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The Impacts of Vegetation Types and Soil Properties on Soil Microbial Activity and Metabolic Diversity in Subtropical Forests

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Abstract: Microbial functional diversity is significantly associated with both nutrient cycling and organic matter decomposition. However, how different forests as well as the soil parent materials influence the soil microbial carbon metabolism remains poorly understood. In this study, a natural secondary forest and a *Pinus yunnanensis* plantation, with similar climatic conditions under contrasting parent materials (clasolite in the non-karst areas and limestone in the karst areas) in Yunnan Province, China, were investigated. The soil microbial carbon metabolism diversity was assessed by the Biolog® ECO-plates. During the dry season, the soil microbial communities used carbon substrate in secondary forest and *Pinus yunnanensis* plantation, showing no significant difference, both in non-karst and karst areas. The microbial communities in the non-karst area were more efficient in utilizing carbon substrates than those in the karst area with the same vegetation types, resulting in the higher accumulation of organic carbon in the karst area. The six categories of most frequently utilized carbon substrates were carbohydrates, carboxylic acids, and amino acids in both the non-karst and the karst areas. The soil basal respiration of the secondary forest was higher than that of the *Pinus yunnanensis* plantation, both in the non-karst and the karst areas. In addition, the driving factors of the soil microbial community functional diversity in the non-karst and karst areas are different. Our findings suggest that soil microbial functional diversity is governed by vegetation types as well as by soil properties in subtropical forests. Moreover, calcareous soil holds a higher proportion of recalcitrant organic carbon, which is difficult to utilize by microorganisms.

Keywords: vegetation types; secondary forest; *Pinus yunnanensis* plantation; subtropical forests; microbial functional diversity; carbon substrate

1. Introduction

Soil microorganisms directly participate in important ecological processes and respond rapidly to environmental changes; they have therefore been identified as the most active part of soils [1]. The composition, structure, diversity, and activity of the microbial community play an important role in

the maintenance of the sustainability of terrestrial ecosystems [2,3]. Microbial functional diversity is significantly associated with nutrient cycling, organic matter decomposition, soil aggregation, and soil productivity [4–6]. As the principal drivers of the soil organic matter decomposition and turnover, soil microorganisms play a significant role in the global carbon cycling [7]. Therefore, the microbial community composition and function are central for soil functioning and forest productivity [8]. The community-level physiological profiles (CLPPs), i.e., the soil microbial metabolic diversity, of heterotrophic soil microbial communities has been widely studied, with a focus on their use of different carbon substrates [9–11]. However, how different forests and soil parent materials influence the soil microbial community in karst ecosystems remains poorly understood.

Since plants have a specific natural history and physiology [11,12], it is possible that different plant species produce distinctive soil conditions, which in turn influences the soil microbial community [13,14]. As a result, forest plants influence the activity, biomass, and composition of soil microbial communities directly based on the production of litter and root exudates and indirectly through changing the physicochemical soil characteristics [11,15]. For instance, deciduous broadleaf trees produce significantly higher litter quantities and decomposition rates compared to coniferous trees, which results in higher nutrient levels [12]. In addition, the litter of coniferous tree species contains higher concentrations of lignin, acids, tannins, and phenolic compounds, which are difficult to decompose and thus strongly affect the soil microbial growth [16–18].

As a factor that influences soil formation, the parent material strongly influences the soil microbial community by impacting the soil physicochemical properties [19,20]. In particular, the parent material chemistry composition and weathering degree controls the soil nutrient availability [21,22]. Different studies have demonstrated that soil characteristics differ between different parent materials [23,24]. The soil microbial community composition of ultrabasic parent materials was found to be significantly higher and more diverse than that on adjacent sandstone [25]. In particular, the soil pH is a central environmental element that shapes the abundances, diversity, composition, and structure of soil microbial communities across various ecosystems [19,26–28]. However, research focusing on the activity and metabolic diversity of the soil microbial community under contrasting parent materials in subtropical restored forests is limited.

The karst landscape is a fragile ecosystem that is distributed throughout the world and accounts for nearly 12% of the world's land surface [29]. The karst region of southwest China covers 0.54 million km^2 and has developed on carbonate bedrock (e.g., limestone and dolomite). Such ecosystems are highly vulnerable due to their thin, coarse, and patchy soil coverage and the resulting low water retention capacity. As a result, karst ecosystems are particularly vulnerable to human activities [29–31]. In such a region, vegetation and soil degradation, as a result of intensive anthropogenic disturbances, are severe, and large areas are often bare. Since the 1990s, large-scale reforestations with *Pinus yunnanensis* Franch have been established in this region [32]. At the same time, the area contains natural secondary forests that are not managed. Several studies have reported that the soil microbial community changes with forest succession and restoration [33,34]. However, specific information about how different forest species and soil parameters influence the soil microbial community in both natural secondary forests and plantations in this subtropical area, in particular in karst regions, is rare.

Therefore, in this study, we selected two forest stands in southern China: the natural secondary forest and *Pinus yunnanensis* plantation, with different parent materials (non-karst and karst areas). We hypothesized that: (i) under different vegetation types, different soil microbial community level physiological profiles exist (Hypothesis I); (ii) the same vegetation type shows a different use of the sole carbon source and biochemical substrates depending on the different parent materials (Hypothesis II); and (iii) the key factors that affect the microbial functional diversity under different vegetation types differ depending on the parent materials (Hypothesis III).

