Community structure and maintenance mechanism of ectomycorrhizal fungi of four coniferous species in eastern Inner Mongolia

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Article

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Abstract

In this study, we focused on four major coniferous species in the eastern part of Inner Mongolia, namely Larix gmelinii var. principis-rupprechtii, Larix gmelinii, Pinus tabulaeformis and Pinus sylvestris var. mongolica, and carry out a systematic study on their ectomycorrhiae (EM) fungi. The present study was based on high-throughput sequencing. Based on the high-throughput sequencing data and analyzed by bioinformatics and statistical methods, the results showed that (1) a total of 150 operational taxonomic units (OTUs) were obtained, which belonged to 26 evolutionary branches of Basidiomycota and Ascomycota, respectively. 26 evolutionary branches. Among them, Tricholoma、Tomentella-thelephora、Suillus-rhizopogon、Wilcoxina、Piloderma、Pustularia、Hygrophorus、Sebacina and Amphinema-tylospora are the EM fungi shared by four conifer species. (2) The species diversity and community composition of EM fungi differed significantly among tree species and sample plots, while soil total nitrogen (N) content and nitrogen/phosphorus (N / P) ratio were the main factors affecting community structure; (3) The Neutral Community Model (NCM) and β-Nearest Taxon Index (β-NTI) showed that stochastic processes dominated the construction of EM fungal communities. The results of this study revealed the geographical distribution pattern and maintenance mechanism of EM fungal communities of four coniferous species in the eastern part of Inner Mongolia, which provides a scientific basis for the restoration practice of disturbed ecosystems and the sustainable development of the regional economy.

Introduction

Ectomycorrhizal (EM) fungi play a crucial role in forest ecosystems, particularly in the growth and nutrient uptake of coniferous tree species. They form symbiotic relationships with plant root systems, helping host plants obtain nutrients and water from the soil while receiving carbon sources from the plants [1]. The structure and maintenance mechanisms of EM fungal communities are influenced by various factors, including climate, soil type, ecological drift, and dispersal limitations [2–5]. Therefore, studying the EM fungal community structure of specific regions and tree species is of great significance for understanding the functions and succession of forest ecosystems.

Understanding the mechanisms underlying microbial community assembly is crucial for comprehending the maintenance of biodiversity and predicting its response to global change. The maintenance of EM fungal communities involves various ecological processes, including deterministic and stochastic processes [6]. The primary factors influencing deterministic processes include abiotic factors (such as climatic conditions and soil physical and chemical properties) and biotic factors (such as host plant specificity) [2], while stochastic processes include ecological drift, dispersal limitations, and EM fungal interspecific interactions (such as competitive exclusion) [3–5]. Ecological drift refers to changes in species abundance caused by random birth, death, and migration events. In EM fungal communities, ecological drift may lead to certain species becoming more common or rare without explicit environmental selection [7]. For example, in *sorghum* systems under drought stress, studies have shown that fungal community assembly is influenced by random forces, particularly during early developmental stages and periods of drought stress [7]. Dispersal limitation refers to the inability of species to reach all potential habitats due to limitations in their dispersal capacity. For EM fungi, spore dispersal may be constrained by factors such as distance, wind, and animal activity, thereby influencing community composition [8]. Studies have shown that at the regional scale, EM fungal community composition is influenced by dispersal limitations, meaning that communities farther apart geographically exhibit lower similarity [9]. However, the specific mechanisms and their interactions in shaping the relative importance of EM fungal communities in different ecosystems require further research to clarify.

The eastern region of Inner Mongolia possesses unique climatic conditions and soil types, providing a special environmental backdrop for studying the diversity and community structure of EM fungi. Studies targeting *Pinus sylvestris* var. *mongolica* in Horqin Sandy Land of Inner Mongolia [10], *Picea mongolica* in the Baiyin'ao Bao Nature Reserve [11], *Pinus sylvestris* var. *mongolica* in Honghuaerji [12], *Larix principis-rupprechtii* in Heili River and Helan Mountain Nature Reserve [13] and *Larix gemelinii Rupr.* in the Great Khingan Mountains [14] have also yielded some results, but these studies were conducted only at the local scale. For the EM fungal communities of key coniferous tree species in this region (such as *Larix gmelinii* var. *principis-rupprechtii*, *Larix gmelinii*, *Pinus tabuliformis*, and *Pinus sylvestris* var. *mongolica*), there is a lack of in-depth research on the systematic structure, geographical distribution patterns, ecological construction processes, and the comprehensive influence of environmental factors at the regional scale.

Given this, this study focuses on the four coniferous tree species mentioned above in the eastern region of Inner Mongolia, utilizing high-throughput sequencing technology combined with bioinformatics and statistical methods, with the aim of: (1) investigating the diversity and community composition of EM fungi in the four coniferous tree species; (2) analyzing the correlation between soil physical and chemical properties, climatic conditions, and EM fungal community structure; identify key influencing factors and their interrelationships; (3) reveal the geographical distribution patterns and maintenance mechanisms of EM fungal communities (i.e., the relative contributions of deterministic and random processes in community assembly). The research findings can provide scientific basis for ecosystem restoration and regional economic development practices, and serve as a reference for studies on the assembly mechanisms and functions of EM fungal communities in different host plants.

Results

The Composition of EM Fungi Species in Four Coniferous Tree Species

A total of 626 OTUs were identified from 596,723 sequences. Among them, 200 OTUs were identified as EM fungi. After sample standardization, 150 OTUs were finally obtained for subsequent analysis. These 150 OTUs belonged to 2 phyla, 4 classes, 4 orders, 10 families and 29 genera. Among them, the dominant genera were *Tricholoma*, *Tomentella* and *Suillus*, accounting for 18%, 17% and 14% of the total sequences respectively.

