Impact of Thinning Intensity on Ectomycorrhizal Fungal Communities in Pinus Massoniana Plantations

Zhongxuan Huang
Sichuan Agricultural University
Xiangjun Li
Sichuan Agricultural University
Xin Zhang
Sichuan Agricultural University
Jingwen Peng
Sichuan Agricultural University
Cheng Huang
Sichuan Agricultural University
Yongqi Xiang
Sichuan Agricultural University
Chuan Fan
Sichuan Agricultural University
Gang Chen
Sichuan Agricultural University
Xianwei Li
lxw@sicau.edu.cn

Sichuan Agricultural University

Research Article

Keywords: ECM diversity, Community structure; Co-occurrence Network, thinning

Posted Date: January 22nd, 2026

DOI: https://doi.org/10.21203/rs.3.rs-8597886/v1

Additional Declarations: No competing interests reported.

Abstract

Ectomycorrhizal (ECM) fungal play an indispensable role in promoting nutrient cycling within forest ecosystems. However, the mechanism by which thinning regulates ECM fungal communities through its effects on soil and plant fine roots are still unclear. To elucidate this, we established a thinning experiment in a 29-year-old low production *Pinus Massoniana* plantation in southwest China, subjected to four thinning intensities in 2018: 0% (CK, control), 10% (low-intensity thinning; LIT), 30% (moderate-intensity thinning; MIT), and 50% (high-intensity thinning; HIT). Results demonstrated that thinning significantly reduced soil pH (1.45%), soil bulk density (9.70%), and available phosphorus (13.59%), while leaving other soil factors unaffected. All thinning intensities (LIT, MIT, HIT) significantly increased fine root biomass (by 32.36%, 54.47%, and 18.78%, respectively) and fine root total nitrogen (by 53.76%, 116.73%, and 107.71%, respectively). Furthermore, it induced significant shifts in the diversity and composition of the ECM fungal community. The complexity of the ECM fungal co-occurrence network initially increased and then decreased with increasing thinning intensity, exhibiting a recurring complexity pattern. The RDA identified the soil C/P ratio as the key factor shaping the community. A partial least squares regression-structural equation model (PLS-SEM) confirmed that thinning directly altered ECM community composition and fine root nutrients, largely independent of soil nutrient changes. In conclusion, our study highlights that thinning regulates Ectomycorrhizal fungal communities primarily through the modification of host fine root traits rather than direct soil nutrient shifts, emphasizing the importance of plant-soil-microbe feedback in forest ecosystem recovery.

INTRODUCTION

Thinning, as the primary measure for density regulation, is one of the key measures for oriented cultivation for large-diameter timber (Qiu et al., 2019; Ren et al., 2024). Thinning enhances soil physicochemical properties, improves understory plant diversity and promotes forest growth by reducing competition among trees within a stand and increasing light and heat penetration. Wang et al (2021) found soil ecosystem functions of low-density plantations are generally better and stable than high-density plantation, which is essential for maintaining soil fertility and improving forest ecosystem productivity. Zhang et al. (2023) found thinning increased soil temperature by 13% and soil moisture by 8.0%. Qiang et al (2023) found the diversity and richness of the entire fungal community and rare taxa increased with the understory vegetation coverage and shrub biomass after thinning, which may accelerate nutrient cycling in forest ecosystems. Thinning increased the fine root yield, biomass, and turnover rate in Chinese Fir (*Cunninghamia lanceolata*) plantation (Wang et al., 2019). High-density stands had lower root length density than low-density stands, implying intense intraspecific competition causing root segregation (Li et al., 2025). However, research on the effects of thinning on soil microorganisms, particularly ectomycorrhizal (ECM) fungi, remains relatively limited. Zhou et al. (2020) revealed that thinning significantly altered soil microbial community structure but had no effect on total microbial biomass though META-analysis. However, Roy (2024) found that thinning has no significant effect on saprophytic fungi or fungal phylogenetic diversity. Therefore, the mechanisms through which thinning affects ectomycorrhizal fungi require further investigation.

Fine roots account for only 2–5% of the total forest biomass but contribute 22–33% of the annual net primary productivity of forest ecosystems (Finér et al., 2019; Kernaghan et al., 2025). Root systems provide unique ecological niches and carbon sources for the colonization and growth of ECM fungi. Changes in root traits and nutrient supply regulate the relative contributions of deterministic and stochastic processes to the assembly of ECM communities (Kou et al., 2024). Plants drive the assembly of root-associated microbial communities by releasing exudates, with glutamine exerting a pronounced chemotactic effect on microorganisms (Tsai et al., 2025). ECM symbiosis positively influences root structure and nutrient absorption, enhancing seeding growth in chestnut and pecan(Chen et al., 2025). Thinning increases the distribution of photosynthates to the underground part (López et al., 2003), and the contents of C, N, P and K in fine roots would inevitably change, among which N was the key limited factor of fine roots, and the amount of N content directly affected turnover and death of fine roots. Studies showed that both mortality and turnover of fine roots increased with the increasing thinning intensity (Wang et al., 2019). Therefore, we hypothesized that changes in ECM community composition had a special relationship with fine roots nutrient.

ECM fungi enhance nutrient utilization efficiency by establishing stable symbiotic relationships with plants, thereby mediating the influence of plants on ecosystem functions (Medina-Vega et al., 2024; Song, 2024). ECM fungi extend their hyphae into the soil, enabling them to acquire nutrients from beyond the immediate vicinity of the roots and facilitating plant absorption of C, N, P, K, and other essential nutrients (Lambers et al., 2008; Luginbuehl et al., 2017; Shi et al., 2023; van der Heijden et al., 2015). ECM fungi can produce phosphatase and phytase to promote the mineralization of Po (Burke et al., 2014), which in turn liberates Pi for plant uptake (Qi et al., 2022; Treseder & Lennonb, 2015). ECM enriches beneficial soil bacteria linked to enhanced plant growth, and it modifies plant root chemistry to enhance stress resistance (Berrios et al., 2024). While existing research has extensively explored the symbiotic relationship between ectomycorrhizal fungi and fine roots, the mechanisms through which different thinning intensities affect this symbiotic system remain unclear.

China has the largest plantation area in the world (FAO, 2020). As an important afforestation and economic tree species in south China, *Pinus Massoniana* was widely distributed in subtropical regions, with a planting area of more than 8 million hectares(Lan et al., 2025). Due to the lack of scientific management measures, the plantation productivity decreases sharply, and diseases and insect pests occur frequently, which seriously restricts the sustainable development of forests (Cheng et al., 2017). In order to promote the productivity of low-efficiency plantations of *P. massoniana* and restore the stability of the ecosystem, we designed three thinning treatments along with a control. Soil physicochemical properties, fine root quadrats, and ECM fungi were measured. This study aims to investigate whether thinning alters the diversity and community composition of ECM fungi, and to further clarify the soil and root factors influencing their diversity and community structure, providing insights for cultivating large-diameter timber and restoring and conserving plantation ecosystems.

MATERIALS AND METHODS

Study Area

The research was conducted in *P. massoniana* plantations on Jinzi Mountain, Yuntai town, Pingchang County (N. 31°37′06"—31°37′20", E. 107°14′40"—107° 15′03"), which is located in the northeast Sichuan Basin. This region belongs to the stepped valley landform, with an altitude of 710–730 m. The region is characterized by a subtropical humid monsoon climate with a mean annual temperature of 16.8 ℃, precipitation of 1138.2 mm, 1365.5 daylight hours, and a frost-free period of 298 days each year. The soil is classified as yellow soil in this study area. The *P. massoniana* plantations were established in 1991. Canopy density was as high as 0.8, average DBH was 17.53 cm, average height was 17.06 m, and stand density was 1500 trees·hm^{-2}. There was no extra management of the plantation, and the understory vegetation mainly relied on natural regeneration, with low plant diversity. The understory dominant shrubs were *Myrsine africana*, *Eurya loquaiana*, and *Rhododendron simsii*, and the dominant herbs were *Miscanthus sinensis, Dicranopteris dichotoma*, and *Pteridium aquilinum.*

Sampling Design and Sample Collection

In June 2018, the stands with generally similar conditions of vegetation and landform were selected for thinning in the study area (Liu et al., 2025). A randomized block design was adopted in this experiment, the four treatments included no thinning (CK, control), 10% of the trees removed (low-intensity thinning; LIT), 30% of the trees removed (moderate-intensity thinning; MIT) and 50% of the trees removed (high-intensity thinning; HIT) (Liu et al., 2025). A total of twelve sample plots (30 × 20 m) were established, with three replicate plots randomly assigned to each of the four treatments. Thinning was implemented using selective cutting method to ensure the uniform distribution of retained trees in the plots, and the felled trees were removed from the plots. In order to lessen latent edge effects, a buffer zone (10 m) was set around each plot. The standard plots were separated by 100 m from each other (Zhang et al., 2023). Basic stand conditions of three different thinning treatment sites were determined (Table 1).

Table 1
Stand features of *P. massoniana* plantations with different thinning treatment.

Stand sites	Thinning intensity	Slope aspect	Gradient (°)	stand density (trees/600 m^2)
CK-1	0	Southeast	4	93
CK-2	0	Southeast	3	92
CK-3	0	Southeast	2	92
LIT-1	10%	South	4	81
LIT-2	10%	Southeast	4	81
LIT-3	10%	South	5	81
MIT-1	30%	Southeast	2	63
MIT-2	30%	Southeast	2	63
MIT-3	30%	South	2	63
HIT-1	50%	Southeast	2	45
HIT-2	50%	Southeast	3	45
HIT-3	50%	Southeast	2	45

In June 2020, mycorrhizal root tips were collected on six randomly selected trees per plot, with a root-cutting knife at a soil depth of 0–20 cm. The samples were placed in an ice box, transported to the laboratory within 48h, and stored in a refrigerator at 4 ℃ for no more than a week. ECM root tips were cleaned with 10 × PBS solution before DNA extraction. The samples were frozen at -80 ℃ Ultra-low Temperature Freezer. Subsequently, the samples were transported to Guangzhou Gedi Bio-Technology Co., Ltd. for DNA extraction.

Roots were collected with a steel auger (10 cm in internal diameter) at soil depths of 0–20 cm at each cardinal point 1 m away from the trunk of the tree and mixed to make a composite sample (Yuan & Chen, 2012). From each selected tree, four primary lateral roots were carefully dug out and traced back to the tree to confirm their origin (Liu et al., 2025). Samples were stored in plastic bags and then transported to the laboratory in ice-filled coolers. In the laboratory, roots of *P. massoniana* were separated from understory vegetation roots based on their morphological characteristics, only the fine roots (diameter ≤ 2 mm) were retained for this research. After the infected root tips were selected, the remaining fine roots were oven-dried at 65 ℃ to a constant weight for subsequent measurement of biomass and nutrient traits.

Soil samples were collected at a depth of 0–20 cm from each sample tree from three different directions. Litter, roots, gravel, and other impurities were removed from the soil. Soil samples for physical property analysis were collected with ring knives (diameter = 5 cm). Soil samples were air-dried indoors, ground and filtered through a 2 mm sieve for the determination of chemical properties.

Analysis of Soil Physicochemical Properties and Fine Roots Nutrient Contents

Soil physicochemical properties were analyzed following conventional methods (Lu, 1999). Soil water content (SWC) was measured by a Thermochron iButton Device (DS1921-G, Maxim Integrated, San Jose, CA, United States). A soil: water (1:5 w/v) suspension was shaken vigorously for 2 min and allowed to stand for 30 min to determine pH by a pH meter (LEICI, China; Bao, 2000). Soil temperature (ST) recorded by Thermochron iButton Device (DS1921-G, Maxim, Integrated, SanJose, CA, USA). Soil bulk density (SBD) was measured by using ring knife. Soil organic carbon (SOC) was measured by wet oxidation with potassium. The total nitrogen (TN) content was determined using the Kjeldahl method, and available nitrogen (AN) was determined by the alkali diffusion method. The total potassium (TK) was measured using the Alkaline fusion-flame photometric method. Available potassium (AK) was measured using the Ammonium acetate - flame photometry method. Total phosphorus (TP) was measured using the alkali fusion-Mo-Sb anti spectrophotometric method, available phosphorus (AP) was measured using the sodium hydrogen carbonate solution-Mo-Sb anti spectrophotometric method. Analysis of fine root organic carbon (ROC), fine root total nitrogen (RTN), fine root potassium kalium (RTK) and fine root total phosphorus (RTP) was the same as soil. Determination of fine root biomass (B): All graded roots were dried at 65℃ to constant weight (48 h) and the dry weight of the fibrous roots was measured with a balance to one ten thousandth of a gram.