2. Materials and Methods

2.1. Study Sites

The study was carried out in Jianshui County (104°53′ E, 24°35′ N), Yunnan Province, southwest China. The area possesses a typical subtropical monsoon climate, which is influenced by the Indian Ocean monsoon, with warm-wet (May to October) and cool-dry seasons (November to April). The mean annual precipitation is 805 mm, the mean annual temperature is 19.8 °C, the mean annual evaporation is 2296.5 mm, and the annual sunshine duration is 2322 h. The region is characterized by typical karst graben basins, karst areas and non-karst areas with a mosaic distribution. In non-karst areas, soils have developed from a clasolite base, while in karst areas, soils have developed from a limestone base. Calcareous soils cover the karst area, and red soils cover the non-karst area (Calcaric Cambisols, FAO).

Both the secondary forest and *Pinus yunnanensis* plantation were selected in the two distinct areas. The naturally regenerated secondary forest (1980) was not subject to any management strategies, and was exposed to little disturbance. The *Pinus yunnanensis* plantation was maintained within the scope of the 'Grain to Green' project at the same time, and was not affected by any human interference after the revegetation planting.

2.2. Sample Collection and Preparation

In March 2017, three 20 m × 20 m plots were established in each forest stand for data collection. The same vegetation types were selected based on the criterion of being more than 100 m away from the forest edge in both the karst and non-karst areas, respectively. To study the characteristics of the various plant communities in each of the vegetation types, quadrats were set up in areas of different forests [35]. The vegetation properties of both the secondary forest and the *Pinus yunnanensis* plantation in the karst and non-karst areas are listed in Table 1. In March 2017 (during the dry season), soil samples were collected from 12 sampling points within each plot, using a 4-cm auger at a depth of 0–15 cm. Prior to sampling, the litter layer was removed; the 12 cores were mixed to obtain one composite sample and sieved through a 2-mm sieve. Subsequently, each soil sample was divided into two parts. After the removal of all visible roots, one part was stored at 4 °C for a microbial analysis, while the other was air-dried and sieved through a 60-mesh sieve for a physicochemical analysis.

Table 1. Basic characteristics of plant communities in non-karst and karst areas in southern China. The results are shown as the mean ± standard errors (SE). The same below.

Parameters		Non-Karst Area		Karst Area	
		Pinus yunnanensis	Secondary Forest	*Pinus yunnanensis*	Secondary Forest
Shrub	Species number (S)	3.333 ± 0.577	6.667 ± 0.577	3.667 ± 0.182	8.667 ± 0.577
	Shannon–Wiener index (H)	0.977 ± 0.260	0.713 ± 0.132	0.875 ± 0.376	1.541 ± 0.094
	Evenness index (E)	0.409 ± 0.054	0.379 ± 0.090	0.457 ± 0.351	0.714 ± 0.022
Herb	Species number (S)	15.000 ± 1.000	11.000 ± 3.606	12.667 ± 2.082	12.333 ± 1.528
	Shannon–Wiener index (H)	2.074 ± 0.153	2.079 ± 0.341	2.091 ± 0.181	1.828 ± 0.267
	Evenness index (E)	0.767 ± 0.066	0.881 ± 0.010	0.828 ± 0.081	0.733 ± 0.138

2.3. Chemical Analysis and Biolog® ECO-plate Technique

The parameters of the soil pH, soil water content (SWC), bulk density (BD), capillary porosity (CP), soil organic carbon (SOC), total nitrogen (TN), total phosphorus (TP), available nitrogen (AN), available potassium (AK), and available phosphorus (AP) were analyzed according to international standard methods [36,37]. Table 2 shows the soil physicochemical characteristics of the study sites.

Microbial biomass carbon (MBC) was determined by the chloroform fumigation-extraction method [38]. MBC was computed by determining the differences between fumigated and unfumigated samples with a conversion factor of 0.45 for MBC [39]. Soil basal respiration (BR) was measured by

alkali absorption [40]. The metabolic quotient was calculated by BR/MBC [41]. The microbial quotient was calculated by MBC/SOC [42].

The soil microbial community and functional diversity were assessed via CLPP analysis, using the Biolog® ECO-plate technique [43]. Each 96-well plate was incubated with three replicates (31 sole carbon sources and one water blank). Briefly, 5 g of fresh soil sample were suspended in 45 ml of sterile 0.85% NaCl solution and shaken for 30 min; subsequently, the supernatant was serially diluted to a concentration of 10^{-2}, and 150 µl of diluted solution were inoculated into each well and incubated at a constant temperature of 25 °C. The light absorbance in each well was recorded as the optical density at 590 nm in 24-h intervals for 168 h [14,44].

Table 2. The mean values of the soil physicochemical properties of two vegetation types in non-karst and karst areas of southern China.

