.88% of all dominant OTUs; The second is SY3, which is 32.86%; SY2 accounts for the lowest proportion, 16.26%, indicating that the distribution of dominant ECM fungi OTUs in *P. massoniana* rhizosphere soil was significantly different among the three sites.

Shown in Table 2, There were 20 OTUs of 11 dominant genera corresponding to 11 dominant families in the three sites, of which the number of dominant OTUs of SY1, SY2 and SY3 were 20, 16 and 19 respectively. The total number of dominant OTUs in the three sites is 15, accounting for 75% of all dominant OTUs, indicating that the composition of dominant ectomycorrhizal fungi in the rhizosphere of Pinus massoniana in the three sites is similar. The first four genera with the largest proportion in SY1 were *Inocybe* (39.65%), *Russula* (27.97%), *Cortinarius* (19.13%) and *Amphinema* (3.31%), accounting for 90.05%; The top four genera with the largest proportion of sy2 are *Suillus* (29.70%), *Cortinarius* (28.54%), *Tricholoma* (12.25%) and *Tomentella* (9.85%), accounting for 80.33%. The first four genera with the largest proportion of sy3 are *Russula* (46.03%), inocybe (20.21%), *Cortinarius* (7.34%), *Scleroderma* (6.42%), accounting for 79.99%; It shows that there are great differences in the composition of dominant ectomycorrhizal fungi OTUs in *Pinus massoniana* rhizosphere soil. Among them, the proportion of some OTUs in the three sites varies greatly. The composition of absolute dominant genera in sampling sites SY1 and sy3 is closer, sy2 is quite different from them, and the ECM fungal community structure of sampling sites SY1 and sy3 is more similar.
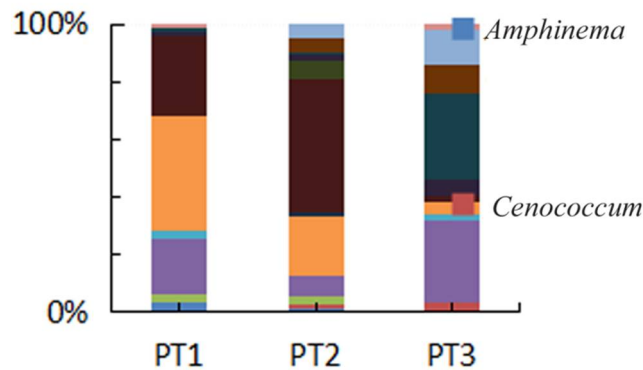

Fig. 2. Ectomycorrhizal fungal community structure of three different sampling sites.

ECM fungi communities in the rhizosphere soil of *P. massoniana* were studied at three sites and α diversity indexes were analyzed (Table 3). According to the ACE index and Chao1 index, the abundance of ECM fungi in SY1 and SY3 was significantly higher than that in SY2; According to Simpson index and Shannon index, there were significant differences in the diversity of ECM fungi in the three sites, SY1 was the highest, SY3 was the second, and SY2 was the lowest. The results showed that there were significant differences in the community structure of ECM fungi in the three sites.

Table 2. Distribution and proportion of dominant operational taxonomic units.

