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Original Research

Structure of Microbial Communities of *Castanopsis Hystrix* Plantations at Different Stand-Ages

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Abstract

Soil microorganisms play an important role in nutrient cycling in forest ecosystems, so it is of great significance to investigate the changes in soil microorganisms with forest age. We collected soil at different soil depths (0-10 cm, 10-20 cm, and 20-30 cm) to investigate the microbial phospholipid fatty acids (PLFAs) and the effects of soil properties and enzyme activities on soil microbial PLFAs. The results showed that: (1) with the increase of forest age, the soil total PLFAs, fungi, and bacteria content showed an increasing and then decreasing trend. (2) Soil fungi and bacteria showed significant positive correlations with soil organic carbon, β-glucosidase activity, acid phosphomonoesterase activity, phenol oxidase activity, peroxidase activity, and microbial biomass phosphorus. (3) Soil total nitrogen, available phosphorus, organic carbon, and total microbial PLFA contents in the 30-year-old *Castanopsis hystrix* plantation forest were higher than in the 6-year-old forest, which indicated that soil quality showed a trend of stable recovery after a longer period of community succession. However, it should also be noted that the microbial PLFAs declined as the stand age increased to 10 years, and fertilizers should be reasonably applied to improve soil fertility in future *C. hystrix* plantation forests.

Keywords: microbial community structure, phospholipid fatty acids, *Castanopsis hystrix*, plantation forest, forest age

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Introduction

China has contributed to the global greening process through intensive afforestation projects and now has the largest planted forest area in the world. Most of the planted forests are in the early and middle-aged stages. Due to the forest age factor, planted forests may still have great potential for carbon (C) sequestration in the future, and scientific management policies for planted forests are of great significance in realizing C neutrality in China [1].

Soil microorganisms play a key regulatory role in plant nutrient acquisition, nitrogen (N) and C cycling, and soil formation [2], and directly or indirectly affect the above-ground vegetation species growth dynamics and soil nutrient cycling turnover, thereby altering the species diversity of terrestrial ecosystems, soil nutrient maintenance, and ecosystem functioning [3]. Weak changes in soil microorganisms may lead to significant changes in aboveground-belowground systems nutrient transformations [1], and the resulting microbial loads are of great interest as indicators of changes in soil C and N pools and soil microbial functions. Soil microbial PLFAs, as a sensitive ecological indicator in terrestrial ecosystems [4-6], can reflect the soil microorganisms' C and N decomposition and mineralization rates, which are affected by soil nutrients and below-ground root residues C and N [5-7].

Previous studies have shown that the soil microbial PLFA content responds differently to vegetation types, mainly originating from the differences in altitudinal gradient, litter biomass, successional stage, and the mutual synergistic effect among soil microorganisms [6, 8]. Many studies focused on the effect of forest age on microbial community characteristics [9-13]. The soil microbial biomass carbon (MBC), bulk density (BD), total porosity, and total potassium (TK) were key factors that affected the bacterial communities [14]. Soil C and N concentrations and PLFA abundances in older plantations increased significantly compared to cropland [15]. Few studies focus on the microbial community characteristics of *Castanopsis hystrix* (*C. hystrix*) plantation forests in southern China.

C. hystrix is a valuable timber species in the southern subtropical zonal forests, with a high growth rate in the tropical, subtropical region, with high economic and ecological value, thus planted on a large scale in forestry production practices and is the main type of ecological public welfare forests.

Our earlier studies have found that *C. hystrix* forest productivity decreases with stand age, soil C stock increases significantly with stand age, and N limitation increases while P limitation is alleviated [9-12]. Six age-sequenced *C. hystrix* forests (6, 10, 15, 25, 30, and 34 years old) operated in the same way at Guangzhou Longyandong Forest Park in Guangdong Province were selected to investigate the trends of soil nutrients and microbial characteristics of different forest ages and soil depths (0-10 cm, 10-20 cm, and 20-30 cm). This study aimed to explore (1) the trends of soil microbial communities in *C. hystrix* plantation forests with forest age and (2) what factors regulate soil microbial communities.

