Postprint: Comparison of Soil Fungal Community Diversity and Functional Groups between Southern Subtropical Native Tree Species and Eucalyptus Plantations

Authors: Xi Shouhong, Ming Angang, Tan Ling, He Jiang, Qin Lin

Date: 2023-05-21T00:00:00+00:00

Abstract

Establishing native tree species plantations and eucalyptus plantations are common practices in forest management in southern subtropical China, yet the response characteristics and mechanisms of soil fungal community diversity and function to these plantation types remain unclear. This study examined four native tree species plantations (Pinus massoniana, Michelia macclurei, Mytilaria laosensis, and Castanopsis hystrix) and an exotic Eucalyptus urophylla × E. grandis plantation in southern subtropical China. Based on 18S rRNA high-throughput sequencing data of soil (0–20 cm) from each stand and using the FUNGuild database, we comparatively analyzed the differential characteristics of soil fungal community diversity and functional groups between native tree species and E. urophylla × E. grandis plantations, as well as the dominant soil environmental factors influencing these differences. The results showed that: (1) The dominant phyla of soil fungi in all five studied stands were Ascomycota and Basidiomycota, but the dominant orders differed between native tree species stands and the eucalyptus plantation. (2) The alpha diversity of soil fungal communities in the eucalyptus plantation was higher than that in native tree species plantations, and its community composition and structure also differed significantly ($P<0.05$). (3) The relative abundance of saprotrophic trophic mode in soils of the four native tree species plantations was higher than that in the eucalyptus plantation. Moreover, the relative abundance of arbuscular mycorrhizal fungi in Michelia macclurei and Mytilaria laosensis plantations was significantly higher than in the eucalyptus plantation, while the relative abundance of symbiotrophic trophic mode, ectomycorrhizal fungi, and wood saprotrophs in the eucalyptus plantation was significantly higher than in native tree species plantations. (4) pH was the primary soil environmental factor causing differences in soil fungal community diversity and functional groups between the eucalyptus

plantation and native tree species plantations. In summary, converting eucalyptus plantations to Michelia macclurei or Mytilaria laosensis plantations in southern subtropical regions can improve soil nutrient levels and enhance soil ecological functions.

Full Text

Preamble

Comparison of Soil Fungal Community Diversity and Functional Groups Between Native Tree Species and Eucalyptus Plantations in South Subtropical China

XI Shouhong[1], MING Angang[2],[3], TAN Ling[1], HE Jiang[1], QIN Lin[1]*

[1]Guangxi Key Laboratory of Forest Ecology and Conservation, College of Forestry, Guangxi University, Nanning 530004, China
[2]Experiment Center of Tropical Forestry, Chinese Academy of Forestry, Pingxiang 532600, Guangxi, China
[3]Guangxi Youyiguan Forest Ecosystem Research Station, Pingxiang 532600, Guangxi, China

Abstract

Establishing plantations of native tree species and eucalyptus are common forest management practices in south subtropical China, yet the response characteristics and mechanisms of soil fungal community diversity and functions to these different plantation types remain unclear. This study investigated four native tree species plantations (*Pinus massoniana*, *Michelia macclurei*, *Mytilaria laosensis*, *Castanopsis hystrix*) and exotic *Eucalyptus urophylla* × *E. grandis* plantations in south subtropical China. Using 18S rRNA high-throughput sequencing data from soil samples (0–20 cm) and the FUNGuild database, we compared and analyzed the differences in soil fungal community diversity and functional groups between native and eucalyptus plantations, and identified the dominant soil environmental factors influencing these differences. The results showed that: (1) Ascomycota and Basidiomycota were the dominant fungal phyla across all five plantation types, but the dominant fungal orders differed between native species plantations and the eucalyptus plantation. (2) The α-diversity of soil fungal communities in the eucalyptus plantation was higher than in native species plantations, with significantly different community composition structures ($P<0.05$). (3) The relative abundance of saprotrophic fungi was higher in native species plantations than in the eucalyptus plantation, while arbuscular mycorrhizal fungi were significantly more abundant in *M. macclurei* and *M. laosensis* plantations. In contrast, the eucalyptus plantation showed significantly higher relative abundances of symbiotrophic fungi, ectomycorrhizal fungi, and wood saprotrophs compared to native species plantations. (4) Soil pH was the primary soil environmental factor driving differences in soil fun-

gal community diversity and functional groups between eucalyptus and native species plantations. Overall, converting eucalyptus plantations to *M. macclurei* or *M. laosensis* plantations in south subtropical China can improve soil nutrient levels and enhance soil ecological functions.

