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## Research Article

# Tree species influences soil microbial community diversity but not biomass in a karst forest in southwestern China

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## Abstract

**Aims** The relationships between plant species and soil microorganisms remain indeterminable in different ecosystems worldwide. In karst ecosystems, soil microbial (SM) community structure and their environmental driving factors are poorly explored, and the relationships between plant species and soil microorganisms are unclear. This study aimed to characterize the general patterns of SM community composition and biomass, and to explore the specific tree species and soil physiochemical properties highly related to SM community diversity and biomass in a karst forest.

**Methods** The effects of tree species on SM community composition and biomass were firstly investigated on the basis of 212 soil samples collected from five dominant tree species (*Lithocarpus confinis* Huang, *Platycarya longipes* Wu, *Itea yunnanensis* Franch., *Machilus cavaleriesi* H. Lév. and *Carpinus pubescens* Burkill) through phospholipid fatty acid (PLFA) analysis of a karst evergreen and deciduous broad-leaved mixed forest in central Guizhou Province, southwestern China. The relationships between SM community structure and tree species and soil physiochemical properties were statistically analysed.

**Important Findings** A total of 132 SM-PLFA biomarkers were detected. The average number of SM-PLFA biomarkers and microbial biomass in each soil sample were 65.97 and 11.22  $\mu\text{g g}^{-1}$ , respectively. Tree species influenced the number of SM-PLFA biomarkers but not the SM biomass. The number of SM-PLFA biomarkers of *C. pubescens* was significantly higher than that of other species ( $P < 0.05$ ); the numbers of SM-PLFA biomarkers amongst other species showed no significant difference. Microbial biomass showed no relationships with the soil physiochemical properties of nutrient-rich surface soils but positively correlated ( $P < 0.05$ ) with soil organic carbon, nitrogen and phosphorus concentrations in deeper soils. The karst forest in the plateau-surface terrain of central Guizhou Province presented a low fungal-to-bacterial ratio, low microbial biomass storage and high microbial community diversity. Specific tree species affect the SM community diversity in this kind of karst forest.

**Keywords** soil microorganisms, tree species, evergreen and deciduous broad-leaved mixed forest, phospholipid fatty acid analysis, karst geomorphology

## 中国西南喀斯特森林树种对土壤微生物群落多样性和生物量的影响

**摘要:** 陆地生态系统中植物种对土壤微生物群落结构的影响不一, 而喀斯特生态系统中植物种对土壤微生物群落结构影响的研究尚未见报道。本研究利用磷酸脂肪酸(PLFA)法, 分析了黔中高原型喀斯特常绿落叶阔叶混交林5个优势树种—窄叶石栎(*Lithocarpus confinis* Huang)、圆果化香(*Platycarya longipes* Wu)、滇鼠刺(*Itea yunnanensis* Franch.)、安顺润楠(*Machilus cavaleriei* H. Lév.)、云贵鹅耳枥(*Carpinus pubescens* Burkill)—与土壤理化性质对土壤微生物群落组成和生物量的影响。在测试的212个土壤样品中共检测出132种PLFA, 每个样品土壤微生物平均PLFA数量和生物量分别为65.97和11.22  $\mu\text{g g}^{-1}$ 。土壤表层(0–10 cm)的土壤微生物PLFA数量与下层(10–20 cm)土壤接近, 但前者土壤微生物生物量显著高于后者( $P < 0.05$ )。树种影响土壤微生物PLFA数量, 但对土壤微生物生物量没有影响。云贵鹅耳枥附近的土壤微生物PLFA数量显著高于其他树种( $P < 0.05$ ), 而其他树种土壤微生物PLFA数量接近。土壤微生物生物量与表层土壤的理化性质无显著相关, 但与下层土壤的有机碳、全氮和全磷含量呈显著正相关( $P < 0.05$ )。总之, 黔中高原型喀斯特森林真菌/细菌生物量比率低, 微生物总生物量低, 但微生物群落多样性高。树种对土壤微生物群落多样性产生影响。

**关键词:** 土壤微生物群落, 树种, 常绿落叶阔叶混交林, 磷脂脂肪酸(PLFA), 喀斯特

## INTRODUCTION

Soil microorganisms consist of all living organisms smaller than 150–200  $\mu\text{m}$ , and they account for less than 0.5% of soil mass (Coleman and Wall 2006; Swift *et al.* 1979). They are highly related not only to soil fertility and structure but also to a series of soil biochemical processes, such as nitrogen fixation, nutrient cycling, organic matter degradation and pollutant decomposition (Falkowski *et al.* 2008; Fierer *et al.* 2013; Schimel 1995). Thus, soil microorganisms are indispensable components of terrestrial ecosystems. With advances in analytical methodologies, the soil microbial (SM) community composition, diversity, functions and the driving factors of various types of ecosystem worldwide have been understood considerably (Frey *et al.* 2003; Gessner *et al.* 2010; Rillig and Mummey 2006). However, the interactions between the aboveground plant species and the belowground soil microorganisms remain indeterminable, with several studies reporting that tree species directly (by altering litter production and root exudates) or indirectly (by changing the soil physiochemical properties, such as soil pH, nutrients and moisture) affects SM communities in certain ecosystems (Georgious *et al.* 2017; Iovieno *et al.* 2010; Prescott and Grayston 2013; van der Heijden *et al.* 2008); other research showed that tree species is a poor predictor of microbial communities in other ecosystems (Barberán *et al.* 2015; McGuire *et al.* 2012; Talbot *et al.* 2014). Furthermore, specific ecosystems,

