Changing Characteristics and Influencing Factors of the Bacterial and Fungal Communities in the Litter of *Casuarina Equisetifolia* Forests of Different Ages in Hainan Island

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Abstract

Casuarina equisetifolia, an important pioneer tree with resistance to typhoon and stress tolerance, is mainly cultivated in the coastal areas of tropical and subtropical zones. *Casuarina equisetifolia* forests have high litter accumulation rates and slow litter decomposition rates. The accumulation of litter may affect the secondary regeneration of *Casuarina equisetifolia*, and the decomposition of litter is largely controlled by microorganisms such as bacteria and fungi. While the characteristics of the microbial diversity in the litter of *Casuarina equisetifolia* forests have not yet been resolved. In order to explore the microbial distribution characteristics and community dynamic changes in the litter of a *Casuarina equisetifolia* forest, we used Illumina Miseq high-throughput sequencing technology to analyze the endophytic and exophytic bacterial and fungal communities of *Casuarina equisetifolia* forests of three ages (young forest, half-mature forest, and mature forest). The results showed that the young and half-mature forests had the highest bacterial diversity, and the half-mature and mature forests had the highest fungal diversity. The community structure and endophytic and exophytic communities of different forest ages were also different. Exophytic bacteria of the phylum Proteobacteria had a gradually decreasing abundance with stand age, while endophytic bacteria of the phylum Actinomycetes had a gradually increasing abundance with stand age; in the half-mature forest, the abundance of nitrogen-fixing microorganisms, such as the genera *Rhizobium*, *Bradyrhizobium*, and *Actinoplanes*, were increased relative to all of the other forest age types. The fungal community structure was different than the bacterial structure. Specifically, the fungal communities of both the young and mature forests were dominated by the class Dothideomycetes of the phylum Ascomycota, while phylum Basidiomycota was dominant in the half-mature forest. The correlation with the physicochemical properties of the litter showed that exophytic bacteria were related to the organic carbon and water content of the litter, endophytic bacteria were related to litter pH and nitrogen content, and fungi were correlated tonitrogen and phosphorus contents. In summary, the community structures of bacteria and fungi in the half-mature forest were more uniform, which was beneficial to the growth of *Casuarina equisetifolia* plants, and the changes observed in the physical and chemical factors of litter were one of the key factors driving microbial community structure.

Introduction

Litter is an important part of nutrient cycling in forest ecosystems, acting as a vector of transport for nutrients from aboveground to belowground[1] The decomposition of litter is significant to nutrient cycles in forest ecosystems [2], and microorganisms are the mechanism for both litter decomposition and the subsequent formation of soil organic matter [3, 4]. The current literature supports an estimate that approximately 90% of the decomposition of organic matter is completed by microorganisms [5]. The soil microorganisms involved in the decomposition of litter mainly include fungi and bacteria. Fungi have a strong ability to decompose litter and are the main driving force for litter decomposition. Additionally, bacteria can decompose lignin, hemicellulose, and cellulose during the decomposition of litter, but the role of bacteria in the decomposition of litter may be underestimated. Microorganisms can colonize not

only the litter surface but also the interior of the litter tissue. Endophytic bacteria can directly degrade the litter, enhancing litter decomposition rates. Alternatively, endophytic bacteria can compete for resources with other microorganisms that colonize the decaying litter, thereby indirectly affecting the rate of litter decomposition [6, 7]. Therefore, the changes in bacterial and fungal community dynamics throughout the litter decomposition process could help reveal the underlying mechanisms controlling litter decomposition, and thus, forest nutrient cycling.

Microorganisms promote the decomposition of litter and the formation of soil organic matter, and the micro-ecological environment during the decomposition of litter in turn can cause changes in the microbial community. For example, the chemical factors released during the decomposition of litter will affect the abundance and composition of bacterial and fungal communities[8]. Available nitrogen and available phosphorus also play a central role in the formation of the microbial community [9, 10], and secondary metabolites can affect the structure and function of the microbial community [11, 12]. For example, there is a negative correlation between fungi and phenolic compounds released during the decomposition of litter [13, 14], and monoterpenoids may stimulate changes in the microbial community [15].

Planted *Casuarina equisetifolia* forests are one of the main types of coastal defense forests on the southeast coast of China [16, 17]. These forests plays an important role in preventing wind damage and sand erosion losses [18, 19, 20]. At present, the coastal defense forests in Hainan are mainly forests composed of a single species, *C. equisetifolia*, and thus the forest floor litter is mainly the litter of *C. equisetifolia* [21]. The growth environment of *C. equisetifolia* is harsh and the soil nutrient content is low. Litter return is an important way to maintain soil nutrients in these *C. equisetifolia* forests [22]. As important biological factors connecting plants and the soil, the activity, community structure, and diversity of soil microorganisms are particularly important in systems with such tight nutrient cycling. Decomposition of *C. equisetifolia* litter is relatively slow, and the litter can accumulate up to a thickness of 20 cm in mature forests. In order to resolve the physical and biochemical mechanisms contributing to the decomposition dynamics of *C. equisetifolia* litter, this study examines the fungal and bacterial communities in the litter along a gradient of forest ages. The differences among the populations of microbial decomposers of *C. equisetifolia* with different forest ages can help determine if the various microbial populations function as barriers to the growth and regeneration of *C. equisetifolia* forests.

Materials And Methods

Study site and sample collection

This study was conducted in the *Casuarina equisetifolia L*. forest on the coast of Guilinyang Development Zone, Haikou City, Hainan Province. The area (20.02°N, 110.52°E) has a tropical ocean monsoon climate with long sunshine hours, abundant heat, and an average annual temperature of 23.8°C. The annual precipitation is 1500–2000 mm [23].

We collected litter from three forest ages: young forest (5–8 years), half-mature forest (15–20 years), and mature forest (30 years and older). We collected *C. equisetifolia* litter from mats with a thickness of 0–10 cm using the “S-type sampling method”. We washed each litter sample with sterile water, then collected the water after washing to detect the exophytic bacteria (samples from the young, half-mature, and mature forests labeled as BY-2, BHM-2, and BM-2, respectively) and exophytic fungi (samples from the young, half-mature, and mature forests labeled as FY-2, FHM-2, and FM-2, respectively); after sterilization with 75% ethanol (1 min), 20% NaClO (10 min), and sterile water, the litter endophytic bacteria (samples from the young, half-mature, and mature forests labeled as BY-1, BHM-1, and BM-1, respectively) and endophytic fungi (samples from the young, half-mature, and mature forests labled as FY-1, FHM-1, and FM-1, respectively) were detected.

