Stochastic processes dominate the community assembly of ectomycorrhizal fungi associated with *Betula platyphylla* in Inner Mongolia, China

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Abstract

The maintenance and driving mechanism of microbial community structure have become important research focuses in microbial ecology. Therefore, clarifying the assembly of EM fungal communities can provide a relevant basis for studying forest diversity, ecological diversity, and ecological evolution. *Betula platyphylla* is a typical EM dependent tree species with the characteristics such as renewal ability and strong competitive adaptability, and it plays a crucial ecological function in Inner Mongolia. However, the research on ~~the diversity and community assembly of EM fungi~~EM fungi's diversity and community assembly is very limited. We investigated EM fungal communities associated with *B. platyphylla* from 15 rhizosphere soil samples across five sites in Inner Mongolia. The fungal rDNA ITS2 region was sequenced using Illumina Miseq sequencing. A total of 295 EM fungal OTUs belonging to 2 phyla, 3 classes, 9 orders, 20 families, and 31 genera were identified, of which *Russula*, *Cortinarius*, and *Sebacina* were the most dominant taxa. ~~There were significant differences~~Significant differences existed in the composition of dominant genera of EM fungi across the five sites, and the relative abundances of dominant genera also showed significant differences among the sites. The β NTI and NCM fitting analyses suggest that ~~the EM fungal community assembly is mainly determined by stochastic processes~~stochastic processes mainly determine the EM fungal community assembly. Our study indicates that *B. platyphylla* harbors a high EM fungal diversity and highlights the important role of the stochastic process in driving community assembly of mutualistic fungi associated with *B. platyphylla* in north China.

Keywords: ectomycorrhizal fungi, *Betula platyphylla*, fungal diversity, community assembly, stochastic process

Introduction

Soil microorganisms are an important component of soil ecosystems, driving and influencing many ecosystem processes such as organic matter decomposition, nutrient cycling, and ecosystem productivity. They are crucial for maintaining global ecosystem functions (*Tedersoo et al., 2014; Crowther et al., 2019*). Mycorrhizal fungi account for approximately 70% of the total microbial population in soil ecosystems, making them one of the most important functional groups in the ecosystem (*Crowther et al., 2019*). Ectomycorrhizal (EM) fungi are certain groups in mycorrhizal fungi, which could form symbiosis with 30 lineages of terrestrial plants. The EM plants (e.g., Pinaceae, Fagaceae, Betulaceae, and Salicaceae) played important roles in the ecosystems (*Tedersoo et al., 2020*). Forest communities are important ecological communities ~~on earth~~ and the foundation of most ecosystems. Therefore, the definition of EM fungal community can provide a relevant basis for forest diversity,

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ecological diversity, and ecological evolution (*Wang et al., 2021*). An in-depth understanding of soil fungal diversity, composition characteristics of different functional groups, and their impact mechanisms is significant for soil health management, sustainable development of ecosystems, and predicting the response and feedback mechanism of microbial communities under changes in environmental factors (*Tedersoo et al., 2014; Bardgett et al., 2014*).

Several studies have attempted to investigate the ~~drivers of the EM fungal community from the aspects of~~EM fungal community's drivers from environmental filtering (i.e., plant, soil, and climate) and dispersal limitation (i.e., spatial distance). For example, research on the geographical distribution of soil fungi on a global scale has shown that climate factors, soil characteristics, and spatial patterns are the best predictive factors for soil fungal richness and community composition. Tree species characteristics are ~~an important determining factor~~important determinants for EM fungal community assembly (*Otsing et al., 2021*). ~~A large number of~~ Several studies have shown that soil pH is an important predictor of microbial diversity in response to global change factors (*Wang et al., 2015*). ~~There are also studies indicating~~Studies indicate that temperature is the most important regulatory factor for microbial diversity in forest soil at a large spatial scale (*Zhou et al., 2016*). It has gradually become a trend to comprehensively understand the mechanism of community construction from different perspectives by combining neutral theory and niche theory. Some studies have shown that the construction of microbial communities ~~are~~ is mainly influenced by deterministic (niche theory) and stochastic processes (neutral theory) (*Chen et al., 2021; Zhang et al., 2021*) e.g. *Liu et al. (2021)* found that the assembly of rhizosphere fungal community associated with *Pinus massoniana* in East Sichuan was determined by a deterministic process. *Wang et al. (2021)* emphasized ~~that~~ a determinant role of dispersal limitation in the stochastic process on EM fungal community assembly associated with common pine species in semiarid and cold temperate forests in Inner Mongolia of China. However, different fungal groups exhibit significant differences in their patterns with changes in climate, soil, and plant parameters (*Tedersoo et al., 2014*). In general, the effect of the deterministic process in defining the EM fungal community could be attributed to the selection of biotic and abiotic environmental variables on EM fungi through their fitness in response to the surrounding conditions (*De Wit & Bouvier, 2006*). These studies have clarified the different ecological