Materials and Methods

Study Area

The study area is located in Guangzhou Longyandong Forest Park, Guangdong Province (23°10'-23°18' N, 113°21'-113°27' E); the soil type is reddish loam, the climate is subtropical monsoon, and the average annual temperature is 21.7ºC. The average annual precipitation is 1,694 mm, the existing woodland area is 1,622 hm^2, the area of ecological public welfare forests accounted for about 932 hm^2, and 63% of the elevation is between 100-538 m.

The sample plots, with trees dominated by *C. hystrix,* had simple stand forest structures. Due to the uniform cessation of anthropogenic disturbance after planting for 5 years in the older *C. hystrix* plantation forests, the native species are beginning to appear, such as *Itea chinensis, Aporusa dioica, Acronychia pedunculata, Ardisia quinquegona, Evodia lepta, Psychotria asiatica, Desmos chinensis, Blechnum orientale, Lophatherum gracile, Adiantum flabellulatum, Dicranopteris dichotoma, Pteris semipinnata, Ottochloa nodosa, and Lindsaea orbiculata.*

Plot Design

Based on preliminary investigations and multiple treadmills, we finally selected representative *C. hystrix* plantation forests at different restoration stages (6-, 10-, 15-, 25-, 30-, and 34-year forest age) as sample plots using a spatial instead of a temporal method (Table 1). Three 20×20 m sample plots were used for each forest age stage. In July-August 2020, to ensure that the selected sample plots had the same or similar stand conditions and forest structural composition, all sample plots were widely distributed spatially, the distance between sample plots of six forest ages was at least 1 km to avoid spatial autocorrelation, and all sample plots were at least 20 m from the forest edge to minimize the forest edge effect [9-12].

Field Sampling and Measurements

Soil samples were collected separately in August 2020 within the *C. hystrix* plantation forest. All soil samples were sieved through a 2 mm sieve, and the mass of each soil sample was about 90 g. The samples were divided into two parts. One sample was stored at -20ºC for determining microbial biomass and PLFAs, and one sample was stored in the refrigerator at 4ºC for the determination of soil physical and chemical properties.

Table 1. Information on the sampling sites.

Site characteristics	Stand Age (years)					
	6	10	15	25	30	34
Location	113.42 E, 23.25 N	113.39 E, 23.23 N	113.42 E, 23.26 N	113.40 E, 23.23 N	113.38 E, 23.26 N	113.40 E, 23.25 N
Altitude (m a.s.l.)	115	120	150	105	175	225
Direction (°)	NW	S	SW	E	N	S
Canopy density (%)	0.7±0.1	0.9±0.1	0.8±0.1	0.8±0.1	0.9±0.1	0.8±0.1
Tree DBH (cm)	16.7±0.1	12.3±0.9	19.1±4.5	19.0±1.5	13.6±2.6	23.9±2.6
Tree height (m)	6.1±0.1	7.9±0.6	9.8±1.6	11.9±1.0	9.4±1.6	13.1±1.5
Basal area (m^2 ha^{-1})	18.3±1.1	22.2±6.2	34.1±0.5	35.7±3.3	28.4±0.5	40.1±2.3
Density (tree ha^{-1})	750.0±43.3	1391.7±376.1	891.7±316.6	1058.3±158.8	1008.3±232.3	633.3±80.4
Shrub cover (%)	-	21.7±14.9	21.1±7.9	20.8±13.5	30.8±12.5	13.4±5.4
Herb cover (%)	-	6.3±4.9	20.4±10.0	28.9±11.3	24.8±9.0	15.6±5.1
Tree species richness	1.0±0.0	2.7±1.5	5.7±3.8	3.3±0.6	6.0±1.0	3.7±1.2
Shrub species richness	-	5.7±0.6	9.7±1.5	5.7±0.6	6.3±1.5	5.7±1.5
Herb species richness	-	5.0±2.0	5.7±0.6	4.7±1.2	3.0±1.2	4.0±1.7