The analysis revealed that 72% of phylogenetic lineages belonged to Basidiomycota, while the remaining 28% were affiliated with Ascomycota. Nine dominant ectomycorrhizal (EM) fungal genera were shared among the four coniferous species: *Tricholoma*, *Tomentella/thelephora*, *Suillus/rhizopogon*, *Wilcoxina*, *Piloderma*, *Pustularia*, *Hygrophorus*, *Sebacina*, and *Amphinema/tylospora* (Fig. 2). Notably, host-specific distribution patterns emerged: *Tricholoma* exhibited its highest relative abundance in *Larix gmelinii* but the lowest in *Pinus tabuliformis*. *Tomentella/thelephora* demonstrated peak abundance in *Larix principis-rupprechtii* while showing minimal presence in *L. gmelinii*. *Suillus/rhizopogon* predominated in *P. tabuliformis* but was least represented in *L. principis-rupprechtii*. *Wilcoxina* reached maximum levels in *Pinus sylvestris* var. *Mongolica* but minimum levels in *L. gmelinii*. *Piloderma* displayed contrasting abundance patterns between *L. gmelinii* (highest) and *P. tabuliformis* (lowest). *Pustularia* showed preferential colonization of *P. sylvestris* var. *Mongolica* over *P. tabuliformis*. *Hygrophorus* exhibited host affinity toward *L. principis-rupprechtii* rather than *P. tabuliformis*. *Sebacina* and *Amphinema/tylospora* both demonstrated significant preference for *P. tabuliformis*, while registering minimal abundance in *L. gmelinii*. These findings collectively indicate substantial interspecific variation in EM fungal community composition across host tree species.

Diversity of EM Fungi in Four Coniferous Species

The species accumulation curves demonstrated that ectomycorrhizal (EM) fungal diversity associated with roots of four coniferous species failed to reach plateau, indicating that additional sampling would yield new EM fungal taxa. Both Shannon and Chao1 indices revealed distinct EM fungal community compositions among the four coniferous species (Fig. 3). Significant interspecific differences in EM fungal diversity were observed, with particularly pronounced disparities between *Pinus tabuliformis* and *Larix gmelinii* ($p < 0.001$), and statistically significant differences between *P. tabuliformis* and *Larix principis-rupprechtii* ($p < 0.05$). Spatial heterogeneity analysis showed significant differences in EM fungal diversity between three sampling sites of *L. principis-rupprechtii* (Saihanwula vs. Huanggangliang, and Saihanwula vs. Wangyezheng; $p < 0.05$). Similarly, notable differences emerged between two sampling locations of *L. gmelinii* (Huanggangliang vs. Saihanwula; $p < 0.05$).

In this study, we conducted PCoA and NMDS ordination analyses to investigate the beta diversity of EM fungi associated with root systems of four coniferous species (Fig. 4). The PCoA results revealed that the first principal coordinate explained 29.97% of variance, while the second accounted for 16.76%. Significant differentiation was detected both among tree species (Adonis test: $R2 = 0.112$, $P < 0.001$) and across sampling sites (Adonis test: $R2 = 0.115$, $P < 0.001$). NMDS ordination combined with envfit analysis further identified soil nitrogen (N) content and N / P ratio as key determinants governing the compositional variation of EM fungal communities in coniferous root systems.

Ecological Process Analysis of EM Fungi in Four Conifer Species.

This study used βNTI to assess the ecological processes driving EM fungal community assembly. Most βNTI values ranged between − 2 and 2 (Fig. 5A), with only 3.5% of cases exhibiting βNTI values exceeding 2, indicating that stochastic processes dominated. Consistent with these findings, NCM analysis further confirmed that community structure was primarily shaped by stochastic mechanisms (Fig. 5B).

Discussion

This study showed that N content and N / P ratio were the most important factors affecting the structure of EM fungal communities, which was consistent with the results of Dong *et al.*, [15] in Hulunbeier sandy *Pinus sylvestris* var. *mongolica*, but it was also shown that effective N was the main factor affecting the composition of EM fungal communities [16], and it was hypothesized that this difference might originate from the changes in physicochemical properties caused by different soil matrices. Thus leading to the existence of different microbial communities in different habitats [17]. Meanwhile, MAT has been found to be the main factor influencing the composition of EM fungal communities [18, 19], whereas the present study did not find a significant effect of MAT, possibly due to the small climatic differences among the four sampling sites in this study.

The dominant EM fungal taxa of the four coniferous species share ecological functions in the ecosystem. *Tricholoma* can promote the uptake of nutrients such as nitrogen and phosphorus by forming a symbiotic relationship with the root system of the plant [20, 21], and enhance the plant's resistance to adversities such as drought, pests and diseases [22–24]. *Tomentella-thelephora* has a strong ability to decompose organic matter and can participate in the carbon and nitrogen cycles in the soil [22, 25], maintaining ecosystem stability as well as material cycling and energy flow [22, 26]. This is in general agreement with the studies of Buée *et al.*, [27], Simard *et al.*, [28], and

Agerer [29]. These results suggest that host plants have a significant effect on EM fungal species composition, presumably due to the conserved phylogenetic ecological niches of host plants [27], e.g., closely related plant species show more similarity than distantly related species in terms of morphological and functional traits, and possess more similar fungal taxa [16]. For example, differences in plant root secretions can affect colonization by EM fungi [30], and there may be differences in root secretions of different conifer species, leading to differences in EM fungal communities [18]. *Tricholoma*、*Tomentella-thelephora*、*Suillus-rhizopogon*、*Wilcoxina*、*Piloderma*、*Pustularia*、*Hygrophours*、*Sebacina* and *Amphinema-tylospora* are the four coniferous species shared dominant taxa of EM fungi, which is inconsistent with previous studies in various host plants and sandy ecosystems, where *Tomentella-thelephora* and *Russula-lactarius* are usually the most dominant EM fungal lineages [28, 29, 31], and it is hypothesized that it may be the host specificity of the present study or the unique local climate resulting from the differences due to host specificity of this study or the unique local climate resulting in a specialized fungal species pool. Future studies should strengthen the functional genomics analysis of these fungi and combine them with field controlled experiments to delve deeper into their roles in the ecosystem.

