



## Guild Patterns of Basidiomycetes Community Associated With *Quercus mongolica* in Mt. Jeombong, Republic of Korea

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### ABSTRACT

Depending on the mode of nutrition exploitation, major fungal guilds are distinguished as ectomycorrhizal and saprotrophic fungi. It is generally known that diverse environmental factors influence fungal communities; however, it is unclear how fungal communities respond differently to environment factors depend on fungal guilds. In this study, we investigated basidiomycetes communities associated with *Quercus mongolica* using 454 pyrosequencing. We attempted to detect guild pattern (ectomycorrhizal or saprotrophic fungal communities) by comparing the influence of geography and source (root and surrounding soil). A total of 515 mOTUs were detected from root (321) and soil (394) of *Q. mongolica* at three sites of Mt. Jeombong in Inje County. We found that patterns of diversity and community structure were different depending on the guilds. In terms of alpha diversity, only ectomycorrhizal fungi showed significant differences between sources. In terms of community structure, however, geography significantly influenced the ectomycorrhizal community, while source appeared to have a greater influence on the saprotrophic community. Therefore, a guild-based view will help to elucidates novel features of the relationship between environmental factors and fungal communities.

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### 1. Introduction

Fungal communities play several important ecological roles in forest ecosystems, especially through their interactions with plants. These interactions, which can be commensal, mutualistic, and pathogenic, can profoundly influence plants in different ways, especially with respect to growth and general health [1,2]. Fungal guilds are distinguished from communities in that guilds are defined by similarities in resource exploitation [3]. Ectomycorrhizal fungi (EMF) and saprotrophic fungi (SPF) are especially important fungal guilds because they are key contributors to forest soil ecosystems. EMF and plants have a mutualistic relationship, with the fungus providing inorganic nutrients such as phosphorous (and often indirectly facilitating the transfer of nitrogen *via* symbiotic bacteria) in exchange for fixed carbon compounds (e.g. carbohydrates) [4]. SPF, on the other hand, decompose wood and litter, returning available nutrients to the soil [5]. Most plant–fungal interaction studies have focused on EMF; however, the importance of SPF in plant physiology through nutrient turn-over in soil may be equally important [6]. A better understanding of

the diversity and distribution of fungal communities within both EMF and SPF guilds will improve our general understanding of forest ecology.

Fungal community structure is affected by both abiotic and biotic factors. Many abiotic factors that influence fungal community structure (e.g. temperature, climate, and soil physiochemistry) are associated with geographical variation [7–9]. Biotic factors, such as competition among fungal species and host plant physiology, are also important in shaping fungal community structure, especially in EMF [9]. While the factors that influence forest fungal communities are extremely complex, important insights can be gained by focusing on differences among and between fungal guilds. For example, EMF species appear to be more heavily influenced by host availability than SPF species. In addition, a recent study demonstrated that plant diversity differentially effects fungal guilds (EMF and SPF) [10]. In general, however, little is known about guild patterns within fungal communities because most studies have focused on a single guild.

Oak trees (*Quercus* spp.) provide important ecological services to forest ecosystems [11] and are an

important source of wood and biocompounds for humans [12], which environmental and economic importance of *Quercus* species led to several studies that focused on associated fungal communities [13–19]. Most of these studies, however, focused only on EMF communities, and other fungal guilds associated with *Quercus* remain poorly understood. *Quercus mongolica* (Mongolian oak) is common in northeastern Asian forests, and is the dominant deciduous tree in forests in Korea, China, Japan, and Russia [20]. Fungal communities associated with *Q. mongolica* have previously been investigated using high throughput sequencing of soil samples [21,22]. However, a recent study showed little overlap between soil and root fungal communities (just 27% of OTUs in soil community) [23]. This result suggests that the soil fungal community may not adequately represent the overall fungal community associated with *Q. mongolica*, and that both root and soil samples should be sampled in order to fully understand fungal guilds associated with *Q. mongolica*.

In this study, we investigated guild related community patterns associated with *Q. mongolica* with samples from both the root system and surrounding soil using 454 pyrosequencing. We focused on the basidiomycetes community because of their relative importance in forest ecology as both EMF and SPF [24]. This strategy also has the advantage of increasing sequencing depth, thus improving the chances of discovering rare taxa. Describing the composition of key fungal guilds of *Q. mongolica* will increase our understanding of fungal–plant interactions and inform oak forest management and conservation.

## 2. Materials and methods

### 2.1. Sampling site information and sample collection

We collected soil and root samples of *Q. mongolica* at Mt. Jeombong in Inje, Republic of Korea (38° 2' 11"N, 128° 28' 21"E). This mountain is in the southern area of Seorak-san National Park which has been designated by Biosphere Reserves as a part of the Man and Biosphere Project of the United Nations Educational, Scientific and Cultural Organization (UNESCO) [25]. The well conserved forest of Mt. Jeombong is a mixed deciduous forest composed primarily of *Acer*, *Carpinus*, *Fraxinus*, *Quercus*, *Abies*, and *Pinus* tree species [26]. Access to Mt. Jeombong is restricted, thus basidiomycetes communities associated with *Q. mongolica* are relatively intact and free of human disturbance. We choose three forest patches (50 m × 50 m) where *Q. mongolica* was dominant and separated by c.a. 5 km. Sampling was conducted twice, once in dry season

in spring (May) and again in high humidity and hot season in late summer (August), 2014. Six trees were selected randomly (more than 5 m interval) in each patch for soil and root collection. After removing litter layers, we collected soil samples under the tree from the top soil where lateral root existed. We also collected samples of lateral roots (more than 30 cm length) connected with ectomycorrhizae. Sampling was permitted by the National Park Authority. A total of 36 pairs of soil and root samples were collected and stored at 4 °C prior to DNA extraction.