Keywords: soil fungal community, Illumina MiSeq high-throughput sequencing, FunGuild, native tree species plantation, eucalyptus plantation

Introduction

Fungi are important components of soil microorganisms that play crucial roles in plant nutrition, organic matter decomposition, and disease mediation (van der Heijden et al., 2016; Aslani et al., 2022). Soil fungal community diversity serves as a key indicator for evaluating soil quality (Martin et al., 2012; Qin et al., 2017; Yu et al., 2021). In forest ecosystems, tree species influence soil fungal community composition because litter and root exudates significantly affect soil properties (Chen et al., 2019), and changes in soil properties drive responses in soil fungal community structure and function (Liang et al., 2017; Wu et al., 2019). To deeply understand soil ecosystem functions, it is essential to emphasize the functional diversification of soil fungal communities (Barbi et al., 2016). Soil fungi exhibit clear functional differentiation: for example, saprotrophic fungi, as important decomposers of soil organic matter, influence element cycling rates (Frey et al., 2019); mycorrhizal fungi provide nutrients to plants through symbiotic associations; and pathogenic fungi affect forest health by infecting plant tissues to obtain energy (Maron et al., 2011). Previous studies have found that fungus-driven ecosystem processes differ among stand types (Chen et al., 2019), and that soil fungal community diversity and function change when external factors such as soil properties and tree species composition are altered (Snajdr et al., 2013; Tedersoo et al., 2014). Therefore, a thorough understanding of the diversity and functional characteristics of soil fungal communities in different tree species plantations and their underlying mechanisms can provide scientific references for tree species selection in afforestation and is important for assessing plantation soil quality.

China has the world's largest plantation area, totaling 79.54 million hectares (Dong et al., 2004). The climate conditions in south subtropical China are favorable, and since the 1980s, large-scale multi-generation continuous planting of fast-growing exotic eucalyptus (*Eucalyptus*) species has made significant contributions to regional economic development, but has also caused ecological problems such as soil fertility decline, biodiversity loss, and reduced ecosystem stability (Deng et al., 2013). As forest management philosophy shifts from single-objective timber production to multi-objective enhancement of ecosystem service quality and benefits, establishing high-value native broadleaf forests (such as *Mytilaria laosensis*, *Michelia macclurei*, *Paramichelia bailonii*, *Castanopsis hystrix*, etc.) has become a development trend for plantation management in subtropical China (Wan et al., 2015; Peng et al., 2018; You et al., 2020). In recent years, scholars have studied soil microbial biomass nitrogen and solu-

ble nitrogen characteristics (Qin et al., 2017), soil phosphorus fraction content and adsorption properties (Zheng et al., 2020), and soil bacterial community diversity (Tan et al., 2014; Qin et al., 2020) in native species versus eucalyptus plantations in south subtropical regions. However, knowledge about soil fungal community diversity and function in these plantations remains limited, which constrains scientific decision-making for tree species selection in afforestation.

This study examined four native species plantations (*Pinus massoniana*, *Michelia macclurei*, *Mytilaria laosensis*, *Castanopsis hystrix*) and *Eucalyptus urophylla* × *E. grandis* plantations in south subtropical China. Based on 18S rRNA high-throughput sequencing data from soil samples (0–20 cm) and FUNGuild functional prediction methods, we aimed to address two questions: (1) Do soil fungal community diversity and functional groups differ significantly between native species plantations and eucalyptus plantations? (2) Are the dominant soil environmental factors influencing these differences consistent? Our objectives were to reveal the changing characteristics and regulatory mechanisms of soil fungal community diversity and function after converting eucalyptus plantations to native species plantations, and to provide a scientific basis for deeply understanding the ecological functions of soil fungal communities in native versus exotic plantations in south subtropical regions.

1.1 Study Area and Soil Sampling

The study site was located at the Experiment Center of Tropical Forestry, Chinese Academy of Forestry (Pingxiang, Guangxi, 106°50 E, 22°10 N). The region has a south subtropical monsoon semi-humid to humid climate, with an average annual temperature of 21°C and average annual precipitation of 1,500 mm, mainly occurring from April to September. The elevation ranges from 130 to 1,045 m, with low mountains and hills as the primary landform types. The zonal soil is mountainous red soil developed from granite (He et al., 2013).