There were significant differences in the α-diversity of the four conifer species analyzed by both Shannon index and Chao1 index. This is basically consistent with the studies of Bao *et al*., [11], Zhao *et al*., [12],Yang *et al*., [13] and Wang *et al*., [18]. For example, there were significant differences in the EM fungal community structures of *Picea mongolica* in the Baiyin'ao Bao Nature Reserve, *Pinus sylvestris* var. *mongolica* in Honghuaerji, *Larix principis-rupprechtii* in Heili River and Helan Mountain Nature Reserve and five species of pines in Inner Mongolia. From the perspective of ecological significance, the differences in α-diversity are of great value. High α-diversity usually means that the ecosystems have greater stability and adaptability. Relevant studies have shown that EM fungal communities with high α-diversity can decompose organic matter more efficiently, promote nutrient cycling, and enhance the nutrient uptake capacity of plants, thus improving the productivity of the whole ecosystem. In addition, diverse EM fungal communities can also provide plants with a wider range of disease and adversity resistance support, which can help to maintain ecosystem health and balance [22–24]. β In diversity, PCoA and NMDS sequencing combined with the PERMANOVA test showed that EM fungal communities differed significantly among tree species and locations. This is consistent with the results of previous studies on plants in the Salicaceae, Betulaceae, and Fagaceae [32–34].

The low NCM analysis m-value (0.001) and βNTI values falling in the range of − 2 to 2 suggest that stochastic processes dominate. One view is that spatial distance poses an obstacle to the dispersal of fungal reproductive offspring (e.g., ascospores, spores, and hyphae of fungi) [35]. At the same time, differences in the dispersal ability of fungal species may lead to changes in the composition of fungal communities due to a priori effects, which show that first-entrant fungi tend to exhibit a more significant dominance in the competition for survival space and resource utilization [36]. However, deterministic processes (e.g., soil physicochemical properties and climatic conditions) still have a significant effect on EM fungal community structure. Future studies could further explore the interactions between deterministic and stochastic processes and how they jointly influence EM fungal community construction.

Combining the results of this study with those of previous studies, it can be seen that both deterministic and stochastic factors play a key role in the construction of EM fungal communities in a specific region of eastern Inner Mongolia where four coniferous species coexist, but stochastic factors playing a dominant role. In this study, soil characteristics were more influential than climatic conditions and geographic distance in terms of abiotic factors influencing the structure of EM fungal communities. This conclusion differs from previous research findings. We speculate that this may be related to ecological drift and random processes that generate diversity, but this needs to be investigated in our future research.

Methods

Study sites and sample collection

The study was conducted in Chifeng City, Inner Mongolia Autonomous Region, with four study sites established: Huanggangliang (E 117°31', N 43°34'), Heilihé (E 118°27', N 41°21'), Saihanwula (E 118°14, N 44°17'), and Wangyedian (E 118°10', N 41°45'). The study area is primarily characterized by hilly and highland terraces, with a northwest-high, southeast-low topography and an average elevation of 1,300–2,000 meters. Climate information for the sampling sites was extracted from the World Clim dataset [37], revealing that the selected sites are located in semi-arid and cold-temperate climate zones, with mean annual temperatures (MAT) ranging from − 0.3 to 5.5°C and mean annual precipitation (MAP) ranging from 374 to 483 mm in Table 1.

Table 1
Information on geographic coordinate, climatic and soil variables in this study.

Sites	Plants	Latitude (°)	Longitude (°)	Altitude (m)	MAT (°C)	MAP (mm)	N (mg.kg-1)	P (mg.kg-1)	Ca (mg.kg-1)	Mg (mg.kg-1)
HGL	Lp	43.5706	117.5217	1496	-0.3	381	7.11	0.59	12.05	5.49
	Lg						6.95	0.57	12.11	5.57
HLH	Lp	41.3606	118.4656	1030	5.48	483	3.76	0.29	1.11	0.63
	Pt						3.38	0.31	1.09	0.64
SHWL	Lp	44.2972	118.2478	1274	0.1	374	31.39	0.81	1.62	0.78
	Lg						32.22	0.77	1.67	0.78
	Pm						31.07	0.81	1.58	0.75
WYD	Lp	41.7508	118.1728	1275	3.62	475	30.47	5.23	1.25	4.01
	Pt						30.83	5.19	1.25	4.13
	Pm						31.92	5.22	1.28	4.05

MAT, mean annual temperature; MAP, mean annual precipitation; N, soil total nitrogen; P, soil total phosphorus; Ca, soil total calcium; Mg, soil total magnesium. HGL, Huanggangliang. HLH, Heilihe. SHWL, Saihanwula. WYD, Wangyedian. Lp, *Larix gmelinii* var. *principis-rupprechtii*. Lg, *Larix gmelinii*. Pt, *Pinus tabuliformis*. Pm, *Pinus sylvestris* var. *mongolica*.