Families	Genera	Percentage %	Dominant OTUs	Percentage (%)	Percentage of total out (%)	Distribution		
						SY1	SY2	SY3
Russulaceae	*Russula*	23.86	OTU_35	18.33	14.07	√	√	√
			OTU_3911	3.62	2.78	√	√	√
			OTU_40	1.91	1.46	√	√	√
Cortinariaceae	*Cortinarius*	19.67	OTU_3074	9.08	6.97	√	√	√
			OTU_15	6.30	4.83	√	√	√
			OTU_1712	4.29	3.30	√	√	√
Suillaceae	*Suillus*	15.38	OTU_62	13.08	10.04	√	√	√
			OTU_250	2.30	1.76	√	√	√
Inocybaceae	*Inocybe*	14.54	OTU_57	6.85	5.26	√	√	√
			OTU_38	5.64	4.33	√	√	√
			OTU_166	2.04	1.57	--	√	√
Tricholomataceae	*Tricholoma*	8.43	OTU_73	6.72	5.16	--	√	√
			OTU_185	1.71	1.31	√	√	√
Thelephoraceae	*Tomentella*	7.10	OTU_106	4.99	3.83	--	√	√
			OTU_255	2.11	1.62	√	√	√
Sebacinaceae	*Sebacina*	3.16	OTU_152	3.16	2.42	√	√	√
Sclerodermataceae	*Scleroderma*	2.63	OTU_414	2.63	2.02	--	√	√
Gloniaceae	*Cenococcum*	2.30	OTU_49	2.30	1.76	√	√	√
Hygrophoraceae	*Hygrophorus*	1.60	OTU_108	1.60	1.23	√		√
Clavulinaceae	*Clavulina*	1.35	OTU_54	1.35	1.03	√	√	√

Notes:√ means this kind of mycorrhiza exists in the sample plot.

Table 3. Alpha diversity indices of the rhizosphere ECM fungi of *Pinus massoniana*.

Sampling site	ACE index	Chaol index	Simpson index	Shannon index	Coverage
SY1	83.76 ± 8.56a	85.25 ± l2.23a	0.08 ± 0.0lb	2.97 ± 0.06a	0.99 ± 0.0003b
SY2	56.74 ± 7.22b	57.59 + 9.05b	0.34 ± 0.08a	1.83 ± 0.22c	0.99 ± 0.0002ab
SY3	86.96 ± 8.88a	89.04 ± 13.81a	0.14 ± 0.03b	2.45 ± 0.09b	0.99 ± 0.0006a

The PCA clustering results of ECM fungal communities of *P. massoniana* in three locations were shown in Fig. 3. The interpretation degree of PC1 coordinate axis was 48.09%, that of PC2 coordinate axis was 33.58%, and the total interpretation degree of the two principal coordinate axes was 81.67%, indicating that the PCA clustering diagram can reflect the differences in the composition of ECM fungal communities in different locations. The samples were clustered in four places with good repeatability. The samples from the three locations were distributed in different quadrants, indicating that there are significant differences in the community structure of ectomycorrhizal fungi in the three locations. From the perspective of PC1 dimension, SY1 and SY3 are in the same dimension, while SY2 and they are distributed in different dimensions, indicating that the community structure of ectomycorrhizal fungi of SY1 and SY3 is similar, but the community composition is quite different from that of SY2.
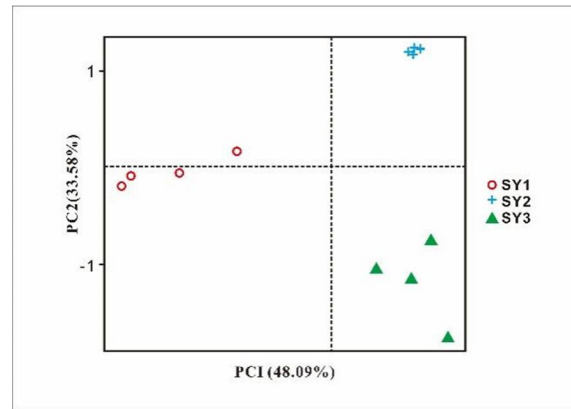
Fig. 3. Principal component analysis of ectomycorrhizal fungal community.

RDA was used to analyze the influence of soil properties on the ECM fungal community composition in the rhizosphere soil of *P. massoniana* at three sites (Table 4). The results showed that all factors explained 97.7% of the difference in the composition of ECM fungi community rhizosphere soil of *P. massoniana* roots. AP, TN, OM and AK all had significant effects on the difference of ectomycorrhizal fungi community composition. Among them, AP and TN had a very significant effect on the difference of community composition, and the interpretation degrees were 59.5% and 30.8% respectively. They were the main factors causing the difference of ECM fungi community structure in the three sites.

Table 4. Redundancy analysis testing effects of soil properties on the ECM fungal communities.

