




## Article

# Impacts of Litter Composition on the Structure and Functional Pathways of Soil Microbial Community during *Phyllostachys Edulis* Expansion

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**Abstract:** Forest ecosystem succession plays an important role in soil microbiota variation, and soil microbes will re-establish ecosystem function after disturbance events. A distinctive disturbance of the expansion of bamboo forest into the surrounding broadleaved ecosystem is the change in litter type input. We conducted a 6-month microcosm experiment to examine the effects of proportional changes in leaf litter composition due to moso bamboo (*Phyllostachys edulis*) invasion into a broadleaved forest on soil microbial community. A series of mixed litters were prepared with bamboo litter occupying at 0%, 33%, 50%, 67% and 100% in proportion (with a decrease in litter carbon (C)/nitrogen (N) from 36.23 to 31.35), and they were then amended into a broadleaved forest soil at a rate of 1%, respectively. Soil bacterial and fungal communities at different incubation stages were determined by high-throughput sequencing. With the increasing proportion of bamboo litter, the broadleaved forest soil exhibited strong changes in microbiome assembly, including reducing bacteria alpha-diversity, the relative abundance of *Acidobacteria* and *Basidiomycota*, while increasing the relative abundance of *Actinobacteria*, *Proteobacteria* and *Ascomycota*. Moreover, the increased proportion of bamboo litter (0% PP→100% PP) increased the relative abundance of membrane transport and carbohydrate metabolism of soil bacteria but decreased the relative abundance of saprotrophic soil fungi. Redundancy analysis showed that bacteria rather than fungal communities changed greatly during incubation ( $p < 0.05$ ). Additionally, the activities of soil pH,  $\text{NO}_3^-$ -N,  $\text{NH}_4^+$ -N, dissolved nitrogen and C- and N- acquiring enzymes were the main factors affecting bacterial and fungal community structure ( $p < 0.05$ ). The soil physicochemical properties were significantly correlated with the composition of microbial phyla among different litter amendment treatments. These results indicated that different proportions of bamboo litter dramatically alter the soil bacterial rather than the fungal community, most likely by changing edaphic patterns. This study has important implications for understanding the litter–soil–microbe synergy during the bamboo forest expansion and provides a basis for assessing the ecological risk of bamboo forest expansion.

**Keywords:** soil incubation; bamboo expansion; litter composition; microbial community; functional pathway



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## 1. Introduction

Litter is an important part of the forest ecosystem and is also a source of organic matter and energy for soil organisms. It has been well recognized that human disturbance and environmental changes can strongly affect forest productivity, thereby changing the input of litter on the ground [1]. Soil microorganisms play an important role in mediating the process of underground biogeochemistry [2]. In the litter–soil ecosystem, soil microorganisms can make an accurate and rapid response to small changes in the forest ecosystem [3–5]. Different litter inputs will change the soil C substrate matrix and then affect the soil

microbial community. For example, changes in litter input can alter the contents of carbon (C), nitrogen (N), phosphorus (P) and other nutrients, as well as the microbial community and enzyme activity [6], which will lead to changes in forest nutrient cycling. Recent studies found that enzyme activity ratios involved in C/N/P cycling are highly variable during litter decay [7]. The changes in soil enzyme activities can reflect the changes in microbial community structure. As already stated, the activities of dehydrogenase, phosphatase and urease have a close relationship with the diversity of microbial community structure [6]. Increasing evidence has indicated that microbial community structures can adapt to nutrient conditions/constraints such as N to control litter decomposition [8], which implies feedback between decomposition and microbial composition when aboveground vegetation changes. Some studies have found that mixed litter may provide more diverse ecological niches for decomposing microorganisms [9–11], thus affecting microbial community structure and function. Therefore, compared with litter decomposition of a single plant species, mixed-litter decomposition can improve soil microbial community richness and diversity [12,13]. So far, there have been few studies on the variation dynamics of soil microbial community structure caused by mixed-litter decomposition, and the interactions between them are still unclear.

Furthermore, bacteria and fungi in soil vary widely in terms of growth strategy competitiveness and how they use resources. In general, bacterial decomposition pathways mainly regulate easily decomposed substrates with high turnover, while fungal communities mainly regulate more complex organic materials with slower turnover [14]. The richness and relative abundance of fungi and bacteria are important characteristics of soil microbial communities, and some studies have shown that the change in litter quality will change soil microbial composition [3,15–18]. Therefore, plant expansion or invasion will undoubtedly change the structure and activities of soil microbial communities through litter (such as stoichiometry [19]). However, the contribution of bacteria and fungi to specific processes in the ecosystem is different. For example, fungi are thought to decompose mainly complex and stubborn plant litters, particularly lignin and cellulose. In contrast, the contribution of bacteria to the decomposition of lignin and humus is not significant. This is because fungi have lower nutritional requirements than bacteria, show higher carbon utilization efficiency on low-quality substrates and have the ability to degrade complex substrates [20]. Bacteria generally have rapid growth and regeneration rates, contain higher amounts of nitrogen, phosphorus and organic compounds and are favorable for survival at lower substrate C/N ratios. In addition, litter diversity will also affect the composition and structure of the microbial community. Litter in a mixture than in a monoculture could decrease *Acidobacteria* and *Solibacteres* but increase *Dothideomycetes* [3]. However, the response of soil microorganisms to litter input remains controversial [15,18]. How forest types with different soil conditions (e.g., whether the forest is disturbed by plant expansion) interact with microbial composition and dynamics during litter decomposition remains elusive.

The rapid expansion of bamboo has been widely reported in many countries due to its fast growth and large-scale clonal reproduction, leading to actual and potential changes in the structure and function of the ecosystem [8]. For example, the expansion of bamboo forest into the surrounding forest ecosystem may lead to increased decomposition of litter and nutrient turnover. The higher the proportion of bamboo in mixed litters, the faster the decomposition rate and nutrient regression of litters [21]. Compared with broadleaf litter, bamboo litter generally has a lower carbon to nitrogen ratio and lignin concentration, as well as lower folic acid alkalinity in litters. In this study, the effects of bamboo litter on the composition and function of soil microbial community in broadleaf forest soil during the expansion process were studied by adding different proportions of bamboo and broadleaf litters in laboratory incubation experiments. Broadleaf litter was used as a positive control, and no litter was used as a negative control. Since soil microorganisms play an important role in ecosystem functions, the study of the microbial community in the bamboo-broadleaved forest ecosystem will provide a theoretical basis for understanding

the nutrient cycling function of bamboo forest during its expansion into the broadleaved forest ecosystem.