Sequencing of litter bacteria and fungi

We extracted metagenomic DNA from an appropriate quantity of each of the pretreated samples. For the bacteria, the 16S region was used as the target DNA sequence, the universal primers 27F (5’-AGAGTTTGATCCTGGCTCAG-3’) and 1492R (5’-GGTTACCTTGTTACGACTT-3’) were used for polymerase chain reaction (PCR) amplification, and the products were purified. The Illumina Miseq PE250 platform was used for sequencing. For the fungi, the ITS (Internal Transcribed Spacer) region was used as the target DNA sequence, the universal primers ITS1F (5’-CTTGGTCATTTAGAGGAAGTAA-3’) and ITS2R (5’-GCTGCGTTCTTCATCGATGC-3’) were used for PCR amplification, and the products were purified. Fluorescence quantification was performed, and after the Miseq library was constructed, the Illumina Miseq PE300 platform was used for sequencing.

Determination of the physicochemical properties of litter

The litter samples were air-dried, ground, passed through a 1-mm sieve and mixed evenly. The drying method was used to determine the water content (WC) of the sample; the potentiometric method was used to determine the pH value (material-to-liquid ratio 1:2.5); the Kjeldahl method was used to determine the total nitrogen (TN); the KCl extraction-colorimetric method was used for the determination of ammonium nitrogen (NH_4-N); the phenol disulfonic acid-colorimetric method was used for the determination of nitrate nitrogen (NO_3-N); the NaOH fusion-molybdenum antimony colorimetric method was used for the determination of total phosphorus (TP); a potassium dichromate-digestion furnace heating method was used to determine the organic matter (C) content.

Data processing and analysis

The paired-end reads obtained by Miseq sequencing were merged according to the overlapping relationship, and the sequence quality was controlled and filtered at the same time. All of the sequences were subjected to OTUs division according to different similarity levels, and the bioinformatics analysis was performed on the OTUs at the 97% similarity level. Mothur software (version v.1.30.1 http://www.mothur.org/wiki/Schloss_SOP#Alpha_diversity) was used to calculate the Alpha diversity of the litter microbial community (Shannon index and Simpson index) and analyze the diversity and abundance of the microbial communities. The R program and Mothur were used to perform Venn

analysis of the OTUs, and the numbers of common and unique OTUs were counted. Based on the data table in the tax_summary_a folder, the R language tool was utilized to plot the microbial community histogram and count the species and proportions of microorganisms contained in each sample at each classification level. PICRUSt software and FUNGuild software were utilized to predict the functions of bacteria and fungi, respectively, and an RDA (redundancy analysis) graph was plotted using Canoco software for Windows 4.5 to analyze the relationship between the composition of the litter microbial community and environmental factors.

Results

Changes in microbial diversity of the litter with different forest ages

The microbial diversity of the litter varied considerably among different forest ages (Figure 1). Both endophytic and exophytic bacteria showed the highest diversity in the half-mature forest and the lowest bacterial diversity in mature forests. The shannon diversity index of endophytic bacteria was 15.76% higher in the half-mature forest than in the young forest and 31.52% higher than the mature forest, while for exophytic bacteria it was 7.56% higher than that of young forests and 11.64% higher than that of mature forests. In the same forest age, the Shannon diversity index of young forest and half-mature forest was similar, while the exophytic bacteria of mature forest was 16.40% higher than that of endophytic bacteria in the same location. The simpson index showed a similar pattern. However, the fungi showed different characteristics. The shannon diversity index of exogenous fungi was 41.67% (young forest), 53.79% (half-mature forest) and 17.81%(mature forest) higher than that of endophytic bacteria in litter of different forest ages. Different from bacteria, the diversity of endophytic fungi in mature forest was higher than that in young forest and half-mature forest, while the diversity of exophytic fungi increased first and then decreased.

BY-1 endophytic bacteria of the young forest BHM-1 endophytic bacteria of the half-mature forest; BM-1 endophytic bacteria of the mature forest; BY-2 exophytic bacteria of the young forest; BHM-2 exophytic bacteria of the half-mature forest; BM-2 exophytic bacteria of the mature forest; FY-1 endophytic fungi of the young forest; FHM-1 endophytic fungi of the half-mature forest; FM-1 endophytic fungi of the mature forest; FY-2 exophytic fungi of the young forest; FHM-2 exophytic fungi of the half-mature forest; FM-2 exophytic fungi of the mature forest

Apparent differences in the microbial community structures in the litter

In the litters of different forest ages, the dominant endophytic and exophytic communities of bacteria at different phylogenetic levels suggest different succession patterns (Figure 2A, 2B). At the phylum level,

the structures of the exophytic and endophytic bacterial communities within different forest ages were substantially different. Among them, the endophytic bacteria were dominated by the phylum Proteobacteria (45.9–55.4%) and the phylum Actinomycetes (48.2–38.6%). Proteobacteria (71.9–65.4%) was the main exophytic bacteria, and the abundance of the phylum Acidobacteria in the endophytic and exophytic bacteria in the half-mature forest and mature forest increased. Exophytic bacteria were more abundant at the phylum level. The phylum Bacteroides accounted for 7.1–14.3% of the exophytic bacterial community, and the unique phyla Cyanobacteria, Firmicutes, and Saccharibacteria appeared. The phylum Armatimonadetes was unique among the endophytic bacteria. At the class level, the main endophytic bacteria were Actinobacteria sp., followed by Alphaproteobacteria sp., whose abundance decreased with an increase in forest age. The abundance of Gammaproteobacteria in the mature forest increased significantly. In exophytic bacteria, the dominant community was Alphaproteobacteria, while in the young and mature forests, Gammaproteobacteria was also a dominant community. Cytophagia had a high abundance in the half-mature forest. The unique norank-p-Saccharibacteria and Clostridia appeared in exophytic bacteria.