Soil properties	Variance explained	P-value
AP	59.5	0.002
TN	30.8	0.002
OM	2.5	0.041
AK	1.7	0.040
AN	1.3	0.165
WC	0.9	0.304
NN	0.8	0.304
pH	0.2	0.662

TN: Total N, AK: Available K, AP: Available P, AN: Ammonium N, NN: Nitrate N,
OM: Organic matter and WC: water content.

The correlation between soil properties and ECM fungal community composition was further analyzed (Table 5). The results showed that AP had a very significant negative correlation with *Russula* and a very significant positive correlation with *Cortinarius*; TN was negatively correlated with *Tricholoma* and *Tomentella*, and positively correlated with *Suillus*; OM has a very significant negative correlation with *Russula*, a very significant positive correlation with *Cortinarius*, AK *Russula* has a very significant negative correlation and a very significant positive correlation with *Cortinarius*. The above results show that the composition of ECM fungi community in *P. massoniana* rhizosphere soil was significantly affected by soil properties.

Table 5. Correlation analysis between properties and ECM fungal community in rhizosphere soil of *Pinus massoniana*.

Genera	TN	AK	AP	AN	NN	OM	pH	WC
Suillus	0.831**	0.361	-0.292	-0.154	-0.278	0.241	-0.604*	-0.365
Tricholoma	-0.607*	-0.034	0.48	0.524	0.246	-0.044	0.354	0.129
Corlinarius	0.395	0.882**	0.847**	0.802**	-0.794**	0.902**	-0.735**	-0.901**
Inocybe	0.039	0.092	0.052	0.052	0.07	0.078	0.081	0.076
Hygmphorus	-0.298	0.104	0.493	0.421	-0.115	0.291	0.031	-0.17
Tomentella	-0.588*	0.042	0.536	0.445	0.052	0.16	0.255	0.037
Russula	0.012	-0.702*	-0.953**	-0.916**	0.474	-0.767**	0.395	0.652*
Sebacina	0.19	0.136	0.075	-0.067	0.018	0.199	-0.147	-0.212

TN: Total N, AK: Available K, AP: Available P, AN: Ammonium N, NN: Nitrate N, OM: Organic matter and WC: water content.

This diversity and community structure characteristics of ectomycorrhizal fungi in *P. massoniana* rhizosphere soil were studied at Xugongdian (SY1), Dishui Guanyin (SY2) and Cherry bay (SY3) in Yuntai Mountain, Guizhou. The community structure of ECM fungi of SY1 and SY3 was similar, but it was very different from SY2. All soil factors explained 97.7% of the differences inECM fungal community composition, among which AP, TN, OM and AK were the main driving factors for the differences in ectomycorrhizal fungal community composition in the three sites.

ECM fungi play an important role in promoting plant growth, nutrient absorption, disease resistance, stress resistance and ecological restoration. *P. massoniana* can coexist with a variety of fungi to form ectomycorrhiza, ensure its healthy growth and maintain the stability of forest ecosystem. (Chen 2019) showed that there was a positive correlation between soil microbial diversity and stability. Therefore, the study of species diversity and community structure of rhizosphere soil microorganisms can provide a theoretical basis for the restoration of forest ecosystem in karst area of Guizhou Province. The diversity and community structure of ECM fungi in *P. massoniana* rhizosphere soil at three sites were analyzed. Based on Illumina miseq high-throughput sequencing platform, 128 ectomycorrhizal fungi OTUs were obtained from three sites, belonging to 2 phyla, 5 classes, 11 orders and 38 genera. The total number of ECM fungi OTUs in the three sites is 42, and the unique OTUs in Xugongdian, Dishui Guanyin and Cherry bay were 17, 15 and 9 respectively, indicating that the community composition of ECM fungi in the three sites was similar, but due to the differences in terrain, vegetation composition and soil properties between different sites, each site had its unique ectomycorrhizal fungi OTUs.

