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RESEARCH ARTICLE

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Fungal Microbiome of Alive and Dead Korean Fir in its Native Habitats

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ABSTRACT

A rapid decline of *Abies koreana* has been reported in most of the natural alpine habitats in Korea. It is generally accepted that this phenomenon is due to climate change even though no clear conclusions have been drawn. Most research has focused on abiotic environmental factors, but studies on the relationships between *A. koreana* and soil fungal microbiomes are scarce. In this study, the rhizoplane and rhizosphere fungal communities in the alive and dead Korean fir trees from its three major natural habitats including Mt. Deogyu, Mt. Halla, and Mt. Jiri in Korea were investigated to identify specific soil fungal groups closely associated with *A. koreana*. Soil fungal diversity in each study site was significantly different from another based on the beta diversity calculations. Heat tree analysis at the genus level showed that *Clavulina*, *Beauveria*, and *Tomentella* were most abundant in the healthy trees probably by forming ectomycorrhizae with Korean fir growth and controlling pests and diseases. However, *Calocera*, *Dacrymyces*, *Gyoerffyella*, *Hydnotrya*, *Microdochium*, *Hyaloscypha*, *Mycosymbioces*, and *Podospora* were abundant in the dead trees. Our findings suggested that *Clavulina*, *Beauveria*, and *Tomentella* are the major players that could be considered in future reforestation programs to establish ectomycorrhizal networks and promote growth. These genera may have played a significant role in the survival and growth of *A. koreana* in its natural habitats. In particular, the genus *Gyoerffyella* may account for the death of the seedlings. Our work presented exploratory research on the specific fungal taxa associated with the status of *A. koreana*.

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1. Introduction

Korean fir (*Abies koreana* Wilson) is a subalpine conifer native to the high mountains of the Korean Peninsula [1–3]. The largest natural habitats in Korea are found on Mt. Halla in Jeju Island, where the original collection in 1917 was made by Ernest Henry Wilson, who introduced *A. koreana* to the West [4]. The Korean name for this fir tree is "Gusang namu," and it comes from "kusalnang" (kusal: sea urchin; nang: tree) in the Jeju language (Figure 1) [5]. *A. koreana* is distributed from 1000 m above sea level (a.s.l.) to the summits on Mt. Halla (1000–1950 m a.s.l.), Mt. Jiri (1050–1900 m a.s.l.), Mt. Deogyu (1350–1590 m a.s.l.), and Mt. Gaya (1350–1420 m a.s.l.) located in the southern regions of the Korean Peninsula [1,6]. Altitude is an important factor for subalpine plant distribution since it is closely correlated with properties such as soil moisture, solar radiation, soil temperature, and soil salinity [1,7].

In the last few decades, Korean fir has been widely used as a living potted Christmas tree and garden tree in Europe and the United States due to its excellent needle retention and desirable pyramid shape. Despite its popularity, it was assessed for the International Union for Conservation of Nature (IUCN) Red List of Threatened Species in 2010 [8], and it has been listed as endangered (EN) under criteria B2ab (ii, iii, v) [9]. In addition, *A. koreana* is also categorized as Endangered B2ab (ii, iii, v) in the Korean Red List of Threatened Species, and is currently being protected under the Endangered Wild Life Protection Law [10]. Moreover, due to complicated environmental factors, increasing habitat loss, forest decline, growth decline, and even withering of

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Figure 1. Image of the cone and leaves of *Abies koreana* on Mt. Halla (photograph taken on June 7 2020).

A. koreana have been observed in all the natural habitats in Korea since the 1980s [11,12]. A variety of hypotheses, including strong typhoon hits [13,14], moisture-related stresses [15–17], and biological life span [2,18] have been proposed in the literature to explain possible causes of the decline. Although no clear conclusions have emerged, there is a general agreement among the scientific societies that the natural habitat loss of *A. koreana* is closely associated with climate change caused by global warming [19], since it has been reported as a biological indicator species vulnerable to climate change [20,21].

Extensive studies have been conducted to investigate the causes of the decline of *A. koreana* in the native habitats of Korea. However, these studies have been primarily focused on the effects of abiotic environmental factors, and limited studies have been carried out on biotic factors such as soil microorganisms in the endemic habitats of Korean fir [22–24]. In the soil, archaea, bacteria, and fungi take up significant fractions of the biomass with high biodiversity and a variety of metabolic activities by forming a microbiome [25,26]. Furthermore, it has been well documented that soil microorganisms play an indispensable role in soil ecology and fertility as they regulate the transformation of nutrients into plants, decompose organic matter (OM), fix atmospheric nitrogen, and detoxify pollutants across various ecosystems [27,28]. Therefore, the plant soil microbiome is termed the dynamic microbial communities associated with both plants and soil [29]. Symbiotic mycorrhizal fungi are able to channel nutrients to their host plants, build up a porous structure in soil that allows air and water penetration, and prevent soil erosion [30–32]. As healthy populations of beneficial mycorrhizae help plants to facilitate immobile nutrient uptake, enhance tolerance to drought and diseases, and improve photosynthesis, the symbiotic partnership of the rhizosphere (RS) is one of the most important biological processes to maintain plant growth and survival in many ecosystems [33].

Arbuscular mycorrhizal (AM) and ectomycorrhizal (ECM) fungi are two major groups of mycorrhizal fungi that are the most prevalent and widespread in forest soils [34–36]. It was also reported that the ECM fungi have a symbiotic relationship with coniferous plants including the genus *Abies* [35,37–40]. However, only limited studies have been conducted on the AM and ECM formed by Korean fir by using recently emerging culture-independent molecular technologies in its natural regions of Korea [23,24,41].

In this study, the rhizoplane (RP) and RS fungal communities in the alive and dead Korean fir tree samples obtained from its three major natural habitats (Mt. Deogyu, Mt. Halla, and Mt. Jiri) in Korea were analyzed by next-generation sequencing (NGS) approaches. We aimed to identify specific fungal taxa that are closely associated with *A. koreana* and to determine and document the contribution of the soil mycorrhizal microbiome to the growth and survival of the plant.

Furthermore, we attempted to reveal possible causes of the decline of the fir tree forest by comprehensively reviewing the soil analysis results obtained from the native *A. koreana* habitats in Korea and comparing these environmental factors with our soil analysis data. In addition, based on this, it was hoped that we could suggest valuable information on specific fungal taxa that are closely related to the restoration or preservation of *A. koreana*.

2. Materials and methods

2.1. Soil sampling

Soil samples of alive and dead Korean fir trees were obtained from Mt. Deogyu, Mt. Halla, and Mt. Jiri, the three largest natural habitats of *A. koreana*, in the southern parts of the Korean Peninsula in 2020 (Figure 2). Figure 3 and Table 1 show the geographical information including the location coordinates for the study sites. A total of six samples from each

Figure 2. Image of alive and dead *Abies koreana* in Mt. Halla (photograph taken on June 7 2020).

site were collected within the range of 15–20 cm from the center of the plant and RP and RS soil samples were taken from 25 to 30 cm deep from the surface where a large proportion of the active root zone exists. RP and RS samples were each collected from three individuals of alive and dead *A. koreana* trees at each study site. Then, samples were immediately transferred to the laboratory under refrigerated conditions at 4 °C and each soil sample was pooled to obtain a composite sample per site for molecular analysis. RP and RS soil samples were labeled as Deogyu_AP, Deogyu_AS, Deogyu_DP, Deogyu_DS, Halla_AP, Halla_AS, Halla_DP, Halla_DS, Jiri_AP, Jiri_AS, Jiri_DP, and Jiri_DS based on their sampling site, plant status, and soil zone (Table 2).

