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Soil Fungal Communities and Enzyme Activities along Local Tree Species Diversity Gradient in Subtropical Evergreen Forest

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Abstract: The majority of studies have found that an increase in tree species diversity can increase the productivity of forest stands thanks to complimentary effects with enhanced resource use efficiency or selection effects; however, it is unclear how tree species diversity affects the soil fungal community and enzyme activities in subtropical evergreen forests. In this study, we used soil high-throughput sequencing to investigate the soil fungal community structure and diversity in the central area of tree clusters in the gradient of tree species richness formed by four possible dominant tree species (*Pinus massoniana* Lamb., *Choerospondias axillaris* Roxb., *Cyclobalanopsis glauca* Thunb. and *Lithocarpus glaber* Thunb.) in subtropical evergreen broad-leaved forest. The results showed that soil organic carbon content and total nitrogen content were significantly higher in mixed tree clusters, and that soil fungal richness and diversity increased with the increase in tree species diversity (1–3 species). Soil acid phosphatase and urease activity were also enhanced with tree species diversity ($p < 0.05$). The relative abundance of soil symbiotic fungi (ectomycorrhizal fungi) decreased, while the relative abundance of saprotrophic fungi increased. Redundancy analysis (RDA) revealed that soil acid phosphatase activity was the main factor affecting soil fungal communities and functional guilds, and that soil water content was the main driving force behind fungal trophic modes. In subtropical forests, changes in tree species diversity have altered the soil fungal community structure and trophic modes and functions, accelerating the decomposition of organic matter, increasing nutrient cycling, and perhaps also changing the nutrient absorption of trees.

Keywords: subtropical forest; species diversity; enzyme activities; fungal community; fungal trophic modes

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1. Introduction

Soil fungi play an irreplaceable and important role in forest ecosystems, participating in the decomposition of plant litter and in the nutrient cycling processes [1,2]. In general, plant species diversity increases stand level productivity from manipulated biodiversity experiments in grassland and forest ecosystems, owing to niche differentiation and/or facilitation between co-occurring plant species with different functional traits [3–5]. More diverse resources and greater spatial heterogeneity can be provided for microbial communities in more diverse stands. Therefore, fungal diversity can be expected to increase and fill a number of niches [6,7]. Nevertheless, previous studies reported inconsistent correlations between plant diversity and fungal diversity, especially at different scales [8]. For example, there was a close correlation between soil microbial diversity and tree species diversity at the landscape scale, but only a poor indirect correlation between soil fungal abundance and tree species diversity at the global scale [8]. At the stand or plot level, many previous studies revealed predominant positive effects of plant diversity on fungal composition and diversity, although some other studies showed otherwise. For example, Chen et al. [9]

reported that soil microbial species richness and community composition are largely influenced by tree species characteristics rather than tree species diversity in subtropical forest. Therefore, it is not clear how tree species diversity affects fungal diversity, composition, and trophic types in subtropical forests.

Soil fungi in forests, including saprotrophic fungi, symbiotrophic fungi and pathogenic fungi, have many different functions in soil that may affect the productivity and structure of plants by changing the decomposition rate and symbiotic relationship, increasing the availability and absorption of nutrients. Accordingly, fungi can be divided into saprotrophic fungi, symbiotic fungi, and pathogenic fungi according to their functional groups. They can have positive, neutral, and negative effects in forest soil [10–12]. For example, symbiotrophic fungi are usually divided into orchid mycorrhizal fungi, ericoid mycorrhizal fungi, ectomycorrhizal fungi and arbuscular mycorrhizal fungi, which form a mutually beneficial symbiotic relationship with plants by promoting the absorption of nutrients in exchange for carbohydrates. Saprotrophic fungi are the main participant in the forest nutrient cycle, assisting in the breakdown (mineralization and decomposition) of the more recalcitrant components of organic matter, accelerating the nutrient cycle in the soil [12,13]. Pathogenic fungi are parasitic on plants and destroy the physiological structure of plants, thus affecting the growth of plants [14]. Therefore, it is of great significance to study the influence of tree species diversity on the composition of soil fungi communities and to improve our understanding of nutrient recycling and utilization by plants in evergreen broad-leaved forest.

Soil enzymes are produced by soil microorganisms and plant roots. Soil enzymes play an important role in the nutrient cycling process, including the decomposition of plant detritus and other physiological metabolic processes [15]. For example, the soil invertase enzyme hydrolyzes sucrose into glucose that is directly absorbed and utilized by plants. Urease and acid phosphatase could accelerate the hydrolysis reaction of urea and organophosphate, respectively, while catalase is closely related to soil respiration and microbial activity [16]. Adamczyk et al. [17] found that enzyme activity was positively related to the number of soil microorganisms. Soil microorganisms maintain the stability of the community structure by regulating their own physiological metabolism and secreting extracellular enzymes [18,19]. Therefore, enzyme activity can be regarded as indicative of microbe performance in the process of nutrient cycling, which is important for understanding links between soil enzymes, fungal communities and plant structures.

The subtropical evergreen broad-leaved forest is one of the main vegetation types in the forest ecosystem, which plays a crucial role in nutrient cycling and in mitigating global warming and carbon emissions [4,20–22]. Here, the overarching aim of our study was to investigate the impact of tree species diversity at a local neighborhood scale on soil fungal diversity, community structure and soil enzyme activity by using high-throughput sequencing technology in an evergreen broad-leaved forest in subtropical China. We hypothesized that: (1) soil fungal diversity and richness is positively associated with local-scale tree species diversity; (2) as more resources and spatial heterogeneity was present in more diverse stands, the soil fungal community structure, trophic modes and functional guilds change in the gradient of tree species diversity by increasing saprotrophic fungi to fill new niches; (3) soil fungal community structure and functional guilds are affected by the micro-environmental condition in the gradient of tree species diversity in this subtropical evergreen forest.

2. Materials and Methods

2.1. Study Site and Experiment Design

The research site was located in Dashanchong Provincial Forest Park (28°23′58″–28°24′58″ N, 113°17′46″–113°19′08″ E), Lukou Town, east of Changsha County, Changsha City, Hunan Province, China. A permanent plot of 1.0 hectare was established in *L. glabra*-*C. glauca* evergreen broad-leaved forest, which was protected from firewood collection and closed for natural generation since the 1950s. The evergreen broad-leaved forest is also the

climax forest type in this subtropical area. This location belongs to a hilly area in central Hunan on the western edge of the remaining veins of Mufu Mountain. It is a landform of low mountains and hills, with an altitude of 225–254 m [23,24]. The climate type in this region is subtropical southeast monsoon climate, the annual average temperature is 17.3 °C, the lowest temperature is −11 °C in January, and the highest temperature is 41 °C in July. Rainfall is mainly concentrated from April to August, with an average annual precipitation of 1412–1559 mm. The soil is mainly a well-drained red soil developed from shale and slate, classified as Alliti–Udic Ferrosols, corresponding to Acrisol in the World Reference Base for Soil Resource [25,26].

