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Microbial Structure and Diversity in Rhizosphere Soil under Different Forest Types in the Subtropical Zone of China

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Abstract

The aim of this study was to explore the soil microbial variability within different forest ecosystems (evergreen broad-leaf forest (EBF), coniferous forest (CF), subalpine dwarf forest (SDF) and alpine meadow (AM) at different altitudes in mid-subtropics of China. The phospholipid fatty acid (PLFA) method was used to analyze the microbial communities in rhizosphere soil under different forest types. The relationships were also analyzed between the microbial diversity and soil nutrients. A total of 27 PLFA biomarkers were detected and the PLFA concentrations decreased in the sequence of bacteria > fungus > actinomycete > protozoa in all forest types. The microbial communities in the soil under all forest types were distinct. The predominant microflora in all soils were 18:1ω9c, 16:1ω7c, cy19:0, a17:0 and 18:0. The indexes of Simpson, Shannon-Wiener and Brillouin of soil microbial community diversity in these four forest types all showed a trend of EBF > CF > SDF > AM. According to principal component analyses (PCA), the variable variances of principal components 1 and 2, which were related to the PLFA biomarkers of soil microorganisms, were 67.67% and 17.91%, respectively. Furthermore, the total PLFAs of different soil microbial groups showed a correlation with soil nutrients and enzyme activities in all forest types. The soil microbial diversity gradually decreased in the order of EBF > CF > SDF > AM in the Daiyun Mountains. Different vegetation types affect soil microbial community composition and diversity by changing the soil physicochemical properties and enzyme activity.

Key Words: Daiyun Mountains; PLFA; Forest types; Elevation; Soil microorganisms

1 Introduction

Microorganisms are one of the most active and influential components of soil ecosystems, and they play an irreplaceable role in soil nutrient transformation and energy cycling (Xue et al. 2008; Zhang et al. 2012; Lerch et al. 2009). Soil microorganisms are sensitive to changes in their

immediate microenvironment. Therefore, the changes in microorganism community structure can reflect both soil ecosystem changes and environmental stress (White and Rice 2009). However, it is difficult to conduct research on the structures of microbial communities by traditional methods because most microbes cannot be artificially isolated and cultured. In recent years, analysis of phospholipid fatty acid (PLFA) content has been employed to identify and quantitatively describe microbial communities (Xue et al. 2008). Evaluation by PLFA has been widely used to study the soil microbial communities due to its high accuracy, stability and sensitivity (Iovieno et al. 2010; Murphy et al. 2011; Zhang et al. 2013; Lazcano et al. 2013; Marcin et al. 2013).

In forest ecosystems, vegetation type is an important factor that affects soil temperature, humidity, nutrients and microbial activity (Zhang et al. 2013). However, there are few studies that have focused on the relationship between soil microbial community and vegetation type in China. To our knowledge, no studies have examined the various characteristics of soil microbial communities under different forest types in mid-subtropics of China. The Daiyun Mountains, located in southeast of China, have received much attention in recent years because they exhibit a complete distribution of vegetation perpendicular band spectra and contain rich primordial plant resources. It is an ideal place to explore the structure and function of a forest ecosystem under different altitudes. In previous studies, we systematically studied the plant resources and soil characteristics in the Daiyun Mountains. The aim of this study was to explore the microbial variability within different forest ecosystems (evergreen broad-leaf forest, coniferous forest, subalpine dwarf forest and alpine meadow) at different altitudes in the Daiyun Mountains.

2 Materials and Methods

2.1 Soil Sample Collection and Processing

This study was conducted in the Daiyun Mountain National Nature Reserve, in the Fujian province of China (latitude 25°38′07″~25°43′40″ N, longitude 118°05′22″~118°20′15″ E), with

an area of approximately 1,3472.4 hm^2 and an altitude ranging from 650 m to 1856 m. It is located between the south subtropics and mid-subtropics, and is considered a transition zone with clear transitional characteristics in climate, soil and plants. The average annual temperature ranges from 15.6°C to 19.5°C. The annual precipitation is about 1700~2000 mm and the average annual air relative humidity is above 80%. It has 220 foggy days and 260 frost-free days per year. The forest types include evergreen broadleaved forest (EBF), coniferous forest (CF), subalpine dwarf forest (SDF) and alpine meadow (AM), and each vegetation zone is dominated the presence of distinct flora. The EBF zone (400~900 m) contains mostly *Castanopsis eyrei*, *Castanopsis fabri*, *Castanopsis carlesii* and *Schima superba,* the CF zone (900m~1400 m) contains *Pinus massoniana* and *Cunninghamia lanceolata*, the SDF zone (1400~1750 m) contains *Rhododendron latoucheae*, *Eurya groffii* and *Eurya rubiginosa,* and the AM zone (1750~1856 m) contains *Miscanthus sinensis*, *Arundinella anomala* and *Eulaliopsis binata.*

