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RESEARCH ARTICLE

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Comparative Analysis of Rhizosphere Fungal Communities in Korean Fir Trees

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ABSTRACT

The Korean fir (*Abies koreana*), a native coniferous species of Korea, predominantly inhabits the subalpine zone. Recently, this species has experienced a significant population decline, primarily attributed to environmental changes in the subalpine zone driven by global warming. Efforts to prevent the extinction of the Korean fir are underway, with a predominant focus on abiotic factors contributing to its decline. However, there is a notable lack of research on the complex interactions between microbial communities and Korean fir, particularly concerning how these interactions vary with the health status of the trees and their impact on population sustainability. Therefore, this study aimed to elucidate the rhizosphere fungal community structure associated with Korean fir trees in Jirisan National Park. We examined different habitat types, including the rhizospheres of native, cultivated, and dead Korean fir and bulk soil. Our findings revealed that the rhizosphere fungal community in the natural habitat of Korean fir predominantly comprises Agaricomycetes. Furthermore, the fungal community structure was more responsive to habitat type variations than seasonal changes. These findings provide basic information for conserving this endangered species and developing alternative habitats for the Korean fir.

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1. Introduction

The Korean fir (*Abies koreana*), a coniferous tree belonging to the Pinaceae family [1], is endemic to the Republic of Korea. Four primary Korean fie tree habitats exist in the Korean Peninsula, including Hallasan, Jirisan, Deogyusan, and Gayasan [2]. These trees predominantly inhabit the subalpine zone, ranging from 1000 to 1900 meters above sea level, characterized by low average annual temperatures, strong winds, and acidic, nutrient-poor soils [3]. The Korean fir's dependency on this unique environment makes it particularly susceptible to environmental changes [4]. Korean fir has been classified as an endangered species by the International Union for Conservation of Nature (IUCN) due to a significant decline in its population [5]. Previous studies have linked this decline to increased temperatures and reduced moisture in the subalpine zone, driven by global warming and decreased snow cover [6–9]. Additionally, other environmental factors such as changes in solar radiation, wind intensity, and the invasion of *Sasa quelpaertensis* have also been identified as threats to the Korean fir's survival [10–13].

While much of the existing research has focused on abiotic factors contributing to the decline of Korean fir [6], there is a growing recognition that biological factors, particularly microorganisms, play a crucial role and have been relatively underexplored. The population decline of Korean fir likely results from a combination of multiple factors, necessitating research across various domains [14]. For instance, surviving Korean firs might exhibit enhanced resilience to environmental changes due to beneficial microorganisms in their rhizosphere [15]. Among these microorganisms, certain fungi form mycorrhizal relationships with plants, potentially increasing the survival chances of Korean fir in adverse conditions [16, 17].

This study aims to investigate the fungal community structure in the rhizosphere and soil from different environments: native Korean fir sites, cultivation sites, dead Korean fir sites, and non-habitat

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soils [18]. By analyzing environmental DNA, we aim to identify fungal taxa that may coexist with Korean fir and explore biological resources for its restoration in Jirisan. Differences in microbial community structures between these environments could provide insights into the factors contributing to Korean fir survival and restoration [14, 18, 19].

2. Materials and methods

2.1. Collection of Korean fir rhizosphere sample

Samples were collected near the Seseok Shelter in Jirisan National Park (35°19'4"N 127°41'36"E). The collection points were April 20, June 23, September 13, and November 11, 2023. Samples were taken from four distinct sites: the native habitat of Korean fir (rhizosphere of native), the cultivation site of Korean fir (rhizosphere of cultivate), the dead site of Korean fir (rhizosphere of dead Korean fir), and non-habitat soil of Korean fir (bulk soil) (Figure 1). From each site, samples were collected from three trees, and the process was repeated three times per tree using a shovel and 50-mL conical tubes ($n = 3$) (Table 1). Rhizosphere samples were taken from the root-contact areas, while bulk soil samples were collected more than one meter from the trees. A total of 48 samples were collected, frozen, and stored in a deep freezer at −80 °C until the total microbial DNA was extracted. We additionally analyzed the physicochemical and physical properties of the four sampling sites (Table 2).

2.2. Extraction of rhizosphere soil DNA and polymerase chain reaction (PCR)

DNA extraction from soil samples was performed using the Fast DNA SPIN Kit for Soil (MP Biomedicals, Irvine, CA, USA), following the manufacturer's instructions. Briefly, 0.5 g of soil was added to the lysing matrix with 978 µL sodium phosphate buffer and 122 µL of molecular transfer buffer (MT buffer). The soil was pulverized for 40 s at 6.0 m/s using a Fest prep device and centrifuged at 18,472×*g*, 4 °C for 10 min using a Micro-Centrifuge 1730 R. The supernatant was transferred to a new E-tube, mixed with 25 µL of Protein precipitate solution (PPS), inverted 10 times, and centrifuged for 5 min. The subsequent supernatant was combined with 1 mL of binding matrix, inverted for 2 min, and left at room temperature for 3 min. The mixture was centrifuged at 12,300 x *g* for 1 min, and this process was repeated until the entire sample was processed. The DNA was washed using 500 µL of Soil extraction washing solution-magnetic bead (SEWS-M) and centrifuged again. The DNA was then dried, eluted with 70 µL of DNA extraction solution (DES), and centrifuged to collect the DNA. The quality and quantity of the extracted DNA were measured using a NanoDrop™ 2000/2000c Spectrophotometer, and all DNA samples were stored at −30 °C. DNA was purified using the ethanol precipitation method. The DNA was mixed with 3 M sodium acetate, 100% ethanol, and cooled on ice for 20 min. After centrifugation and removing the supernatant, the DNA was