## 2. Materials and Methods

### 2.1. Soil and Litter Sampling

The experimental soils were collected from a broadleaved forest, located at the Lingfeng Mountain Reserve, An'ji County, Zhejiang province, China (30°36' N, 119°63' E). The site has a monsoon subtropical climate with a mean air temperature of 16.1 °C and an average annual precipitation of 1431 mm [22]. The soil is classified as a Ferrasols in the Food and Agriculture Organization of the United Nations (FAO) soil classification system. The region is rich in vegetation resources with woody plants from 64 families and 231 species. Common conifer species are *Pinus massoniana* and some artificial wetland pine forest. The deciduous broadleaved trees are *Quercus acutissima* Carruth and *Quercus chenii* Nakai. Evergreen broadleaved trees include *Cyclobalanopsis glauca*, *Castanopsis sclerophylla* and *Schima superba* Gardn. All of the trees are over 50 years old, with a high stand density of undergrowth shrubs and weeds.

In October 2018, five replicate soil blocks (25 m<sup>2</sup> × 20 cm depth) were collected randomly from the broadleaved forest and pooled to form a composite soil sample. All the dead or live roots and plant debris were removed from the soil. The soil sample was then passed through a 2 mm sieve, and the resultant sample was mixed thoroughly to form a homogenized bulk sample. The soil had a pH (H<sub>2</sub>O) of 4.20, soil organic carbon (SOC) concentration of 33.45 g kg<sup>-1</sup> and total N (TN) concentration of 1.35 g kg<sup>-1</sup>. Plant litter was collected from nylon mesh bags randomly distributed in the pure broadleaved and bamboo forest and their mixed forest. Including bamboo leaves, a mixture of broadleaved leaves, *Cyclobalanopsis glauca*, *Castanopsis sclerophylla* and *Quercus acutissima* Carruth, were collected. Litters were washed carefully with ultrapure water to remove impurities and then dried at 60 °C for 12 h. One part of the dried litters was ground (<0.5 mm) to measure its characteristics, such as C and N contents. The litters of bamboo, *Cyclobalanopsis glauca*, *Castanopsis sclerophylla* and *Quercus acutissima* Carruth had a C content of 359.1, 427.8, 436.3 and 463.6 mg C g<sup>-1</sup> dry mass and a N content of 11.5, 14.4, 9.9 and 13.5 mg N g<sup>-1</sup> dry mass. The litter properties among different treatments are shown in Table 1.

**Table 1.** Litter C, N and C/N ratio of different leaf litter treatments.

Treatment	Litter Composition	Quantity of Litter/g 100 g <sup>-1</sup> soil	Total C/mg g <sup>-1</sup>	Total N/mg g <sup>-1</sup>	C/N	Lignin/%	Cellulose/%
0% PP	Broadleaved	1	442.58 ± 10.78 d	12.57 ± 1.38 a	36.23 ± 4.27 a	49.15 ± 1.15 b	11.83 ± 0.53 a
33% PP	Broadleaved + Bamboo	0.667 + 0.333	414.76 ± 12.13 c	12.21 ± 0.96 a	34.61 ± 2.80 a	48.48 ± 1.15 ab	11.58 ± 0.49 a
50% PP	Broadleaved + Bamboo	0.5 + 0.5	400.84 ± 5.12 bc	12.04 ± 0.78 a	33.79 ± 2.16 a	48.15 ± 1.15 ab	11.46 ± 0.48 a
67% PP	Broadleaved + Bamboo	0.333 + 0.667	386.93 ± 3.23 b	11.86 ± 0.64 a	32.98 ± 1.64 a	47.82 ± 1.15 ab	11.33 ± 0.46 a
100% PP	Bamboo	1	359.10 ± 0.55 a	11.50 ± 0.59 a	31.35 ± 1.60 a	45.15 ± 0.29 a	10.45 ± 0.20 a

0% PP, bamboo litter occupying at 0%; 33% PP, bamboo litter occupying at 33%; 50% PP, bamboo litter occupying at 50%; 67% PP, bamboo litter occupying at 67%; 100% PP, bamboo litter occupying at 100%; the same below. Data are means ± S.D., *n* = 3. Different letters in a single row indicate significantly different means according to Duncan's test at *p* < 0.05 level.

## 2.2. Experimental Design

To eliminate spatial variation in microclimate, soil heterogeneity and the rhizospheric effect, we used a microcosm approach to investigate the short-term effects of change in litter quality induced by bamboo invasion into the broadleaved forest on soil microbiome. We prepared a series of mixed-species litters with bamboo litter occupying at the 0%, 33%, 50%, 67% and 100% proportion (with a decrease in litter C/N from 36.23 to 31.35) to generally simulate the proportional changes in leaf litter composition under bamboo invasion. Six treatments, including a non-amended negative control (CK), 0% PP (broadleaved leaf litter only), 33% PP (broadleaved leaf litter/bamboo leaf litter = 2:1), 50% PP (broadleaved leaf litter/bamboo leaf litter = 1:1), 67% PP (broadleaved leaf litter/bamboo leaf litter = 1:2) and 100% PP (bamboo leaf litter only) were set in this experiment. Respective oven-dried litter (1 g dry mass) was amended into 100 g of dry soil (at a rate of 1% according to the annual litter input of the bamboo forest) [23], and then treated soils were incubated in sterilized sealed glass jars (1 L) at 25 °C in the dark for 6 months. Soil moisture was adjusted to 60% of water-holding capacity with deionized water and was maintained throughout the incubation period by weighing the jars twice a week and adding deionized water when necessary. In total, there were 15 replicates per treatment constructed in this study, which permitted the destructive sampling of three replicates for each treatment at Days 5, 15, 30, 90 and 180 for microbiome analyses. One subsample from each sample (15 g) was frozen and stored at −80 °C for later DNA extraction and amplicon sequencing. Another subsample around 50 g of soil was stored at 4 °C for no longer than 1 week prior to soil enzyme activity, soil microbial biomass, soil nitrate ( $\text{NO}_3^-$ -N) and soil ammonium ( $\text{NH}_4^+$ -N) analyses. The bulk of the remaining soil was air-dried at room temperature for characterization of physicochemical properties.

