



Diversity of Ectomycorrhizal Fungal Communities Associated with *Tuber koreanum* in Korea

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ABSTRACT

Ectomycorrhizal fungi (EMF) are crucial for the formation of *Tuber* fruiting bodies, including the newly discovered *T. koreanum*, in Korea. This study explores the diversity and distribution of EMF communities associated with *T. koreanum* across various regions in Korea and assesses the effects of soil physicochemical properties on these communities. Soil analysis indicated that *T. koreanum* habitats have a lower pH compared to *T. melanosporum* habitats documented in other studies, with sandy loam texture being optimal for fruiting body development. Nonmetric multidimensional scaling analysis revealed significant positive correlations between the relative abundances of certain EMF genera and exchangeable potassium and calcium. These findings offer valuable insights into the ecological requirements of *T. koreanum* and support future conservation and cultivation strategies for truffle species in Korea.

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1. Introduction

Truffles, members of the genus *Tuber*, are highly valued edible fungi that form distinctive underground fruiting bodies, known for their unique aroma and flavor [1]. These ectomycorrhizal fungi form symbiotic relationships with tree roots, primarily of those belonging to the Fagaceae (oak) and Pinaceae (pine) families [2].

Globally, truffles are considered a delicacy with significant culinary and economic value. The genus *Tuber* encompasses approximately 180–220 species [2], each with unique ecological preferences and distribution patterns [3]. For instance, *T. melanosporum* prefers alkaline soils while *T. japonicum* and *T. oregonense* thrive in acidic conditions. Many species also favor sandy loam soils each adapting to distinct soil chemical properties. These diverse ecological preferences influence the distribution and interactions of truffle species with their environment.

Studies on truffles in Korea are still underdeveloped. Historically, only *Tuber aestivum* var. *uncinatum* and *T. borchii* were documented [4, 5]. However, additional species, such as *T. huidongense* and *T. himalayense*, have been found recently [6, 7]. Particularly, the discovery of *T. koreanum* represents a noteworthy advancement in understanding the diversity of truffles in this region [8]. *T. koreanum* is characterized by the presence of white fruiting

bodies and its association with *Q. aliena*, a tree commonly found in Korean forests.

The formation of truffle fruiting bodies is a complex process influenced by both abiotic and biotic factors [9]. Abiotic factors include soil physicochemical properties, such as pH, nutrient content, and soil texture, whereas biotic factors involve interactions among various ectomycorrhizal fungal (EMF) species [10–13]. The optimal conditions for *T. koreanum* remain unclear, with initial studies indicating a preferred soil pH of 6 for mycelial growth in culture [14]. Because of the recent discovery of *T. koreanum*, knowledge regarding its ecological requirements, factors influencing its distribution, and its ecological niche are limited.

This study aimed to address these gaps by assessing the diversity and distribution of *T. koreanum*-associated EMF communities across two regions in Korea. Additionally, it assesses how soil physicochemical properties affect these fungal communities, thereby contributing to the essential knowledge required for the conservation and potential commercial cultivation of *Tuber* species in Korea. Understanding the diversity and structure of EMF communities associated with *T. koreanum* and the role of soil properties in shaping these communities can provide valuable insights into the ecological dynamics of this species, and our study is anticipated

to contribute to broader efforts to conserve and sustainably cultivate truffle species in Korea, aligning with global trends in truffle agriculture.

2. Materials and methods

2.1. Study sites and sample collection

The study was conducted in the two primary habitats of *Tuber koreanum* in Korea: Gyeongju and Uljin. These sites were selected based on the previous observations of *T. koreanum* fruiting bodies and their associations with *Q. aliena* trees. Sampling was conducted every three months from July 2021 to April 2022 to capture seasonal variations. At each site, the humus beneath the trees where *T. koreanum* fruiting bodies were observed was removed. A circular area with a diameter of 30 cm and depth of 20 cm was excavated around the base of each tree. One kg of soil and root samples were collected in polyethylene bags. To minimize contamination, tools were sterilized between the samples. Soil samples were sieved through a 2 mm mesh to remove debris and gravel and subsequently dried in a sterile environment for 24 h before further analysis. The roots were washed with tap water to remove surface soil (without damaging the mycorrhizae) and subsequently placed in Petri dishes for identification under a dissection microscope. Random root tips were placed in 2 mL e-tubes for DNA extraction.

2.2. Soil physicochemical analysis

The soil samples were sent to the Jeil Analysis Center (Seoul, Korea) for a comprehensive physicochemical analysis. The measured parameters included pH, total nitrogen, available phosphorus, exchangeable potassium (K) and calcium (Ca), organic matter content (%), moisture content, and sand, silt, and clay proportions.