whose relationships between plant species and soil microorganisms remain undetermined, still exist in remote or certain topographical areas. An example of such ecosystems is the karst forest located in tropical and subtropical regions.

Karst is a highly special geomorphology formed from the dissolution of soluble rocks, such as limestone, dolomite and gypsum. Karst geomorphology is scattered in various areas worldwide, with a total area of approximately  $22 \times 10^6 \text{ km}^2$ , accounting for 14.8% of the global land area (Jiang *et al.* 2014). Southwestern China is one of the three regions with the largest and continuous distribution of karst ecosystems. The two other regions are Southern America and the Mediterranean coasts. Existing studies on soil microorganisms of various vegetation types in this region indicate that microbial biomass carbon, nitrogen and phosphorus of forests, especially primary forests, are significantly higher, and their microbial quantity is larger than those of shrublands, grasslands, farmlands and bare lands (Lu *et al.* 2013; Song *et al.* 2013; Tan *et al.* 2014a; Xu *et al.* 2013). Bacteria rather than fungi and actinomycetes are the dominant microorganisms in karst soils (Su *et al.* 2013; Wu *et al.* 2019). Karst forests present high bacterial diversity (Chen *et al.* 2019). Elevation, slope, soil characteristics (pH, nutrients, moisture and depth), plant dispersal limitation, tree diversity and density can be drivers of SM quantity, community composition, diversity and biomass (Feng *et al.* 2013; Lu *et al.* 2013; Peng *et al.* 2019; Song *et al.* 2013, 2018; Zhou *et al.* 2017).

However, the effects of aboveground plant species on belowground SM community composition, diversity and biomass have not been explored.

The influences of species on SM biomass, community composition and diversity have often been studied at the forest scale, i.e. in different forest in studies with only one dominant tree species considered for each forest type but not within a single forest with multiple dominant tree species (Huygens *et al.* 2011; Kang *et al.* 2018; Xiao *et al.* 2016). In the present study, 212 soil samples of different species and diameters at breast height (DBHs) were collected from a typical karst evergreen and deciduous broad-leaved mixed forest in southwestern China and subjected to phospholipid fatty acid (PLFA) analysis. Specifically, we (i) examined the SM community composition and biomass of the karst forest based on vast soil samples and (ii) explored the specific tree species and soil physiochemical properties highly related to SM community diversity and biomass in such forest type. This study will not only broaden the understanding of the general patterns of SM communities and their driving factors but also provide guiding significance for vegetation restoration and reconstruction in karst regions.

## MATERIALS AND METHODS

### Study area description

This study was conducted at Tianlong Mountain (105°45'50" E, 26°14'40" N, 1550 m), which is a typical and representative mountain in the plateau surface-type karst morphological region in Puding County, central Guizhou Province, southwestern China. This region has a subtropical humid monsoon climate. The mean annual air temperature in 1961–2013 (Puding weather station, 105°45' E, 26°19' N, 1244 m) was 15.2 °C, with the lowest monthly mean in January (5.2 °C) and the highest in July (23.0 °C). The mean annual precipitation is 1341 mm with a distinct seasonal pattern. More than 1000 mm precipitation occurs between May and September, and the rest occurs between October and April. The sky is cloudy, resulting in a low sunshine percentage of 26.3%. The parent rock is limestone, and the soil type is brown limestone soil.

The vegetation type is a secondary evergreen and deciduous broad-leaved mixed forest, which was naturally restored from clear cutting in the late 1950s and is currently in the succession stage of subclimax.

According to complete vegetation surveys conducted in the summer of 2012 and 2015, *Lithocarpus confinis* Huang, *Platycarya longipes* Wu (*Platycarya longipes* Wu and *Platycarya strobilacea* Sieb. were combined into *P. strobilacea* in the latest Flora of China edition), *Itea yunnanensis* Franch., *Machilus cavaleriei* H. Lév. and *Carpinus pubescens* Burkill are the five dominant tree species with the highest biomass and largest number of individuals (Liu *et al.* 2016b, 2018a).