DNA Extraction and PCR Amplification

Total genomic DNA was extracted from plant samples using the E.Z.N.A.® soil DNA Kit (Omega Bio-tek, Norcross, GA, U.S.) according to manufacturer's instructions. The DNA extract was checked on 1% agarose gel, and DNA concentration and purity were determined with NanoDrop 2000 UV-vis spectrophotometer (Thermo Scientific, Wilmington, USA). The fungal ITS region was amplified by PCR using primers ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3'). Amplifications were performed on an ABI GeneAmp® 9700 PCR thermocycler (Applied Biosystems, CA, USA). The PCR amplification of ITS RNA gene was performed as follows: initial denaturation at 95 ℃ for 2 min, followed by 27 cycles at 95 ℃ for 2 min, followed at 98 ℃ for 10 s, 62 ℃ for 30 s, and 68 ℃ for 30 s and a final extension at 68 ℃ for 10 min. The PCR mixtures contain 5 × TransStart FastPfu buffer 4 μL, 2.5 mM dNTPs 2 μL, forward primer (5 μM) 0.8 μL, reverse primer (5 μM) 0.8 μL, TransStart FastPfu DNA Polymerase 0.4 μL, template DNA 10 ng, and finally ddH_2O up to 20 μL. PCR reactions were performed in triplicate. The PCR product was extracted from 2% agarose gel and purified using the

AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) according to manufacturer's instructions and quantified using Quantus™ Fluorometer (Promega, USA).

Purified amplicons were pooled in equimolar and paired-end sequenced on an Illumina MiSeq PE300 platform/NovaSeq PE250 platform (Illumina, San Diego,USA) according to the standard protocols by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China). The clean tags were clustered into operational taxonomic units (OTUs) at a 97% similarity threshold using UPARSE (Edger, 2013; version 9.2.64) pipeline. All chimeric sequences were removed using UCHIME algorithm (Edger, 2011) and finally obtained effective sequences for further analysis. The sequence with highest abundance was selected as representative sequence within each cluster. The raw reads were deposited into the NCBI Sequence Read Archive (SRA) database (BioProject: PRJNA770507).

Statistical Analysis

All data is uploaded to the Omicsmart cloud platform (https://www.omicsmart.com), where ECM α diversity, community abundance, and correlations with environmental factors were calculated separately. Putative functions of the ECM fungi were predicted using the FunGuild software. The graphical representations, including box plots of soil physicochemical properties, bar charts of fine root nutrients and box plots of microbial diversity, were generated with Origin 2021 Pro software. For mean comparison, the Paired Comparison Plot plugin was applied with the Bonferroni method at a significance level of 0.05 to label significance levels. Principal Coordinate Analysis (PCA) was performed using the online platform ChiPlot (https://www.chiplot.online/), and their corresponding graphical representations were generated. Redundancy Analysis (RDA) was employed in Canoco 5 to examine how environmental variables relate to ECM fungal diversity and abundance, with the goal of pinpointing the key factors driving shifts in community composition (Ren et al., 2016). Microbial co-occurrence networks were constructed in R based on Spearman correlations (using the psych and Igraph packages; relative abundance > 0.5%). Subsequently, the networks were visualized in Gephi, and their topological metrics were calculated. Final image formatting was performed in Adobe Photoshop CS6.

Partial Least Squares Regression (PLSR) was employed to analyze the effects of thinning on soil physicochemical properties, fine root nutrients, and microbial communities. All indicators were imported into SmartPLS 4.0 to construct the initial model, and a bootstrap analysis was performed with a subsample size of 5000, a specified number of iterations, and a two-tailed test. The PLS algorithm was configured with the path weighting scheme, setting the maximum iterations to 5000 and the termination threshold for inner model changes (10^{-}□) to 10. Following this, the final model was estimated. After removing indicators with smaller path coefficients and performing multiple calculations, the final structural equation model (SEM) was obtained. The structural equation model diagram was then created in Microsoft Office PowerPoint 2016.

RESULTS

Soil Physicochemical Properties and Fine Roots Nutrient Contents.

Thinning led to significant reductions in soil pH (1.45%), SBD (9.70%), and AP (13.59%). Conversely, its effects on SWC, SOC, TN, TP, and TK were not significant (Fig. 1). Relative to CK, MIT treatment significantly altered soil properties compared to CK, lowering pH and the C/P ratio (by 1.87% and 47.85%, respectively), while elevating TN and TP (by 43.62% and 48.72%, respectively). HIT resulted in a significant 18.45% decrease in AP ($P < 0.05$).

Different lowercase letters indicate significance among four different treatments at the $P < 0.05$ level, based on Tukey by Origin 2021 Pro. The values are mean ± standard error. SBD, soil bulk density; ST, soil temperature; SWC, soil water content; SOC, soil organic carbon; TN, soil total nitrogen; TP, soil total phosphorus; TK, soil total potassium; AN, soil available nitrogen; AP, soil available phosphorus; AK, soil available potassium; C/N, Ratio of SOC/TN; C/P, Ratio of SOC/TP; N/P, Ratio of TN/TP.

Thinning significantly affected *P. massoniana* B, ROC, and RTP, but had no significant effect on RTK (Fig. 2). All thinning intensities significantly increased B and RTN. Specifically, LIT, MIT, and HIT increased B by 32.36%, 54.47%, and 18.78%, respectively, and increased RTN by 53.76%, 116.73%, and 107.71%, respectively. Conversely, these treatments reduced ROC by 15.95%, 30.07%, and 30.41%, respectively ($P < 0.05$).

Different lowercase letters indicate significance among four different treatments at the $P < 0.05$ level, based on Tukey by Origin 2021 Pro. The values are mean ± standard error. B, fine root biomass; ROC, root organic carbon; RTN, root total nitrogen; RTP, root total phosphorus; RTK, root total potassium.

Diversity and Composition of ECM Community

Thinning significantly affected the Sobs, Simpson, Shannon, and Pielou indices ($P < 0.01$), but had no significant effect on the Chao1 and ACE indices (Fig. 3). Compared with CK (203.67 ± 17.79), HIT (241.33 ± 16.56) significantly increased Sobs ($P < 0.01$); relative to MIT (186.67 ± 10.26), HIT extremely significantly increased Sobs ($P < 0.001$). Compared with CK (3.62 ± 0.17), LIT (5.06 ± 0.08) and HIT (4.05 ± 0.02) significantly increased the Shannon index ($P < 0.001$), while MIT (3.32 ± 0.07) significantly decreased the Shannon index ($P < 0.01$). Compared with CK (0.80 ± 0.02), LIT (0.94 ± 0.00) and HIT (0.86 ± 0.00) significantly increased the Simpson index ($P < 0.001$), while MIT (0.70 ± 0.02) significantly decreased it ($P < 0.001$). Compared with CK (0.47 ± 0.03), LIT (0.66 ± 0.01) significantly increased the Pielou index ($P < 0.001$) and HIT (0.51 ± 0.00) significantly increased the Pielou index ($P < 0.01$), while MIT (0.44 ± 0.01) significantly decreased the Pielou index ($P < 0.05$).

Thinning significantly altered the abundance patterns of ECM fungi (Fig. 4). At the genus level, MIT significantly increased the relative abundance of *Trichoderma* ($P < 0.05$) while extremely significantly decreasing the relative abundances of LIT and HIT ($P < 0.001$). Thinning significantly reduced the relative abundance of *Penicillium* ($P < 0.05$) and also decreased the relative abundance of *Aspergillus* ($P > 0.05$). LIT and MIT significantly increased the relative abundance of *Lactarius* and *Russula* ($P < 0.05$), while HIT

significantly reduced the relative abundance of *Lactarius* ($P < 0.05$) and had no significant effect on *Russula* ($P > 0.05$). HIT and LIT significantly increased the relative abundance of *Chloridium*, while MIT significantly decreased the relative abundance of *Chloridium* ($P < 0.05$).

At the species level, compared to CK, LIT significantly increased the relative abundance of *Russula sanguinea*, *Lactarius salmonicolor*, and *Lactuca virosa*, but decreased the relative abundance of *Trichoderma spirale* ($P < 0.001$). MIT significantly increased the relative abundance of *Russula sanguinea* and *Lactarius salmonicolor* ($P < 0.001$), while having no significant effect on *Lactuca virosa* and *Meliniomyces bicolor* ($P > 0.05$). HIT significantly reduced the relative abundance of *Trichoderma spirale*, *Penicillium arenicola*, and *Lactarius salmonicolor* ($P < 0.05$), increased the relative abundance of *Meliniomyces bicolor* ($P < 0.05$), and had no significant effect on the abundance of *Russula sanguinea* and *Lactuca virosa* ($P > 0.05$). Overall, thinning significantly altered the community composition of ECM.

We obtained 1380083 fungal effective tags from the raw dataset and clustered them into 744 OTUs, of which 664 OTUs were identified to ECM. PCA analysis revealed that the first axis explained 54.73% of the variance, and the second axis accounted for 34.79%, with both axes collectively explaining 89.52% of the variance, which indicate effective dimensionality reduction. Significant separation of ECM microbial communities was observed among treatments ($P = 0.001$), with MIT and CK showing relatively similar microbial diversity (Fig. 5).

Co-occurrence networks of ECM fungal communities in *P. massoniana* plantations under different thinning treatments were constructed, and the topological characteristics of these networks were analyzed (Fig. 6, Table 3). These networks predominantly featured positive associations, ranging from 50.12% to 67.02% for ECM. The number of nodes and the number of edges for LIT and HIT were significantly higher than those for CK and MIT. HIT had the highest number of nodes (228), while LIT had the highest number of edges (8238). In co-occurrence networks, positive cohesion significantly outnumbers negative cohesion, with MIT exhibiting the highest positive cohesion (67.02%). Compared to other treatments, the co-occurrence network of LIT exhibits the highest Average Degree, Average Weighted Degree, Density, and Average Clustering Coefficient, along with the lowest Average Path Length. This indicates that interactions among ECM communities under this treatment are more tightly interconnected, demonstrating higher connectivity efficiency and stability.

Table 3
The co-occurrence network topological properties of soil ECM communities with four different thinning treatments in *P. massoniana* plantations.

Topological properties	CK	LIT	MIT	HIT
Average Degree	56.248	73.883	58.447	62.298
Average Weighted Degree	6.605	20.087	8.529	4.757
Density	0.28	0.333	0.313	0.274
Modularity	0.049	0.016	0.039	0.068
Eigenvector Centrality	0.0517	0.0213	0.011	0.0135
Average Clustering Coefficient	0.747	0.803	0.782	0.744
Average Path Length	1.72	1.667	1.687	1.726
The number of nodes	202	223	188	228
The number of edges	5681	8238	5494	7102
Positive cohesion	63.99	50.12	67.02	66.38
Negative cohesion	25.45	23.48	19.48	27.25

Relationship Between Environmental Variables and Fungal Functional Groups.

For subsequent analysis, the 15 fungal functional groups exhibiting the highest relative abundances were selected (Fig. 7). LIT and HIT significantly increased the abundance of the Plant Saprotroph-Wood Saprotroph functional group, while MIT significantly decreased its abundance ($P < 0.05$). LIT and MIT significantly increased the abundance of ECM functional groups ($P < 0.05$). LIT and HIT significantly increased the abundance of the Ectomycorrhizal-Undefined Saprotroph and Endophyte functional groups, while HIT significantly increased the abundance of the Endomycorrhizal-Plant Pathogen-Undefined Saprotroph functional group ($P < 0.05$). LIT and MIT significantly increased the abundance of the Endophyte-Plant Pathogen-Undefined Saprotroph functional group ($P < 0.05$).

Using the ECM diversity index and ECM species abundance as response variables, and soil physicochemical properties and fine root nutrients as explanatory variables, we removed variables with low contribution, then performed RDA ordination on 12 plots across four thinning intensities and plotted the explanatory power of different variables (Fig. 8). The red arrows represent the environmental factors of soil physicochemical properties and fine root nutrients, while the blue arrows indicate the ECM diversity index and ECM species abundance in the figure. Results indicate that axes I and II explain 96.70% of the variance, effectively reflecting the relationship between the ECM diversity index and soil physicochemical properties as well as fine root nutrients (Fig. 8A). pH showed significant positive correlations with Pielou, ACE, and Shannon indices; AN and SOC exhibited significant positive

correlations with Simpson and Chao1 indices; C/P demonstrated significant positive correlation with Sobs; while TN and B displayed significant negative correlations with Simpson, Chao1, and Sobs indices. C/P and AK explained 38.1% ($P < 0.05$) and 22.9% ($P < 0.05$) of the variance, respectively, were the primary factors influencing the ECM diversity index.

Figure 8C shows that axes I and II explain 94.52% of the variance, effectively reflecting the relationship between ECM genera abundance and soil physicochemical properties and fine root nutrients. ROC showed significant positive correlations with *Aspergillus* and *Penicillium*; *Chloridium* exhibited significant positive correlations with RTP, *Trichoderma* demonstrated significant positive correlations with TN and significant negative correlations with C/N. TN and ROC explained 44.7% ($P < 0.01$) and 25.0% ($P < 0.01$) of the variance, respectively, and were the primary factors influencing ECM species abundance. Besides, B, SWC and RTP were important factors, which explained 17.9% ($P < 0.01$), 5.8% ($P < 0.01$) and 0.9% ($P < 0.05$) of the variance, respectively.

The goodness-of-fit (GOF) value for the structural equation model was 0.7043 > 0.7, indicating good model fit (Fig. 9). Partial Least Squares Structural Equation Modeling (PLS-SEM) showed that thinning positively influenced the soil nutrient, fine root nutrients and ECM community diversity and composition. Thinning significantly influence fine root nutrients ($R^2 = 0.593$) and ECM community composition ($R^2 = 0.959$) ($P < 0.05$), but have no significantly influence on soil nutrient and ECM α-diversity ($P > 0.05$). Soil nutrient positively influenced ECM α-diversity and community composition.