2.3. Community analysis of ectomycorrhizal fungi

Root samples in 2-mL e-tubes were cleaned by adding sterile water and vortexing for 1 min. This process was repeated 4–5 times. The washed root samples were macerated using an Automil (Tokken Inc., Chiba, Japan) and homogenized. DNA was extracted using the HiGene™ Genomic DNA Prep Kit (BIOFACT, Daejeon, Korea) for root samples and the DNeasy PowerSoil Kit (Qiagen, Hilden, Germany) for soil samples, following the manufacturer's protocols. The internal transcribed spacer (ITS) region of ribosomal DNA, specifically ITS2, was amplified

using ITS3-Mi and ITS4-Mi primer sets [15]. The PCR conditions included an initial denaturation at 95°C for 2 min, followed by 35 denaturation cycles at 95°C for 20 s, annealing at 55°C for 40 s, and extension at 72°C for 1 min, with a final extension at 72°C for 5 min. The amplified products (2 µL) were loaded onto a 1.5% agarose gel and electrophoresed for 20 min to confirm the bands of 300–400 bp. Subsequently, the samples were sent to CJ Bioscience (Suwon, Korea) for NGS analysis (Illumina MiSeq). Raw sequence data were deposited on NCBI Sequence Read Archive (SRA) under Project ID PRJNA1179355. The raw sequence reads that were obtained from NGS were analyzed using EzBioCloud [16]. 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 [17]. Secondary analysis of the generated datasets was performed using the Microbiome Taxonomic Profiling software provided by CJ Bioscience Co. (Seoul, Korea). Only fungal sequences that were identified at the genus level obtained from NGS results were used for the analysis [18]. EMF communities were analyzed using the FUNGuild database [19] and references [20–22] to assign ecological guilds.

2.4. Statistical analysis

The relative abundance of fungal genera was calculated, and diversity indices (Shannon's index, evenness, and genera number) were computed using the MVSP 3.2 software (KCS, Pentraeth, UK). Nonmetric multidimensional scaling (NMS) was conducted using the PC-ORD 6.22 software (Wild Blueberry Media LLC, Corvallis, USA) to explore community similarity and environmental correlations.

3. Results and discussion

3.1. Soil properties

The average soil pH values over the four seasons were measured as 4.74 in Gyeongju and 4.46 in Uljin. The average total phosphorus (%) was 0.74 in Gyeongju and 0.78 in Uljin and available phosphate (mg/kg) was 318.2 in Gyeongju and 185.9 in Uljin. Exchangeable potassium (meq/100 g) was 0.48 in Gyeongju and 0.31 in Uljin, exchangeable calcium (meq/100 g) was 7.11 in Gyeongju and 3.09 in Uljin, and organic matter content (%) was 8.93 in Gyeongju and 11.21 in Uljin (Table 1). The average soil temperature was 13.3°C and 11.9°C in Gyeongju and Uljin, respectively, while the soil moisture content

Table 1. Soil chemical component values of *Tuber koreanum* habitats.

		pH	Total nitrogen (%)	Available phosphorus (mg/kg)	Exchangeable potassium (meq/100 g)	Exchangeable calcium (meq/100 g)	Organic matter (%)
Gyeongju	Mean	4.74	0.74	318.2	0.48	7.11	8.93
	(Min.-Max.)	(4.63–4.91)	(0.50–1.16)	(133.7–572.4)	(0.31–0.88)	(4.08–11.41)	(0.93–17.70)
Uljin	Mean	4.46	0.78	185.9	0.31	3.09	11.21
	(Min.-Max.)	(3.87–5.61)	(0.68–0.92)	(64.6–403.9)	(0.17–0.50)	(1.69–6.09)	(9.71–14.16)

Table 2. Soil physical component values of *Tuber koreanum* habitats.

		Soil temperature (°C)	Water contents (%)	Sand (%)	Silt (%)	Clay (%)	Soil texture
Gyeongju	Mean	13.3	20.4	67.7	18.0	14.3	Sandy loam
	(Min.-Max.)	(4.9–23.4)	(14.1–27.4)	(66.3–68.6)	(11.4–25.7)	(6.0–20.0)	
Uljin	Mean	11.9	22.11	65.3	16.5	18.2	Sandy loam
	(Min.-Max.)	(0.5–23.9)	(17.1–25.1)	(59.3–70.9)	(9.7–25.1)	(15.4–22.5)	

