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RESEARCH ARTICLE

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Diversity and community structure of ectomycorrhizal mycorrhizal fungi in roots and rhizosphere soil of *Abies koreana* and *Taxus cuspidata* in Mt. Halla

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ABSTRACT

In this study, the roots and rhizosphere soil of *Abies koreana* and *Taxus cuspidata* were collected from sites at two different altitudes on Mt. Halla. Ectomycorrhizal fungi (EMF) were identified by Illumina MiSeq sequencing. The proportion of EMF from the roots was 89% in *A. koreana* and 69% in *T. cuspidata*. Among EMF in rhizosphere soils, the genus *Russula* was the most abundant in roots of *A. koreana* ($p < 0.05$). The altitude did not affect the biodiversity of EMF communities but influenced fungal community composition. However, the host plants had the most significant effect on EMF communities. The result of the EMF community analysis showed that even if the EMF were isolated from the same altitudes, the EMF communities differed according to the host plant. The community similarity index of EMF in the roots of *A. koreana* was higher than that of *T. cuspidata* ($p < 0.05$). The results show that both altitude and host plants influenced the structure of EMF communities. Conifers inhabiting harsh sub-alpine environments rely strongly on symbiotic relationships with EMF. *A. koreana* is an endangered species with a higher host specificity of EMF and climate change vulnerability than *T. cuspidata*. This study provides insights into the EMF communities, which are symbionts of *A. koreana,* and our critical findings may be used to restore *A. koreana*.

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1. Introduction

Abies koreana E. H. Wilson is a Korean endemic species that inhabits alpine and sub-alpine regions. It is vulnerable to recent climate changes [1] and was recently designated as an endangered species by the International Union for Conservation of Nature (IUCN) [2]. The distribution of the species is limited to altitudes between 1,000 and 1,900 m in Mt. Halla and Mt. Jiri [3]. The populations of *A. koreana* have declined in almost all habitats [4]. *Taxus cuspidata* Siebold et Zucc is distributed in the Korean peninsula, Japan, northeastern China, and the Russian far east. In South Korea, it inhabits altitudes between 700 to 2,500 m, from Mt. Halla to Mt. Seungjeok [5]. It is classified as a species of least concern by the IUCN, and the degree of damage due to climate change is not as high as that for other conifers. However, the number of deaths in this species has also been increasing on Mt. Halla and Mt. Deogyu [5]. The cause of this decline in alpine coniferous species has not yet been identified, but it could be attributed to complex interactions between various environmental factors caused by global warming [4,6].

Global warming, the leading cause of climate change, continues to progress, and the result has been a general increase in temperature in alpine regions, with temperature distribution varying according to altitude, and shifting habitats [7]. In addition, alpine and sub-alpine zones are unfavorable environments for plants because of the high annual difference in various climatic factors, such as temperature, and poor soil quality. In particular, highland tree species are isolated at the top of mountains, making it difficult to move to a new habitat. As the temperate plants of lowlands gradually expand to the highlands, the highland trees are pushed out because of competition and risk extinction. In the case of Mt. Halla, as the temperature increased at higher altitudes, the distribution of competitive pine trees expanded even at relatively high temperatures. At the same time, the number of evergreen conifers in the sub-alpine zone decreased significantly. The growth of highland tree species, including *A. koreana,* is highly related to climate, and as environmental changes, such as climate change, accelerate, the growth rate decreases, and the frequency of dead trees increases [4]. Conifers in the sub-alpine zone have been classified as the

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most vulnerable to climate change because they are sensitive to temperature change. As the number of *A. koreana* has recently decreased significantly due to extremely high temperatures and drought, studies are being conducted to identify the cause of deaths and help restore the plants.

Previous studies on the effects of climate change have mainly focused on vascular plants and animals, although the potential response of soil fungal communities to climate change has also been investigated [8,9]. Ectomycorrhizal fungi (EMF) and saprotrophic fungi in the soil play an important role in organic matter decomposition and the carbon cycle [10]. EMF are a major component of forest ecosystems and constitute up to 80% of the total fungal biomass in forest soil. These fungi form symbiotic relationships with various plants such as Pinaceae, Fagaceae, and Betulaceae [11,12]. These relationships increase the efficiency of root absorption of soil moisture and various nutrients and promote the growth of host plants by protecting plant roots from pathogens and heavy metals [11,13]. These fungal symbionts are also known to play an important role in the survival and growth of seedlings [14,15]. Soil microbes, including EMF, influence plant responses to climate change. Changes in microbial diversity and community structure not only disturb ecosystem functions and balance but also negatively affect soil productivity and cause plant death [16,17]. Plants in harsh alpine environments rely heavily on symbiotic relationships with mycorrhizal fungi [18]. Therefore, studies on the interaction between EMF and their host plants are needed to conserve endangered coniferous species.