2.2. Physicochemical analyses of RS soil samples

RS soil samples from each site were pooled to obtain a 1 kg composite sample and soil physicochemical properties were analyzed by the Korea Forestry Promotion Institute (Seoul, South Korea) using standard protocols [42]. Soil texture analysis was carried out using the hydrometer method. Soil pH and soil electrical conductivity (EC) were measured using a pH meter and an EC meter, respectively. Cation exchange capacity (CEC) was analyzed by the ammonium acetate method. Soil OM content and total nitrogen (TN) were measured by the dry combustion method. Available phosphate (AP) analysis was performed using the Lancaster method. Exchangeable cation contents (K^+, Ca^{2+}, Na^+, and Mg^{2+}) were measured by atomic absorption spectrophotometer.

2.3. DNA extraction and NGS library construction

DNA extraction from RP and RS soil samples was performed using the DNeasy PowerSoil Pro Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocols. Briefly, 0.25 mg of each soil sample was used for DNA extraction. The extracted DNA was stored at −20 °C until further use. The first polymerase chain reaction (PCR) run was carried out with the inner primer set, including Rd1SP and Rd2SP. The outer primer set, including adaptor and index, was used in the second PCR in order to construct an NGS library. The Emerald Amp PCR Master Mix (Takara, Shiga, Japan) was used to amplify the fungal internal transcribed spacer (ITS) 2 region with a primer set of ITS86F (5′-ACA CTC TTT CCC TAC ACG ACG CTC TTC CGA TCT

Figure 3. Three soil sampling sites in Mts. Deogyu, Halla, and Jiri (the map was downloaded from Google Earth version 9.169.0.0, accessed on August 31 2022).

Table 1. List of sampling sites and their geographic coordinates.

		GPS coordination			
Study site	Plant status	Latitude	Longitude	Altitude (a.s.l.)	Sampling date
Mt. Deogyu (Seolcheonbong)	Alive	N35°51′42.4″	E127°44′42.8″	1590.2 m	May 31 2020
	Dead	N35°51′40.0″	E127°44′42.1″	1599.6 m	
Mt. Halla (Witseoreum)	Alive	N33°21′44.8″	E126°31′09.2″	1694.8 m	June 7 2020
	Dead	N33°21′45.1″	E126°31′08.5″	1697.7 m	
Mt. Jiri (Nogodan)	Alive	N35°17′46.3″	E127°31′49.1″	1441.7 m	July 6 2020
	Dead	N35°17′46.3″	E127°31′46.8″	1442.9 m	

GTG AAT CAT CGA ATC TTT GAA-3′) and ITS4R (5′-GTG ACT GGA GTT CAG ACG TGT GCT CTT CCG ATC TCC TCC GCT TAT TGA TAT GC-3′) [43]. The first PCR amplification conditions were 3 min at 95 °C, followed by 30 cycles of 30 s at 95 °C, 30 s at 58 °C, and 1 min in at 72 °C, with a final extension of 3 min at 72 °C in a thermocycler (Eppendorf, Hamburg, Germany). The second PCR consisted of 3 min at 95 °C, followed by 30 cycles of 30 s at 95 °C, 30 s at 58 °C, and 1 min at 72 °C, with a final extension of 3 min at 72 °C. The total volume of each PCR reaction was 25 µl. The sizes of the amplicons were verified by the E-Gel Precast Agarose Electrophoresis System (Thermo Fischer Scientific, Waltham, MA). Approximately, 450–500 bp PCR products were selected for NGS library construction. The selected libraries were quantified with the Qubit dsDNA HS Assay Kit (Thermo Fischer Scientific, Waltham, MA) on the Qubit 2.0 Fluorometer (Thermo Fischer Scientific, Waltham, MA) and diluted to 0.5 ng μl^{-1}. DNA quality and integrity were validated by the Agilent 2000 Bioanalyzer Platform (Agilent Technologies, Santa Clara, CA).

2.4. NGS sequencing

Illumina MiSeq sequencing (Illumina, San Diego, CA) was performed using the MiSeq Reagent Kit v3 (Illumina, San Diego, CA). NGS sequencing was carried out at the KNU NGS Core Facility at Kyungpook

Table 2. List of samples obtained from the natural habitats of *Abies koreana*.

Sample name	Sampling site	Plant status	Soil zone	Number of samples[a]
Deogyu_AP[b]	Mt. Deogyu	Alive	Rhizoplane	3
Deogyu_AS[c]			Rhizosphere	3
Deogyu_DP[d]		Dead	Rhizoplane	3
Deogyu_DS[e]			Rhizosphere	3
Halla_AP[f]	Mt. Halla	Alive	Rhizoplane	3
Halla_AS[g]			Rhizosphere	3
Halla_DP[h]		Dead	Rhizoplane	3
Halla_DS[i]			Rhizosphere	3
Jiri_AP[j]	Mt. Jiri	Alive	Rhizoplane	3
Jiri_AS[k]			Rhizosphere	3
Jiri_DP[l]		Dead	Rhizoplane	3
Jiri_DS[m]			Rhizosphere	3

[a]Each rhizoplane and rhizosphere sample was collected from three individuals of both alive and dead Korean fir trees at each study site and pooled into one representative samples.
[b]Rhizoplane sample from alive Korean fir trees on Mt. Deogyu.
[c]Rhizosphere sample from alive Korean fir trees on Mt. Deogyu.
[d]Rhizoplane sample from dead Korean fir trees on Mt. Deogyu.
[e]Rhizosphere sample from dead Korean fir trees on Mt. Deogyu.
[f]Rhizoplane sample from alive Korean fir trees on Mt. Halla.
[g]Rhizosphere sample from alive Korean fir trees on Mt. Halla.
[h]Rhizoplane sample from dead Korean fir trees on Mt. Halla.
[i]Rhizosphere sample from dead Korean fir trees on Mt. Halla.
[j]Rhizoplane sample from alive Korean fir trees on Mt. Jiri.
[k]Rhizosphere sample from alive Korean fir trees on Mt. Jiri.
[l]Rhizoplane sample from alive Korean fir trees on Mt. Jiri.
[m]Rhizosphere sample from alive Korean fir trees on Mt. Jiri.

National University (Daegu, South Korea). All the fastq files obtained in this study were deposited to the NCBI Sequence Read Archive (SRA, http://www.ncbi.nlm.nih.gov/sra) under the accession number PRJNA884490.

2.5. Bioinformatics and statistical analysis

All the sequence raw data were processed using QIIME2 version 2021.04 [44]. Raw sequence reads were filtered with DADA2, and removed sequences belonged to unassigned, chloroplasts, and mitochondria. Filtered reads were rarefied at 10,100 without any discarded samples. UNITE database version 8.0 was used as a reference database for fungal taxonomic assignment [45]. The resulting biom, Excel, mapping, and metadata files were used in Calypso [46] and R language to analyze fungal communities. Fungal community composition and biodiversity, which are associated with soil physiochemical properties, were analyzed with the phyloseq package. Shannon, Chao1, and Inverse Simpson (InvSimpson) indices for alpha diversity analysis were computed using the "vegan" R package [47]. Beta diversity calculation was carried out using principal coordinate analysis as reported by Jeong et al. [43]. Heatmap was plotted to visualize hierarchical clustering with Euclidean distance using pheatmap R package [48]. Heat trees that are related to the status of the Korean fir trees were analyzed with the R package Metacoder v.0.3.5.1 [49]. The final visualization was conducted using the ggplot2 package.

3. Results and discussion

3.1. Physiochemical properties of rhizosphere soil

The physiochemical properties of RS soil samples taken from the natural habitats of both alive and dead *A. koreana* trees are summarized in Table 3. There were no differences in soil textures of each RS samples from alive and dead trees at each study site. pH levels of Deogyu_AS and Jiri_AS were slightly higher than those of Deogyu_DS and Jiri_DS, respectively. However, the opposite values were observed in Mt. Halla RS samples. OM contents of Deogyu_DS and Halla_DS were lower than those of Deogyu_AS and Halla_AS, respectively, while the opposite results were observed in Mt. Jiri RS samples. Especially, a noticeable decrease in OM was observed in Halla_DS compared to Halla_AS. Similar trends in TN were observed in each RS sample, as TN levels are, in general, closely related to OM contents in soil. There was not much difference in AP

Table 3. Physiochemical properties of rhizosphere soil.