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processes and their relative importance in controlling some microbial communities. However, there is still an important question regarding the ecological processes underlying the community assembly of EM fungi. It remains uncertain and ~~deserves~~ warrants further exploration, considering their crucial role in ecosystems.

Betula platyphylla, as a pioneer species in the succession process of natural secondary forest ecosystems plays a crucial role in temperate deciduous broad-leaved forests. It is of great significance in maintaining regional ecological balance. *B. platyphylla* is a typical EM-dependent tree species. It has been reported that EM fungi associated with *B. platyphylla* mainly include Russulaceae, Amanitaceae, Boletaceae, Cortinariaceae, Tricholomataceae, and other families, as well as *Gomphidius*, *Suillus*, *Pisolithus*, *Boletus*, *Cortinarius*, *Inocybe*, *Sebacina*, *Piloderma*, *Hebeloma*, *Cenococcum*, *Tuber*, *Geopyxis*, and *Amanita*, etc (*Bai et al., 2006; Fan et al., 2013*). The findings mentioned above were primarily based on the identification of EM morphology. However, relying solely on EM morphology ~~for the identification of~~to identify EM fungi poses several challenges. Firstly, it brings a heavy workload and low efficiency. Secondly, it cannot accurately reflect EM fungal diversity because some EM fungi may ~~be~~ not form EM root tips when ~~sample~~ sampling collections. Therefore, to better understand the structure of the EM fungal community of *B. platyphylla*, *Yang et al. (2018)* have studied the composition of the EM fungal community of *B. platyphylla* using high-throughput sequencing technology in Heilihe Natural Reserve, Saihanwula Natural Reserve and Helanshan Natural Reserve of Inner Mongolia (i.e. a local scale). The results showed that *B. platyphylla* has a high diversity of EM fungi. In summary, the current research on the mycorrhizal fungi of *B. platyphylla* mainly focuses on the symbiotic mechanism between fungi and host plants~~, as well as~~ and the classification and identification of fungi. As several studies indicated that EM fungal communities were significantly different across sites, even for the single plant species, which mirrors the site/geographic effect on EM fungal communities (*Hackel J, et al., 2022*). ~~And we still know less about the EM fungal diversity, community structure and their differences at~~We still know less about the EM fungal diversity, community structure, and differences on a large scale in ~~the~~ Inner Mongolia. Therefore, this study ~~carried out the investigation and research on~~investigated and researched EM fungi of *B. platyphylla* at a large scale (spanning about 2400 km from east to west and 1700km from north to south). There is a significant zonal distribution of water, heat, and vegetation across the sites from east to west. In terms of climate and temperature zones, it successively crosses the cold ~~temperate zone, medium temperate zone, and warm temperate zone~~, medium, and warm temperate zones. From the perspective of humidity, it presents a climate change characteristic of moist, semi-humid, and semi-arid in sequence. So, this paper intends to answer the following questions: (1) ~~If there a~~are there any corresponding changes in EM fungi with this zonal distribution environment? (2) What are the determining factors for EM fungal diversity and community assembly of *B. platyphylla* under cross-climatic conditions~~.~~?