### Field sampling

Field sampling was conducted in summer 2016. A total of 106 individuals from the five dominant tree species, namely, 21 *L. confinis* (DBH: 1.0–34.0 cm), 25 *P. longipes* (DBH: 2.1–30.6 cm), 20 *I. yunnanensis* (DBH: 1.4–21.4 cm), 20 *M. cavaleriei* (DBH: 1.1–18.4 cm) and 20 *C. pubescens* (DBH: 1.8–16.7 cm), distributed in a 30 m horizontal × 100 m vertical plot at Tianlong Mountain were selected. The plot was an extension of the permanent monitoring plot (200 m horizontal × 100 m vertical) of Puding Karst Ecosystem Research Station, and it was established to conduct destructive research. The destructive and permanent monitoring plots at Tianlong Mountain of Puding Karst Ecosystem Research Station were selected after investigating the whole central Guizhou Province; the vegetation, soil and karst morphology in the plots were representative of this region. After the organic layer was removed, a soil pit was dug around each tree stem base, and two mineral soils in each soil pit were sampled from two layers (0–10 cm and 10–20 cm). A total of 212 soil samples were collected. Any visible plant roots and stones in soils were sieved (2 mm). Then, each soil was subsampled for physiochemical and PLFA analyses.

### Soil physiochemical properties and PLFA analyses

Soil total nitrogen (N) was determined using an elemental analyser (vario EL III CHNOS Elemental Analyser, Elementar Analysensysteme GmbH, Germany). Soil organic carbon (SOC) was determined using oil bath  $K_2Cr_2O_7$  titration. Soil pH was measured with a pH metre (PB-10, Sartorius, Germany). Other total soil element concentrations, namely, phosphorus (P), potassium (K), calcium (Ca) and magnesium (Mg), were measured using an inductively coupled plasma atomic emission spectrometer (iCAP 6300 ICP-OES Spectrometer, Thermo Scientific, USA).

PLFAs were extracted from 8 g freeze-dried soil to estimate the microbial biomass and community

structure. The specific procedure was described in previous reports (Bossio and Scow 1998; Liu *et al.* 2012). The concentration of each PLFA was calculated using the following equation:

$$\text{PLFA} = \left( \frac{\text{response of unknown PLFA}}{\text{response of 19:0 internal standard}} \right) \times \text{concentration of 19:0 internal standard} \times \left( \frac{\text{volume of sample}}{\text{mass of soil}} \right) \quad (1)$$

The units of PLFA, the concentration of 19:0 internal standard, sample volume and soil mass were nanogram per gram dry soil, nanogram per microliter and microliter and gram, respectively.

The PLFAs were further grouped as bacteria, fungi, actinomycetes and protozoa (Francisco *et al.* 2016; Frostegård and Bååth 1996; Liu *et al.* 2010; Qian *et al.* 2010; Ruess and Chamberlain 2010; Stefanowicz *et al.* 2016) (Table 1).

### Statistical analyses

Statistical tests were performed using SPSS 19.0 and CANOCO 5. Independent-sample *t* test was used to determine differences in soil physiochemical properties and microbial community structure between the two soil layers. One-way analysis of variance (ANOVA) was performed to analyse the differences in soil physiochemical properties and microbial community structure amongst various tree species. Furthermore, one-sample *t* test was performed to determine the differences in microbial biomass and the number of SM-PLFA

biomarkers between the karst forest and other types of vegetation in China and across the world. Pearson's correlation and redundancy analyses were performed to evaluate the relationships between tree DBH and soil physiochemical properties and between microbial community structure and plant and soil physiochemical properties.

## RESULTS

### Soil physiochemical properties

Soil N, SOC and P concentrations and C/P, C/N and N/P ratios decreased with soil depth. Conversely, pH, Ca, K and Mg concentrations increased with soil depth (Table 2). Soil physiochemical properties differed significantly ( $P < 0.01$ ) between the two soil layers except pH and P concentration. Soil pH slightly varied as indicated by small variable coefficients. However, other soil physiochemical properties showed substantially variations as shown by high variable coefficients, especially those of Ca and Mg concentrations (Table 2).

Species can be an important influencing factor of soil physiochemical properties. Soil pH and N, SOC, Ca, Mg and P concentrations of *C. pubescens* were significantly ( $P < 0.05$ ) higher, and K concentration was significantly ( $P < 0.05$ ) lower than those of other species (Table 3). Soil physiochemical properties amongst other species were not significantly different (Table 3). Furthermore, tree DBH exhibited no correlation with soil physiochemical properties.