In a narrow geographic range, altitude correlates with average annual temperature [19]. The present study aimed to determine the diversity of EMF in the roots and rhizosphere soil of *A. koreana* and *T. cuspidata* at different altitudes on Mt. Halla. The difference in EMF diversity and community structure according to altitude and host plants was compared using next generation sequencing (NGS) [20,21].

2. Materials and methods

2.1. Sample collection

The roots and rhizosphere soil of *A. koreana* and *T. cuspidata* were collected from Mt. Halla in June 2018. Along the Gwaneumsa trail, altitudes of 1,510–1,560 m are designated as the lower part (N33°37′, E126°53′), and the coniferous forest begins here. The area at 1,860–1,910 m above sea level is designated as the upper part (N33°36′, E126°53′), and the coniferous forest end here. A total of 24 samples were collected from the lower and upper areas, three from the roots and rhizosphere soil of each host plant. The collected roots were carefully rinsed with tap water to remove the soil and then stored at −70 °C until use. The collected soil was sieved through a 2 mm sieve to remove rocks and root fragments and then stored at −70 °C until further use.

2.2. DNA extraction and amplification

To extract root DNA, after treatment in 70% ethanol for 1 min and 30% H_2O_2 for 30 s, the root surface was sterilized by washing it with sterile water several times. The moisture was then removed using sterile filter paper. Ectomycorrhizal root tips were collected and ground in a mortar using liquid nitrogen to extract genomic DNA using the DNeasy Plant mini kit (Qiagen, Redwood City, CA, USA). Soil samples were ground with a grinder (FastPrep-24^{TM}; MP Biomedicals, Santa Ana, CA, USA) for 5 min. Total genomic DNA in the soil was extracted from the 250 mg soil samples and stored at −20 °C until needed for PCR. The extracted DNA was measured with Epoch^{TM} Spectrometer (BioTek, Winooski, VT, USA), and a sample of 20 ng/μl or more was used for PCR. PCR primer pairs, ITS3-Mi (5′-TCGTC GGCAG CGTCA GATGT GTATA AGAGA CAGGC ATCGA TGAAG AACGC AGC-3′) and ITS4-Mi (5′-GTCTC GTGGG CTCGG AGATG TGTAT AAGAG ACAGT CCTCC GCTTA TTGAT ATGC-3′) were used to amplify the internal transcription space 2 (ITS2) region of fungal ribosomal DNA. PCR was performed under the following conditions: denaturation (95 °C, 30 s), annealing (55 °C, 1 min), and extension (72 °C, 30 s) for a total of 25 cycles after predenaturation (95 °C, 3 min), followed by a final extension (72 °C, 5 min) [22]. The PCR product was electrophoresed on 1.5% agarose gel to confirm the amplified band of 500–800 bp ITS region. We then performed sequencing using the Illumina MiSeq sequencing platform at ChunLab Co. (Seoul, South Korea).

2.3. Sequencing and data analysis

The row reads that were obtained from NGS were analyzed using EzBioCloud [23]. The sequences were classified into operational taxonomic units (OTUs) based on 97% sequence similarity in the database after selecting sequence reads for analysis through quality checks [24]. Secondary analysis of the generated datasets was performed using the Microbiome Taxonomic Profiling software provided by ChunLab Co. Among the OTUs, only those corresponding to EMF at the genus level were separately classified to calculate the relative abundance of EMF. The Shannon's diversity index, evenness, and

similarity index were calculated using Multi-Variate Statistical Package 3.22 (Kovach Computing Services, Pentraeth, UK) [25]. In addition, principal component analysis was performed using the PC-ORD 10 software (Wild Blueberry Media LLC, Corvallis, OR, USA) [26]. Similarities between communities were analyzed using the SPSS software (SPSS Inc., Chicago, IL, USA).

3. Results

A total of 1,890,884 reads were obtained from the roots and rhizosphere soil of *A. koreana* and *T. cuspidata*, and a total of 19,801 OTUs were identified at 97% sequence similarity. The Good's coverage estimator for all samples was more than 99%, indicating that they were sufficient to represent the collected fungal community. The number of reads, number of OTUs, abundance-based coverage estimator, Chao's species richness estimator, and Shannon's index were all higher in the soil than the roots regardless of host plant ($p < 0.005$) and showed no significant difference in roots and soil according to altitude or host plant (Table 1).