Materials & Methods

Site Description and Sampling

Five typical secondary forests of *B. platyphylla* were selected along over a 2300-km west-east transect across Inner Mongolia in north China (**Supplementary Fig.1**).~~,~~ ~~which~~ The selected forests were included from eastern temperate continental monsoon climate to western temperate continental climate, with mean annual temperature (MAT) ranging from 4.75 to 5.97 ℃ and mean annual precipitation (MAP) from 267 to 506 mm based on the climate data extracted from the WorldClim dataset at 30-arc-second resolution (*Hijmans et al., 2005*). The forests were approximately 20 years old and ~~with~~ had no disturbance. Five sample sites from west to east are Helan Mountain National Nature Reserve (HLS), Erlongshitai National Forest Park (ELST), Hademen National Forest Park (HDM), Wulanba National Nature Reserve in Inner Mongolia (WLB), and Genhe (GH) (**Supplementary Table 1**). At each site, 4-7 tree individuals were selected from each pure birch forest, and the individuals were >10 m away from each other to ensure sample independence (*Lilleskov et al., 2004*). Three rhizosphere soil cores were collected from each individual and mixed as one composite sample (~200g). The fresh soil samples were sieved through a 2 mm sieve to remove the roots and debris. Subsamples used for soil physical and chemical properties tests were stored at 4℃ prior to the analyses, and the subsamples for DNA extraction were frozen at -80℃.

Analysis of Soil Properties

Soil physical and chemical properties including soil total nitrogen (TN), total organic carbon (TOC), available potassium (AK), available phosphorus (AP), soil water content (SWC), pH value, and soil electrical conductivity (EC) were analyzed in this study. ~~The test should be conducted directly using a soil nutrient analyzer (LASA AGRO 1900 ， STEPS, Germany) according to agricultural standards~~According to agricultural standards, the test should be conducted directly using a soil nutrient analyzer (LASA AGRO 1900， STEPS, Germany).

Rhizosphere Soil DNA Extraction, PCR, and MiSeq Sequencing

Genomic DNA was extracted from 0.25g frozen soil using the PowerSoil DNA isolation kit (Mobio Laboratories, Inc. USA), according to the manufacturer's instructions. The detailed method for DNA extraction can refer to the method described by Gao et al. (2013), and other specific methods such as the PCR protocol ~~is~~ available ~~in~~ by *Ihrmark et al. (2012)* and *Wang et al. (2019)*. Briefly, the fungal internal transcribed spacer 1(ITS2) region was amplified using the PCR primers of ITS1F and ITS2. The PCR products of each sample were purified and mixed at equimolar amounts (200ng) and then sequenced on an Illumina MisSeq PE250 platform (Illumina, San Diego, CA, United States). High throughput sequencing work ~~were~~ was ~~all~~ completed by Shanghai Personal Biotechnology Co., Ltd (Shanghai, China).

Bioinformatics analysis

A detailed description of methods ~~about~~ for the raw data ~~were~~ was processed and analyzed, and it was completely referenced ~~from~~ by *Fan et al. (2023)*. ~~And t~~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: CRA013683) that are publicly accessible at https://ngdc.cncb.ac.cn/gsa/browse/CRA013683.

Statistical analysis

All statistical analysis was performed in R4.3.1. In our study, the taxonomic composition of EM fungi was visualized using Krona's (v.2.6) pie chart. At the same time, EM fungal OTU accumulation curves for each site, alpha diversity for EM fungal analysis including OTU richness, Shannon-Wiener, Simpson, Chao1, and abundance-based coverage estimator (ACE) index, beta diversity for EM fungal analysis including the principal coordinate analysis (PCoA) which Based on Bray–Curtis dissimilarity matrices, permutational multivariate ANOVA (PerMANOVA) with 999 permutations, the nonmetric multidimensional scaling (NMDS) analysis, and community assembly for EM fungal analysis among including the Neutral Community Model (NCM) and a β-mean nearest taxon distance (βMNTD) were all carried out. The specific methods and detailed description of specific criteria for analyzing all the above content can be found in ~~zhang~~ Zhang *et al. (2023)* and *Wang et al. (2021a)*.

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Results

Ectomycorrhizal Fungal Database Summary

377,969 sequences were obtained de-redundancy and chimera. These sequences were classified into 2,039 OTUs based on the 97% similarity level, and after identification, 1,132 OTUs (331,960) were identified as fungi. Finally, 295 EM fungal OTUs were obtained after identification and flattening of EM fungi for analysis of fungal community composition (**Supplementary Tab.2**). Among the 295 OTUs, 22 OTUs belonged to Ascomycota (3.01% of total EM fungal reads), 273 OTUs to Basidiomycota (96.99%).