Samples were collected in the Daiyun Mountains during December of 2018. We selected three 20 m ×20 m sample plots with similar topography, landform, slope direction and gradient in different forest types of EBF (650 m), CF (1000 m), SDF (1500 m) and AM (1850 m). During sampling, 20 samples were collected using a 2 cm diameter soil sampler and consolidated into one bulk sample corresponding to the soil sample location. Each sample depth was collected from the topsoil to 20 cm below the topsoil. After each soil sample was consolidated and thoroughly mixed, it was split into two subsamples. One split was passed through a 2 mm sieve and stored at 4°C in a refrigerator to test soil microbial community characteristics and enzymatic activity determination. The second split was naturally dried and sieved and stored for testing soil physico-chemical properties.

2.2 Soil Physicochemical Properties and Enzymatic Activity Determination

Soil physicochemical properties were measured using protocols outlined in *Methods of forest*

soil analysis (State Forestry Administration 2000). The soil pH, total nitrogen (TN), total phosphorus (TP), total potassium (TK) were measured and the soil organic matter (OM) was determined. The soil enzyme activity was tested following the methods described by Guan (1986). Each sample was measured in triplicate and reported as the arithmetic mean.

2.3 Phospholipid Fatty Acid Separation and Gas Chromatography

The PLFA biomarker method was used to analyze soil microbial community structure. Briefly, 4 g fresh soil samples were measured into 50 mL centrifuge tubes with 20 mL 0.2 moL·L^{-1} of a KOH methanol solution and oscillated for 5 minutes. The centrifuge tubes were then heated for one hour in a 37°C water bath and oscillated once every 10 minutes during the heating process. After heating, 3 mL of a 1.0 mol·L^{-1} acetic acid solution was added to each centrifuge tube to neutralize the base and was shaken well. Following neutralization, 10 ml of n-hexane was added to each centrifuge tube, each tube was shaken well to encourage PLFAs to partition into the organic phase, and the tubes were then centrifuged for 15 minutes at 1000 r·min^{-1}. Following centrifugation, 5 ml of the hexane solution was taken into clean glass tubes and solvent-evaporated by nitrogen purging. The substrate was then re-dissolved in 1 mL of a hexane and methyl butyl ether (v/v = 1: 1) solution, transferred to a Gas Chromatography vial, and stored at -20°C until analysis by Gas Chromatography Mass Spectrometry (GC-MS). All organic solvents used were High Performance Liquid Chromatography (HPLC) grade reagents. PLFAs were detected using a Varian240 GC-MS. The oven temperature program started at 70°C for 1 minute (inlet temperature of 280°C and a split ratio of 20:1). The temperature was then increased to 170°C at a rate of 20°C per minute, held at 170°C for 2 minutes, increased to 280°C at a rate of 5°C per minute, held at 280°C for 5 minutes, increased to 300°C at a rate of 40°C per minute, and held at 300°C for 1.5 minutes (Lin et al. 2013).

2.4 Naming and Content Determination of Phospholipid Fatty Acids

The naming convention of PLFA is X:YωZ as described in Frostegård et al. (1993) and Ponder et al. (2009), where X represents the total number of carbon atoms in the main chain of the fatty acid. Starting from the carboxyl group, Y, Z and ω represent the number of unsaturated double bonds, the position of the double bonds (away from the end of methyl) and the existence of double bonds, respectively. In addition to this nomenclature for the PLFA backbone, “c” and “t” denote *cis*- and *anti*-double bonds, “i” and “a” denote isomeric and trans-isomeric methyl branch groups, “br” indicates the presence of methyl branch groups of unknown position, “Me” represents the position of methyl side groups, “cy” represents a cyclopropyl group, and the number before OH represents the position of the hydroxyl group.

PLFAs exhibit structural diversity and biological specificity, and thus can be used as biomarkers in different populations in microbial communities. The microbial biomass and the microbial community structure can be identified via quantitative determination of PLFAs. For example, a16:0, i16:0, a17:0, i17:0 and i18:0 is indicative of Gram-positive bacteria; i15:0, 16:1ω9c, i17:0 and 17:1ω8c are indicative of Gram-negative bacteria; 10Me17:0 and 10Me18:0 are indicative of Actinomycetes; 18:3ω6c (6, 9, 12) and 18:1ω9c are indicative of Fungi (Anne et al. 2013). The peak area and internal standard curve method were used to test fatty acid quantification with methyl esterification 19:0 and concentrations were reported in ug g^{-1} (Zou et al. 2013).

