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Extracellular Enzyme Activity and Nutrient Characteristics of *Pinus massoniana* Lamb. Families with Different Growth Levels: Insights into the Ectomycorrhizal Fungal Community and Rhizosphere Soil

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Abstract: The symbiosis between ectomycorrhizal (ECM) fungal species and the root system of *Pinus massoniana* is important for its growth. However, the characteristics of ECM fungal communities and their relationships with extracellular enzyme activities and nutrients in the rhizosphere soil of different *P. massoniana* genotypes have not been well characterized. In this study, *P. massoniana* families (groups of offspring from different parents) with different levels of growth were selected for investigating ECM fungal communities, soil nutrients, extracellular enzyme activity, and leaf nutrient concentrations to explore the relationships between *P. massoniana* and the composition of the ECM fungal community. The high-growth (HG) family of *P. massoniana* had more different ECM fungal communities than the medium-growth (MG) and low-growth (LG) families; each family had a unique and dominant genera (HG: *Amphinema* and *Pseudoclathrosphaerina*; MG: *Russula* and *Auricularia*; and LG: *Russula* and *Amanita*). *Amphinema* was the main contributor to the differences among the three families (contribution: HG-MG 0.225 and HG-LG 0.17) and had rich extramatrical mycelium, which favored the growth of the HG family and positively affected the accumulation of soil organic carbon. Structural equation modelling showed that the dominant genera in the HG family had significant positive effects on the activity of three extracellular enzymes (BG, NAG, and AP) (weak to moderate positive effects of *Amphinema* on BG, NAG, and AP and moderate positive effects of *Pseudoclathrosphaerina* on BG, NAG, and AP), which might have contributed to the differences in extracellular enzyme activities among the families with different growth levels. Redundancy analysis indicated that *P. massoniana* growth traits (tree height, diameter at breast height, and timber volume), soil total nitrogen, and the N/P ratio significantly influenced ECM fungal communities. The study revealed the characteristics of ECM fungal communities, soil extracellular enzyme activity, and nutrient features of *P. massoniana* with different growth levels, which help improve our understanding of the relationship between *P. massoniana* genotype and ECM fungal communities.

Keywords: *P. massoniana* family; ECM fungi; soil extracellular enzymes; rhizosphere soil

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1. Introduction

Soil microbes play a critical role in driving terrestrial ecosystem processes by connecting aboveground biomass with belowground ecosystems [1]. As an important source of soil microbiota, extraradical mycelium (ECM) fungal communities are essential for plant productivity and soil nutrient cycling in forest ecosystems. ECM fungi can enhance the host plant's nutrient acquisition, pathogen resistance, and drought tolerance and in return

obtain carbon from the host [2] while producing various extracellular oxidases and hydrolytic enzymes that mobilize and release smaller organic molecules from soil organic matter (SOM) [3]. As previously mentioned, ECM fungi can enhance nutrient cycling in rhizospheric environments and their species richness and composition are also associated with soil physicochemical properties and nutrient availability [4]. Meanwhile, soil physicochemical properties can drive the community structure of fungi, thereby improving the nutrient environment of the soil [5]. For example, increased nitrogen availability in soils decreases ECM fungal colonization and alters their species richness [6]. Host plants also influence soil fungi, with recent studies showing that plants can directly affect their own root colonization through intra- and inter-specific variation [7–9]. While it is true that some mycorrhizal fungi have broad host ranges [10], it is important to note that within a single tree species, the composition of mycorrhizal fungal communities can be highly dynamic during stand development. Additionally, the diversity and composition of mycorrhizal fungi in the subterranean parts of the same species can vary across different developmental stages and ages. These variations are often associated with changes in soil enzyme activity [11]. Furthermore, stands of the same tree species with different growth rates exhibit differences in the range of ECM symbionts in their roots, as fast-growing stands allocate more energy and photosynthetic carbon to fine roots than slow-growing stands [12–14].

Soil extracellular enzymes, as crucial functional products of fungi, can be modulated by soil microorganisms to regulate the balance of necessary nutrients, thereby enabling adaptation to climate change [15] and affecting soil carbon sequestration and emission [16]. As a key factor in nutrient mobilization and acquisition [17], extracellular enzyme activity is a useful indicator for evaluating microbial nutrient demand [18,19]. As a proximate factor in the decomposition of soil organic matter and litter, soil extracellular enzymes exert effective control over the cycling of carbon, nitrogen, phosphorus, and other nutrients in terrestrial soil ecosystems [20–22]. Soil extracellular enzyme activity is influenced by several factors, including stand age [23], fungal community evolution time [24], and changes in both altitudinal and altitudinal gradients [25]. These variations are caused by the presence of ECM fungi, which are the key producers of extracellular enzymes [26,27], within different soil microenvironments [28,29]. Therefore, it is still unclear as to how soil extracellular enzyme activity is influenced by ECM fungal communities differ under different growth-rate genotypes of trees.

Pinus massoniana Lamb. [30], widely planted in southern China due to its adaptability, rapid growth, and high utilization rate, is used for timber and resin production and accounts for 20% of the national afforestation area [31]. Different *P. massoniana* families (groups of offspring from different parents) show different mechanisms of response to environmental changes [32–34]. This study examines the ectomycorrhizal fungal community and soil extracellular enzyme activity characteristics in the rhizosphere of *P. massoniana* trees with high, medium, and low growth levels selected according to progeny test data for 9-year-old stands. The aim of this study was to determine whether there are differences in the ECM fungal community and soil extracellular enzyme activity among families with the three different growth levels and to reveal the relationship between the ECM fungal community composition and the growth performance and soil nutrients of *P. massoniana*. The results can provide a theoretical basis for studying the interaction between *P. massoniana* aboveground growth and the belowground environment.

2. Materials and Methods

2.1. Study Region

The study area is located in Mochong Town, Duyun City, Guizhou Province (107°26′46″ E, 26°4′34″ N). The minimum temperature of the seed orchard is −6.9 °C, with an average annual temperature of 15.8 °C and an average annual precipitation of over 1400 mm, with the precipitation concentrated from May to October accounting for more than 80% of the annual precipitation. The average relative humidity is approximately 80%: the area is a

subtropical humid area. According to the Chinese Soil Classification, the soil type in the study area is yellow soil, mainly composed of loamy sand, and with a pH of 4.77.