The results showed that basidiomycetes were the absolute dominant fungi of ECM fungi of *P. massoniana*, accounting for 96.20% of all ECM fungi. The rests were ascomycetes. The results of this study were similar to the community composition of *P. massoniana* rhizosphere microorganisms in other areas of Guizhou reported by other researchers (Chen 2020). Basidiomycetes were one of the most important decomposers of lignified vegetation debris. Ascomycetes can decompose plant residues and degrade organic matter (Bcimfordc *et al.* 2014). Because the litter of *P. massoniana* forms rich humus under its forest, which increases the content of organic matter and cellulose in the soil. At the same time, *P. massoniana* can also provide a large amount of carbohydrates for ECM fungi. Therefore, it provides a good living environment

for basidiomycetes in ectomycorrhizal fungi and promotes it to become the dominant ectomycorrhizal fungi group in the soil. The abundant ectomycorrhizal fungi in the rhizosphere also provide a large number of nutritional elements for *P. massoniana*. Together, they strengthen the recycling of nutrients in the forest ecosystem and maintain the stability of the ecosystem (Wang and Bau 2014).

There were 11 dominant genera of ECM fungi present in the rhizosphere of *P. massoniana*, namely *Russula, Cortinarius, Suillus, Inocybe, Tricholoma, Tomentella, Sebacina, Scleroderma, Cenococcum, Hygrophorus* and *Clavulina*. Among them, *Russula, Cortinarius* and *Suillus* fungi fruiting bodies were found in the investigation of ECM fungi resources in Shibing Yuntai Mountain, However, no fruiting bodies of other dominant fungi were found in the investigation (Luo 2013). The amount of rainfall and temperature have a great impact on the formation of fruiting bodies.

Chen (2020) studied the community structure of ECM fungi in the rhizosphere soil of *P. massoniana* in Longli forest farm and Leigongshan National Nature Reserve in Guizhou. It showed that about 70% of the ECM fungi of *P. massoniana* in Guizhou were endemic species (genera). *Russula* and *Tomentella* were the most important common genera, and the dominant ECM fungi were *Russula, Tomentella, Hyloscypha, Amphinema* and *Piloderma*. The common dominant genera in this study were *Russula, Tomentella*. However, the dominant genera are different, which indicated that there were significant differences in the community structure of ECM fungi of *P. massoniana* in different places. *Russula* and *Tomentella*s are the absolute dominant genera of ectomycorrhizal fungi of *P. massoniana* reported by Chen and others (2020). The relative abundance of these two genera in this study was also very high, indicating that although there are significant differences in the ecological environment of different places, the dominant ECM fungi of *P. massoniana* forest were basically the same. This also showed that the mutual selection between ECM fungi and hosts had a certain specificity. Soil contains a large number of ECM fungal hyphae, which plays an important role in the healthy growth of *P. massoniana* e and maintaining the stability of forest ecosystem.

By analyzing the community structure of ECM fungi in three sites, it was found that the community structure of Cherry bay and Xugongdian was similar, but different from that of Dishui Guanyin, and the diversity and richness of ECM fungi in Cherry bay and Xugongdian were significantly higher than that in Dishui Guanyin. It may be related to the significant differences of soil properties in the three sites. On the other hand, the differences of associated shrubs and herbs under *P. massoniana* forest in the three sites may also be an important reason for the differences of ECM fungi community structure. Researches showed that vegetation types (Han 2015) and plant root exudates (Hu 2018, Zhalnina *et al.*2018) also have significant effects on the structure of soil microbial community. The type and quantity of root exudates determine the diversity and abundance of rhizosphere microorganisms and affect the growth, reproduction and metabolic process of microorganisms (Hu 2018). Therefore, different shrubs and herbs under the forest have different root exudates, which will also affect the community structure of ectomycorrhizal fungi to a certain extent.