2.5 Statistical Processing of Data

Microsoft Office 2013 was used for initial data processing and mapping. Single factor analysis of variance, principal component analysis, and diversity index analysis were determined by Statistical Package for the Social Science (SPSS 13.0).

3 Results and Discussion

3.1 PLFA Analysis of Soil Microbes in Different Forest Types

In all soil samples, 27 PLFA biomarkers were detected that showed significantly different distributions among the different forest types (Table 1). There were significant differences in the concentrations of 18 microbial groups found in the soils from the different forest types (e.g., No. 5, 7, 8, 10 and 11, Table 1). Some PLFA biomarkers appeared in all forest, for example, 10Me16:0, 16:0 and 18:3ω6c (No. 6, 9, and 12), which belong to fully distributed biomarkers. Other biomarkers existed in only some forest type soil samples, which were attributed to incomplete biomarker distribution. For example, 10Me19:0 and i18:0 were only found in EBF soil. As shown in Table 1, most of the microbial species and total PLFA concentrations were higher in low altitude samples than those in high altitude samples. There were 27, 25, 21 and 19 species of PLFA biomarkers in the EBF, CF, SDF and AM forest type soil samples, respectively. The concentration of PLFA biomarkers in the soil samples from these four forest types were 118.60 ± 1.27 ug g^{-1}, 88.90 ± 1.10 ug g^{-1}, 70.32 ± 0.86 ug g^{-1} and 49.92 ± 0.63 ug g^{-1}, respectively. According to the linear relationship between total microbial concentrations and total PLFA biomarkers, we concluded that the concentration of soil microbes gradually decreased in the order of EBF > CF > SDF > AM in the Daiyun Mountains.

3.2 Dominant Microflora PLFA Distribution in Different Forest Types

The highest PLFA biomarkers contents in the soil of different forest types of Daiyun Mountain were 18:1ω9c, 16:1ω7c, cy19:0, a17:0 and 18:0, which were dominant microflora and played major roles in the soil. The concentration of 5 PLFA biomarkers descended in the sequence of EBF > CF > SDF > AM in the Daiyun Mountains, indicating that dominant microbial flora presented a similar distribution trend at different elevation. Additionally, the content of biomarker 18:1ω9c (an indicator for fungi) was highest in the soil of different forest types, followed by 16:1ω7c (an indicator for Gram-negative bacteria), and a17:0 accounts for the lowest content (an

173 indicator for thermophilic bacillus).

174 **3.3 Characteristic Microorganisms PLFA Distribution in Different Forest Types**

175 The characteristic peaks of 16:0, 10Me16:0 and 18:3ω6c are the main PLFA biomarkers for
176 bacteria, actinomycetes and fungi, respectively (Johansen et al. 2005; Sullivana et al. 2006). As
177 seen in Table 1, the relative biomass of bacteria, fungi and actinomycetes were different in the
178 soil samples from different forest types, of which bacteria was the largest, followed by fungi,
179 followed by actinomycetes. The relative biomass of bacteria and fungi as indicated by PLFA
180 biomarker concentration decreased in the sequence of EBF > CF > SDF > AM, while that of the
181 relative biomass actinomycetes as indicated by PLFA biomarker concentration decreased in the
182 sequence of EBF > AM > SDF > CF.

183 PLFA concentrations indicative of Gram Bacteria (G+ & G-), fungi, actinomycetes and the
184 proportions between them are listed in Table 2. The highest concentrations of Gram-positive
185 bacteria (G+) biomarkers (19.64 ug g^{-1}) and Gram-negative bacteria (G-) biomarkers (37.03
186 ug·g^{-1}) were found in EBF soil. The lowest concentrations of G^+ biomarkers and G^- biomarkers
187 in SDF and AM soil were the lowest values with 7.06 ug·g^{-1} and 19.31 ug·g^{-1}, respectively. The
188 sequence of G+/G- and fungi are EBF > CF > SDF > AM. The ratio of fungi and Gram bacterium
189 declining as the following sequence: SDF > EBF > CF > AM, and the concentration of
190 actinomycetes decreased following the sequence of EBF > SDF > CF > AM.

191 **3.4 The Diversity of Soil Microbial Communities in Different Forest Types**

192 Diversity indices of soil microbial communities in different forest types are shown in Table 3.
193 The Simpson index, Shannon-Wiener index and Brillouin index values showed a similar trend
194 among different forest types, descending in the order of EBF > CF > SDF > AM. The
195 Shannon-Wiener index revealed the species dominance, composition and distribution. The
196 highest Shannon index was found in EBF soil, indicating that the microbial community in this

soil exhibited the most homogenous distribution. The Brillouin index further weakens the non-random sampling. The significant differences among the calculated Brillouin indexes indicated a high sampling randomness. Simpson index reflects the dominant species of most common species in soil microbial communities. The Simpson indices among the forest type soil samples decreased following the sequence EBF > CF > SDF > AM, but no significant differences were detected. The value of McIntosh index in the AM soil was significantly higher than those in the EBF and CF soil samples.