The fungal community structure of litter showed obvious changes between different forest ages (Figure 2C, 2D). The main endophytic fungi were the phyla Basidiomycota (29.8–69.6%) and Ascomycota (25.5–65.2%). The relative abundance of Basidiomycota showed a decreasing trend after an initial increase, and it was dominant in the half-mature forest (69.6%), while the phylum Ascomycota was the dominant community in the mature forest (65.2%). For exophytic fungi, the phylum Ascomycota was dominant (61.8–83.6%) regardless of *C. equisetifolia* forest age. At the class level, the general community structures of the young forest and mature forest were relatively similar. For endophytic fungi, the abundance of the class Dothideomycetes increased from 3.5% in the half-mature forest to 40.0% in the mature forest, and the relative abundance of the class Agaricomycetes in the half-mature forest reached 69.1%. At the same time, the class Leotiomycetes was observed in the half-mature forest, but did not appear in the young and mature forests. For exophytic fungi, the abundance of the class Dothideomycetes in the young and mature forests was about 55%, but dropped to 32.7% in the half-mature forest. The distribution of communities in the half-mature forest was relatively balanced at the phyla and class level.

The effect of forest age on the dominant bacterial and fungal communities of litter

We compared the distribution of 16 dominant genera in the litter bacterial communities with different forest ages (Table 1), and the results showed that the abundances of dominant genera exhibited greatly differences between forest ages. For endophytic bacteria, in the young and half-mature forests, the dominant genus was *Sphingomonas*. In the young forest, there were large numbers of acidic microorganisms, such as *Acidothermus* (6.46%) and unclassified_f_Acetobacteraceae (1.28%). In the half-mature forest, the number of acidic microorganisms decreased, while the number of nitrogen-fixing

microorganisms increased, such as unclassified_f_Bradyrhizobiaceae (4.96%), *Actinoplanes* (4.27%), and *Rhizobium* (4.05%). In the mature forest, the bacterial community had undergone significant changes. The dominant bacterial genera changed to *Curtobacteria* (13.01%), *Massilia* (8.59%), *Kineococcus* (7.84%), and unclassified_f_Enterobacteriaceae (5.12%). For exophytic bacteria, the dominant bacterial genera in different forest ages were the same; they were unclassified_f_Enterobacteriaceae and *Sphingomonas*, and the relative contents of the two reached more than 20%. In the half-mature forest, the abundance of the acidic microorganism norank_f_Acetobacteraceae increased to a certain extent (4.83%), while in the mature forest, its abundance decreased significantly (0.74%). In addition, for both endophytic bacteria and exophytic bacteria, the number of dominant communities (relative abundance > 4.00%) in the mature forest increased.

The abundance of dominant genera in the fungal community structure differed significantly among forest ages (Table1), and there were shifts in the dominant fungal communities that appeared in the different forest ages. The dominant genera of endophytic fungi from the young, half-mature, and mature forests were unclassified_k_Fungi, unclassified_c_Agaricomycetes, and unclassified_o_Xylariales, respectively. The dominant genera of exophytic fungi from the young, half-mature, and mature forest were *Mycosphaerella*, unclassified_o_Auriculariales, and unclassified_o_Pleosporales, respectively. Among the endophytic fungi, there were a large number of dominant bacterial genera in the mature forest, and the relative abundance of six genera reached more than 10%. The distribution of dominant bacterial communities in the half-mature forest of exophytic fungi was relatively even, and the abundances of five dominant fungal genera reached about 10%.

Table 1
The relative abundances of dominant bacteria and fungi at the genus level in the litter of *Casuarina equisetifolia* within different forest ages

	Genus	endophytic			exophytic		
		young forest	half-mature forest	mature forest	young forest	half-mature forest	mature forest
Dominant bacterial community	Sphingomonas	**7.36**	**7.84**	3.03	**7.74**	**9.79**	**8.25**
	Curtobacterium	1.49	2.22	**13.01**	0.49	0.95	1.49
	Unclassified-f-Enterobacteriaceae	0.30	1.09	**5.12**	**20.87**	**11.67**	**12.24**
	Acidothermus	**6.46**	0.94	0.22	0.15	0.09	0.21
	Jatrophihabitans	**4.27**	2.86	1.12	0.35	0.42	0.73
	Unclassified-f-Bradyrhizobiaceae	2.49	**4.96**	/	0.38	0.60	0.10
	Actinoplanes	3.70	**4.27**	0.40	2.03	0.62	0.43
	Rhizobium	3.15	**4.05**	1.71	2.54	2.44	1.08
	Massilia	1.20	0.83	**8.59**	0.58	0.52	**4.67**
	Kineococcus	3.58	1.12	**7.84**	1.95	2.08	2.23
	Norank-f-Acetobacteraceae	1.28	1.02	0.24	1.24	**4.83**	0.74
	Nocardioides	0.39	2.26	**4.96**	0.25	0.61	1.72
	Stakelama	/	/	4.32	/	0.80	1.88
	Salinicola	/	/	3.93	/	0.69	4.40
	Salinisphaera	/	/	**4.98**	/	/	1.80
	Mucilaginibacter	/	/	/	2.68	2.52	**4.23**
Dominant fungal community	Unclassified-k-Fungi	**29.56**	4.87	1.40	9.34	**9.04**	**7.44**
	Unclassified-c-Agaricomycetes	6.11	**32.54**	**12.56**	0.75	/	0.81
	Unclassified-o-Xylariales	5.10	3.93	**16.19**	1.23	2.30	3.26
	Mycosphaerella	3.02	0.38	**10.81**	**32.30**	5.38	6.03
	Unclassified-o-Auriculariales	0.14	2.97	**16.06**	0.74	**12.68**	2.30
	Unclassified-o-	0.54	0.15	1.33	4.27	3.05	**19.74**

Pleosporales						
Unclassified-c-Dothideomycetes	**13.51**	0.10	0.06	1.71	1.12	1.03
Unclassified-p-Ascomycota	4.66	4.52	1.64	7.82	**12.45**	2.43
Unclassified-p-Basidiomycota	4.10	0.28	0.87	3.38	**10.69**	1.25
Circinotrichum	1.00	1.08	0.27	2.60	1.49	**9.86**
Toxicocladosporium	0.78	0.11	0.17	5.37	**9.68**	4.51
Devriesia	0.43	0.77	**10.03**	1.42	6.30	6.53
Trechispora	**16.16**	**14.51**	/	/	/	/
Unclassified-f-Marasmiaceae	/	**14.88**	2.44	/	1.92	6.64
Hortaea	/	/	**11.02**	/	/	5.57

The influence of environmental factors on the structure of bacterial and fungal communities

The physicochemical properties of the litter in the *C. equisetifolia* forests are presented in Table 2. The litters of different forest ages were acidic (pH 5.30–5.59). As the forest age increases, the pH of litter increases gradually, and the pH value in the mature forest was significantly higher than that of the young forest ($P < 0.05$). The water content of litter was the highest in the half-mature forest, and the water content of the mature forest was 45.04% lower than that of the half-mature forest. The contents of TN, NH_4_N and NO_3_N were the highest in the mature forest, and the total nitrogen content in the mature forest was significantly different from that in the half-mature forest, which was 26.47% higher than that in the half-mature forest, and 19.47% higher than that in the young forest. There was no significant difference between the content of P and C in different forest ages. The P content of half-mature forest and mature forest was similar, which is 35.39% higher than that of young forest. The content of C continuously decreased, and the content of C in mature forest was 58.92% lower than that in young forest.