Figure 1. Appearance of the sites where samples were collected. Soil samples were collected separately from the Korean fir (A) rhizosphere of native (N 35.318, E 127.694), (B) rhizosphere of cultivate (N 35.317, E 127.693), (C) rhizosphere of dead Korean fir (N 35.316, E 127.692), and (D) bulk soil (N 35.316, E 127.691). Rhizosphere samples were collected from soil in contact with the roots of the Korean fir, and bulk soil was collected more than 1 m away from the Korean fir. Samples were collected in 50 ml from three Korean fir in each area, and the individuals from which samples were collected were marked. Samples were collected from the same individuals during the following sample collection ($n = 3$).

Table 1. Samples collection location and date.

Location	GPS	Sampling date			
Bulk soil (BS)	N 35.316, E 127.691	Apr 20, 2023	June 23, 2023	Sep 13, 2023	Nov 11, 2023
Rhizosphere of cultivate (RC)	N 35.317, E 127.693				
Rhizosphere of native (RN)	N 35.318, E 127.694				
Rhizosphere of dead Korean fir (SD)	N 35.316, E 127.692				

Table 2. Soil physicochemical and physical properties for four sites of Korean fir trees.

					cmolc/kg						
Site	pH	Av.P_2O_5 (mg/kg)	OM (%)	T-N (%)	Ex. K	Ex. Ca	Ex. Mg	CEC	clay (%)	sand (%)	silt (%)
Bulk soil (BS)	5.93	59.54	4.76	0.16	6.11	3.67	3.66	15.71	7.47	75.33	17.20
Rhizosphere of cultivate (RC)	5.12	73.28	7.04	0.29	6.44	4.70	12.13	24.14	6.93	72.50	20.57
Rhizosphere of native (RN)	3.94	13.74	16.63	1.05	6.99	2.95	10.32	38.30	5.33	31.17	63.50
Rhizosphere of dead Korean fir (SD)	4.71	47.33	28.52	1	5.91	3.58	5.88	28.13	10.67	47.50	41.83

washed with 70% ethanol, dried, and dissolved in Tris-EDTA (TE) buffer. The purified DNA was stored at −80 °C.

PCR was conducted targeting the internal transcribed spacer 2 (ITS2) region using a T100 Thermal Cycler (BIO-RAD, Hercules, California, USA). Primers for amplifying the ITS2 region were MiSeq ITS3 (5′-TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG GCA TCG ATG AAG AAC GCA GC-3′) and MiSeq ITS4 (5′-GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA GTC CTC CGC TTA TTG ATA TGC-3′) diluted to a concentration of 10 pmol. The reagent conditions for PCR were 1 µL of DNA, 1 µL of MiSeq ITS3, 1 µL of MiSeq ITS4, 12.5 µL of KAPA HiFi Hot Start ReadyMix (Roche, Basel, Switzerland), and 4.5 µL of sterile water, so the total volume was set to 20 µL. The PCR conditions included an initial denaturation at 95 °C for 3 min, followed by 24 cycles of 95 °C for 30 s, 58 °C for 30 s, and 72 °C for 30 s, and a final extension at 72 °C for 5 min.

For samples where PCR failed using the method mentioned above, PCR was performed using the Touchdown PCR method. The reagent for Touchdown PCR was mixed with 1 µL of DNA, 2 µL of MiSeq ITS3, 2 µL of MiSeq ITS4, 25 µL of KAPA HiFi Hot Start ReadyMix (Roche, Basel, Switzerland), and 10 µL of sterile water, and the final volume was set to 40 µL. The settings of the PCR machine were 95 °C for 3 min (1 cycle), 95 °C for 30 s, 60 °C for 30 s (−0.5 °C per cycle), 72 °C for 30 s (29 cycles), 95 °C for 30 s, 55 °C for 30 s, 72 °C for 30 s (29 cycles), and 72 °C for 7 min (1 cycle). The PCR products were then subjected to gel electrophoresis and purified using a gel elution kit.

2.3. Metagenome of Korean fir rhizosphere fungal community

Metagenome amplicon sequencing of the DNA samples was outsourced to Macrogen (Seoul, Korea). The sequencing was performed using the Herculase II Fusion DNA Polymerase Nextera XT Index V2 Kit, following the 16S Metagenomic sequencing library preparation protocol (Part # 15044223 Rev. B). The Illumina system was employed for sequencing. The generated data were converted to FASTQ format and analyzed using the DADA2 package (version 1.28.0) [20] in R software (version 4.0.3) [21]. The error rates were initially assessed using the divisive amplicon denoising algorithm 2 (DADA2). Sequences with an average quality score below 20 were trimmed, cutting forward reads to 260 bp and reverse reads to 200 bp. The forward and reverse reads were subsequently merged using the DADA2 algorithm to form amplicon sequence variants (ASVs). Chimeric ASVs were removed during this process. Utilizing the IDTAXA algorithm [22] and UNITE [23] referencing the sh_general_release_dynamic_s_all_10.05.2021 database (https://unite.ut.ee/) Non-fungal eukaryotic ASVs were excluded from the dataset. The DADA2 package was further used to remove barcode sequences attached to the primers (MiSeq ITS3 and MiSeq ITS4) across the 48 samples. The percentage of fungal sequences in each sample was assessed to verify the suitability of the samples for fungal community analysis. It was confirmed that more than 60% of sequences in all samples, except for one bulk soil sample collected in June and another in November, were fungal. Good's coverage analysis and Chao's coverage analysis were