In this permanent study, 100 subplots with 10 × 10 m size were subdivided for floristic composition and spatial distribution investigation. For each tree species, the coordinate position of each tree, DBH, tree height, and crown width was recorded for all trees with a diameter at breast height (DBH) greater than 1 cm. Therein, four typical subtropical tree species, including two early successional tree species, *P. massoniana* and *C. axillaris*, and two late succession tree species, *C. glauca* and *L. glaber*, were the top four dominant tree species according to the relative importance value, which was calculated by summing up the relative density, relative frequency, and relative basal area of each species on a percentage basis [23]. In this experiment we used the method of Jacob et al. [27] and Xiang et al. [28] to identify tree clusters. Given that the interactions between tree species diversity and soil microorganisms are long-time processes, only trees with DBH > 10 cm were selected. Three target trees were selected to form a small-scale triangle as tree clusters of variable tree species richness, in ranges of one-, two-, and three-species combinations. The tree clusters with a gradient of tree species diversity were set up in the adjacent area with these four possible dominant tree species. Therefore, there were four combinations of one tree species, six combinations of two tree species irrespective of abundance, and four combinations of three tree species, with three replicates for each combination, resulting in 42 tree clusters in total. The spatial distribution of these four tree species with DBH are shown in Figure 1.

2.2. Soil Sampling

In July 2017, after litter removal, three soil cores were extracted from the central area of each tree cluster with a soil corer to a depth of 10 cm, then combined to form a single composite sample. These 42 composite soil samples were passed through a 2 mm mesh sieve to remove roots, stones and litter fragments, then divided into three sections for further analysis. The first part of the fresh soil samples was air-dried for chemical analysis, the second was stored in a 4 °C refrigerator to determine enzyme activity within 14 days, and the third part was immediately stored at −80 °C in a freezer for DNA extraction and molecular analysis. In addition, one 200 cm^3 ring knife was used to collect soil at the center point of soil depth for soil water content and bulk density measurement.

2.3. Soil Physicochemical Characterization and Enzyme Activity Measurements

Soil water content and bulk density were determined by oven-drying at 105 °C to a constant mass. Soil pH was measured using a composite electrode acidity meter (FE20, Mettler Toledo, Shanghai, China) after a soil and water suspension was shaken at a ratio of 1:2.5. The determination of soil organic carbon (SOC) used the $K_2Cr_2O_7$ oxidation method described by Walkley [29]. Soil total nitrogen was digested with concentrated acid, then the Kjeldahl semi-micro nitrogen determination method was used and soil total phosphorus was measured using the molybdenum antimony colorimetric method.

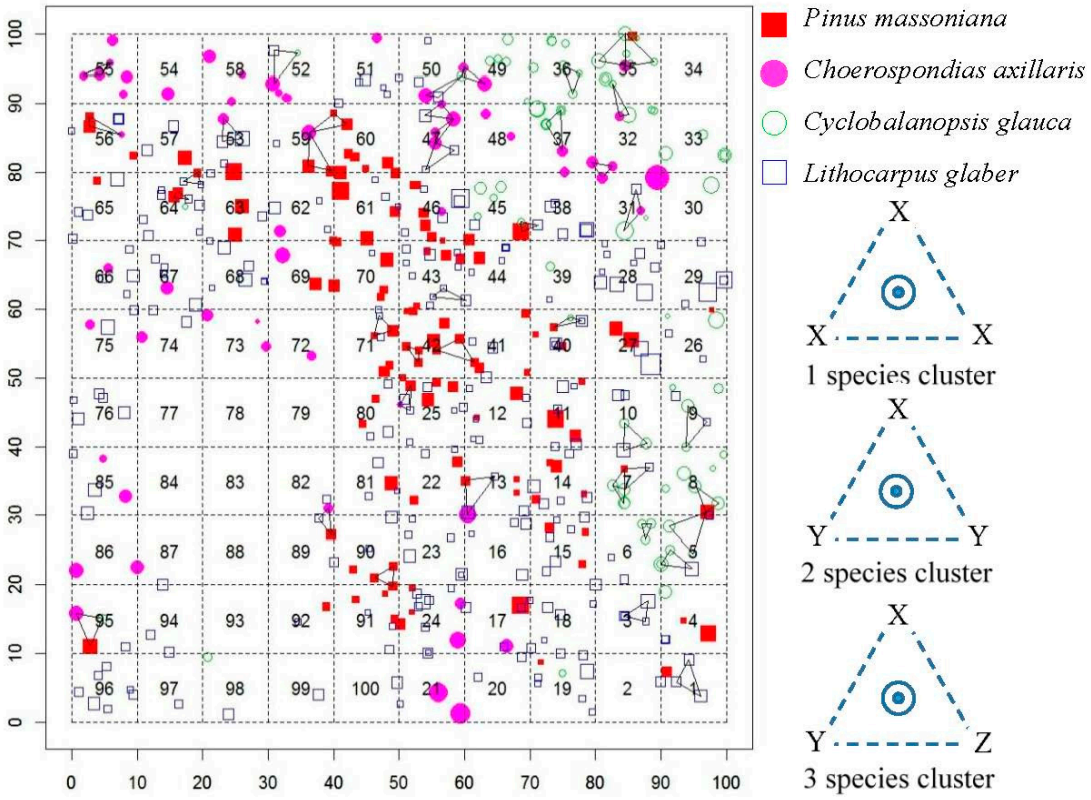

Figure 1. Experimental design and layout of tree clusters in gradient of tree species richness, ranging from one to two and three tree species combinations in *L. glaber*-*C. glauca* forest. X, Y and Z represent the different species of *P. massoniana*, *C. axillaris*, *C. glauca* or *L. glaber.* The central filled circle represents the soil sampling point. The size of the colorful symbol represents the DBH size, DBH > 10 cm.