2.2. Mycorrhiza, Rhizosphere Soil, and Leaf Sampling

The experimental materials were obtained from the progeny test stand of *P. massoniana* established in the spring of 2013. The progeny test stand, which was planted using a completely randomized block design, included 46 families with 5 individuals each planted in a single row within each plot. Based on years of growth data, three families with different growth rates were selected: a high growth (HG), medium growth (MG), and low growth (LG), as shown in Table 1. Three blocks were randomly selected and then one individual from each of the three families was chosen randomly for tree samples. In August 2021, the leaves located in the southern part of the crown of each sample tree were collected and stored at −20 °C for subsequent determination of leaf carbon (leaf C), leaf nitrogen (leaf N), and leaf phosphorus (leaf P). Fine roots were excavated in the main root extension direction at the base of the tree trunk and rhizosphere soil was collected at the site of root sampling. The mixed roots and soil samples from each tree (with at least two samples per tree) were packaged and stored in an ice box to prevent loss of activity; please refer to Dong et al. [11] for specific sampling methods. After transportation to the laboratory, the soil samples were divided into two groups: one group was kept at −20 °C for soil extracellular enzyme activity analysis and identification of rhizosphere microbial community composition within one week [33], while the other group was air-dried at room temperature for determination of the soil organic carbon (SOC), total nitrogen (TN), and total phosphorus (TP).

Table 1. Growth statistics of family sample trees with different growth rates. Note: mean ± standard error where different lowercase letters indicate significant differences ($p < 0.05$) based on Duncan's multiple range test. $V = 0.000094602 \times (D^{(1.88156-0.0030651\times(D+H))}) \times (H^{(0.7684+0.0046574\times(D+H))})$.

Parameter List	High Growth (HG)	Medium Growth (MG)	Low Growth (LG)
Height/(m)	9.37 ± 0.57 a	8.90 ± 0.13 a	4.77 ± 0.20 b
DBH/(cm)	17.62 ± 0.39 a	16.60 ± 0.15 a	9.59 ± 0.94 b
$V/(m^3)$	0.122 ± 0.007 a	0.105 ± 0.003 b	0.023 ± 0.004 c
year	9	9	9

2.3. Soil and Leaf Nutrient Determination

After being air-dried at room temperature in a shaded area for one week and sieved, the pine leaves were dried and ground with a ball mill and 0.1 g samples (accurate to 0.0001 g) were taken for analysis. The total nitrogen of rhizosphere soil and pine leaves were determined by the sulfuric acid digestion and sodium salicylate method; the total phosphorus was determined by the sulfuric acid digestion and molybdate-antimony anti-method. Total nitrogen and total phosphorus analyses were performed using a fully automated chemical sequential analyzer (De Chem-Tech. GmbH, Hamburg, Germany). The organic carbon of rhizosphere soil and total carbon in leaves were determined by potassium dichromate oxidation; the soil pH was measured by potentiometry. The specific methods were consistent with those described by Bao [35].

2.4. Determination of Soil Extracellular Enzyme Activity

β-1,4-Glucosidase (BG, EC 3.2.1.21), *β*-1,4-N-acetylglucosaminidase (NAG, EC 3.2.1.14), and acid phosphatase (AP, EC 3.1.3.1) activities were determined in this study. These three enzymes are resistant to denaturation and have relatively long half-lives [36]. After soil sample processing, the enzyme activity was measured based on the fluorescence method using a standard set reported by German [37] with a mesh size of 30–50.

2.5. Molecular Identification of the Ectomycorrhizal Community

2.5.1. DNA Extraction, PCR Amplification, and Illumina Sequencing

DNA extraction, PCR amplification, and Illumina sequencing of mycorrhizal fungi were completed by Novogene Co., Ltd. (Beijing, China). The genomic DNA of the samples was extracted by the CTAB method [38] and then the extracted DNA purity and concentration were detected by agarose gel electrophoresis. Appropriate amounts of DNA were then placed into a centrifuge tube and diluted with sterile water to 1 ng/μL. The ITS1-1F region of fungi was amplified by PCR using the diluted genomic DNA as a template. The primers were ITS5-1737F (F: CTTGGTCATTTAGAGGAAGTAA and R: GCTGCGTTCTTCATCGATGC) [39]. The PCR products were examined by agarose gel electrophoresis at a 2% concentration, mixed thoroughly in equal amounts according to the concentration of the PCR products, and then examined again using agarose gel electrophoresis at a 2% concentration after which the target bands were recovered. Libraries were constructed using the NEBNext® Ultra™ IIDNA Library Prep Kit and the libraries were quantified by a Qubit instrument and Q-PCR; after the libraries were qualified, they were sequenced using NovaSeq6000.

2.5.2. Sequencing Data Processing

The samples were split from the downstream data according to the PCR amplification primer sequences and the reads were spliced using FLASH (V1.2.11, http://ccb.jhu.edu/software/FLASH/, accessed on 11 January 2023) software to obtain raw tags [40]. Finally, the clean tags were compared with the database using Vsearch software (version 2.8.1) to detect chimaera and removed to obtain the final effective tags [41].

2.5.3. Amplicon Sequence Variation (ASV) Noise Reduction and Species Annotation

For the effective tags obtained above, the DADA2 module in QIIME2 software (version 2) was used to reduce noise and filter out sequences with abundances less than five to obtain the final amplicon sequence variants (ASVs) and the feature list [42]. The obtained ASVs were subjected to species annotation by aligning them with a pretrained naive Bayes classifier using the classify-sklearn module in the QIIME2 software [43], thereby obtaining species-level information for each ASV based on the UNITEv8.2 database.

2.6. Data Analyses

Differences in rhizospheric soil nutrients, leaf nutrients, and extracellular enzyme activity among the three families of *P. massoniana* were analysed using one-way analysis of variance (ANOVA) in SPSS software (version 22.0, SPSS Inc. 2018) followed by Duncan's multiple range test at a significance level of 0.05. Pearson correlation analysis ($p < 0.05$, $p < 0.01$, and $p < 0.001$) was performed using the corrplot package in R software (version 4.0) to examine the correlations among the rhizospheric soil nutrients, leaf nutrients, extracellular enzyme activity, and growth performance of *P. massoniana*. Simper analysis (similarity percentage) [44] was conducted using the vegan package in R software (version 4.0) to quantify the contribution of ECM fungal species to the differences among the three families of *P. massoniana.* A structural equation model was constructed using AMOS software (version 4.0) to examine the relationship between the dominant ECM genera of *P. massoniana* and extracellular enzyme activity. Bar charts were drawn using Origin 8.9.0 (version 2021, Origin Inc. 2021) software. The results of denoised ASVs were visualized using the Draw Venn Diagram platform (https://bioinformatics.psb.ugent.be/webtools/Venn/, accessed on 11 January 2023) to generate a Venn diagram illustrating the shared and unique ASVs of ECM fungal communities in the three families of *P. massoniana*. Redundancy analysis (RDA) was conducted using Canoco5.0 [45] software (Microcomputer Power, Inc., Ithaca, NY, USA) to study the effects of *P. massoniana* growth performance and rhizosphere soil nutrient content on the composition of ectomycorrhizal fungal communities.