Soil microbial community composition was determined by phospholipid fatty acid methods (PLFAs), according to Wu et al. (2020) [16]. According to the method mentioned by Wan et al. 2021 [14, 17], the soil pH was determined by air-drying with a water-soil ratio of 2.5:1; the soil water content (SWC) was determined by drying and weighing; SOC was measured by oxidation with potassium dichromate and external heating; total nitrogen (TN) was analyzed by the Kjeldahl method; total phosphorus (TP) was determined by alkaline solubilization of sodium hydroxide and molybdenum antimonimony antithesis colorimetric assay; and the ammonium nitrogen (NH_4^+-N), nitrate nitrogen (NO_3^--N) were determined by potassium chloride leaching-indophenol blue colorimetric method; available phosphorus (AP) was determined by molybdenum antimony colorimetric method after leaching with ammonium fluoride-hydrochloric acid [14].

Statistical Analysis

One-way analysis of variance (ANOVA) was used to test the effect of stand age on soil properties, soil microbial biomass, and microbial PLFAs. Data were processed and analyzed using Excel 2020 and SPSS 22.0 software and graphed using GraphPad Prism 8 software. Redundancy analysis (RDA) was performed using R Studio 4.1.1 (*vegan* package). Pearson's correlation analysis and Mantel's test were performed using R Studio 4.1.1 (*hmisc, corrplot, vegan, dplyr, ggcor,* and *ggplot2* packages). The SEM model was performed using R Studio 4.1.1 (*piecewiseSEM, QuantPsyc*, and *lme4* packages).

Results

Characterization of PLFAs, Fungi, Bacteria, and Fungi:Bacteria Ratio

Soil microbial PLFA, bacteria, fungi, and fungi:bacteria (F:B) were not significantly affected by stand age in the *C. hystrix* plantation forest (Fig. 1). In the 0-10 cm soil layer, there were no significant differences between the fungi of different stand ages. In the 10-20 cm soil layer, the F:B of the 34 -year-old stand was significantly higher than the other stand stages ($p<0.05$). In the 20-30 cm soil layer, the F:B of the 34-year-old stand was significantly higher than the 6, 10, 15, and 30-year-old stands ($p<0.05$).

Characterization of Microbial Community Characterization

In the 0-10 cm soil layer, G^+:G^- in the 6-year-old stand was significantly higher ($p<0.05$) than in the other stands (Fig. 2). In the 10-20 cm soil layer, G^+:G^- in the 34-year-old stand was significantly lower ($p<0.05$) than the 6-, 10-, and 30-year-old stands. In the 20-30 cm soil layer, G^+:G^- in the 34-year-old stand was significantly lower ($p<0.05$) than in the 6- and 30-year-old stands.

Redundancy Analysis of Soil Microbial Community and Soil Properties

Redundancy analysis (RDA) was used to analyze the interrelationships between soil microbial communities and soil properties in different stand ages of *C. hystrix*