**Table 1:** PLFAs used as microbial indicators

Microbial groups	PLFAs
Bacteria	10:0, 11:0, 12:0, 13:0, 14:0, 15:0, 16:0, 17:0, 18:0, 19:0, 20:0, 22:0, 24:0, 10:0 2OH, a11:0, 12:0 2OH, a12:0, 12:0 DMA, a13:0, i13:0, 13:1ω3, 5c, a14:0, i14:0, i14:0 3OH, 14:1ω5, 8, 9c, a15:0, 15:0 DMA, i15:0, i15:0 DMA, a15:1ω9c, i15:1ω6c, 15:1ω5, 6, 7, 8, 9c, 16:0 2OH, a16:0, i16:0, 16:1ω3, 6, 7, 9c, 16:1ω7c alcohol, 16:1ω7c DMA, 16:1ω9c aldehyde, 16:1ω9c DMA, 16:2 DMA, a17:0, cy17:0ω7c, 17:0 DMA, i17:0, a17:0ω7c, i17:1ω9c, 17:1ω8c, 18:0 DMA, i18:0, 18:1ω5, 7c, 18:1ω7, 9c DMA, 18:2 DMA, a19:0, cy19:0 9,10 DMA, cy19:0ω6, 7, 9c, i19:0, 19:1ω8, 9c, cy20:0ω6c, i20:0, 20:1ω4, 6, 8c, 21:1ω3, 4, 5, 6, 8, 9c, i22:0, 22:1ω3, 5, 6, 8, 9c, 23:1ω5c, 24:1ω3, 7, 9c
Fungi	21:0, 23:0, 16:1ω5c, 18:1ω9c, 18:2ω6c, 20:1ω9c
Actinomycetes	10Me 16:0, 10Me 17:0, 10Me 17:1ω7c, 10Me 18:0, 10Me 18:0ω7c, 10Me 19:1ω7c, 10Me 20:0, 10Me 22:0
Protozoa	15:4ω3c, 16:3ω6c, 18:3ω6c, 19:3ω3, 6c, 19:4ω6c, 20:2ω6c, 20:3ω6c, 20:4ω6c, 20:5ω3c, 21:3ω3, 6c, 22:2ω6c, 22:4ω6c, 22:5ω3, 6c, 22:6ω3c, 23:1ω4c, 23:3ω3, 6c, 23:4ω6c, 24:3ω3, 6c, 24:4ω6c

**Table 2:** Soil physiochemical properties of a karst forest in southwestern China

Soil layer		pH	N	SOC	P	K	Ca	Mg	C/P	C/N	N/P
0–10 cm	Mean	7.51 a	6.77 A	77.46 A	0.57 a	6.01 A	34.37 A	30.48 A	140.22 A	11.46 A	12.12 A
	SD	0.31	2.06	25.09	0.17	1.27	33.88	22.83	41.50	1.46	2.73
	CV (%)	4.06	30.33	32.39	29.34	21.14	98.60	74.91	29.60	12.72	22.51
10–20 cm	Mean	7.67 a	3.62 B	38.62 B	0.43 a	6.30 B	40.00 B	39.05 B	90.44 B	10.89 B	8.49 B
	SD	0.30	1.56	15.58	0.15	1.88	48.93	32.74	21.04	1.71	2.83
	CV (%)	3.91	43.08	40.34	34.80	29.77	122.33	83.84	23.27	15.75	33.36

The unit of element concentration is milligram per gram. Mean values of soil physiochemical properties with different letters between two soil layers were significantly different ( $t$  test,  $P > 0.05$  or  $P < 0.01$ ).

**Table 3:** Physiochemical properties in surface soils (0–10 cm) of different tree species in a karst forest in southwestern China

Species	pH	N	SOC	P	K	Ca	Mg
<i>Lithocarpus confinis</i> Huang	7.48 ab	6.62 ab	76.72 ab	0.55 b	6.46 a	21.72 b	22.74 b
<i>Platycarya longipes</i> Wu	7.55 ab	6.77 ab	74.51 ab	0.57 b	6.46 a	35.76 ab	28.35 ab
<i>Itea yunnanensis</i> Franch.	7.53 ab	5.85 b	66.16 b	0.53 b	6.19 ab	31.54 ab	32.01 ab
<i>Machilus cavaleriei</i> H. Lév.	7.40 b	7.02 ab	85.08 a	0.52 b	5.53 bc	32.32 ab	28.99 ab
<i>Carpinus pubescens</i> Burkill	7.61 a	7.52 a	85.58 a	0.69 a	5.33 c	52.71 a	42.83 a

The unit of element concentration is milligram per gram. Soil physiochemical properties with different letters amongst tree species were significantly different (one-way ANOVA,  $P < 0.05$ ).