## 2.3. Analyses of Soil Properties and Soil Enzyme Activities

Soil pH was measured in a 1:2.5 soil-to-water ratio using a pH meter (Mettler-Toledo, Switzerland). SOC and TN were determined by wet digestion using  $\text{K}_2\text{CrO}_7$  oxidation and the Kjeldahl method, respectively, according to Lu [24]. Dissolved organic carbon (DOC) and dissolved N (DN) were extracted by the method of Justine [25], in which the DOC was extracted by the TOC-L CPH CN200 (Tokyo, Japan), and DN in the extracts was determined by the semi-micro-Kjeldahl method. Soil nitrate ( $\text{NO}_3^-$ -N) and ammonium ( $\text{NH}_4^+$ -N) contents were extracted by 50 mL of 0.5 M KCl and then determined by the Skalar SAN++ (WestCo Scientific Instruments, Brookfield, CT, USA) [26]. Net N mineralization for each treatment from each stage was quantified by subtracting the N content of extractable minerals found in each treatment from the N content of extractable minerals found in the unmodified control soil [27].

Activities of four C-acquiring enzymes, including  $\alpha$ -1,4-glucosidase (AG),  $\beta$ -1,4-glucosidase (BG),  $\beta$ -D-cellobiosidase (CB), and  $\beta$ -1,4-xylosidase (XYL); two N-acquiring enzymes, including N-acetyl- $\beta$ -D-glucosaminidase (NAG) and leucine aminopeptidase (LAP); and one P-acquiring enzyme (acid phosphomonoesterase (AP)) were assayed according to the methods described previously by Chen [28].

## 2.4. DNA Extraction and Amplicon Sequencing

DNA was extracted from 0.25 g of frozen soil using the PowerLyzerPowerSoil Kit (QIAGEN) according to the manufacturer's protocol. The extracted DNA concentration and purity were determined with the NanoDrop2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). DNA integrity was examined on 1% agarose gels and then stored at −40 °C until use.

Bacterial and fungal communities were assessed through variation in their 16S and ITS rRNA gene sequence, in which the specific primer pairs were 338F/806R and ITS3F/ITS4R, respectively. Paired-end sequencing was performed on an Illumina HiSeq 400 platform (Illumina, San Diego, CA, USA) at Shanghai Majorbio Bio-Pharm Technology Co., Ltd. These sequences were assigned to the sample using a specific barcode and stored in a

FASTQ-formatted file. Raw data FASTQ files were analyzed using the software pipeline Vsearch version 2.8.1 [29].

Raw overlapping 16S paired-end reads were merged, and merged sequences with an overlap length smaller than 200 bp and with more than 50 mismatches were discarded. After primer and barcode trimming, merged reads with an expected error rate higher than 1% were removed. Sequences with the smallest single base number less than 10 were discarded. Filtered sequences were clustered into operational taxonomic units (OTUs) at 97% identity and RDP-based chimera detected using Usearch version 11.0 (<http://www.drive5.com/usearch/> accessed on 1 December 2021). OTUs were taxonomically classified using the RDP database. With 0.6 as the confidence threshold, the OTU representative sequence was compared with the RDP database to obtain the taxonomic information of the representative OTU sequence. The representative sequences of each OTU were annotated to obtain the corresponding species information and relative abundance distribution. PICRUSt was used to standardize the OTU abundance table and obtain the corresponding KEGG Orthology (KO) information. KEGG Pathway information was obtained based on KEGG database information, and the abundance of each functional category was calculated based on OTU abundance.

Raw overlapping ITS paired-end reads were merged, and merged sequences with an overlap length smaller than 100 bp and with more than 30 mismatches were discarded. After primer and barcode trimming, merged reads with an expected error rate higher than 1% were removed. Sequences with the smallest single base number less than 10 were discarded. Filtered sequences were clustered into operational taxonomic units (OTUs) at 97% identity, and with 0.6 as the confidence threshold, the OTU representative sequences were taxonomically classified with the UNITE database [30]. Sequences that were not in the UNITE database were indicated as “Unassigned”, while sequences that were present in the UNITE database but without taxonomic information available were classified as “unclassified\_k\_Fungi” for the taxonomic level. The FUNGuild microecological tool was used to predict and analyze the function of soil fungi according to the nutrient pattern.