EMF diversity analysis was performed by selecting sequences corresponding to EMF from the sequences obtained through NGS. The ratio of EMF colonized with the roots was 88% (±4.5) in *A. koreana* and 69.9% (±21.6) in *T. cuspidata,* showing a higher trend in *A. koreana*. The relative frequency of EMF found in the roots of *A. koreana* was the highest for Basidiomycota at 82.7%, followed by Ascomycota at 16.0% (Table 2). For the roots of *T. cuspidata*, the relative frequency of Basidiomycota was 85.9% and that of Ascomycota was 13.1%. At the class level, regardless of host plant and altitude, Agaricomycetes and Leotiomycetes accounted for the highest proportion. At the genus level, *Russula, Clavulina,* and *Tomentella,* accounted for more than 90% of the EMF in the roots of *A. koreana* in the lower region. *Clavulina* and *Russula* accounted for significantly higher proportions of the EMF in the roots of *A. koreana* in the upper region. For the roots of *T. cuspidata*, *Tomentella* and *Lactarius* had the highest proportion in the lower region and *Russula* and *Tomentella* had the highest proportion in the upper region. The genera common to all samples of *A. koreana* and *T. cuspidata* roots, regardless of altitude, were *Clavulina* and *Russula*.

Total fungi from the rhizosphere soil of *A. koreana* appeared in the order Basidiomycota, Mortielrellomycota, and Ascomycota. At the class level, Agaricomycetes, Mortierellomycetes, and Leotiomycetes showed a high proportion (Table 3). The proportion of fungi in the rhizosphere soil of *T. cuspidata* was in the following order: Basidiomycota > Mortierellomycota > Ascomycota, and at the class level, the order was: Agaricomycetes > Agaricostilbomycetes > Aphelidiomycetes. These results confirmed a difference in the fungi present in the rhizosphere soil between *A. koreana* and *T. cuspidata*. The common EMF, regardless of altitude, were *Entoloma*, and *Laccaria* and *Melniomyces. Russula* and *Clavulina* were dominant in the lower region, and *Clavulina* and *Cortinarius* were dominant in the upper region in the rhizosphere soil of the *A. koreana*. In the rhizosphere soil of *T. cuspidata*, the fungi *Inocybe, Clavulina,* and *Russula* accounted for a high proportion in the lower region in that order. Meanwhile, *Russula, Sebacina*, and *Clavulina* showed a high proportion in the upper region. These results highlighted the differences according to altitude.

Shannon's index, evenness, number of genera, and number of OTUs of the EMF community from the roots and rhizosphere soil of the host plants were analyzed at the genus level. The results showed no significant difference according to altitude or host plant. We compared the number of EMF OTUs in the roots with that in the rhizosphere soil and found more EMF colonization in the roots of *A. koreana* than in *T. cuspidata*, and this was higher in the lower region than in the upper region. Furthermore, *Russula* showed a significant difference ($p < 0.05$, Figure 1). The results of principal component analysis highlighted that the community structure of EMF was

Table 1. Illumina MiSeq sequencing results and diversity estimates for each sample.

			Sequencing results (Mean ± SE)		Diversity estimates (Mean ± SE)[a]		
	Altitude	Sample Type	Number of valid reads	Number of OTUs	ACE	Chao	Shannon's Index
Abies koreana	Lower	Root	71734.67 ± 3225.91	360.67 ± 62.20	365.32 ± 63.19	361.43 ± 62.45	1.32 ± 0.35
		Soil	71546.33 ± 1066.62	1185.00 ± 76.30	1200.45 ± 76.53	1189.21 ± 76.19	3.87 ± 0.25
	Upper	Root	71867.33 ± 4795.05	302.67 ± 33.45	264.53 ± 10.57	260.91 ± 10.57	1.70 ± 0.55
		Soil	75209.00 ± 4847.46	1052.33 ± 220.17	1072.11 ± 219.64	1058.84 ± 219.08	3.69 ± 0.80
Taxus cuspidata	Lower	Root	67218.00 ± 5771.87	320.00 ± 126.72	327.79 ± 128.67	321.83 ± 126.94	1.98 ± 0.67
		Soil	96624.67 ± 7361.21	1451.67 ± 121.58	1478.42 ± 121.58	1460.19 ± 120.92	4.31 ± 0.41
	Upper	Root	80545.00 ± 2099.36	414.67 ± 103.51	424.14 ± 106.45	417.77 ± 104.64	2.35 ± 0.47
		Soil	95549.67 ± 7517.73	1513.33 ± 234.24	1536.22 ± 236.85	1519.33 ± 234.68	4.46 ± 0.41

[a]Diversity estimates at species level. Threshold of 97% similarity was used to define OTUs.
ACE: abundance-based coverage estimator; Chao: Chao's species richness estimator; OTU: operational taxonomic unit.