### 2.2. DNA extraction, PCR amplification, and pyrosequencing

Genomic DNA was extracted in triplicate from 250 mg of soil sample using the PowerSoil DNA isolation kit (MoBio, Carlsbad, CA, USA) following the manufacturer's instructions. Roots were rinsed carefully with distilled water until all soil and debris were removed before DNA extraction. After drying, lateral roots were divided into 10 sections (c.a. 2 cm) and ground using a mortar and pestle with 2 ml of CTAB buffer. Then genomic DNA was extracted using a modified CTAB method [27].

We used the basidiomycetes specific primer set ITS1F and ITS4B [28] for primary PCR amplification. Sequencing adapters and multiple index delimitation (MID) sequences were attached to ITS1F and ITS4 primers and a second PCR was carried out for pyrosequencing. PCR amplifications were performed using C1000 thermal cycler (Bio-Rad, Richmond, CA, USA) under the following conditions: 94 °C for 5 min, followed by 30 cycles of 94 °C for 30 s, 55 °C for 30 s, 72 °C for 40 s, and 72 °C for 5 min for final extension. PCR products were checked using 1% agarose gel electrophoresis and purified using the Expin PCR Purification Kit (GeneAll, Korea). Pyrosequencing was conducted using the 454 GS FLX plus platform at Macrogen (Seoul, Korea) in the reverse direction. Sequence data were deposited in the NCBI Sequence Read Archive (SRA) (PRJNA362705).

### 2.3. Bioinformatic analysis

Raw data from pyrosequencing were filtered using QIIME v1.8.0 [29]. Molecular operational taxonomic units (mOTUs) were clustered (98.5% similarity and single linkage methods), and chimeric sequences were filtered using Usearch 5.2.236 [30] in QIIME. In each mOTU, the most abundant sequence was chosen as a representative, which were then used for taxonomic assignment using BLAST against the UNITE v7.1 (22.08.2016) database [31] as well as sequences from the Seoul National University Fungal Collection. We assigned mOTUs to the

genus level. After removing non-fungal and singleton mOTUs, we rarified the number of sequences to the minimum reads number for further analysis. Alpha diversity indices and community distance (Bray–Curtis) were calculated in QIIME. Further statistical tests (ANOVA, and Constrained Analysis of Principal Coordinates [CAP]) were conducted in R [32], and graphical plots (boxplot, barplot, PCoA plot) were drawn using ggplot2 [33] and phyloseq [34] packages in R. Fungal guild was determined using FUNGuild [35], and classified as EMF or SPF. When mOTUs were not classified to EMF or SPF by FUNGuild, we attempted classification refinement by BLAST searching sequences in GenBank. If the BLAST search matched “ectomycorrhizal fungi” with high sequence similarity (>95%), we classified the mOTUs as EMF.

### 3. Results

#### 3.1. Basidiomycetes diversity and guild classification associated with *Q. mongolica*

We obtained 107,336 sequence reads, and used 90,408 reads for analysis after filtering. In order to normalize the data, we rarified the sequences of all samples to 420 reads. Five samples (three root and two soil samples) were discarded from the analysis because of the low number of reads. All samples exhibited high Good's coverage (0.96–1.00), thus, we determined that the sequencing effort was sufficient for further analysis. All mOTUs belonged to Agaricomycetes except *Cryptococcus* (Tremellomycetes). Among 515 mOTUs detected in this study, 473 mOTU were classified into 53 basidiomycetes genera (two classes, 15 orders, and 30 families) and the other 42 mOTU were not classified. Three hundred and twenty-one mOTUs and 394 mOTUs were found in root and soil, respectively.

FUNGuild classified 368 mOTUs (31 genera) as EMF and 104 mOTUs (21 genera) as SPF. In terms of relative abundance, the major EMF genera found in both sample types (soil and root) were *Sebacina* (28.9%), *Russula* (12.7%), *Tomentella* (7.5%), *Amphinema* (4.2%), *Cortinarius* (3.5%), and *Amanita* (3.4%) (Table 1). *Cryptococcus* (6.2%) was the most abundant SPF genus found in both sample types, followed by *Mycena* (4.9%), *Marasmiellus* (2.6%), *Marasmius* (1.8%), *Galerina* (1.4%), and *Trechispora* (1.4%). In both root and soil samples, the proportion of EMF was higher than that of SPF, while there was no difference in guild proportion depending on sampling site and season (Table 1). In a comparison of all basidiomycetes communities, 33 genera were found in both sample sources, while eight genera were found only in root samples

and 12 genera were found only in soil (Table 1). Among the three sampling sites and both guilds, 15 genera were commonly found in all root communities, and considered as core genera (Table 1; Figure 1) and, based on relative abundance, *Sebacina*, *Russula*, *Tomentella*, *Cryptococcus*, and *Mycena* were the major core genera associated with *Q. mongolica* (Table 1). Most genera were overlapped together between spring and summer (Table 1). *Hygrophorus* was the only major EMF genus found exclusively in one type of sample source and season (soil in summer season, Table 1).

Among the 15 core genera, nine were EMF and six were SPF. In root samples, the major EMF genus was *Sebacina*, which was more than twice as abundant as the next most common genus, *Russula* (relative abundance: 25.5% and 10.4%, respectively). The next most common EMF genera found in root samples were *Tomentella* (6.6%) and *Cortinarius* (3.9%). In soil samples, *Sebacina* was also dominant, and more than twice as abundant, as *Russula* (relative abundance: 32.1% and 14.8%, respectively). The next most common EMF genera found in soil samples were *Tomentella* (8.3%), *Amphinema* (5.9%), and *Amanita* (5.5%) (Table 1). In terms of presence frequency, the core EMF was well represented in both root and soil samples with similar presence frequency (Figure 1).