In this study, four species of secondary forests at the peak growth stage were selected. Sampling was completed in July 2021. At each site, 3–7 individual plants of each conifer species were selected (with a spacing between plants of > 10 m), and 300 g of fine roots from each plant were collected from each of the three directions along the main trunk of the tree using the rooting method. These samples were then combined to form a single root sample, which was preserved in an ice box and brought back to the laboratory immediately to be frozen in a refrigerator set at -80 ℃. Additionally, fine root samples were collected from each individual inter-root (approximately 200 g), air-dried, and sieved (through a 2 mm mesh) for soil physical and chemical analysis. Concurrently, soil samples (approximately 200 g) were obtained from the inter-root region of each individual plant specimen, subsequently air-dried, and sieved (through a 2 mm mesh) for soil physicochemical characterization.

Soil properties analysis

The soil physical and chemical properties measured include total nitrogen (N), total phosphorus (P), total calcium (Ca), total magnesium (Mg), and the nitrogen/phosphorus ratio (N / P). The specific operations involved are outlined as follows: N content was determined by the semi-micro Kjeldahl method, P content was determined using a Thermo Fisher Scientific iCAP 6300 inductively coupled plasma emission spectrometer, the N / P ratio was calculated, and Ca and Mg content were determined using an Optima 8000 PerkinElmer inductively coupled plasma emission spectrometer (Appendix 1).

DNA Extraction, PCR, and MiSeq Sequencing

In the laboratory setting, root samples were meticulously washed with tap water to ensure their cleanliness and integrity. Subsequently, intact root samples were meticulously divided into segments measuring approximately 2 centimeters in length. The selection of intact mycorrhizae for analysis was conducted at random, with 200 slices from each plant selected for examination. Morphological characteristics such as shape, color, dendrites, fungal hyphae, surface texture, transparency, and the presence or absence of vesicles were documented. The 10,200 mycorrhizae samples were then preserved in a refrigerator set at − 80 ℃.The extraction of DNA was performed using a modified cetyltrimethylammonium bromide (CTAB) method [38]. and the ITS2 region was amplified by polymerase chain reaction (PCR) using a two-step method [39–41]. First, the first round of PCR was performed with the fungus-specific primers ITS1f (5' - CTTGGTCATTTAGAGGAAGTAA − 3') and ITS4 (5' - TCCTCCCCTCCTTAGAGGAAGTAA − 3') were utilized as primers to amplify the entire ITS region, and subsequently, fITS7 (5' - GTGARTCATCGAATCTTTG − 3') and ITS4 with base tag sequences were employed as primers to amplify the fungal ITS2 region. The purified samples were then subjected to sequencing on the Illumina Miseq double-end sequencing platform at the Chengdu Institute of Biology, Chinese Academy of Sciences.

Bioinformatics Analysis

Initially, low-quality sequences were filtered using QIIME software [42]. Subsequently, sequences in the ITS2 region of the quality control data were extracted using ITSx software [43], and sequences in the Unified Fungal Species Database [44] were referenced. Chimeric sequences in the ITS2 region were examined and removed using Usearch v11. The UPARSE pipeline [45] was then used to classify the non-chimeric ITS2 region sequences into OTUs according to 97% sequence similarity. Then, the representative sequences (with the highest number of sequences) of each OTU were classified into OTUs using the NCBI (National Center for Biotechnology Information) and UNITE reference databases for BLAST (Basic Local Alignment Search Tool) [46]. Each OTU was identified to the best taxonomic position by referring to the taxonomic criteria of Tedersoo *et al.* [35] and the EM fungi were identified according to the method of Kõljalg *et al.*, [44].The raw sequence data reported in this paper have been deposited in the Genome Sequence Archive in National Genomics Data Center, China National Center for Bioinformation/Beijing Institute of Genomics, Chinese Academy of Sciences (GSA: CRA028857) that are publicly accessible at CRACRA028857.

Statistical Analysis

The classification of EM fungi was performed using R-3.6.1 [47], and alpha diversity metrics, including Chao1 and Shannon indices, were computed with the vegan package [48]. The Chao1 index serves as an estimator for the total number of operational taxonomic units (OTUs) based on the observed OTU count. In contrast, the Shannon index reflects the diversity of OTUs across various samples. This study employed two approaches - cmdscale and metaMDS - to integrate Principal Co-ordinates Analysis (PCoA) and Non-Metric Multi-Dimensional Scaling (NMDS),

avoiding metric-based measurements. By utilizing the ordiellipse function, the 95% confidence intervals for EM fungal communities associated with different coniferous tree species were determined. Furthermore, the envfit command was applied to analyze the influence of geographical locations, climate, and soil factors, thereby identifying the primary drivers shaping the structure of the EM fungal community. The adonis command was used to validate these findings through Permutational Multivariate Analysis of Variance (PERMANOVA) analysis. To investigate the assembly mechanisms of the EM fungal community, the β-Nearest Taxon Index (βNTI) was adopted. Specifically, the comdistnt function in the picante package [49] was utilized to quantify pairwise phylogenetic turnover among communities based on the β-Mean Nearest Taxon Distance (βMNTD). The mntd and ses.mntd functions were employed to calculate the Mean Nearest Taxon Distance (MNTD) and the Beta Taxonomic Index (BTI). A value of |βNTI| >2 indicates that the phylogenetic turnover rate significantly exceeds (or falls below) the spatial distribution expectation, implying a deterministic process dominates. Conversely, when |βNTI| < 2, no significant difference exists between the phylogenetic turnover rate and the expected spatial distribution, suggesting stochastic processes play a more significant role in community assembly. Additionally, Sloan's Neutral Community Model (NCM) [50] was applied to assess the assembly process by correlating the migration rate "m" of individual fungal OTUs with their occurrence frequency and abundance (after logarithmic transformation). All results were visualized using ggplot2.