Table 2. Relative abundance (%) of major genera of fungi found in the roots of *Abies koreana* and *Taxus cuspidata*.

	Relative abundance (Mean ± SE)			
	Lower		Upper	
Genera	*A. koreana*	*T. cuspidata*	*A. koreana*	*T. cuspidata*
Cenococcum	0.004 ± 0.004		0.114 ± 0.106	2.517 ± 1.510
Ceratobasidium		0.003 ± 0.003		0.015 ± 0.015
Chroogomphus		23.823 ± 23.823		
Clavaria		0.020 ± 0.020		0.009 ± 0.009
Clavulina	32.035 ± 32.027*	4.858 ± 2.548*	44.211 ± 26.273*	3.513 ± 3.499*
Cortinarius	0.014 ± 0.013	0.076 ± 0.074	5.601 ± 5.494*	1.264 ± 1.243
Endogone			0.003 ± 0.003	0.174 ± 0.174
Entoloma	0.016 ± 0.010	0.093 ± 0.070	0.007 ± 0.004	1.779 ± 1.771
Hydnotrya		0.002 ± 0.002	0.036 ± 0.036	3.367 ± 1.690
Hygrophorus			0.012 ± 0.012	0.137 ± 0.137
Inocybe	0.003 ± 0.003	0.096 ± 0.094	0.003 ± 0.003	2.182 ± 2.180
Laccaria	0.175 ± 0.175	0.093 ± 0.073	3.397 ± 3.397*	5.177 ± 5.144
Lactarius	0.013 ± 0.012	26.103 ± 18.271	4.124 ± 4.124	0.003 ± 0.003
Meliniomyces	4.241 ± 2.443*	0.027 ± 0.015	7.267 ± 4.016*	4.592 ± 1.686*
Piloderma		3.451 ± 3.447	0.006 ± 0.006	
Pseudotomentella	0.001 ± 0.001	0.410 ± 0.410	1.194 ± 1.193	
Russula	33.227 ± 31.337*	7.098 ± 4.200*	28.658 ± 15.572*	40.226 ± 24.094*
Sebacina	2.605 ± 2.605	4.186 ± 4.181	2.794 ± 2.123*	4.104 ± 2.891*
Tomentella	27.619 ± 27.614	29.621 ± 16.553	2.167 ± 1.068*	30.596 ± 22.481*
Shannon's index	0.32 ± 0.15[a]	1.01 ± 0.0.09[b]	1.12 ± 0.47	1.15 ± 0.34
Species evenness	0.15 ± 0.05[a]	0.46 ± 0.07[b]	0.44 ± 0.19	0.45 ± 0.12
Number of genera	8.33 ± 2.60	11.67 ± 5.86	12.00 ± 0.58	12.67 ± 0.88
Number of OTUs	61568.00 ± 2339.96	11636.04 ± 6721.52	60311.33 ± 5827.89	39863.67 ± 14442.12

Major genera were selected based on the relative abundance in the total sample (≥0.5%).
*Fungal strains that were found at all sites.
OTU: operational taxonomic unit.

Table 3. Relative abundance (%) of major genera of fungi found in the rhizosphere soil of *Abies koreana* and *Taxus cuspidata*.