3. Results

3.1. Rhizosphere Soil and Leaf Nutrients, Extracellular Enzyme Activities, and Ratio Characteristics of P. massoniana Families

The results revealed that the nitrogen and phosphorus content in the leaves of the HG family was significantly higher than that of the MG and LG families (Figure 1B,C). Specifically, the leaf nitrogen content (leaf N) in all three families ranged from 9.53 to 12.27 g/kg. Moreover, the patterns of soil organic carbon (SOC) and total nitrogen (TN) in the rhizosphere soil exhibited a similar trend, with the HG family displaying significantly higher content compared to the MG and LG families (Figure 1A,B). However, no significant differences were observed in terms of the content of leaf carbon (Leaf C) and total phosphorus (TN) in rhizosphere soil (Figure 1A,C).

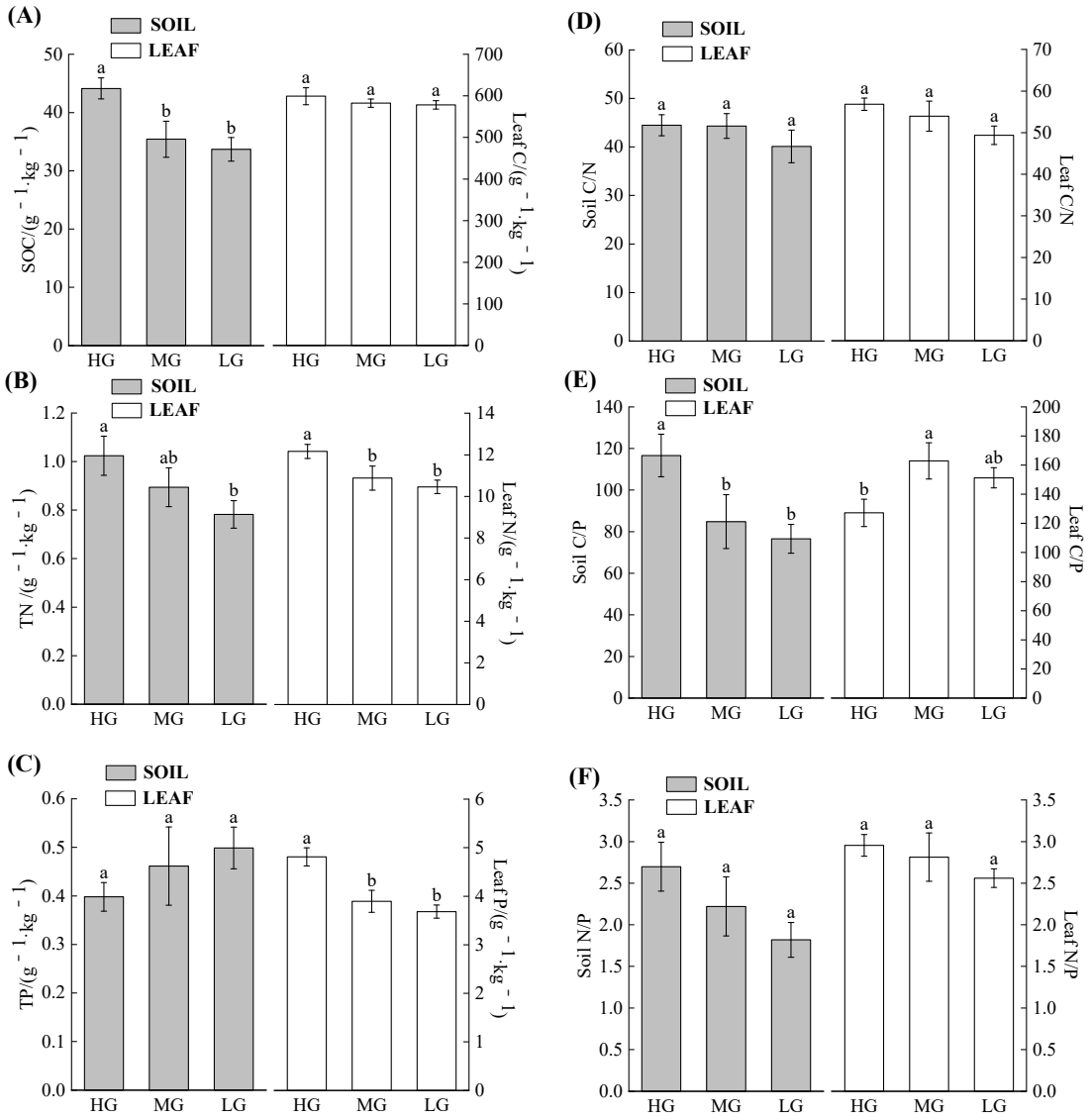

Figure 1. Rhizosphere soil and leaf nutrients and their ratios in *P. massoniana* families with different growth levels. Note: mean ± standard error, different lowercase letters indicated significant differences ($p < 0.05$) based on Duncan's multiple range test. Note: TN, total nitrogen of the rhizosphere soil; TP, total phosphorus of the rhizosphere soil; C, carbon; C/P, carbon-to-phosphorus ratio; N/P, nitrogen-to-phosphorus ratio; C/N, carbon-to-nitrogen ratio; HG, high growth; MG, medium growth; and LG, low growth. The subgraphs letters denote (**A**) SOC and C (carbon); (**B**) TN, total nitrogen of the rhizosphere soil; (**C**) TP, total phosphorus of the rhizosphere soil; (**D**) C/N, carbon-to-nitrogen ratio; (**E**) C/P, carbon-to-phosphorus ratio; (**F**) N/P, nitrogen-to-phosphorus ratio.

In terms of nutrient ratios, only the carbon-to-phosphorus ratio (C/P) exhibited significant differences among the three families, as depicted in Figure 1D–F. Specifically, the rhizosphere soil carbon-to-phosphorus ratio (Soil C/P) was found to be higher in the HG family compared to the other two families. Conversely, the carbon-to-phosphorus ratio in the leaves (Leaf C/P) was considerably lower in the HG family in comparison to the MG family, with no significant difference observed in relation to the LG family. It was noteworthy that the nitrogen-to-phosphorus ratio (N/P) in the leaves of all three families were found to be below 10 (Figure 1F). This observation implied the existence of potential variations in nutrient allocation strategies among the families of *P. massoniana*.