Table 2
Physicochemical properties of Casuarina equisetifolia litter

	WC (%)	pH	TN (mg/kg)	TP (mg/mL)	C (g/kg)	NH_4_N (mg/kg)	NO_3_N (mg/kg)
Young forest	13.25 ± 0.67 ^{ab}	5.30 ± 0.11 ^{b}	18937.61 ± 2138.33 ^{ab}	14.72 ± 5.98	2.31 ± 1.17	0.11 ± 0.02	2.41 ± 0.84
Half-mature forest	16.34 ± 3.06 ^{a}	5.48 ± 0.06 ^{ab}	17890.18 ± 1810.20 ^{b}	19.93 ± 6.78	2.06 ± 0.77	0.11 ± 0.02	3.23 ± 0.0.81
Mature forest	8.98 ± 2.51 ^{b}	5.59 ± 0.17 ^{a}	22625.25 ± 2160.45 ^{a}	19.93 ± 6.78	1.69 ± 0.42	0.17 ± 0.08	3.83 ± 1.63

Different letters in the same column indicate significant differences ($p \leq 0.05$)

The redundancy analysis (RDA) results of the dominant communities of endophytic and exophytic bacteria and the physicochemical properties of the litters of different forest ages are shown in Figures 3A and 3B. For endophytic bacteria, the combination of the first two axes explained 63.79% of the total variance of the RDA. From the length of the arrow in the Figure 3A, it can be seen that total nitrogen (TN) had the greatest impact on the structure of the endophytic bacterial community ($p < 0.05$). From the projection distance of the sample to the litter quality factors, it can be seen that pH, NH_4_N, NO_3_N, and TN had the strongest relationship to the community structure of the mature forest. *Curtobacterium*, *Kineococcus*, and *Massilia* showed the closest relationship with pH and NH_4_N. The water content (WC) was strongly correlated with the endophytic bacterial community structure in the half-mature forest and the young forest. Among half-mature forest endophytes, *Sphingomonas* and *Rhizobium* had the closest relationship with organic carbon, while *Actinoplane* and *Acidothermus* had the closest relationship with WC. For exophytic bacteria, the combination of the first two axes explained 85.03% of the total variance of the RDA. WC and TN had the strongest relationship with the exophytic bacterial community, and both reached a significant level ($p < 0.05$). In the young and the half-mature forests, organic carbon and WC had a relatively high correlation with the community composition of exophytic bacteria, and the most abundant unclassified_f_Enterobacteriaceae had the closest relationship with organic carbon. In the mature forests, the community structure of exophytic fungi was strongly related to the pH and N-related factors. Among them, *Massilia* had a relatively high abundance and showed the closest relationship with ammonium nitrogen. In general, between the young forest period and the mature forest period, the changes in the litter nitrogen, phosphorus, and pH were related to the community structure of exophytic bacteria.

The RDA analysis results of the dominant communities of the endophytic and exophytic fungi at the level of order, and the physicochemical properties of litter, are shown in Figures 3C and 3D. The analysis of the physicochemical properties of the endophytic fungi and litter showed that pH was most strongly linked to the endophytic fungal community ($p < 0.05$). As the forest age increased, the community structure of fungi showed relatively large differences. Among them, Capnodiales and Xylariales, which had relatively

high abundances in the mature forest, were positively correlated with the litter pH and nitrogen. The unclassified fungi and Trechisporales with high abundances in the young and half-mature forests were not highly correlated with litter pH. For exophytic fungi, total nitrogen and ammonium nitrogen both had strong relationships with the exophytic fungal community ($p < 0.05$). The fungal community structures of the three forest ages were considerably different. Among them, Capnodiales showed the shortest distance to the young forest, was the dominant species in this period, and had a negative correlation with environmental factors. Pleosporales, another dominant species, exhibited strong, positive correlations with TN, ammonium nitrogen, and total phosphorus (TP).

Changes in the function of bacterial communities of different forest ages

In order to explore the functional differences of the dominant endophytic and exophytic bacterial communities in the litter, we used the PICRUSt software to predict and analyze the function of the characterized bacterial communities. Based on the KEGG database (Kyoto Encyclopedia of Genes and Genomes), the dominant bacterial communities contained in both endophytic and exophytic bacteria—*Sphingomonas*, *Kineococcus*, *Massilia*, *Amnibacterium*, and *Rhizobium*—were selected for predicting results and comparison (Figure 4). A total of 21 sub-functions of endophytic bacteria and exophytic bacteria in the second-level functional layer showed significant differences. Specifically, carbohydrate metabolism, biosynthesis of other secondary metabolites, membrane transportation, and metabolism of litter endophytic bacteria were considerably stronger in the endophytic community than in the exophytic bacteria community in our system. However, environmental adaptation, lipid metabolism, and purine nucleotide metabolism of exophytic bacteria were considerably stronger than those of endophytic bacteria. From the third-level functional layer, it can be seen that endophytic and exophytic bacteria produced a variety of secondary metabolites including alkaloids, and there were significant differences.

The fungal community function in the litter of the *C. equisetifolia* forests was composed of three types: pathotrophic, saprotrophic, and symbiotrophic (Figure 5). For exophytic fungi, the relative abundance of *Plant Pathogen* in the young forest was relatively high, at 36.1%, and it decreased to 14.5% in the half-mature forest. Correspondingly, *Endophyte-Plant Pathogen* increased to 9.7%. The abundances of *Undefined Saprotroph* and *Endophyte-Plant Pathogen-Undefined Saprotroph* in the mature forest both increased substantially. For endophytic fungi, woody saprophytes (16.2%) were mainly found in the young forest, followed by undefined saprophytes and plant pathogens, and the distribution of these was relatively even. The three trophic modes with a high abundance in the half-mature forest were *Wood Saprotroph* (14.6%), *Undefined Saprotroph* (9.2%), and *Endophyte-Plant Pathogen-Undefined Saprotroph* (14.9%). It can be seen that these dominant trophic modes in the half-mature forests were more abundant than those in the young forest. The abundance of *Plant Pathogen* in the mature forest increased greatly, reaching 34.9%.