3.5 Principal Component Analysis of Soil Microbial Community in Different Forest Types

The principal components analysis (PCA) of soil PLFA biomarkers in different forest types is illustrated in Figure 1. PCA indicated that the variance was primarily explained by principal component (PC) factors 1 and 2 at 67.67% and 17.91%, respectively. The EBF soil plotted at the positive terminal of PC1 and the intersection point of positive and negative terminals of PC2, the CF soil plotted at the positive terminals of PC1 and PC2, the SDF soil plotted at the negative terminals of PC1 and PC2, and the AM soil plotted at the negative terminals of PC1 and the positive terminal of PC2. PC1 and PC2 were clearly able to distinguish the diversities among the soil microbial communities from different forest types. Based on the correlative analysis between PCA and PLFA biomarkers, 11 PLFA biomarkers appeared to positively correlate with PC1: 16:0, 16:1ω7c, 16:1w7t, 16:1ω5c, 18:0, 18:1ω9c, 18:3ω6c (6,9,12), 20:4ω6c (6,9,12,15), 9Me18:0, a16:0, cy17:0, cy19:0 and i17:0 (Table 1). However, only two PLFA biomarkers correlated with PC2: 16:1w9 (positive correlation) and i14:0 (negative correlation).

3.6 Soil Physicochemical Property and Enzyme Activity in Different Forest Types

The physicochemical properties of the soil samples from different forest types in the Daiyun Mountains are shown in Table 4. Significant physicochemical differences were detected among all measured parameters except pH. The soil water content ranged from 17.73 ± 0.24% to 22.96 ±

0.63%, while soil pH ranged from 4.51 ± 0.02 to 4.70 ± 0.05, and demonstrated that soil acidity reduced with increasing altitude in the typical southern acid soil. The TN, TP, TK and OM concentrations in the soil samples decreased following the sequence of EBF > CF > SDF > AM. The TN, TP, TK and OM concentrations in AM were 45.23%, 56.67%, 37.57% and 30.38% of those in EBF, demonstrating that soil nutrition gradually decreased with increasing altitude.

Significant differences in soil enzyme activities were observed (Table 5). In four different forest types, enzyme activities of soil phosphomonoesterase, invertase, polyphenol oxidase, urease and catalase decreased in order of EBF > CF > SDF > AM. AM soil enzyme activities were only 67.39%, 50.87%, 37.80%, 41.57% and 78.06% of those in EBF. Soil enzyme activities were significantly different among the soils, except for PMEase in EBF or CF and polyphenol oxidase (PPO) in SDF, which exhibited a common characteristic that soil enzyme activities gradually decreased with increasing altitude.

3.7 Correlation between Microbial PLFA and Soil Nutrients

The differences in positive correlation coefficient between the total concentrations of PLFA biomarkers for soil bacteria, fungi, actinomycete, protozoa and soil nutrients among the samples are shown in Table 6. The total PLFA biomarkers for bacteria correlated positively with OM, TN and TP. In addition, there was an extremely significant positive correlation between total PLFA biomarkers for bacteria and TK. The total PLFA biomarkers for fungi showed an extremely significant positive correlation with OM, TN and TK. The total PLFA biomarkers for actinomycetes showed a significant positive correlation with OM and TK. The total PLFA biomarkers for protozoa showed a significant positive correlation with OM and TN and showed an extremely significant positive correlation with TK. Correlations also existed among total PLFA biomarkers for each microorganism (Table 6). Extremely significant positive correlations were detected between bacteria and fungi as well as total PLFA biomarkers for protozoa. There was a

significant positive correlation between bacteria and actinomycetes, as well as significant positive correlations among the total PLFA biomarkers for fungi, actinomycetes and protozoa.

3.8 Correlation between Microbial PLFA and Soil Enzyme Activity

The correlation coefficient comparisons between soil enzyme activity and the total concentration of PLFA biomarkers for soil bacteria, fungi, actinomycetes, protozoa are shown in Table 7. The total PLFA biomarkers for bacteria showed a significantly positive correlation with PMEase and Ure. In addition, there were extremely significant positive correlations between total PLFA biomarkers for bacteria and PPO or Catalase (CAT). An extremely positive significant correlation was observed between total PLFA biomarkers for fungi and PMEase, and a significant positive correlation was observed between total PLFA biomarkers for fungi and PPO. Extremely significant positive correlations were observed between total PLFA biomarkers for actinomycetes and PMEase and significant positive correlations were observed between total PLFA biomarkers for actinomycetes and PPO. There were also extremely significant positive correlations observed between total PLFA biomarkers for protozoa and PMEase, PPO, Ure or CAT.