Of the six major SPF genera, all were detected in root samples, and 50% were detected in both soil and root samples. *Mycena* and *Marasmiellus* were the dominant SPF genera in root samples (relative abundance: 9.2% and 5.3%, respectively), followed by *Marasmius* (3.7%), *Cryptococcus* (3.1%), *Galerina* (2.9%), and *Trechispora* (2.5%). In soil, *Cryptococcus* was by far the most abundant genus with a relative abundance (9.2%) of more than an order of magnitude higher than the other two genera, *Mycena* and *Trechispora* (0.6% and 0.5%, respectively) (Table 1). With the exception of *Trechispora*, core SPF genera did not exhibit consistent presence frequency between the root and soil samples. *Athelopsis*, *Mrasmiellus*, *Mycena*, and *Scopuloides* were more frequently detected in roots, while *Cryptococcus* was more common in soil samples (Figure 1).

#### 3.2. Analysis of diversity and community structure

The number of mOTUs ( $p = .003$ ), chao1 richness ( $p = .016$ ), and Shannon's diversity ( $p = .031$ ) were significantly higher in the soil than in root samples, Shannon's evenness was not significant ( $p = .249$ ). No indices were significantly different between root and soil samples with the exception of Shannon's diversity between sampling season ( $p = .031$ ). When we separated the fungal communities by guild,

**Table 1.** Relative abundance<sup>a</sup> (%) and mOTU number of basidiomycetes genera (53 genera, 515 mOTU) associated with *Quercus mongolica*.