The zonal vegetation is subtropical evergreen broadleaf forest. In the 1950s, Chinese fir was planted on clear-cut sites of evergreen broadleaf forest. Native species plantations of *Mytilaria laosensis*, *Michelia macclurei*, *Castanopsis hystrix*, and *Pinus massoniana* were established in the 1980s on clear-cut sites of Chinese fir plantations (initial planting density of 2,500 trees $\cdot$ hm^{-2}). The *Eucalyptus urophylla* × *E. grandis* plantation was planted in 2008 on a clear-cut Chinese fir site (initial density of 2,500 trees $\cdot$ hm^{-2}) and clear-cut with stump retention in 2014 to form a second-generation coppice forest. In February 2017, three 20 m × 20 m plots were randomly established in each plantation type for stand surveys, with distances between plots of at least 20 m. The site conditions and stand characteristics of the five plantations are shown in Table 1 .

Within each plot, three sampling points were randomly selected along the left diagonal, and surface soil (0–20 cm) was collected using a soil auger with an inner diameter of 5 cm. Soils from the three sampling points were mixed to form one composite sample, yielding 15 soil samples across the five plantations. Soil

samples were placed in polyethylene bags, stored with ice packs, and transported to the laboratory. Fresh soil samples were passed through a 2 mm stainless steel sieve and divided into three portions: one stored at -80°C for DNA extraction, one refrigerated at 4°C for determination of nitrate and ammonium nitrogen, and one air-dried, passed through a 0.25 mm sieve, and stored for measurement of basic chemical properties.

1.2 Soil Physicochemical Property Determination

Soil water content (SWC) was determined using the oven-drying method. Soil pH was measured with a pH meter (Prtavo 907 MULTI-N, Germany) in a water suspension (soil:water = 1:2.5). Soil organic carbon (SOC) content was determined by the potassium dichromate external heating method. Nitrate nitrogen (NO_3^--N) content was measured by phenoldisulfonic acid colorimetry, and ammonium nitrogen (NH_4^+-N) by the diffusion method. Available phosphorus (AP) content was determined by double acid extraction-molybdenum antimony colorimetry (Lu, 2000). Total nitrogen (TN) and total phosphorus (TP) contents were measured by H_2SO_4-$HClO_4$ digestion followed by analysis using a SmartChem200 automatic chemical element analyzer (Alliance, France). The soil carbon-to-nitrogen ratio (C/N) was calculated as the ratio of SOC to TN content.

1.3 DNA Extraction, PCR Amplification, and Illumina Miseq Sequencing

Soil microbial total DNA was extracted using the PowerSoil® DNA Isolation Kit (MoBio, USA). Genomic DNA integrity was checked by 1% agarose gel electrophoresis, and purity and concentration were measured using a NanoDrop2000 micro-volume UV spectrophotometer (Thermo Fisher Scientific). Fungal 18S rRNA genes were amplified using primers 1196R (5 -TCTGGACCTGGTGAGTTTCC-3) and SSU0817F (5 -TTAGCATGGAATAATRRAATAGGA-3) (Rousk et al., 2010). PCR amplification was performed using TransStart Fastpfu DNA Polymerase in a 20 L reaction system containing: 4 L 5×FastPfu Buffer, 2 L dNTPs (2.5 mmol $\cdot$ L^{-1}), 0.8 L forward primer (5 mol $\cdot$ L^{-1}), 0.8 L reverse primer (5 mol $\cdot$ L^{-1}), 0.4 L FastPfu Polymerase, 0.2 L BSA, 10 ng template DNA, and ddH_2O to 20 L. Thermal cycling conditions were: 95°C for 3 min; 35 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 45 s; final extension at 72°C for 10 min; and cooling to 10°C. Each sample had three replicates, and PCR products from the same sample were pooled and detected by 2% agarose gel electrophoresis. PCR products were purified using the AxyPrep DNA Gel Extraction Kit (AXYGEN) and quantified with the QuantiFluor™-ST blue fluorescence quantification system (Promega). Library construction and high-throughput sequencing were performed by Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China) using the Illumina Miseq PE300 platform. All high-throughput sequencing data have been submitted to NCBI SRA (https://www.ncbi.nlm.nih.gov/) under accession number

PRJNA936188.