3.9 Correlation between Soil Contents and Microbial Diversity

The correlation coefficient comparisons between the soil contents and microbial diversity among the samples are shown in Table 8. Simpson index showed a significant positive correlation with PPO and Ure. Shannon index showed a significant positive correlation with TN, TP, OM, PMEase and Ure and showed an extremely significant positive correlation with TK, PPO, and CAT. Brillouin index showed a significant positive correlation with TN, TP, Inv and Ure and showed an extremely significant positive correlation with TK, OM, PMEase, PPO, and CAT. McIntosh index showed a significant positive correlation with TK, OM and PMEase and showed an extremely significant positive correlation with pH, TN, and TP.

3.10 Discussion

Many factors affect microbial community diversity, and vegetation type was found to be one of the most important factors. Soil microbial diversity arose from the collective effects of soil nutrients, litter and root exudates, etc. Different vegetation types affect soil physical and chemical properties by changing the amount and composition of litter, which leads to different soil microbial composition, quantity and distribution (Han et al. 2007; Wang et al. 2013; Zhang et al. 2013). In the present study, the order of forest soil microbial community diversity was EBF > CF > SDF > AM, which showed that vegetation type affected the characteristics of the underground soil microbial community. The forest community varied across elevation gradients in the Daiyun Mountain Nature Reserve which possesses a clear plant succession zone spectrum, resulting in differences in biomass and litter. This study demonstrated that the community diversity in the EBF soil was the highest and the litter concentration was also highest, and decreased in the soil samples from the CF, SDF, and AM vegetation zones, respectively. Reduction of vegetation diversity and litter will inevitably affect soil properties of forest stand. The measurements of soil physical-chemical properties and enzyme activities under different forest types showed that the soil nutrient indexes subsequently changed with forest type, which reflected significant differences between soil moisture content, TN, TK, OM, Inv, Ure and CAT, and thus the species and quantities of soil microorganisms. According to the analysis of soil microbial community structure using PLFA technology, the soil physical-chemical properties, enzyme activity and microbial community structure showed a similar trend, which descended in the order of EBF > CF > SDF > AM. Therefore, different vegetation types may lead to different soil microbial community structures and functions under the same site conditions. In general, the species and amount of microbial PLFA biomarkers in EBF were significantly higher than those found in the AM soil. Additionally, the Simpson index, Shannon-Wiener index, and Brillouin index values for soil microbial communities showed the same trend observed for

physical-chemical properties, enzyme activity and microbial community structure (EBF > CF > SDF > AM). PCA indicated that PC1 and PC2, which were related to the PLFA biomarkers of soil microorganisms, could explain 67.67% and 17.91% of the variance, respectively, and thus could distinguish the majority of the diversity among the microbial communities found in soil from different forest types.

From the analysis of soil microbial community diversity indexes, soil moisture content, Shannon-wiener index and Simpson index of soil microbial communities in EBF and CF were relatively high. High moisture content could accelerate the rate of litter decomposition and enhance the OM concentration, thus benefiting the growth and metabolism of microbes in the soil. The SDF and AM vegetation diversity and coverage were relatively low, and the soil moisture content was low, which would limit the living environmental conditions of soil microbes and thus reduce soil microbial community diversity. Changes in soil moisture led to changes in microbial activity, which agrees with previous research by Gordon et al. (2008) who found that high soil moisture content increased soil microbial activity.

Vegetation secretion and litter are the major sources of carbon and nitrogen to microbes. Variations in the amount of root exudates and litter are driven by vegetation differences in the four studied forest types, and thus affected microbial diversity in these samples. EBF and CF soils were mainly dominated by arbor. They exhibited high species diversity, high root biomass, and high root exudates. These soil characteristics could provide rich carbon and nitrogen sources for soil microbes and thus improved the soil microbial diversity. However, SDF and AM soils were mainly dominated by shrubs and herbage. They exhibited unitary community structure, simple vegetation root systems, low coverage area, little root biomass, and little root exudates. These soil characteristics could result in the reduction of carbon and nitrogen sources required for microbial growth and reproduction and resulted in a decline in the observed soil microbial diversity.

Soil enzyme activity is an important factor in forest soil ecosystems, and is a key link between the plant and soil nutrient systems, potentially promoting the transformation of material to energy in the ecosystem. Important soil enzymes, such as PMEase, Inv, PPO, Ure and CAT, have been observed to play a significant role in the remobilization of soil nitrogen, phosphorus, potassium and other compounds, thus affecting the microbial diversity indirectly (Alkorta et al. 2003). Moreover, there was a significant relationship between the content of TK, PMEase, PPO, Ure, CAT and soil microbial diversity in different vegetation types based on the correlation analysis between microbial community structure diversity and soil physical-chemical properties.