Sample		Particle size distribution							AP[c]		Exchangeable cation ($cmol_c\ kg^{-1}$)					
Site	Plant status	Sand (%)	Silt (%)	Clay (%)	Soil textile classification	pH	OM[a] (%)	TN[b] (%)	(mg kg^{-1})	CEC[d] ($cmol_c\ kg^{-1}$)	K^+	Na^+	Ca^{2+}	Mg^{2+}	EC[e] ($ds\ m^{-1}$)	NaCl (%)
Mt. Deogyu (Seolcheonbong)	Alive	44.3	47.1	8.7	L (loam)	4.5	18.41	0.93	23.0	25.52	0.65	0.11	1.47	0.88	0.86	0.018
	Dead	44.2	47.5	8.4	L (loam)	4.3	15.72	0.82	23.7	23.10	0.48	0.09	1.59	0.69	0.97	0.003
Mt. Halla (Witseoreum)	Alive	48.0	47.2	4.8	SL (sandy loam)	4.9	27.25	1.50	42.4	29.85	0.81	0.15	1.15	1.29	0.67	0.009
	Dead	58.7	35.8	5.5	SL (sandy loam)	5.4	8.45	0.38	25.8	14.81	0.17	0.08	0.57	0.40	0.27	0.003
Mt. Jiri (Nogodan)	Alive	56.1	35.4	8.5	SL (sandy loam)	5.4	12.53	0.58	40.5	19.07	0.30	0.07	3.28	0.81	0.40	0.003
	Dead	56.8	34.7	8.5	SL (sandy loam)	4.9	18.26	0.84	29.6	22.81	0.41	0.06	1.09	0.71	0.37	0.004

[a]Organic matter.
[b]Total nitrogen.
[c]Available phosphate.
[d]Cation exchange capacity.
[e]Electrical conductivity.

between Deogyu_AS and Deogyu_DS. However, significant reductions in AP were observed in Halla_DS and Jiri_DS compared to Halla_AS and Jiri_AS, respectively. Overall, lower AP levels were recorded in all DS samples than those in AS ones across all the study sites. Slightly lower CEC value was found in Deogyu_DS than that of Deogyu_AS. On the other hand, a slight increase in CEC was observed in Jiri_DS compared to Jiri_AS. Likewise to OM and TN, a distinguishable decrease in CEC was also recorded in Halla_DS compared to Halla_AS. Patterns of exchangeable cations were accordingly similar to those of CEC across all RS samples. Slightly lower EC value was found in Jiri_DS than that of Jiri_AS. On the contrary, a slight increase in EC was observed in Deogyu_DS compared to Deogyu_AS. However, a significant decrease in EC was also recorded in Halla_DS compared to Halla_AS.

The soil textures of the alive trees at each study site were the same as those of the dead trees as shown in Table 3. Lower pH values were found in the dead tree samples than those in alive tree samples from Mt. Deogyu and Mt. Jiri, while pH was a little bit higher in the dead tree sample compared to the alive sample in Mt. Halla. Lowered OM contents were found in the dead tree samples in Mt. Deogyu and Mt. Halla, but the opposite results were observed in Mt. Jiri. The tendencies of TN, CEC, and exchangeable cations levels were similar to those of OM contents at each site. Lower AP values were observed in the dead soil samples compared to the alive samples in all the study sites. EC values were lower in the dead soil samples than those of the alive samples from Mt. Halla and Mt. Jiri, while the opposite results were recorded in Mt. Deogyu. NaCl concentrations were generally lowered in the dead tree samples than those of the alive samples from Mt. Deogyu and Mt. Halla, but NaCl levels of the alive and dead tree samples were almost the same as each other in Mt. Jiri.

The soil textures of the three study sites were loam and sandy loam (SL). A previous survey on the distribution patterns of Korean fir in the southern parts of the Korean Peninsula indicated that *A. koreana* trees were intensively distributed in SL (51%) and silt loam (SiL, 36%) soils at over 1200 a.s.l., which normally allow for good drainage [50]. Furthermore, a recent study by Park et al. [51] showed that the major soil textures of *A. koreana* were loam (Mt. Jiri) and SiL (Mts. Deogyu and Halla). In addition, SL and SiL are typically considered as the most suitable soil textures for Korean fir [13,51,52] since they possess excellent nutrient and water holding capacities. Moreover, it was found that two of our study sites on Mt. Halla (Halla_AS and Halla_DS) and Mt. Jiri (Jiri_AS and Jiri_DS) also hold favorable soil textures for *A. koreana*. However, *A. koreana* forest soils over 1093 m a.s.l. in Georim Valley – Seseok Shelter on Mt. Jiri were sand (S) soil (Supplementary Table 3).

The pH values of the RS soil samples obtained from the study sites varied from 4.3 to 5.4, which are slightly higher than the average pH values of the natural *A. koreana* habitats in Korea; Mt. Deogyu (pH 4.1), Mt. Halla (pH 4.4), and Mt. Jiri (pH 4.1) [51]. However, the average pH value of Korean forest soils is 5.5 [53]. The low pH values of Korean fir forest soils may have resulted from the typical characteristics of the subalpine forests in Korea under the humid microthermal climate due to the reduced degradation of litterfalls and the accumulation of organic acids. Previous studies showed that slightly higher pH values were observed in the soils of unhealthy or declining *A. koreana* forests in Witseoreum, Yeongsil, and Seongpanak on Mt. Halla and Banyabong on Mt. Jiri (Supplementary Tables 2 and 3). Similar results were also obtained in the soil samples from our Witseoreum site on Mt. Halla (Halla_AS and Halla_DS) and Nogodan site on Mt. Jiri (Jiri_AS and Jiri_DS). However, contrary results were also observed in Seolcheonbong on Mt. Deogyu. Hence, no adverse effects of the soil pH can be observed on the growth and survival of Korean fir. However, the pH levels should be monitored regularly since too acidic conditions may lead to dieback or even death of coniferous trees, as the optimal pH values for conifers in Korea have been reported between pH 4.8 and pH 5.5 [54].

Soil OM is one of the crucial components of soil composition that affects chemical reactions and the availability of nutrient elements to plants. Table 3 shows that the OM contents of the soils ranged from 8.45% to 27.5% and these results were higher than the average OM content (4.5%) of forest soil in Korea [53]. Park et al. [51] also reported that the average OM values in 121 natural habitats of *A. koreana* forests were 10.4% in Mt. Deogyu, 13.5% in Mt. Jiri, and 13.9% in Mt. Halla. The high OM contents may be attributed to the low annual temperatures and large numbers of annual cloudy days in the natural habitats of *A. koreana* due to high altitudes [55] as temperature and moisture strongly affect microbial biomass and soil OM mineralization [56,57]. The OM contents in Mt. Halla soils were higher than those in Mt. Deogyu and Mt. Jiri. This may have resulted from aluminum–humus complex formation in volcanic ash soil of Jeju Island that aid the accumulation of soil organic carbons [18,58]. There was a decreasing tendency in the RS soils of the dead Korean fir trees

(Deogyu_DS and Halla_DS) compared to those in the alive ones in Mts. Deogyu and Halla (Deogyu_AS and Halla_AS), but the opposite results were observed in Mt. Jiri (Jiri_AS and Jiri_DS). Decreased OM contents in the soils of unhealthy *A. koreana* trees in Witseoreum, Yeongsil, and Seongpanak on Mt. Halla (Supplementary Table 2) were reported by Kwon [59] and Lim et al. [60]. On the contrary, Park [61] reported that there was a slight increase in OM in the declining *A. koreana* forests compared to the healthy ones in Banyabong on Mt. Jiri (Supplementary Table 3). In general, soil OM can have several important effects such as nutrient availability, water retentions, and temperature regulation on *A. koreana* forests. Furthermore, it can influence the decomposition rate of dead *A. koreana* trees. Additionally, it can also impact the overall health and status of alive *A. koreana* trees through improved nutrient cycling, soil structure, and microbial activity. According to the previous studies conducted in Korea (Supplementary Tables 1–3), *A. koreana* habitats tend to have higher soil OM contents compared to the average OM of Korean forest soils (4.49%). Park et al. [51] conducted a correlation analysis between alive and dead Korean fir trees and soil environmental factors in the representative native habitats of *A. koreana*, but a positive correlation between OM and alive trees was observed only in Mt. Halla. However, statistically significant correlations of OM and status of Korean fir trees were not found in other regions. Even though it was difficult to derive meaningful results from our study with only a limited sample size, it is assumed that the decline in *A. koreana* population is to some extent related to the decrease in OM content especially in case of Mt. Halla. Therefore, monitoring the quality and quantity of soil OM in the natural habitats would be an essential practice for maintaining healthy *A. koreana* forests.