	Relative abundance (Mean ± SE)			
	Lower		Upper	
Genera	*A. koreana*	*T. cuspidata*	*A. koreana*	*T. cuspidata*
Cenococcum	0.298 ± 0.219	1.150 ± 0.954*	0.187 ± 0.146*	0.149 ± 0.078
Ceratobasidium		0.013 ± 0.008	1.243 ± 1.243	2.206 ± 1.995*
Chroogomphus		0.041 ± 0.041		
Clavaria	0.105 ± 0.084	0.251 ± 0.251	0.574 ± 0.411	1.452 ± 1.329*
Clavulina	32.506 ± 30.696	22.152 ± 2.450*	32.156 ± 19.166*	14.173 ± 14.010*
Cortinarius	2.444 ± 2.436*	5.637 ± 3.654*	28.952 ± 26.832	2.373 ± 1.763*
Endogone		0.009 ± 0.009	0.913 ± 0.904	0.132 ± 0.118
Entoloma	0.553 ± 0.142*	1.868 ± 1.309*	0.703 ± 0.438*	0.494 ± 0.177*
Hydnotrya	0.789 ± 0.789	1.386 ± 0.800	1.624 ± 1.204*	1.133 ± 1.018*
Hygrophorus		0.049 ± 0.049		0.990 ± 0.985
Inocybe	5.533 ± 5.501	25.845 ± 12.401*	0.559 ± 0.351*	7.684 ± 6.529*
Laccaria	10.481 ± 10.237*	6.079 ± 3.658*	12.624 ± 9.402*	6.851 ± 4.633*
Lactarius	0.067 ± 0.067	0.879 ± 0.710	0.075 ± 0.055	0.212 ± 0.189
Meliniomyces	0.306 ± 0.016*	0.536 ± 0.195*	0.941 ± 0.129*	1.172 ± 0.453*
Piloderma	1.595 ± 1.595	6.556 ± 5.516	1.831 ± 1.798*	0.003 ± 0.001
Pseudotomentella	2.477 ± 2.477	2.232 ± 2.184	1.398 ± 1.384*	0.006 ± 0.006
Russula	33.824 ± 31.385*	12.425 ± 10.936	4.66 ± 2.522	33.632 ± 28.569*
Sebacina	7.216 ± 7.216	8.380 ± 7.354*	5.921 ± 1.257*	23.507 ± 11.778*
Tomentella	1.415 ± 0.781*	2.741 ± 1.259	1.747 ± 1.382	2.486 ± 1.260*
Trichophaea	0.107 ± 0.066	1.120 ± 1.120	1.007 ± 0.694*	0.851 ± 0.503
Wilcoxina		0.004 ± 0.004	1.742 ± 1.742	
Shannon's index	0.82 ± 0.58	1.77 ± 0.03	1.42 ± 0.40	1.31 ± 0.42
Species evenness	0.29 ± 0.19	0.60 ± 0.02	0.29 ± 0.19	0.43 ± 0.13
Number of genera	13.33 ± 3.18	19.33 ± 2.33	13.33 ± 3.18	21.67 ± 2.60
Number of OTUs	24754.67 ± 1503.04	26834.67 ± 6748.14	30505.67 ± 6762.64	33287.33 ± 13486.08

Major genera were selected based on the relative abundance in the total sample (≥0.5%).
*Fungal strains that were found at all sites.
OTU: operational taxonomic unit.

similar in the roots and not in the rhizosphere soil, and there was a difference depending on the host plant but not altitude (Figure 2). In both roots and rhizosphere soil samples, it was found that both the EMF community similarity of *A. koreana* was higher than that of *T. cuspidata*. In addition, to compare the difference in EMF communities between samples, a two-way cluster analysis was performed at the genus level using the gradient method. In this result, it was also confirmed that even though the EMF community of *A. koreana* rhizosphere soil was different at the two altidudes, the EMF community of *A. koreana* roots was the most similar in both altitudes (Figure 3). The similarity index of the roots was higher than that of

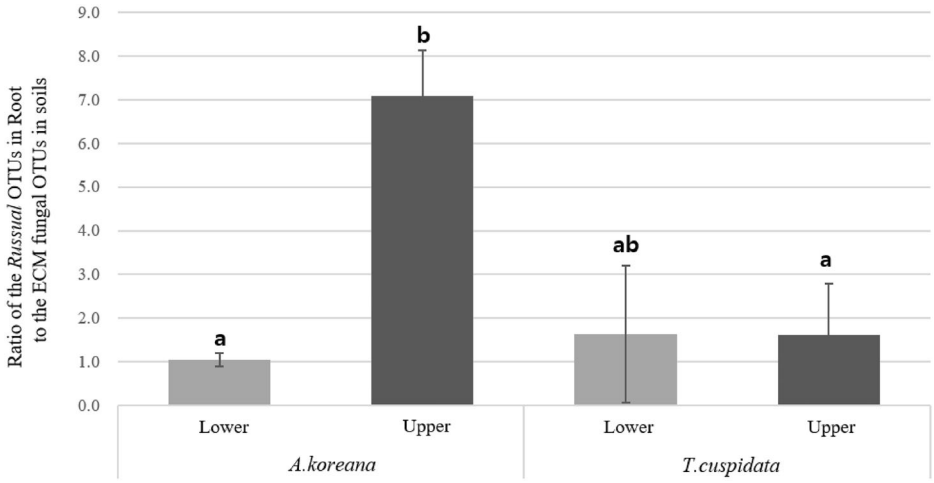

Figure 1. Ratio of the *Russula* OTUs in the root to the ectomycorrhizal fungal OTUs in soils affected by altitude (lower and upper) and host (*Abies koreana* and *Taxus cuspidata*) in Mt. Halla. Statistical significance is indicated by letters ($p \leq 0.05$). ECM: ectomycorrhizal; OTU: operational taxonomic units.