1.4 Bioinformatics Analysis

Trimmomatic software was used to filter adapter sequences and low-quality reads from the raw sequences. Usearch software (version 7.1, http://drive5.com/uparse/) was used to cluster non-repetitive sequences (excluding singletons) into operational taxonomic units (OTUs) at 97% similarity, with chimeras removed during clustering to obtain representative OTU sequences. The RDP classifier Bayesian algorithm was used on the Qiime1 platform to assign taxonomic information to OTU representative sequences at 97% similarity by comparing against the Silva database (Release 123, http://www.arb-silva.de) with a confidence threshold of 0.7. After merging the OTU table and species information table, Python 3.8 was used to compare against the FUNGuild v1.1 database to parse the trophic modes and functional guilds of fungal communities in each sample. To ensure reliable interpretation of fungal functional guilds, only "highly probable" and "probable" confidence levels were retained (Nguyen et al., 2016).

1.5 Data Processing

The Kruskal-Wallis rank sum test was used to examine significant differences in soil fungal phyla and orders among different plantations. Alpha diversity of fungal communities was characterized using Chao1, Shannon, and Simpson indices based on OTUs (with lower Simpson values indicating higher diversity), calculated using Mothur software (Patrick et al., 2017). Differences in soil physicochemical properties and fungal alpha diversity among plantations were tested by one-way ANOVA with Duncan's multiple comparison. Spearman rank correlation was used to test relationships between alpha diversity and soil physicochemical factors. All analyses were performed using SPSS 26.0 (SPSS, Inc, Chicago, IL).

Beta diversity of fungal communities was analyzed by non-metric multidimensional scaling (NMDS) based on Bray-Curtis distances using the "metaMDS()" function in the R package vegan. Permutational multivariate analysis of variance (PERMANOVA) with 999 permutations was used to test significant differences in beta diversity among soil samples using the "adonis()" function in vegan. Generalized dissimilarity modeling (GDM) (Ferrier et al., 2007) was used to analyze the effects of soil physicochemical properties on beta diversity, with the weighted Bray-Curtis dissimilarity matrix as the response variable and soil properties as predictors. In GDM outputs, the magnitude of change on the y-axis represents the relative intensity of OTU turnover in fungal communities under the influence of soil physicochemical properties; larger y-axis changes indicate greater influence of soil properties on fungal community composition. Only variables with significant GDM fits were plotted. Redundancy analysis (RDA) was used to explore relationships between soil fungal functional groups

and physicochemical properties, with significance tested by Monte Carlo permutation test (999 permutations) using the "rda()" function in vegan.

2.1 Soil Physicochemical Properties

ANOVA revealed that soil water content in the eucalyptus plantation did not differ significantly from the *Michelia macclurei* plantation (P>0.05), but was significantly higher than in the *Pinus massoniana* and *Castanopsis hystrix* plantations and significantly lower than in the *Mytilaria laosensis* plantation (P<0.05) (Table 2). Soil pH in the eucalyptus plantation was significantly higher than in the *M. macclurei*, *M. laosensis*, and *C. hystrix* plantations, but not significantly different from the *P. massoniana* plantation. The other seven soil physicochemical properties (SOC, TN, NO_3^--N, NH_4^+-N, TP, AP, and C/N ratio) showed no significant differences between the eucalyptus plantation and the four native species plantations.

2.2 Soil Fungal Community Composition at Taxonomic Level

At 97% similarity, species annotation of OTU representative sequences revealed 33 phyla, 54 classes, 84 orders, 109 families, and 110 genera across all soil samples. The dominant fungal phyla (relative abundance $ $10%) in all five plantations were Ascomycota (40.6%–76.1%) and Basidiomycota (15.4%–41.2%) (Figure 1 [Figure 1: see original paper]A). Kruskal-Wallis tests showed no significant differences in the relative abundances of these two dominant phyla between the eucalyptus plantation and native species plantations (P>0.5).