Four soil enzyme activities (acid phosphatase, invertase, urease, catalase) were assayed using a modification of the method described by Guan et al. [30]. The acid phosphatase activity was measured by the colorimetric method of phenyl disodium phosphate. One gram of air-dried soil was cultured at 37 °C for 12 h and expressed as the number of milligrams of phenol released. The invertase activity was determined by the 3, 5-dinitrosalicylic acid colorimetric method, expressed by the milligrams of glucose released within 24 h of culturing 1 g of soil at 37 °C in air-dried soil. For urease activity, the sodium phenate-sodium hypochlorite colorimetric method was adopted. One gram of air-dried soil was incubated at a constant temperature for 24 h and expressed as the number of milligrams of ammonia nitrogen released. The catalase activity was determined by ultraviolet spectrophotometry, expressed in milligrams of hydrogen peroxide decomposed by 1 g of air-dried soil in 20 min.

2.4. DNA Extraction, Amplification and Sequencing

According to the manufacturer's instructions, an EZNA® Soil DNA Isolation Kit (Omega Bio-tek, Norcross, GA, USA) was used to extract total soil genomic DNA from 0.25 g of 0–15 cm fresh soil samples. DNA was extracted from each soil sample three times and then mixed well. A NanoDrop-2000c ultraviolet-visible spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA) was used to quantify the quality and concentration of the extracted DNA. The primers ITS1F (CTTGGTCATTTAGAGGAAGTAA) and ITS2R (GCTGCGTTCTTCATCGATGC) were used for PCR amplification of the fungal ITS r DNA gene ITS1 variable region. The PCR amplification system was 20 μL system, 4 μL 5*FastPfu buffer, 2 μL 2.5 mM dNTPs, 0.8 μL primers (5 μM), 0.4 μL FastPfu polymerase, 0.2 μL BSA; 10 ng DNA template, and finally made up to 20 μL with ddH2O. The PCR instrument used was ABI Geneamp® 9700. The amplification procedures were as follows: pre-denaturation

at 95 °C for 3 min, 35 cycles (denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, extension at 72 °C for 4 s), and finally extension at 72 °C for 10 min. The extracted DNA from each soil sample was amplified three times by PCR. Amplification products were separated by electrophoresis on 2% agarose gels and then purified using an AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA). The PCR products were eluted by Tris-HCl and detected by 2% agarose electrophoresis. Quantification was performed using the Quantifluor ™-ST (Promega, Madison, WI, USA). According to the Illumina MiSeq platform (Illumina, San Diego, CA, USA) standard operating procedures, the purified amplified fragments were constructed into a PE 2*300 library. The resultant PCR products' high-throughput sequencing was executed on an Illumina Miseq PE300 sequencing platform at the Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China) [26].

2.5. Bioinformatic Analyses

Trimmomatic software was used for quality control of the original sequences, and Flash software was used to splice the sequence read assembly. First, a threshold of 50 bp was set. If the average quality value in the window was less than 20, the back-end base was cut off from the window to remove the sequence whose length was lower than 50 bp after quality control. Second, to accurately match the barcode, primers allowed two base mismatches discarded to remove fuzzy bases. Third, sequences on both ends were spliced according to overlap base, as overlap should be greater than 10 bp. Operational taxonomic unit out-clustering was performed on the sequence according to 97% similarity using UPARSE software (version 7.1 http://drive5.com/uparse (accessed on 1 November 2020)) [31] after discarding mismatch sequence and singletons. Using UCHIME software to eliminate chimeras, Classifier (http://rdp.cme.msu.edu/) (accessed on 1 November 2020) was employed to classify and annotate each sequence. The fungal ITS R DNA sequence was compared with the UNITe7.0/iti_fungi database, and the comparison threshold set to 70%.

2.6. Statistical Analysis

All the analyses were performed at the tree cluster level. All data were tested for normal distribution with the Shapiro–Wilk test. Differences among different tree species richness levels were compared with one-way analysis of variance (ANOVA), followed by Tukey–Kramer post hoc test for soil physiochemical properties, enzyme activities and soil fungi community richness index, including the Chao1 estimator (Chao) index and the observed richness (Sobs) index [32], and diversity index (Shannon index and Simpson index) at 0.05 level. Venn diagrams for graphical descriptions of unique and shared fungal OTUs between different tree species diversity were calculated using the "VennDiagram" package in R (Version 4.0.2 software). General linear regression analysis was performed to test the relationship between the diversity of different tree species and the diversity of fungi (Shannon index and Simpson index) and the richness of fungi (Chao index and Sobs index). Pearson correlation analysis was applied to test the relationship between biotic and abiotic parameters as well as soil fungal community composition, trophic modes, and functional guilds. In order to test whether biotic and abiotic factors affect the soil fungal community, distance-based redundancy analysis (RDA) and Monte Carlo testing were performed after all influencing factor data was standardized. All the analyses were conducted with R (R development Core Team, Vienna, Austria).

3. Results

3.1. The Composition of the Soil Fungal Community in the Gradient of Tree Species Diversity

In total, 2,563,342 fungal sequences were generated from 42 soil samples by high-throughput Illumina sequencing after standardization. Overall, there were 2035 OTUs, with 97% identity according to the classified fungal sequences in the natural regenerated forest investigated here (Figure 2). Among them there were 846 OTUs in common among the tree species combinations; the unique OTUs numbered 112, 173 and 306 in one tree species, two

tree species, and three tree species clusters, respectively (Figure 2). In all soil samples, the dominant fungal phylum was *Ascomycota* (50.9%, 951 OTUs); Unclassified Fungi (33.5%, 665 OTUs), *Chytridiomycota* and *Rozellomycota* were the smaller phyla, accounting for 0.21% and 0.23% of the total fungal sequence, respectively (Figure 3). The relative abundance of *Basidiomycota* decreased with the increase of species diversity, accounting for 12.36%, 12.20% and 11.84% at one-, two- and three-species combinations, respectively (Figure 3).