(%) was 20.4 and 22.11 in Gyeongju and Uljin, respectively. The sand (%) content was 67.7 in Gyeongju and 65.3 in Uljin, while silt (%) was 18.0 in Gyeongju and 16.5 in Uljin, and clay (%) was 14.3 in Gyeongju and 18.2 in Uljin. Both regions exhibited a sandy loam texture throughout the year (Table 2). Black truffle (*T. melanosporum*) prefers alkaline soils (pH \geq 7) for cultivation [9], whereas *T. koreanum* exhibits optimal mycelial growth at pH 6 in the culture medium. However, the soil pH in Gyeongju and Uljin, inhabited by *T. koreanum* was more acidic [14]. This aligns with the optimal soil pH of 5.3 for the Japanese white truffle (*T. japonicum*), which is more acidic than that for *T. himalayense* [23]. Similarly, white truffles in Oregon, USA (*T. oregonense* and *T. gibbosum*) thrive at a soil pH of 4.5–5.4 [24], whereas *T. thailandicum* in Thailand grows at a soil pH of 4.5 [25]. In this study, the soil texture was sandy loam that is optimal for the formation of tuber fruiting bodies [9].

3.2. Molecular identification and diversity analysis

A total of 580,733 reads were obtained from the Illumina MiSeq results, with 58,879 reads (7,789–20,195) from Gyeongju root samples, in addition to 180,911 reads (35,872–54,756) from Gyeongju soil samples, 124,682 reads (21,181–57,188) from Uljin root samples, and 216,261 reads (44,238–67,038) from Uljin soil samples. Of these, 466,716 reads identified at the genus level were used for analysis. The proportion of ectomycorrhizal fungi among the total fungi in the Gyeongju root and soil samples was 60.33 and 5.52%, respectively, while that in the Uljin root and soil samples was 86.67 and 7.23%, respectively. Across both regions, 45 genera of ectomycorrhizal fungi were identified, with 19, 19, 38, and 33 genera in Gyeongju roots, Uljin roots, Gyeongju soils, and Uljin soils, respectively (Figures

1 and 2). *Scleroderma* exhibited the highest relative abundance in Gyeongju root samples year-round, specifically in autumn and winter, when the fruiting bodies were formed [26]. *Scleroderma* is a common ectomycorrhizal genus in *Tuber* study areas and is associated with tuber fruiting body formation. Uljin roots exhibited a high relative abundance of *Melanogaster*—a genus commonly observed in *Tuber* study areas—in summer, winter, and spring [26]. *Tomentella* and *Pseudotomentella* were identified in both the Gyeongju and Uljin root and soil samples, aligning with previous records from various *Tuber* study areas [26].

3.3. Fungal community structure and soil factors

All three diversity indices (Shannon's index, genus evenness, and genera number) were higher in the soil samples than that in the root samples (Table 3). Shannon's index and genus evenness were higher in Uljin roots than that in Gyeongju roots, except in summer. All three diversity indices were higher in the soil samples from Gyeongju than in those from in Uljin. Shannon's index in Gyeongju root samples was the highest in summer and lowest in winter, whereas in Uljin, it was the highest in winter. Shannon's index was the lowest in spring in both regions. These findings align with studies on the diversity of EMF that are symbiotic with *Quercus mongolica* in Korea [27].

Analyzing the relationship between soil environmental factors and genera with relative abundance over 0.5% using NMS community composition indicated a closer association by region and DNA extraction source rather than by season (Figure 3). The relative abundances of *Astraeus*, *Boletus*, *Tylopilus*, *Scleroderma*, and *Sebacina* exhibited positive correlations with exchangeable potassium and calcium. This aligns with the findings of studies on ectomycorrhizal fungi in limestone areas in Korea,