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Ectomycorrhizal Fungal Diversity

The accumulation curves of each site did not show any signs of reaching an asymptote, suggesting that further sample collection may result in ~~more~~ unknown EM fungal OTUs (**Fig.1A**). The abundance and frequency ranking of EM fungal OTU shows that the EM fungal community contains a small number of dominant lineage and a large number of rare lineage (**Supplementary Fig.2**). We analyzed the alpha diversity of the EM fungal community of *B. platyphylla* in five sites, and the t-test method was used to calculate the significant difference of species richness between different sites (P<0.05). The nonparametric Wilcoxon test showed that EM fungal OTUs' richness (log transformed), Shannon and Chao1 indices significantly differed across five sites, and were 55.600±6.807 (mean±SE), 3.808±0.261 (mean±SE) and 68.575±31.397 (mean±SE), respectively. The HSD turkey tests further indicated that the indices of ELST were significantly lower than those in GH, HDM, HLS and WLB (**Wilcoxon: p < 0.05, Figure 1B, C, D**). The above results indicated that there were significant differences in the diversity of EM fungi in the five sites, and multiple comparisons showed that the diversity of EM fungi in GH was significantly higher than that in the other four sites.

Ectomycorrhizal Fungal Community Composition and Assembly

A total of 30 lineages were found in 15 soil samples of five sites in the current study, of which */Russula-Lactarius*(26.65%), */Sebacina*(18.66%), */Cortinarius*(17.44%), */Inocybe*(9.25%), */Hygrophorus*(8.3%), */Amanita*(4.49%), */Tomentella-Thelephora*(3.83%), */Clavulina*(3.02%), */Piloderma*(2.71%) and */Terfezia-Peziza*(1.51%) were the dominant evolutionary lineages (**Supplementary Tab.3, >1% of total sequences**). The EM fungal community composition ~~were~~ was different in different sites, */Wilcoxina*, */Laccaria*, */Tomentellopsis* and */Serendipita1* were only detected of GH; */Cantharellus* were only detected of WLB; */Ceratobasidium3* and */Tuber-Helvella* were only detected of HDM; */Otidea* and */Genea-Humaria* were only detected of ELST; */Paxillus-Gyrodon* and */Marcelleina-*

Peziza were only detected of HLS. Comparing the relative abundance of EM fungi at the genus level (listing the top 10 genera with relative abundance, >1% of total sequences) can provide a clearer explanation of the differences between different regions (**Figure 2**).

The venn diagram showed that the number of OTUs was different in the five sites, from west to east ~~was~~ HLS-83, ELST-70, HDM-77, WLB-71, and GH-106, respectively. The proportion of OTUs number in different regions is 28.14%, 23.73%, 26.10%, 24.07%, and 35.93% of total OTUs number, respectively, indicating significant differences among fungal communities in different regions, with HLS and GH having the higher number of OTUs, and of which only 1 OTU were shared by the five sites. Furthermore, each of the sites harbors unique OTUs, 36, 19, 3, 39 and 69 fungal OTUs only existed on HLS, ELST, HDM, WLB, and GH, respectively, accounting for 56.27% of total OTUs number (**Figure 3**). The analysis suggests that the reason may be due to significant differences in water and thermal conditions due to the large span between the east and west (**Supplementary Tab.1**). This indicates that the community composition of EM fungi has a certain correlation with different geographical locations and environmental conditions.

The EM fungal community structure with the different sites was analyzed using PCoA based on the Bray-Curtis distance **(Fig.4)**. PCoA ordination analysis showed that the PCoA first-axis explanation rate was 24.46%, the second-axis explanation rate was 22.36%, in which clear area separation in terms of sampling sites. Moreover, PerMANOVA agreed with the PCoA in that there were significant differences in the EM fungal communities between different sampling sites **(Fig.4, Adonis: R^2=0.89, P=0.001).** NMDS ordination revealed that EM fungal communities of the five sites were clearly separated, particularly the community in GH **(Fig.5).** In addition, an environmental fitting test demonstrated climate conditions~~,~~ and soil properties were significantly correlated with the EM fungal community **(Fig 5 and Supplementary Tab. 4).** In terms of geographical location, GH is located in a low altitude area, and is also the easternmost area among the five sites, which is closer to the coast compared