Parameters	Non-Karst Area		Karst Area	
	Pinus yunnanensis	Secondary Forest	*Pinus yunnanensis*	Secondary Forest
pH	4.633 ± 0.091	4.850 ± 0.052	5.983 ± 0.099	5.917 ± 0.133
SWC (%)	25.456 ± 0.000	24.835 ± 0.000	23.481 ± 2.179	34.933 ± 3.043
BD (g cm^{-3})	1.269 ± 0.049	1.413 ± 0.059	1.073 ± 0.046	1.126 ± 0.088
CP (%)	47.9 ± 0.012	46.7 ± 0.020	45.540 ± 3.260	50.202 ± 2.563
SOC (g kg^{-1})	6.709 ± 0.753	7.569 ± 1.195	28.271 ± 4.020	42.348 ± 5.808
TN (g kg^{-1})	4.225 ± 1.058	3.233 ± 1.727	4.975 ± 0.135	5.275 ± 1.267
TP (g kg^{-1})	0.182 ± 0.025	0.528 ± 0.209	0.598 ± 0.070	1.341 ± 0.143
AP (mg kg^{-1})	1.160 ± 0.418	8.107 ± 4.565	1.267 ± 0.303	3.247 ± 0.660
AK (mg kg^{-1})	20.000 ± 0.000	103.333 ± 5.774	78.667 ± 6.855	83.333 ± 16.116
NO_3^--N (mg kg^{-1})	10.880 ± 1.924	10.433 ± 0.424	11.907 ± 0.551	12.527 ± 0.243
NH_4^+-N (mg kg^{-1})	4.987 ± 2.039	28.813 ± 6.128	4.755 ± 2.072	20.350 ± 5.324

Notes: SWC, soil water content; BD, bulk density; CP, capillary porosity; SOC, soil organic carbon; TN, total nitrogen; TP, total phosphorus; AP, available phosphorus; AK, available potassium; NO_3^--N, nitrate nitrogen; and NH_4^+-N, ammonium nitrogen.

2.4. Biolog® ECO-plate Analysis

The metabolic capacity of the microbial community was calculated according to the average well color development (AWCD) as:

$$AWCD = \sum(C - R)/N \quad (1)$$

where C represents the absorbance of each well, R represents the absorbance value of the control (water blank), and N represents the number of substrates (31) [43]. The negative values were set to zero [45]. In this study, the 72-h optical density value for each sample was used to calculate the carbon source use. The microbial functional diversity was calculated via the Shannon–Wiener diversity index (H′), the Pielou index (E), the Simpson index (D), and the McIntosh index (U), as follows:

$$H' = -\sum P_i \times \ln P_i \quad (2)$$

$$E = H'/\ln S \quad (3)$$

$$D = 1 - \sum P_i^2 \quad (4)$$

$$U = [\sum (C_i - R)^2]^{1/2} \quad (5)$$

where P_i represents the relative abundance of the i-th species (n_i/N), n_i represents the AWCD of the i-th substrate, and N represents the sum of AWCD of all substrates at t = 72 h; S represents the mean number of substrates (S), which represents the carbon source use on the ECO-plate (AWCD > 0.2).

The soil microbial CLPP was calculated as the ratio of AWCD to the sum of (C−R). Here, 3% of the total use values of each ECO-plate were selected as the base of the carbon source [46].

2.5. Statistical Analysis

The results of the AWCD, Shannon–Wiener diversity index (H'), Pielou index (E), Simpson index (D), and McIntosh index (U) were analyzed using SPSS 19.0 (SPSS Inc., Chicago. IL, USA). To test the significant differences of the soil physicochemical properties and the soil microbial community diversity indicators, a one-way analysis of variance (ANOVA) was utilized at a significance level of 0.05. A principal components analysis (PCA) was used to identify a subset of the original variables for a Biolog data analysis. A redundancy analysis (RDA) was used to explore the relationship between the soil microbial carbon source use patterns and their relationships with environmental factors (soil physico-chemical properties). A Monte Carlo permutation test (999 permutations) was applied to determine and sort factors that significantly influence the carbon source use. PCA and RDA were performed using the Canoco 4.5 for Windows. The figures were generated by Origin 8.5 (Origin Lab., Hampton, MA, USA).

3. Results

3.1. Soil Microbial Community Characteristics of the Two Different Vegetation Types

In the non-karst area, only the McIntosh index (U) of the secondary forest was higher than that of the *Pinus yunnanensis* plantation. In the karst area, all the diversity indicators of the soil microbial community showed no significant differences under both two different vegetation types (Table 3). In the non-karst area, the average use ratio of the six investigated carbon source categories was higher in the secondary forest than in the *Pinus yunnanensis* plantation; however, the differences were not significant. A similar trend was also observed in the karst area (Figure 1).

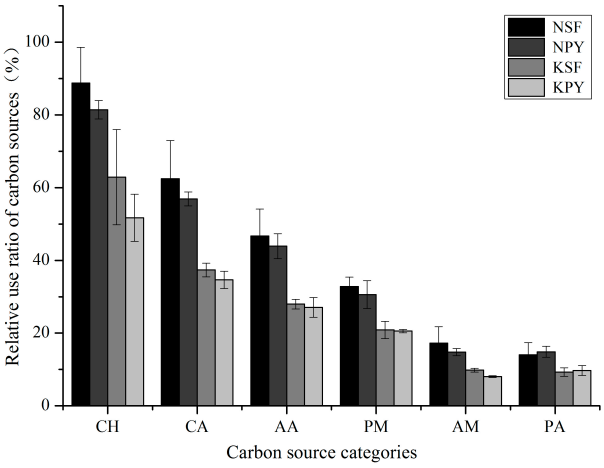

Figure 1. The relative use ratio of six carbon substrate categories for the two vegetation types in the non-karst and karst areas of southern China. NSF—non-karst secondary forest; NPY—non-karst *Pinus yunnanensis* plantation; KSF—karst secondary forest; KPY—karst *Pinus yunnanensis* plantation; CH—Carbohydrates; CA—Carboxylic acids; AA—Amino acids; PM—Polymers; AM—Amines amides; and PA—Phenolic acids. Bars indicate standard error.