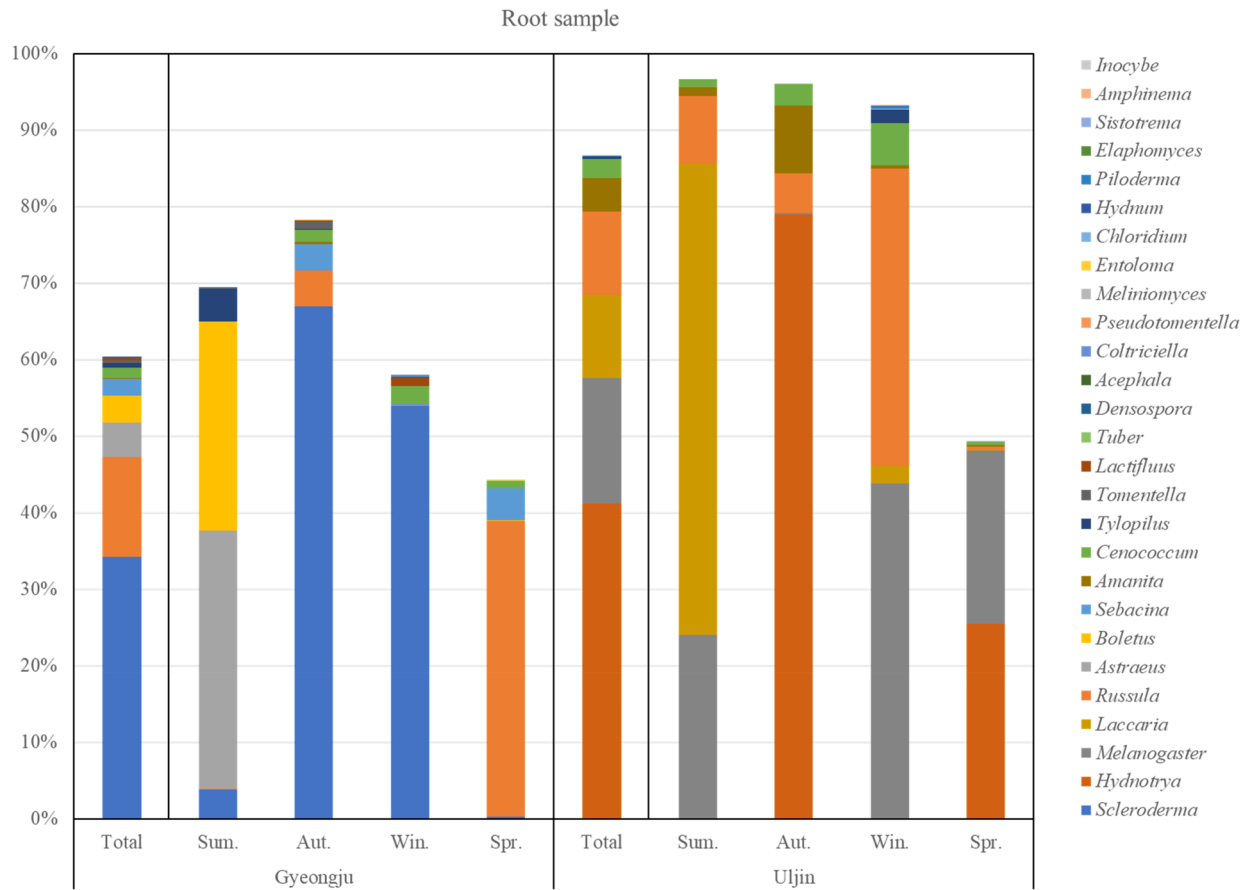


Figure 1. Ectomycorrhizal fungal composition and relative abundance based on season in the root samples (Sum.: summer, Aut.: autumn, Win.: winter, and Spr.: spring).