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to other sites. ~~In terms of~~Regarding environmental factors, GH is affected by the southeast monsoon, which ~~leads to a large amount of water vapor being brought~~brings a large amount of water vapor into ~~from~~ the sea surface, resulting in the highest MAP, lowest MAT, and the largest proportion of SWC. Therefore, there is a significant difference in microbial communities compared to inland areas. Due to the close distance and the small differences in soil rhizosphere environment between ELST and HDM, ~~and~~ the natural conditions are ~~basically~~ the same, resulting in smaller community differences. After comparing ELST with HLS and HDM, the difference between HLS and HDM is greater due to the geographical location of HLS is closer to inland areas, resulting in lower temperatures and less precipitation. The significant differences in soil microbial communities between ELST and GH are mainly due to different ecological environments.

The EM fungal community ecological process ~~were~~ was fitted to the NTI and *β*NTI. The NTI values ranged from −1.8 to 0.22, and the mean NTI value across all samples was not significantly different from 0 **(*P* > 0.05, Fig.6A)**, indicating the phylogenetic relatedness in the communities was stochastic. Whereas, most *β*NTI values fall in the range of -2 to 2 **(87.74%; Fig.6B)**, while the case of |*β*NTI|>2 is only 12.26%, which also indicates that the assembly process of EM fungal communities is mainly affected by stochastic processes. Meanwhile, The result of calculating the distribution of |RC_{bary}| shows ecological drift, dispersal limitation and homogeneous dispersal account for 42%, 42% and 16% respectively **(Supplementary Fig.3)**, indicating that stochastic processes had a stronger effect on the community assembly of EM fungi than the deterministic processes in the present study.

Discussion

Structure and diversity of EM fungal community of *B. platyphylla*

A total of 2039 fungal OTUs were obtained in this study, of which 295 were EM fungal, belonging to 2 phyla, 3 classes, 9 orders, 20 families and 31 genera, indicating that there are abundant EM fungal communities in the rhizosphere of *B. platyphylla*. Previous studies have shown that ~~the~~ forests with a high fungal diversity commonly harbor ~~the~~ a strong ability to resist ecosystem disturbances and remediation (*Guo Y., et al. 2018*). Studies have shown a significant positive correlation between soil biodiversity and various ecosystem functions (nutrient cycling, decomposition, plant production, and reducing potential pathogenicity) (*Delgado-Baquerizo 2020*). In addition, tree species characteristics are an important determining factor in the composition of fungal communities (*Otsing, et al., 2021*). The abundant EM fungal community in the rhizosphere of *B. platyphylla* plays an important role in maintaining the stability of the forest ecosystem and nutrient cycling, which may be an important reason for its widespread distribution in northern China.

At the phylum level, Basidiomycota has the highest relative abundance at 97%, while the rest are Ascomycota. Basidiomycetes contain laccase with a strong degradation effect on lignin, and some of which can also use oxidase to ~~inorganic~~ inorganic-organic nutrients in leaf litter~~,~~ and then convert nutrients into usable forms of plants (*Shah et al., 2016; Cheeke et al., 2017; Steidinger et al., 2019*). Some EM ~~fungal~~ fungi can produce extracellular enzymes, such as cellulase, hemicellulase and polyphenol oxidase, to promote the degradation of plant litter components (*Read et al., 2003; Shah et al., 2013*). Other studies have found that Basidiomycetes can effectively degrade stubborn hydrocarbons (such as phenols, halogenated hydrocarbons, aromatic hydrocarbons, and persistent organic pollutants) in the ecological environment, playing a positive role in the sustainable development of the ecological environment (*Treu, et al, 2017*). Ascomycota fungi are mostly saprophytes, which can decompose refractory organic matter, such as lignin and keratin, and play an important role in the nutrient cycle of forest ecosystems (*Paungfoo-Lonhienne et al., 2015*). Fungi in the soil decompose the litter of understory vegetation, providing nutrients for the soil, which in turn provide nutrients for the growth and reproduction of fungi, and subsequently, nutrient elements form a cycle between fungi and understory vegetation.