The quantity and quality of soil OM are very closely linked to soil nitrogen [62–64]. Since OM is one of the primary sources of forest soil TN [65], areas with high OM contents have been found to have relatively high TN [18,55]. Furthermore, higher TN contents ranging from 0.38% to 1.50% were recorded in all our study sites and these values were higher than the average TN content (0.19%) of typical Korean forest soils [53]. Moreover, lower concentrations of TN were found in unhealthy or declining *A. koreana* forests compared to the healthy ones in Seolcheonbong sites (Deogyu_AS and Deogyu_DS) on Mt. Deogyu and Witseoreum (Halla_AS and Halla_DS), Yeongsil, and Seongpanak sites on Mt. Halla (Supplementary Tables 1–3). However, Nogodan (Jiri_AS and Jiri_DS) and Banyabong areas on Mt. Jiri showed the opposite results. Moreover, TN does not directly indicate plant-available nitrogen such as NO_3^--N and NH_4^+-N [66].

AP is another essential parameter that directly affects plant growth and development and it is an important indicator to evaluate the level of soil phosphorus supply. AP levels in most of the *A. koreana* forest soils were higher than the average AP concentration (25.6 mg kg^{-1}) of Korean forest soils [53] (Supplementary Tables 4–6). However, it was reported that the optimal AP ranges for most plants in Korean forests were typically between 100 and 200 mg kg^{-1} [67]. Moreover, phosphorus availability is lower under acidic soil conditions since phosphorus precipitates with Al^{3+} and Fe^{3+} ions at a soil pH of below 5.5 [68,69]. This may account for the low AP contents of *A. koreana* forest soils in the natural habitats of Korea where soil pH values were mostly below 5.5. Therefore, AP could be the most limiting nutrient for the growth and survival of *A. koreana* in Korea. Our findings are also in agreement with the previous study by Jeong et al. [43].

CEC is a fundamental property that influences the ability of the soil to hold onto positively charged ions for plant growth since exchangeable cations are the most important source of immediately available plant nutrients. The CEC values of the soil samples from the study sites ranged from 14.81 $cmol_c$ kg^{-1} to 29.85 $cmol_c$ kg^{-1} (Table 3). The optimal CEC content of the Korean forest soils for plants and vegetation is around 12.00 $cmol_c$ kg^{-1} [70] and all the CECs were well above the optimal CEC level. It is generally considered that CEC is closely associated with the OM contents of soil [71]. Therefore, it can be assumed that the soil CEC level was not a limiting factor in the proliferation of *A. koreana* in the natural habitats of Korea. However, all the *A. koreana* soils in the study sites had higher values than the average CEC (16.50 $cmol_c$ kg^{-1}) of the Korean forest soils [60], except for the RS soils of the dead Korean fir trees on Mt. Halla (14.81 $cmol_c$ kg^{-1}).

The order of the exchangeable cations in the *A. koreana* soils at the study sites was $Ca^{2+} > Mg^{2+} > K^+ > Na^+$ and this tendency was consistent with that of general Korean forest soils [53]. However, the order of the substitutional cations in the RS soils of the healthy *A. koreana* on Mt. Halla (Halla_AS) was $Mg^{2+} > Ca^{2+} > K^+ > Na^+$ and subsequently we were unable to infer any conclusion from these results.

Soil EC is directly proportional to the nutrient concentration because cations (Ca^{2+}, Mg^{2+}, K^+, Na^+, and NH_4^+) and anions (SO_4^{2-}, Cl^-, NO_3^-, and HCO_3^-) in soil water dissolved from salts that carry electrical charge. Soil EC does not directly affect plant growth,

but it can be used as an indirect indicator of the amount of nutrients available for plant uptake and salinity levels. EC values ranged from 0.27 to 1.29 ds m^{-1} in all the RS samples, and these values were similar to those obtained from previous studies (Supplementary Tables 1–3). All the EC results were <2.0 ds m^{-1} which is generally regarded as the optimal soil EC that does not impede plant growth and microbial activity.

NaCl concentrations varied from 0.003% to 0.018%. To our best knowledge, there is no available information on NaCl levels in the natural habitats of *A. koreana* in Korea. Therefore, no comparison can be made between samples. It is noteworthy that the soil salinity of the RS on Mt. Halla (Halla_AS) was not influenced by the oceanic location of Jeju Island.

3.2. Microbiome of RP and RS in the study sites

We obtained 571,725 reads for 12 samples. Rarefaction curves for the samples are shown in Supplementary Figure 1. Ascomycota, Basidiomycota, and Mortierellomycota were the most dominant ones in the *A. koreana* study sites (Figure 4, Table 4). Basidiomycota was the most abundant phylum in the RP and RS soils of Mt. Deogyu (Deogyu_AS and Deogyu_DS) regardless of the status of the Korean fir tree, followed by Ascomycota and Mortierellomycota. However, Ascomycota was the most dominant in most cases of the Mt. Halla soil samples, except for the RP soil (Halla_DP), where Basidiomycota showed the highest relative abundance. In the case of Mt. Jiri, the most dominant phylum in the soils of alive *A. koreana* (Jiri_AP and Jiri_AS) was Ascomycota, but it was shifted to Basidiomycota in the soils of dead Korean fir trees (Jiri_DP and Jiri_DS). Overall, it was found that in the RS soils, there was a tendency for relative abundance Ascomycota to increase while relative abundance of Basidiomycota decreased. Additionally, these findings support recent research by Jeong et al. [43].

At the family level, Mortierellaceae, Russulaceae, Sebacinaceae, Serendipitaceae, and Other (the unassigned fungal group) were the major constituents of each fungal community (Figure 5). The dominant families in the fungal communities in the AP and AS samples were mostly Mortierellaceae and Other. Furthermore, Russulaceae and Sebacinaceae were dominant in the DP and DS samples, whereas Other was the most abundant taxonomic group in the AP and AS samples. However, taxonomic information on Other is ambiguous because many operational taxonomic units (OTUs) were placed into the unassigned group and represented as Other due to the lack of information in the public databases.