### 2.5. Statistical Analyses

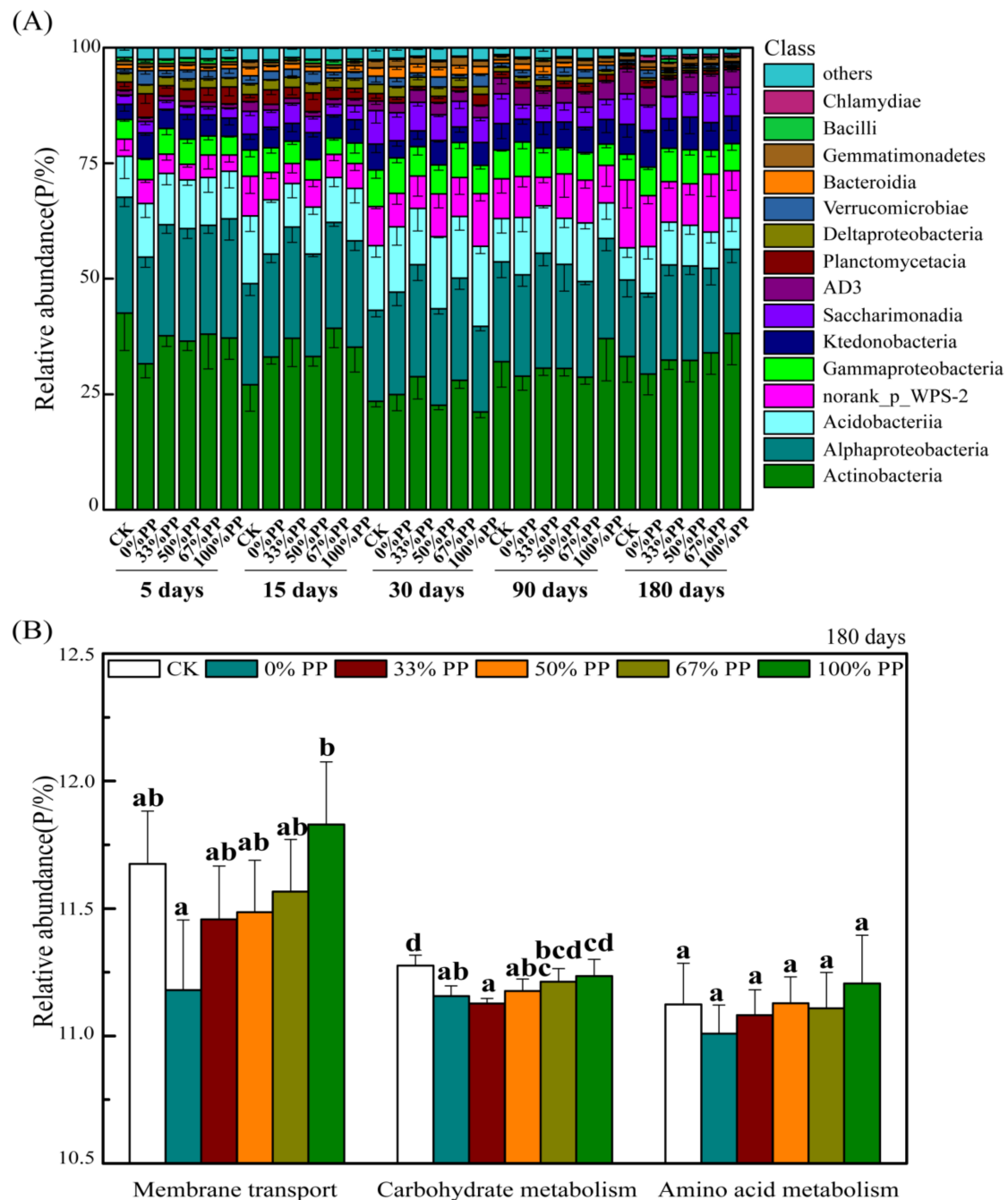
Simple files were imported into R v.3.5.0 [31] and Rstudio for downstream statistical analysis. To compensate for the different sequencing depths, the samples were sparser at 9000 depths in 16S and 32,000 depths in ITS sequences. Alpha-diversity was calculated using the Chao1, Shannon and OTU number index. Beta-diversity was calculated using the Bray–Curtis (BC) distance. Based on the BC dissimilarity index, redundancy analysis (RDA) and analysis of similarity (ANOSIM) with 999 permutations were conducted in the “vegan” package. The significance of environmental variables was fitted with “envfit”, via which the correlation test of the combination of microbial matrix and environment variables was carried out. Repeated measurement analysis of variance (RMANOVA) was performed to identify the temporal effects of leaf litter addition on microbial alpha-diversity. A value of  $p < 0.05$  was considered to be statistically significant.

## 3. Results

### 3.1. Bacterial Community Composition and Functional Pathways

As shown in Supplementary Materials Figure S1A, *Actinobacteria* (32.1%) and *Proteobacteria* (29.1%) were dominant in all six treatments. Compared with the 0% PP treatment, the relative abundance of *Actinobacteria* and *Proteobacteria* increased, while the relative abundance of *Acidobacteria* decreased (except on the 30th day of incubation) with the increasing proportion of bamboo litter. At the class level (Figure 1A), *Actinobacteria* (21.27–42.51%), *Alphaproteobacteria* (16.56–25.81%) and *Acidobacteria* (6.81–17.35%) were the dominant bacterial classes in all six treatments. With the increase in incubation time, the relative abundance of *Saccharimonadia*, *Bacteroidia* and *Deltaproteobacteria* in each treatment increased at the early stage and then decreased after 30 days of incubation. The relative abundance of *Actinobacteria* showed opposite variation in the 100% PP and 0% PP treatments that de-

creased in 0% PP (from 35.92% to 32.01%) but increased in 100% PP (from 37.13% to 38.15%) during incubation.



**Figure 1.** The community composition (A) and functional pathways (B) of bacteria at the level of class in the broadleaved forest soil during incubation. CK, with no litter; 0% PP, bamboo litter occupying at 0%; 33% PP, bamboo litter occupying at 33%; 50% PP, bamboo litter occupying at 50%; 67% PP, bamboo litter occupying at 67%; 100% PP, bamboo litter occupying at 100%. Bars with different letters among different treatments are significantly different ( $p < 0.05$ ).

Based on the KEGG function classification, we obtained seven bacteria metabolic functional pathways, which have a total of 41 subfunctions. At the end of incubation (180 days), the most abundant functional pathways were carbohydrate metabolism and amino acid metabolism, followed by genetic information processing and environmental information processing (membrane transport) (Figure 1B). The results showed that different

bamboo litter proportions had significant effects on membrane transport and carbohydrate metabolism ( $p < 0.05$ ). Moreover, the relative abundance of the functional pathways of membrane transport, carbohydrate metabolism and amino acid metabolism in 100% PP treatment was higher than that in other treatments (Figure 1B).

### 3.2. Fungal Community Composition and Functional Pathways

As shown in Supplementary Materials Figure S1B, *Ascomycota* (53.88–84.17%), *Basidiomycota* (8.88–27.25%) and *Mucoromycota* (4.07–10.72%) were the dominant fungi during litter decomposition in the six treatments. Thirty days after incubation, the relative abundance of *Ascomycota* was significantly increased, and that of *Basidiomycota* was significantly decreased with the addition of bamboo litter compared with the 0% PP treatment. At the class level (Figure 2A), *Sordariomycetes* (9.49–38.41%), unclassified\_p\_*Ascomycota* (8.61–39.64%) and *Eurotiomycetes* (9.44–25.80%) dominated the fungal community. The addition of litters significantly increased the relative abundance of *Sordariomycetes* and *Dothideomycetes* and decreased that of *Eurotiomycetes*. In particular, the relative abundance of *Dothideomycetes* decreased (from 13.54% to 5.5%) in the 67% and 100% PP treatments but increased (from 6.45% to 11.14%) in 0% and 33% PP treatments.