Discussion

It is generally believed that the soil microbial community plays an important role in the decomposition of litter. However, the microbial community in the litter itself also plays an important role in litter decomposition. We observed that in a mature *C. equisetifolia* forest the diversities of endophytic and exophytic bacteria were considerably reduced compared to younger forests. While the diversity of endophytic fungi increased and the diversity of exophytic fungi decreased to a certain extent with forest age, there was no significant difference among different forest ages. This supports that “the rapid establishment of bacterial diversity is driven by simple compounds obtained in the early stages of decomposition” [24]. The bacterial decomposer community is suitable for degrading simple compounds, while the diversity of the fungal community depends on the remaining litter tissues.

In the exophytic bacteria, the phylum Proteobacteria was the dominant community, while the phylum Proteobacteria and the phylum Actinomycetes in the endophytic bacteria were the common dominant bacteria. It can be seen that the phylum Actinomycetes mainly acted inside the litter. Symbiotic nitrogen-fixing microorganisms, such as the genera *Rhizobium*, *Bradyrhizobium*, and *Actinoplanes*, were dominant in the communities of endophytic bacteria in the half-mature forest, which also explains the strong nitrogen-fixing ability of *C. equisetifolia*. Gauthier et al. found that the amount of fixed nitrogen of *C. equisetifolia* grown on coastal sands was 40–60 kg/(hm^2.a) [25]. This was consistent with the community distribution characteristics of nitrogen-fixing microorganisms we obtained. We also observed that the relative abundances of the phyla Proteobacteria, Bacteroides, and Acidobacteria—in the young and half-mature forests were high, and their abundances gradually decreased as the forest age increased. However, the abundance of the phylum Actinomycetes increased, which indicates that when the forest age is young, the bacteria participate in the decomposition of cellulose, while in the half-mature forest, the number of microorganisms involved in biological nitrogen fixation increases. Proteobacteria, Bacteroidetes and acidobacteria are generally considered to be eutrophic communities, while Actinobacteria are oligotrophic communities [26–30]. Juottonen *et al*. [31]also found that Firmicutes, Proteobacteria, and Acidobacteria could degrade cellulose. Our results indicate that the diversity of bacterial communities in the litter of *C. equisetifolia* based on resource acquisition and nutritional status, changes with the age of the forest.

The phylum Ascomycota of litter fungi is one of the main participants in the decomposition of litter. AnZhou et al. found the cellulose-degrading communities in the phylum Ascomycota [32]. Our results also indicate that Ascomycota plays an important role in the decomposition of *C. equisetifolia* litter, as its relative abundance in exophytic fungi reached 60–80%. This is consistent with Golebiewski et al.’s results of the decomposition of pine litter. At the class level, the dominance of Dothideomycetes in the mature and young forests was obvious, while the classes Agaricomycetes and Dothideomycetes were the dominant species in the half-mature forest. Voriskova *et al.*’s research [33] found that the class Dothideomycetes was dominant in senescent oak leaves. Ascomycota was also dominant in the early stages of decomposition of pine litter [34]. It is worth noting that the Basidiomycota fungal community are abundant in the half-mature forest of *C. equisetifolia* with good growth status.

RDA analysis showed that when the forest age was young, the organic carbon and water content showed a strong correlation with the community composition of exophytic bacteria. For endophytic bacteria, the pH and nitrogen-related factors of litter were strongly correlated. Combined with the PICRUSt analysis (Figure 4), we found that the same dominant bacterial communities were involved in mainly membrane transport and plant metabolism within the plant, while those on the surface of the litter were more involved in the adaptation to the environment, such as decomposing aromatic compounds, organic acids, and polyols in the environment.

The dominant communities of fungi had a strong correlation with the nitrogen and phosphorus contents of the litter, indicating that the change in the chemical composition of the litter during the decomposition process, especially the change in the nitrogen content, were most likely to shape the communities of litter microbial decomposers of the *C. equisetifolia* forest lands with different forest ages. Ascomycota was the dominant fungal phyla in the young and mature forests, while the majority of the organisms were leaf surface saprophytes, among which *Trichoderma*, *Penicillium*, and *Aspergillus* have been shown to play a key role in degrading complex organic matter, especially cellulose. In addition to Ascomycota, Basidiomycota was also dominant in the half-mature forest. Many species of Basidiomycota, such as white-rot polypore and brown-rot polypore, can decompose lignin in plant litter [35]. This suggests that the decomposition of litter by fungi in the half-mature forest is more comprehensive and thorough. However, due to the low-resolution limitation of high-throughput sequencing in the classification of fungi, it will be necessary in the future to conduct systematic studies on cultivable fungi and multiple scales of *C. equisetifolia* forest land to investigate the full potential of functions.

Conclusions

In *C. equisetifolia* forests of different ages, the bacteria and fungi in the litter had different diversity distributional patterns, and the species of endophytic and exophytic bacteria and fungi were also different. In the young and half-mature forests, the bacterial community diversity was relatively high, while the diversity of fungal communities was relatively high in the half-mature and mature forests. In general, the bacterial and fungal community structures in the half-mature forest were more uniform, which was beneficial to the growth of *C. equisetifolia* plants. Both the bacterial community and the fungal community were correlated to litter physical and chemical factors, most especially the litter nitrogen content.

Declarations

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Competing Interests The authors declare no competing interests.