Furthermore, the acid phosphatase (AP) activity was found to be the highest among the extracellular enzymes analyzed in this study, with values averaging around 2000 $u^{-1} \cdot g^{-1} \cdot h^{-1}$ (Figure 2). Notably, the AP activity in the rhizosphere soil of the HG family exhibited a significantly higher level compared to the MG and LG families, with an increase of approximately 40%. A similar pattern was observed in the activities of *β*-1,4-N-acetylglucosaminidase (NAG), with a much greater increase observed in the HG family ranging from 77% to 129% (Figure 2). It is worth mentioning that the HG family also exhibited higher activity of β-1,4-glucosidase (BG) compared to the LG family but showed no significant difference compared to the MG family (Figure 2).

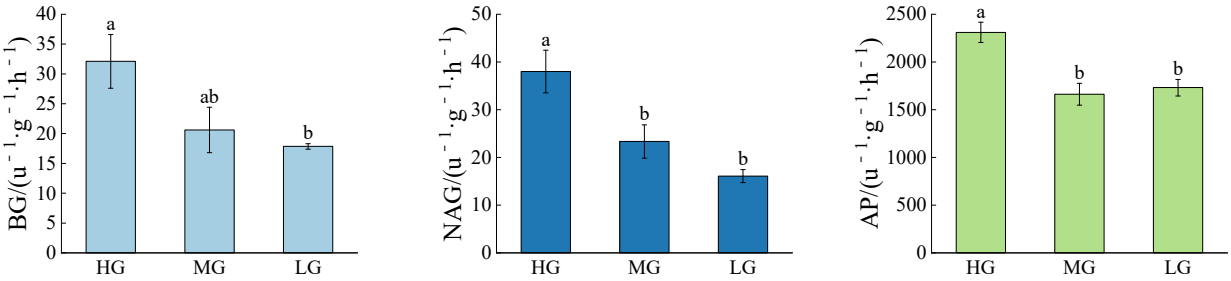

Figure 2. Extracellular enzyme activities in the rhizosphere soil of *P. massoniana* families with different growth levels. Note: mean ± standard error, where different lowercase letters indicate significant differences ($p < 0.05$) based on Duncan's multiple range test. Note: BG, *β*-1,4-glucosidase; NAG, *β*-1,4-N-acetylglucosaminidase; AP, acid phosphatase; HG, high growth; MG, medium growth; and LG, low growth.

3.2. Community Composition of ECM Fungi

Based on the ASVs' results, a Venn diagram was constructed to illustrate the distribution of ASVs among different families of *P. massoniana*. The diagram revealed that, in addition to 81 shared ASVs, the HG, MG, and LG families possessed 148, 132, and 132 unique ASVs, respectively (Figure 3). These findings highlighted the presence of unique microbial populations within each family. Additionally, the most dominant ectomycorrhizal (ECM) fungal genera in the HG family were *Amphinema* P. Karst. (30.5%) and *Pseudoclathrosphaerina* Voglmayr. (13.4%) (Figure 4). In the MG family, the ECM fungal genera *Auricularia* Bull. and *Russula* Pers. were found to be the most abundant, representing 14.0% and 10.2% of the total community, respectively. *Russula* was also dominant in ECM fungal community in the LG family, followed by *Amanita* Pers. (15.6%) (Figure 4).

Using simper analysis, the Bray–Curtis dissimilarity index of ECM fungal community composition among the three families of *P. massoniana* was decomposed. The results indicated that the main contributor to this dissimilarity index was *Amphinema* (contribution: HG-MG 0.225, HG-LG 0.17). Additionally, other genera with an abundance of less than 0.1% and unidentified genera also made substantial contributions (contribution: HG-MG 0.25 and HG-LG 0.225) (Figures 5 and 6). These findings underscored the distinctive composition and contribution patterns exhibited by ECM fungal genera within the HG family.

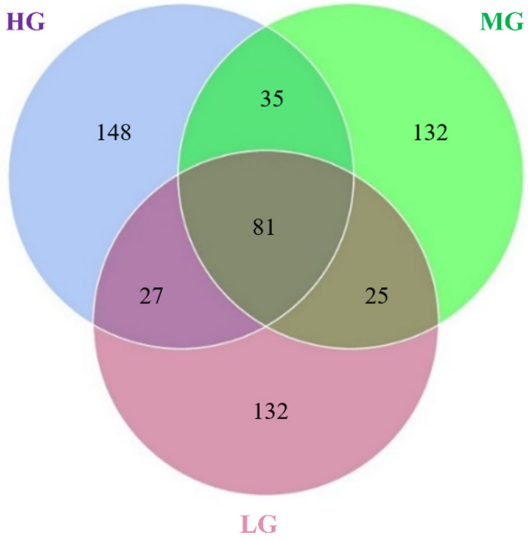

Figure 3. ASV Venn diagram of *P. massoniana* families with different growth levels. Note: HG, high growth; MG, medium growth; and LG, low growth.

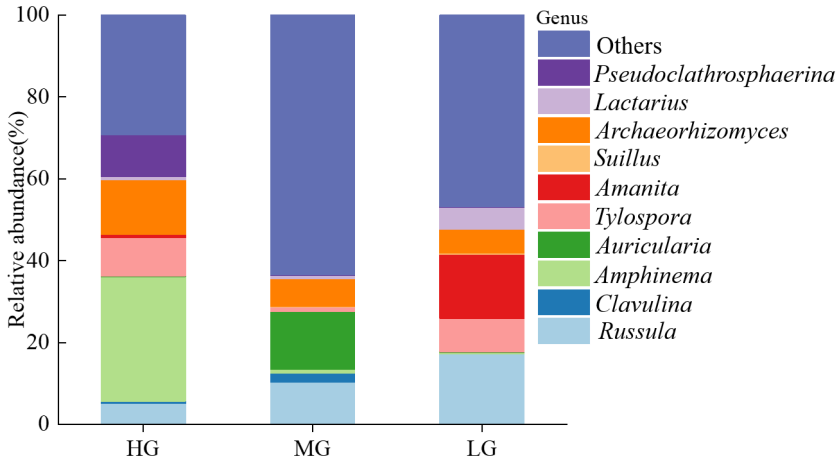

Figure 4. Relative abundance distribution of ECM fungal communities (genus level) of *P. massoniana* families with different growth levels. Note: All were the top 10 genera with average abundance > 0.1%. Note: HG, high growth; MG, medium growth; and LG, low growth.