The top 10 most abundant fungal orders across all plantations were: Agaricomycetes_{unclassified}, Hypocreales, Eurotiales, Archaeorhizomycetales, Onygenales, Sordariales, Tremellales, Ascomycota_{unclassified}, Incertae_{Sedis}, and Sordariomycetes_{unclassified} (Figure 1B). Kruskal-Wallis tests revealed that the eucalyptus plantation had significantly lower relative abundance of Archaeorhizomycetales than the *M. laosensis* plantation, significantly lower Onygenales than the *P. massoniana* and *C. hystrix* plantations, but significantly higher Tremellales and Hypocreales than the *C. hystrix* and *M. laosensis* plantations, and significantly higher Sordariales than the *C. hystrix* plantation (P<0.05).

2.3 Soil Fungal Community Diversity Based on OTU Level

2.3.1 Alpha Diversity

A total of 580,568 optimized sequences were obtained from all soil samples, with an average of 38,704 sequences per sample and an average length of 401 bp. Rarefaction curves gradually plateaued with increasing sequencing depth, indicating that the sequencing data adequately captured the fungal community

composition in the soil samples (Figure 2 [Figure 2: see original paper]). Clustering at 97% similarity yielded 440 OTUs, with 114 OTUs shared among all five plantations. The eucalyptus plantation had the highest total number of OTUs (279) and unique OTUs (57), compared to the native species plantations (*P. massoniana*: 215 total, 17 unique; *M. macclurei*: 263 total, 21 unique; *M. laosensis*: 250 total, 16 unique; *C. hystrix*: 181 total, 8 unique).

ANOVA showed that the Chao1 index of soil fungal communities in the eucalyptus plantation was significantly higher than in the *C. hystrix* plantation ($P<0.05$), but not significantly different from the other three native species plantations ($P>0.05$) (Figure 3 [Figure 3: see original paper]A). The Shannon index did not differ significantly among plantations (Figure 3B), while the Simpson index was significantly lower in the eucalyptus plantation than in all native species plantations (Figure 3C). Overall, soil fungal community α-diversity was significantly higher in the eucalyptus plantation than in native species plantations. Spearman rank correlation analysis revealed that only the Simpson index was significantly negatively correlated with soil pH ($r=-0.549$, $P=0.034$).

2.3.2 Beta Diversity

NMDS analysis based on Bray-Curtis distances effectively characterized differences in fungal community structure among plantations (Stress=0.072) (Figure 4 [Figure 4: see original paper]). PERMANOVA analysis indicated that the eucalyptus plantation had significantly different soil fungal community structures compared to *P. massoniana* (Fpseudo=5.06, $P=0.001$), *M. macclurei* (Fpseudo=2.92, $P=0.043$), *M. laosensis* (Fpseudo=2.28, $P=0.045$), and *C. hystrix* (Fpseudo=4.56, $P=0.001$) plantations ($P<0.05$). GDM analysis revealed that soil physicochemical factors influencing fungal community composition could be divided into three categories: (1) factors with greater influence at low gradients, including SOC (Figure 5 [Figure 5: see original paper]A) and TP (Figure 5E); (2) factors with greater influence at high gradients, where TN showed significantly increased effects on fungal community structure at values above approximately 2.1 g/kg (Figure 5B); and (3) factors with gradually increasing effects on community change as values increased, including pH (Figure 5C), NO_3^--N (Figure 5D), and SWC (Figure 5F).

2.4 Functional Groups of Soil Fungal Community

Based on FUNGuild v1.1 database classification, 75 OTUs (18.2% of total OTUs) were assigned to six trophic modes and ten functional guilds. The six trophic modes were saprotroph, symbiotroph, pathotroph, pathotroph-saprotroph, pathotroph-symbiotroph, and pathotroph-saprotroph-symbiotroph (Figure 6 [Figure 6: see original paper]A). Saprotrophs were the dominant trophic mode in native species plantations (21.7%–76.3%), while symbiotrophs had the highest relative abundance in the eucalyptus plantation (45.7%). The ten functional guilds were: endophyte-fungal parasite-plant pathogen, endophyte-plant pathogen-wood saprotroph, plant pathogen-undefined

saprotroph, animal pathogen-fungal parasite-undefined saprotroph, stem saprotroph-wood saprotroph, ectomycorrhizal fungi, arbuscular mycorrhizal fungi, wood saprotroph, dung saprotroph-soil saprotroph, and plant pathogen (Figure 6B). The eucalyptus plantation had markedly higher relative abundances of ectomycorrhizal fungi (44.7%) and wood saprotrophs (11.0%) than native species plantations, while arbuscular mycorrhizal fungi were significantly more abundant in *M. macclurei* (17.5%) and *M. laosensis* (20.8%) plantations than in the eucalyptus plantation.