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Figures

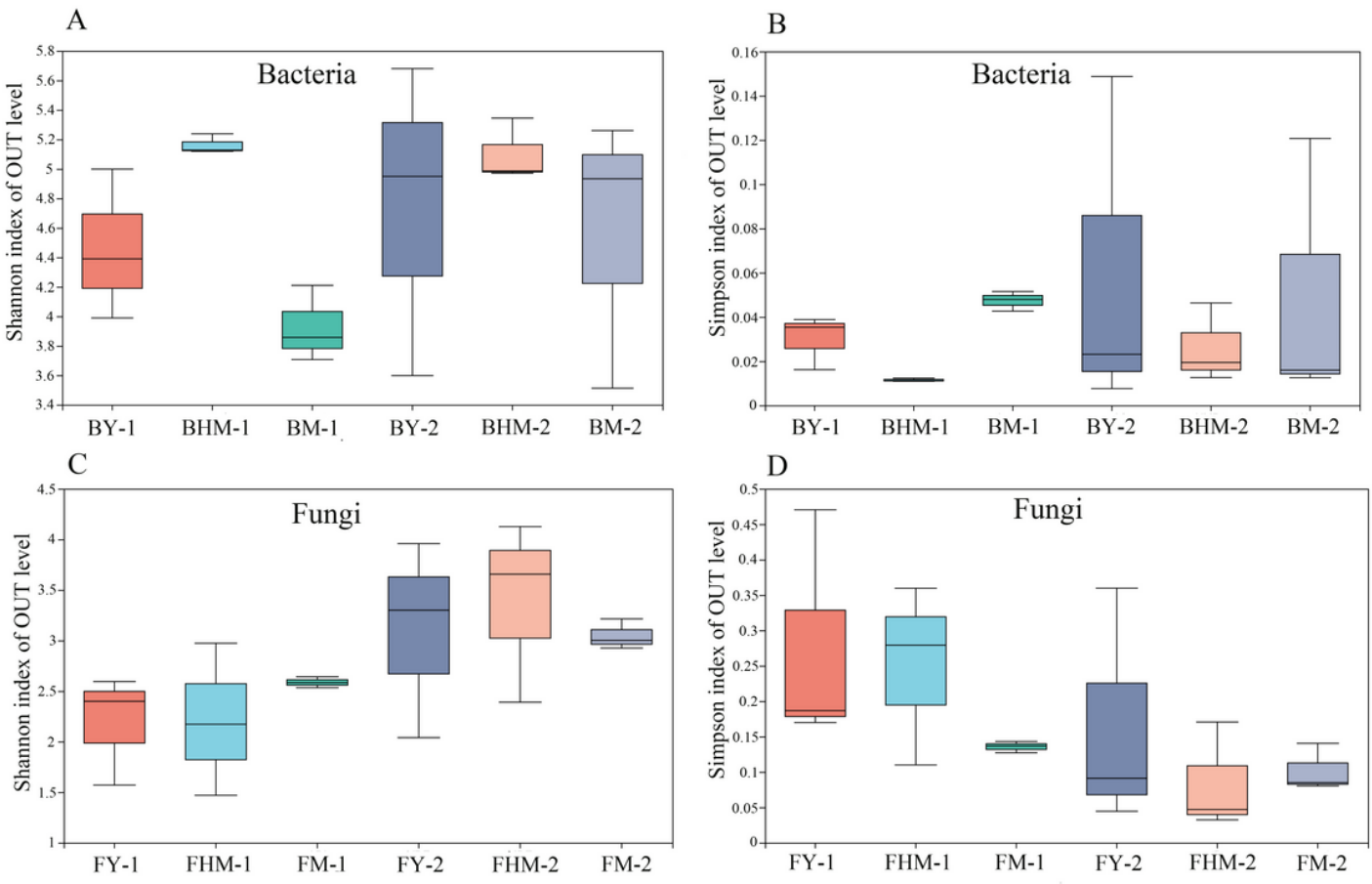

Figure 1

Boxplots of the Shannon index and Simpson index for the bacterial and fungal communities within different forest ages (A: Shannon index of bacteria; B: Simpson index of bacteria; C: Shannon index of fungi; D: Simpson index of fungi)

BY-1 endophytic bacteria of the young forest BHM-1 endophytic bacteria of the half-mature forest; BM-1 endophytic bacteria of the mature forest; BY-2 exophytic bacteria of the young forest; BHM-2 exophytic bacteria of the half-mature forest; BM-2 exophytic bacteria of the mature forest; FY-1 endophytic fungi of the young forest; FHM-1 endophytic fungi of the half-mature forest; FM-1 endophytic fungi of the mature forest; FY-2 exophytic fungi of the young forest; FHM-2 exophytic fungi of the half-mature forest; FM-2 exophytic fungi of the mature forest