The map of soil microbial CLPP showed that the different vegetation types also influenced the soil carbon source utilization patterns (3% of the total use values of each ECO-plate were selected as the base of the carbon source) (Figure 2). Under different vegetation types, the main carbon source types that contributed to the CLPP differences were five carbohydrates, two amino acids, two polymers, two amides, and one phenolic acid in the non-karst area. However, in the karst area, these were D–cellobiose, α–D–lactose, D,L–α–glycerol phosphate, D–galactonic acid γ–lactone, α–ketobutyric acid, D–malic acid, L–serine, Tween 40, glycogen, and 4–hydroxy benzoic acid, respectively.

Carbon sources	Chemical formula	Vegetation types			
		NSF	NPY	KSF	KPY
Carbohydrates					
D-Cellobiose	$C_{12}H_{12}O_{11}$				
α-D-Lactose	$C_{12}H_{12}O_{11}$				
β-Methyl-D-Glucoside	$C_7H_{14}O_6$				
D-Xylose	$C_5H_{10}O_5$				
i-Erythritol	$C_4H_{10}O_4$				
D-Mannitol	$C_6H_{14}O_6$				
N-Acetyl-D-Glucosamine	$C_8H_{15}NO_6$				
Glucose-1-Phosphate	$C_6H_{13}O_9P$				
D,L-α-Glycerol Phosphate	$C_6H_9O_6P$				
D-GalactonicAcid γ-Lactone	$C_6H_{10}O_6$				
Carboxylic acids					
Pyruvic Acid Methyl Ester	$C_4H_6O_3$				
D-Glucosaminic Acid	$C_6H_{13}NO_6$				
D-Galacturonic Acid	$C_6H_{10}O_7$				
γ-Hydroxybutyric Acid	$C_4H_8O_3$				
Itaconic Acid	$C_5H_6O_4$				
α-Ketobutyric Acid	$C_4H_6O_3$				
D-Malic Acid	$C_4H_6O_5$				
Amino acids					
L-Arginine	$C_4H_{14}N_4O_2$				
L-Asparagine	$C_4H_8N_2O_3$				
L-Phenylalanine	$C_9H_{11}NO_2$				
L-Serine	$C_3H_7NO_3$				
L-Threonine	$C_4H_9NO_3$				
Glycyl-L-Glutamic Acid	$C_7H_{12}N_2O_5$				
Polymers					
Tween 40	Tween40				
Tween 80	Tween80				
α-Cyclodextrin	$C_{36}H_{60}O_{30}$				
Glycogen	$(C_6H_{10}O_5)n$				
Amines amides					
2-Hydroxy Benzoic Acid	$C_7H_6O_3$				
4-Hydroxy Benzoic Acid	$C_7H_6O_3$				
Phenolic acids					
Phenylethyl-amine	$C_8H_{11}N$				
Putrescine	$C_4H_{12}N_2$				
Length of highly utilized carbon source＞3%		21	17	18	14

Legend: ≤3%; 3-3.5%; 3.5-4%; ≥4%

Figure 2. Use pattern of 31 carbon substrates by the microbial community under a *Pinus yunnanensis* plantation and a secondary forest in non-karst and karst areas in southern China.

The soil basal respiration of the secondary forest was higher than that of the *Pinus yunnanensis* plantation, both in the non-karst and the karst areas (Figure 3a). The soil microbial quotient of the secondary forest was higher than for the *Pinus yunnanensis* plantation in the non-karst area (Figure 3b), while the soil metabolic quotient of the secondary forest was lower than that of the *Pinus yunnanensis* plantation in the non-karst area (Figure 3c). The soil microbial quotient and metabolic quotient of the secondary forest and *Pinus yunnanensis* plantation showed no significant difference in the karst area (Figure 3b,c).

3.2. Soil Microbial Community Characteristics between Non-Karst and Karst Areas

All the diversity values of the soil microbial community showed no significant differences between the non-karst and karst areas, both in the secondary forest and the *Pinus yunnanensis* plantation (Table 3). However, the average use ratio of the six carbon source categories was higher in the non-karst area than in the karst area, both for the secondary forest and the *Pinus yunnanensis* plantation (Figure 1).

Table 3. Diversity indicators of the soil microbial community under different forests in non-karst and karst areas of southern China.