then performed to compare the track file and input file, ensuring that the fungal presence exceeded 60%. Both Good's coverage and Chao's coverage were determined to be over 99%, reaffirming that the data were appropriate for biological community analysis [24]. This meticulous approach to sequencing and data analysis ensures the reliability and accuracy of the fungal community structure data, facilitating comprehensive insights into the fungal ecology associated with the Korean fir.

2.4. Data visualization

Data visualization was carried out using R software packages including ggplot2 (version 3.4.2) [25], ggh4x (version 0.2.6) [26], and vegan (version 2.6.4) [27]. The initial step involved rarefaction curve analysis to visualize the increase in amplicon sequence variants (ASVs) with the number of reads. The x-axis of the rarefaction curve represents the number of reads, while the y-axis denotes the number of ASVs.

2.5. Diversity analysis

Alpha diversity was assessed using the Observed index, Shannon index, and Simpson index to determine community composition differences due to seasonal changes among the four types of regions. The Observed index analyzed species diversity, while the Shannon and Simpson indices considered species diversity and abundance [28, 29]. For regional comparisons within each season, analysis of variance (ANOVA) was employed for data meeting normality and equal variance assumptions, whereas the Kruskal–Wallis test was used otherwise. Post-hoc analysis for groups with significant differences (p-value < 0.05) was conducted using the Benjamini–Hochberg procedure of the Conover–Iman test, and statistically significant samples were visualized with different alphabets. The relative abundance of taxa within a sample was calculated, and the top 10 taxa were visualized in bar graphs, with taxa not in the top 10 classified as "others." This analysis was performed at the phylum and class levels.

2.6. Principal coordinate analysis of fungal communities

Principal coordinate analysis (PCoA) was used to examine differences between fungal communities of Korean fir by region and season. The Bray–Curtis dissimilarity formula quantified community changes, with PCoA 1 and PCoA 2 results visualized on the x-axis and y-axis, respectively. Regional differences were distinguished by node color, and seasonal differences by node shape. The average value coordinates of sample data collected three times in each region were represented as closed nodes. Structural differences between fungal communities were statistically analyzed using permutational multivariate analysis of variance (PERMANOVA) of the pairwiseAdonis (version 0.4.1) R package [30].

2.7. Analysis of specific fungal taxa

We further analyzed whether specific fungal taxa in the rhizosphere of Korean fir maintained high abundance despite seasonal changes. Taxa with potential mycorrhizal associations were selected, focusing on Agaricomycetes, Dothideomycetes, Pezizomycetes (ectomycorrhizae formers), and Glomeromycetes, Archaeosporomycetes, Paraglomeromycetes, and Endogonomycetes (arbuscular mycorrhiza formers) [31–35]. Relative abundances were statistically analyzed for regional differences within a season. ANOVA was used for data meeting normality and equal variance assumptions; otherwise, the Kruskal-Wallis test was used. Post-hoc tests for groups with significant differences (p-value < 0.05) employed the Benjamini–Hochberg procedure of the Conover-Iman test. Additionally, a 1:1 comparison of Agaricomycetes abundance between seasons was performed using t-tests.

2.8. Heatmap visualization

A heatmap was generated using the package 'pheatmap' of R software [32] to analyze the clustering of taxa based on relative abundance. The analysis was conducted separately for regions and seasons at the class level. The x-axis dendrogram represented clustering by region and season, while the y-axis dendrogram represented clustering of taxa. This detailed visualization and statistical analysis provide a comprehensive understanding of the fungal community structure and dynamics in different environments and seasons, shedding light on the ecological interactions affecting the Korean fir.

3. Result

To analyze regional and seasonal differences in the fungal community of the Korean fir rhizosphere, PCR was conducted targeting the ITS2 region of DNA extracted from soil samples, followed by metagenomic analysis. This approach led to the identification of 13,722 taxa from 48 samples (Tables S1–S4). Subsequently, DNA sequences from all samples were analyzed using rarefaction curves to ensure

that most species' DNA had been detected. The rarefaction curves confirmed that the sampling depth was sufficient for capturing the majority of species, allowing for the next stage of analysis to be conducted (Figure S1).

3.1. Diversity of the rhizosphere fungal community structure of the Korean fir

Alpha diversity analysis of the Korean fir rhizosphere fungal community was conducted using the Observed, Shannon, and Simpson indexes. The Observed index considers only the diversity of taxa within the community, while the Shannon and Simpson indices consider both the diversity and abundance of taxa. No statistically significant differences were detected between regions in any of the indices (Observed, Shannon, and Simpson) for the samples collected in April, June, and September (p-value ≥ 0.05) (Figure 2A–C). In November, while the Observed and Simpson indices showed no statistically significant differences between regions, the Shannon index revealed a statistically significant difference (p-value = 0.0349) (Figure 2D).