Genus	Total	mOTU no.	Guild	Source <sup>b</sup>		Site <sup>c</sup>						Season <sup>c</sup>				
				R	S	R1	S1	R2	S2	R3	S3	M-R	M-S	A-R	A-S	
<i>Agaricomycetes</i>																
<i>Amanita</i> *	3.4	26	EMF	1.2 (14)	5.5 (23)	0.1	0.1	0.9	3.4	2.4	12.7	0.4	1.0	1.8	10.6	
<i>Amphinema</i>	4.2	7	EMF	2.5 (2)	5.9 (7)	0.0	0.0	0.0	0.0	7.0	16.7	0.2	4.9	4.8	7.0	
<i>Astraeus</i>	1.2	1	EMF	2.0 (1)	0.4 (1)	0.0	<0.1	0.0	0.0	5.5	1.2	1.2	0.0	2.7	0.9	
<i>Boletus</i> *	0.8	9	EMF	0.4 (6)	1.2 (9)	0.2	0.3	1.1	3.4	0.1	0.3	0.5	2.1	0.4	0.3	
<i>Byssocorticium</i>	0.4	3	EMF	0.2 (2)	0.6 (3)	0.6	<0.1	0.0	1.9	0.0	0.0	0.0	1.0	0.4	<0.1	
<i>Ceratobasidium</i>	<0.1	1	EMF	<0.1 (1)	0.0 (0)	0.0	0.0	<0.1	0.0	0.0	0.0	<0.1	0.0	0.0	0.0	
<i>Chroogomphus</i>	<0.1	1	EMF	0.0 (0)	<0.1 (1)	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.1	0.0	0.0	
<i>Clavulina</i> *	0.9	11	EMF	0.9 (6)	1.0 (7)	0.2	0.0	2.6	0.3	0.2	2.5	1.2	0.2	0.7	1.9	
<i>Cortinarius</i>	3.5	20	EMF	3.9 (10)	3.2 (16)	0.1	0.8	12.7	9.9	0.0	0.0	2.5	4.9	5.2	1.3	
<i>Entoloma</i> *	<0.1	3	EMF	<0.1 (2)	<0.1 (2)	<0.1	0.0	<0.1	0.1	<0.1	0.0	<0.1	0.1	<0.1	<0.1	
<i>Hydnellum</i>	<0.1	3	EMF	0.1 (3)	0.0 (0)	0.0	0.0	0.0	0.0	0.2	0.0	0.1	0.0	<0.1	0.0	
<i>Hygrophorus</i>	1.1	6	EMF	<0.1 (1)	2.1 (6)	0.0	0.2	<0.1	4.5	0.0	2.0	<0.1	0.0	0.0	4.4	
<i>Inocybe</i>	1.4	9	EMF	1.0 (2)	1.8 (8)	<0.1	4.5	0.0	0.0	2.8	0.5	2.1	3.0	0.0	0.3	
<i>Laccaria</i>	1.2	5	EMF	1.3 (3)	1.0 (5)	3.8	0.0	0.0	2.6	0.0	0.7	2.6	1.5	0.0	0.6	
<i>Lactarius</i> *	0.6	6	EMF	0.5 (5)	0.6 (4)	0.7	<0.1	0.8	2.0	0.1	0.1	0.6	0.3	0.4	1.0	
<i>Lactifluus</i>	2.4	4	EMF	1.3 (2)	3.5 (4)	0.0	0.3	2.3	8.8	1.6	2.2	0.3	1.8	2.2	5.4	
<i>Leccinum</i>	0.1	2	EMF	0.0 (0)	0.3 (2)	0.0	0.0	0.0	0.2	0.0	0.7	0.0	0.5	0.0	0.1	
<i>Phylloporus</i>	<0.1	1	EMF	0.0 (0)	<0.1 (1)	0.0	0.1	0.0	0.0	0.0	0.0	0.0	<0.1	0.0	<0.1	
<i>Piloderma</i> *	2.1	6	EMF	1.1 (4)	3.0 (6)	0.9	1.3	0.1	3.0	2.0	4.7	1.9	5.1	0.3	0.7	
<i>Protoglossum</i>	<0.1	1	EMF	0.0 (0)	<0.1 (1)	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.1	
<i>Ramaria</i>	0.1	2	EMF	0.0 (0)	0.1 (2)	0.0	0.0	0.0	0.0	0.0	0.3	0.0	0.2	0.0	0.0	
<i>Rhizopogon</i>	0.1	3	EMF	<0.1 (1)	0.2 (2)	0.0	0.0	0.0	0.0	<0.1	0.7	<0.1	0.4	0.0	0.0	
<i>Russula</i> *	12.7	75	EMF	10.4 (51)	14.8 (68)	10.0	15.6	9.6	22.5	11.4	7.7	7.6	20.0	13.1	9.1	
<i>Sebacina</i> *	28.9	87	EMF	25.5 (50)	32.1 (74)	45.3	53.6	17.4	21.2	14.2	19.6	20.6	32.1	30.2	32.1	
<i>Sistotrema</i>	0.1	4	EMF	0.0 (0)	0.2 (4)	0.0	<0.1	0.0	0.0	0.0	0.6	0.0	0.1	0.0	0.4	
<i>Suillus</i>	0.4	4	EMF	0.6 (3)	0.3 (2)	0.0	0.0	0.0	0.0	1.7	0.7	1.1	0.0	0.1	0.6	
<i>Tomentella</i> *	7.5	59	EMF	6.6 (37)	8.3 (48)	6.9	8.5	7.9	4.5	5.3	11.3	4.6	5.9	8.5	11.0	
<i>Tomentellopsis</i>	<0.1	1	EMF	<0.1 (1)	<0.1 (1)	<0.1	<0.1	0.0	0.0	0.0	0.0	0.0	0.0	<0.1	<0.1	
<i>Tricholoma</i>	1.0	6	EMF	1.9 (5)	0.1 (3)	3.8	<0.1	2.0	0.0	0.0	0.2	0.1	0.2	3.6	<0.1	
<i>Tyloporus</i>	0.4	1	EMF	0.8 (1)	<0.1 (1)	2.4	0.1	0.0	0.0	0.0	0.0	0.0	0.1	1.6	<0.1	
<i>Tylospora</i>	<0.1	1	EMF	0.1 (1)	<0.1 (1)	0.0	0.0	0.0	0.0	0.2	0.1	0.0	<0.1	0.1	<0.1	
<i>Agaricus</i>	<0.1	1	SPF	<0.1 (1)	<0.1 (1)	0.0	0.0	0.0	0.0	0.1	<0.1	0.1	<0.1	0.0	0.0	
<i>Athelia</i>	<0.1	2	SPF	<0.1 (2)	<0.1 (1)	0.1	<0.1	<0.1	0.0	0.0	0.0	<0.1	0.0	<0.1	<0.1	
<i>Athellopsis</i> *	0.1	2	SPF	0.1 (2)	<0.1 (2)	<0.1	<0.1	0.2	0.1	0.2	<0.1	0.2	<0.1	0.1	0.1	
<i>Auricularia</i>	0.1	2	SPF	0.3 (2)	0.0 (0)	0.0	0.0	0.0	0.0	0.8	0.0	0.5	0.0	0.1	0.0	
<i>Clitocybe</i>	<0.1	1	SPF	0.0 (0)	<0.1 (1)	0.0	0.0	0.0	0.0	0.0	<0.1	0.0	<0.1	0.0	0.0	
<i>Clitocybula</i>	<0.1	1	SPF	0.1 (1)	<0.1 (1)	0.0	0.0	0.2	<0.1	0.0	0.0	0.1	<0.1	<0.1	0.0	
<i>Clitopilus</i>	<0.1	2	SPF	0.0 (0)	<0.1 (2)	0.0	0.0	0.0	<0.1	0.0	<0.1	0.0	0.1	0.0	0.0	
<i>Delicatula</i>	0.1	1	SPF	0.1 (1)	0.0 (0)	0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.2	0.0	
<i>Galerina</i>	1.4	5	SPF	2.9 (5)	0.0 (0)	0.0	0.0	9.5	0.0	0.0	0.0	3.8	0.0	2.0	0.0	
<i>Ganoderma</i>	<0.1	1	SPF	0.0 (0)	0.1 (1)	0.0	0.1	0.0	<0.1	0.0	0.2	0.0	0.1	0.0	<0.1	
<i>Gymnopilus</i>	0.1	1	SPF	0.1 (1)	0.0 (0)	0.0	0.0	0.0	0.0	0.3	0.0	0.2	0.0	0.0	0.0	
<i>Leucoagaricus</i>	0.1	3	SPF	<0.1 (1)	0.1 (2)	0.0	0.0	0.0	0.0	<0.1	0.4	0.0	0.1	<0.1	0.2	
<i>Lycoperdon</i>	<0.1	2	SPF	0.0 (0)	0.1 (2)	0.0	0.1	0.0	0.0	0.0	<0.1	0.0	<0.1	0.0	0.1	
<i>Marasmiellus</i> *	2.6	2	SPF	5.3 (2)	0.0 (0)	0.9	0.0	3.6	0.0	10.6	0.0	7.4	0.0	3.2	0.0	
<i>Marasmius</i>	1.8	1	SPF	3.7 (1)	<0.1 (1)	0.0	0.0	6.8	<0.1	4.5	<0.1	5.3	<0.1	2.2	<0.1	
<i>Mycena</i> *	4.9	34	SPF	9.2 (32)	0.6 (9)	15.2	<0.1	1.7	0.1	10.0	1.5	8.0	1.0	10.4	0.1	
<i>Onnia</i>	<0.1	1	SPF	0.0 (0)	<0.1 (1)	0.0	0.0	0.0	0.0	0.0	0.1	0.0	<0.1	0.0	0.0	
<i>Scopuloides</i> *	0.3	2	SPF	0.4 (1)	0.2 (2)	0.9	0.4	0.2	0.1	<0.1	0.0	<0.1	0.1	0.7	0.3	
<i>Sphaerobolus</i>	1.5	2	SPF	0.9 (2)	0.0 (0)	0.0	0.0	3.0	0.0	0.0	0.0	1.9	0.0	0.0	0.0	
<i>Trechispora</i> *	0.4	12	SPF	2.5 (8)	0.5 (9)	<0.1	0.6	0.3	0.5	6.5	0.3	2.8	0.3	2.2	0.7	
<i>Asterophora</i>	<0.1	1	Parasitic	0.0 (0)	<0.1 (1)	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.1	
Unidentified	5.8	42	Unknown	8.9 (30)	2.8 (26)	4.3	0.5	11.8	6.6	10.8	2.1	15.7	1.6	2.5	4.2	
<i>Tremellomycetes</i>																
<i>Cryptococcus</i> *	6.2	26	SPF	3.1 (15)	9.2 (20)	3.4	12.8	5.3	4.3	1.1	9.5	6.3	11.4	0.2	6.6	