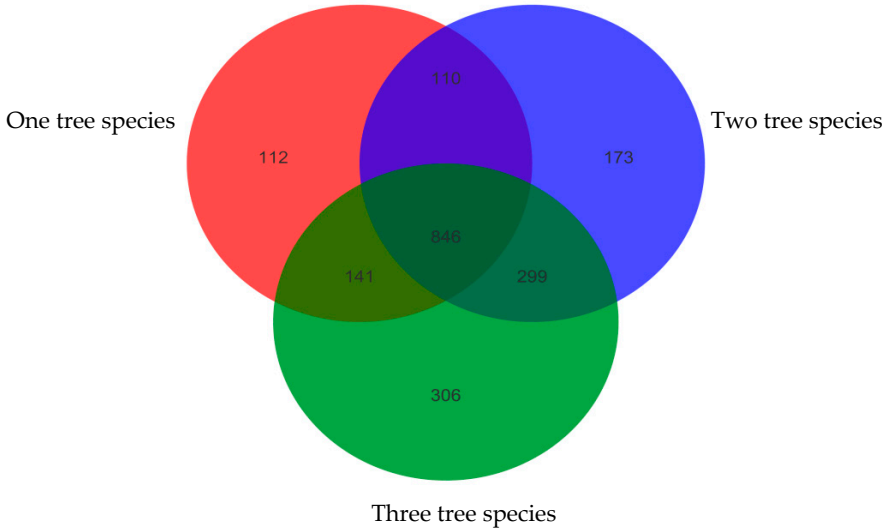

Figure 2. Venn diagram of the number of shared and unique OTUs in tree clusters of varying tree species richness (with one, two, and three tree species combinations), after normalizing the number of reads. The sequence similarity of fungal OTU was 97%. Species 1, Species 2 and Species 3 represent a tree species richness of one, two, and three, respectively (for more descriptions see Figure 1).

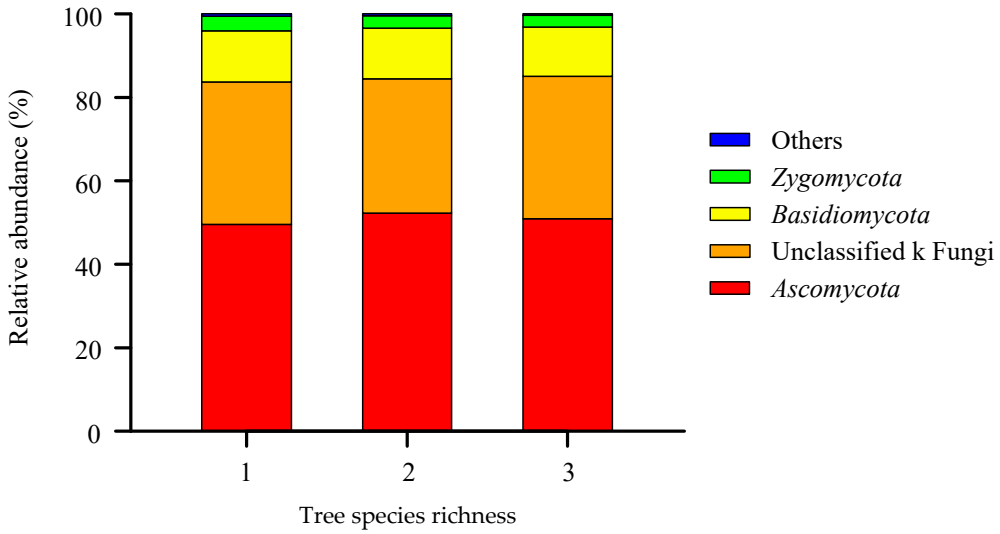

Figure 3. Composition of soil fungal communities (at phylum level) in tree clusters of varying tree species richness, with one, two, and three tree species combinations.

Fungal OTUs were used to infer trophic modes to functional groups; ten main functional groups were detected in the Pathotroph, Saprotroph and Symbiotroph trophic groups. The relative abundance of ectomycorrhizal fungi was the highest in each species combination (Figure 4). The relative abundance of ectomycorrhizal fungi decreased with the increase of tree species richness, accounting for 82.38%, 78.20%, and 63.15% in one tree species, two tree species, and three tree species clusters, respectively. In contrast, the relative abundance of saprotrophic fungi increased, accounting for 0.13%, 1.27% and 1.67%, respectively (Table 1).

Table 1. The relative abundance (%) of Symbiotroph, Saprotroph and Pathotroph in soils of tree clusters varying in tree species richness, with one, two, and three tree species combinations.

Tree Species Richness	AP	FP	PP	DS-PS	SS	US	WS	Ec	EO-MRAB	En
1	0.03 ± 0.01^{b}	0.002 ± 0.001^{b}	0.04 ± 0.01^{b}	0.97 ± 0.96^{a}	0.37 ± 0.18^{a}	15.70 ± 6.02^{a}	0.13 ± 0.05^{a}	82.38 ± 5.81^{a}	0.36 ± 0.28^{a}	0.03 ± 0.01^{a}
2	0.04 ± 0.01^{b}	0.002 ± 0.001^{b}	0.05 ± 0.01^{b}	6.58 ± 5.61^{a}	0.10 ± 0.08^{a}	11.89 ± 2.64^{a}	1.27 ± 1.08^{a}	78.20 ± 8.83^{a}	1.82 ± 1.39^{a}	0.04 ± 0.01^{a}
3	0.12 ± 0.03^{a}	0.000 ± 0.001^{a}	0.22 ± 0.03^{a}	0.63 ± 0.62^{a}	0.24 ± 0.19^{a}	32.24 ± 10.23^{a}	1.67 ± 0.78^{a}	63.15 ± 10.32^{a}	1.59 ± 1.58^{a}	0.13 ± 0.05^{a}

Different lowercase letters indicate significant differences in tree species diversity ($p < 0.05$). AP: Animal Pathogen, FP: Fungal Parasite, PP: Plant Pathogen, DS-PS: Dung Saprotroph-Plant Saprotroph, SS: Soil Saprotroph, US: Undefined Saprotroph, WS: Wood Saprotroph, Ec: Ectomycorrhizal, EO-MRAB: Ectomycorrhizal-Orchid Mycorrhizal-Root Associated Biotroph, En: Endophyte.