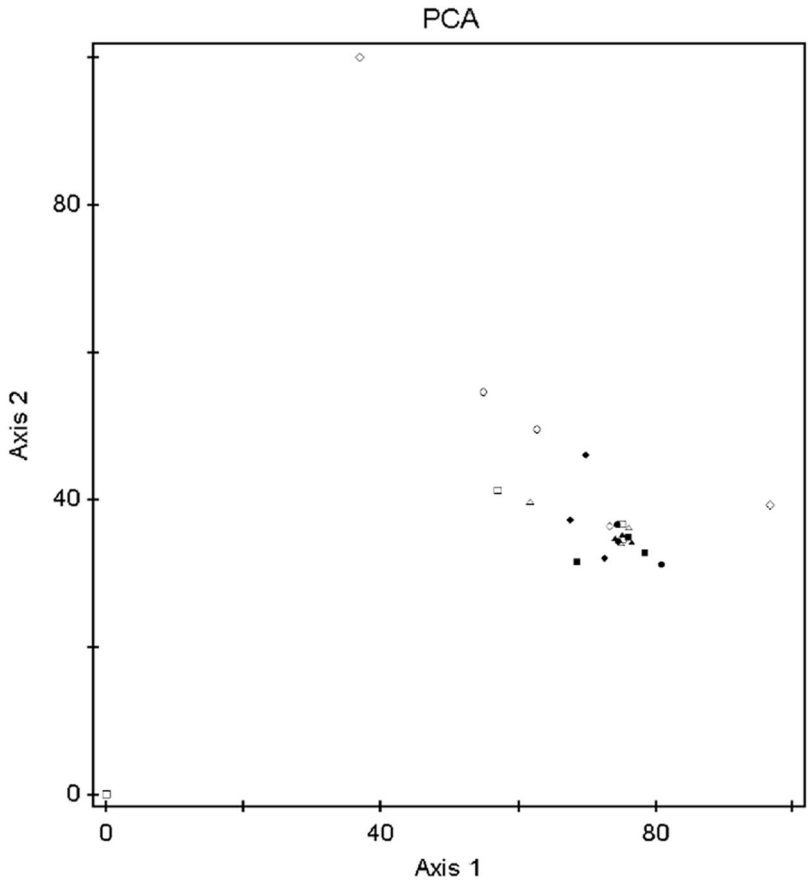

Figure 2. Principal component analysis plot for the ectomycorrhizal fungi communities colonizing the roots and rhizosphere soil samples of *Abies koreana* and *Taxus cuspidata* collected from the lower and upper parts of Mt. Halla. Black triangles represent roots of *A. koreana,* and black squares represent *T. cuspidata* in the lower parts. White triangles represent soils of *A. koreana,* and white squares represent *T. cuspidata* in the lower parts. Black circles represent roots of *A. koreana,* and black rhombus represents *T. cuspidata* in the upper parts. White circles represent rhizosphere soils of *A. koreana,* and (white rhombus represents *T. cuspidata* in the upper parts.

the soil, the similarity index of the *A. koreana* root community was higher than that of the *T. cuspidata* root community, and the EMF community similarity in the rhizosphere soil of *A. koreana* was higher than that of *T. cuspidata* ($p < 0.05$) (Figure 4).

4. Discussion

Through molecular identification of the EMF root tips from *A. koreana* and *T. cuspidata* inhabiting Mt. Halla, it was reported that the genus *Clavulina* was dominant in the roots of *A. koreana* in both