### SM community structure

A total of 132 SM-PLFA biomarkers were detected in all soil samples. The number of SM-PLFA biomarkers in each soil sample ranged from 37 to 90, with an average of 65.97. The biomarkers consisted of 46.10 bacteria, 4.80 fungi, 6.40 actinomycetes and 8.67 protozoa. Exactly 30 SM-PLFA biomarkers (14:0, 15:0, 16:0, 17:0, 18:0, 19:0, i14:0, a15:0, 15:0 DMA, i15:0, i15:1 $\omega$ 6c, 10Me 16:0, i16:0, 16:1 $\omega$ 5, 7, 9c, 16:3 $\omega$ 6c, 10Me 17:0, a17:0, cy17:0 $\omega$ 7c, i17:0, 10Me 17:1 $\omega$ 7c, 17:1 $\omega$ 8c, 10Me 18:0, 18:1 $\omega$ 7, 9c, 10Me 18:1 $\omega$ 7c, 18:2 $\omega$ 6c, cy19:0 $\omega$ 7c and 10Me 20:0) were included in all the 212 soil samples, and 7 SM-PLFA biomarkers (13:1 $\omega$ 5c, i15:0 DMA, 16:1 $\omega$ 9c aldehyde, cy19:0 $\omega$ 6c, 19:1 $\omega$ 9c, cy20:0 $\omega$ 6c and 21:1 $\omega$ 5c) appeared only once. Furthermore, the five dominant species shared 108 mutual SM-PLFA biomarkers. The biomarker representative included 21:1 $\omega$ 5c (PLFA biomarker appearing only in the soils of a certain species was defined as its representative biomarker) for *L. confinis*; i15:0 DMA, cy19:0 $\omega$ 6c and cy20:0 $\omega$ 6c for *P. longipes*; 16:1 $\omega$ 9c aldehyde and 19:1 $\omega$ 9c for *I. yunnanensis*; 13:1 $\omega$ 5c for *C. pubescens*.

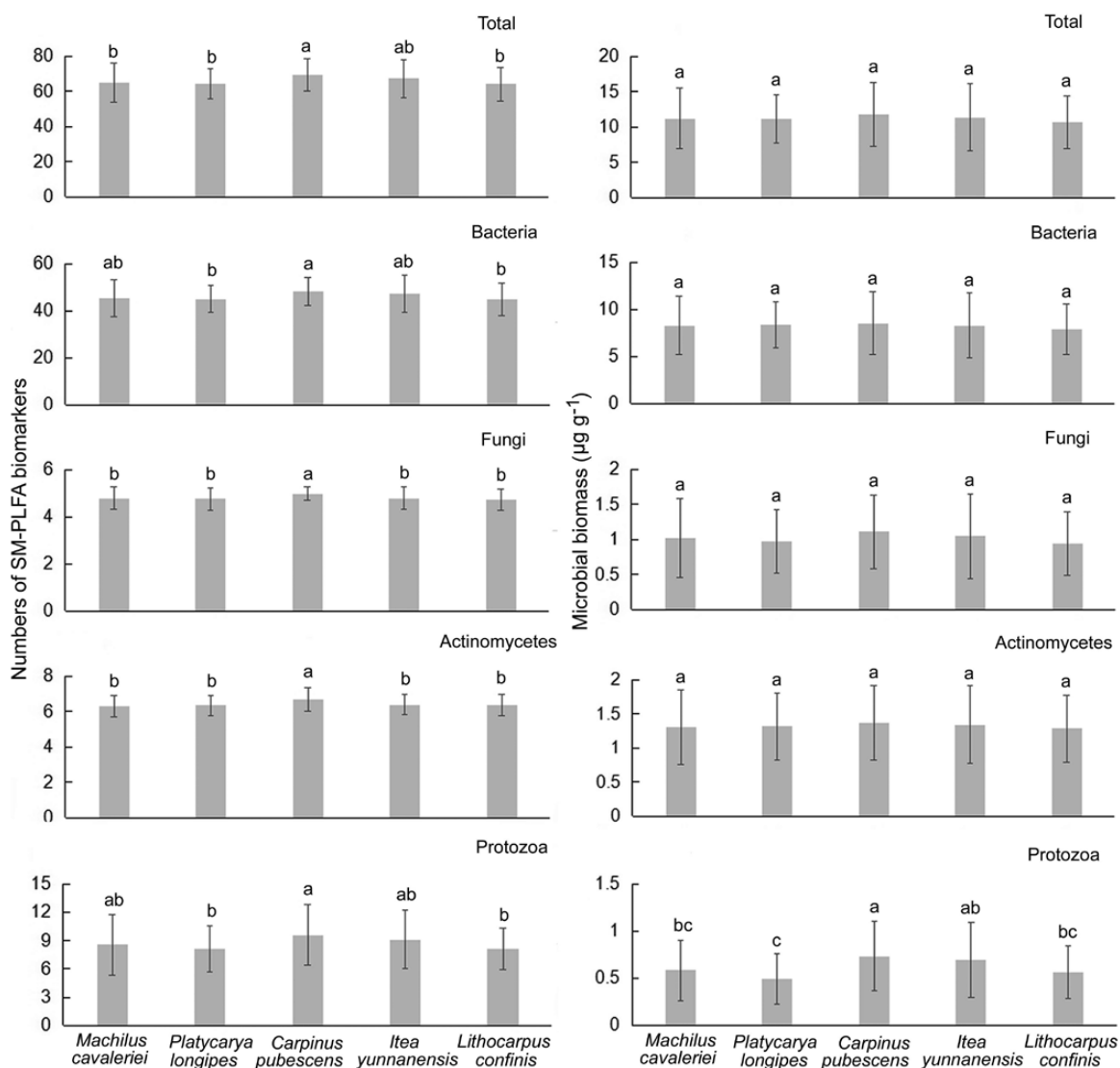
The microbial biomass in each soil sample ranged from 3.75 to 23.31  $\mu\text{g g}^{-1}$ , with an average of 11.22  $\mu\text{g g}^{-1}$ .