DISCUSSION

Alterations in Soil Physicochemical Properties and Fine Roots Nutrients After Thinning

Thinning has no discernible effect on soil physicochemical properties. MIT significantly increased TN and TP, which indicated that MIT treatment could better improve soil nutrients. Our previous study on the same plots showed that short-term thinning did not significantly change soil physical and chemical properties, which may be caused by different thinning intensities (Liu et al., 2021). In this experiment, intensity settings featured a steeper gradient and longer thinning cycle. MIT was more conducive to increase organophosphorus input from plant or microorganism remains and increased soil P content (Dang et al., 2018). Therefore, MIT treatment in this area could be more conducive to improve site conditions in *P. massoniana* plantations where the soil is generally deficient in phosphorus. Additionally, after thinning, MIT significantly reduced soil pH, consistent with the research findings of Cheng et al. (2015) and Zeng et al. (2023). Thinning increases soil respiration (Zhang et al., 2022), leading to higher CO_2 emissions. CO_2 dissolves in water to form H_2CO_3, and the dissolution of H_2CO_3 releases H^+ ions, thereby lowering soil pH. Additionally, thinning reduces canopy interception of precipitation (Niu et al., 2023) and increases rainfall infiltration (Liu et al., 2024). The resulting increase in precipitation leaching accelerates the loss of alkaline base cations (such as Ca^{2+}, Mg^{2+}, K^+, Na^+) from the soil, thereby lowering

pH. This effect is one of the ecological consequences that must be comprehensively considered in forest management activities.

Thinning significantly increased fine root B, RTN, and RTK. ROC decreased with increasing thinning intensity, while RTN increased with increasing thinning intensity (Fig. 2). The death and decomposition of tree roots releases organic matter after thinning, promoting the growth of understory vegetation and root systems (Li et al., 2020). Meanwhile, additional light and space into the stand and promoted the growth of remaining trees by thinning treatment (Ares et al., 2009; Dang et al., 2018; Lei et al., 2018). Increasing of B indicated that the ability of absorbing water and nutrients were improved after thinning. Studies on the horizontal distribution of fine root biomass of *P. massoniana* showed that the release of competitive space would increase the distal fine root biomass (Xiangjun Li et al., 2020), which also supported this conclusion.

Following thinning, a slight increase in soil organic carbon and a significant increase in fine root biomass were observed, accompanied by a decrease in organic carbon within the fine roots. This phenomenon may arise because after thinning, abundant litter produced by understory vegetation covers the ground surface, forming a new litter layer. Through leaching, fragmentation, and microbial decomposition, this litter is gradually converted into soil organic carbon (Qu et al., 2025). Additionally, the release of biomass carbon from fine root mortality, coupled with slower decomposition rates, also contributes to increased soil organic carbon accumulation (Ma et al., 2025; Witzgall et al., 2024). Conversely, thinning alters the carbon allocation strategy of *P. massoniana*: the tree may direct more carbon toward above-ground growth (trunk and branches) (Poorter et al., 2012) rather than allocating it to the below-ground portion (root system) to promote root growth—which is the primary objective of thinning. Therefore, even with increased fine root biomass, its turnover rate changes, directly affecting soil carbon flux. Turnover and death of fine roots were significantly correlated with N content (Ma et al., 2012), higher RTN meant more vigorous activity of fine roots, and higher fine roots turnover tends to benefit from changes in soil nutrients (Asaye & Zewdie, 2013), which is consistent with our findings that thinning increased TN and TP. Thinning also increased the species diversity of understory vegetation and microbial diversity(Wang et al., 2023), increased the root activity and the root exudation rate, which improve the ecological process of plantation underground (Wang et al., 2019). Therefore, thinning alters the carbon allocation strategy of *P. massoniana*, redirecting carbon from the root system to the aboveground parts, thereby promoting its growth.

Thinning Affected Composition and Diversity of ECM Fungal Communities

Thinning significantly altered the abundance patterns of ECM fungi, with different thinning intensities producing distinct effects on their abundance. At the Genus level, thinning significantly reduced the relative abundance of *Penicillium, Aspergillus*, increased the relative abundance of *Russula*, and exhibits varying effects to the relative abundance of *Trichoderma*, *Lactarius*, and *Chloridium* depending on the intensity of thinning. *Trichoderma* and *Penicillium* are the dominant genera in *P. massoniana* forests,

consistent with the findings of Yang et al. (2023) across different forest ages. *Trichoderma* belong to natural-plant-growth promoting fungi, since they colonize the roots through penetration, utilize compounds released by the host plant, and promote plant growth and photosynthetic rate(Dourou & La Porta, 2023), and improving plant performance and productivity (Lopez-Coria et al., 2023). Our research found that MIT increased the abundance of *Trichoderma* (specifically *Trichoderma spirale*) and promoted the accumulation of fine root biomass. *Trichoderma* can effectively diminish both disease occurrence and intensity through the production of enzymes that degrade fungal cell walls, secondary metabolites with antimicrobial activities, mycoparasitism, induction of plant defense responses, and competition for resources and space in the rhizosphere or within tissues as endophytic fungi (Barbosa et al., 2024). Zheng et al. (2025) found that *Trichoderma spirale* effectively enhances the activity of defense enzymes and the accumulation of reactive oxygen species, thereby strengthening its disease resistance. Chen et al. (2024) discovered that o-aminobenzoic acid secreted by *Trichoderma* promotes lateral root development by regulating plant root growth through auxin signaling and RBOHF-induced endodermis cell wall remodeling.

Penicillium exhibits strong ecological adaptability (Torres-Cruz et al., 2018), promoting host growth and enhancing the stress resistance of host plants (Chen et al., 2022; He et al., 2016). Certain *Penicillium* species (e.g., *Penicillium oxalicum*) can also secrete organic acids such as glucose and oxalic acid to chelate metal ions like Fe^{3+}, Ca^{2+}, and Al^{3+}, converting insoluble phosphorus into available forms and increasing soil phosphorus availability (Xue et al., 2019). As thinning intensity increases, *Penicillium* abundance gradually decreases, reducing phosphate conversion efficiency in soil and consequently diminishing available phosphorus content. Thus, thinning may weaken phosphorus metabolic processes. *Russula* fungi were enriched under P. pinaster (less basic pH) (Perez-Izquierdo et al., 2020), as thinning lowered soil pH, thereby promoting *Russula* growth. *Lactarius* species form symbiotic mycorrhizae with *P. massoniana*, secreting metabolites like flavonoids and phenolics during mycorrhizal association. These compounds significantly enhance the tree's resistance to drought and oxidative stress (Zhang et al., 2024). LIT and MIT significantly increased the relative abundance of *Lactarius* species, indicating that these treatments enhanced *P. massoniana*'s stress resistance while promoting nutrient uptake and growth regulation.

Thinning significantly affected the Sobs, Simpson, Shannon, and Pielou indices ($P < 0.01$), while it had no significant effect on the Chao1 and ACE indices (Fig. 5). Simpson and Shannon indexes comprehensively reflected the community richness and evenness (Crist et al., 2003), indicating that thinning increased the richness and diversity of ECM communities. Research on fungal community diversity in plantations showed that thinning did not affect alpha diversity. These results indicated that ECM fungi were more susceptible to environmental disturbances and were particularly sensitive to habitat changes (Rinaldi et al., 2008). Although there was no significant changes in community evenness, thinning may have opened a niche for ECM fungi (Segnitz et al., 2020) and promoted their proliferation (Averill & Hawkes, 2016; Bödeker et al., 2016). Chao1 and ACE represent the number of OTUs predicted, and the results showed that thinning measures did not make a species disappear or appear suddenly, and the whole ECM fungal community tends to be stable. LIT and HIT both increased the complexity of the ECM co-occurrence

network, whereas MIT reduced its complexity. This phenomenon rejects the traditional moderate disturbance hypothesis and aligns with the findings of Jordi Sola et al. (2025): higher heterogeneity does not necessarily promote community stability but is accompanied by more complex ecological processes. LIT imposes minimal disturbance on forest environments (Fan et al., 2025), thereby preserving the original complexity of the ECM co-occurrence network. MIT, by removing some trees, prevents sufficient development of understory vegetation. This shifts resource inputs from diverse tree litter to litter and residues from a single dominant species, thereby reducing resource heterogeneity. Consequently, ECM food sources diminish, leading to decreased complexity in the ECM co-occurrence network (Li et al., 2024). Conversely, HIT increases resource heterogeneity (Feng et al., 2022). This includes retained wood litter, litter and root exudates from rapidly developing understory vegetation, as well as enhanced light and thermal resources. This diverse resource base supports ECMs in forming diverse communities and more complex functional networks. As thinning intensity increases, the complexity of the ECM functional network first increases, then decreases, and then increases again. This supports the “intermediate trough” hypothesis of resource heterogeneity and the view that different thinning intensities have different effects (Hartmann et al., 2014). Biodiversity can enhance ecosystem stability at lower levels but weaken it at higher levels (Pennekamp et al., 2018), suggesting that MIT may have strengthened ecosystem stability(He et al., 2025; Liu et al., 2025).

Thinning Drived ECM Fungal Functional Groups with Environmental Factors Changed

FunGuild analysis showed that thinning increased the relative abundance of ECM fungi, but with the increasing of thinning intensity, the relative abundance of ECM fungi gradually decreased, which suggested that short-term thinning promoted the colonization of ECM fungi. Some studies have proposed the opposite view. Studies on the effects of thinning on ECM and saprophytic fungi in *Larch* plantation showed that thinning promoted the colonization of saprophytic fungi, but inhibited ECM fungi (Zhou et al., 2020), which is in agreement with previous work in the Gulf Coastal Plain, USA (Mushinski et al., 2018). Therefore, we speculated that the ECM fungal communities would be affected by time for thinning, thinning intensity and tree species. RDA showed that B was the key limiting one in all fine roots factors to affect the differentiation of ECM fungi function groups. Within a suitable range, forest gap facilitated production and circulation of the fine roots (Lyu et al., 2021). In addition, increased understory vegetation diversity would make the root exudates increased, and the root system of *P. massoniana* grew more rapidly due to the "Allelopathy" (Bais et al., 2003; Lyu et al., 2021), and eventually increased hosts for ECM fungi. SEM indicates that thinning significantly affected fine root nutrients, primarily represented by fine root biomass, which in turn influenced soil nutrients. The main sources of ROC were the distribution of aboveground photosynthates and the decomposition of soil stubborn carbon (Kwaśna et al., 2017). Even if SOC was always maintained at a low level, fine roots will obtain more C in the soil through ECM mycelium to ensure the continuation of life activities, so we can infer that ROC is more important than SOC for ECM fungi. There was a significant negative correlation between RTN and ECM fungal community. Previous findings showed that the increase of RTN caused higher mortality of the fine root and promoted the succession of ECM fungal community (Chen et al., 2019). In this process,

saprophytic fungi had the opportunity to inhibit ECM by grabbing common niches, which was consistent with the conclusion that high-intensity thinning reduced the relative abundance of ECM fungi. Although thinning had no significant effect on soil pH, it still had a high degree of explanation for the functional groups of ECM fungi. A few researches have shown that pH had a positive effect on the diversity of ECM fungi in acidic soil (Benucci et al., 2016; Wang et al., 2017) and soil acidification lead to the decrease of soil fertility and the absorption capacity of fine roots (van Breemen et al., 1997). At the same time, ECM fungi absorb nutrients from the soil and transport them to the aboveground part through a huge mycelial network to maintain the normal growth of plants (Hawkins et al., 2023). *P. massoniana* still grow vigorously in acidic soil, which depends on the strong ability of ECM fungi to absorb water and nutrients in an environment which is not conducive to growth. Generally speaking, in this study, short-term thinning did not directly affect the differentiation of fungal functional groups by changing soil nutrients, and fine root biomass and nutrients played a more important role in ECM fungal communities.

CONCLUSION

To sum up, thinning significantly affected ECM community in *P. massoniana* plantation by changing fine root biomass and nutrient contents, while short-term thinning could not significantly change soil physicochemical properties of the forests. Fine root biomass is the main driving factor of ECM fungal community succession. This conclusion proves that thinning has a significant effect on ECM community from the point of view of belowground ecology. The process of thinning to improve low productive plantations is long and complicated. Overall, MIT increased the diversity of ECM fungi in *P. massoniana* plantations, promoted ECM colonization and fine root nutrient accumulation. The findings provide a scientific basis for the near-natural management of *P. massoniana* plantations.

Declarations

CONFLICTS OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

FUNDING

This study was supported by the National Key Research and Development Program of China (Grant No. 2023YFD2200901, 2017YFD060030205), the German Government loans for Sichuan Forestry Sustainable Management (Grant No. G1403083), and the “Tianfu Ten Thousand Talents Plan” of Sichuan Province (Grant No. 1922999002).