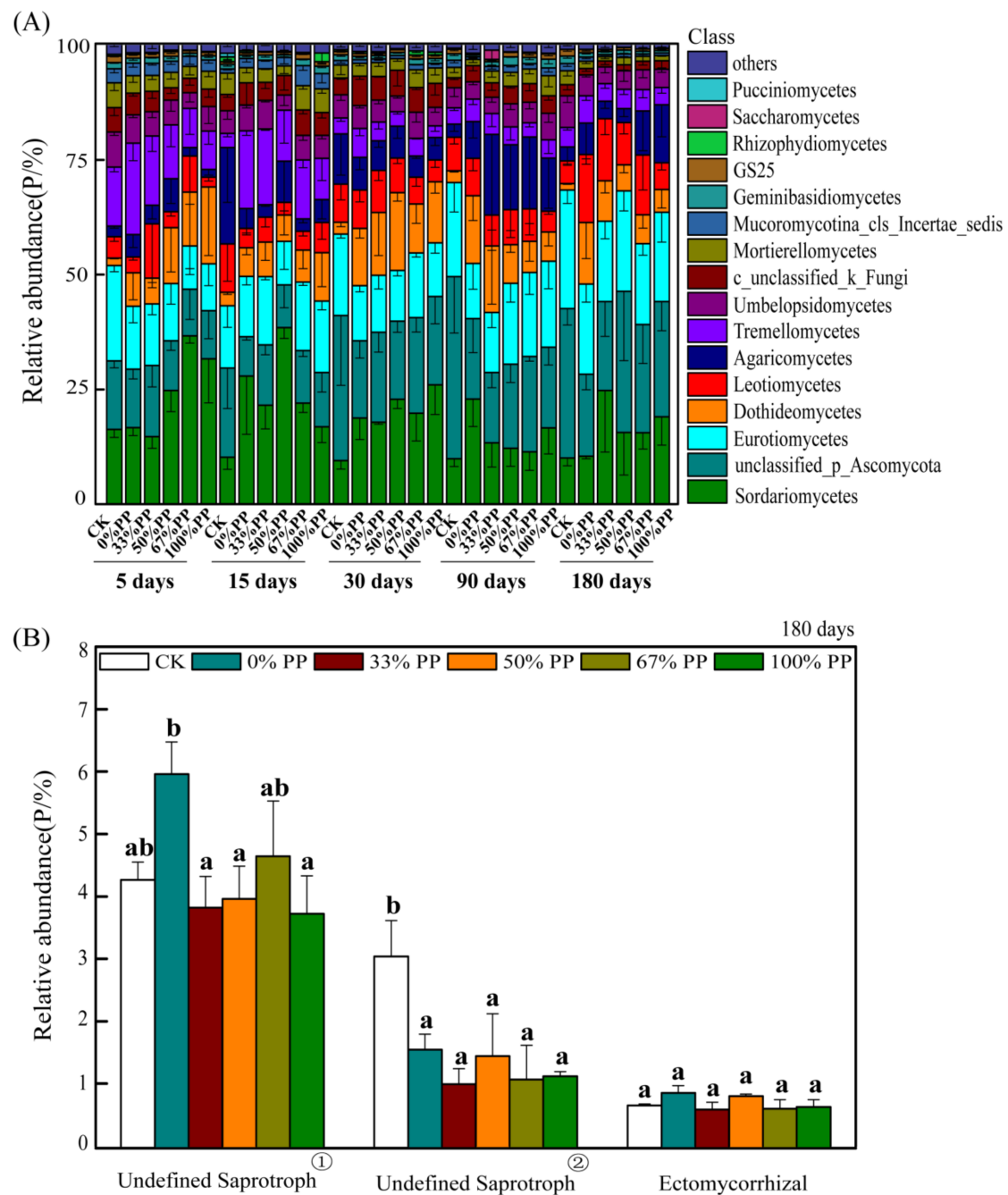
Based on the FUNGuild function classification, we obtained three functional groups based on how they acquire their energy. At the end of incubation (180 days), the most abundant functional pathway was saprotroph (fungal parasite-undefined saprotroph and endophyte-litter saprotroph-Soil saprotroph-Undefined saprotroph), followed by symbiotroph (ectomycorrhizal) (Figure 2B). Bamboo litter proportion had no effect on fungal functional pathways ( $p > 0.05$ ), except for fungal parasite-undefined saprotroph. However, bamboo litter decreased the relative abundance of these three functional pathways (Figure 2B).