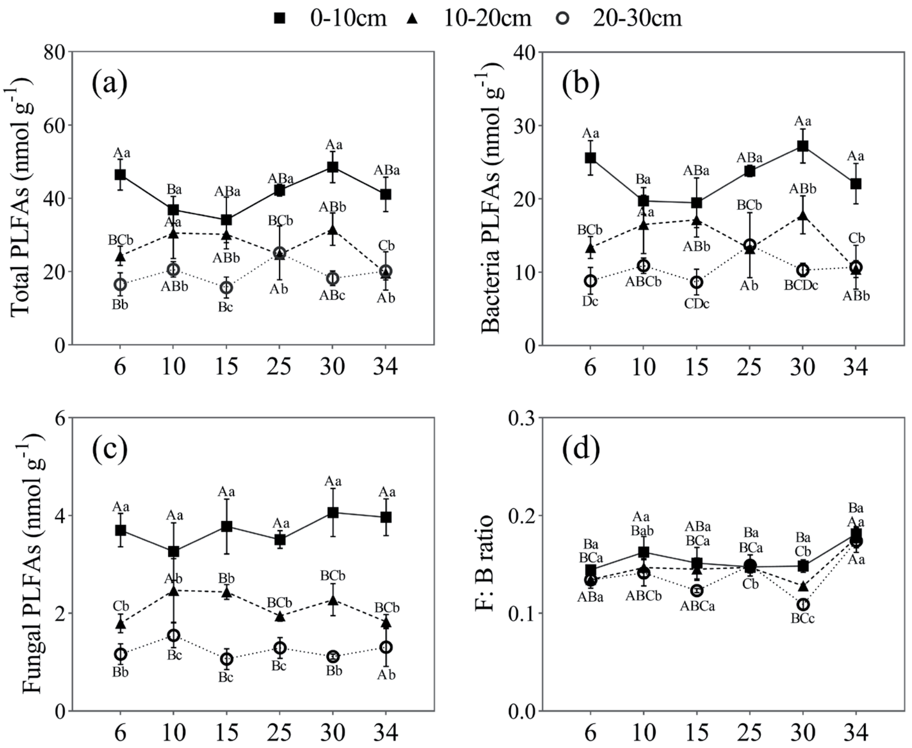

Fig. 1. a) Characterization of soil PLFAs, b) fungi, c) bacteria, and d) fungi:bacteria ratio in *Castanopsis hystrix* plantations of six forest ages (6a, 10a, 15a, 25a, 30a, and 34a). Different capital letters represent significant differences between stand ages on the same soil layer ($p<0.05$). Different lowercase letters represent significant differences between soil layers (0-10 cm, 10-20 cm, and 20-30 cm) on the same stand age ($p<0.05$).

plantation forests (Fig. 3). The RDA analysis results showed that the explanatory rates of RDA1 and RDA2 were 51.22% and 27.61%, respectively, with a cumulative explanatory rate of 78.83%. The soil pH was negatively correlated with the soil NO_3^--N, AN, TN, AP, and SOC.

Relationship between Soil Microbial Communities and Environmental Factors

Soil pH showed a highly significant negative correlation with SOC, TN, NO_3^--N, AP, MBC, microbial biomass nitrogen (MBN), microbial biomass phosphorus (MBP), cellobiohydrolase activity (CBH enzyme), phenol oxidase activity (PhOx enzyme), and peroxidase activity (Perox enzyme) ($p<0.001$), while SOC and TN showed a significant positive correlation ($p<0.01$) with soil enzyme activities (BG, NAG, ACP, CBH, PhOx, and Perox enzyme) as well as microbial biomass (MBC, MBN, and MBP), and NO_3^--N and AP showed significant positive correlation ($p<0.01$) with microbial biomass carbon, nitrogen, and phosphorus (MBC, MBN, and MBP) were significantly and positively correlated ($p<0.01$), and NO_3^--N and AP were significantly and positively correlated ($p<0.001$) with microbial biomass carbon, nitrogen, and phosphorus (MBC, MBN, and MBP). The Mantel's test results showed that the bacteria were significantly and positively correlated with soil BD, pH, SWC, SOC, TN, AN, NO_3^--N, AP, MBC, MBN, MBP, BG enzyme, ACP enzyme, PhOx enzyme, and Pero enzyme ($p<0.05$) (Fig. 4). There was a significant positive correlation ($p<0.05$) between fungi and soil pH, SOC, TN, AN, NO_3^--N, AP, MBC, MBN, MBP, BG enzyme, ACP enzyme, PhOx enzyme, and Perox enzyme. Besides, the soil TN was significantly associated with soil fungi and bacteria ($p<0.01$).

Furthermore, the study underscores that total PLFAs showed a highly significant positive correlation with enzyme activities (BG, NAG, AP, CBH, PhOx, and Perox), and soil properties (SOC, AN, NH_4^+-N, NO_3^--N, TN, TP, DOC, and AP) ($p<0.05$) in the 0-10 cm soil layer; plant diversity showed a non-significant correlation with enzyme activities (Fig. 5).