Declarations

Author contributions

Y.F. conceived the project. Y.F. and J.L.(Jinyan Li) designed experiments and analysed all data in the paper. J.L.(Jinyan Li) Z.Y., X.L., L.W. and J.L.(Jiani Lu) finished the experiments and obtained the relevant experimental data. J.L.(Jinyan Li) and Z.Y. wrote the manuscripts, Y.F. revised it. All authors have read and agreed to the published version of the manuscript.

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Competing interests

The authors declare no competing interests.

Field Study Permissions

The following information was supplied relating to field study approvals (i.e., approving body and any reference numbers):

Field experiments were approved by the National Field Scientific Observation and Research Statlon of Forest Ecosystem.

Voucher specimen

Larix gmelinii var. principis-rupprechtii, China, Inner Mongolia, Saihanwula, 44.29 N 118.24 E, 1274m, 21 July 2021, Fan Yongjun #NMG-2021-026(IMUST-00526)

Larix gmelinii, China, Inner Mongolia, Saihanwula, 44.29 N 118.24 E, 1274m, 21 July 2021, Fan Yongjun #NMG-2021-027(IMUST-00527)

Pinus tabuliformis, China, Inner Mongolia, Heilihe, 41.36 N 118.46 E, 1030m, 11 July 2021, Fan Yongjun #NMG-2021-028(IMUST-00528)

Pinus sylvestris var. Mongolica, China, Inner Mongolia, Saihanwula, 44.29 N 118.24 E, 1274m, 21 July 2021, Fan Yongjun #NMG-2021-029(IMUST-00529)

DNA Deposition

The following information was supplied regarding the deposition of DNA sequences:

The raw sequence data reported in this paper have been deposited in the Genome Sequence Archive (Genomics, Proteomics & Bioinformatics 2021) in National Genomics Data Center (Nucleic Acids Res 2022), China National Center for Bioinformation/Beijing Institute of Genomics, Chinese Academy of Sciences (GSA: CRA028857) that are publicly accessible at https: e at https://ngdc.cncb.ac.cn/gsa/browse/CRA028857.

Data Availability

The following information was supplied regarding data availability:

The raw sequence data is available in the Genome Sequence Archive: CRA028857.

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Figures

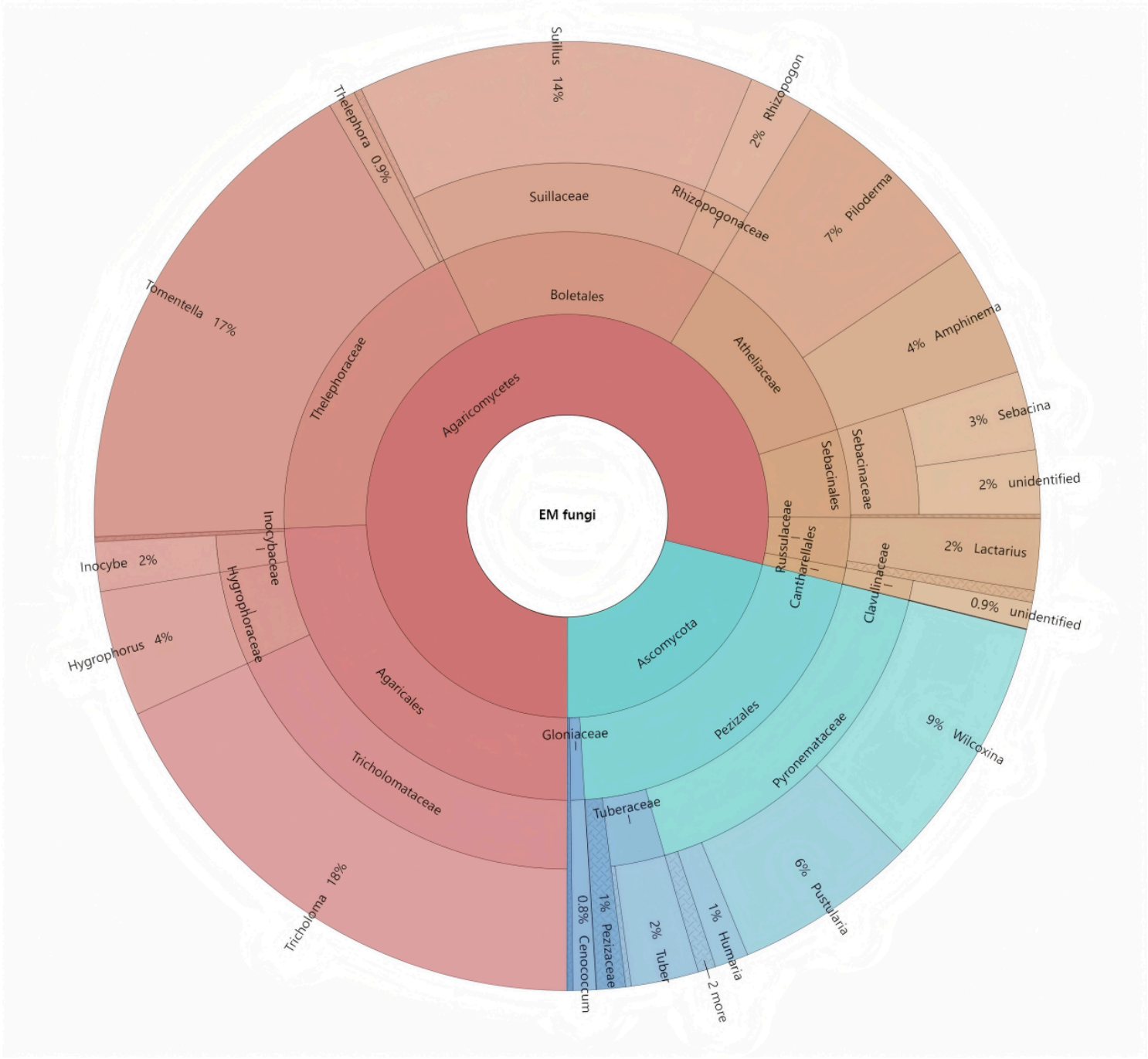

Figure 1

Krona chart of taxonomic affiliation of ectomycorrhizal fungi and their relative abundances. Inner circlerepresent higher taxonomic ranks and more detailed taxonomic ranks are presented in outer circles.