Author Contribution

All authors contributed to the study conception and design. Zhongxuan Huang revised the text and figures in the manuscript. Xiangjun Li conducted field work, laboratory analysis and drafted the manuscript. Xin Zhang, Jingwen Peng, Cheng Huang, Yongqi Xiang carried out the data analysis and literature search. Chuan Fan, Gang Chen and Xianwei Li designed this research and revised the manuscript critically. All the authors commented on the analysis and gave final approval for publication.

ACKNOWLEDGMENTS

We also thank all professors who provided helpful guidance in this research.

Data Availability

The original contributions presented in the study are publicly available. This data can be found here: https://www.ncbi.nlm.nih.gov/sra/PRJNA770507.

References

1. Ares, A., Berryman, S. D., Puettmann, K. J., 2009. Understory vegetation response to thinning disturbance of varying complexity in coniferous stands. Applied Vegetation Science, 12(4), 472-487. https://doi.org/https://doi.org/10.1111/j.1654-109X.2009.01042.x
2. Asaye, Z., Zewdie, S., 2013. Fine root dynamics and soil carbon accretion under thinned and un-thinned Cupressus lusitanica stands in, Southern Ethiopia. Plant and Soil, 366(1-2), 261-271. https://doi.org/10.1007/s11104-012-1420-3
3. Averill, C., Hawkes, C. V., 2016. Ectomycorrhizal fungi slow soil carbon cycling. Ecol Lett, 19(8), 937-947. https://doi.org/10.1111/ele.12631
4. Bais, H. P., Vepachedu, R., Gilroy, S., Callaway, R. M., Vivanco, J. M., 2003. Allelopathy and exotic plant invasion: from molecules and genes to species interactions. Science, 301(5638), 1377-1380. https://doi.org/10.1126/science.1083245
5. Barbosa, L. O., Conceicao, T., Neves, A. O., Rocha, W. Z. B., Damasceno, B. S., Fonseca, P. L. C., Ribeiro, P. R., Tome, L. M. R., Bortolini, D. E., Martins, F. M., Raya, F. T., Goes-Neto, A., Soares, A. C. F., 2024. Native and Non-Native Soil and Endophytic Trichoderma spp. from Semi-Arid Sisal Fields of Brazil Are Potential Biocontrol Agents for Sisal Bole Rot Disease. J Fungi (Basel), 10(12). https://doi.org/10.3390/jof10120860
6. Benucci, G. M., Lefevre, C., Bonito, G., 2016. Characterizing root-associated fungal communities and soils of Douglas-fir (Pseudotsuga menziesii) stands that naturally produce Oregon white truffles (Tuber oregonense and Tuber gibbosum). Mycorrhiza, 26(5), 367-376. https://doi.org/10.1007/s00572-015-0677-9
7. Berrios, L., Bogar, G. D., Bogar, L. M., Venturini, A. M., Willing, C. E., Del Rio, A., Ansell, T. B., Zemaitis, K., Velickovic, M., Velickovic, D., Pellitier, P. T., Yeam, J., Hutchinson, C., Bloodsworth, K., Lipton, M. S.,

Peay, K. G., 2024. Ectomycorrhizal fungi alter soil food webs and the functional potential of bacterial communities. mSystems, 9(6), e0036924. https://doi.org/10.1128/msystems.00369-24

8. Bödeker, I. T. M., Lindahl, B. D., Olson, Å., Clemmensen, K. E., Treseder, K., 2016. Mycorrhizal and saprotrophic fungal guilds compete for the same organic substrates but affect decomposition differently. Functional Ecology, 30(12), 1967-1978. https://doi.org/10.1111/1365-2435.12677
9. Burke, D. J., Smemo, K. A., Hewins, C. R., 2014. Ectomycorrhizal fungi isolated from old-growth northern hardwood forest display variability in extracellular enzyme activity in the presence of plant litter. Soil Biology & Biochemistry, 68, 219-222. https://doi.org/10.1016/j.soilbio.2013.10.013
10. Chen, L., Swenson, N. G., Ji, N., Mi, X., Ren, H., Guo, L., Ma, K., 2019. Differential soil fungus accumulation and density dependence of trees in a subtropical forest. Science, 366(6461), 124-128. https://doi.org/doi:10.1126/science.aau1361
11. Chen, T., Zhu, C., Li, S., Xia, Y., Huang, J., Wang, W., Lian, C., Chen, Y., Zhao, Y., Zhang, S., 2025. Impact of ectomycorrhizal symbiosis on root system architecture and nutrient absorption in Chinese chestnut and pecan seedlings. Plant and Soil, 513(2), 2689-2705. https://doi.org/10.1007/s11104-025-07332-7
12. Chen, X., Zhu, B., Chen, D., Zhang, X., Tao, J., Xue, Q., Chen, Y., Niu, X., 2022. The Antagonism of *Penicillium griseofulvum* CF3 Against the Pathogens and Its Growth-promoting Effect on Cassava. Molecular Plant Breeding, 20(24), 8231-8236. https://doi.org/10.13271/j.mpb.020.008231
13. Chen, Y., Fu, Y., Xia, Y., Miao, Y., Shao, J., Xuan, W., Liu, Y., Xun, W., Yan, Q., Shen, Q., Zhang, R., 2024. Trichoderma-secreted anthranilic acid promotes lateral root development via auxin signaling and RBOHF-induced endodermal cell wall remodeling. Cell Rep, 43(4), 114030. https://doi.org/10.1016/j.celrep.2024.114030
14. Cheng, C. P., Wang, Y. D., Fu, X. L., Xu, M. J., Dai, X. Q., Wang, H. M., 2017. Thinning effect on understory community and photosynthetic characteristics in a subtropical plantation. Canadian Journal of Forest Research, 47(8), 1104-1115. https://doi.org/10.1139/cjfr-2017-0082
15. Cheng, X., Kang, F., Han, H., Liu, H., Zhang, Y., 2015. Effect of thinning on partitioned soil respiration in a young Pinus tabulaeformis plantation during growing season. Agricultural and Forest Meteorology, 214-215, 473-482. https://doi.org/10.1016/j.agrformet.2015.09.016
16. Crist, Thomas O., Veech, Joseph A., Gering, Jon C., Summerville, Keith S., 2003. Partitioning Species Diversity across Landscapes and Regions: A Hierarchical Analysis of α, β, and γ Diversity. The American Naturalist, 162(6), 734-743. https://doi.org/10.1086/378901
17. Dang, P., Gao, Y., Liu, J., Yu, S., Zhao, Z., 2018. Effects of thinning intensity on understory vegetation and soil microbial communities of a mature Chinese pine plantation in the Loess Plateau. Sci Total Environ, 630, 171-180. https://doi.org/10.1016/j.scitotenv.2018.02.197
18. Dourou, M., La Porta, C. A. M., 2023. A Pipeline to Investigate Fungal-Fungal Interactions: Trichoderma Isolates against Plant-Associated Fungi. J Fungi (Basel), 9(4). https://doi.org/10.3390/jof9040461

19. Fan, C., Zhou, G., Chen, H., Du, Z., Liu, R., He, Y., Huang, C., Qiu, S., Zhu, Y., Li, J., Zhou, X., 2025. Thinning intensity influences the C:N:P stoichiometry in forest ecosystems: A global synthesis. Geoderma, 460. https://doi.org/10.1016/j.geoderma.2025.117435
20. FAO, 2020. Global Forest Resource Assessment 2020. Retrieved from Rome:
21. Feng, Y. H., Schmid, B., Loreau, M., Forrester, D., Fei, S. L., Zhu, J. X., Tang, Z. Y., Zhu, J. L., Hong, P. B., Ji, C. J., Shi, Y., Su, H. J., Xiong, X. Y., Xiao, J., Wang, S. P., Fang, J. Y., 2022. Multispecies forest plantations outyield monocultures across a broad range of conditions. SCIENCE, 376(6595), 865-+. https://doi.org/10.1126/science.abm6363
22. Finér, L., Zverev, V., Palviainen, M., Romanis, T., Kozlov, M. V., 2019. Variation in fine root biomass along a 1000 km long latitudinal climatic gradient in mixed boreal forests of North-East Europe. Forest Ecology and Management, 432, 649-655. https://doi.org/10.1016/j.foreco.2018.09.060
23. Hartmann, M., Niklaus, P. A., Zimmermann, S., Schmutz, S., Kremer, J., Abarenkov, K., Luscher, P., Widmer, F., Frey, B., 2014. Resistance and resilience of the forest soil microbiome to logging-associated compaction. ISME J, 8(1), 226-244. https://doi.org/10.1038/ismej.2013.141
24. Hawkins, H. J., Cargill, R. I. M., Van Nuland, M. E., Hagen, S. C., Field, K. J., Sheldrake, M., Soudzilovskaia, N. A., Kiers, E. T., 2023. Mycorrhizal mycelium as a global carbon pool. Curr Biol, 33(11), R560-R573. https://doi.org/10.1016/j.cub.2023.02.027
25. He, G., Wang, X., Liao, G., Huang, S., Wu, J., 2016. Isolation, Identification and Characterization of Two Aluminum-Tolerant Fungi from Acidic Red Soil. Indian J Microbiol, 56(3), 344-352. https://doi.org/10.1007/s12088-016-0586-4
26. He, T., Lei, J., Peng, Y., Wang, R., Chen, X., Liu, Z., Gao, X., Dang, P., Yan, W., 2025. Thinning Intensity Enhances Soil Multifunctionality and Microbial Residue Contributions to Organic Carbon Sequestration in Chinese Fir Plantations. Plants (Basel), 14(4). https://doi.org/10.3390/plants14040579
27. Kernaghan, G., LeFait, B., Hussain, A., 2025. Dynamics of pine ectomycorrhizae following root disturbance. Mycorrhiza, 35(2), 12. https://doi.org/10.1007/s00572-025-01190-y
28. Kou, Y., Ding, J., Yin, H., 2024. Temperature governs the community assembly of root-associated ectomycorrhizal fungi in alpine forests on the Qinghai-Tibetan Plateau. Sci Total Environ, 955, 176820. https://doi.org/10.1016/j.scitotenv.2024.176820
29. Kwaśna, H., Mazur, A., Kuźmiński, R., Jaszczak, R., Turski, M., Behnke-Borowczyk, J., Adamowicz, K., Łakomy, P., 2017. Abundance and diversity of wood-decay fungi in managed and unmanaged stands in a Scots pine forest in western Poland. Forest Ecology and Management, 400, 438-446. https://doi.org/10.1016/j.foreco.2017.04.023
30. Lambers, H., Raven, J. A., Shaver, G. R., Smith, S. E., 2008. Plant nutrient-acquisition strategies change with soil age. Trends Ecol Evol, 23(2), 95-103. https://doi.org/10.1016/j.tree.2007.10.008
31. Lan, Z., Jiang, X., Li, G., Lu, Y., Yao, H., Lu, D., 2025. Modeling pine forest growing stock volume in subtropical regions of China using airborne Lidar data. GIScience & Remote Sensing, 62(1). https://doi.org/10.1080/15481603.2025.2477869

32. Lei, L., Xiao, W., Zeng, L., Zhu, J., Huang, Z., Cheng, R., Gao, S., Li, M. H., 2018. Thinning but not understory removal increased heterotrophic respiration and total soil respiration in Pinus massoniana stands. Sci Total Environ, 621, 1360-1369. https://doi.org/10.1016/j.scitotenv.2017.10.092
33. Li, L., Fang, W., Ma, L., An, Y., Pan, C., Xian, L., Dong, Z., Wei, D., Xiong, X., 2024. Effects of Thinning Measures on Soil Microbial Diversity of *Pinus massoniana* Forest in Pine Wilt Disease Endemic Areas. Forest Engineering, 40(05), 82-93. https://doi.org/10. 7525/j. issn. 1006-8023. 2024. 05. 009
34. Li, X., Li, Y., Zhang, J., Peng, S., Chen, Y., Cao, Y., 2020. The effects of forest thinning on understory diversity in China: A meta-analysis. Land Degradation & Development, 31(10), 1225-1240. https://doi.org/10.1002/ldr.3540
35. Li, X., Su, Y., Yin, H., Liu, S., Chen, G., Fan, C., Feng, M., Li, X., 2020. The Effects of Crop Tree Management on the Fine Root Traits of Pinus massoniana in Sichuan Province, China. Forests, 11(3). https://doi.org/10.3390/f11030351
36. Li, X., Zeng, D.-H., Zhang, Y., Mao, Z., Sun, Y., Sheng, Z., Shi, K., Wang, G., Lin, G., 2025. Complementarity of Fine Roots and Ectomycorrhizal Fungi in Nitrogen Acquisition Along a Gradient of Intraspecific Competition Intensity. Plant, Cell & Environment, 48(7), 4873-4885. https://doi.org/https://doi.org/10.1111/pce.15487
37. Liu, S., Yin, H., Li, X., Li, X., Fan, C., Chen, G., Feng, M., Chen, Y., 2021. Short-Term Thinning Influences the Rhizosphere Fungal Community Assembly of Pinus massoniana by Altering the Understory Vegetation Diversity. Front Microbiol, 12, 620309. https://doi.org/10.3389/fmicb.2021.620309
38. Liu, S., Yin, H., Su, Y., Li, X., Fan, C., 2025. Early Response of Rhizosphere Microbial Community Network Characteristics to Thinning Intensity in Pinus massoniana Plantations. Microorganisms, 13(6). https://doi.org/10.3390/microorganisms13061357
39. Liu, X., Jiao, L., Cheng, D., Liu, J., Li, Z., Li, Z., Wang, C., He, X., Cao, Y., Gao, G., 2024. Light thinning effectively improves forest soil water replenishment in water-limited areas: Observational evidence from Robinia pseudoacacia plantations on the Loess Plateau, China. Journal of Hydrology, 637. https://doi.org/10.1016/j.jhydrol.2024.131408
40. Lopez-Coria, M., Guzman-Chavez, F., Carvente-Garcia, R., Munoz-Chapul, D., Sanchez-Sanchez, T., Arciniega-Ruiz, J. M., King-Diaz, B., Sanchez-Nieto, S., 2023. Maize plant expresses SWEET transporters differently when interacting with Trichoderma asperellum and Fusarium verticillioides, two fungi with different lifestyles. Front Plant Sci, 14, 1253741. https://doi.org/10.3389/fpls.2023.1253741
41. López, B., Sabate, S., Gracia, C., 2003. Thinning effects on carbon allocation to fine roots in a Quercus ilex forest. Tree Physiol., 23(17), 1217-1224. https://doi.org/10.1093/treephys/23.17.1217.
42. Lu, R. (1999). *Soil Agricultural Chemical Analysis Methods*. Beijing: China Agricultural Science and Technology Press.
43. Luginbuehl, L. H., Menard, G. N., Kurup, S., Van Erp, H., Radhakrishnan, G. V., Breakspear, A., Oldroyd, G. E. D., Eastmond, P. J., 2017. Fatty acids in arbuscular mycorrhizal fungi are synthesized by the

host plant. Science, 356(6343), 1175-1178. https://doi.org/10.1126/science.aan0081