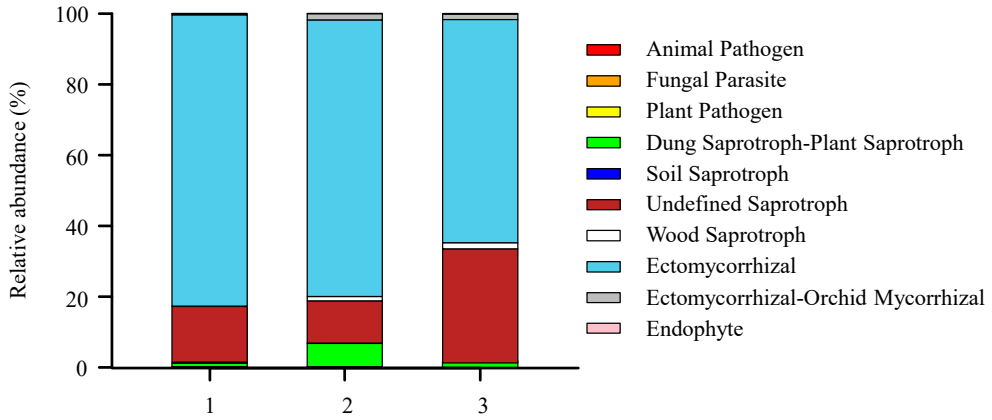

Figure 4. Compositions of fungal functional groups (guild) inferred by FUNGuild in tree clusters varying in tree species richness, with one, two, and three tree species combinations.

3.2. Correlation between Soil Fungal Diversity and Local Tree Species Diversity

A general linear regression analysis was performed on tree species diversity, fungal diversity and fungal richness, and the results showed that tree species diversity had a very significant positive correlation with fungal richness (Sobs index) and fungal diversity (Shannon index) ($p < 0.001$) (Figure 5a,b); there was also a significant positive correlation between tree species diversity and fungal richness (Chao index) ($p < 0.05$) (Figure 5c). Tree species diversity and fungal diversity (Simpson index) had a very significant positive correlation ($p < 0.01$) (Figure 5d).

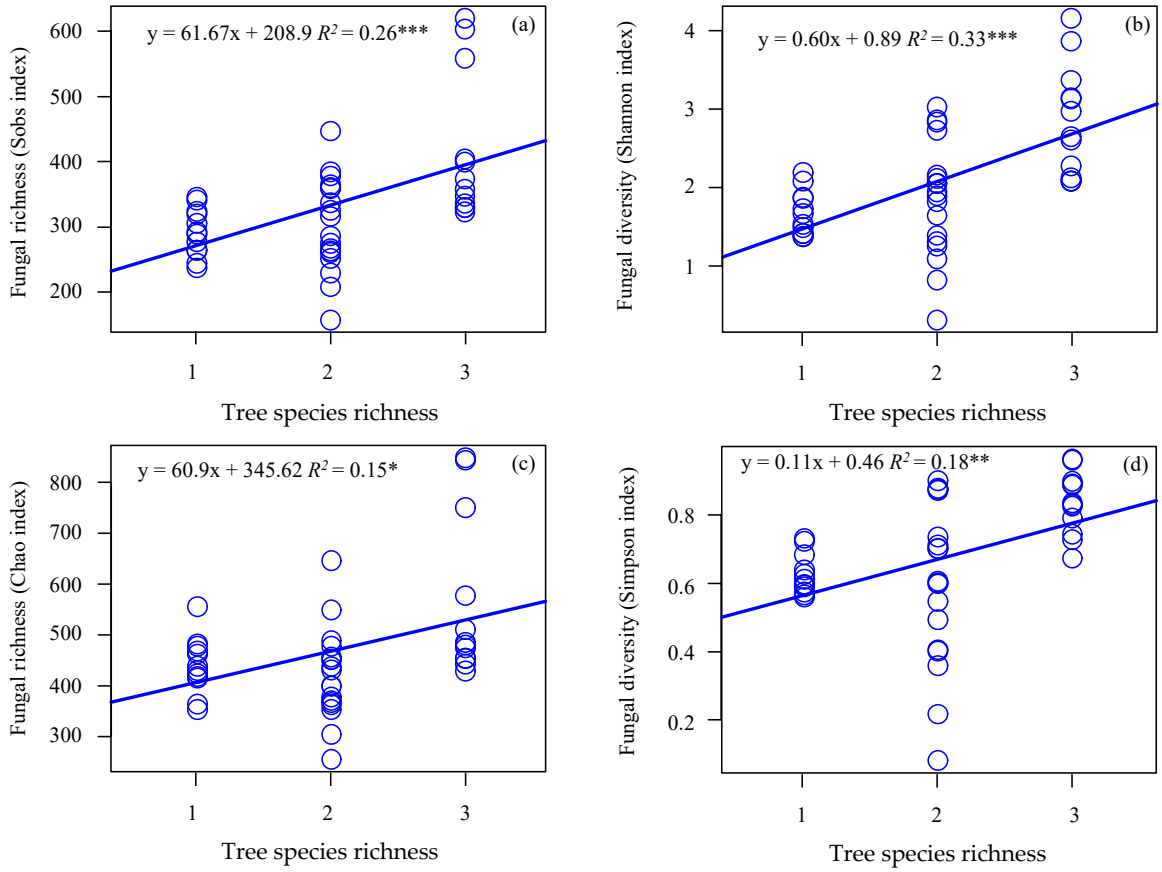

Figure 5. Correlation between soil fungal diversity and richness and tree species richness in tree clusters with varying tree species richness of one, two, and three tree species combinations in subtropical evergreen broad-leaved forest. Soil fungal richness: (**a**) Sobs index, (**c**) Chao index. Soil fungal diversity: (**b**) Shannon index, (**d**) Simpson index. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

3.3. Soil Physicochemical Properties and Soil Enzymatic Activities

Soil bulk density and water content showed no significant differences with different tree species richness. Soil organic carbon was significantly higher in two and three tree species clusters than in one species clusters. The soil total nitrogen concentration was the highest in the three tree species combination, and the difference between the three tree species and the two tree species was statistically significant ($p < 0.05$). The soil total phosphorus concentration fluctuated, and no significant differences were detected among different tree species diversity levels ($p > 0.05$) (Table 2).

Table 2. Soil physical and chemical properties in tree clusters varying in tree species richness, from one, two, and three tree species combinations.

Tree Species Richness	Bulk Density (g/cm^3)	Water Content (%)	pH	SOC (g/kg)	TN (g/kg)	TP (g/kg)
1	1.05 ± 0.04^{a}	14.20 ± 0.63^{a}	3.99 ± 0.06^{a}	18.68 ± 1.78^{a}	1.46 ± 0.34^{b}	0.27 ± 0.01^{a}
2	1.12 ± 0.03^{a}	14.32 ± 0.51^{a}	3.99 ± 0.03^{a}	28.06 ± 2.54^{b}	1.32 ± 0.23^{b}	0.30 ± 0.01^{a}
3	1.06 ± 0.04^{a}	13.57 ± 1.29^{a}	3.96 ± 0.06^{a}	28.55 ± 3.20^{b}	2.58 ± 0.29^{a}	0.24 ± 0.01^{a}

Different lowercase letters indicate significant differences tree species diversity ($p < 0.05$). SOC: Soil organic carbon, TN: Total nitrogen, TP: Total phosphorus.