<sup>a</sup>Relative abundance represents proportion of sequence number of genus divided by total sequence number of each categories.

<sup>b</sup>The number in parenthesis represents number of mOTU.

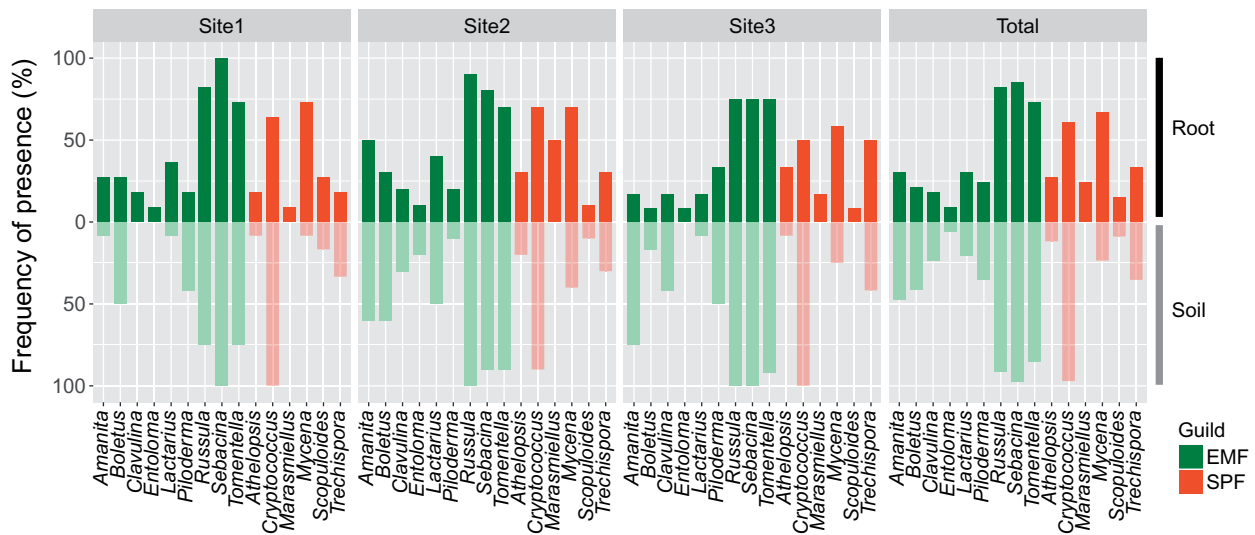
<sup>c</sup>R, root; S, soil; M, May; A, August.

Asterisk represents core genera associated with *Quercus mongolica*.

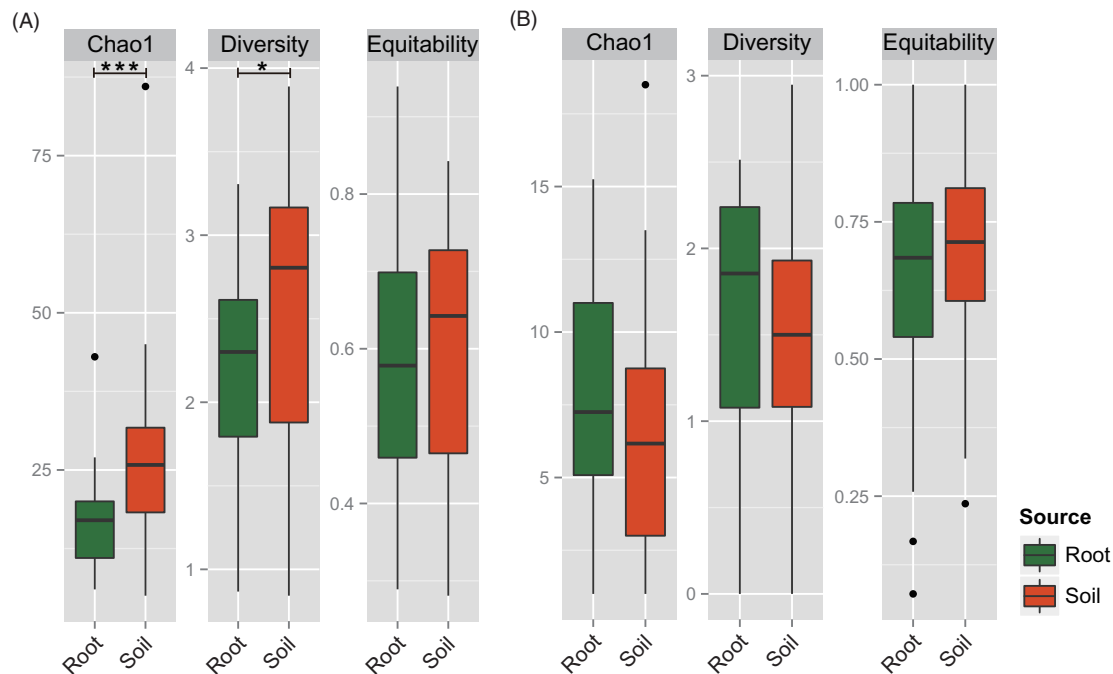
we found guild specific difference in alpha diversity. In EMF communities, indices of richness (chao1) ( $p < .001$ ) and diversity (Shannon) ( $p = .027$ ) were significantly higher in soil than in root samples, while evenness was not significantly different ( $p = .773$ ) (Figure 2(A)). However, none of the diversity indices in EMF communities were significantly different by sampling site and season. In SPF communities, none of the diversity was different in

any category (source, sampling site, and season) (Figure 2(B)).