The five sites in this study are approximately 2000km from east to west, with significant differences in climate, geography, soil, and other aspects. The genera of *Russula*, *Cortinarius*, *Sebacina*, *Inocybe*, and *Hygrophorus* are all distributed in the five sites. This is consistent with the conclusions of other researchers. Their results showed that *Russula* and *Hymenomyces* are widely distributed in temperate regions and can coexist with a variety of trees or shrubs (*Jang et al.*, *2012; LeDuc et al.*, *2013*). They are the dominant EM fungi of *B. platyphylla*. It has been ~~ever~~ reported that the relative abundances of these two genera ~~is~~ are linearly correlated (*Xing et al.*, *2020*). Previous ~~study~~ studies ~~has~~ have shown that *Cortinarius* can secrete a large amount of peroxidase (*Bödeker et al.*, *2014*), which may play an important role in degrading dead branches and leaves. In addition, *Sebacina* is also widely distributed in various forest ecosystems, with almost no host specificity (*Oberwinkler et al.*, *2013*).

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At the same time, our study found that the community structure of EM ~~fungal~~ fungi in the five sites ~~were different to some extent, and the dominant genera and their relative abundance in each site were different~~differed to some extent, and the dominant genera and their relative abundance in each site differed. *Russula* has the highest relative abundance in the eastern GH and central ELST of Inner Mongolia. *Cortinarius* is the dominant genus in the HDM of central Inner Mongolia, while *Sebacina* is the dominant genus in the HLS of western Inner Mongolia. *Otidea* only exists in ELST. Each site has its ~~own~~ unique species, and there are also species shared by two or more sites, which better illustrates the diversity and differences between fungal communities. This is because the five sites in this study have a great span in geographical distance, and there are significant differences in ecological environmental factors among different sites, while a large number of studies show that the community structure of EM fungal is affected by multiple environmental factors (*Tedersoo et al.*, *2014; Crowther et al.*, *2019*). In addition, it is also related to the different microenvironments of EM ~~fungal~~ fungi in different sites. Although the constructive species in each sample in this study are pure birch forest, the complexity of litter resources available to EM ~~fungal~~ fungi is different due to different associated shrubs and herbs under the forest (*Urbanová et al.*, *2015*), leading to certain differences in their community structure. Other studies also believe ~~that there is a group of relatively abundant fungi~~a group of relatively abundant fungi exists in a specific environment (*Fitzpatrick et al.*, *2020*).

Correlation between EM fungal community and soil physicochemical factors

In this study, all factors, including TN, TOC, AK, pH, longitude, and latitude, ~~have a very significant impact on~~significantly impact the distribution of EM fungi. The soil microbial community is a dynamically changing self-organizing system, and its community structure and diversity are influenced by various environmental factors, such as vegetation factors, climate factors, and soil factors.

Research has shown that pH value is an important factor affecting soil microbial diversity on a global scale (*Steidinger et al.*, *2019*). The analysis of the community structure of EM fungi in the rhizosphere soil of *B. platyphylla* at a large scale in this study also supports the above conclusions. It spans about 2400km from east to west in Inner Mongolia, and the soil pH value in the site shows a significant trend of change, gradually transitioning from the alkaline soil of the HLS in the western (pH 7.51-8.57) to the acidic soil of the GH in the eastern (pH 4.84-4.91). In addition, pH value is positively correlated with altitude, MAT, and EC, and negatively correlated with AK, SWC, longitude, latitude, AP, TOC, and TN. The research shows that these factors ~~have influence on~~influence the community structure of EM fungi (*Miyamoto et al.*, *2015*). In this study, pH ~~has a significant impact on~~significantly impacts the community composition of EM fungi. This may be because EM fungi produce a lot of organic acids in the process of mycorrhizal symbiosis with the host, which reduces the soil pH value, changes environmental factors (such as nutrient availability, organic carbon) and undergrowth vegetation structure, and then affects the community structure of EM fungi (*Hedwall et al.*, *2018*).