Figure 6 shows that the orders of relative abundance at the genus level were *Sebacina* (56%) and *Clavulina* (11%) in the Deogyu_AP sample, and *Cortinarius* (40%), *Clavulina* (26%), and *Sebacina* (15%) in the Deogyu_AS sample. However,

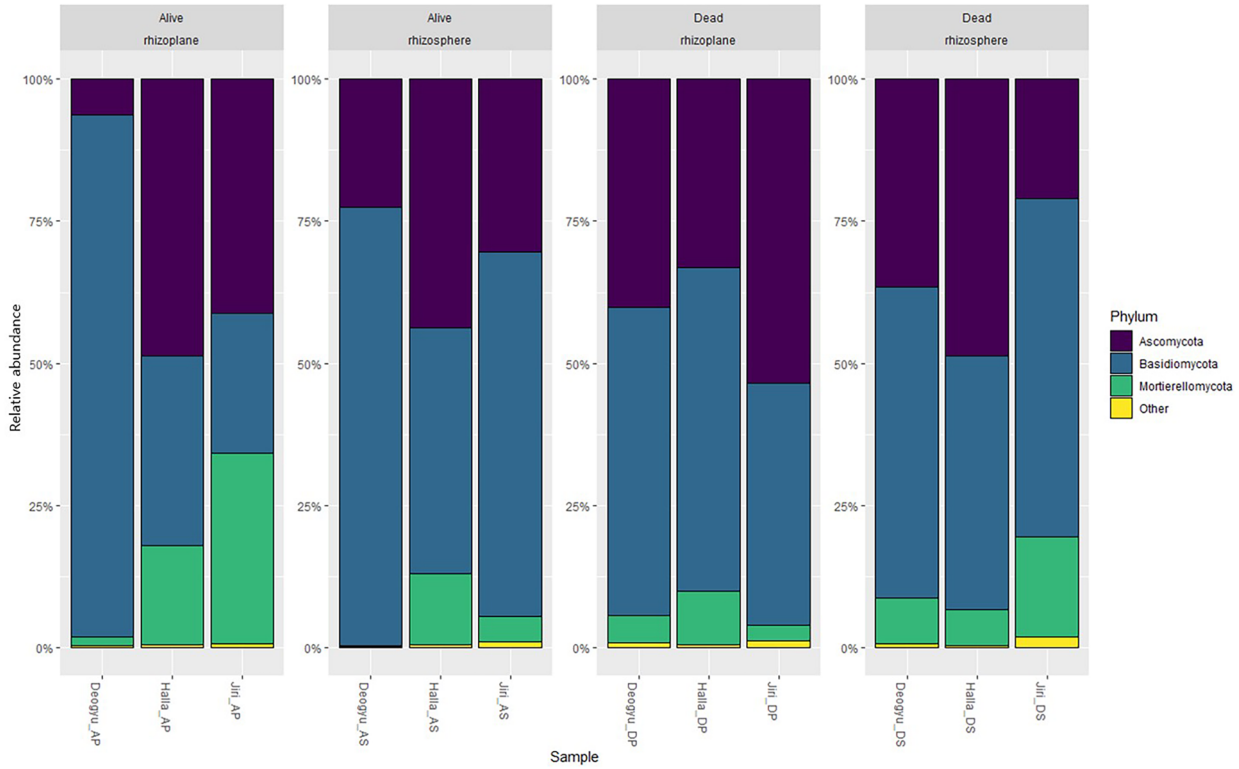

Figure 4. Relative abundance of the top three dominant phyla (Ascomycota, Basidiomycota, Mortierellomycota) in alive and dead Korean fir soils from Mt. Deogyu, Mt. Halla, and Mt. Jiri.

Mortierella (12%) and *Serendipita* (9%) were the most abundant genera in the Deogyu_DP sample, and *Serendipita* (10%) and *Mortierella* (7%) were the most abundant genus in the Deogyu_DS. In addition, *Clavulina* (28%) and *Mortierella* (21%) were the major genera in the Halla_AP sample, while *Clavulina* (29%), *Mortierella* (28%), and *Solicoccozyma* (13%) were the most dominant groups in the Halla_AS sample. In the case of Mt. Jiri, the orders of relative genus abundance were *Laccaria* (29%) and *Russula* (12%) in the Jiri_AP sample and *Mortierella* (41%) and *Leohumicola* (8%) in the Jiri_AS sample. Additionally, *Russula* (59%) and *Mortierella* (21%) were the two most dominant genera in the Jiri_DP sample, and *Russula* (56%) and *Mycopappus* (6%) were the most abundant ones in the Jiri_DS sample. The hierarchical clustering results at genus level showed that the soil samples from the healthy Korean fir trees in each study location were clustered together, while those from the dead Korean fir trees in each study site were also grouped together (Figure 7).

Table 4. Comparison of taxonomic groups identified by Illumina MiSeq sequencing analysis.

Sample name	Phylum	Class	Order	Family	Genus
Deogyu_AP[a]	6	12	26	45	51
Deogyu_AS[b]	3	6	12	37	37
Deogyu_DP[c]	3	15	28	41	39
Deogyu_DS[d]	6	14	29	29	29
Halla_AP[e]	5	10	16	15	15
Halla_AS[f]	3	9	12	25	25
Halla_DP[g]	5	14	28	39	46
Halla_DS[h]	7	14	28	48	51
Jiri_AP[i]	5	13	24	34	37
Jiri_AS[j]	5	12	26	36	39
Jiri_DP[k]	4	7	10	28	29
Jiri_DS[l]	5	13	28	37	35

[a]Rhizoplane sample from alive Korean fir trees on Mt. Deogyu.
[b]Rhizosphere sample from alive Korean fir trees on Mt. Deogyu.
[c]Rhizoplane sample from dead Korean fir trees on Mt. Deogyu.
[d]Rhizosphere sample from dead Korean fir trees on Mt. Deogyu.
[e]Rhizoplane sample from alive Korean fir trees on Mt. Halla.
[f]Rhizosphere sample from alive Korean fir trees on Mt. Halla.
[g]Rhizoplane sample from dead Korean fir trees on Mt. Halla.
[h]Rhizosphere sample from dead Korean fir trees on Mt. Halla.
[i]Rhizoplane sample from alive Korean fir trees on Mt. Jiri.
[j]Rhizosphere sample from alive Korean fir trees on Mt. Jiri.
[k]Rhizoplane sample from alive Korean fir trees on Mt. Jiri.
[l]Rhizosphere sample from alive Korean fir trees on Mt. Jiri.

As shown in Table 5, the Shannon, Chao1, and InvSimpson indices were calculated for all the samples. The Shannon index was the highest (2.94) in the Deogyu_DS sample, while the Chao1 index was the highest (57.00) in the Halla_DP sample. The highest InvSimpson index of 11.99 was observed in the Deogyu_DP sample. Furthermore, alpha diversity was compared based on the study sites (Deogyu, Halla, and Jiri), plant status (alive and dead), and soil types (RP and RP), and there was no significant difference ($p < 0.05$) among these groups (Supplementary Figures 2–4). In addition, beta diversity was also compared among the regions, health status of the Korean fir, and soil types. It was found that the estimated diversity in each study site was significantly different from another (Figure 8), while beta diversity was very similar to each other based on the status of the plant and soil types (Supplementary Figures 5 and 6). The variation in