Discussion

PLFAs reflect the transient state of microbial changes and are rapidly decomposed after cell death [18]. In this study, the total PLFAs, fungal, and bacterial content in the 0-10 cm soil layer were higher than the 10-20 cm soil layer and the 20-30 cm soil layer, which may be due to the topsoil (0-10 cm layer) containing rich and diverse understory vegetation, and the input of litter may stimulate the proliferation of microorganisms.

With the increase in forest age, the PLFA content in the topsoil showed a trend of decreasing, increasing, and then decreasing, among which the highest PLFA content was found in the 30-year-old *C. hystrix* forest, consistent

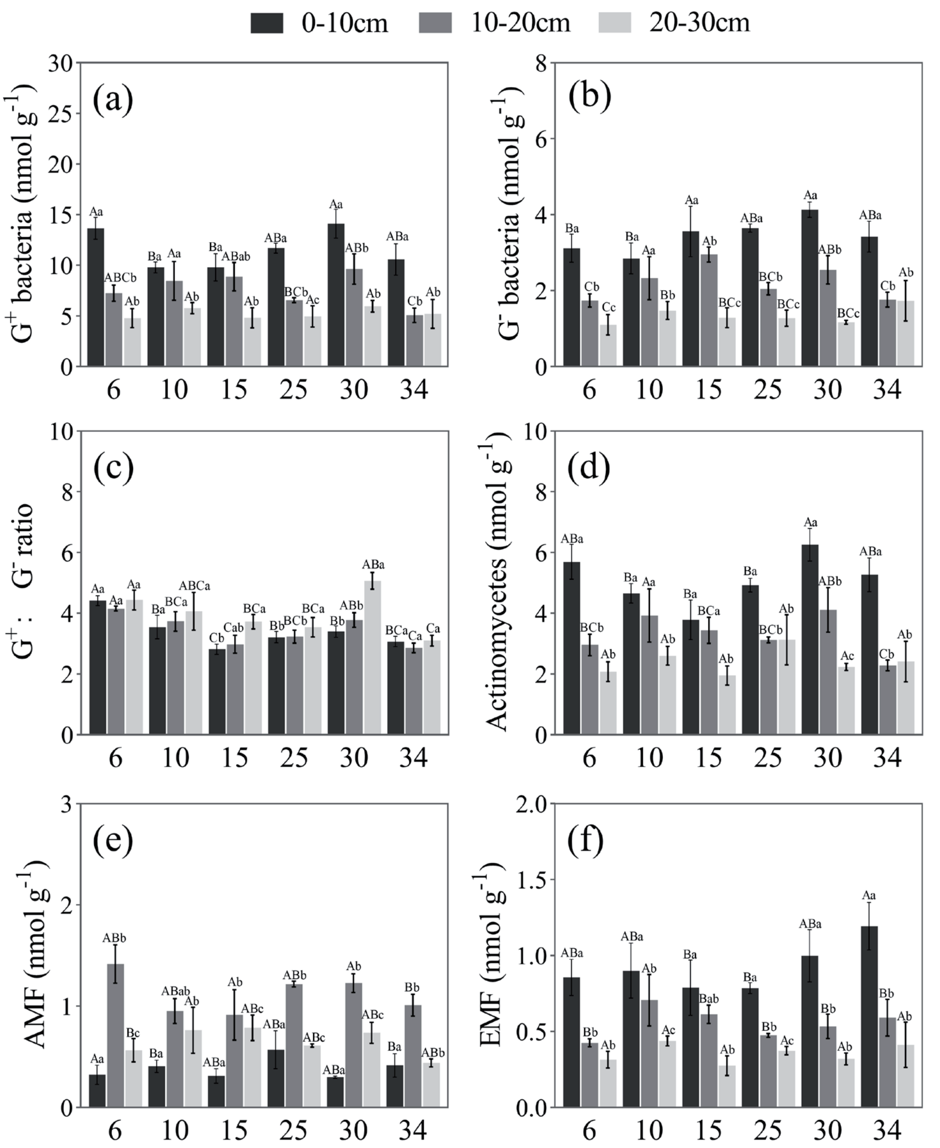

Fig. 2. a) Characterization of soil G^+ bacteria, b) G^- bacteria, c) G^+:G^- ratio, d) actinomycetes, e) arbuscular mycorrhizal fungi (AMF), and f) ectomycorrhizal fungi (EMF) in *Castanopsis hystrix* plantations of six forest ages (6a, 10a, 15a, 25a, 30a, and 34a). Different capital letters represent significant differences between stand ages on the same soil layer ($p<0.05$). Different lowercase letters represent significant differences between soil layers (0-10 cm, 10-20 cm, and 20-30 cm) on the same stand age ($p<0.05$).

with the results of previous studies [19]. Additionally, bacteria prefer environments with better soil fertility, while fungi are more able to survive in environments with poorer ground fertility, and the B:F ratio can reflect soil quality conditions and ecosystem stability [15]. The B:F ratio in the *C. hystrix* plantation forest was the highest in the 34-year-old forest, confirming that soil fertility was greatly improved under the 34-year *C. hystrix* plantation forest. Further, soil G^+ and G^- bacteria use difficult-to-decompose and easy-to-decompose soil organic matter as energy sources, respectively, and