RDA of soil fungal functional groups and physicochemical properties showed that the first two axes explained 95.43% of the total variation (87.62% and 7.81% for RDA1 and RDA2, respectively) (Figure 7 [Figure 7: see original paper]). Soil samples from the eucalyptus plantation were located on the positive side of RDA1, while native species plantations were on the negative side. Monte Carlo permutation tests identified pH as the dominant factor (P=0.045) significantly influencing differences in soil fungal functional groups between the eucalyptus plantation and native species plantations.

3.1 Effects of Different Stand Types on Soil Fungal Community Diversity

Ascomycota and Basidiomycota can degrade recalcitrant substances such as lignin and cutin, and are considered core microorganisms in forest soils that play important roles in soil nutrient cycling and the function and stability of microbial flora (Qiao et al., 2017; Wang et al., 2018). This study found that Ascomycota and Basidiomycota were the dominant fungal phyla across all five plantations, consistent with findings for native species plantations formed by natural recovery after artificial block planting (Song et al., 2020) and *Eucalyptus urophylla* × *E. grandis* plantations (Chen et al., 2020) in south subtropical regions. Generally, nutrient-rich soils favor copiotrophic fungi, while oligotrophic fungi increase in relative abundance in relatively nutrient-poor soils (Schneider et al., 2012). In this study, the significantly higher relative abundance of Archaeorhizomycetales in *M. laosensis* plantation soil compared to the eucalyptus plantation may be because Archaeorhizomycetales typically colonize rhizospheres of species that provide organic matter for saprotrophic fungi (Meng et al., 2020), and *M. laosensis* plantation soil had higher organic carbon than the eucalyptus plantation. Additionally, the significantly higher relative abundance of Onygenales in *P. massoniana* and *C. hystrix* plantations compared to the eucalyptus plantation may be due to their significantly lower soil pH and water content, as studies have shown that lower pH and moisture can increase Onygenales abundance (Claudia et al., 2022). Furthermore, previous research suggests that the relative abundances of Sordariales and Tremellales are positively correlated with pH (Man et al., 2021) and litter content (Chen et al., 2020), which is closely related to stand density (Zhou, 2019). The higher soil pH and stand density in the eucalyptus plantation compared to native species plantations may explain the enrichment of Sordariales and Tremellales.

Compared with native species plantations, the eucalyptus plantation had higher total OTU numbers, unique OTU numbers, Chao1 and Shannon indices, and lower Simpson index, indicating higher soil fungal community α-diversity. Previous studies have found significant positive correlations between forest soil fungal α-diversity and soil pH (Shen et al., 2014), as different pH values alter soil environments and consequently affect fungal community diversity (Green et al., 2004). Spearman rank correlation analysis in this study showed that Simpson index was significantly negatively correlated with soil pH ($P<0.05$), and soil pH was higher in the eucalyptus plantation than in native species plantations, indicating that pH is the main factor regulating the higher α-diversity in the eucalyptus plantation. However, Song et al. (2020) found that soil carbon and nitrogen content were key factors affecting soil fungal diversity in different plantations in south subtropical China, while Yang et al. (2020) reported that soil fungal diversity in Loess Plateau plantations was controlled by soil C/N ratio. This study did not find soil carbon/nitrogen content or C/N ratio as dominant factors affecting differences in soil fungal community diversity between native and eucalyptus plantations, likely because these properties did not differ significantly between the two plantation types. NMDS and PERMANOVA analyses showed significant differences in soil fungal community structure between the eucalyptus plantation and native species plantations. Combined with GDM and ANOVA results, we infer that soil water content and pH were the main causes of these differences. Numerous studies have demonstrated that pH is an important factor affecting forest soil fungal community β-diversity; for example, Ping et al. (2017) found that pH had strong effects on soil fungal community β-diversity in Changbai Mountain forests, and Chen et al. (2020) reported that changes in eucalyptus plantation soil fungal communities in response to fertilization were negatively correlated with pH. Additionally, studies have shown that soil water content is closely related to soil fungal community structure (Yang et al., 2017; Chen et al., 2019). In summary, pH is the primary soil environmental factor causing differences in soil fungal community diversity and structure between eucalyptus and native species plantations.