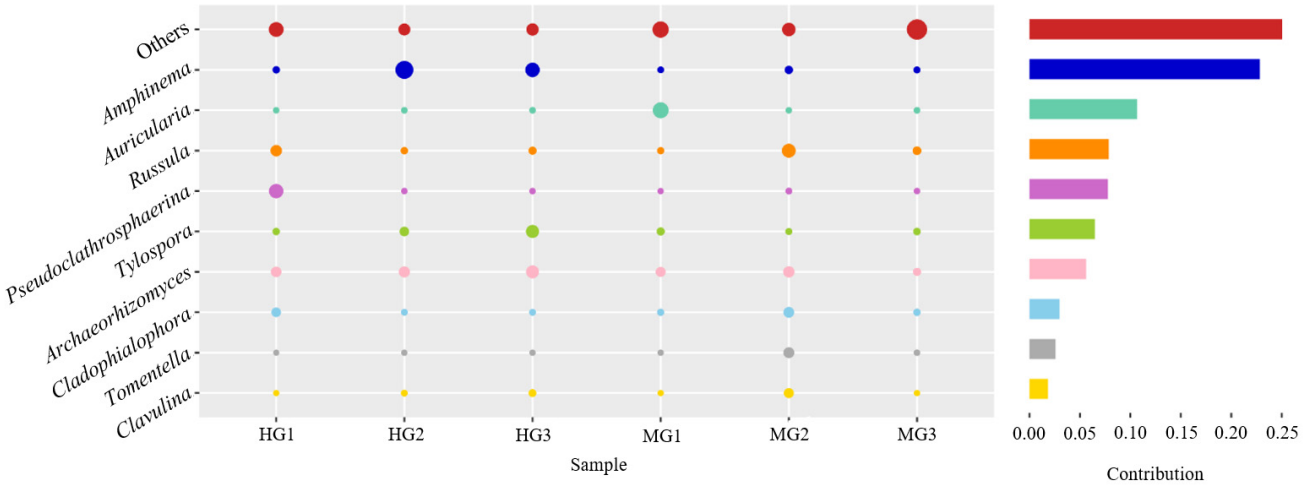

Figure 5. Contribution map of ECM fungal community composition in the HG and MG families of *P. massoniana*. Note: HG, high growth and MG, medium growth.

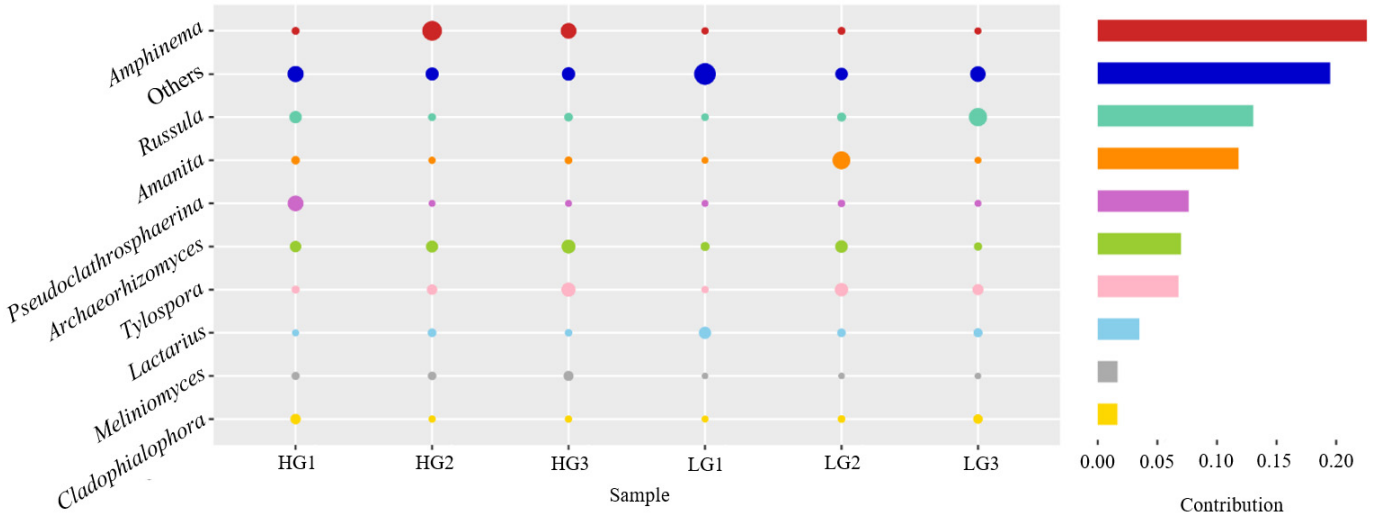

Figure 6. Contribution map of ECM fungi community composition in the HG and LG families of *P. massoniana*. Note: HG, high growth and LG, low growth.

3.3. Relationships between the Soil Extracellular Enzyme Activities, Nutrients, and Growth Performance of P. massoniana

Pearson correlation analysis showed significant correlations between the three soil enzyme activities and the growth performance, rhizosphere soil nutrients, and leaf nutrient contents (Figure 7). Extracellular enzyme activities in the rhizosphere soil displayed a moderate to strong positive correlation with the growth performance of *P. massoniana*, such as Height, DBH and V. There was no significant correlation observed between BG and NAG or between BG and AP. However, NAG exhibited a significant correlation with AP. Extracellular enzyme activities in the rhizosphere soil exhibited a non-significant correlation with leaf and rhizosphere soil nutrients for BG. However, AP and NAG demonstrated a significant positive correlation with Leaf P, SOC, and Soil N while displaying a negative correlation with Leaf C/P and Leaf N/P. Similar trends were also observed in the growth performance parameters. These findings implied that NAG and AP may have a more prominent role in nutrient dynamics.

3.4. Relationships between ECM Fungal Communities and Extracellular Enzyme Activity, Soil Nutrients, and Growth Performance

Redundancy analysis (RDA) was conducted with the ECM fungal abundance of *P. massoniana* as the response variable and soil chemical properties and tree growth performance as explanatory variables. The results showed that the first two axes of the RDA explained 44.16% of the total microbial variation (Figure 8). The Monte Carlo test indicated significant correlations ($p < 0.05$) between tree growth performance (Height, DBH, and V), soil total nitrogen (TN), soil N/P, and ECM fungal community composition (Table 2).