Different soil properties significantly affect the community structure of ECM fungi. On the contrary, the physiological activities of ECM fungi and their own activities (such as secretion) can change the soil properties of to a certain extent (Tedersoo *et al.*2014). Therefore, exploring the community structure of ECM fungi in a certain environment is positive significance to evaluate the health and stability of the ecosystem under this environmental condition. The results showed that soil properties had a significant effect on the community structure of ECM fungi in the rhizosphere soil of *P. massoniana*. All factors explained 97.7% of the difference in the composition of ECM fungi community. AP, TN, OM and AK all had significant effects on the

differences of ECM fungi community composition. Among them, AP and TN had significant effect on the differences of ECM fungi community composition. The explanatory degrees of the differences of ectomycorrhizal fungi community composition in the three sites were 59.5% and 30.8% respectively, which was the main reason for the differences of ECM fungi community structure in three different sites.

In most Karst areas, the TP in soil is very high, but most of them exist in insoluble state that cannot be used, and the AP in soil is very low. ECM fungi can convert organic phosphorus in soil into available phosphorus through complex mechanism for plants to absorb and use. It is found that phosphorus is closely related to fungal community structure and diversity (He *et al.*2017). The results of this study also confirmed this point. The content of soil AP contributed the most to the difference of ECM fungal community composition among the three sites. The distribution of fungal communities in ecosystems was closely related to soil nitrogen content (Cox *et al.*2010, Shen *et al.* 2019). Most studies believe that higher soil nitrogen content will reduce biodiversity (Simkin *et al.*2016, Zhang *et al.* 2018), and ECM fungi are also very sensitive to soil nitrogen content (Leff *et al.* 2015, Zhang *et al.*2018, Liu *et al.* 2020). In this study, soil TN content was also an important factor affecting the community structure of ectomycorrhizal fungi, and its content is significantly negatively correlated with ectomycorrhizal fungi, *Russula* are highly sensitive to soil nitrogen content.

The distribution of soil fungi is also closely related to soil pH and water content (Bahram *et al.* 2018). In this study, pH has a very significant negative correlation with *Rhizoctonia*, indicating that the *Cortinarius* of *P. massoniana* in dolomite karst area prefer to grow in acidic environment. Fungal communities were also sensitive to changes in soil water content. In this study, there was a very significant negative correlation between *Cortinarius* and soil water content, indicating that the rhizosphere fungi of *P. massoniana* in dolomite karst area may have stronger drought resistance. The results of redundant analysis (RDA) of soil properties and ECM fungi community structure in this study show that soil pH value and water content have no significant contribution to the difference of ECM fungi community composition in the three sites, which may be due to the small change range in dolomite Karst area despite some differences in pH value and soil water content, not enough to cause significant differences in the community structure of ectomycorrhizal fungi in various sites. On the other hand, it may also be related to the relatively small sensitivity of the distribution of some ECM fungi to soil pH.

References

Bahram M, Hildebrand F, Forslund SK, Jennifer LA, Nadejda AS, Peter MB 2018. Structure and function of the global topsoil microbiome. Nature **560**: 233-237.

Bcimfordc C, Fcidbcrg K, Nyiindcr S, Rikkinen J, Tuovila H, Dorfelt H, Gube M, Jackson DJ, Reitner J, Seyfullah LJ, Schmidt AR 2014. Estimating the phancrozoic history of the Ascomycota lineage combining fossil and molecular data. Mol. Phylogen. Evol. **78**: 386-398.

Chen FL, Zhang K, Xiang D, Wu AP, Li YZ, Zou D, Zheng H 2019. Impacts of litter decomposition of eucalyptus on soil microbial community. A microcosm study. Acta Pedolog. Sin. **56**(2): 432-442.

Chen J 2020. Diversity and distribution of ectomycorrhizal fungi of *pinus massoniana* in Guizhou, Guizhou University, China. pp. 1-21.

Cox E, Barsoum N, Lilleskov EA, Bidartondo MI 2010. Nitrogen availability is a primary determinant of conifer mycorrhizas across complex environmental gradients. Ecol. Lett. **13**: 1103-1113.

Han SZ, Gao R, Li AP, Ma HL, Yin YF, Si YT, Chen SD, Zheng QR 2015. Soil microbial community structure of two types of forests in the mid-subtropics of China. Chin. J. Appl. Ecol. **26**: 2151-2158.