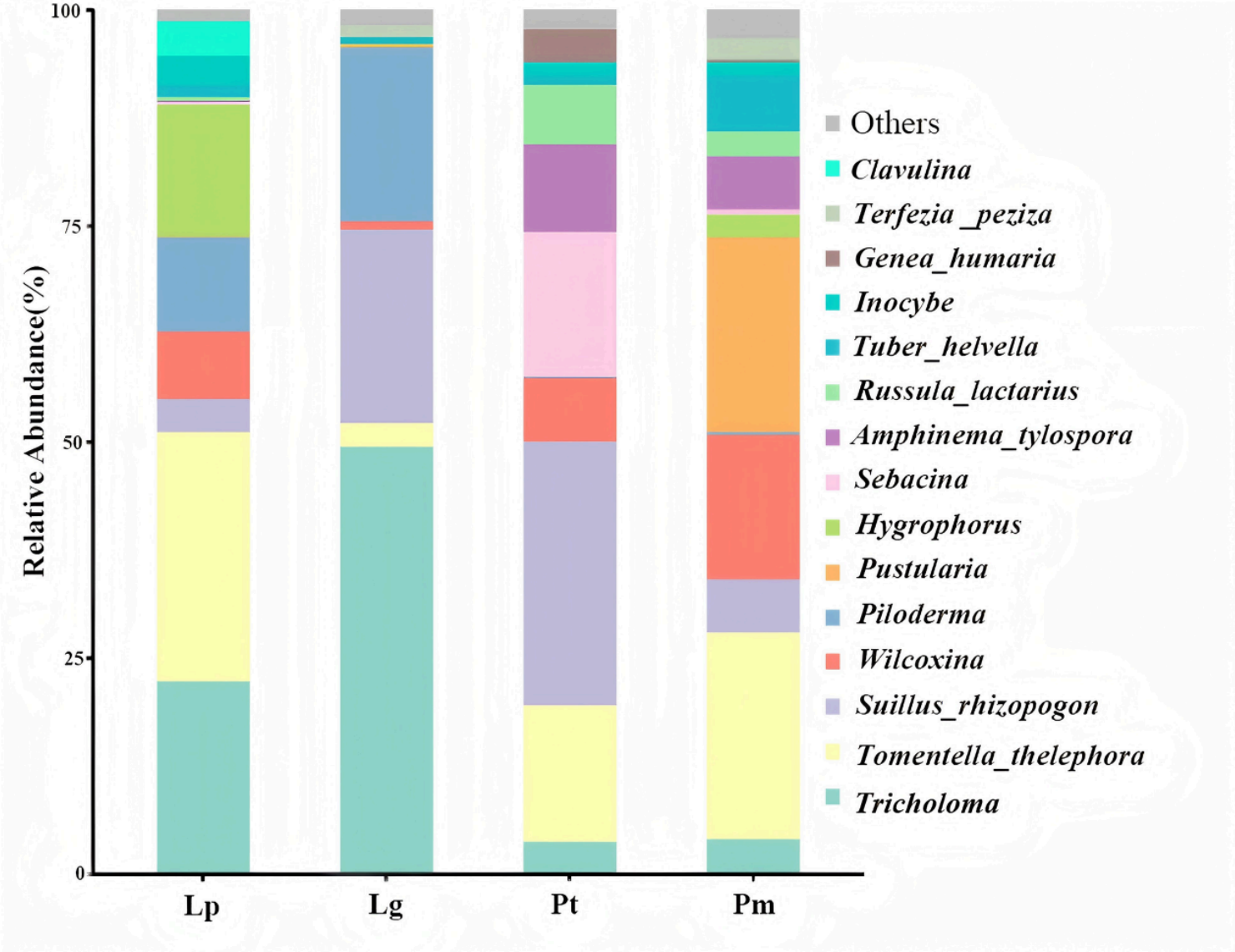

Figure 2

Species composition of root EM fungi of four conifer species. Note: Lp, *Larix gmelinii* var. *principis-rupprechtii*. Lg, *Larix gmelinii*. Pt, *Pinus tabuliformis*. Pm, *Pinus sylvestris* var. *mongolica*.