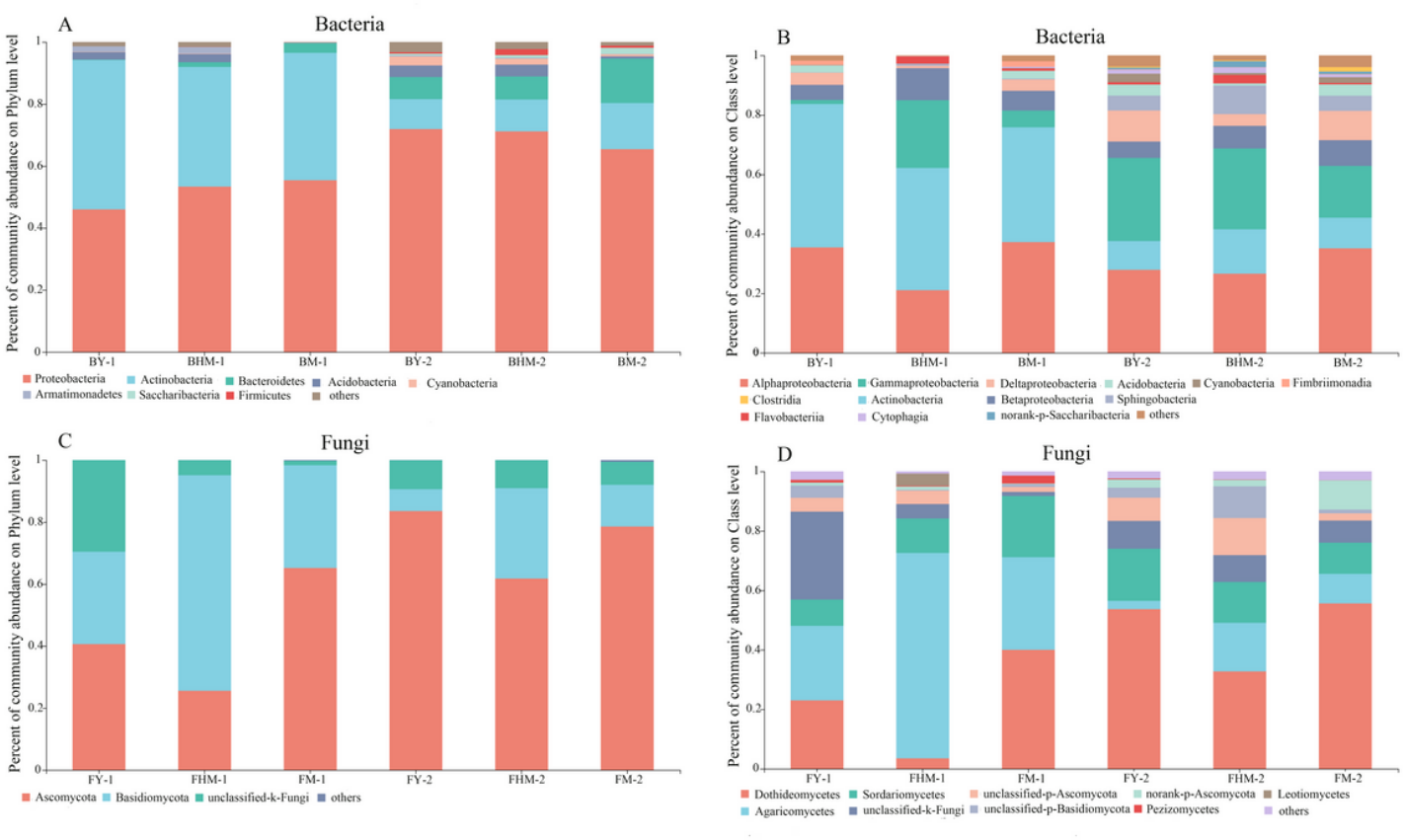

Figure 2

The relative abundances of endophytic and exophytic bacteria and endophytic and exophytic fungi in the litters of different forest ages: Bacterial phylum (A), Bacterial class (B), Fungal phylum (C), Fungal class (D).

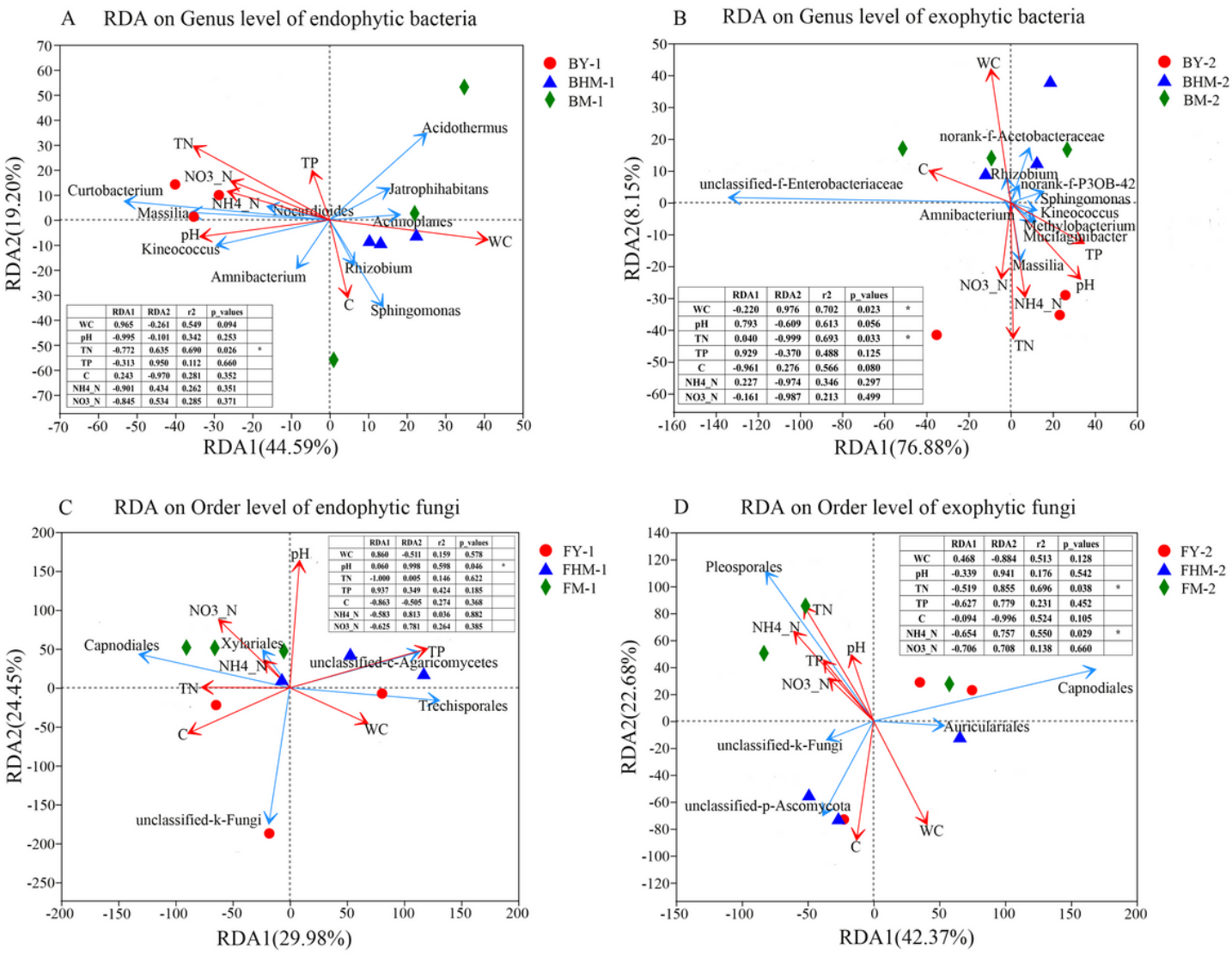

Figure 3

Redundancy analysis (RDA) of the bacterial and fungal communities in the litter of *Casuarina equisetifolia* and the physicochemical properties of the litter