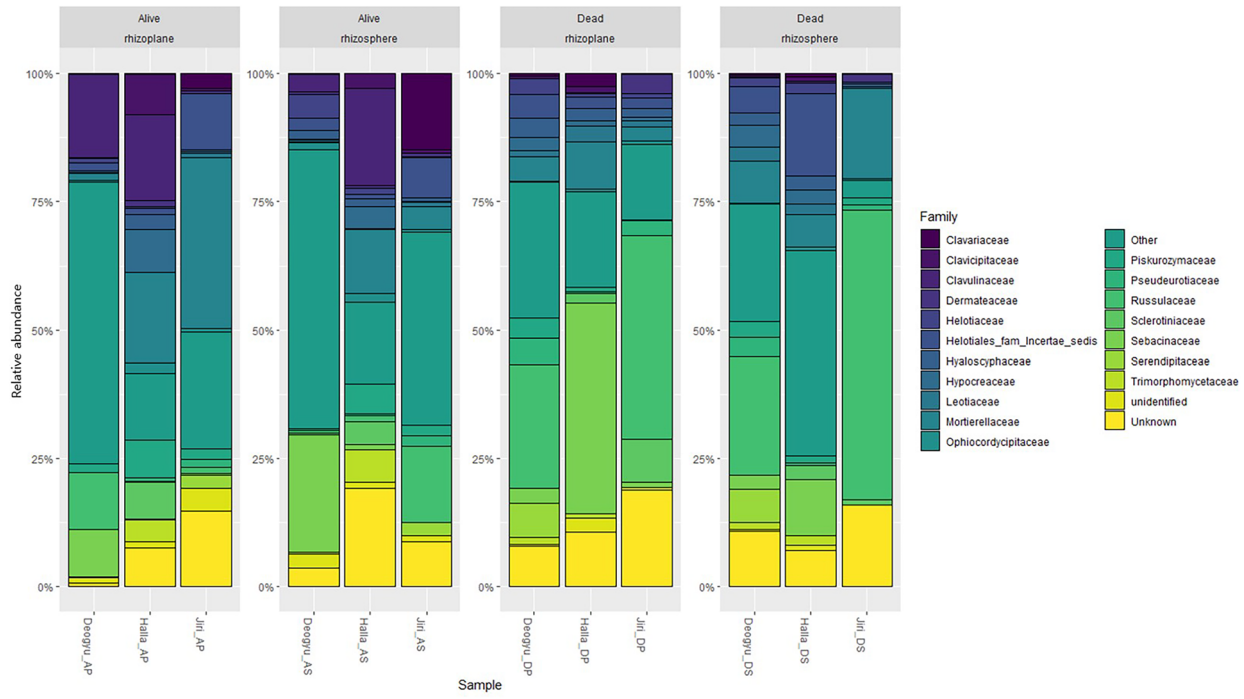

Figure 5. Relative abundance of major families in alive and dead Korean fir soils from Mt. Deogyu, Mt. Halla, and Mt. Jiri. Mortierellaceae, Russulaceae, Sebacinaceae, and Serendipitaceae were the major constituents of each fungal community.

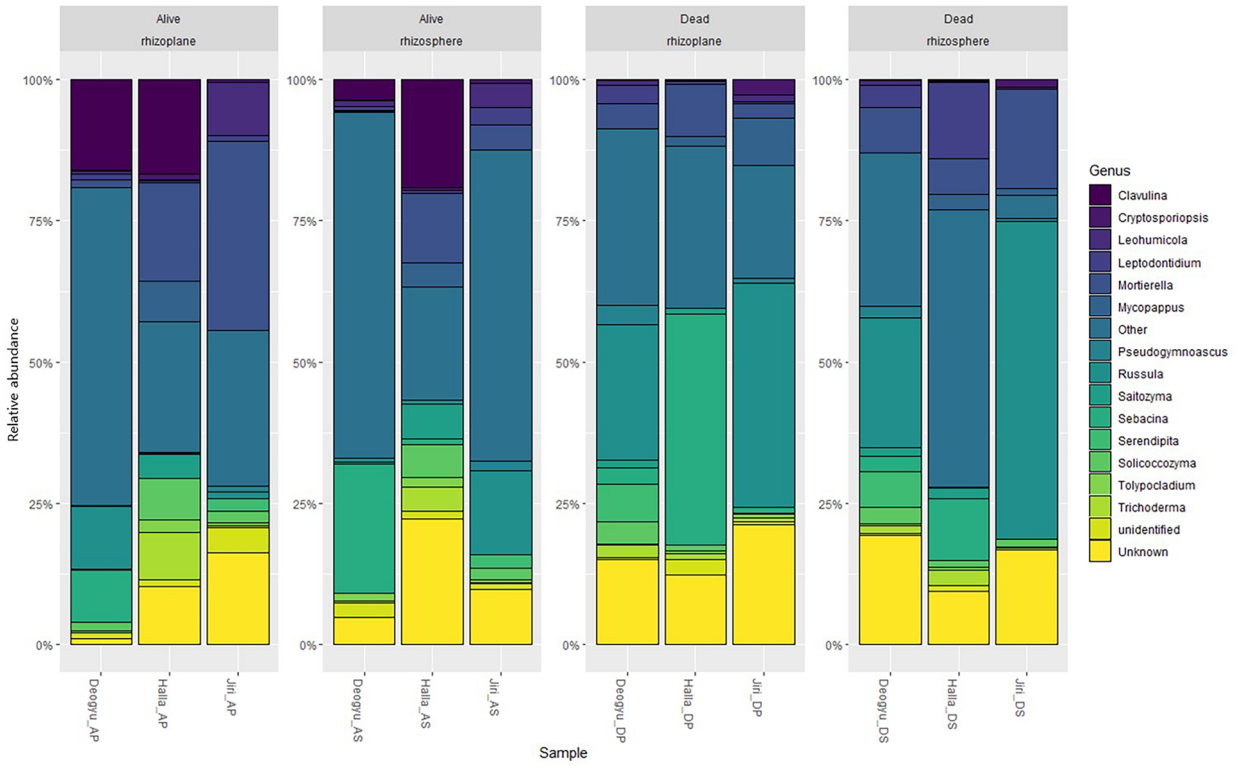

Figure 6. Relative abundance of major genera in alive and dead Korean fir soils from Mt. Deogyu, Mt. Halla, and Mt. Jiri.

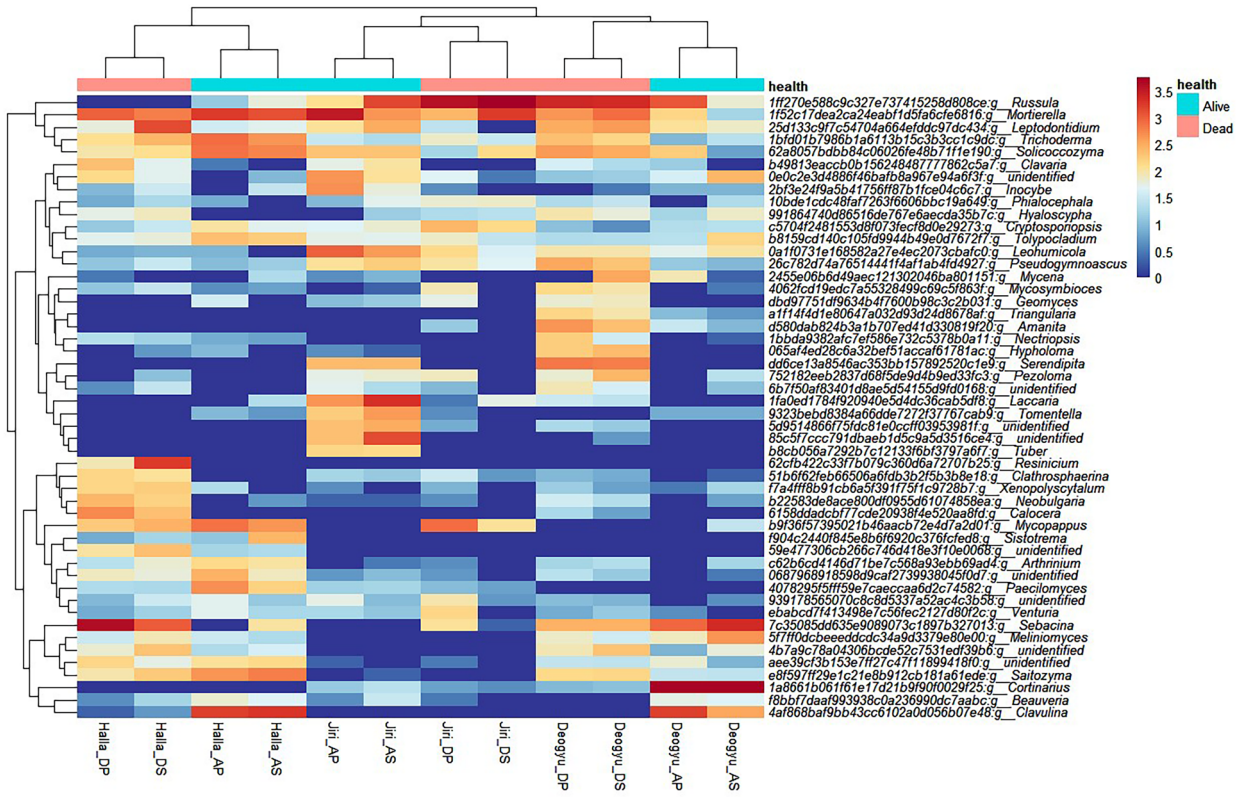

Figure 7. Hierarchical clustering of sequences classified at the genus level. The soil samples from the healthy trees in each study location were clustered together, while those from the dead trees in each study site were also grouped together.

species identities was only observed from one study site to another site according to the beta diversity calculations.