He D, Shen WJ, Eberwein J, Zhao Q, Ren L, Wu QL 2017. Diversity and co-occurrence network of soil fungi are more responsive than those of bacteria to shifts in precipitation seasonality in a subtropical. Soil Biol. Biochem. **115**: 499-510.

Hu LF, Robert CAM, Cadot S, Zhang X, Ye M, Li B, Manzo D, Chervet N, Steinger T, van der Heijden MGA 2018. Root exudate metabolites drive plant-soil feedbacks on growth and defense by shaping the rhizosphere microbiota. Nature Commun. **9**: 2738.

Leff JW, Jones SE, Prober SM, Barberan A, Borer ET, Firn JL, Harpole WS, Hobbie SE, Hofmockel KS, Knops JMH, McCulley RL, La Pierre K, Risch AC, Seabloom EW, Schuetz M, Steenbock C, Stevens and Noah F 2015. Consistent responses of soil microbial communities to elevated nutrient inputs in grasslands across the globe. Proceedings of the National Academy of Sciences of the United States of America **112**: 10967-10972.

Li M 2016. Ectomycorrhizae fungi diversity in main community of *Pinus massoniana* in Guizhou, Guizhou University. pp. 5-27.

Liu SG, Wang H, Tian P 2020. Decoupled diversity patterns in bacteria and fungi across continental forest ecosystems. Soil Biol. Biochem. **144**: 107763.

Luo GT, Wang JX, Li XY 2013. Ecological study on macrofungi of Yuntai mountain in Shibing, Guizhou Province. J. Fungal Res. **11**:166-171.

Ren CJ, Zhang W, Zhong ZK, Ren C, Zhang W, Zhong ZK, Han X, Yang G, Feng Y, Ren G 2018. Differential responses of soil microbial biomass, diversity and compositions to altitudinal gradients depend on plant and soil characteristics. Sci. Total Environ. **160**: 750-758.

Rillig MC, Ryo M, Lehmann A, Aguilar-Trigueros CA, Buchert S, Wulf A, Iwasaki A, Roy J, Yang GW 2019. The role of multiple global change factors in driving soil functions and microbial biodiversity. Science. **366**(467): 886-890.

Shen FF, Liu Y, Luo CT, Kong YL, Zhu CQ 2019. Research progress on response and adaptation of plant and soil microbial community diversity to global change in terrestrial ecosystem. Ecol. Environ. Sci. **28**: 2129-2140.

Simkin SM, Allen EB, Bowman WD. Clark CM, Belnap J, Brooks ML, Cade BS, Collins SL, Geiser LH, Gilliam FS, Jovan SE, Pardo LH, Schulz BK, Stevens CJ, Suding KN, Throop HL, Waller DM 2016. Conditional vulnerability of plant diversity to atmospheric nitrogen deposition across the United States. Proceedings of the National Academy of Sciences of the United States of America **113**: 4086-4091.

Tedersoo L, Bahram M, Polme S, Koljalg U, Yorou NS 2014. Global diversity and geography of soil fungi. Science **346**: 1256688.

Veresoglou SD, Halley JM, Rillig MC 2015. Extinction risk of soil biota. Nature Communications **6**: 8862.

van Dam NM, Bouwmeester HJ 2016. Metabolomics in the rhizosphere: Tapping into belowground chemical communication. Trends in Plant Sci. **21**: 256-265.

Wang F, Bau T 2014. Research advances in the diversity of soil fungi. J. Fungal Res. 12: 178-185.

Zhang TA, Chen YH, Ruan HH 2018. Global negative effects of nitrogen deposition on soil microbes. The ISME J. **12**: 1817-1825.

Zhalnina K, Louie KB, Hao Z, Mansoori N, Rocha UN, Shi SJ, Cho HJ, Karaoz U, Loque D, Bowen BP, Firestone MK, Northen TR, Brodie EL 2018. Dynamic root exudate chemistry and microbial substrate preferences drive patterns in rhizosphere microbial community assembly. Nature Microbiol. **3**: 470-480.