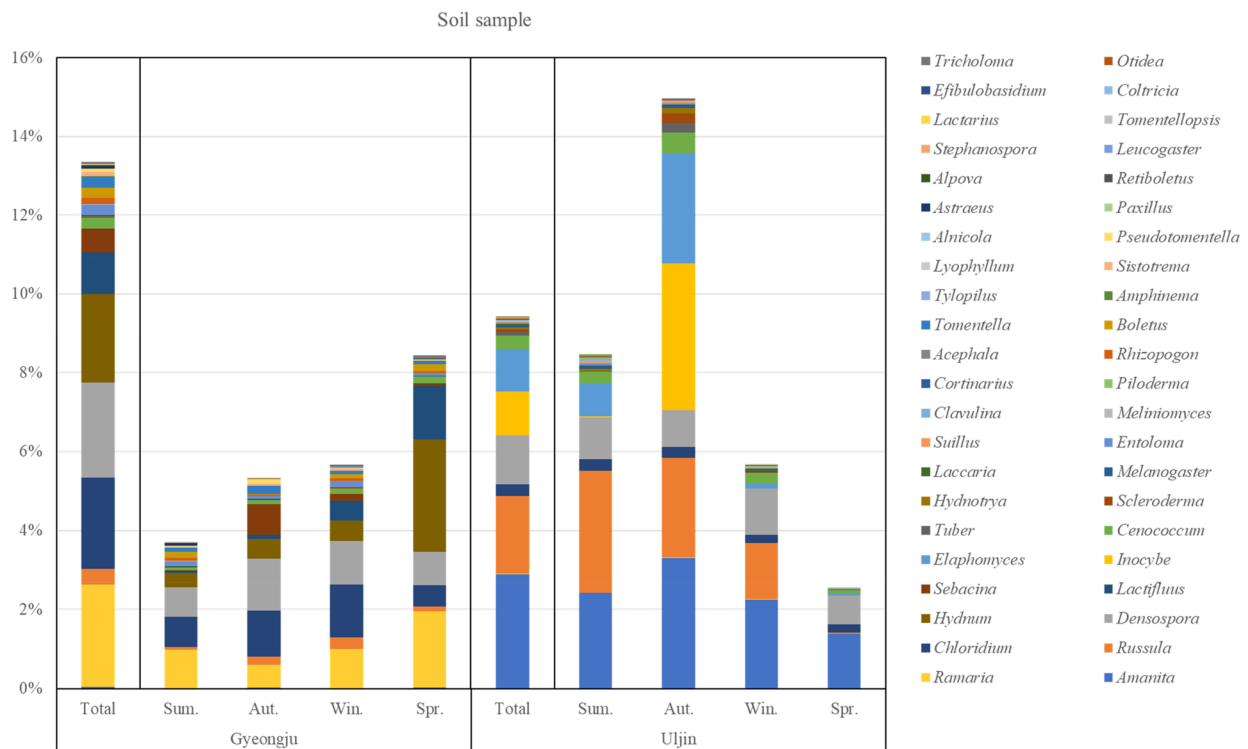


Figure 2. Ectomycorrhizal fungal composition and relative abundance based on season in the soil samples (Sum.: summer, Aut.: autumn, Win.: winter, and Spr.: spring).

Table 3. Alpha diversity indices based on season.

Site	Season	Gyeongju				Uljin			
		Sum.	Aut.	Win.	Spr.	Sum.	Aut.	Win.	Spr.
Shannon's index	Root	1.072	0.618	0.317	0.499	0.956	0.667	1.118	0.819
	Soil	2.178	2.155	2.208	1.911	1.766	1.945	1.633	1.267
Genus evenness	Root	0.488	0.258	0.132	0.18	0.435	0.26	0.424	0.342
	Soil	0.623	0.687	0.695	0.601	0.536	0.59	0.501	0.447
Genera number	Root	9	11	11	16	9	13	14	11
	Soil	33	23	24	24	27	27	26	17

(Sum.: summer, Aut.: autumn, Win.: winter, Spr.: spring).

**Figure 3.** NMS coordination plot using Sørensen distance measure of ectomycorrhizal fungal communities (ECa: exchangeable calcium, EK: exchangeable potassium; GJ: Gyeongju, UJ: Uljin; Sum.: summer, Aut.: autumn, Win.: winter, Spr.: spring; R.: root, S.: soil).

where *Sebacina* exhibited positive correlations with exchangeable calcium [28]. In contrast to previous studies where *Tuber* exhibited positive correlations with pH and exchangeable calcium, this study exhibited no significant correlation, likely because it focused solely on *T. koreanum* habitats.

In this study, we found that *T. koreanum* thrives in acidic soil conditions, in contrast to black truffles including *T. melanosporum*, which prefer alkaline soils [9]. Additionally, the dominance of EMF genera such as *Scleroderma* and *Melanogaster* in *T. koreanum* habitats, along with their positive correlation with potassium and calcium levels, underscores the importance of these soil nutrients in supporting

fungal diversity and fruiting body formation [26]. *Scleroderma*, a genus commonly associated with truffle formation, further supports its critical role in truffle ecosystems. These findings highlight key ecological relationships between *T. koreanum* and EMF species, as well as the role of soil conditions in shaping fungal community structure. Furthermore, this study contributes to a growing body of studies on truffle ecology beyond *T. melanosporum*, emphasizing the diversity of truffle species and their unique ecological niches.

While this study provides valuable insights, it was conducted in only two primary habitats of *T. koreanum*, which may not fully capture the range of



environmental conditions in which this species thrives. The limited study locations are due to the fact that *T. koreanum* has been found in very few sites to date, with the most recent discovery made by authors in Mungyeong, Gyeongbuk. As *T. koreanum* is identified in more regions, expanding the geographic scope of future studies will provide a more comprehensive understanding of its distribution. Additionally, the study was conducted over a relatively short period, and although seasonal variations were captured, longer-term studies could provide more robust data on the dynamics of EMF communities over multiple years.

Future studies should explore additional *T. koreanum* habitats across Korea, as well as the effects of soil manipulation on fungal communities. Long-term monitoring and metagenomic sequencing could provide more detailed insights into the fungal dynamics that support *T. koreanum* growth. These findings provide essential knowledge for the conservation of *T. koreanum* and contribute to a broader understanding of the ecological dynamics that support truffle species in Korea, particularly highlighting the importance of soil properties and fungal community composition.

Disclosure statement

The authors declare that there are no conflicts of interest.

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