In the ordination analysis (CAP) based on the Bray–Curtis distance, community structure showed clustering by categories. Sample source ( $p = .001$ ; 2.64% explanatory power) and sampling site ( $p = .001$ ; 7.65% explanatory power) significantly affected the community structure, while sampling season was not significant ( $p = .306$ ) (Figure 3).



**Figure 1.** Frequency of 15 core basidiomycetes genera found in the root and soil.

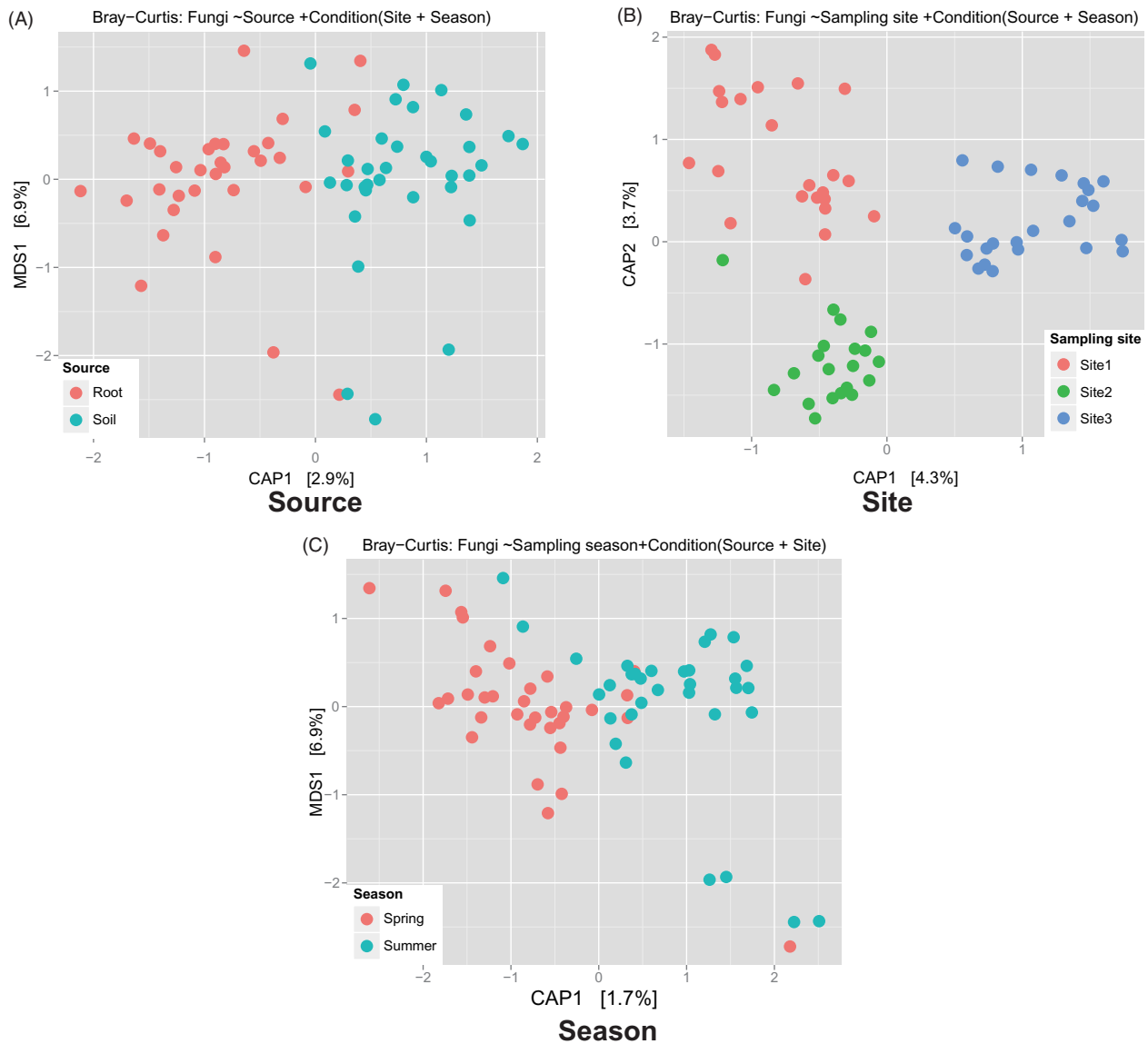


**Figure 2.** Alpha diversity of (A) EMF and (B) SPF community in the root and soil of *Quercus mongolica*. Significance of difference was tested using Wilcoxon test (\*:  $p < .05$ , \*\*\*:  $p < .001$ ).

Similar to alpha diversity, guild specific community structure pattern was also different between EMF and SPF. For EMF, sampling site significantly affected only community structure ( $p = .001$ ; 7.88% explanatory power), while sample source ( $p = .446$ ) and sampling season were not significant ( $p = .437$ ) (Figure 4(A)). In SPF communities, however, both sample source ( $p = .001$ ; 11.1% explanatory power) and sampling site ( $p = .001$ ; 5.60% explanatory power) were significantly different, while sampling season was not ( $p = .153$ ) (Figure 4(B)).