Abundances of the eight phyla based on the status of the plant and soil types were estimated. However, there was no significant difference between the fungal taxon and samples (Supplementary Figures 7 and 8). In addition, a heat tree was generated to graphically display the most abundant taxa among the samples (Supplementary Figure 9). Figure 9

Table 5. Comparison of fugal diversity indices of each representative sample.

Sample name	Shannon	Chao1	InvSimpson
Deogyu_AP[a]	1.54	49.00	2.51
Deogyu_AS[b]	1.59	13.00	3.75
Deogyu_DP[c]	2.90	53.00	11.99
Deogyu_DS[d]	2.94	54.00	11.85
Halla_AP[e]	2.32	28.00	6.20
Halla_AS[f]	2.15	22.00	6.06
Halla_DP[g]	2.59	57.00	7.05
Halla_DS[h]	1.73	51.00	2.22
Jiri_AP[i]	2.23	47.00	4.71
Jiri_AS[j]	2.77	56.00	10.46
Jiri_DP[k]	1.21	16.00	2.19
Jiri_DS[l]	2.11	47.00	3.75

[a]Rhizoplane sample from alive Korean fir trees on Mt. Deogyu.
[b]Rhizosphere sample from alive Korean fir trees on Mt. Deogyu.
[c]Rhizoplane sample from dead Korean fir trees on Mt. Deogyu.
[d]Rhizosphere sample from dead Korean fir trees on Mt. Deogyu.
[e]Rhizoplane sample from alive Korean fir trees on Mt. Halla.
[f]Rhizosphere sample from alive Korean fir trees on Mt. Halla.
[g]Rhizoplane sample from dead Korean fir trees on Mt. Halla.
[h]Rhizosphere sample from dead Korean fir trees on Mt. Halla.
[i]Rhizoplane sample from alive Korean fir trees on Mt. Jiri.
[j]Rhizosphere sample from alive Korean fir trees on Mt. Jiri.
[k]Rhizoplane sample from alive Korean fir trees on Mt. Jiri.
[l]Rhizosphere sample from alive Korean fir trees on Mt. Jiri.

illustrates the family Clavulinaceae and the genus *Clavulina*, the family Cordycipitaceae and the genus *Beauveria*, and the genus *Tomentella*, including two unidentified groups, were positively related to the healthy Korean fir trees, while the class Eurotiomycetes, the class Dacrymycetes, the order Dacrymycetales, the family Dacrymycetaceae, and the genera *Calocera* and *Dacrymyces*, the family Discinaceae and the genus *Hydnotrya*, the family Microdochiaceae and the genus *Microdochium*, the family Leotiaceae, the genus *Gyoerffyella*, the genus *Hyaloscypha*, the genus *Mycosymbioces*, and the genus *Podospora* were significantly related to the unhealthy *A. koreana* trees.

Clavulina is a genus of clavarioid fungi containing around 83 species [72]. The members of *Clavulina* are mainly tropical, but some are also distributed in temperate regions and other parts of the world [73–75]. *Clavulina* species can be recognized by their distinctive clavarioid to coralloid basidiomata and basidia with two cornute sterigmata [76,77]. There is extensive evidence that *Clavulina* forms ECM associations with a variety of plants [78–80] including the genus *Abies* [39,81]. Recently, Kim et al. [24] demonstrated that the dominance of *Clavulina* spp. in the alive *A. koreana* soil samples on Mt. Halla (63%) was reduced to 10% in the dead Korean fir soil samples. This reduction in the relative abundance of *Clavulina* was also observed in the RP and RS samples of Mt. Deogyu and Mt. Halla from our study

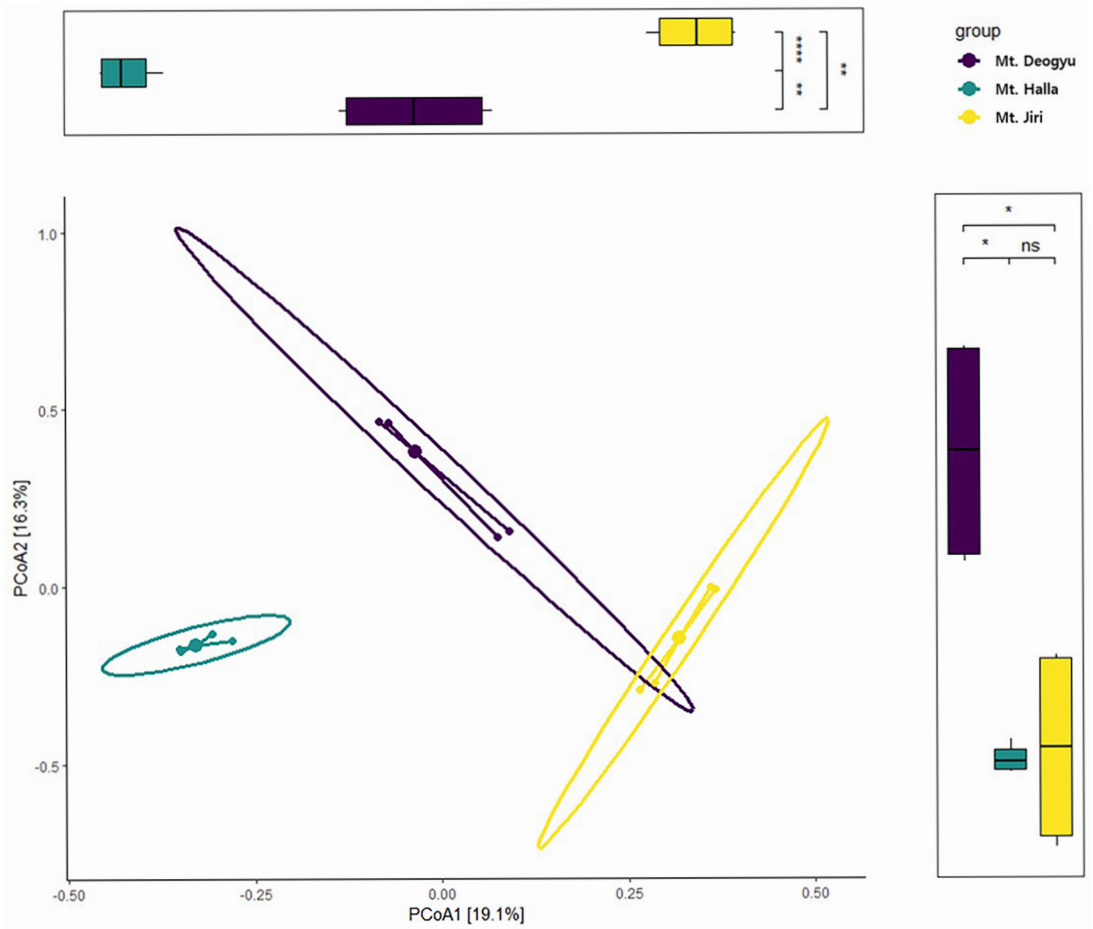

Figure 8. Beta diversity among the study sites (*$p < 0.05$; **$p < 0.01$; ****$p < 0.0001$).