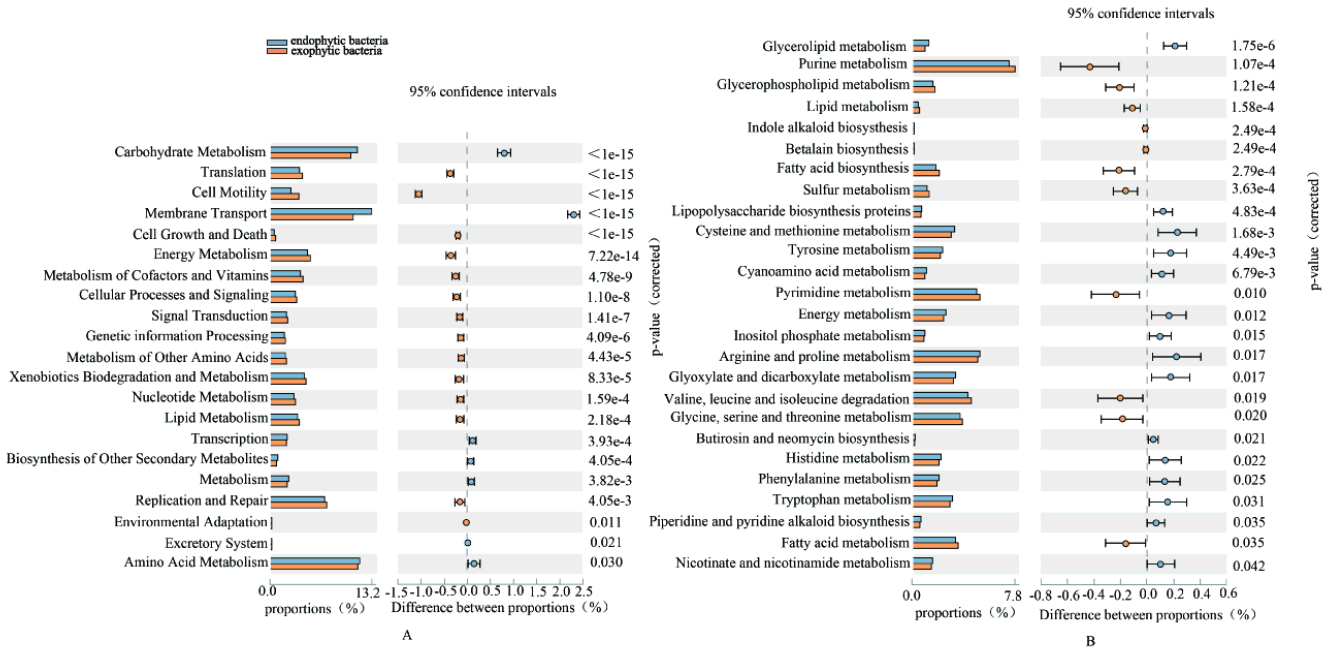

Figure 4

Prediction of the difference in the functional genes among different bacteria. A:Pathway level 2; B: Pathway level 3;

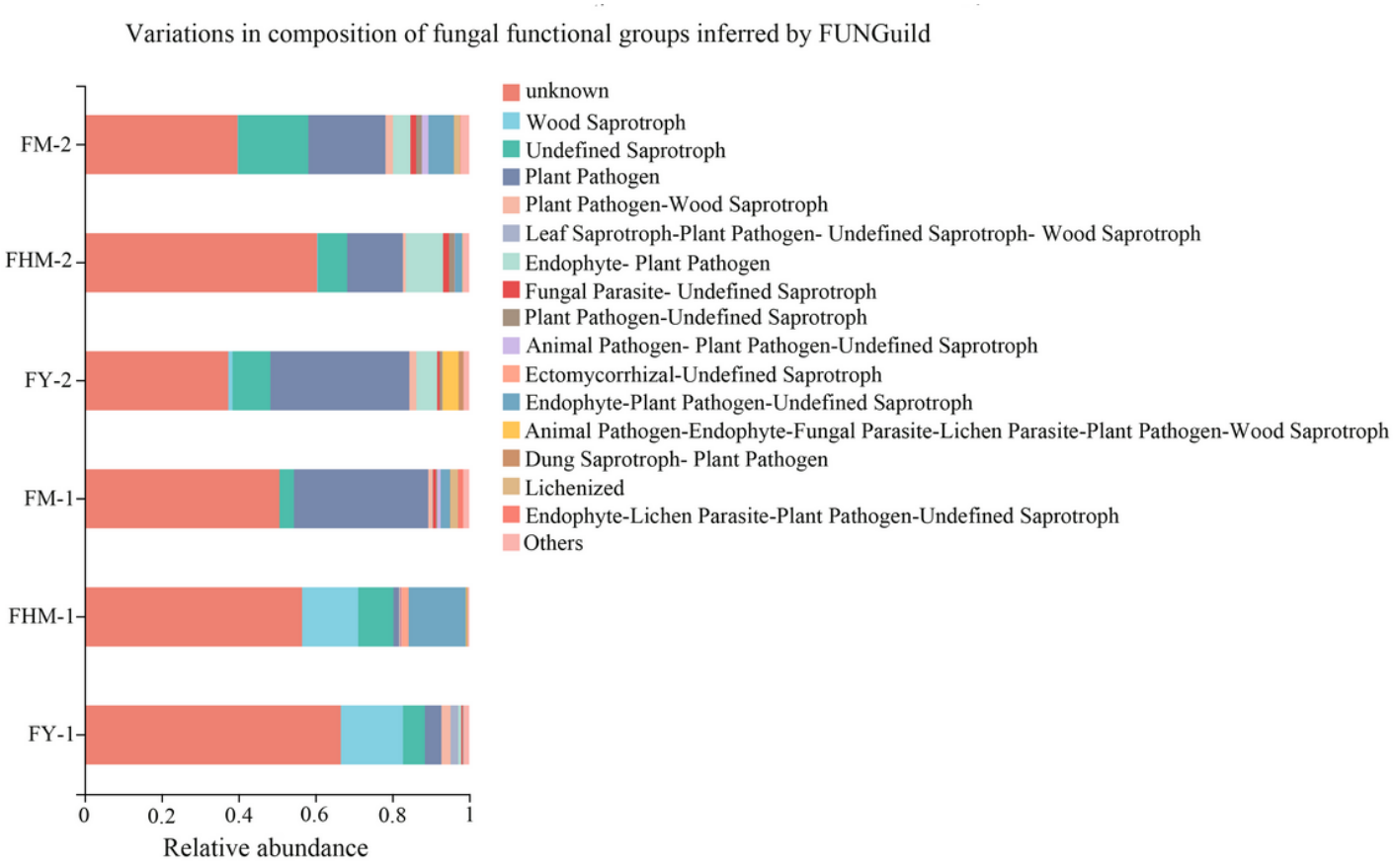

Figure 5

Prediction of the difference in fungal functions of litters within different forest ages