Soil microorganism plays a vital role in the forest sustainable development. The study of soil microorganism community structure and function diversity is important for understanding the relationship between microbes, the environment, and plants. This is especially true with respect to the ways in which soil microbial diversity and vegetation diversity influence each other. The soil microbial diversity had significant impact on vegetation diversity in the Daiyun Mountain forest ecosystems. Under the same site conditions, different vegetation types resulted in variable soil properties, and thus affected the soil microbial composition and diversity, providing a theoretical basis for sustainable management in forest ecosystems. There are many factors that affect the structure and functional diversity of soil microbial community (Iovieno et al. 2010; Murphy et al. 2011). The PLFA biomarker method is a quick method to effectively determine the diversity of soil microbial community structure. In this study, the PLFA biomarker method was employed to analyze the characteristics of soil microbial community structure in different forest types of the Daiyun Mountains and a satisfactory result was achieved. This analysis demonstrated that the concentrations of SOM, TK, PMEase, PPO, Ure, CAT were the main factors affecting the soil microbial diversity.

4 Conclusion

This study demonstrated that soil microorganisms and plant types were closely connected to each other in a forest ecosystem. The order of forest soil microbial community diversity was EBF > CF > SDF > AM, which showed that vegetation type affected the characteristics of the underground soil microbial community. The measurements of soil physical-chemical properties and enzyme activities under different forest types showed that the soil nutrient indexes subsequently changed with forest type, which reflected significant differences between soil moisture content, TN, TK, OM, and thus the species and quantities of soil microorganisms. This study reveals the interactions between the composition and structure of the microbial community and soil nutrients in rhizosphere soil, and explores the roles of microorganisms involved in biogeochemical cycling of nutrient elements under different forest types. Our results provide clues to help researchers to more deeply understand the ecological linkages between soil microorganisms and plant types.

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Author contributions： Zeyan Wu, Wenxiong Lin, Yanlin Zhao, Chengzhen Wu and Liuting Zhou conceived the ideas and designed methodology; Jianjuan Li and Chen Zhang collected the data; Liuting Zhou, Wei Chu and Xinlai Guo analysed the data; Liuting Zhou and Zeyan Wu led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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Table 1 PLFA Types and concentrations (ug·g^{-1}) in soils from different forest types in the Daiyun Mountains

No.	Biomarkers	Microbial group	Forest types			
			EBF	CF	SDF	AM
1	10Me16: 0	Actinomycetes	4.12±0.08a	3.23±0.01c	3.32±0.05bc	3.40±0.04b
2	10Me18: 0	Actinomycetes	2.98±0.04a	2.33±0.04b	1.82±0.01c	-
3	10Me19: 0	Actinomycetes	4.22±0.05	-	-	-
4	14: 1ω5c	*Pseudomonas* sp.	2.69±0.01a	1.77±0.02c	2.32±0.01b	1.70±0.01c
5	15: 0	Aerobic bacteria	2.32±0.03b	1.97±0.03d	2.02±0.03c	2.48±0.03a
6	16: 0	Gram-negative bacteria	6.27±0.12a	5.44±0.05b	5.15±0.10c	3.87±0.05c
7	16: 1ω7c	Gram-negative bacteria	14.31±0.18a	11.49±0.20b	8.61±0.14c	5.76±0.03d
8	16: 1ω7t	Aerobic bacteria	1.43±0.04a	1.24±0.02b	1.18±0.01c	1.10±0.01d
9	16: 1ω9	Gram-negative bacteria	2.02±0.02a	2.09±0.03a	1.05±0.01b	2.07±0.03a
10	16: 1ω5c	Methane-oxidizing bacteria	0.94±0.01a	0.81±0.01b	-	-
11	18: 0	Hydrogenobacter	8.67±0.13a	7.89±0.13b	6.38±0.07c	4.97±0.04d
12	18: 1ω9c	Fungi	18.48±0.12a	12.92±0.20b	9.82±0.18c	5.68±0.06d
13	18: 3ω6c (6, 9, 12)	Fungi	5.82±0.02a	4.90±0.03b	4.07±0.04c	2.28±0.01d
14	20: 4ω6 (6, 9, 12, 15)	Protozoa	1.19±0.04c	0.61±0.01b	0.34±0.01c	0.18±0.01d
15	22: 6	Barophilic/psychrophilic bacteria	1.68±0.03b	1.93±0.02a	-	-
16	23: 0	Fungi	0.54±0.01d	0.95±0.02b	1.01±0.01a	0.63±0.01c
17	9Me18: 0	Actinomycetes	0.91±0.01a	0.42±0.01b	0.34±0.01c	
18	a15: 0	Gram-positive bacteria	3.87±0.07a	3.05±0.04c	3.50±0.02b	1.96±0.01d
19	a16: 0	Gram-positive bacteria	0.88±0.01a	0.76±0.01b	-	-
20	a17: 0	Gram-positive bacteria	7.02±0.10a	7.22±0.06a	4.67±0.04b	3.79±0.05c
21	cy17: 0	Gram-negative bacteria	4.89±0.03a	3.52±0.03b	1.75±0.01c	1.57±0.02c
22	cy19: 0	Gram-negative bacteria	9.54±0.13a	7.64±0.11b	5.26±0.06c	4.94±0.03d
23	cy19: 0ω8c	*Burkholderia* sp.	2.40±0.02a	0.82±0.01c	1.43±0.02b	0.55±0.01d
24	i14: 0	Aerobic bacteria	3.56±0.04b	2.48±0.03c	4.60±0.08a	1.67±0.22d
25	i16: 0	Gram-positive bacteria	1.18±0.01c	1.55±0.01a	1.03±0.02d	1.32±0.02b
26	i17: 0	Gram-positive bacteria	2.88±0.02a	1.86±0.01b	-	-
27	i18; 0	Gram-positive bacteria	3.81±0.02	-	-	-
Total concentration of different PLFA biomarkers			118.60±1.28a	88.90±1.10b	70.32±0.86c	49.92±0.64d