Parameter	Non-Karst Area		Karst Area	
	Pinus yunnanensis	Secondary Forest	*Pinus yunnanensis*	Secondary Forest
Shannon H′	2.214 ± 0.076	2.110 ± 0.578	2.468 ± 0.700	2.735 ± 0.380
Pielou E	0.648 ± 0.018	0.751 ± 0.222	0.925 ± 0.324	1.179 ± 0.385
Simpson D	0.856 ± 0.040	0.806 ± 0.054	0.869 ± 0.076	0.890 ± 0.051
McIntosh U	0.210 ± 0.120	0.509 ± 0.329	0.369 ± 0.272	0.478 ± 0.181

The map of soil microbial CLPP showed that the same vegetation types in the non-karst area were more efficient in utilizing carbon substrates than in the karst area (Figure 2). The soil basal respiration of both the secondary forest and *Pinus yunnanensis* plantation was higher in the non-karst area than in the karst area (Figure 3a). The soil microbial quotient of the secondary forest was higher in the non-karst area than in the karst area (Figure 3b). The soil metabolic quotient of the *Pinus yunnanensis* plantation was higher in the non-karst area than in the karst area (Figure 3c). The soil microbial quotient of the *Pinus yunnanensis* plantation and the metabolic quotient of the secondary forest showed no significant difference between the non-karst and karst areas (Figure 3b,c).

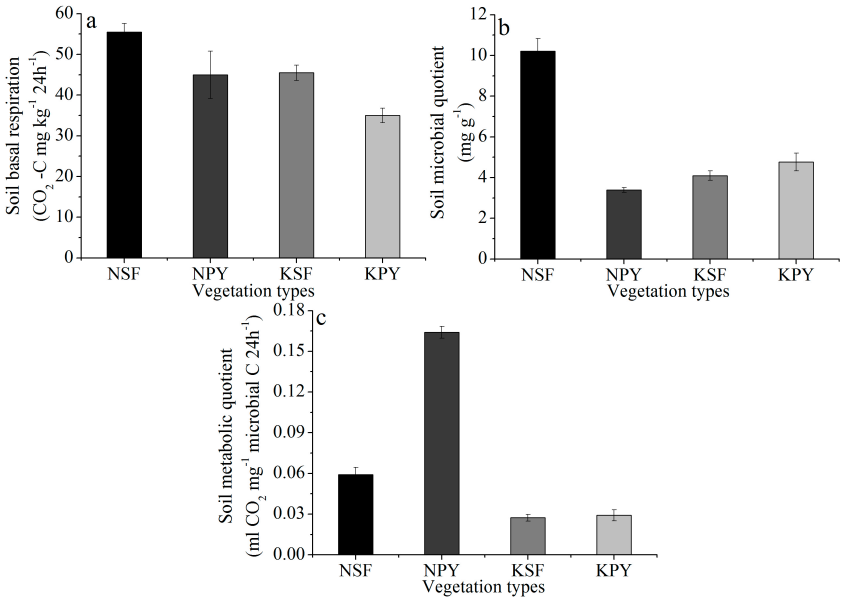

Figure 3. The soil microbial activities under different vegetation types. (**a**) Soil basal respiration; (**b**) Soil microbial quotient; and (**c**) Soil metabolic quotient.

Figure 4 presents the average utilization intensity of six categories of carbon substrates in the Biolog® ECO-plate. Different vegetation types showed various performances on different carbon substrates, and the results also indicated that the six categories of carbon substrates most frequently utilized were carbohydrates, carboxylic acids, and amino acids both in the non-karst and karst areas (Figure 4). The principal component analysis (PCA), based on the carbon source usage at 72 h of incubation, clearly separated the non-karst areas from the karst areas (Figure 5).

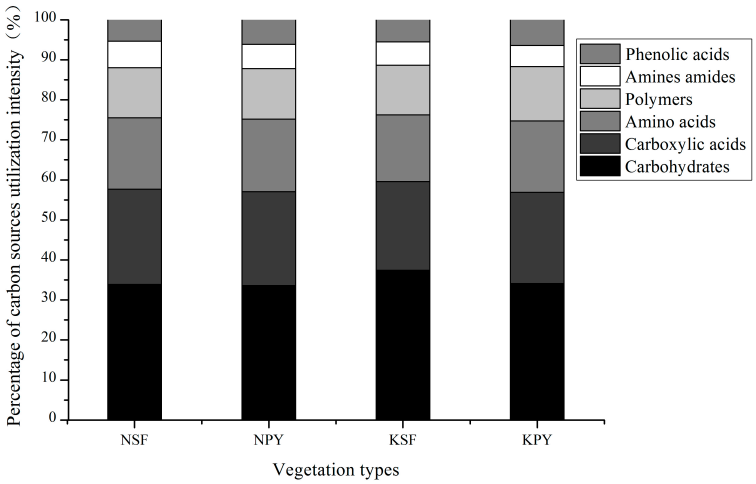

Figure 4. The utilization percentages of six categories of carbon substrates under different vegetation types.

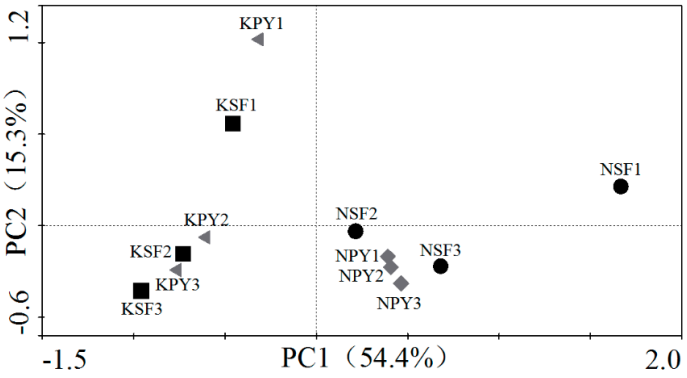

Figure 5. The principal components analysis of carbon source usage by soil microbial communities under different vegetation types.