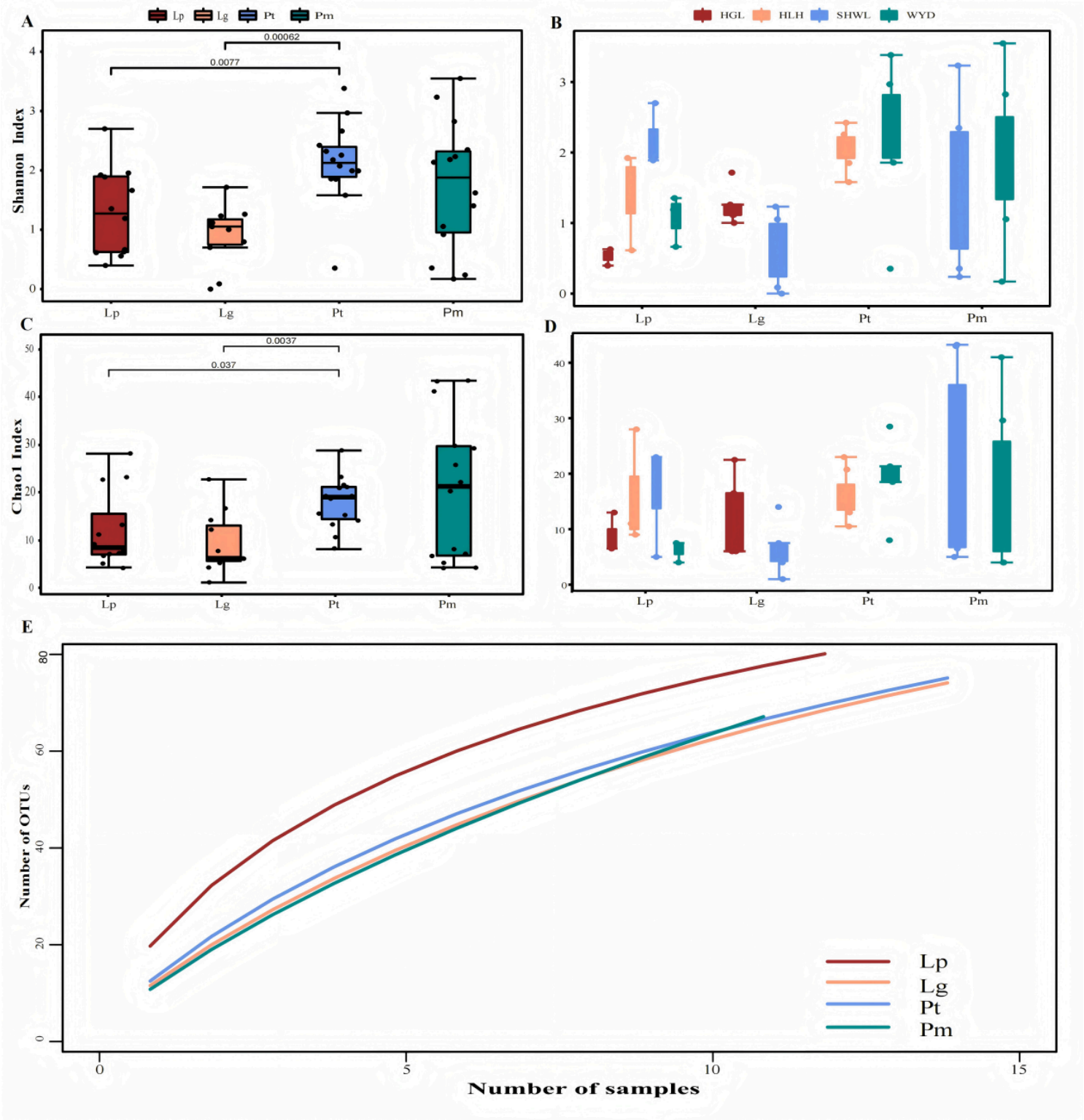

Figure 3

Differences between two species were tested according to wilcox.test. Cumulative curves (E) of EM fungi Shannon indexes (A, B), Chao1 indexes (C, D) and operational taxonomic units (OTUs) of four conifer species. Note: HGL, Huanggangliang. HLH, Heilihe. SHWL, Saihanwula. WYD, Wangyedian. Lp, *Larix gmelinii* var. *principis-rupprechtii*. Lg, *Larix gmelinii*. Pt, *Pinus tabuliformis*. Pm, *Pinus sylvestris* var. *mongolica*.