A large number of studies have shown that TN has a significant impact on the community structure of EM fungi, and the results of our study also support the above conclusions. TN may affect soil microbial community structure by affecting the diversity and dominance of aboveground vegetation (*Avis et al.*, *2003*). Corrales' research suggests that to some extent, nitrogen deposition reduces the colonization of EM fungi and alters their community structure, and with the increase of nitrogen, the abundance of some EM fungal groups increases while some decreases (*Corrales et al., 2017*). Soil water content is another important factor affecting soil microbial abundance on a global scale (*Steidinger et al.*, *2019*). The samplinge site in this study has a great span in longitude, and the soil water conditions at each site are significantly different.~~;~~ ~~so~~ Hence, the community composition of EM fungi at each site is significantly different. Soil water content may affect the content of chemical ions in the soil~~, while~~. In contrast, the water was affected by various chemical process and biological processes in the soil, thus affecting the pH value of the soil. These findings suggest~~s~~ that different EM fungal taxa harbor distinct ecological niches and thus prefer~~red~~ to exist~~ed~~ in certain habitats.

In this study, GH has the most unique OTUs, and the richness and diversity of EM fungal OTUs are significantly higher than other sites, while the MAT of GH is significantly lower than other sites. The abundance of EM fungi is negatively correlated with MAT, and the research results are consistent with those reported by *Miyamoto et al. (2018)*. ~~And t~~The research results show that some EM fungi prefer to survive in lower temperature environments, possibly due to the high organic carbon content in cold habitats, which increases the diversity of EM fungi (*Rosling et al., 2003; Buée et al., 2007*). Moreover, compared with other soil fungi, EM fungi have significant competitive advantages in ~~terms of~~ nutrition and carbon acquisition in cold habitats (*Fernandez & Kennedy, 2016*). GH is located in a low altitude area with higher longitude and is also the easternmost area among the five sites, which is closer to the coast compared to other sites. The influence of the southeast monsoon on GH results in a large amount of water vapor being brought into the sea surface, resulting in the highest MAP, lowest MAT, and the largest proportion of SWC. Therefore, there are significant differences in microbial communities compared to inland areas.

Common fungal species grow well under suitable soil nutrients and water content conditions. For example, the relative abundance of certain fungi decreases with an increase in pH value, while others exhibit opposite trends (*Wang et al.*, *2015*). Similarly, certain fungal groups increase with high ~~the increase of~~ nutrients levels, while others are inhibited (*Paungfoo-Lonhienne et al.*, *2015*). As is well known, different EM fungal species occupy different growth environments and respond differently to environmental changes such as climate change or nitrogen deposition. For example, many fungal OTUs belonging to *Cortinarius*, *Tylospora*, and *Piloderma* tended to occupy cold habitats; conversely, *Russula* and *Lactarius* were found across wider temperature ranges and in warmer habitats (*Miyamoto et al.*, *2018*). These results indicate that different fungal groups often have different ecological niches. In conclusion, the results of this study indicate that ecological environmental factors ~~have a significant impact on~~significantly impact the community structure of EM fungi. Most ~~of the~~ EM fungi have a significant correlation with ecological environment factors, which ~~indicated~~ indicates ~~that~~ the importance of EM fungi as underground indicator species of forest status and environmental conditions (*Suz et al., 2014*), and also shows the ecological specificity of EM fungi. The above research shows that ~~the~~ future global warming may ~~lead to the reduction of~~ reduce the diversity of EM fungi. Therefore, determining the global distribution characteristics of EM fungi and determining the factors controlling their distribution are necessary ~~conditions~~ for understanding the current and future functions of forest ecosystems (*Steidinger et al., 2019*).

Conclusions

A total of 2039 fungal OTUs were obtained in this study, 295 of which were EM fungi, belonging to 2 phyla, 3 classes, 9 orders, 20 families and 31 genera. It can be divided into three classes, Agaricomycetes is the absolute advantage class. *Russula*, *Cortinarius* and *Sebacina* are the dominant genera in the rhizosphere soil of *B. platyphylla*. The EM fungal community is affected differently by the soil physical and chemical properties, in which TN, TOC, AK, pH, longitude, and latitude have a very important influence on the EM fungal composition, and other factors also have significant influence. Neutral model analysis (NCM) and βNTI index found that the community structure was mainly affected by the stochastic process, which could provide a better basis for the community assembly of EM fungi. The results of this study can provide a reference for the research on the adaptability of EM fungi to the environment in different regions, and also provide a direction for the research on plant stress resistance in Inner Mongolia, China.

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