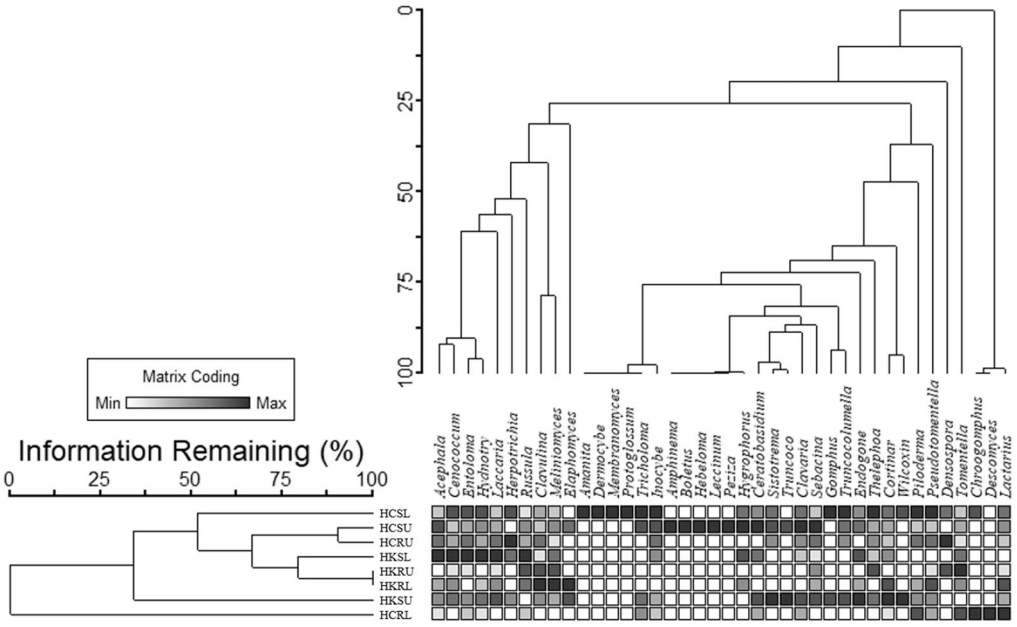

Figure 3. Two-way cluster analysis of ectomycorrhizal fungal communities isolated from roots and rhizosphere soils of *Abies koreana* and *Taxus cuspidata* in the lower and upper parts of Mt. Halla. HKSL: rhizosphere soils of *A. koreana* in the lower parts; HKSU: rhizosphere soils of *A. koreana* in the upper parts; HKRL: roots of *A. koreana* in the lower parts; HKRU: roots of *A. koreana* in the upper parts; HCSL: rhizosphere soils of *T. cuspidata* in the lower parts; HCSU: rhizosphere soils of *T. cuspidata* in the upper parts; HCRL: roots of *T. cuspidata* in the lower parts; HCRU: roots of *T. cuspidata* in the upper parts.

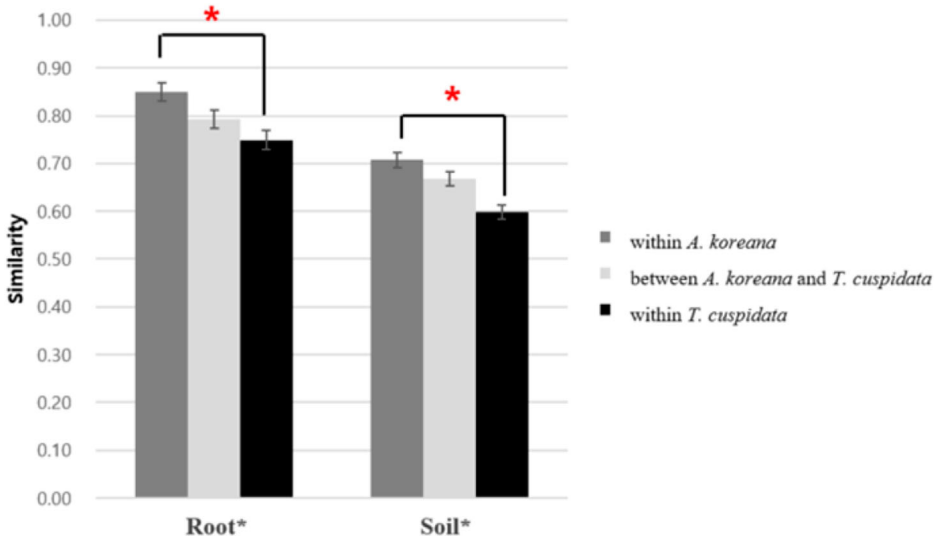

Figure 4. Similarity index of ectomycorrhizal fungal communities of roots and rhizosphere soil of *Abies koreana* and *Taxus cuspidata* collected on Mt. Halla and subjected to next-generation sequencing analysis. Significance is indicated by asterisks ($^*p < 0.05$).

upper and lower regions, and genus *Tomentella* was dominant in the roots of *T. cuspidata* [27]. *Clavulina* is widely distributed in temperate and tropical rainforests [28,29]. *Russula* contains approximately 750 species of mycorrhizal mushrooms, has a diverse host range, and is the most abundant and most widely distributed boreal EMF [30]. *Tomentella* is commonly found in broadleaved and coniferous forests, and it has been reported as a symbiotic EMF of gymnosperms and angiosperms, including orchid plants [31–33]. Kim et al. [34] analyzed the diversity of Ascomycota and Basidiomycota in the rhizosphere soil of *A. koreana* in Mt. Halla. They reported that species belonging to *Clavulina* accounted for more than 50% of the EMF recorded.