44. Lyu, Q., Liu, J., Liu, J., Luo, Y., Chen, L., Chen, G., Zhao, K., Chen, Y., Fan, C., Li, X., 2021. Response of plant diversity and soil physicochemical properties to different gap sizes in a Pinus massoniana plantation. PeerJ, 9, e12222. https://doi.org/10.7717/peerj.12222
45. Lyu, Q., Shen, Y., Li, X., Chen, G., Li, D., Fan, C., 2021. Early effects of crop tree management on undergrowth plant diversity and soil physicochemical properties in a Pinus massoniana plantation. PeerJ, 9, e11852. https://doi.org/10.7717/peerj.11852
46. Ma, C., Zhang, W., Wu, M., Xue, Y., Ma, L., Zhou, J., 2012. Effect of aboveground intervention on fine root mass, production, and turnover rate in a Chinese cork oak (Quercus variabilis Blume) forest. Plant and Soil, 368(1-2), 201-214. https://doi.org/10.1007/s11104-012-1512-0
47. Ma, N., Li, S., McCormack, M. L., Freschet, G. T., Ciais, P., Wang, H., Niu, S., Reich, P. B., Zhang, M., Zhao, R., Zhao, B., Gao, D., Gessler, A., Huang, Y., Gu, J., Fu, X., Dai, X., Meng, S., Zheng, J., Yang, F., Kou, L., 2025. Substantial forest soil carbon accrual from absorptive fine roots over decadal timescales. Nature Geoscience. https://doi.org/10.1038/s41561-025-01790-5
48. Medina-Vega, J. A., Zuleta, D., Aguilar, S., Alonso, A., Bissiengou, P., Brockelman, W. Y., Bunyavejchewin, S., Burslem, D., Castano, N., Chave, J., Dalling, J. W., de Oliveira, A. A., Duque, A., Ediriweera, S., Ewango, C. E. N., Filip, J., Hubbell, S. P., Itoh, A., Kiratiprayoon, S., Lum, S. K. Y., Makana, J. R., Memiaghe, H., Mitre, D., Mohamad, M. B., Nathalang, A., Nilus, R., Nkongolo, N. V., Novotny, V., O'Brien, M. J., Perez, R., Pongpattananurak, N., Reynolds, G., Russo, S. E., Tan, S., Thompson, J., Uriarte, M., Valencia, R., Vicentini, A., Yao, T. L., Zimmerman, J. K., Davies, S. J., 2024. Tropical tree ectomycorrhiza are distributed independently of soil nutrients. Nat Ecol Evol, 8(3), 400-410. https://doi.org/10.1038/s41559-023-02298-0
49. Mushinski, R. M., Gentry, T. J., Boutton, T. W., 2018. Organic matter removal associated with forest harvest leads to decade scale alterations in soil fungal communities and functional guilds. Soil Biology and Biochemistry, 127, 127-136. https://doi.org/10.1016/j.soilbio.2018.09.019
50. Niu, X., Fan, J., Du, M., Dai, Z., Luo, R., Yuan, H., Zhang, S., 2023. Changes of Rainfall Partitioning and Canopy Interception Modeling after Progressive Thinning in Two Shrub Plantations on the Chinese Loess Plateau. Journal of Hydrology, 619. https://doi.org/10.1016/j.jhydrol.2023.129299
51. Pennekamp, F., Pontarp, M., Tabi, A., Altermatt, F., Alther, R., Choffat, Y., Fronhofer, E. A., Ganesanandamoorthy, P., Garnier, A., Griffiths, J. I., Greene, S., Horgan, K., Massie, T. M., Machler, E., Palamara, G. M., Seymour, M., Petchey, O. L., 2018. Biodiversity increases and decreases ecosystem stability. Nature, 563(7729), 109-112. https://doi.org/10.1038/s41586-018-0627-8
52. Perez-Izquierdo, L., Zabal-Aguirre, M., Verdu, M., Buee, M., Rincon, A., 2020. Ectomycorrhizal fungal diversity decreases in Mediterranean pine forests adapted to recurrent fires. Mol Ecol, 29(13), 2463-2476. https://doi.org/10.1111/mec.15493
53. Poorter, H., Niklas, K. J., Reich, P. B., Oleksyn, J., Poot, P., Mommer, L., 2012. Biomass allocation to leaves, stems and roots: meta-analyses of interspecific variation and environmental control. New Phytol, 193(1), 30-50. https://doi.org/10.1111/j.1469-8137.2011.03952.x

54. Qi, X. X., Chen, L., Zhu, J. A., Li, Z., Lei, H. M., Shen, Q., Wu, H. L., Ouyang, S., Zeng, Y. L., Hu, Y. T., Xiang, W. H., 2022. Increase of soil phosphorus bioavailability with ectomycorrhizal tree dominance in subtropical secondary forests. Forest Ecology and Management, 521. https://doi.org/ARTN 120435 10.1016/j.foreco.2022.120435
55. Qiang, W., Gunina, A., Kuzyakov, Y., Luo, R., Zhang, Y., Liu, B., Pang, X., 2023. Shifts of understory vegetation induced by thinning drive the expansion of soil rare fungi. J Environ Manage, 342, 118119. https://doi.org/10.1016/j.jenvman.2023.118119
56. Qiu, X., Peng, D., Wang, H., Wang, Z., Cheng, S., 2019. Minimum data set for evaluation of stand density effects on soil quality in Larix principis-rupprechtii plantations in North China. Ecological Indicators, 103, 236-247. https://doi.org/10.1016/j.ecolind.2019.04.010
57. Qu, Q., Xu, H., Xu, L., You, C., Tan, B., Li, H., Zhang, L., Wang, L., Liu, S., Xu, Z., Xue, S., Wang, M., 2025. Forest thinning effects on soil carbon stocks and dynamics: Perspective of soil organic carbon sequestration rates. Catena, 250. https://doi.org/10.1016/j.catena.2025.108759
58. Ren, C., Zhao, F., Kang, D., Yang, G., Han, X., Tong, X., Feng, Y., Ren, G., 2016. Linkages of C:N:P stoichiometry and bacterial community in soil following afforestation of former farmland. Forest Ecology and Management, 376, 59-66. https://doi.org/10.1016/j.foreco.2016.06.004
59. Ren, Y., Li, X., Cui, Z., Liu, J., He, Q., Zeng, S., Liu, X., 2024. Research Progress in the Cultivation of Large-diameter Timber Plantation in China. World Forestry Research, 37(03), 86-93. https://doi.org/10.13348/j.cnki.sjlyyj.2024.0043.y
60. Rinaldi, A. C., Comandini, O., Kuyper, T. W., 2008. Ectomycorrhizal fungal diversity: separating the wheat from the chaff. Fungal Diversity, 33, 1-45.
61. Roy, M.-È., Surget-Groba, Y., Rivest, D., 2024. Long-term effects of different harvesting intensities on soil microbial communities in a hardwood temperate forest. Forest Ecology and Management, 559. https://doi.org/10.1016/j.foreco.2024.121810
62. Segnitz, R. M., Russo, S. E., Davies, S. J., Peay, K. G., 2020. Ectomycorrhizal fungi drive positive phylogenetic plant-soil feedbacks in a regionally dominant tropical plant family. Ecology, 101(8), e03083. https://doi.org/10.1002/ecy.3083
63. Shi, J., Wang, X., Wang, E., 2023. Mycorrhizal Symbiosis in Plant Growth and Stress Adaptation: From Genes to Ecosystems. Annu Rev Plant Biol, 74, 569-607. https://doi.org/10.1146/annurev-arplant-061722-090342
64. Sola, J., Fairchild, T. P., Perkins, M. J., Bull, J. C., Griffin, J. N., 2025. Counteracting Cascades Challenge the Heterogeneity-Stability Relationship. Ecol Lett, 28(8), e70158. https://doi.org/10.1111/ele.70158
65. Song, W., 2024. Ectomycorrhizal fungi: Potential guardians of terrestrial ecosystems. mLife, 3(3), 387-390. https://doi.org/10.1002/mlf2.12127
66. Torres-Cruz, T. J., Hesse, C., Kuske, C. R., Porras-Alfaro, A., 2018. Presence and distribution of heavy metal tolerant fungi in surface soils of a temperate pine forest. Applied Soil Ecology, 131, 66-74. https://doi.org/10.1016/j.apsoil.2018.08.001

67. Treseder, K. K., Lennonb, J. T., 2015. Fungal Traits That Drive Ecosystem Dynamics on Land. Microbiology and Molecular Biology Reviews, 79(2), 243-262. https://doi.org/10.1128/Mmbr.00001-15

68. Tsai, H. H., Tang, Y., Jiang, L., Xu, X., Denervaud Tendon, V., Pang, J., Jia, Y., Wippel, K., Vacheron, J., Keel, C., Andersen, T. G., Geldner, N., Zhou, F., 2025. Localized glutamine leakage drives the spatial structure of root microbial colonization. Science, 390(6768), eadu4235. https://doi.org/10.1126/science.adu4235

69. van Breemen, N., Finzi, A. C., Canham, C. D., 1997. Canopy tree-soil interactions within temperate forests: effects of soil elemental composition and texture on species distributions. Canadian Journal of Forest Research, 27(7), 1110-1116. https://doi.org/10.1139/x97-061

70. van der Heijden, M. G. A., Martin, F. M., Selosse, M. A., Sanders, I. R., 2015. Mycorrhizal ecology and evolution: the past, the present, and the future. New Phytol, 205(4), 1406-1423. https://doi.org/10.1111/nph.13288

71. Wang, C., Xue, L., Dong, Y., Jiao, R., 2021. Effects of stand density on soil microbial community composition and enzyme activities in subtropical Cunninghamia lanceolate (Lamb.) Hook plantations. Forest Ecology and Management, 479. https://doi.org/10.1016/j.foreco.2020.118559

72. Wang, D., Olatunji, O. A., Xiao, J., 2019. Thinning increased fine root production, biomass, turnover rate and understory vegetation yield in a Chinese fir plantation. Forest Ecology and Management, 440, 92-100. https://doi.org/10.1016/j.foreco.2019.03.012

73. Wang, H., Sun, X., Chen, D., Wu, C., Zhang, S., 2023. Effects of Moderate Thinning on Biological Diversity and Soil Multifunctionality in *Larix kaempferi* Plantation. Scientia Silvae Sinicae, 59(06), 1-11. https://doi.org/10.11707/j.1001-7488.LYKX20220508

74. Wang, X., Liu, J., Long, D., Han, Q., Huang, J., 2017. The ectomycorrhizal fungal communities associated with Quercus liaotungensis in different habitats across northern China. Mycorrhiza, 27(5), 441-449. https://doi.org/10.1007/s00572-017-0762-3

75. Wang, Y., Wei, X., del Campo, A. D., Winkler, R., Wu, J., Li, Q., Liu, W., 2019. Juvenile thinning can effectively mitigate the effects of drought on tree growth and water consumption in a young Pinus contorta stand in the interior of British Columbia, Canada. Forest Ecology and Management, 454. https://doi.org/10.1016/j.foreco.2019.117667

76. Witzgall, K., Steiner, F. A., Hesse, B. D., Riveras-Muñoz, N., Rodríguez, V., Teixeira, P. P. C., Li, M., Oses, R., Seguel, O., Seitz, S., Wagner, D., Scholten, T., Buegger, F., Angst, G., Mueller, C. W., 2024. Living and decaying roots as regulators of soil aggregation and organic matter formation—from the rhizosphere to the detritusphere. Soil Biology and Biochemistry, 197. https://doi.org/10.1016/j.soilbio.2024.109503