### 3.3. Bacterial and Fungal $\alpha$ -Diversity Vary with Litter Type and Incubation Time

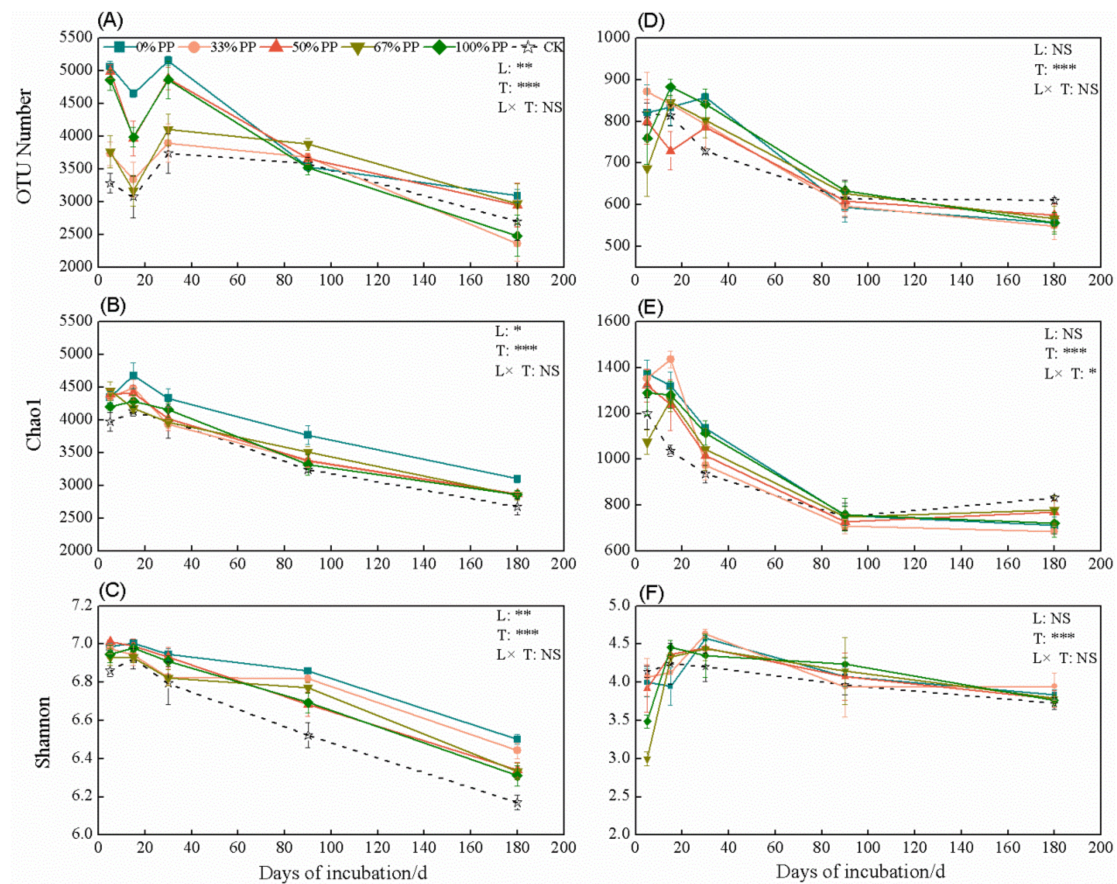
The microbiome richness, or  $\alpha$ -diversity, varied greatly across different litter ratios and incubation times for both bacteria and fungi (Figure 3). The Chao1, OTU number and Shannon index of the bacteria community increased with litter addition, while richness and evenness gradually decreased during incubation (Figure 3A–C). Compared with the 0% PP treatment, the increased proportion of bamboo litter (33% PP→100% PP) decreased the Chao1, OTU number and Shannon index of the bacterial community during incubation. For the fungal community, litter type had a non-significant effect on fungal  $\alpha$ -diversity (Figure 3D–F), but incubation time significantly affected fungal  $\alpha$ -diversity. The addition of litter increased the Chao1 index and OTU number compared with CK before 90 days of incubation.

### 3.4. Contributions of Environmental Factors to Microbial Community Structure

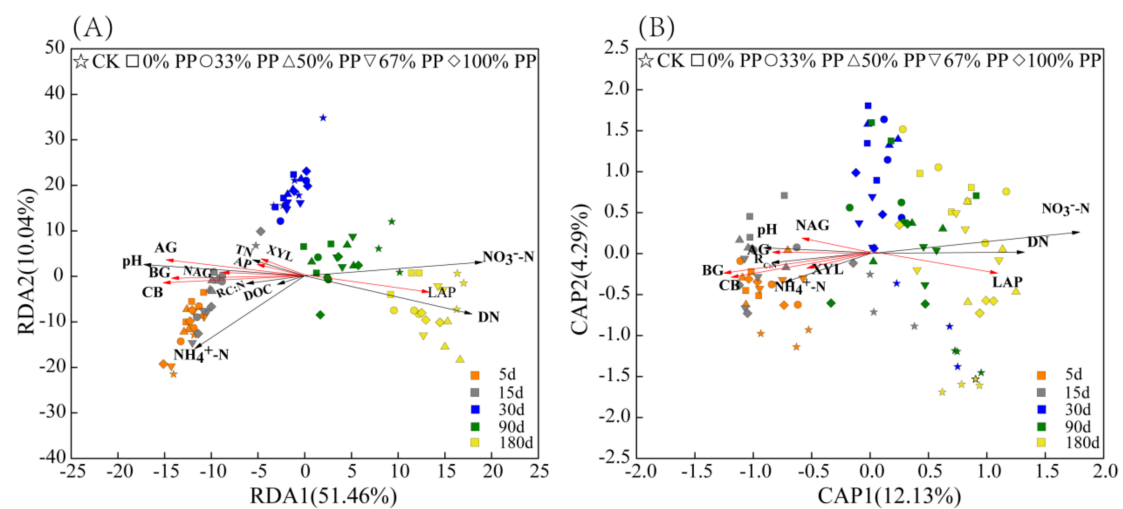
Redundancy analysis showed that there were significant differences in soil bacterial community structure at different incubation stages (Figure 4A), which were greatly affected by soil chemical properties (Supplementary Materials Table S1). The main variables significantly affecting bacterial community structure were soil pH,  $\text{NO}_3^-$ -N and DN ( $p < 0.001$ ). In particular, the soil bacterial communities of the different litter treatments were negatively correlated with  $\text{NO}_3^-$ -N, DN and LAP activities but positively correlated with pH, C- and N-acquiring enzyme activities (Supplementary Materials Figure S2) at the early stage of incubation.



**Figure 2.** The community composition (A) and functional pathways (B) of the fungal community at the level of class in the broadleaved forest soil during incubation. Undefined Saprotroph (①): Fungal Parasite-Undefined Saprotroph; Undefined Saprotroph (②): Endophyte-Litter Saprotroph-Soil Saprotroph-Undefined Saprotroph. CK, with no litter; 0% PP, bamboo litter occupying at 0%; 33% PP, bamboo litter occupying at 33%; 50% PP, bamboo litter occupying at 50%; 67% PP, bamboo litter occupying at 67%; 100% PP, bamboo litter occupying at 100%. Error bars indicate standard deviation. Different letters show significant differences ( $p < 0.05$ ).



**Figure 3.** Effects of litter type (0 % PP, 33% PP, 50% PP, 67% PP, 100% PP) on bacterial (A–C) and fungal (D–F) alpha-diversity. Error bars indicate standard deviation. *p*-values from RMANOVA on the effects of the litter type (L) and incubation time (T) are also presented. CK, with no litter; 0% PP, bamboo litter occupying at 0%; 33% PP, bamboo litter occupying at 33%; 50% PP, bamboo litter occupying at 50%; 67% PP, bamboo litter occupying at 67%; 100% PP, bamboo litter occupying at 100%. Error bars indicate standard deviation.