the G^+:G^- ratio can also better characterize the changes in soil fertility. In this study, the G^+:G^- ratio of the 34-year-old *C. hystrix* plantation forest was relatively lower than that of other forest ages, which also indicated that the soil nutrient status of the *C. hystrix* forest was better than that of the young forest after entering the mature forest stage [20].

The correlation analysis results showed significant positive correlations between soil fungal and bacterial contents and SOC, BG, ACP, PhOx, Perox, and MBP, further confirming that soil enzyme activities are important factors driving changes in microbial communities' biomass under different forest ages. SOC showed a significant positive correlation with fungal and bacterial content, which indicated that SOC, as a major energy source required by microorganisms, is considered a key factor in regulating soil microbial biomass and community structure [15, 21-23]. Additionally, soil AN was significantly and positively correlated with fungal and bacterial content, which indicated that the higher the soil AN content, the higher the microbial activity

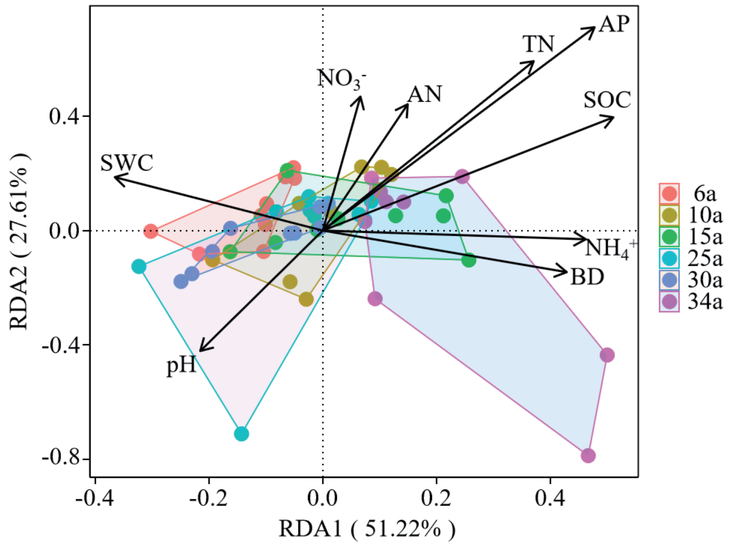

Fig. 3. Redundancy analysis (RDA) of soil microbial communities and soil properties in *Castanopsis hystrix* forests of six stand ages (6a, 10a, 15a, 25a, 30a, and 34a). The arrows represent soil factors, and the dots of different colors represent samples of different forest ages. The angle between the arrow line and the sorting axis indicates the correlation between a soil indicator and the sorting axis; the smaller the angle, the higher the correlation. SOC, soil organic carbon; TN, total nitrogen; TP, total phosphorus; NH_4^+-N, ammonium nitrogen; NO_3^--N, nitrate nitrogen; AP, available phosphorus; AN, available nitrogen; BD, bulk density; SWC, soil water content.