Soil acid phosphatase activity and urease activity showed a significant positive correlation with tree species diversity under different tree species diversity gradients ($p < 0.05$) (Figure 6a,c, Table S1). Soil catalase activity was negatively correlated with tree species diversity ($p < 0.05$) (Figure 6d) and correlation between soil invertase activity and tree species diversity was not significant (Figure 6b).

3.4. Relationship between Soil Properties, Enzyme Activities and Fungal Communities

Redundancy analysis and the Monte Carlo test were used to determine the effects of environmental factors and soil enzyme activities on soil fungal community composition (at the phylum level), trophic modes, and functional groups. For fungal community composition, the first and second principal component axes accounted for 54.20% and 26.48%, respectively (Figure 7). Soil bulk density, water content, acid phosphatase, urease, and catalase activities explained 3.12%, 3.16%, 6.37%, 3.46%, 3.70%, respectively. For the fungal trophic modes, the first principal component axis and the second principal component axis explain 48.71% and 35.71%, respectively (Figure 8a), and the soil water content, pH, organic carbon, acid phosphatase activities explained separately 28.45%, 10.38%, 11.41%, 15.35%. For functional groups, the first and second principal component axes accounted for 60.23% and 29.26% (Figure 8b), respectively. Soil water content, acid phosphatase, urease, catalase activities explained 17.45%, 20.66%, 8.54%, 7.83%, respectively.

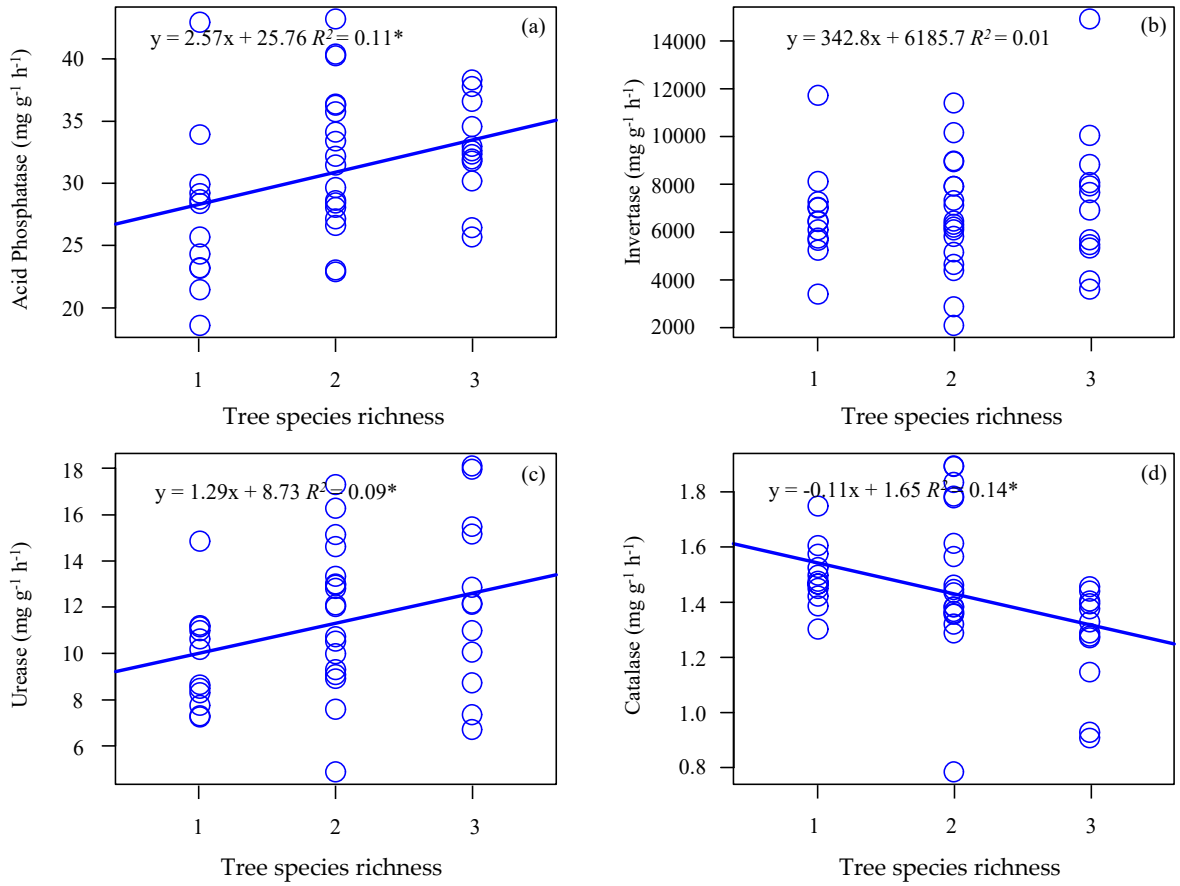

Figure 6. Correlation between soil enzyme activity and tree species richness in tree clusters varying in tree species richness, from one, two, and three tree species combinations in subtropical evergreen broad-leaved forests. (**a**) acid phosphatase, (**b**) invertase, (**c**) urease, (**d**) catalase. Statistical significance is denoted by * $p < 0.05$.