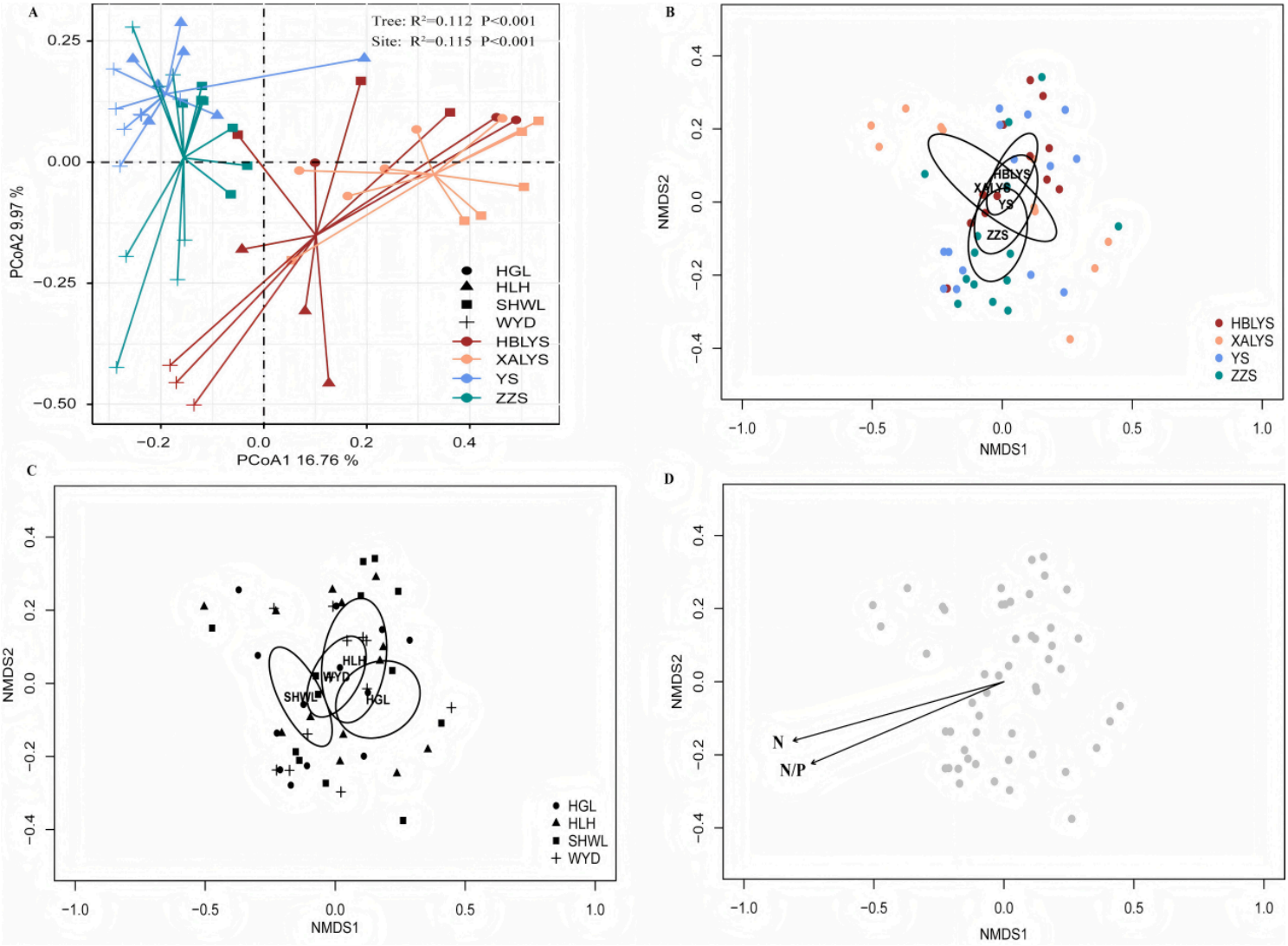

Figure 4

PCoA (A) and NMDS (B, C) ordination analyses of differences between four conifer EM fungal species and sample sites. Ellipses represent 95% confidence intervals around each site centroid. Soil variables conform to the NMDS ranking (D). N, total nitrogen content, N/P, nitrogen to phosphorus ratio. Note: HGL, Huanggangliang. HLH, Heilihe. SHWL, Saihanwula. WYD, Wangyedian. Lp, *Larix gmelinii* var. *principis-rupprechtii*. Lg, *Larix gmelinii*. Pt, *Pinus tabuliformis*. Pm, *Pinus sylvestris* var. *mongolica*.

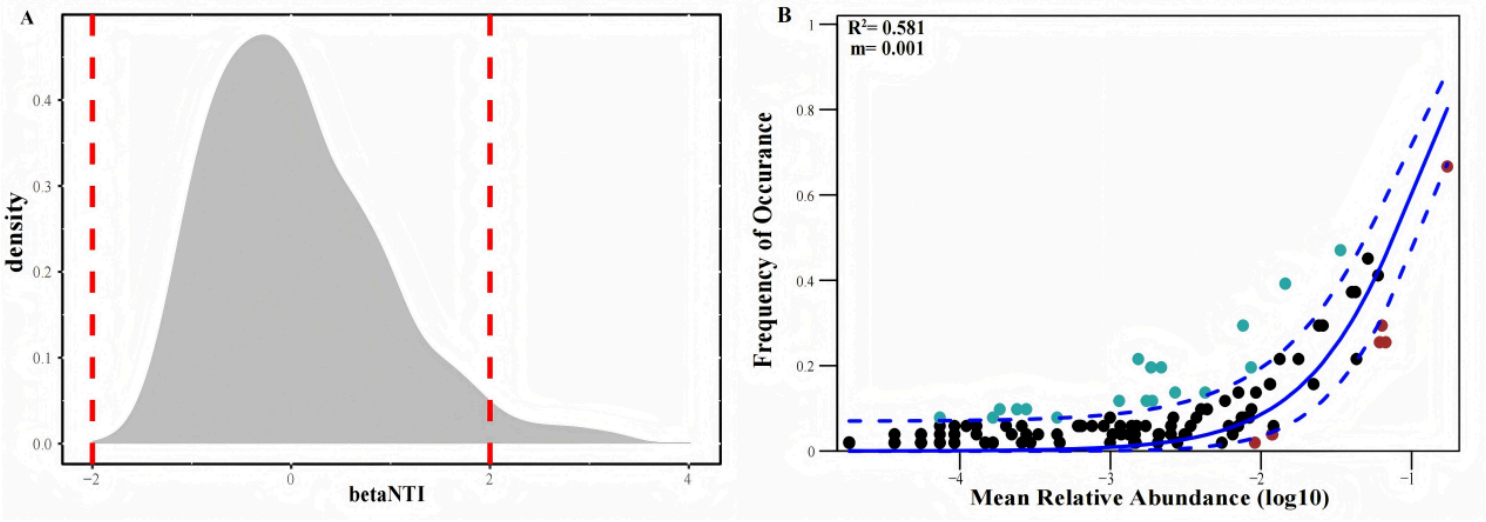

Figure 5

βNTI of fungal OTUs (A) and NCM Index (B). Solid blue line represents the predicted occurrence frequency, and dashed blue lines represent 95% confidence intervals around the model prediction. Light blue and red dots indicate the OTUs that occur more and less frequently than given by the model; R2 indicates the fit to the neutral model; m indicates immigration rate.