77. Xue, Y., Ye, W., Yang, S., Li, P., Xu, B., 2019. Isolation and identification of P-dissolving fungi strain and its effects on phosphate-solubilizing and plant growth promotion. Agricultural Research in the Arid Areas, 37(04), 253-262. https://doi.org/10.7606 /j.issn.1000-7601.2019.04.34

78. Yang, X., Xu, M., Chen, J., Zhang, J., 2023. Effects of stand ages on ectomycorrhizal fungal diversity in Pinus massoniana forests. Journal of Forest and Environment, 43(01), 76-83. https://doi.org/10.13324/j.cnki.jfcf.2023.01.010
79. Yuan, Z. Y., Chen, H. Y., 2012. A global analysis of fine root production as affected by soil nitrogen and phosphorus. Proc Biol Sci, 279(1743), 3796-3802. https://doi.org/10.1098/rspb.2012.0955
80. Zeng, L., Xiao, W., Liu, C., Lei, L., Jian, Z., Shen, Y., Li, M.-H., 2023. Effects of thinning and understorey removal on soil extracellular enzyme activity vary over time during forest recovery after treatment. Plant and Soil, 492(1-2), 457-469. https://doi.org/10.1007/s11104-023-06187-0
81. Zhang, H., Ying, B., Hu, Y., Wang, Y., Yu, X., Tang, C., 2022. Response of soil respiration to thinning is altered by thinning residue treatment in Cunninghamia lanceolata plantations. Agricultural and Forest Meteorology, 324. https://doi.org/10.1016/j.agrformet.2022.109089
82. Zhang, H., Zha, T., Yu, Y., Zhang, Z., Zhang, X., Zhang, H., Ji, X., 2023. Functional vegetation community responses to soil and topographic factors in the Loess Plateau of China. Land Degradation & Development, 34(17), 5355-5372. https://doi.org/https://doi.org/10.1002/ldr.4849
83. Zhang, S., Geng, Y., Liu, Y., Wang, J., Hu, B., 2024. Comparison and analysis of widely targeted metabolomics of mycorrhizal of *Lactarius akahatsu* and *Pinus massoniana*. Journal of Central South University of Forestry & Technology, 44(11), 11-21+57. https://doi.org/10.14067/j.cnki.1673-923x.2024.11.002
84. Zhang, X., Chen, L., Wang, Y., Jiang, P., Hu, Y., Ouyang, S., Wu, H., Lei, P., Kuzyakov, Y., Xiang, W., 2023. Plantations thinning: A meta-analysis of consequences for soil properties and microbial functions. Sci Total Environ, 877, 162894. https://doi.org/10.1016/j.scitotenv.2023.162894
85. Zheng, X., Huang, X., Jiang, J., Chen, Y., Chen, J., 2025. Biocontrol potential of *Trichoderma spirale* JSAFC 2090 against pecan diseases. Forest Pest and Disease, 44(02), 12-20. https://doi.org/10.19688/j.cnki.issn1671-0886.20250004
86. Zhou, T., Wang, C., Zhou, Z., 2020. Impacts of forest thinning on soil microbial community structure and extracellular enzyme activities: A global meta-analysis. Soil Biology and Biochemistry, 149. https://doi.org/10.1016/j.soilbio.2020.107915
87. Zhou, Z., Wang, C., Ren, C., Sun, Z., 2020. Effects of thinning on soil saprotrophic and ectomycorrhizal fungi in a Korean larch plantation. Forest Ecology and Management, 461. https://doi.org/10.1016/j.foreco.2020.117920

Figures

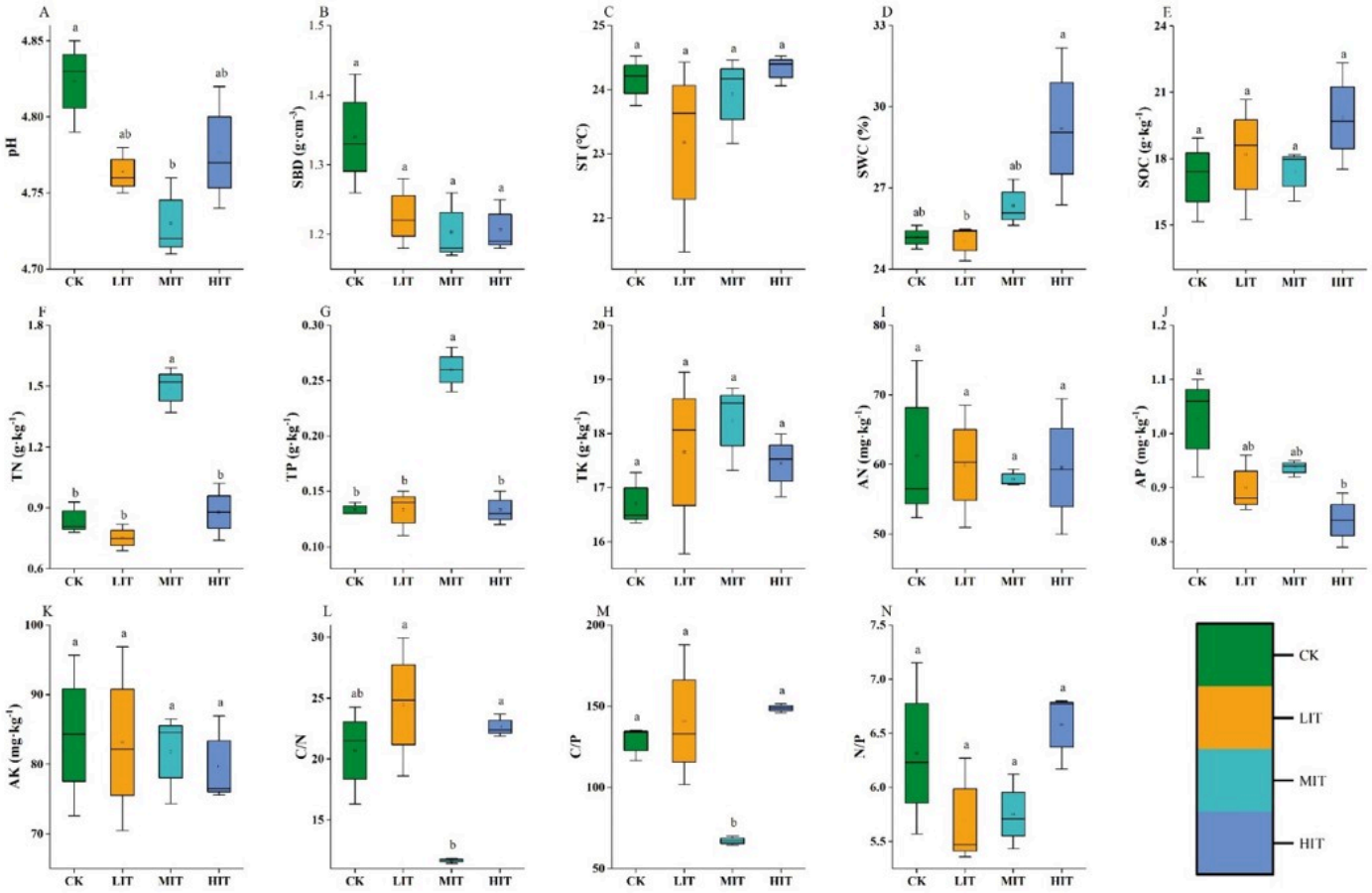

Figure 1

Soil properties for the four different thinning treatments in *P. massoniana* plantations.

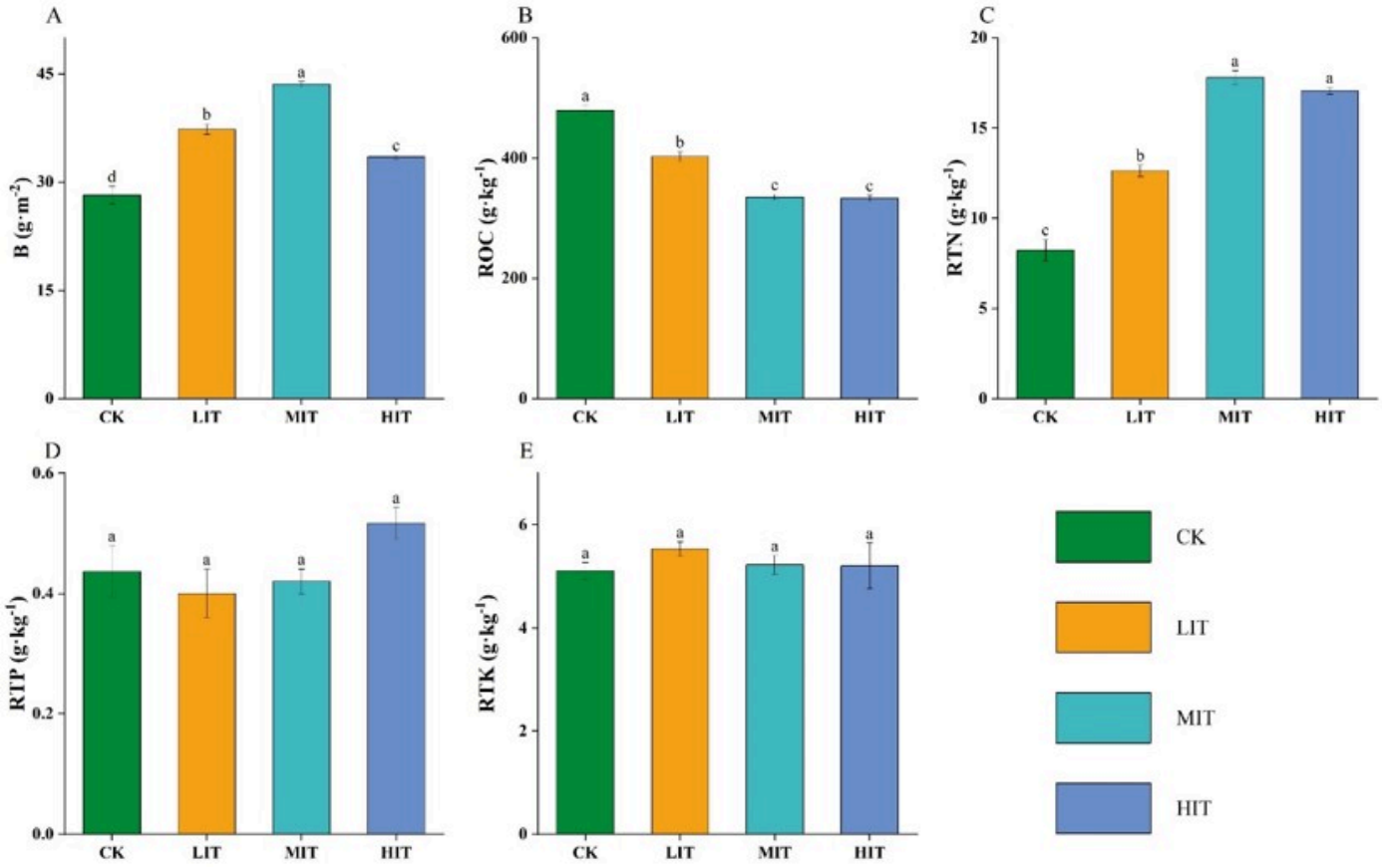

Figure 2

Fine roots nutrient contents with four different thinning treatments in *P. massoniana*plantations.