3.3. Relationship between Soil Microbial Community Characteristics and Soil Physicochemical Properties

The soil physicochemical properties were the main environmental factors that influenced the soil microbial community functional diversity. In the non-karst area, the first and second axes explained 65.2 and 13.7% of the respective variance. The accumulated changes of the relationship between the carbon source use and environmental factors were 68.7 and 83.1%, respectively. In the karst area, the first and second axes represented 55.6 and 30.5% of the variance, and the accumulated changes of the relationship between the carbon source use and environmental factors were 58.6 and 90.8%, respectively (Table 4).

Table 4. Eigenvalues and cumulative variances of the redundancy analysis ordination of the soil microbial community.

Parameters	Non-Karst Area				Karst Area			
Axes	**I**	**II**	**III**	**IV**	**I**	**II**	**III**	**IV**
Eigenvalues	0.652	0.137	0.090	0.070	0.556	0.305	0.057	0.030
Soil microbial-physicochemical properties	1.000	0.987	0.999	0.965	0.983	1.000	1.000	1.000
Cumulative percentage variance of species data (%)	65.2	79.0	88.0	95.0	55.6	86.1	91.8	94.9
Cumulative percentage variance of soil Microbial-physicochemical properties (%)	68.7	83.1	92.6	100	58.6	90.8	96.8	100
Sum of all canonical eigenvalues	0.950				0.949			
Sum of all eigenvalues	1.000				1.000			

The RDA ordination plot (Figures 6 and 7) suggests that all of the selected soil properties significantly influenced the carbon source utilization. In the secondary forest, BD, NH_4^+-N, AK, AP, and N:P correlated positively with the carbon source utilization, while in the *Pinus yunnanensis* plantation BD, N:P, SWC, and AK correlated positively with the carbon source utilization (Figure 6a,b). The soil properties that significantly influenced the carbon source use in the non-karst area followed the order of TP, C:P, TN, NH_4^+-N, SWC, SOC, AK, N:P, C:N, BD, and pH. In the karst area, the order was CP, NO_3^--N, C:N, NH_4^+-N, SOC, SWC, TN, BD, N:P, pH, and TP (Table 5). The results also demonstrated environmental factors that explained the similarity of the carbon source utilization in different parent materials of NH_4^+-N, SOC, and SWC, and the dissimilarity of TP, CP, C:P, NO_3^--N, TN, and C:N (Figure 7a,b). Among these, chemical properties were significant variables for the carbon source utilization variation in the non-karst area, while in the karst area these were soil chemical properties and physical characteristics.

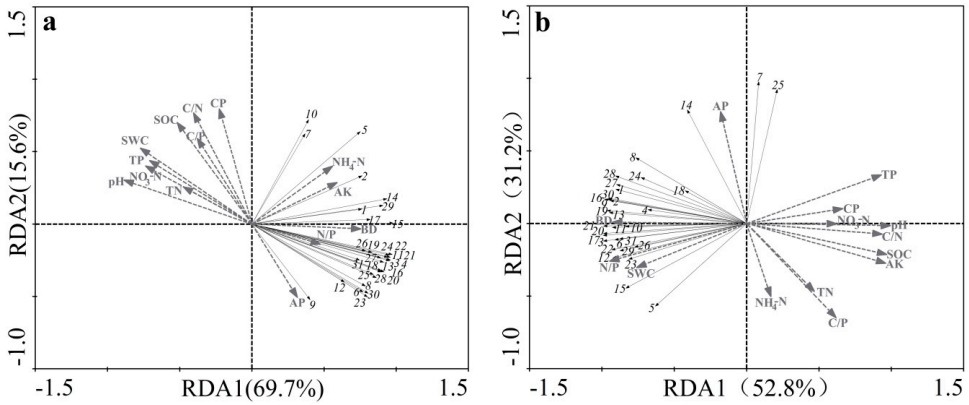

Figure 6. Redundancy analysis of the carbon source use patterns of microbial communities under (**a**) the secondary forest and (**b**) the *Pinus yunnanensis* plantation. The carbon substrates are: 1: D–Cellobiose, 2: α–D–Lactose, 3: β–Methyl–D–Glucoside, 4: D–Xylose, 5: i–Erythritol, 6: D–Mannitol, 7: N–Acetyl–D–Glucosamine, 8: Glucose–1–Phosphate, 9: D,L–α–Glycerol Phosphate, 10: D–Galactonic Acid γ–Lactone, 11: Pyruvic Acid Methyl Ester, 12: D–Glucosaminic Acid, 13: D–Galacturonic Acid, 14: γ–Hydroxybutyric Acid, 15: Itaconic Acid, 16: α–Ketobutyric Acid, 17: D–Malic Acid, 18: L–Arginine, 19: L–Asparagine, 20: L–Phenylalanine, 21: L–Serine, 22: L–Threonine, 23: Glycyl–L–Glutamic Acid, 24: Tween 40, 25: Tween 80, 26: α–Cyclodextrin, 27: Glycogen, 28: 2–Hydroxy Benzoic Acid, 29: 4–Hydroxy Benzoic Acid, 30: Phenylethyl–amine, and 31: Putrescine. The same below.