Pairwise beta diversities based on the Bray–Curtis distance were compared within and between sources (root and soil) using Kruskal–Wallis test (Figure 5). Within sources, both of EMF and SPF showed

significantly different beta diversities between the sources ( $p < .001$ ) with high community difference within the root. However, SPF showed significantly low beta diversities within the soil (Figure 5(A)). Between sources, we compared the diversities of three groups (within individuals, within sites, and between sites) (Figure 5(B)). EMF showed significantly different community between groups ( $p < .001$ ), which showed lowest community differences within same individual tree (individual group), followed by it within same sampling site (within sites group) and between sampling site (between sites group). In SPF, however, community differences did not show significant difference between groups ( $p = .242$ ).



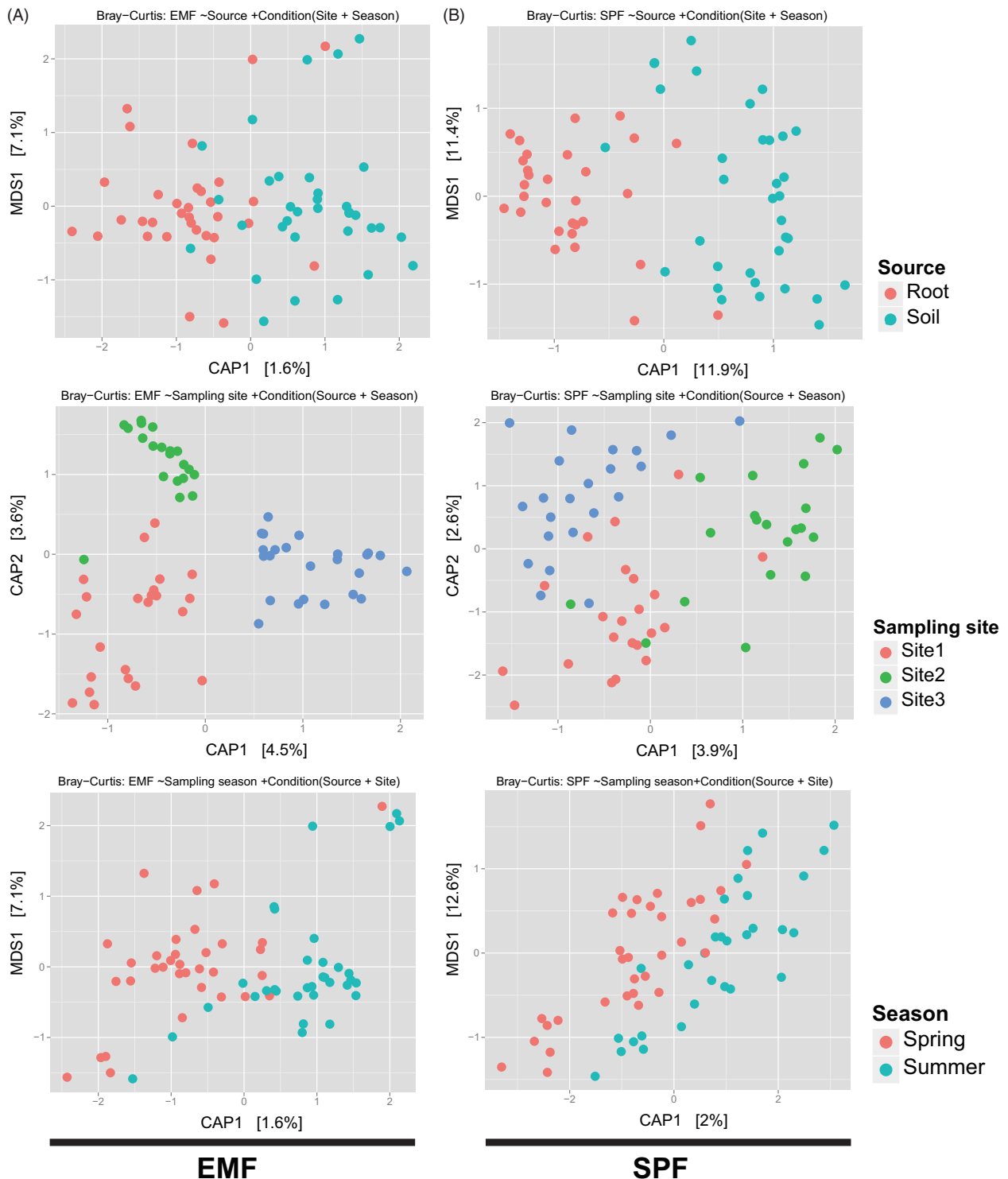
**Figure 3.** Constrained Analysis of Principal coordinates (CAP) plots based on the Bray–Curtis distance for total basidiomycetous community. CAP model for communities (A) constrained by source and conditioned by sampling site and season (2.64% explanatory power;  $p = .001$ ), (B) constrained by sampling site and conditioned by source and sampling season (7.65% explanatory power;  $p = .001$ ), and (C) constrained by sampling season and conditioned by source and sampling site (1.53% explanatory power;  $p = .306$ ).