3.2 Effects of Different Stand Types on Soil Fungal Community Functional Groups

Fungi have complex life histories, and some fungi actively adopt multiple nutritional strategies to adapt to their environment, representing a relatively advanced survival strategy (Xiong et al., 2020). This study found that saprotrophic fungi were the dominant trophic mode in native species plantations, indicating that Ascomycota and Basidiomycota in these soils were primarily saprotrophic. Previous studies have also shown that saprotrophic fungi dominate in native species plantations in other regions of China (Deng et al., 2020; Chen et al., 2022), and that saprotrophic fungi, as important decomposers in soils, play major roles in nutrient cycling (Nie et al., 2018; Sun et al., 2019). However, symbiotrophic fungi dominated in the eucalyptus plantation, indicating that Ascomycota and Basidiomycota in its soil were mostly symbiotrophic. There-

fore, establishing native species plantations to replace eucalyptus plantations in south subtropical China is beneficial for improving soil fertility and quality.

This study found that arbuscular mycorrhizal fungi were significantly more abundant in *M. macclurei* and *M. laosensis* plantations than in the eucalyptus plantation. Arbuscular mycorrhizal fungi can promote plant growth, improve soil structure, and enhance plant resistance to adverse environments (AI-Yahya'ei et al., 2011; Wilson et al., 2016), suggesting that converting eucalyptus plantations to *M. macclurei* or *M. laosensis* plantations would enhance soil ecological functions. Notably, the eucalyptus plantation had significantly higher ectomycorrhizal fungi and wood saprotrophs than native species plantations, which directly led to symbiotrophic fungi being dominant, as both groups belong to the symbiotrophic trophic mode (Ge et al., 2021; Gilmartin et al., 2022). Liu (2020) found that symbiotrophic fungi were most abundant in multi-generation continuously planted eucalyptus plantations in south subtropical China, with wood saprotrophs as the dominant functional guild. Chen et al. (2020) also reported that ectomycorrhizal fungi were most abundant in *E. urophylla* × *E. grandis* plantations in south subtropical China, consistent with our results. Ectomycorrhizal fungi can enhance plant nutrient and water uptake (Nasholm et al., 1998), while wood saprotrophic fungi conduct water and also improve plant water absorption capacity (Gilmartin et al., 2022). Therefore, planting eucalyptus plantations in south subtropical China can increase the abundance of ectomycorrhizal fungi and wood saprotrophs, enhancing plant water and nutrient absorption, but may consequently lead to soil fertility decline. Monkai et al. (2022) found that decreased soil pH reduces ectomycorrhizal fungal formation. The higher soil pH in the eucalyptus plantation compared to native species plantations may explain the enrichment of ectomycorrhizal fungi. Soil fungal functions are closely related to stand litter, and wood saprotrophic fungi generally exist within plants (Gilmartin et al., 2022). Their enrichment in the eucalyptus plantation may be due to higher wood saprotroph content in eucalyptus litter compared to native species plantations, though further research is needed to confirm this. Redundancy analysis and Monte Carlo permutation tests showed that pH was the main soil environmental factor causing differences in soil fungal functional groups between eucalyptus and native species plantations, consistent with research demonstrating that pH significantly affects fungal functional guilds during forest conversion in Southeast Asia (Monkai et al., 2022).

4 Conclusion

Soil fungal community diversity and functional groups differ between native species plantations and eucalyptus plantations in south subtropical China, primarily due to soil pH. Both plantation types were dominated by Ascomycota and Basidiomycota, but showed differences in dominant orders. The eucalyptus plantation had higher soil fungal community α-diversity and significantly different community composition compared to native species plantations. Saprotrophic

fungi were most abundant in native species plantations, with arbuscular mycorrhizal fungi being significantly more abundant in *M. macclurei* and *M. laosensis* plantations than in the eucalyptus plantation. In contrast, symbiotrophic fungi dominated the eucalyptus plantation, which had significantly higher relative abundances of ectomycorrhizal fungi and wood saprotrophs than native species plantations. Overall, replacing eucalyptus plantations with native species plantations (especially *M. macclurei* and *M. laosensis*) in south subtropical China can improve soil fertility and enhance soil ecological functions.

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Note: Figure translations are in progress. See original paper for figures.