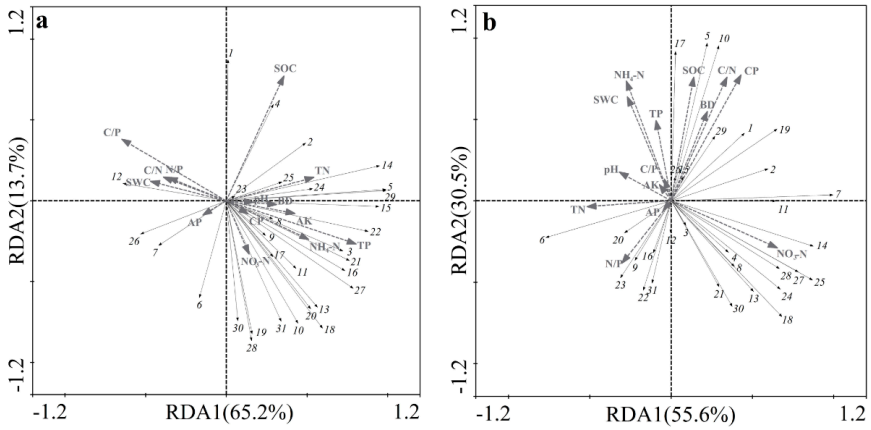

Figure 7. Redundancy analysis of the carbon source use patterns of microbial communities under the (**a**) non-karst area and (**b**) karst area.

Table 5. Importance order of the explanation of the physical and chemical factor variation.

Parameter	Non-Karst Area		Karst Area	
Importance Ranking	Physicochemical Properties	Explained Variation (%)	Physicochemical Properties	Explained Variation (%)
1	TP	45.2	CP	30.2
2	C/P	31.8	NO_3^--N	29.4
3	TN	25.4	C/N	26.2
4	NH_4^+-N	22.9	NH_4^+-N	22.8
5	SWC	20.9	SOC	20.5
6	SOC	19.3	SWC	19.4
7	AK	18.7	TN	17.7
8	N/P	17.2	BD	14.9
9	C/N	16.3	N/P	13.1
10	BD	14.1	pH	11.5
11	pH	10.1	TP	11.4
12	CP	9.9	C/P	5.8
13	AP	9.7	AP	5.5
14	NO_3^--N	8.8	AK	5.4

4. Discussion

4.1. Differences in Soil Microbial Community Characteristics under Both Vegetation Types

The Biolog® ECO-plates is an appropriate and sensitive method for the investigation of the soil microbial function diversity and activity [47]. Different function diversity index values and the use ratios of the six carbon source categories indicated that the microbial diversity and activity differed slightly between the two vegetation types. The soil microbial community of the secondary forest had a slightly higher carbon source use efficiency than that of the *Pinus yunnanensis* plantation (Figure 1). This is because soil moisture is a dominant controller of the microbial community composition and function, and because soil microorganisms regulate many crucial ecosystem processes such as the litter decomposition and biogeochemical cycle, which may potentially affect C and nutrient cycling [48,49]. According to previous studies, a decreased rainfall or soil water content may alter the microbial communities' composition and functioning by restricting the substrate diffusion and by increasing the physiological stress experienced by microbes [50]. Specifically, a limited soil water availability decreases the solute mobility, constrains the substrate supply to the decomposers, and directly inhibits microbial growths. Our findings indicate that during the dry season in subtropical forests, drought or drying might induce soil water stress and reduce the substrate availability for microbes, resulting in no significant difference in the soil microbial functional diversity among different vegetation types. The results also indicate that future experiments involving the soil microbial activity and metabolic diversity in subtropical forests should consider both seasonal rainfall variations.

Previous studies also showed that the soil microbial community functional diversity differs between deciduous and pine forests [8,51]. The reason for this discrepancy remains unclear. One explanation might be that the soil physicochemical properties influence the soil microbial activity [20]. Higher nutrient contents, especially N and P, support a higher microbial biomass and may indirectly facilitate an increase of the soil bacteria [10,52]. Under the same temperature and precipitation of both areas, deciduous broadleaf trees produce more litter quantities and have a more rapid decomposition than coniferous trees, thus resulting in higher nutrient levels of deciduous broadleaf trees compared to soils under coniferous tree species [12]. Another reason might be that the litter of evergreen coniferous tree species contains higher lignin, acids, and tannins levels, which are difficult to decompose and thus strongly affect soil microorganisms. In particular, tannins exert stronger depressive effects on soil bacteria than on fungi [16,18]. In addition, drought-tolerant tree species might have developed physiological adaptations to soil water shortage (such as a higher deaminase activity), which partly alleviates the effect of drought [11,53]. At the investigated study sites, the broadleaf species *Quercus*

variabilis Bl. and *Quercus baronii Skan* in the natural secondary forest are sclerophyllous species and therefore more adapted to water deficiency than the *Pinus yunnanensis* plantation during the dry season, which might also explain the difference in the soil microbial activity between both forest stands. All these results corroborate the previous studies, according to which deciduous broadleaf forests have different soil microbial biological activities from coniferous forests [15,54].