Note: Values in the same line followed by a different letter are significantly different (α<0.05)

Table 2 PLFA concentrations (ug g^{-1}) in G^+, G^-, Fungi, Actinomycetes and the PLFA proportions found in soils from different forest types in the Daiyun Mountains

Characteristics Microbial communities	Forest type			
	EBF	CF	SDF	AM
Gram-positive bacteria (G^+)	19.64±0.23	14.45±0.13	9.21±0.08	7.06±0.08
Gram-negative bacteria (G^-)	37.03±0.48	30.17±0.42	21.82±0.32	19.31±0.16
G^+/ G^- (%)	53.04	47.88	42.19	36.57
Fungi	24.83±0.15	18.77±	14.89±0.25	8.58±0.08
Fungi/Gram bacteria (%)	43.83	42.08	48.01	32.56
Actinomycete	12.23±0.18	5.99±0.06	6.47±0.07	3.40±0.04

Table 3 Diversity indices for soil microbial communities in different forest type of the Daiyun Mountains

Forest type	Simpson index	Shannon index	Brillouin index	McIntosh index
EBF	0.93±0.02a	4.24±0.04a	3.94±0.05a	0.81±0.02b
CF	0.92±0.04a	4.10±0.04a	3.74±0.02b	0.81±0.08b
SDF	0.92±0.06a	3.96±0.03ab	3.60±0.04c	0.82±0.02ab
AM	0.92±0.03a	3.93±0.08b	3.51±0.078c	0.85±0.04a

Note; Values in the same row followed by a different letter are significantly different ($\alpha<0.05$), the same below

Table 4 Soil chemical properties in different forest type of the Daiyun Mountains

Forest type	Water content / (%)	pH	TN/ ($g \cdot kg^{-1}$)	TP/ ($g \cdot kg^{-1}$)	TK/ ($g \cdot kg^{-1}$)	OM/ ($g \cdot kg^{-1}$)
EBF	22.96±0.63a	4.51±0.02a	3.10±0.12a	0.26±0.05a	15.33±0.47a	47.79±0.62a
CF	21.42±0.75b	4.50±0.01ab	2.85±0.03b	0.25±0.03a	11.39±0.09b	35.07±0.84b
SDF	18.93±0.49c	4.58±0.02ab	2.09±0.04c	0.20±0.01b	9.06±0.23c	30.24±0.51c
AM	17.73±0.24d	4.70±0.05b	1.40±0.02d	0.14±0.01c	5.76±0.16d	14.52±0.15d

Table 5 Soil enzyme activities at different forest type of the Daiyun Mountains

Types	PMEase ($mg \cdot g^{-1} \cdot h^{-1}$)	Inv ($mg \cdot g^{-1} \cdot d^{-1}$)	PPO ($mg \cdot g^{-1} \cdot 2h^{-1}$)	Ure ($mg \cdot g^{-1} \cdot d^{-1}$)	CAT ($ml \cdot g^{-1} \cdot 20min^{-1}$)
EBF	1.48±0.09a	17.42±0.15a	0.08±0.005a	0.70±0.08a	1.67±0.06a
CF	1.24±0.05b	15.13±0.12b	0.05±0.002b	0.38±0.05b	1.48±0.09b
SDF	1.18±0.02b	14.15±0.06c	0.03±0.003c	0.33±0.03c	1.35±0.05c
AM	0.99±0.03c	8.86±0.03d	0.03±0.001c	0.29±0.02d	1.30±0.05d