Post-hoc analysis of the Shannon index for the November samples indicated that the bulk soil samples had a more even distribution of various taxa compared to the native site samples and the dead Korean fir site samples. The cultivated site samples exhibited taxon diversity that was intermediate between the bulk soil, native site, and dead Korean fir site samples. These findings suggest that while seasonal changes do not significantly affect the alpha diversity of the rhizosphere fungal community in April, June, and September, there is a notable difference in taxon distribution in November (Figure 2). Specifically, the bulk soil samples in November showed a diversity of fungi members in fungal

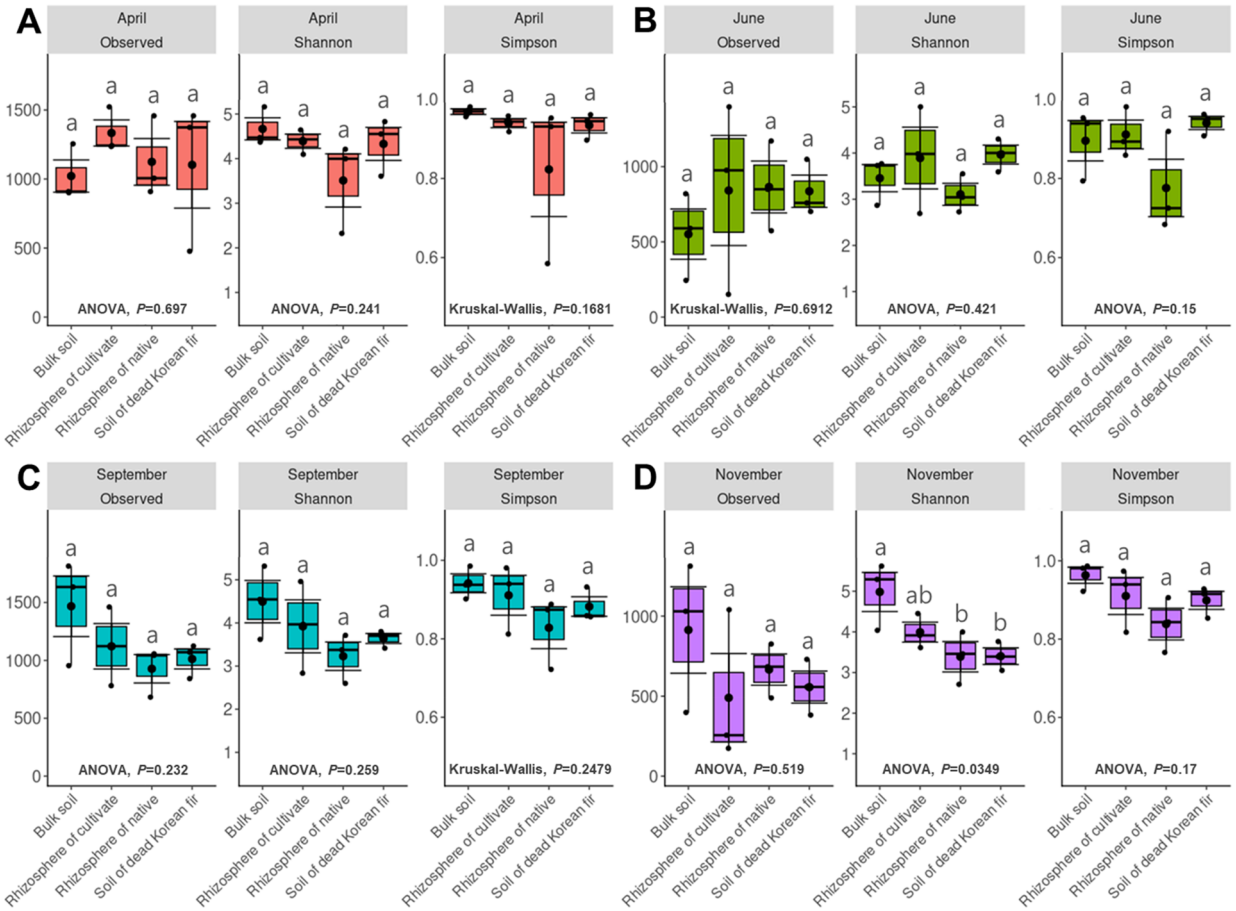

Figure 2. Alpha diversity graph showing changes in fungal communities according to Korean fir sample collection sites and seasonal changes. Alpha diversity analysis was performed by comparing trimmed amplicon sequence variants (ASVs) with the sh_general_release_dynamic_s_all_10.05.2021 database using divisive amplicon denoising algorithm 2 (DADA2). Afterward, changes in fungal community structure in (**A**) April, (B) June, (C) September, and (D) November were analyzed using the Observed, Shannon, and Simpson index. Statistical analysis of the data was performed using analysis of variance (ANOVA) if the data between regions followed both normality and equal variance, and Kruskal–Wallis test otherwise. Lastly, data with statistically significant differences between regions were post hoc tested using the Benjamini–Hochberg procedure of the Conover–Iman test to analyze the presence or absence of differences between specific regions.