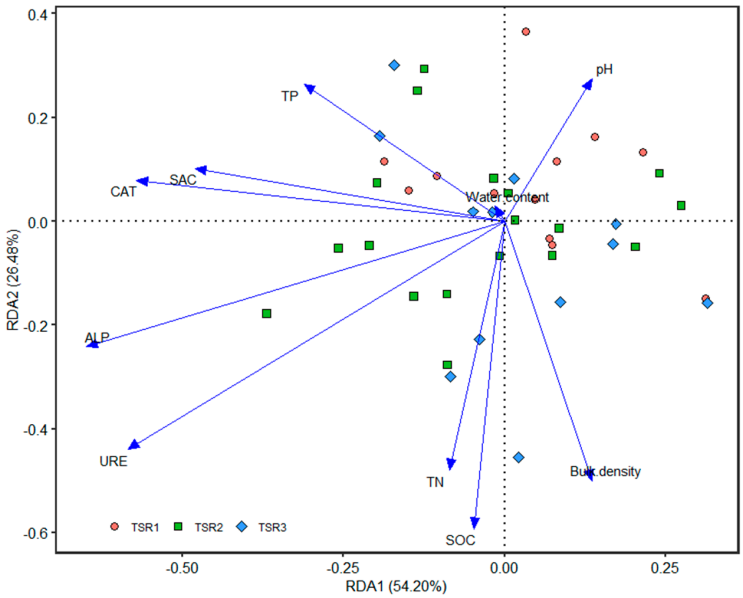

Figure 7. Redundancy analysis (RDA) illustrates the relationship between the phylum composition of soil fungal communities and environmental variables in tree clusters of varying tree species richness with one, two, and three tree species combinations in subtropical evergreen broad-leaved forest. SOC: Soil organic carbon, URE: urease, ALP: Acid phosphatase, SAC: Invertase, CAT: Catalase. TSR1: tree species richness 1, TSR2: tree species richness 2, TSR3: tree species richness 3.

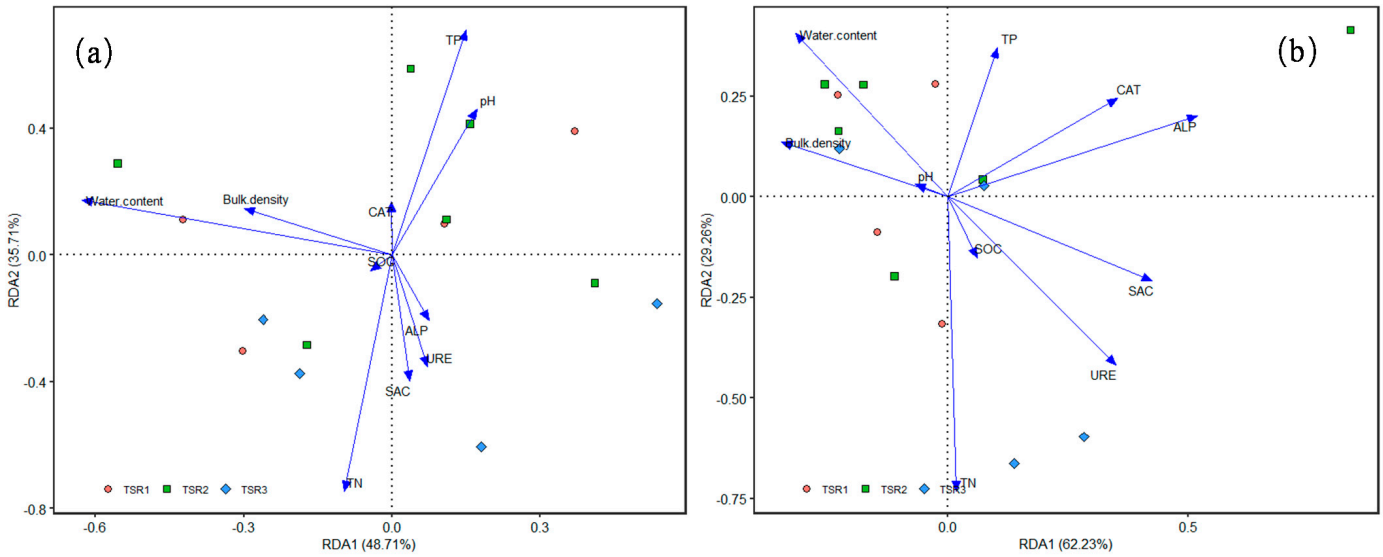

Figure 8. (**a**) Redundancy analysis (RDA) illustrates the relationship between soil fungal trophic modes and environmental variables under tree species combinations. (**b**) Redundancy analysis (RDA) illustrates the relationship between soil fungal functional groups (guild) and environmental variables in tree clusters varying in tree species richness, from one, two, and three tree species combinations in subtropical evergreen broad-leaved forest. SOC: Soil organic carbon, URE: urease, ALP: Acid phosphatase, SAC; Invertase, CAT: Catalase. TSR1: tree species richness 1, TSR2: tree species richness 2, TSR3: tree species richness 3.

Acidic phosphatase was positively correlated with soil water content, *Ascomycota*, Dung Saprotroph-Plant Saprotrophs, ectomycorrhizal-orchid mycorrhizal-root Associated Biotrophs, and relative abundance of Wood Saprotrophs, and was negatively correlated with Unclassified fungi (Tables S2–S4). There was a positive correlation between the relative abundance of *Basidiomycota* and invertase (Table S3). Urease was positively correlated with organic carbon, total nitrogen and fungal diversity (Shannon index), and negatively correlated with *Zygomycota* and Unclassified fungi. Catalase was positively correlated with a relative abundance of *Basidiomycota*, and negatively correlated with Pathotrophs, Pathotroph-Saprotrophs, and Fungal Parasites (Table S5). Soil water content was negatively correlated with a relative abundance of Pathotroph-Symbiotrophs and Endophytes, while soil water content was positively correlated with an abundance of Symbiotrophs. Soil organic carbon was positively correlated with a relative abundance of *Zygomycota*. Soil total phosphorus was negatively correlated with fungal richness (Chao index and Sobs index), fungal diversity (Shannon index and Simpson index), and Unclassified fungi (Table S3).

4. Discussion

4.1. Soil Fungal Community Structure in the Gradient of Tree Species Diversity

Trees may affect the characteristics of the ecosystem through a variety of ecological processes, including litter generation, microclimate changes, root secretion of chemicals, and root symbiotic fungi that affect the soil fungal community structure [33]. In our results, soil fungal community composition patterns changed with tree species diversity, and different tree species combinations had different enzyme activities and soil nutrient contents, which also led to different microbial composition [34]. The soil carbon concentration was significantly higher in mixed tree clusters than in pure species tree clusters. Different combinations of tree species may have different types and weights of litters, and the leaf litter of different tree species may be decomposed by specific fungal communities, which may lead to differing effects of litter from different tree species on fungal communities. Therefore, different tree species combinations have different effects on soil fungi. Soil fungal communities were relatively stable at the phylum level under different tree species diversity gradients. The dominant phyla under the gradients of tree species diversity were

Ascomycota, Unclassified fungi, and *Basidiomycota*. Among them, the *Ascomycota* were not significantly different in the diversity gradient of different tree species, and the relative abundance was high. Thus, *Ascomycota* was the dominant fungal community in subtropical forests; these results are similar to those of previous researchers [11,21,35].