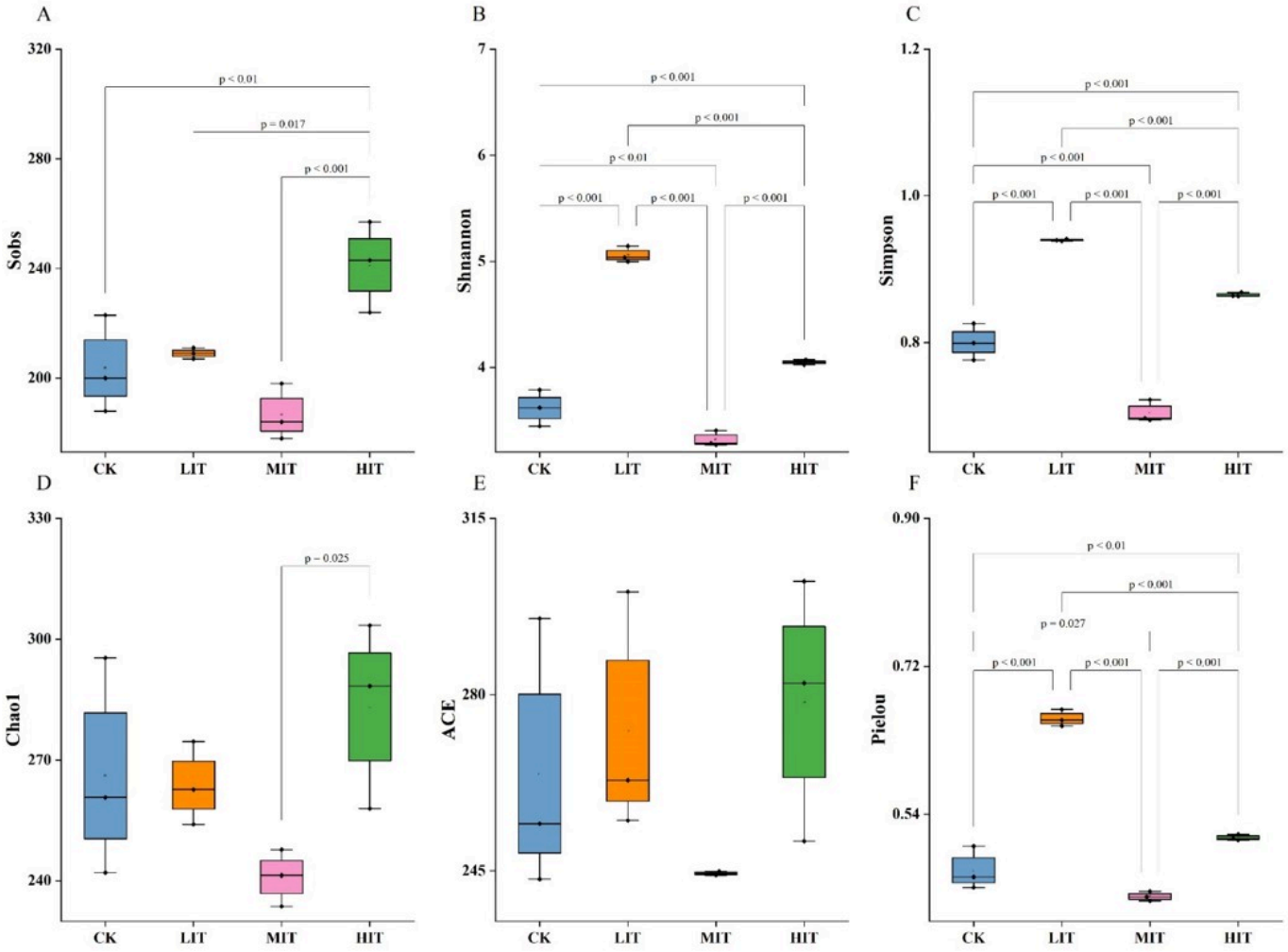

Figure 3

Alpha diversity indices of the ECM fungal communities with four different thinning treatments in *P. massoniana* plantations.

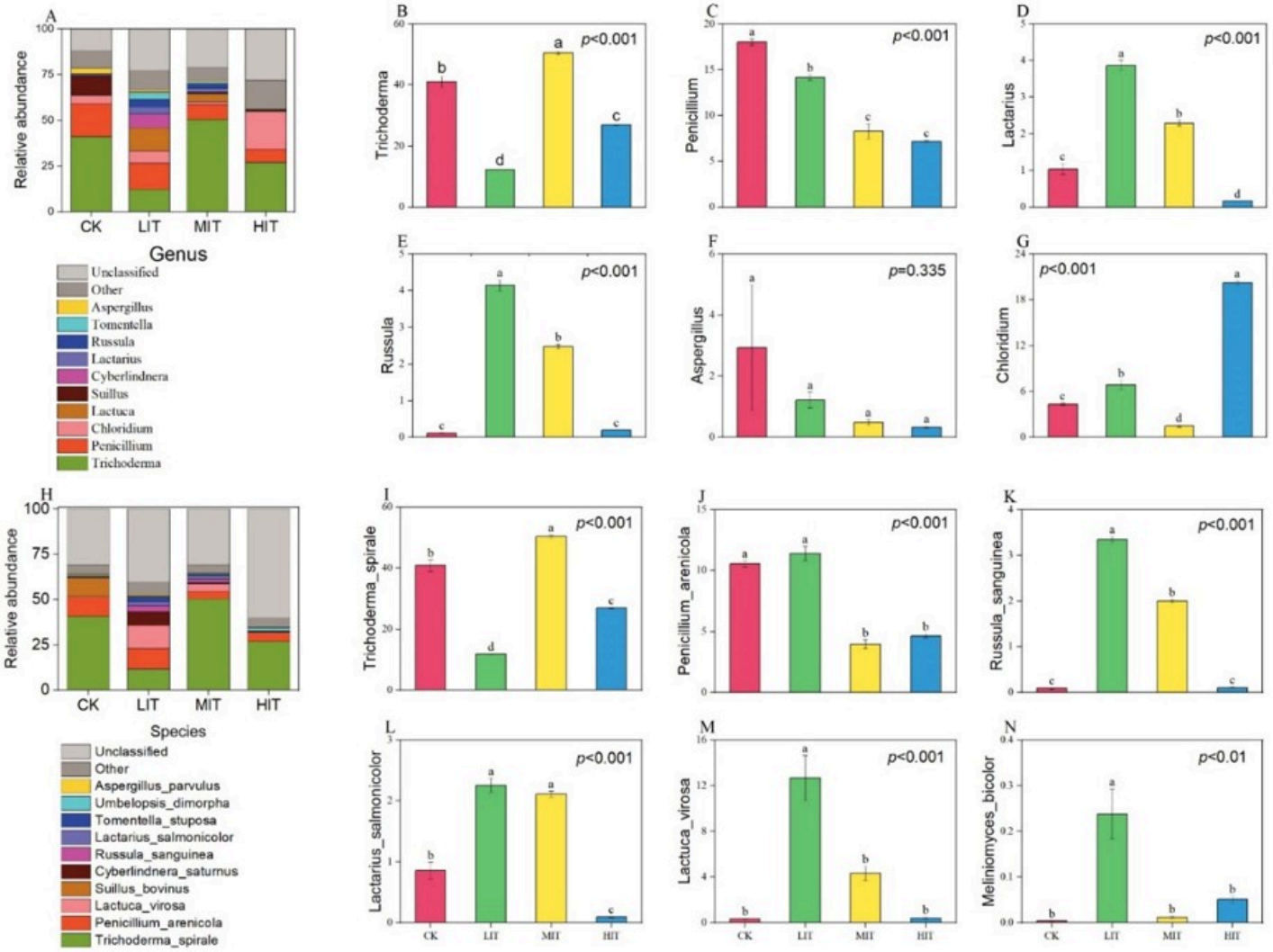

Figure 4

Relative abundance of fungal taxa at the different level. A: Relative abundance of fungal taxa at the Genera level. B-G: Relative abundance of dominant Genera. H: Relative abundance of fungal taxa at the Species level. I-N: Relative abundance of species. Only the taxa with average relative abundance of >0.1% are shown as dominant Genera.

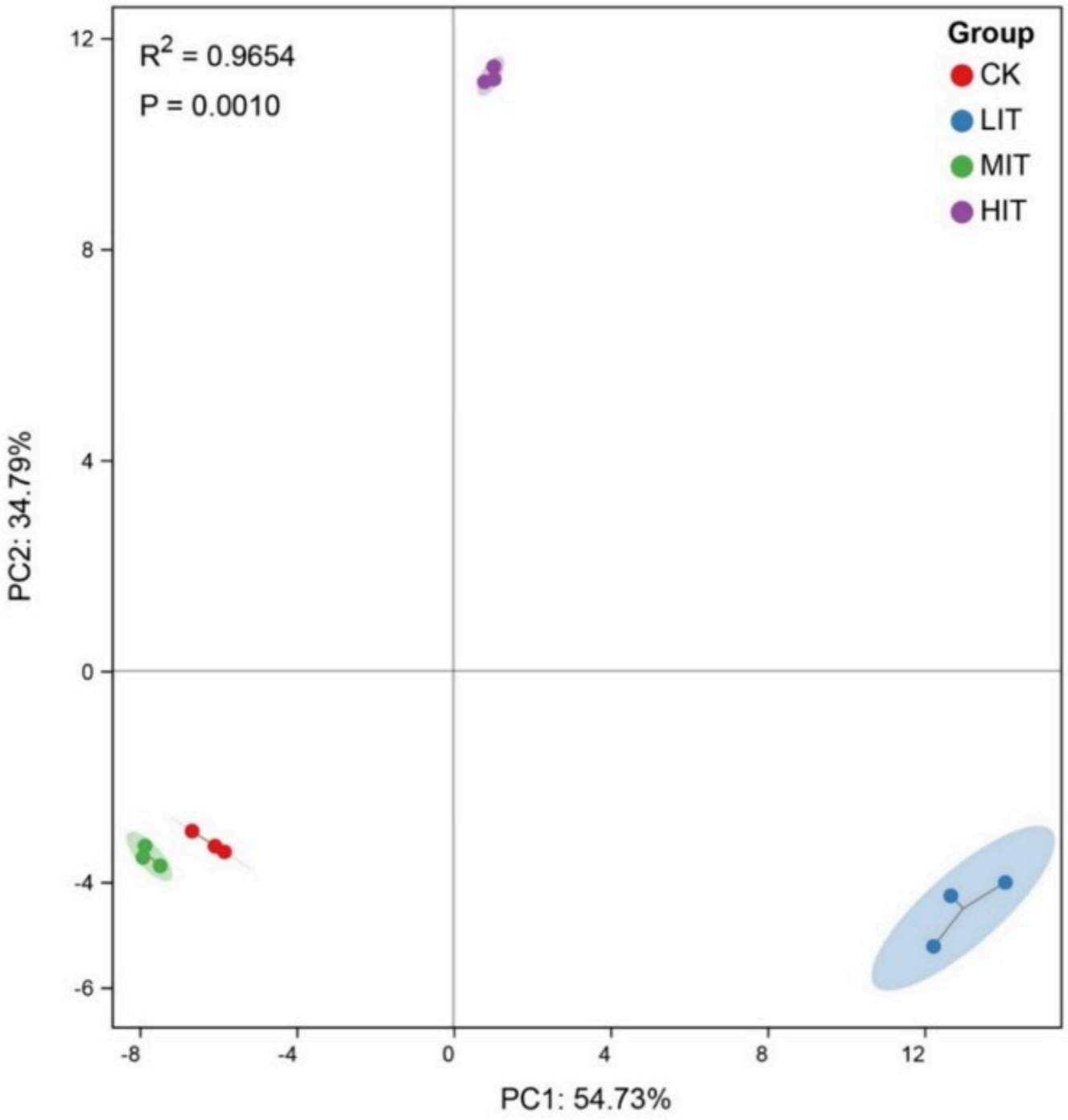

Figure 5

Principal coordinate analysis of the ECM fungal community based on Unweighted_unifrac distance among different groups. The circle represents the 95% confidence interval.

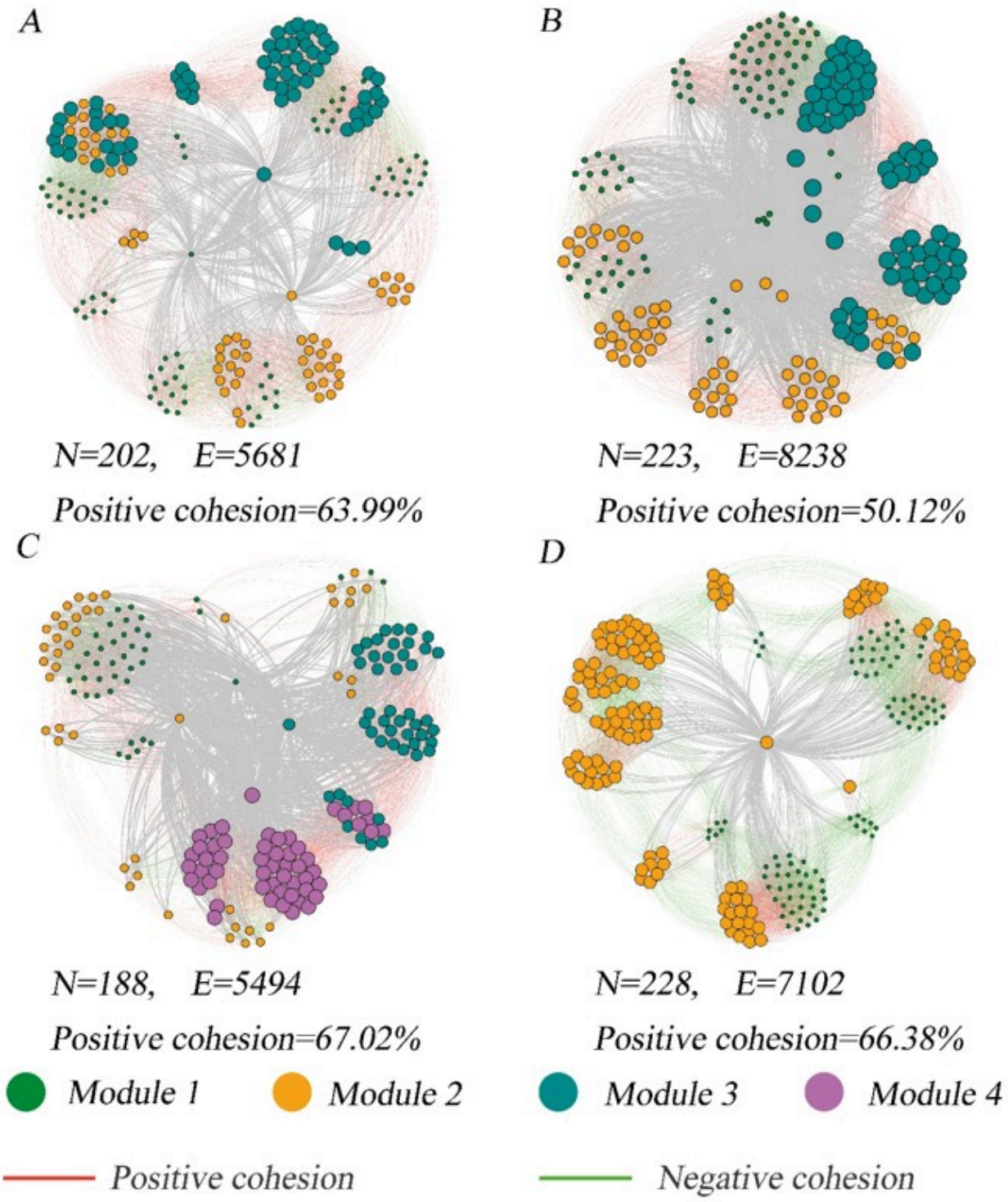

Figure 6

The co-occurrence network of soil ECM communities with four different thinning treatments in *P. massoniana* plantations.