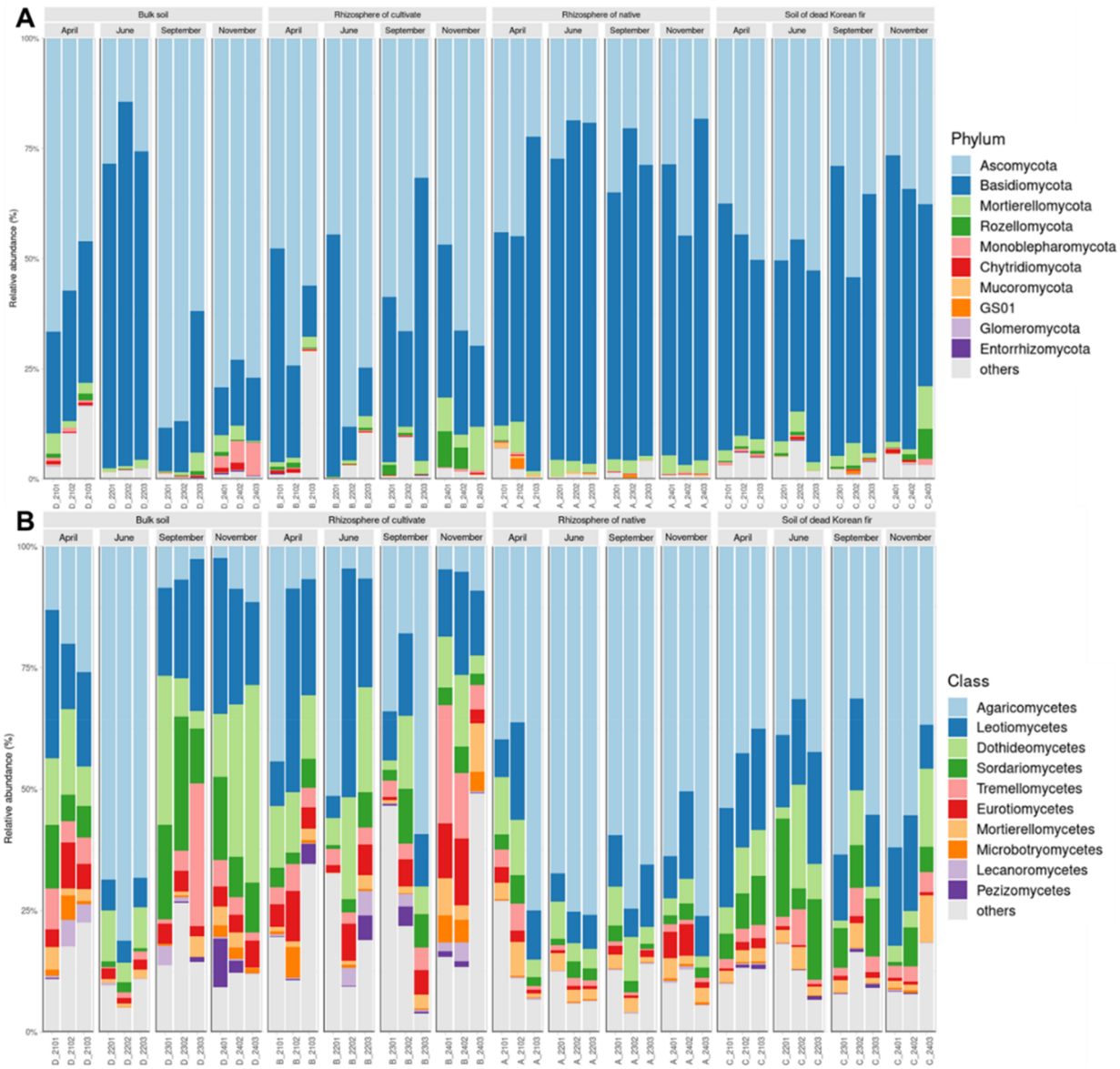

Figure 3. Beta diversity analysis to identify the types of taxa that make up the Korean fir rhizosphere fungal communities. The beta diversity analysis clustered amplicon sequence variants (ASVs) of collected samples. Afterward, the ASVs of each sample were converted into percentages to calculate the relative abundance of taxa. Finally, the top 10 taxa with relative abundance were identified in the classification system at the (A) phylum and (B) class, and taxa with other low relative abundances were classified as others.

community structure compared to other sites, possibly reflecting unique environmental conditions during this period.

3.2. Relative abundance and diversity of taxa comprising the Korean fir rhizosphere fungal community

Relative abundance analysis was performed at the fungal classification system's phylum and class levels to evaluate the relative abundance and diversity of taxa within the Korean fir rhizosphere fungal community. At the phylum level (Figure 3A), Ascomycota and Basidiomycota were the dominant taxa, collectively accounting for approximately 80% of the fungal community in all samples. Following these, Mortierellomycota and Rozellomycota displayed relatively higher abundance, though their overall proportion in the community remained below 10%. Other taxa constituted a very small fraction of the community.