Specifically, the growth performance of *P. massoniana* exhibited a positive correlation with the following genera: *Amphinema*, *Archaeorhizomyces* Rosling and T.Y. James, *Clavulina* J. Schroöt., *Pseudoclathrosphaerina*, and *Russula* (Figure 8). Furthermore, soil TN and soil N/P demonstrated a positive correlation with *Amanita*, *Tylospora* Donk., *Amphinema*, *Archaeorhizomyces*, and *Clavulina* but displayed a negative correlation or no correlation with other genera.

Structural equation modeling was used to analyze the relationship between the dominant ECM genera and three extracellular enzyme activities. The results showed that the dominant genera *Amphinema* and *Pseudoclathrosphaerina* had a positive effect on the activities of the three enzymes (Figure 9).

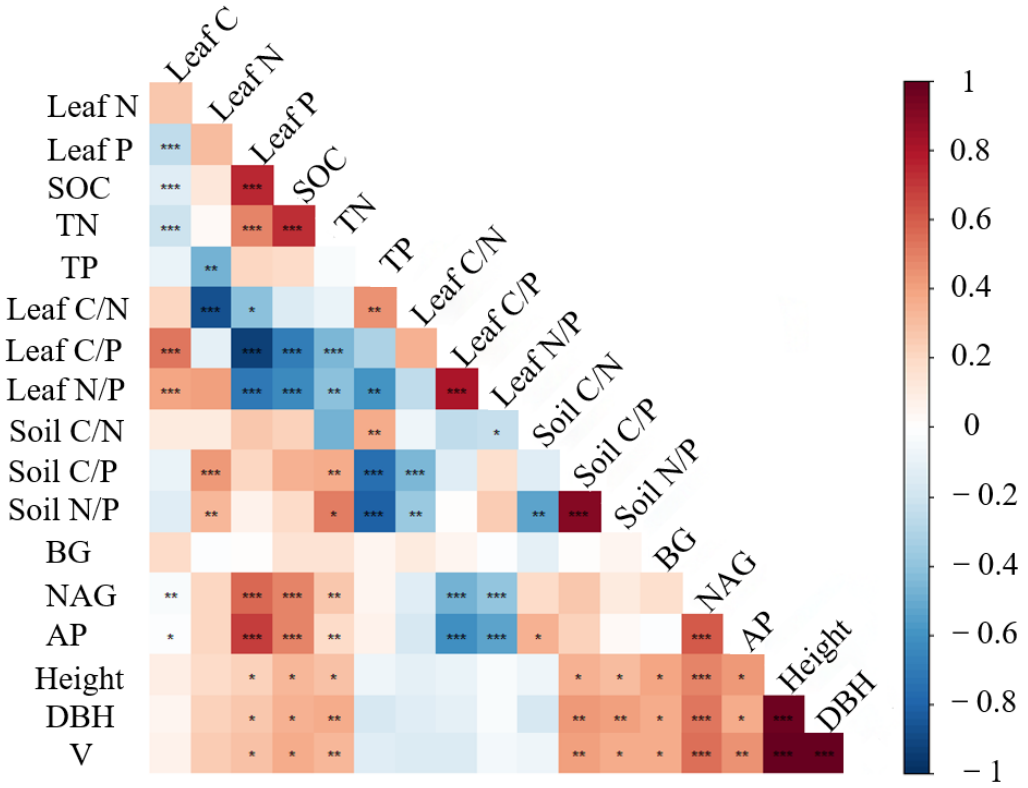

Figure 7. Pearson correlation test. Note: V, timber volume; Height, tree height; DBH, diameter at breast height; BG, β-1,4-glucosidase; NAG, β-1,4-N-acetylglucosaminidase; AP, acid phosphatase; TN, total nitrogen of the rhizosphere soil; TP, total phosphorus of the rhizosphere soil; C, carbon; C/P, carbon-to-phosphorus ratio; N/P, nitrogen-to-phosphorus ratio; C/N, carbon-to-nitrogen ratio. * represents $p < 0.05$, ** represents $p < 0.01$, *** represents $p < 0.001$.

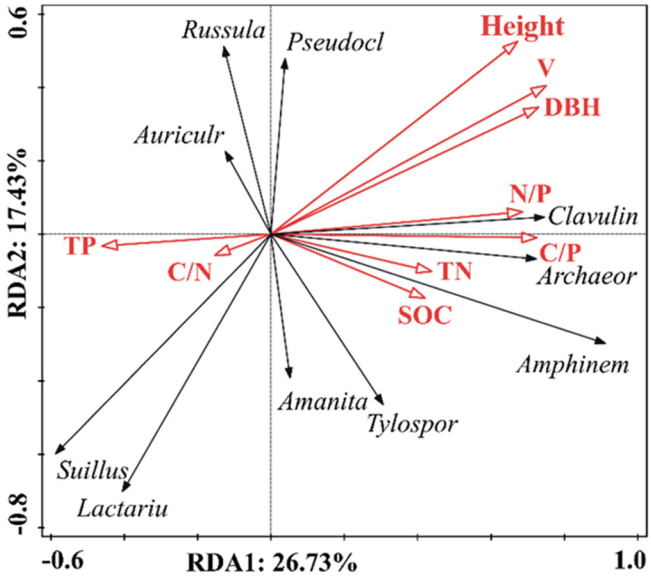

Figure 8. Relationship between the top 10 ECM fungi (>0.01%) in terms of relative abundance, soil nutrients, and growth performance of *P. massoniana*. Note: *Clavulina* (*Clavulin*), *Amphinema* (*Amphinem*), *Auricularia* (*Auriculr*), *Tylospora* (*Tylospor*), *Archaeorhizomyces* (*Archaeor*), *Lactarius* (*Lactariu*), *Pseudoclathrosphaerina* (*Pseudocl*); V, timber volume; Height, tree height; DBH, diameter at breast height; TN, total nitrogen of the rhizosphere soil; TP, total phosphorus of the rhizosphere soil; C/P, carbon-to-phosphorus ratio; N/P, nitrogen-to-phosphorus ratio; C/N, carbon-to-nitrogen ratio.

Table 2. **Table 2.** Conditional effects of soil nutrients and growth performance on ECM fungal community composition. Explanation (%): the explanation rate of a single variable relative to the total variation. Note: V, timber volume; Height, tree height; DBH, diameter at breast height; TN, total nitrogen of the rhizosphere soil; TP, total phosphorus of the rhizosphere soil; C/P, carbon-to-phosphorus ratio; N/P, nitrogen-to-phosphorus ratio; C/N, carbon-to-nitrogen ratio. * $p < 0.05$, ** $p < 0.01$.