4.2. Soil Fungal Richness and Diversity in the Gradient of Tree Species Diversity

In our study, the general linear regression analysis revealed that fungal diversity and fungal richness, including soil fungal richness (Sobs index and Chao index) and fungal diversity (Simpson index and Shannon index) were significantly correlated with tree species diversity, which supports our first hypothesis. The reasons for this difference can be found in both the biotic and abiotic environments [21]. As the diversity of tree species increases, environmental conditions and resource heterogeneity increase [6,36,37]; however, as the diversity of tree species increases, leaf and root litter may transport different types of organic matter to the soil and provide more niche space for saprotrophic fungi. Different tree species combinations increase the species of root Symbiotrophic fungi, thus increasing the soil fungal richness [33,38]. The high soil organic carbon and total nitrogen concentration in the mixed-species tree clusters is favourable for the reproduction of soil fungi, leading to higher fungal diversity, in agreement with Yang et al. [39] who concluded that fungal diversity was positively correlated with soil urease activity. Soil urease catalyzes the decomposition of urea into inorganic nitrogen which is well absorbed and utilized by microorganisms, which may cause the inorganic nitrogen content of the soil to accumulate rapidly. This could partly explain why fungal diversity increased with tree species diversity. A similar trend of increased fungal diversity was consistent with previous reports from mixed tropical forest sites with high tree diversity in the Amazon region and in the temperate forests in the Czech Republic [38,40].

4.3. Soil Fungi Trophic Modes and Functional Guilds in the Gradient of Tree Species Diversity

The trophic modes and function of soil fungi changed from being dominated by symbiotic fungi to saprotrophic fungi with the increase of tree species diversity. Fungi have various trophic modes and functional guilds, and different trophic modes and functional guilds have different functions [41]. According to the results of our research, saprotrophic fungi, saprotrophic-symbiotrophic fungi, symbiotrophic fungi and unidentified fungi account for a higher proportion of the trophic modes. Among them, saprotrophic fungi was one of the heterotrophic strategies of many fungal groups. Responsible for decomposing complex compounds such as cellulose, hemicellulose and lignin derived from litter inputs and decomposition in the ecosystem, these play a vital role in the decomposition of organic matter, nutrient cycling and carbon cycling [14]. As the diversity of tree species increases, the relative abundance of saprotrophic fungi increases, which may be due to the increase in the heterogeneity of resources and the environment. With a higher diversity gradient of tree species come higher spatial heterogeneity, better utilization of spatial light resources, faster growth of trees, and more litter. The complementarity of niches and the increase in the types of plant residues and rhizosphere deposits that enter the soil organic matrix mean that more saprotrophic fungi fill the niche [42]. The relative abundance of symbiotic fungi decreased with the increase of tree species diversity, but the difference was not significant. Different tree species diversity gradients and differences among regions and regional species pools will change the soil fungi trophic modes and functional guilds [7,21,40]. Pathogenic fungi can attack host cells and obtain nutrients from them, but they can also control the population structures of other plants, animals, fungi and harmful insects [43]. Meanwhile, Gonthier et al. [44] demonstrated that increased ectomycorrhizal fungi richness may inhibit the invasion and reproduction of pathogenic fungi richness. The fact that ectomycorrhizal fungi decreased with the increase of species diversity could result in the increased abundance of pathogenic fungi.

4.4. Different Reactions of Soil Fungal Community Structure with Soil Properties and Enzyme Activities

Soil physicochemical properties in association with diversity of tree species can have an effect on the community structure of soil fungi, and soil fungi secrete enzymes to promote carbon and nutrient cycling, which plays an important role in the forest ecosystem [1,45]. Soil enzyme activity was closely related to the decomposition of organic matter, and soil fungi promote the decomposition and transformation of organic compounds by secreting enzymes [46]. According to the RDA and Pearson correlation analysis, soil acid phosphatase activity was the main driving force of fungal community structure and functional guilds, whereas soil water content was the main driving force of fungal trophic modes. Soil acid phosphatase activity was strongly correlated with soil moisture content, pH and organic carbon. The synthesis of soil acid phosphatase accelerates the decomposition of soil organic matter and changes soil physicochemical properties, which in turn changes the proliferation of soil fungi, promoting the ability of soil fungi to secrete soil acid phosphatase [47]. It may be that in the process of soil formation and weathering in subtropical regions microorganisms were more likely to be restricted by phosphorus, while microorganisms were more likely to be restricted by nitrogen in temperate zones [21]. Previous studies have reported that soil total phosphorus had an important effect on fungal diversity and richness in subtropical regions [48]. *Ascomycota* was generally considered to be a group with decomposition functions in the soil and which participates in the decomposition process of many nutrients in the forest ecosystem [49]. *Basidiomycota* was significantly positively correlated with catalase and invertase. White-rot fungi in *Basidiomycota* have genes encoding extracellular enzymes and can secrete catalase and some enzymes related to lignin decomposition [49].

5. Conclusions

Our analysis of soil microbiota and functional enzymes within a three-species tree diversity gradient showed that soil fungi richness and diversity and enzyme activities (acid phosphatase and urease) were positively correlated with tree species diversity, which supports our first hypothesis. Furthermore, soil organic carbon content and total nitrogen content were higher in mixed tree clusters than in pure species clusters. According to the RDA results, soil acid phosphatase activity was the main driving force of fungal community structure and functional guilds, while soil water content was the main driving force of fungal trophic modes. In conclusion, these findings could shed light on plant-soil–microbial interactions in subtropical forests, and provide future researchers with a better understanding of the relationship between soil fungal community structure, trophic modes and functional guilds, soil properties, and enzyme activities in the gradient of tree species diversity.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/f12101321/s1. Table S1: Soil enzyme activities among different tree species diversities. Table S2: Physical and chemical properties and enzyme activities of soil in different tree species diversity. Table S3: Results of pearson correlation between soil fungal communities and soil characteristics as well as enzyme activities in tree clusters varying in tree species richness from 1, 2, and 3 tree species combinations in subtropical evergreen broad-leaved forests. Table S4: Correlations coefficients between fungal trophic modes and soil characteristics as well as enzyme activities in tree clusters varying in tree species richness from 1, 2, and 3 tree species combinations in subtropical evergreen broad-leaved forests. Table S5: Correlation coefficients among fungal trophic modes and soil characteristics as well as enzyme activities by diversity of different tree species.

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