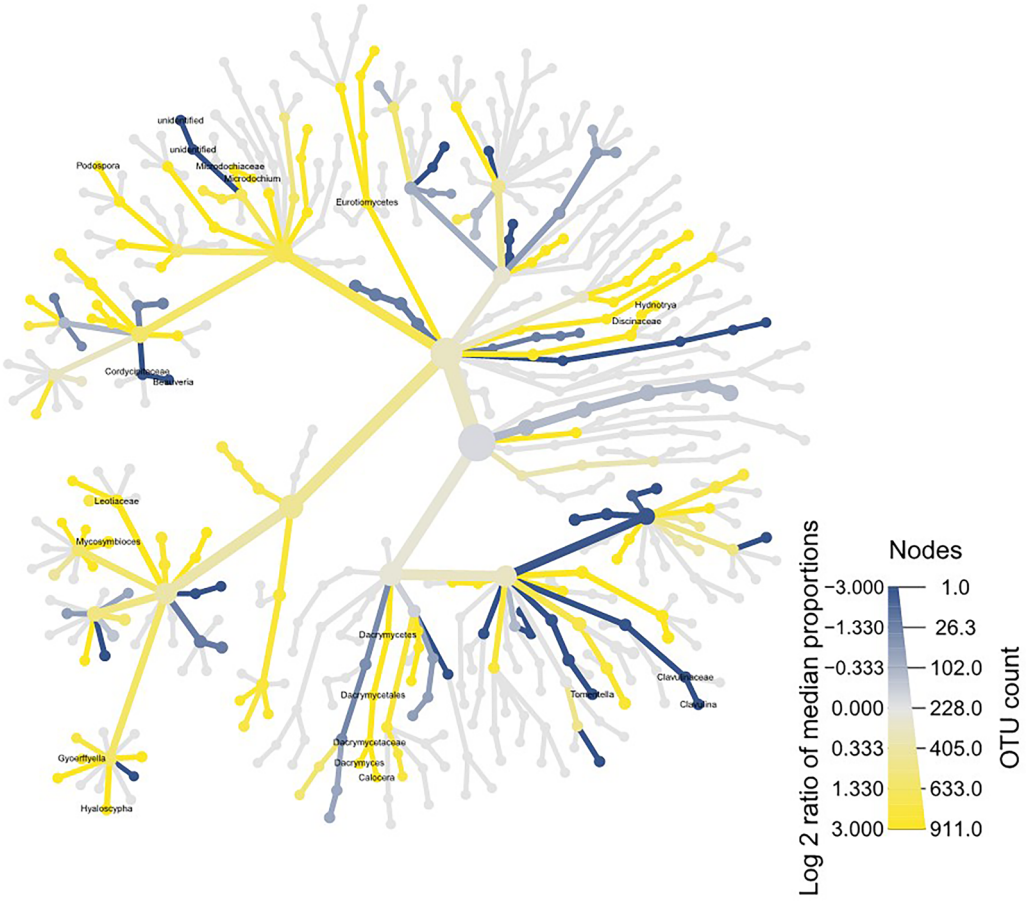

Figure 9. Heat tree showing the abundance of fungal genera with $p < 0.05$ based on the plant's status (alive and dead). The color represents the percent of operational taxonomic units (OTUs) assigned to each taxon (blue represents an alive tree and yellow represents a dead tree, respectively). Node diameter is proportional to the out counts and edge width is proportional to the number of reads.

(Figure 6). This indicates that the *Clavulina* genus may play an indispensable role in *A. koreana* root growth, health, tolerance, and resistance to various environmental stresses by providing essential nutrients and protection. It was also shown that *Clavulina* had significantly higher proportions of the ECM fungi in the roots of *A. koreana* on Mt. Halla [23,82]. Therefore, *Clavulina* could be considered in future *A. koreana* reforestation programs to establish ECM networks between the fungus and plant in their natural habitats. Further studies should initially focus on inoculating *Clavulina* spp. with *A. koreana* seedlings to form successful ECM associations in nurseries at greenhouse and field scales.

Beauveria is a filamentous soil fungus belonging to the entomopathogenic fungi and it is found all around the world. Some *Beauveria* species such as *Beauveria bassiana* can act as pathogens in more than 700 insect species [83]. However, this species can also form a mutualistic interaction with a wide range of plants [84–86] as it can establish itself as an endophyte in various plant species and show protective activity against insect pests and plant pathogens [87–89]. Hence, it could be presumed that *Beauveria* spp. may have promoted the growth of *A. koreana* and biologically controlled insect pests and plant diseases.

Tomentella is a genus of corticioid fungi that can be usually found ubiquitously on fallen branches, leaf litter, rotten wood, and soil in forests. It can be characterized by its resupinate and thin basidiocarps, colored hymenophore, and generative hyphal system with simple septa or clamps [90–92]. Most *Tomentella* spp. form mycorrhizal associations with a wide variety of host plants [93–97] and many of them act as mycobionts of alpine ECM plants for plant establishment and development under extreme conditions [98]. Furthermore, *Tomentella* was also one of the most dominant ECM fungi in the roots of *A. koreana* on Mt. Halla in culture-dependent [23] and culture-independent [82] studies. Hence, it can be assumed that *Tomentella* spp. play an important role in the survival and growth of *A. koreana* as symbionts.

The genus *Calocera* usually grows on dead and dying trees and is generally characterized by cylindrical basidiocarps, branched fruiting bodies, and an amphigenous hymenium [99,100]. Moreover,

Dacrymyces species are saprotrophs as they also usually occur on dead wood. Their distribution is worldwide and the genus *Dacrymyces* can be recognized by its cylindrical basidiocarps and hyphae with clamp connections [99,101]. *Hydnotrya* is a genus of hypogeous fungi producing macroscopic fruit bodies partially or completely embedded in the soil. *Hydnotrya* is distributed across the northern hemisphere including Asian, European, and North American countries [102]. *Microdochium* species are commonly known as plant pathogens and saprophytes isolated from diseased plant hosts such as grasses and cereals [103]. The genus is characterized by its spherical and erumpent stromata and solitary transparent spindle-shaped to oval conidia [104]. Furthermore, *Gyoerffyella* species are known as aquatic and terrestrial saprobes and pathogens normally found in decaying leaves [105–107]. It was demonstrated that naturally regenerating *A. alba* seedlings were infected by *Gyoerffyella* species causing seedling decline [108]. Therefore, this genus may account for the decline of Korean fir seedlings in their natural habitat. Moreover, the genus *Hyaloscypha* has 35 recognized species which includes a variety of saprobes formed on decaying plant matter, and there are several asexual species that can form ectomycorrhizae in plant roots [109,110]. However, this kind of relationship was not observed in this study. *Mycosymbioces* species are terrestrial saprotrophs, and they are found in the root of plants [111]. In addition, *Podospora* species are saprophytic endophytes that commonly occur on trees and grasses as well as soil [112]. Therefore, the mentioned saprotrophic fungal genera are responsible for the decomposition of OM from dead *A. koreana* plants.

4. Conclusions

Soil analysis results showed that AP was the only limiting nutrient for the growth and survival of *A. koreana* across all the study sites. It also seemed the *A. koreana* habitats of Mt. Halla were more vulnerable to the decline in OM content level than those of Mt. Deogyu and Mt. Jiri probably due to their geographical and climatic characteristics based on our findings and comprehensive review of the publications on the analysis of environmental factors that have been conducted in Korea's native fir tree habitats. According to beta diversity estimations, it was found that the soil fungal diversity at each study site was significantly different from each other. Heat tree analysis indicated that entomopathogenic *Beauveria* and ECM *Clavulina* and *Tomentella* showed a positive correlation with the alive *A. koreana*. The saprophytic and pathogenic genera including *Calocera*, *Dacrymyces*, *Gyoerffyella*, *Hydnotrya*, *Microdochium*, *Hyaloscypha*, *Mycosymbioces*, and *Podospora* showed a significantly negative relationship with *A. koreana*. In conclusion, our work demonstrated the significant difference in the RP and RS fungal diversity of the alive and dead Korean fir trees. Our findings may contribute to the prevention of the decline of the Korean fir forest by ensuring the stability of the forest fungal ecosystems. Moreover, significant information on the specific fungal taxa that are closely related to the status of *A. koreana* and could be used for a better understanding of the cause of the forest decline as a biological indicator. This study gives comprehensive insights into the dominant fungal species closely associated with the health and growth of *A. koreana* in three representative Korean fir trees native habitats. Future work on the precise mechanisms between the ECM fungi and *A. koreana* at different scales is still required, and further study of this topic may be profitable.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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Data availability statement

All fastq files have been deposited into an NCBI SRA under the accession number PRJNA884490.

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