In bulk soil, the relative abundance of Ascomycota showed a sharp decrease in June compared to April, followed by an increase in September and November. Rhizosphere of cultivated exhibited the highest overall relative abundance of Ascomycota, a trend consistent across all four seasons. In the rhizosphere of native, Basidiomycota maintained a high relative abundance of around 70% throughout the four seasons. Both Ascomycota and Basidiomycota were maintained at similar relative abundances across all seasons in the rhizosphere of dead Korean rir.

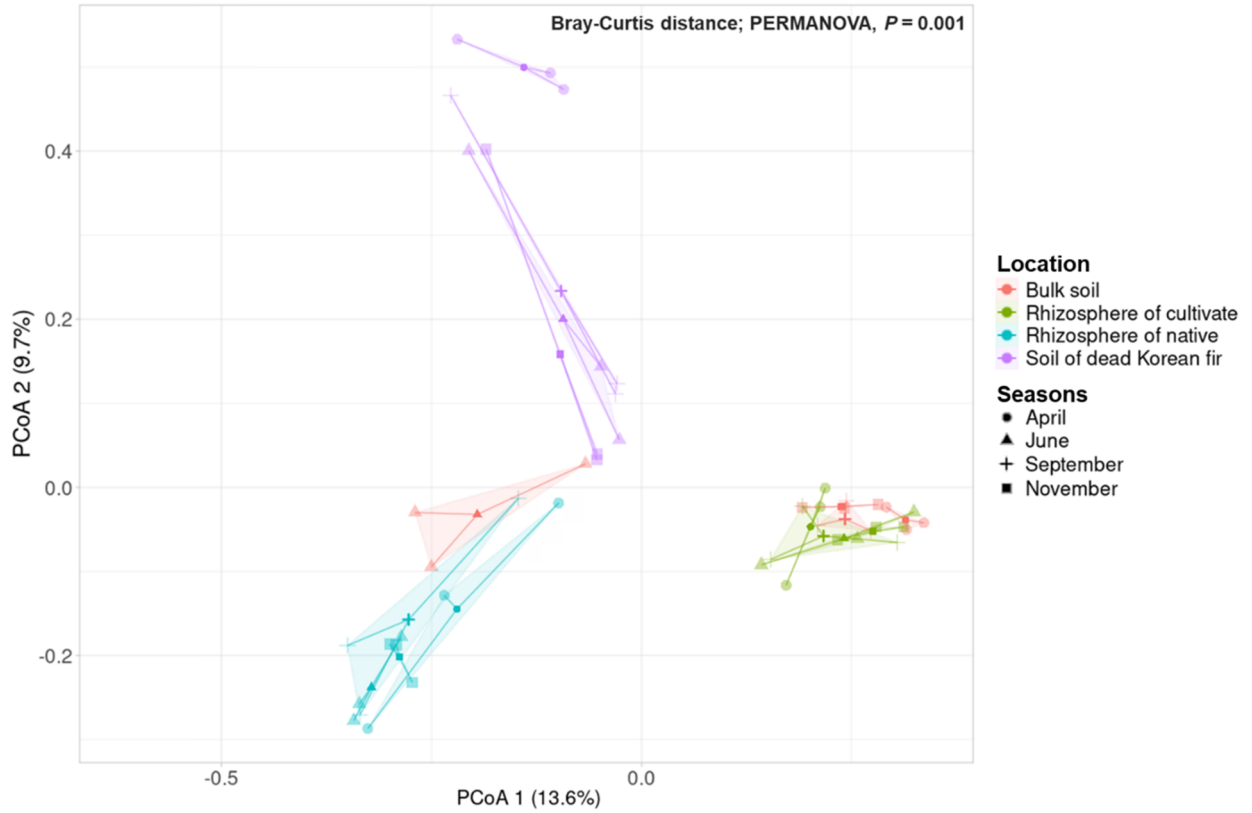

Figure 4. Principal coordinates analysis (PCoA) to identify changes in the structure of the fungal community in the rhizosphere of Korean fir according to regional and seasonal changes. PCoA was performed by reprocessing the amplicon sequence variants (ASVs) of the sample using the Bray-Curtis dissimilarity formula to quantify the diversity of fungi constituting the community. Next, permutational multivariate analysis of variance (PERMANOVA) statistical analysis was used to statistically analyze differences in fungal community structure between regions. On the PCoA graph, color differences between nodes represent differences in region, and differences in shape represent differences in seasons. The coordinates of the average value of three repeated data in each region were expressed as closed nodes.

At the class level (Figure 3B), Agaricomycetes, Leotiomycetes, Dothideomycetes, and Sordariomycetes were dominant, comprising approximately 70% of the total fungal abundance. Agaricomycetes showed high abundance in the rhizosphere of native and rhizosphere of dead Korean fir, with particularly high dominance in the rhizosphere of native (close to 70%). Conversely, its abundance was low in the rhizosphere of cultivation and showed high abundance in bulk soil only in June. However, Leotiomycetes maintained a consistent relative abundance of around 20% across all regions and seasons (Figure 3).

These results highlight significant regional and seasonal influences on the fungal community structure within the Korean fir rhizosphere. The dominance of Ascomycota and Basidiomycota underscores their critical roles. At the same time, the dynamic changes observed in classes such as Agaricomycetes and Leotiomycetes reflect the complex interactions and environmental responses within these fungal communities.