4.2. Differences in Soil Microbial Characteristics between Non-Karst and Karst Areas

Various modes of carbon source utilization suggest a different availability and quality of carbon sources in the soils [55]. The microbial communities in the non-karst area were more efficient in utilizing the six carbon substrates categories than those of the karst area with the same vegetation types (Figure 1). This was confirmed by previous studies, according to which the calcareous soil holds a higher proportion of recalcitrant organic carbon, which cannot be effectively used by the soil microbial community [56].

It has been suggested that the parent material exerts an indirect but strong effect on the microbial community and structure, which is likely mediated through the modification of the base cation status, soil moisture, pH, organic compounds, and nutrient availability [57–59]. In addition, the determination of the soil properties can also be based on the parent material [60], which influences the microbial form and function [22,61]. We speculate that the differences are due to the following reasons.

First, soil moisture may be the leading factor that reduces the soil microbial community diversity as well as the activity under conditions of water-limitation [62]. According to previous studies, water availability differs significantly between the non-karst and karst areas [63–65]. This was also the case in the present study, where at the peak of the dry season, the soil water content in the karst area was significantly lower than in the non-karst area. Second, the presence of different organic compounds in the soil might influence microbial communities through a priming effect [66]. Third, P deficiency significantly decreases the soil microbial biomass, functional diversity, metabolic activity, and basal respiration [67]. In other words, the complex interaction between the optimal nutrient availability and microbial growth is consistent with the soil organic carbon content [67]. Microorganisms in soils with balanced nutrient levels have a higher carbon source use efficiency and metabolisms. Consequently, decreases in the microbial growth are mainly due to low P levels, followed by decreased N and K concentrations [68]. Previous studies have demonstrated that in karst ecosystems, P deficiencies are much more common than in non-karst ecosystems [30,55,69,70]. In addition, N or P in excess or limitation plays key roles in organic carbon dynamics and microbial dynamics [71]. This would explain the decreased carbon source use rates in the karst area that were found in this study, resulting in the accumulation of higher levels of organic carbon.

4.3. Impacts of Soil Physico-Chemical Properties on Microbial Communities

Soil microbial communities are influenced by numerous factors [52]. The physico-chemical characteristics of the soil affect the soil microbial composition, structure, and functional diversity [14,20,72]. In this study, the soil parameters TP, C:P, TN, NH_4^+-N, SWC, and SOC were the main variables that were found to influence carbon source use variation in the non-karst area, while in the karst area the most important parameters in this regard were CP, NO_3^--N, C:N, NH_4^+-N, SOC, and SWC.

In both the non-karst and the karst areas, SWC seemed to be the leading factor that influenced the microbial functional diversity, which was consistent with previous studies in semiarid areas [62,73]. An obvious connection between the microbial functional diversity and SOC was also found. This corroborated the results of previous studies [67,74,75]. In contrast, no clear correlation was found between SOC and the microbial functional diversity of a sandy loam soil influenced by long-term agricultural activities [62]. The soil pH is a significant factor, which has been reported to influence the composition and diversity of soil microbial communities, either directly [19,76] or indirectly based on the changes in carbon and nutrient availability [77]. The optimum living environment of fungi and bacteria differ significantly. Fungi prefer acidic soils with low nutrient availability and high contents of

difficult-to-decompose organic matter [78], while bacteria prefer soil containing abundant nutrients that are highly decomposable [79,80]. However, this was not the case in the present study, possibly because the pH values of the soils differed over a comparatively narrow range, and a large proportion of the soils were acidic.

Our results also indicated that SOC, TN, and TP affected the diversity of the soil microbial communities. Previous studies have shown that variations in the soil microbial community composition were associated with both the nutrient ratios (i.e., ecological stoichiometry) and the dissolved organic matter in litter [81,82]. The elemental ratios may affect the microbial community composition due to differences in life strategies (r or K strategies) [83]. When environmental resources are sufficient, r-strategy microbes are stimulated, while K-strategy microbes survive when resources are deficient [83]. In this study, high nutrient levels changed the carbon use patterns, which led to differences in the utilized carbon sources between the secondary forest and the *Pinus yunnanensis* plantation.

5. Conclusions

The activity and metabolic diversity are the two metrics of the soil microbial community function. Soil microbial communities of the secondary forest used carbon substrates slightly more efficiently than the communities of the *Pinus yunnanensis* plantation, both in the non-karst and karst areas. The use efficiencies of the six investigated carbon substrate categories were higher in the non-karst than in the karst areas, resulting in a higher accumulation of organic carbon in the karst areas. The soil basal respiration of the secondary forest was higher than that of the *Pinus yunnanensis* plantation both in the non-karst and karst areas. The soil chemical properties significantly impacted the carbon source use in the non-karst area, while in the karst area the soil physical characteristics also significantly affected the microbial communities. Our findings clarify the impacts of the dominant forest species and soil properties on the soil microbial community metabolic diversity and carbon storage in subtropical forests.

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Abbreviations

CLPP	Community level physiological profile

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