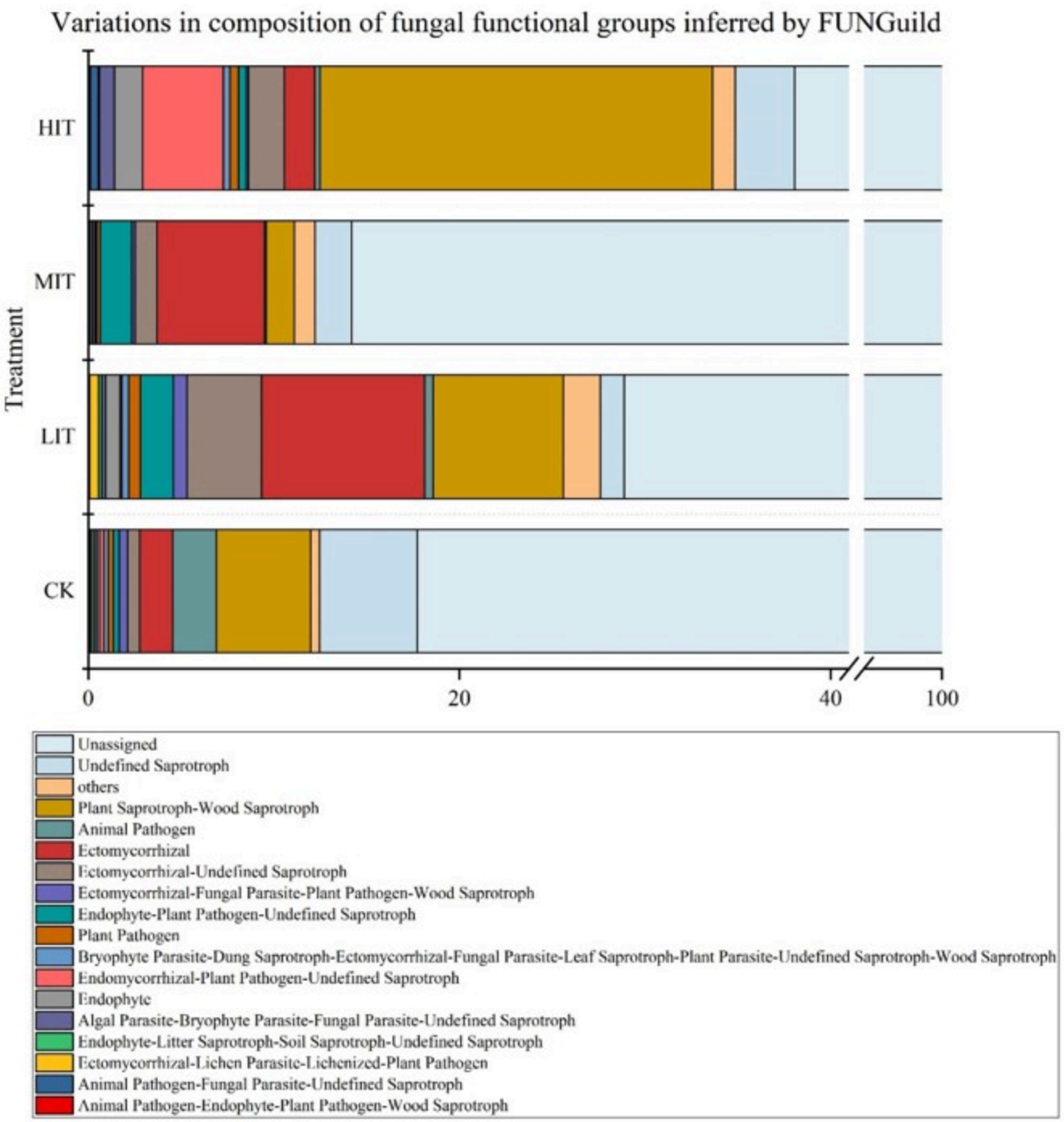

Figure 7

Variations in composition of fungal functional groups inferred by FUNGuild

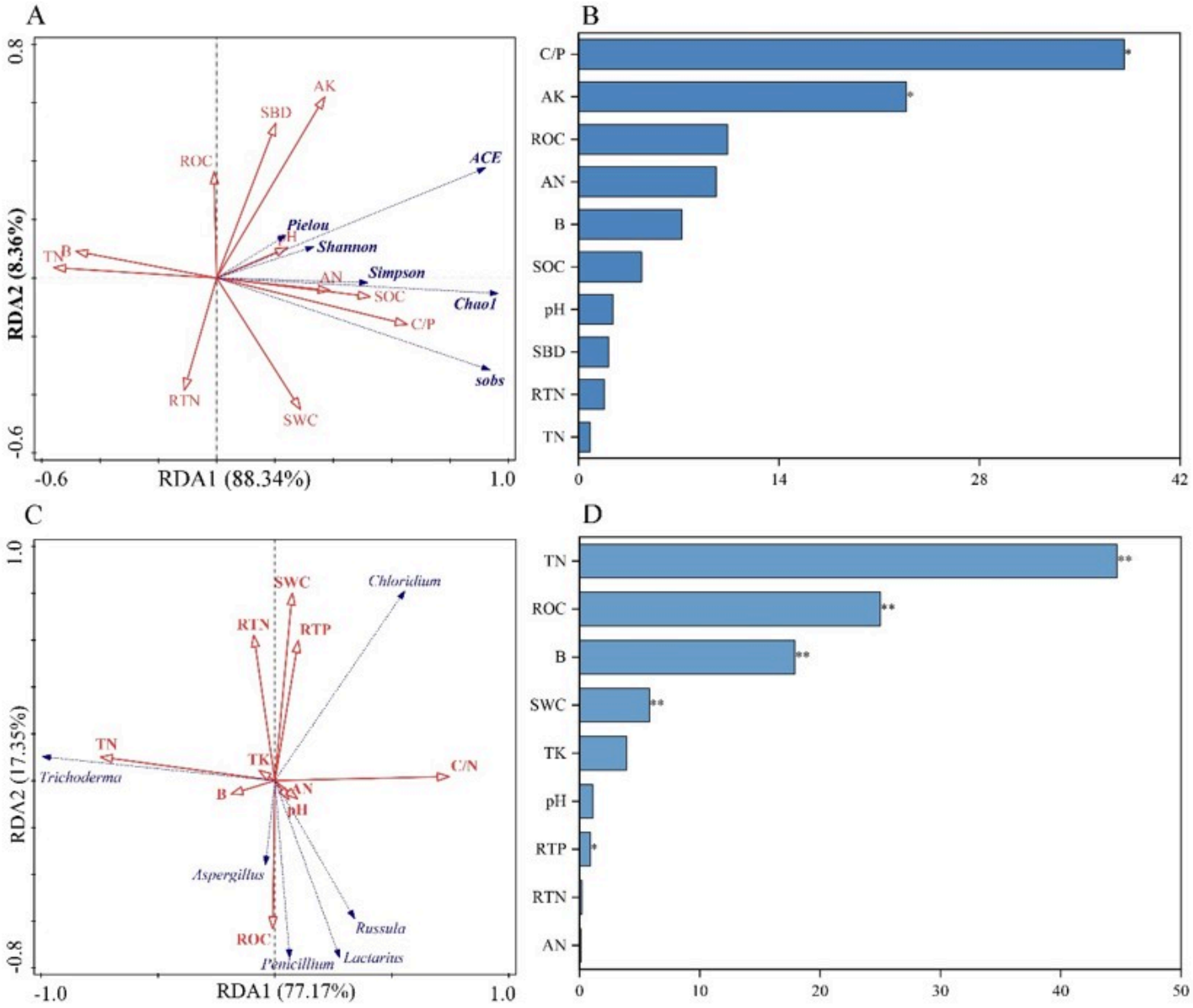

Figure 8

Redundancy analysis (RDA) ordination map of ECM diversity index (A) and Abundance of ECM Fungi (C) with soil physicochemical factors and fine roots nutrient contents. B: RDA results of percentage variation of ECM diversity, D: RDA results of percentage variation of ECM genera abundance.

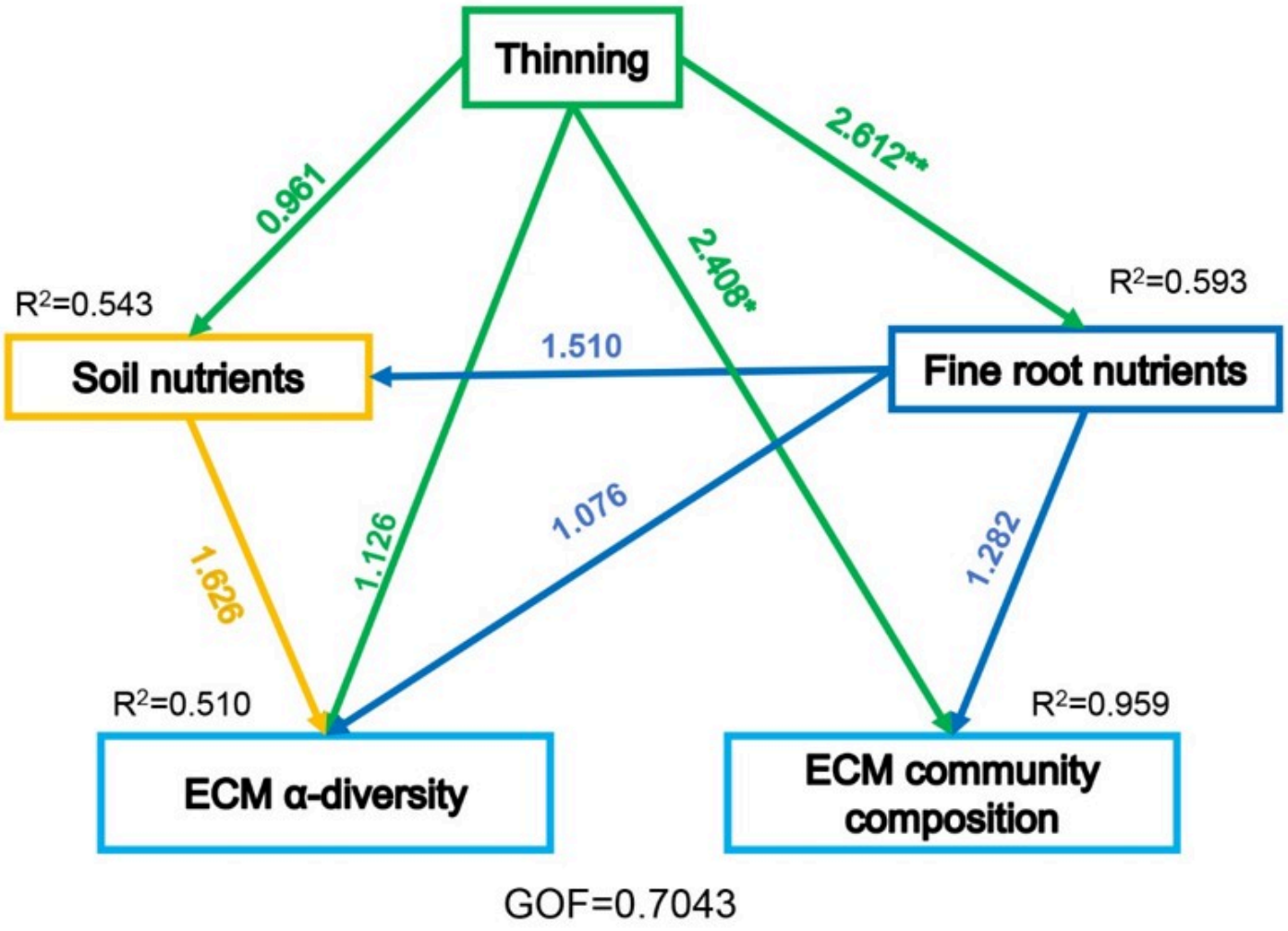

Figure 9

Partial Least Squares Structural Equation Modeling (PLS-SEM) shows the Interaction between Soil physicochemical properties and Fine root nutrients and Community structure of ECM. *, $P < 0.05$; **, $P < 0.01$, ***, $P < 0.001$.