Variables	Explanation %	*F* Value	*p* Value
V	19	5.9	0.002 **
Height	18.8	7.3	0.002 **
DBH	6.8	3.1	0.026 *
TN	8	3.4	0.012 *
TP	3.5	1.9	0.11
SOC	0.1	<0.1	0.992
N/P	7.3	3.8	0.008 *
C/P	1.3	0.7	0.5
C/N	0.6	0.3	0.82

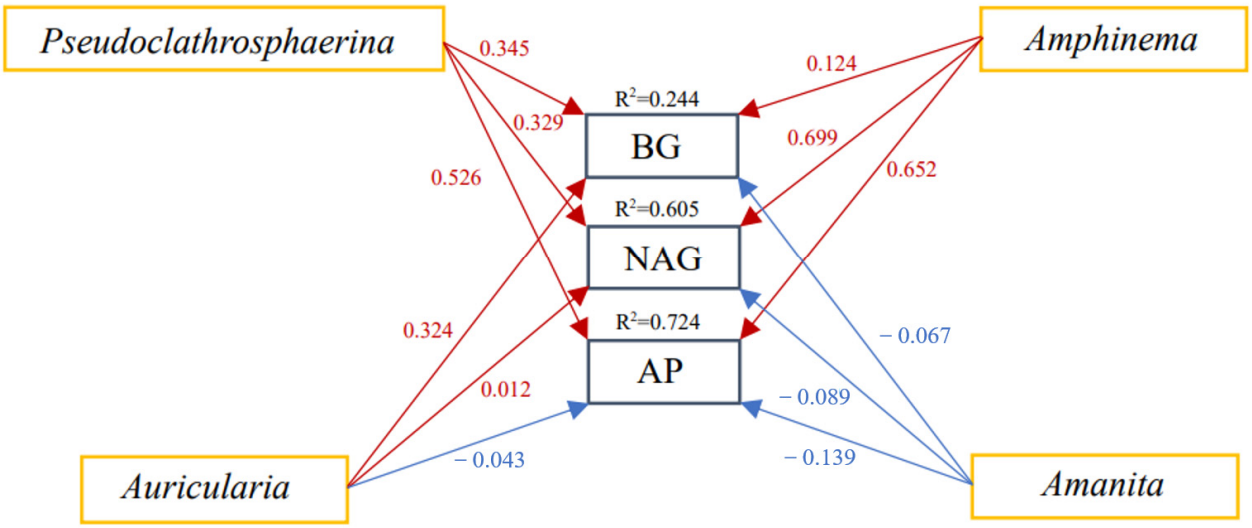

Figure 9. Structural equation model of the dominant genera and extracellular enzyme activities of *P. massoniana*. Note: x2 = 8.752, DF = 9, $p = 0.460$, GFI = 0.925, RMSEA = 0.000. The red and blue arrows represent positive and negative pathways, respectively. The numbers adjacent to the arrows are standardized path coefficients. Note: BG, β-1,4-glucosidase; NAG, β-1,4-N-acetylglucosaminidase; AP, acid phosphatase.

4. Discussion

4.1. Effects of Different Growth Families of P. massoniana on Rhizosphere Soil and Leaf Nutrients

In this study, the range of leaf total nitrogen (leaf N) contents in *P. massoniana* was 9.53–12.27 g/kg (Figure 1B), lower than the global average of 20.06 g/kg [46] and the average of Chinese plants measured at 20.20 g/kg [47]. The N/P ratio has been widely used to predict nutrient limitation of plant growth [48]. All N/P ratios of *P. massoniana* leaves in this study (Figure 1F) were less than 10 (ranging from 2.56 to 2.95), indicating a N limitation [49] consistent with the findings of previous studies [50]. Although N/P ratios have been widely used for determining nutrient limitations in plants, an accurate prediction of the optimal N limitation for *P. massoniana* requires further investigation due to variations in plant N nutrient levels over time and differences in optimal N/P ratios among different tree species [51]. Among the three families of *P. massoniana*, the leaf total nitrogen and phosphorus contents of the HG family were significantly higher than those of the MG and LG families (Figure 1B,C) and positively correlated with tree growth performance to varying degrees. It has been previously reported that plants with a higher growth rate have

lower leaf C/P ratios than slower-growing individuals [52]. An increase in the leaf C/P ratio indicates a decrease in the plant growth rate [53]. The significantly lower leaf C/P ratio in the HG family (Figure 1E) suggests that the higher growth rate of the HG family results in a large amount of nitrogen and phosphorus being stored in the leaves to support rapid growth, supporting the growth rate hypothesis [54].

In this study, the rhizosphere soil SOC content and three extracellular enzyme activities were significantly higher in the HG family than in the MG and LG families (Figures 1A and 2) and the growth performance (height, DBH, and volume) of *P. massoniana* was significantly positively correlated with rhizosphere soil nutrients (SOC and TN) and the activities of three soil extracellular enzymes (BG, NAG and AP) (Figure 8). Previous studies have shown that there are significant differences in root biomass allocation patterns among different genotypes (provenances, clones, or cultivars) of the same species [55,56] and, as an important component of soil organic carbon (SOC) [57,58], root biomass plays an important role in the input and storage of soil carbon [46]. The three different growth families of *P. massoniana* selected in this study showed differences in height, DBH, and volume (Table 1) and, according to the "quality ratio effect", taller and more extensive plant individuals have higher productivity and more complex underground functions than shorter and less extensive individuals, producing greater biomass [59–61]. Therefore, the high-growth family of *P. massoniana* could input more carbon into the rhizosphere soil, providing a large amount of substrate for extracellular enzymes, which in turn can affect their activities. The increase in extracellular enzyme activity can promote the release of nutrients in the rhizosphere soil and benefit the growth of *P. massoniana*. This study also showed that the rhizosphere soil C/P ratio of the HG family was significantly higher than that of the other two families and that the soil total nitrogen content of the HG family was significantly higher than that of the LG family. Previous studies have shown that a higher nitrogen content and C/P ratio can promote the accumulation of soil organic matter (SOM) [62]. SOM in forest soil mainly comes from plant inputs on the surface (plant litter) or underground (plant roots); soil organic carbon (SOC) is one of its main sources [63]. Therefore, it is speculated that the differences in rhizosphere soil organic carbon among the families with different growth levels are likely to be caused by their growth differences. The difference in rhizosphere soil C/P ratio between the HG family and the other two families also indicates that the mineralization ability of phosphorus varies between high-growth and low-growth families of *P. massoniana* [64]. The significant positive correlations between the rhizosphere soil C/P ratio and the height and DBH of *P. massoniana* confirm this inference.