The biomass was composed of 8.28  $\mu\text{g g}^{-1}$  bacteria (accounting for 73.97% of the total microbial biomass), 1.01  $\mu\text{g g}^{-1}$  fungi (8.63%), 1.32  $\mu\text{g g}^{-1}$  actinomycetes (11.77%) and 0.61  $\mu\text{g g}^{-1}$  protozoa (5.64%). The 30 dominant SM-PLFA biomarkers accounted for 76.6%  $\pm$  6.6% of the total microbial biomass.

Surface soils (0–10 cm) showed similar numbers of SM-PLFA biomarkers (all: 66.40  $\pm$  10.35 to 65.54  $\pm$  9.52; bacteria: 46.31  $\pm$  7.23 to 45.89  $\pm$  6.71; fungi: 4.78  $\pm$  0.41 to 4.82  $\pm$  0.47; actinomycetes: 6.46  $\pm$  0.65 to 6.33  $\pm$  0.56; protozoa: 8.84  $\pm$  2.92 to 8.50  $\pm$  2.79) with deeper soils (10–20 cm). However, the former presented significantly ( $P < 0.05$ ) higher total microbial (12.20  $\pm$  4.50 to 10.24  $\pm$  3.42  $\mu\text{g g}^{-1}$ ), bacterial (9.02  $\pm$  3.29 to 7.53  $\pm$  2.44  $\mu\text{g g}^{-1}$ ) and fungal (1.12  $\pm$  0.57 to 0.91  $\pm$  0.43  $\mu\text{g g}^{-1}$ ) biomass than the latter.

### Relationships between the structure of SM community and the properties of plant and soil

Tree species affected the number of SM-PLFA biomarkers but did not influence the SM biomass except protozoan biomass (Figs 1 and 2). The number of SM-PLFA biomarkers in *C. pubescens* was significantly ( $P < 0.05$ ) higher than that in other species; the



**Figure 1:** Relationships between tree species and the number of SM-PLFA biomarkers (left) and microbial biomass (right) of a karst forest in southwestern China. Letters not shared across columns represent significant difference via one-way ANOVA.

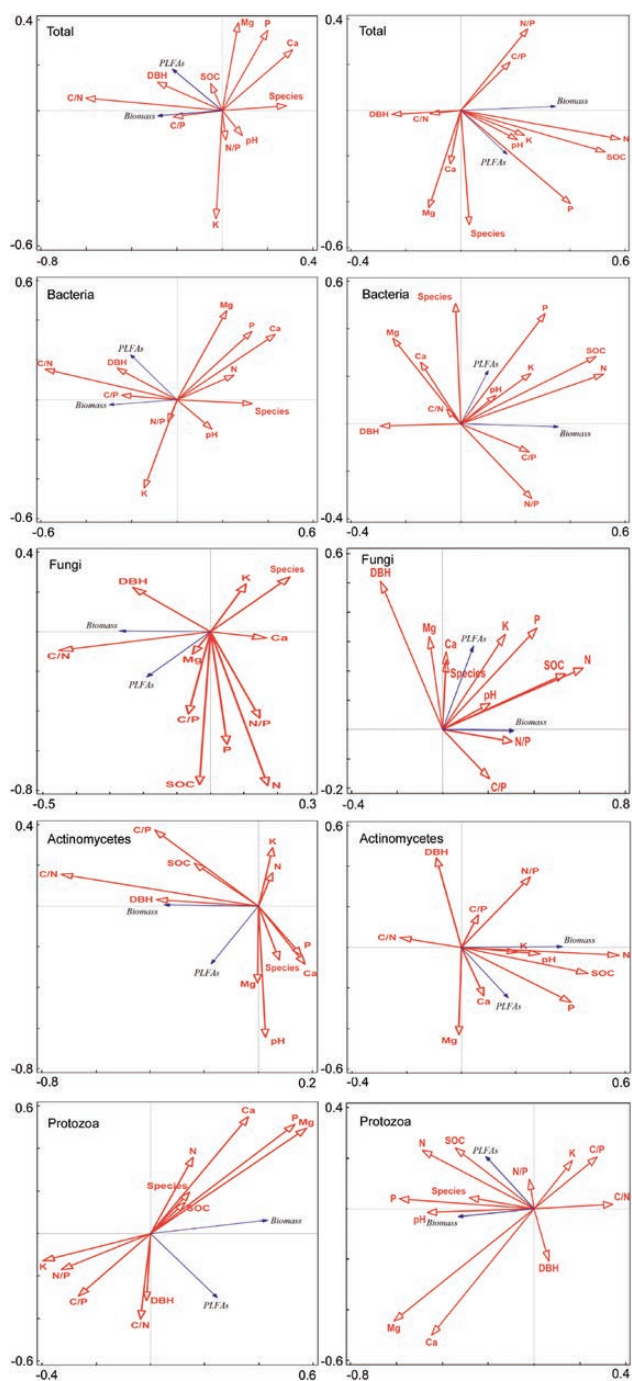
numbers of SM-PLFA biomarkers amongst other species showed no significant difference (Fig. 1). Tree DBH was not related to microbial biomass nor the number of SM-PLFA biomarkers.