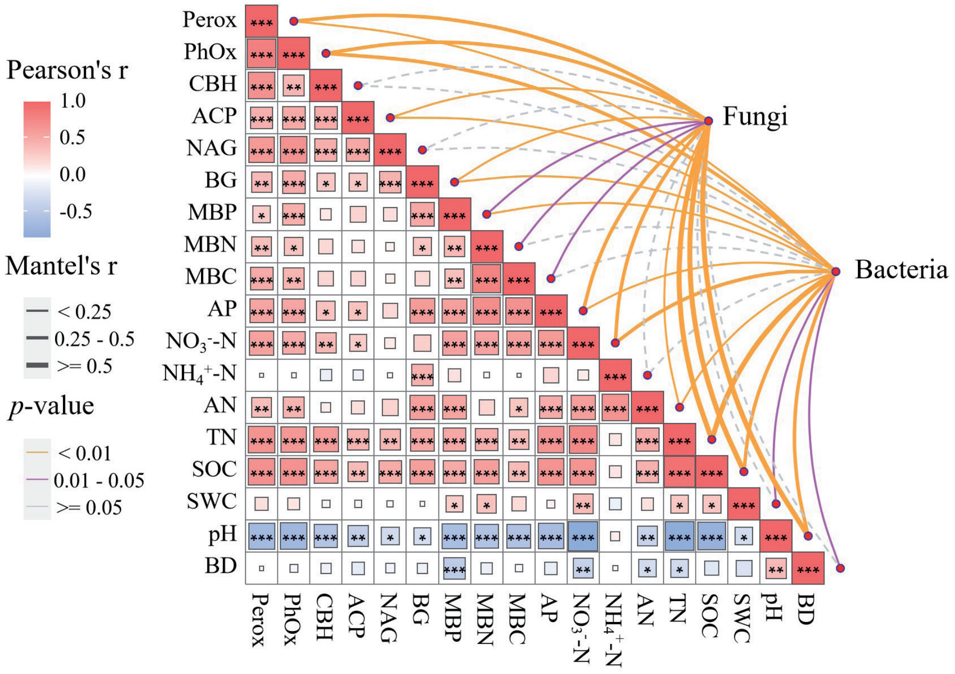

Fig. 4. Correlation of soil fungi and bacteria with soil and microbial factors. Bacteria, bacteria; Fungi, fungi; BD, bulk density; SWC, soil water content; SOC, soil organic carbon; TN, total nitrogen; AN, available nitrogen; NH_4^+-N, ammonium nitrogen; NO_3^--N, nitrate nitrogen; AP, available phosphorus; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; MBP, microbial biomass phosphorus; ACP, acid phosphomonoesterase activity; BG, β-glucosidase activity; NAG, N-acetylglucosaminidase activity; PhOx, phenol oxidase activity; Perox, peroxidase activity; CBH, cellobiohydrolase activity. ***$p<0.001$; **$p<0.01$; *$p<0.05$.

and reproduction capacity.

This study also found that the soil organic carbon and TN contents of 30-year and 34-year *C. hystrix* plantation forests were higher than those of other forest ages, indicating that *C. hystrix* plantation forests have stronger soil fertility after entering the mature forest stage, which may be due to the differences in community structure, understory vegetation, and species diversity, which lead to differences in the soil physicochemical properties of plantation forests at different forest ages [9-12].