Table 6 Correlation analysis of microbial PLFA concentrations and soil nutrients in different forest types of the Daiyun Mountains

Factor	Bacteria	Fungi	Actinomycetes	Protozoa	OM	TN	TP
Fungi	0.97**	1					
Actinomycete	0.89*	0.93*	1				
Protozoa	0.98**	0.96*	0.95*	1			
OM	0.94*	0.99**	0.94*	0.93*	1		
TN	0.95*	0.97**	0.82	0.89*	0.96*	1	
TP	0.91*	0.96*	0.78	0.85	0.95*	0.99**	1
TK	0.98**	1.00**	0.94*	0.97**	0.99**	0.96**	0.94*

Note; *means $P < 0.05$, significant correlation;**means $P < 0.01$, extremely significant correlation.

Table 7 Correlation analysis of microbial PLFA concentrations and soil enzyme activity in different forest types of the Daiyun Mountains

Factor	Bacteria	Fungus	Actinomycetes	Protozoa	PMEase	Inv	PPO	Ure
Fungi	0.97**	1						
Actinomycete	0.89*	0.93*	1					
Protozoa	0.98**	0.96*	0.95*	1				
PMEase	0.95*	0.99**	0.97**	0.97**	1			
Inv	0.88	0.97**	0.87	0.86	0.95*	1		
PPO	0.98**	0.93*	0.92*	0.99**	0.94*	0.8	1	
Ure	0.92*	0.89*	0.97**	0.98**	0.93*	0.78	0.98**	1
CAT	0.99**	0.96*	0.93*	1.00**	0.96**	0.85	1.00**	0.96**

Table 8 Correlation analysis of soil contents and microbial diversity in different forest types of the Daiyun Mountains

Factor	PH	TN	TP	TK	OM	PMEase	Inv	PPO	Ure	CAT
Simpson index	-0.38	0.59	0.51	0.77	0.7	0.79	0.56	0.91*	0.96*	0.87
Shannon index	-0.8	0.93*	0.89*	0.97**	0.92*	0.94*	0.85	0.99**	0.93*	0.99**
Brillouin index	-0.83	0.94*	0.91*	0.99**	0.96**	0.98**	0.90*	0.98**	0.95*	0.99**
McIntosh index	0.99**	-0.97**	-0.99**	-0.91*	-0.94*	-0.89*	-0.98**	-0.74	-0.67	-0.8

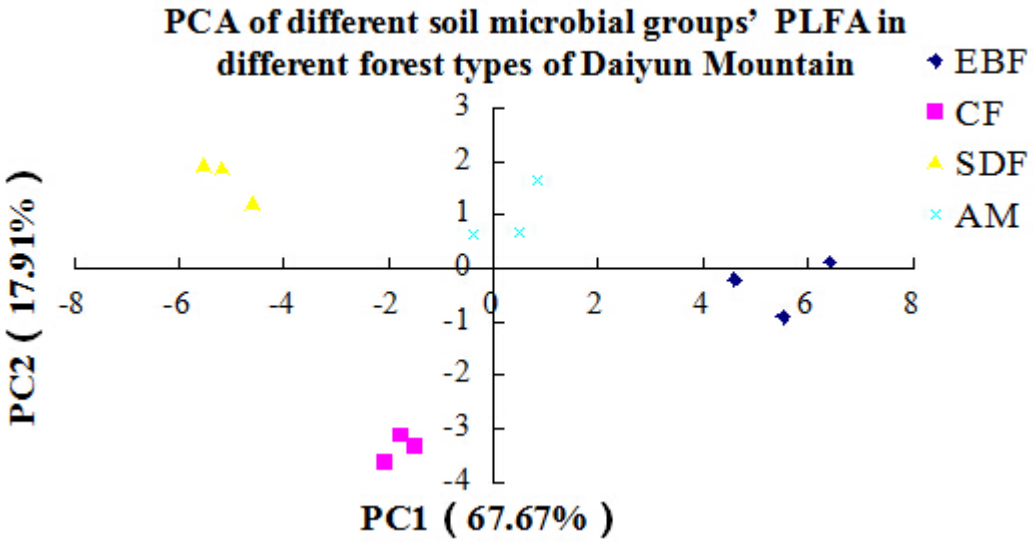

Fig 1 PCA of different soil microbial groups' PLFA in different forest types of the Daiyun Mountains