In surface soils (0–10 cm), microbial biomass exhibited no relation to soil physiochemical properties in most cases. Only protozoan biomass showed significantly positive relationships with Mg ( $P < 0.01$ ), P ( $P < 0.01$ ) and Ca ( $P < 0.05$ ) concentrations. Actinomycete biomass was positively related to the C/N ratio ( $P < 0.01$ ) (Fig. 2). However, in deeper soils (10–20 cm), total microbial and actinomycete biomass significantly ( $P < 0.05$ ) increased with the increase in N and SOC concentrations, and bacterial and fungal

biomass significantly ( $P < 0.05$ ) increased as the N concentration increased. The protozoan biomass significantly ( $P < 0.05$ ) increased as the P concentration increased (Fig. 2). The number of SM-PLFA biomarkers was not related to soil physiochemical properties in neither soil layer.

## DISCUSSION

Karst habitats are considerably heterogeneous (Zhu 2003). In the present study, soil physiochemical properties showed considerable variations as indicated by high variable coefficients (Table 2). Liu *et al.* (2019) observed that soil N and P concentrations of the same depth from different soil pits in the same karst forest



**Figure 2:** Biplots of the number of SM-PLFA biomarkers, microbial biomass and plant and soil properties from redundancy analyses. Left: surface soils (0–10 cm); right: deeper soils (10–20 cm).

differed significantly; the highest N concentration was approximately 56-fold of the lowest. Although soil element concentrations, stoichiometry characteristics and their vertical distribution patterns in karst ecosystems have been widely investigated (Du *et al.* 2017; Liu *et al.* 2019; Zhang *et al.* 2013), previous studies were all based on small soil sample quantities

and presented inconsistent results. The results from such studies may present significant uncertainties or mistakes. Liu *et al.* (2019) demonstrated that soil C/N and C/P ratios increased with soil depth based on nine soil samples. Tan *et al.* (2014b) demonstrated that deeper soils (10–20 cm) displayed higher C/N ratio and lower K concentration than surface soils (0–10 cm) based on 15 soil samples. Their findings were inconsistent with our results, that is, soil C/N and C/P ratios decreased, and K concentration increased with soil depth based on vast soil samples. Therefore, studies on soil element concentrations and stoichiometry characteristics in karst ecosystems should be based on vast soil samples to ensure the accuracy of results. A study on SOC in the same karst forest indicated that 29, 46 and 71 soil samples were required to reach 80%, 90% and 99% accuracies, respectively, by random sampling (Li 2015).

Plant species is a significant predictor of SM communities in certain studies (Georgious *et al.* 2017; Iovieno *et al.* 2010; Prescott and Grayston 2013; van der Heijden *et al.* 2008), but other research were unable to find the same result (Barberán *et al.* 2015; McGuire *et al.* 2012; Talbot *et al.* 2014). Previous studies were often performed in separate vegetations. In the present study, we first investigated the relationships between tree species and SM community structure in a karst forest and observed that tree species can be an important influencing factor of SM community diversity, but it depends on specific tree species. The number of SM-PLFA biomarkers of *C. pubescens* was significantly ( $P < 0.05$ ) higher than that of other species; the numbers of SM-PLFA biomarkers amongst other species presented no significant difference (Fig. 1). Tree species caused no effect on the SM biomass, as indicated by the lack of significant difference in microbial biomass amongst all species (Figs 1 and 2).

Soil physiochemical properties of *C. pubescens* were significantly ( $P < 0.05$ ) different with those of other species, but no significant differences were found amongst other species (Table 3). Low lignin concentration and high nitrogen concentration were tested in the litter of *C. pubescens* (Liu *et al.* 2016a); a high litter necromass was observed in the same karst forest where *C. pubescens* was aggregately distributed (Liu *et al.* 2011). Nutrient-rich soils and high litter necromass determine the high potential nutrient amounts for soil microorganism coexistence; low lignin concentration and high nitrogen concentration in litter accelerate litter decomposition, which enhances the nutrient amount and availability for



soil microorganism coexistence (Aguilera *et al.* 2016; Georgious *et al.* 2017; Liu *et al.* 2016a). These results all indicate that *C. pubescens* directly (by altering litter quality and quantity) and indirectly (by changing the soil physiochemical properties) affects SM communities in the karst forest. Nevertheless, the effects of root exudates of different tree species on SM communities should be determined in future studies.