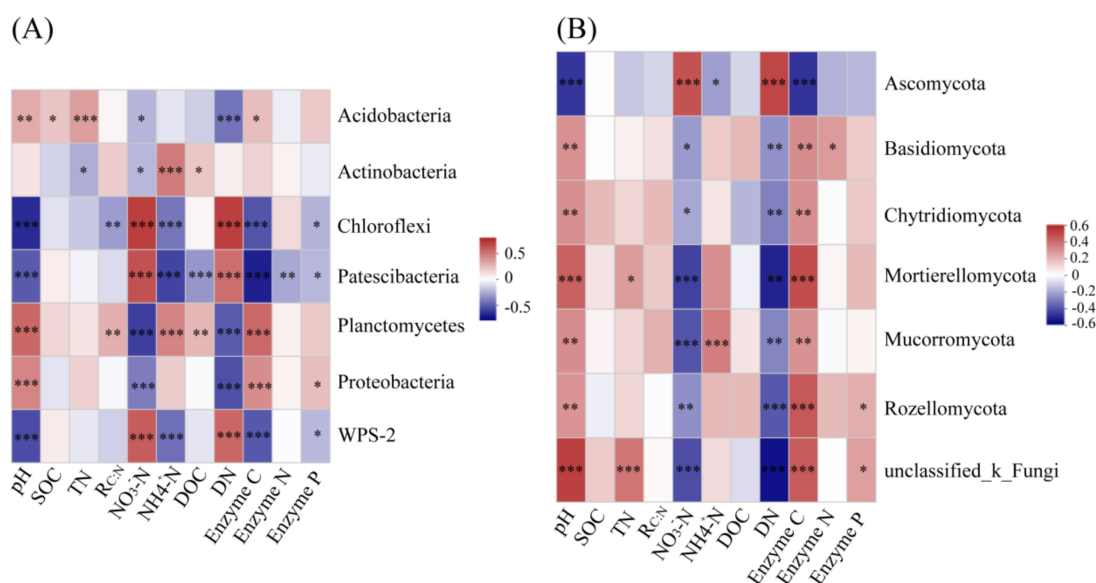


**Figure 4.** The redundancy analysis (RDA) ordination plots of the community compositions of bacteria (A) and fungal (B) communities. CK, with no litter; 0% PP, bamboo litter occupying at 0%; 33% PP, bamboo litter occupying at 33%; 50% PP, bamboo litter occupying at 50%; 67% PP, bamboo litter occupying at 67%; 100% PP, bamboo litter occupying at 100%.

Neither litter treatments nor incubation stages had a significant effect on soil fungal community structure (Figure 4B). The main variables significantly affecting fungal community structure were soil pH,  $\text{NO}_3^-$ -N,  $\text{NH}_4^+$ -N, DN and C- and N-acquiring enzyme activities ( $p < 0.001$ ).

### 3.5. Impact of Environmental Factors on Bacterial and Fungal Taxa

The correlation heat map results showed that *Chloroflexi*, *Patescibacteria* and *Planctomycetes* were the three most significant bacterial groups affected by environmental factors (Figure 5A). Notably, *Acidobacteria*, *Planctomycetes* and *Proteobacteria* were positively related to pH and C-acquiring enzyme activities, while *Chloroflexi*, *Patescibacteria* and WPS-2 were negatively related to pH,  $\text{NH}_4^+$ -N and C-acquiring enzyme activities ( $p < 0.001$ ). The main variables significantly affecting bacterial community composition were pH, mineral-N ( $\text{NO}_3^-$ -N and  $\text{NH}_4^+$ -N), DN and C-acquiring enzyme activities.



**Figure 5.** The heat map of the relationship between species and environmental factors at the phylum level of bacteria (A) and fungal (B) communities. Significance level: \*,  $p < 0.05$ ; \*\*,  $p < 0.01$ ; \*\*\*,  $p < 0.001$ .

However, fungal community composition at the phylum level was not significantly affected by SOC and  $R_{C:N}$  (the ratio of SOC to TN) (Figure 5B). Besides *Ascomycota*, all fungal phyla were positively correlated to pH and C-acquiring enzyme activities but negatively correlated to  $\text{NO}_3^-$ -N and DN. The main variables significantly affecting fungal community composition were pH,  $\text{NO}_3^-$ -N, DN and C-acquiring enzyme activities.

## 4. Discussion

### 4.1. Effects of Litter on Soil Bacterial and Fungal Community Structure

Litter is an important source of soil organic matter, and changes in the input of litter can affect the soil microbial community structure by changing soil available nutrient content [32]. The addition of different types of litter and their decomposition process will change the quality of litter and the content and availability of nutrients to the native microorganisms in the soil. Thus, the soil microbial community will make corresponding adjustments to respond to these changes. In this study, the OTU number of bacteria in the soil changed significantly after the addition of different proportions of bamboo litter. Moreover, with the increase in bamboo litter proportion, the OTU number of soil bacteria increased significantly. This may be due to the faster decomposition rate of bamboo litter and the faster return of nutrients in litter. Soil bacteria have more nutrient sources, allowing them to proliferate. The results of this study also showed that the richness index and

uniformity index of the soil bacterial and fungal communities increased significantly in litter addition compared with CK (Figure 3). Different proportions of bamboo litter changed the bacterial diversity but had no effect on the fungal diversity. With the increase in the proportion of bamboo litter, the Chao1 index, OTU number and Shannon index of the bacterial community decreased. However, this study found that litter quality had no significant effect on the  $\alpha$ -diversity of soil fungal communities ( $p > 0.05$ ). These results support the findings of Wang et al. [33] and Xing et al. [34], which reported the fungal community composition converged from the early to the late stage. However, this is inconsistent with previous studies reported in changes in litter quality induced by N deposition [18], in which changes in litter quality increased the diversity of the fungal community, and higher fungal diversity was observed in soil with mixed litter.