In this study, the 30-year-old the soil TN, AP, and

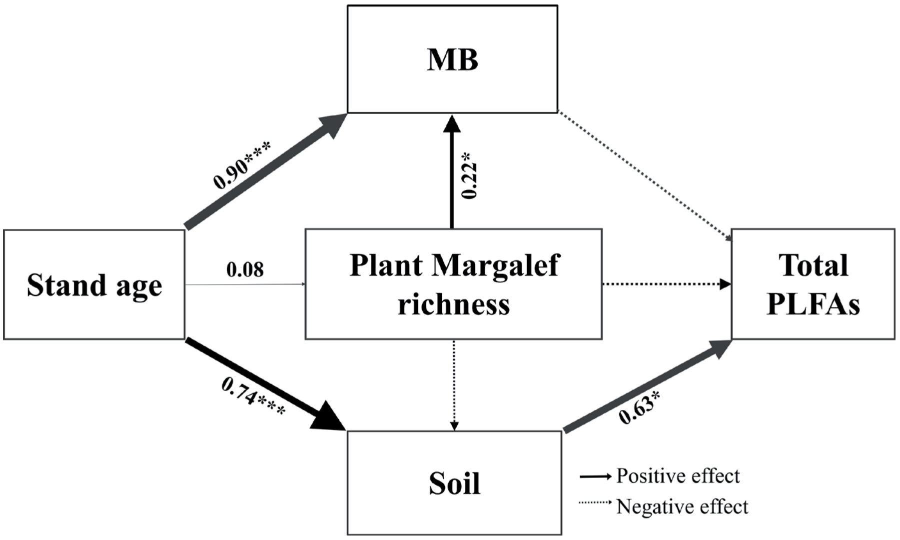

Fig. 5. The partial least squares path model of stand age, soil properties (SOC, DOC, AN, TN, NH_4^+-N, NO_3^--N, TP, and AP), plant Margalef richness (shrub and herb), MB (MBC, MBN, MBP, BG, NAG, AP, CBH, PhOx, and Perox), and total PLFAs in *Castanopsis hystrix* forests of six stand ages (6a, 10a, 15a, 25a, 30a, and 34a). $^{**}p<0.01$; $^{*}p<0.05$. Numbers near the lines represent the standard path coefficients. Solid arrows indicate positive flows of causality (p<0.05); dashed lines indicate non-significant ($p>0.05$) pathways; arrow widths denote the magnitude of these effects.

SOC contents of *C. hystrix* plantation forests were higher than the 6-year-old forest, and the soil microor-ganisms had the same trend. The soil total microbial PLFAs of the 30-year-old *C. hystrix* plantation forest was higher than the 6-year-old forest. This indicates that the soil fertility of the *C. hystrix* plantation forest showed a stable rebound trend after a longer period of community succession.

This study revealed that soil microbial activity is closely related to enzyme activities and soil properties. Plant communities tend to input more easily decomposable low-molecular organic matter into the soil through leaf litter, root systems and leaf secretions, thereby increasing soil nutrient content and providing soil microorganisms with a better quality food source, thus promoting microbial growth [12]. Soil enzyme activity plays multiple roles in forest ecosystems, including promoting organic matter decomposition and participating in soil nutrient cycling [12].

This study underscores that the total PLFAs was positively correlated with enzyme activities (BG, NAG, AP, CBH, PhOx, and Perox) and soil properties (SOC, DOC, AN, TN, NH_4^+-N, NO_3^--N, TP, and AP) in the 0-10 cm soil layer. Therefore, it is suggested that fertilization can be used to accelerate the community evolution of *C. hystrix* plantation forests in southern subtropics, and improve soil fertility to accelerate the promotion of soil nutrient cycling. Enhance soil microbial activity by monitoring and regulating soil nutrients, such as soil TN, SOC, AN, NO_3^--N, and AP.

Conclusions

This study showed that the soil total PLFAs, fungi, and bacteria content showed an increasing and then decreasing trend with the increase of forest age. Soil fungal and bacterial contents showed significant positive correlations with soil organic carbon, BG, ACP, PhOx, Perox, and MBP. Soil TN, AP, organic carbon contents, and total microbial PLFAs in the 30-year-old *C. hystrix* plantation forest were higher than in the 6-year-old forest, which indicated that the soil quality showed a trend of stable recovery after a longer period of community succession. However, it should also be noted that the microbial content declined as the stand age increased to 10 years, and fertilizers should be reasonably applied to improve soil fertility in future *C. hystrix* plantation forests operated up to 10 years.

Acknowledgments

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Conflict of Interest

The authors declare no conflict of interest.

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