Previous studies on SM community composition in karst ecosystems indicated that bacteria are the dominant microorganisms in karst soils (Su *et al.* 2013; Wu *et al.* 2019). In the present study, the fungal-to-bacterial ratio (fungal biomass divided by bacteria biomass) was relatively low (only 0.12). This finding was partly because alkaline soils in karst ecosystems favour bacteria rather than acid-tolerant fungi (Högberg *et al.* 2003; Joergensen and Wichern 2008; Rousk *et al.* 2009). Furthermore, the SOC concentration and nutrient availability influence the SM community composition. In nutrient-poor soils, fungi are the most dominant because they can decompose refractory substrates or absorb nutrients from eutrophic patches by using mycelium to remit their nutrient limitation (Strickland and Rousk 2010; Williamson *et al.* 2005). In the karst forest, SM biomass showed no relation to the physiochemical properties of surface soils in most cases, indicating that nutrient condition (nutrient rich) in surface soils is not a limiting factor. High soil element concentrations favour bacteria in the karst forest.

Considerably heterogeneous habitats in karst soils create diverse local niches for soil microorganism appearance (Chen *et al.* 2019; Peng *et al.* 2019). Nutrient availability may also greatly influence the SM community diversity; high SM community diversity occurs in nutrient-rich soils (Aguilera *et al.* 2016). A total of 132 SM-PLFA biomarkers were detected in the karst soil; this number was significantly ( $P < 0.01$ ) higher than those (21–42) of nonkarst forests in subtropical China (Luo *et al.* 2014, 2018; Ni *et al.* 2018; Su *et al.* 2018; Zhang *et al.* 2018) and significantly ( $P < 0.01$ ) higher than those of other Chinese and global vegetation types (18–98) (Chatterjee *et al.* 2009; Chi *et al.* 2018; Gu *et al.* 2017; Huygens *et al.* 2011; Liu *et al.* 2018b; Sun *et al.* 2018; Wei *et al.* 2018; Yang *et al.* 2017). The average SM biomass was  $11.22 \mu\text{g g}^{-1}$  in the karst forest, showing that this biomass was not significantly different ( $P > 0.05$ ) from those of other Chinese and global vegetation types ( $2.16$ – $152.12 \mu\text{g g}^{-1}$ ) (Chatterjee *et al.* 2009; Francisco *et al.* 2016; Liu *et al.* 2018b; Ni *et al.* 2018; Su *et al.* 2018; Zhang *et al.* 2018).

However, the karst forest exhibited shallow soils, a high rate of outcrops and a low soil reserve volume. Therefore, the karst forest in plateau-surface terrain of central Guizhou Province is a forest with low microbial biomass storage.

Our results revealed that deeper soils (10–20 cm) showed high microbial community diversity, similar to that on surface soils (0–10 cm). These soils also presented a relatively high microbial biomass, although the value was significantly ( $P < 0.05$ ) lower than the latter. Thus, considerable research on soil microorganisms should focus not only on surface soils but also on deeper soils (>10 cm) in karst and other types of ecosystem.

Degraded shrublands, tussocks and grasslands rather than forests are currently the dominant vegetation types in karst regions (ECVMC 2007); however, mixed forest should be the potential vegetation here, thus indicating the need for forest restoration and reconstruction. Understanding SM community structure and their environmental driving factors and the effects of plant species on soil microorganisms is essential to vegetation restoration and reconstruction. Planting tree species that favour the survival of soil microorganisms and anthropogenically improve soil conditions accordingly may potentially combat rocky desertification through promoting vegetation restoration and reconstruction in karst regions in southwestern China.

## CONCLUSIONS

SM community composition and biomass and their environmental driving factors, together with the effects of tree species on SM community structure, were explored through PLFA analysis in a karst forest in southwestern China. A total of 132 SM-PLFA biomarkers were detected. The average number of PLFA biomarkers and the average microbial biomass in each soil sample were 65.97 and  $11.22 \mu\text{g g}^{-1}$ , respectively. Microbial biomass was highly related to SOC, N and P concentrations in deeper soils. Tree species affected the number of SM-PLFA biomarkers but not the SM biomass. The karst forest in plateau-surface terrain of central Guizhou Province presented a low fungal-to-bacterial ratio, low microbial biomass storage and high microbial community diversity. The present study is important for understanding SM community structure and restoring and reconstructing vegetation in karst regions. More advanced methodologies, such as high-throughput sequencing, are recommended for the exploration

of the characteristics of soil microorganisms in karst ecosystems in future studies.

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