The structure of the soil bacteria community is more sensitive to the change in soil chemical properties than the fungal community. However, the addition of different litter types does not have a unified conclusion on the changes in soil bacteria community structure [15,16,18,33,35]. In this study, we found that there were significant differences in the community structure of bacteria rather than fungi at different incubation stages (Figure 4). Moreover, soil bacteria communities were significantly different between the 0% PP and 100% PP treatments at the end of incubation, suggesting that differences in litter species constructed different soil bacterial communities. The main reason for the above differences may be that the input of aboveground litter of different tree species changed the effective nutrient content, leading to the change in available carbon source for microorganisms [15]. Redundancy analysis (RDA) showed that soil bacterial community structure was significantly correlated with pH, DOC,  $\text{NH}_4^+$ -N and C- and N-acquiring enzyme activities at the early stage of incubation (30 d) but significantly correlated with  $\text{NO}_3^-$ -N and DN at the late stage (90 d) (Figure 3A). It showed that the microorganisms were mainly limited by N source in the early stage of culture, while the N limit gradually decreased with the increase in the proportion of bamboo litter. At the later stage of incubation, as the proportion of bamboo litter increases, the imbalance of C and N may promote the metabolic control of the soil–litter system and play a key role in shifting from N restriction to C restriction [36]. In this study, pure broadleaved forest soil was used to investigate the effects of litter type and bamboo litter proportion on the native soil microbial communities during the invasion of bamboo forest. There was no significant difference in fungal community structure, which may be due to the fact that fungi mainly decompose complex and stubborn plant litter [14,20]. However, since the incubation time of this experiment was only 180 days, the decomposition of litters was still dominated by easily decomposed substances during the incubation period, resulting in no significant difference in the structure of the fungal community from litter treatment.

#### 4.2. Effect of Bamboo Litter on Bacterial and Fungal Community Composition and Function Pathways

Changes in microbial communities mediated by leaf litter quality also affect soil ecosystem functions [34,37,38]. Many studies have found that *Proteobacteria*, *Actinobacteria* and *Ascomycota* are the most abundant phyla in the early stage of litter decomposition and are considered to be the main decomposers [3]. In this study, we found that in the five decomposition stages, *Actinobacteria* and *Proteobacteria* are dominant in the bacterial community, and *Ascomycota* is dominant in the fungal community (Figures 1 and 2). With the increasing proportion of bamboo litter, the relative abundance of *Actinobacteria* and *Proteobacteria* was higher than 0% PP, while the relative abundance of *Acidobacteria* was lower than 0% PP. *Acidobacteria* are typical oligotrophic bacteria, while *Proteobacteria* are eutrophic bacteria, which are suitable for soil environments with high nutrient content. This indicates that the decomposition rate of litter with the higher proportion of bamboo litter results in the rapid return of litter nutrients and the improvement of soil nutrient availability. This leads to an increase in the relative abundance of *Proteobacteria* and *Actinobacteria* and a decrease in the relative abundance of *Acidobacteria*. Previous studies showed that *Acidobacteria*

plays an important role in lignin degradation, and bamboo litter usually contains less than broadleaf litter. This study also found that compared with 0% PP, the relative abundance of *Ascomycota* was significantly increased, while *Basidiomycota* was significantly decreased with the increasing proportion of bamboo litter in the first 30 days of incubation (Figure 2). We can hypothesize that the decomposition of litter will reduce the number of readily available compounds, leaving more difficult-to-use compounds. Nevertheless, *Basidiomycota* may be easier to use refractory compounds than *Ascomycota*. The quality of plant litter is considered to be the dominant factor affecting nutrient release [39]. The ratio of carbon to nitrogen is usually an important attribute of litter quality, which has a certain impact on the composition of soil microbial communities [18,33]. In addition, through correlation analysis, we found that soil pH,  $\text{NO}_3^-$ -N, DN and C-acquisition enzyme activity have a significant impact on the composition of the soil bacteria and fungi community ( $p < 0.05$ ). It is well known that soil pH has a significant effect on microbial community composition and functional pathways [3]. In this study, soil pH was positively correlated with the three dominant bacterial taxa (*Actinobacteria*, *Proteobacteria* and *Acidobacteria*) and *Basidiomycota* while negatively correlated with *Ascomycota* (Figure 5). This indicated that the different availability of nutrients released into soil during litter decomposition can affect soil pH and result in the variation of dominant soil microbial species.

The effect of vegetation on soil microbial community structure will further affect the functional pathway of soil bacteria and fungi. In this study, soil bacteria mainly involved 7 primary functional layers and 41 secondary functional layers. Among them, the metabolic function of soil bacteria is active and extremely important. For example, the membrane transport function is conducive to maintaining various life activities of the bacteria, which is the basis for the normal function of soil bacteria [40]. Carbohydrate metabolism is closely related to the effects of nitrogen fixation and phosphorus solubilization, which is beneficial to the nitrogen and phosphorus cycles of plants [41]. Amino acid metabolism can help bacteria absorb amino acids, which is conducive to the survival and reproduction of bacteria [42]. In this study, we found that the addition of bamboo litter increased the relative abundance of amino acid and carbohydrate metabolism genes. This further reflects an important adaptive strategy of bacteria to cope with the shortage of resources; namely, bamboo litter makes the microorganisms switch from N to C limitation. FUNGuild was used to predict the ecological functions of soil fungi in this study. From the perspective of nutrition type, the saprophytic type was the most important nutrition type, which may be related to *Ascomycota*, the most dominant phylum. *Ascomycota*, mostly saprophytes, is an important decomposer in soil, which can decompose difficult-to-degrade organic matter and plays an important role in nutrient cycling.

## 5. Conclusions

Our study provides direct evidence of the impact of bamboo expansion on the soil microbial community in broadleaved forest soil by adding different proportions of bamboo litter. We found changes in soil microbial community structure and function pathway caused by mixing bamboo with broadleaved litter in different proportions, which was attributed to the increase in soil N availability as bamboo litter decomposes, resulting in the switch of microorganisms from N to C restriction. This means that microorganisms need to change their community structure in response to changes in the soil environment and relieve their own nutrient pressure. Soil bacteria rather than fungi respond significantly to both litter quality and incubation time, indicating that soil microbial composition was determined by easily decomposed substrates with a high turnover rate during the 180 days incubation. Our study highlights that the change in proportions of bamboo and broadleaved litter during bamboo expansion will affect the native microbial community structure and functional pathways of broadleaved forest soil and have important impacts on soil C sequestration and nutrient cycling. Besides the aboveground litter, roots may also affect the microbial community structure of broadleaved forest soil during bamboo expansion.

Therefore, further consideration should be given to the effects of both root and litter, as well as the soil basic parameters, mineralogy and quality, on the microbial community.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agronomy12010220/s1>. Figure S1: The community composition of bacteria and fungal at the level of phylum; Figure S2: C-, N- and P-acquiring enzyme activities; Table S1: Treatment effects on soil chemical properties during the incubation.

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