3.3. Regional and seasonal correlation of Korean fir rhizosphere fungal communities

PCoA was performed to assess the regional and seasonal correlation of the rhizosphere fungal communities associated with Korean fir (Figure 4). The analysis revealed that all samples' node positions, except those from bulk soil, remained relatively stable across different seasons. The node positions for samples from native, cultivated, and dead Korean fir sites did not exhibit significant changes with seasonal variations, suggesting a stable fungal community structure across different seasons. However, the nodes representing samples collected in June were notably distant from those collected in April, September, and November, indicating a significant seasonal shift in the bulk soil fungal community.

To statistically validate these observations, PERMANOVA was conducted. The results demonstrated a highly significant difference in the cluster structure of all samples, with a p-value of 0.001. This indicates that the variations observed, particularly in

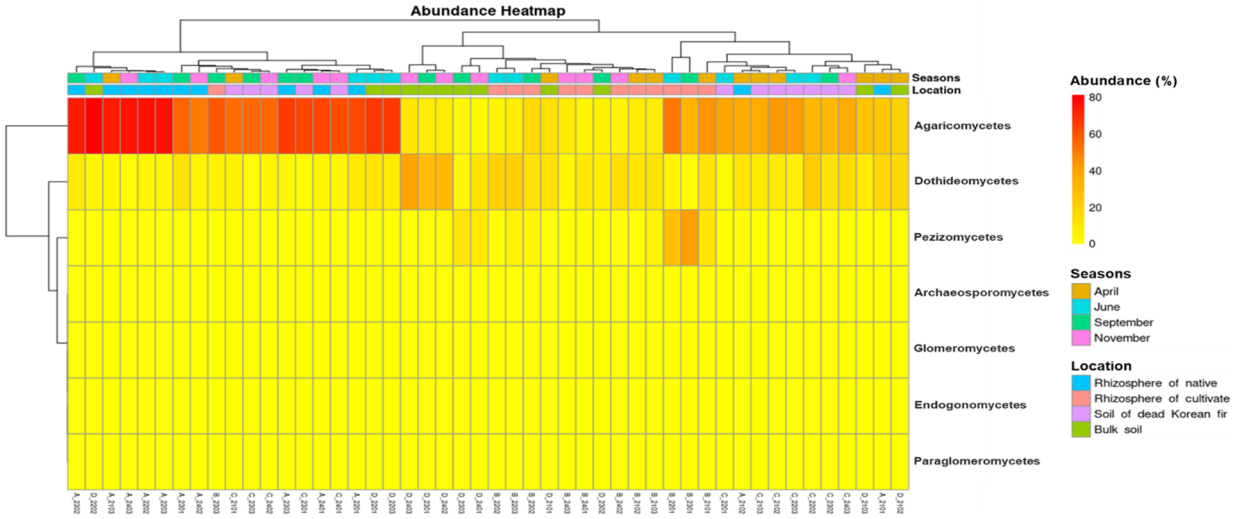

Figure 5. Abundance heatmap analysis by samples of class-level taxa that are likely to be mutual with the Korean fir. The abundance of specific taxa likely to coexist with fir trees was analyzed by location and season. The dendrogram on the *x*-axis is a similarity analysis of the community structure according to the location and seasons of the collected samples, and the dendrogram on the y-axis is an analysis of the abundance similarity between taxa.

the bulk soil samples, are statistically significant and not due to random chance. These findings highlight the pronounced seasonal influence on the fungal community structure in bulk soil, contrasted with the more stable community structures in other regions of the Korean fir rhizosphere. This stability in non-bulk soil samples suggests that the rhizosphere environment may buffer against seasonal fluctuations, maintaining a more consistent fungal community composition

3.4. Mycorrhiza taxa with significant abundance in the rhizosphere of native Korean fir

An investigation was conducted to identify class-level taxa that maintain high abundance across all four seasons in the rhizosphere of native Korean fir. The taxa examined included Agaricomycetes, Dothideomycetes, Pezizomycetes, Glomeromycetes, Archaeosporomycetes, Paraglomeromycetes, and Endogonomycetes. Among the examined taxa, only Agaricomycetes consistently maintained the highest abundance in the rhizosphere of native Korean fir throughout all four seasons (Figure S2A). In contrast, the relative abundance of Agaricomycetes exhibited significant fluctuations in other regions, including the rhizosphere of cultivated Korean fir, rhizosphere of dead Korean fir, and bulk soil. To further analyze the variability in Agaricomycetes abundance, a t-test was performed comparing samples collected in April and September. In bulk soil, there was a significant decrease in the abundance of Agaricomycetes from April to September. The abundance of Agaricomycetes remained relatively stable in the rhizosphere of native Korean fir and other regions (Figure S2B).

These findings highlight the unique stability of Agaricomycetes in the rhizosphere of native Korean fir, suggesting a potentially crucial role for this class in maintaining the health and stability of these ecosystems. The significant fluctuations observed in other regions underscore the dynamic nature of fungal communities and the potential influence of environmental factors and interactions on their composition.