4.2. Effects of Different Growth Families of P.massoniana on ECM Community Composition and Extracellular Enzyme Activity

Figure 4 revealed differences in the dominant ECM fungal genera and their abundances at the genus level among the three families, with different dominant genera for each. The dominant genus in the ECM fungal community of the high-growth family (HG) was *Amphinema* (30.5%) followed by *Pseudoclathrosphaerina* (13.4%); *Auricularia* (14.0%) was the dominant genus in the medium-growth family (MG) and *Russula* (17.2%) in the low-growth family (LG) of *P. massoniana*. Although the ECM fungal communities had similarities in their function as mycorrhizae, there were differences in the colonization habits and decomposition and utilization of soil nutrients among different ECM fungal species [65]. Moreover, differences in extracellular enzyme activities can also reflect differences in the composition of ECM fungal communities [66]. In this study, the NAG and AP extracellular enzyme activities in the rhizosphere soil of the HG family were significantly higher than those in the MG and LG families, while the BG activity was significantly higher in the LG family than in the MG family but not significantly different from that in the HG family (Figure 2), indicating a distinct ECM fungal community composition and stronger nutrient decomposition and release ability in the rhizosphere of the HG family which were beneficial for its growth. Furthermore, beyond the top 10 abundant ECM fungal genera shared among the three families of *P. massoniana*, the MG and LG families harbored other

fungi at abundances lower than 0.1%, accounting for 63.53% and 46.80%, respectively, while the representation of other fungi with abundances less than 0.1% in the HG family was 29.30% (Figure 4), possibly related to the carbon source supply of the host plant to its own ECM mycorrhizae [2]. The superior growth performance of the HG family was attributed to its unique ECM fungal community, which was different from that of the MG and LG families, enabling it to achieve greater aboveground and belowground biomass. Therefore, compared with the MG and LG families, the HG family provided more colonization space and carbon sources (for fungi such as *Amphinema* and *Pseudoclathrosphaerina*) for the ECM fungal community, resulting in differences in the shared and dominant genera of ECM fungi among the three families with different growth levels. The RDA also showed that the growth performance (volume, height, and DBH) of *P. massoniana* was an important factor affecting the abundance of ECM fungi (Figure 8).

Differences in ECM fungal communities and extracellular enzyme activity in the rhizosphere soil of *P. massoniana* may reflect different nutrient acquisition modes. Prior research indicates that fungi with widespread hyphal growth provide less nitrogen to host plants [67], which may explain the higher NAG activity in the rhizosphere soil of the HG family. As a dominant fungal genus in the HG family, *Amphinema* possesses abundant external mycelia [68] and produces a large number of nitrogen-acquiring enzymes by oxidizing SOM to meet its own and the host plant's nitrogen requirements. The positive effect of *Amphinema* on NAG in the structural equation model also confirms this mechanism (Figure 9). Compared to those of the MG and LG families, the ECM fungal community and extracellular enzyme activity in the HG family appear to better match the nutrient acquisition model proposed by Hupperts et al. [69].

In addition, the rhizosphere soil SOC of the HG family was significantly higher than that of the other two families (Figure 1), which may also be related to the impact of ECM fungal changes on soil carbon accumulation. In the study by Weight et al. [70], long-distance exploring ECM fungal groups, namely those capable of producing abundant external mycelia, can provide more biomass than short-distance exploring ECM fungi and their hyphae typically have hydrophobic surfaces and relatively low rhizosphere soil contact areas [71], resulting in their hyphae being more persistent than those of short-distance groups [72]. Therefore, in this study, the differences in the ECM fungal community structure of *P. massoniana* families with different growth levels may have had an impact on the input of fungal necromass into the soil carbon pool, where the ECM fungal community of the HG family, dominated by *Amphinema*, may be more conducive to SOC accumulation in the soil compared to the ECM fungal communities of the MG and LG families.

This study also revealed evidence of carbon sources for *P. massoniana* ECM fungal communities other than host plants. In the correlation analysis (Figure 7), rhizosphere soil organic carbon had varying degrees of positive correlation with three extracellular enzymes, as enzyme activity has a strong dependence on soil carbon and is sensitive to changes in soil SOC [23,73]. Although there was a certain positive correlation between organic carbon and the BG enzyme, it was not significantly associated with NAG and AP enzymes which may be related to the carbon source of soil microorganisms. The soil carbon pool is one of the carbon sources for soil microorganisms [74] where the extracellular enzymes BG and NAG can help microorganisms obtain carbon by decomposing different substrates. When cellulose decomposed by BG is the main carbon resource in the soil, microorganisms use more cellulose than chitin and peptidoglycan degraded by NAG. However, under conditions limited by environmental factors, such as chitin and peptidoglycan, cellulose would become the main substrate for microorganisms to utilize [75]. In this study, we explored the impact of nitrogen limitation on *P. massoniana*. We found that the NAG enzyme plays a critical role in catalyzing chitin decomposition into carbohydrates and inorganic nitrogen [76]. As a result, *P. massoniana* may release more NAG enzymes through root exudates, influencing ECM fungal communities and obtaining more nitrogen to alleviate its own nitrogen stress. Additionally, this alleviation mechanism provides a source of carbon for the ECM fungal community by decomposing a significant amount of chitin and peptidoglycan that NAG can break down.

5. Conclusions

This study explored the ECM fungal community composition, rhizosphere soil extracellular enzyme activity and nutrient characteristics, and growth performance of different growth families of *P. massoniana*. The high-growth family of *P. massoniana* displayed a higher leaf nutrient content (leaf N and leaf P), allowing it to cope with the high nutrient demand from its rapid growth. The application of appropriate nitrogen fertilizer can help alleviate *P. massoniana*'s nitrogen limitations in the study area. Furthermore, the high-growth family of *P. massoniana* displayed a higher abundance of ECM fungal communities that favor its growth and enable soil SOC accumulation. This family allocated more carbon sources to promote the secretion of more extracellular enzymes by ECM fungal communities, resulting in higher enzyme activity in the rhizosphere and an increase in phosphorus mineralization. In this way, the high-growth family provided a source of carbon for the ECM fungal communities beyond the host plants. Overall, this study offers important insights into the ECM fungal community structure, rhizosphere soil extracellular enzyme activity, and nutrient characteristics of *P. massoniana* with different growth levels. The findings provide guidance for a deeper understanding of the relationship between growth performance and the belowground environment of *P. massoniana*.

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