In this study, the Shannon's index of EMF in the roots and rhizosphere soil showed a tendency to be higher in the upper region than in the lower region, which is thought to be related to the increase in temperature at higher altitudes. As the temperature increases in the upper region, tree species that usually inhabit the upper region are declining, and growth decline and death have been observed in *A. koreana* in the lower regions. In Mt. Halla, it was reported that the number of *A. koreana* decreased

while the number of *T. cuspidata* increased [35]. This could be expected because *A. koreana* find it increasingly difficult to grow as climate change progresses because this species initiates cambium activity at a lower Winkler index than *T. cuspidata* [36]. Furthermore, it is known that *A. koreana* growth positively correlates with photosynthesis and water-use efficiency. Therefore, it can be estimated that the imbalance of water due to the increase in solar radiation and the lack of soil moisture during the spring drought period due to the rise in temperature caused by global warming has a negative effect on the growth of *A. koreana* [37,38].

EMF not only increases the network between host plants in forest ecosystems but also significantly contributes to the growth of host plants through the supply of nutrients and moisture [11]. However, since the fallen leaves and underground tubers of bamboo, which have the highest coverage in the central and lower parts of Mt. Halla, thickly cover the ground, the diversity of the lower vegetation is rapidly decreasing, and it has a negative effect on the seed germination and seedling growth of *A. koreana* [39]. In addition, the diversity of EMF has decreased because the lower region was more affected by climate change than the upper region, which is presumed to have affected the decrease in the number of host plants. However, since *A. koreana* in the upper region has a symbiotic relationship with various species of EMF, it is thought that the negative impact of climate change can be reduced. Furthermore, in *A. koreana*, two or three genera of EMF dominate regardless of altitude, whereas in *T. cuspidata,* there is a significant difference in species composition depending on the sample even at the same altitude, indicating a lower EMF specificity compared to that of *A. koreana*.

Russula is an EMF widely distributed in temperate forests [40]. Lee et al. [27] analyzed the EMF root tips of *A. koreana* and *T. cuspidata* from Mt. Halla and discovered *Russula favrei* only in *A. koreana.* In the results of Lee et al. [27], similar to the results of this study, the similarity between EMF communities of *A. koreana* roots was higher than that of EMF communities of *T. cuspidata* roots. This could be because *Clavulina* and *Russula* show strong dominance and high frequency in all samples of *A. koreana* in both the upper and lower regions. Although there is some difference depending on the altitude, it was found that the distribution of the EMF community is greatly affected by the host plant. Jarvis et al. [41] reported that there was no significant difference in the species richness of EMF according to altitude, but there was a substantial difference in the community structure of EMF. In the present study, the diversity of EMF in the lower and upper regions did not show a significant difference depending on the altitude, but there was a difference in the community structure, suggesting similarities with the results of the previous study. The community analysis results also confirmed that the range of EMF coexisting with *T. cuspidata* was wider than that of *A. koreana*. Therefore, the results showed that the EMF communities of *A. koreana* are more specific to this species than those of *T. cuspidata*. Hence, it can be concluded that *A. koreana* is highly dependent on EMF.

In response to recent global warming, alpine and sub-alpine ecosystems are undergoing significant changes. Previous research has predicted that drought stress and temperature increase due to warming will reduce coniferous forests and expand deciduous forests [42]. These will change the structure and species richness of EMF communities, but the nature of this response is difficult to predict. The sub-alpine region of Mt. Halla is relatively barren because of its poor soil layer and environmental conditions. The alpine plants inhabiting the region are highly dependent on symbiotic relationships with EMF [43]. Since *A. koreana* are highly sensitive to temperature and moisture stress because of the environmental characteristics of their native places, studies on the characteristics of symbiotic EMF and their community structures must precede the maintenance and preservation of *A. koreana*. The above results could serve as fundamental data for understanding the EMF community coexisting with *A. koreana* and restoring the *A. koreana* population. In subsequent studies, it is necessary to investigate the differences in the analyzed EMF community more closely and to monitor the trends over time. In addition, it is considered essential to analyze the EMF community through various methods from various samples considering various climatic factors, the physical environment of the soil, and the growth status of *A. koreana*.

Disclosure statement

No potential conflict of interest was reported by the authors.

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