3.5. Clustering of Korean fir location and season samples according to taxa type

Korean fir rhizosphere and soil samples were classified based on collection location and season. The similarity between samples, according to the type and abundance of taxa, was visualized using dendrograms (Figure 5). Agaricomycetes exhibited a significantly higher abundance compared to other taxa. This is reflected in the *y*-axis dendrogram, where Agaricomycetes are distinctly separated from other taxa due to their high abundance. The remaining taxa demonstrated low abundance and were closely clustered in the *y*-axis dendrogram, indicating their relative similarity in abundance and type. On the *x*-axis dendrogram, samples from the same region tended to cluster together, indicating regional similarities in the fungal community structure. In contrast, seasonal samples did not exhibit a clear clustering pattern, suggesting that seasonal variations had less influence on the overall community structure compared to regional differences. These findings indicate that while the fungal community composition is strongly influenced by the collection

region, the seasonal variations play a lesser role. The dominance of Agaricomycetes further underscores its significant role in the rhizosphere of Korean fir, distinguishing it from other fungal taxa in terms of abundance and ecological importance.

4. Discussion

The decline in the population of Korean fir is hypothesized to be associated with recent climate change, although the precise causative factors remain unidentified. Consequently, due to the limitations of existing studies in fully elucidating the decline of Korean fir populations, this study was undertaken from a biological perspective, a relatively underexplored area. Specifically, the investigation focused on analyzing the structural changes in the rhizosphere fungal community under varying seasonal and regional conditions. This community, which possesses substantial biomass within forest soils, maintains a close symbiotic relationship with trees. The analysis revealed no significant differences in alpha diversity of the fungal community within the rhizosphere of Korean fir across different regions. However, beta diversity analysis indicated notable variations in the relative abundance of the constituent taxa. This suggests that while the taxa composition remains largely consistent across regions, their relative abundances vary considerably. Notably, the phylum Basidiomycota and its subgroup Agaricomycetes exhibited higher abundance in the rhizosphere of native fir trees compared to other regions, indicating that the habitat significantly influences the fungal community structure. Conversely, seasonal changes did not markedly affect the fungal community structure in most areas, except for bulk soil. In bulk soil, where the fungal community structure exhibited substantial fluctuations with seasonal changes, the absence of trees and soil exposure to direct sunlight were noted. This implies that the observed changes in the fungal community structure are likely attributable to stronger influences from external environmental factors compared to other regions [35].

Among the native, cultivated, and deceased areas of Korean fir, where no significant changes in the fungal community structure were observed despite seasonal variations, the high abundance of Basidiomycota and Agaricomycetes in the native habitat may be associated with the stability of the fungal community structure in this environment. Similar to other members of the Pinaceae family, Korean fir forms ectomycorrhizal associations with various higher fungi belonging to the Basidiomycota, enabling survival in nutrient-poor and acidic forest soils [36, 37]. Notably, many taxa within Agaricomycetes, such as *Tricholoma*, *Russula*, *Amanita*, and *Sebacina*, are known to form ectomycorrhizae with conifers [38–41]. Given that the soil from the native site of Korean fir has been established for a long period, it is plausible that this enduring symbiotic relationship has allowed ectomycorrhizal taxa to become dominant within the fungal community [42]. Interestingly, the fungal community structure in cultivated sites of Korean fir significantly differed from that in the native sites. This disparity may arise because the soil in cultivated areas is relatively recent, leading to heightened competition among species and the absence of a dominant species [43].

In the soil of dead Korean fir, Basidiomycota and Agaricomycetes were more abundant compared to cultivated sites, even after the trees had perished. This fungal community structure likely emerged during the decomposition process of the dead Korean fir. Numerous taxa within Basidiomycota and Agaricomycetes are white rot fungi, which decompose lignin in wood, potentially maintaining high abundances by outcompeting other microorganisms through more active wood decomposition [44, 45]. This hypothesis is further supported by the analysis results, which showed higher abundances of Ascomycota and Leotiomycetes—groups that include many saprophytic and phytopathogenic fungi—in the dead sites compared to the native sites of Korean fir [14].

These regional differences in fungal community structure were distinctly observable through principal coordinates analysis (PCoA). However, it is important to note that this study investigated only three Korean fir per region, which may be insufficient to represent the entire population confidently. Therefore, future research necessitates a larger sample size to enhance the reliability of the findings. Additionally, rhizosphere samples were collected from thick roots near the soil surface rather than from the root tips, where root exudates are actively secreted to minimize damage to the nationally protected Korean fir. Consequently, the results may not fully represent the fungal community across the entire rhizosphere of Korean fir [46, 47]. In the microbiome analysis of the rhizosphere fungal community, classification below the Order level was not conducted. This decision was made due to the unreliability of the analysis results, as most species remained unidentified and were not present in the database. Therefore, this study is limited by its inability to identify species and elucidate the roles of each taxon within the community. Given these limitations, future research should consider additional

approaches, such as comparing and analyzing the number and types of fruiting bodies formed in the native site of Korean fir with those in other regions. This approach leverages the characteristics of Agaricomycetes, which are abundant in the native site, to form fruiting bodies [44, 45]. By addressing these issues, we can move closer to securing microbial resources critical for preserving the endangered Korean fir.

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Disclosure statement

No potential conflict of interest was reported by the authors.

Data availability statement

All data are incorporated into the article and its online supplementary materials. The project has been deposited at NCBI under the BioProject (PRJNA11120381)

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