## 4. Discussion

### 4.1. Diversity of basidiomycetes EMF and SPF associated with *Q. mongolica*

The pattern of dominant EMF genera found in *Q. mongolica* was different than that of other oak species. Generally, *Tomentella* is the most dominant genus associated with oak species [13–19]. However, fungal communities of *Q. mongolica* in two previous studies [21,22], as well as this study (Table 1), do not follow this pattern. Although we detected *Tomentella* frequently in our study, the abundance was low in both root and soil samples, and the most abundant EMF genera in this study were *Sebacina* and *Russula*. Thus, *Q. mongolica* may favor associations with *Sebacina* or *Russula* over *Tomentella* compared to other oak species. The high abundance of *Sebacina* found in this study also differed from

reports of previous studies of fungal communities associated with *Q. mongolica* [21,22]. Both of the studies showed low abundance of *Sebacina*, while *Russula* [21] or Cortinariaceae [22] were dominant. *Sebacina* is globally distributed and associated with diverse tree species, usually as mycorrhizal symbionts [36]. However, there are reports of endophytic *Sabacina* species in various herbaceous plants [37], and species that exist as endophytes in *Q. mongolica*, such as *Sebacina*, may have gone undetected in the soil samples of previous studies (although we note that we did recover *Sebacina* in our soil samples). An alternate explanation for the unusually high abundance of *Sebacina* in this study was the sampling site characteristics. Mt. Jeombong is a well preserved and protected national park, and shows late successional and climax forest stages. Because *Sebacina* has been shown to be abundant in



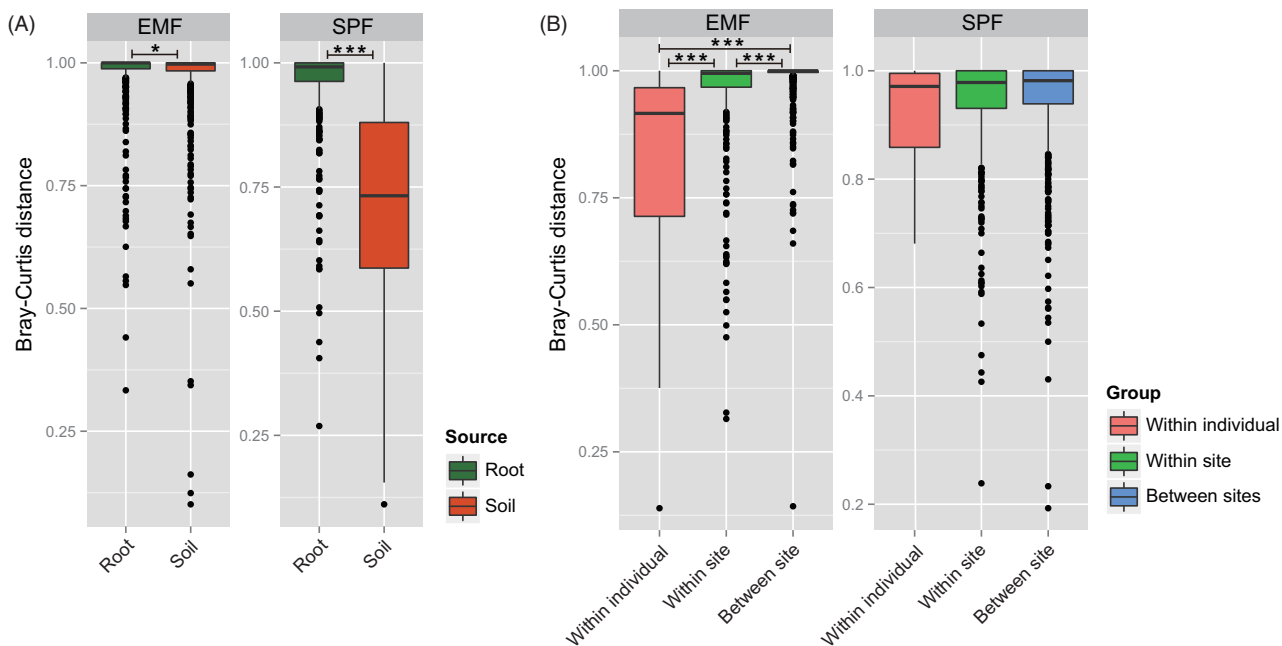
**Figure 4.** Constrained Analysis of Principal coordinates (CAP) plots based on the Bray–Curtis distance. CAP model constrained by source and conditioned by sampling site and season for (A) EMF and (B) SPF communities.

the late-stage forests compared to early stage forests [38], the relatively high abundance reported here may reflect the relatively pristine site conditions.

The major SPF genera associated with *Q. mongolica* were *Cryptococcus* and *Mycena*, although there were striking differences between these genera depending on sample source. In soil samples, *Cryptococcus* was far more abundant than any other genera (>an order of magnitude), while in root samples, *Mycena* was the most abundant genus, however, the relative

abundance of other major SPF genera in root samples was not dramatically lower (range: 2.5–5.3%). Thus, *Cryptococcus* appears to be distinctly dominant in soil while several different SPF genera were generally equally common in roots.

Because there are limited number of other studies for SPF communities in oaks, it is difficult to compare SPF communities in the roots of *Q. mongolica* and other oak species; however, the high abundance of *Cryptococcus* and *Mycena* observed in our study



**Figure 5.** Pairwise beta diversities based on the Bray–Curtis distance (A) within source (root and soil), and (B) between source. Significance of difference was tested using Kruskal–Wallis test (\*:  $p < .05$ , \*\*\*:  $p < .001$ ).

is consistent with a previous study that used soil samples from *Q. mongolica* [22]. In addition, fruiting bodies of *Mycena* are commonly found in other oak species [39]. Moreover, *Cryptococcus* is the most abundant species in various forest soils, include those of oak forests [40]. Therefore, these two SPF genera are probably widely distributed in *Q. mongolica* forests.

While the importance of EMF in oak forests are well known, our results suggest that diverse SPF are also important contributors to *Q. mongolica* forest soil systems. SPF can suppress plant pathogens [41,42], induce plant immune response [43], promote growth [42], and increase nutrient uptake [44,45]. *Cryptococcus*, for example, has been shown to enhance root growth in the medicinal shrub, *Agathosma betulina* by supplying nutrients [46]. In addition, recent studies showed that SPF often have facultative biotrophic capacity colonizing root [47,48]. Among the 201 wood decaying basidiomycetes tested, 34 species successfully colonized roots of *Picea abies* or *Pinus sylvestris* seedlings [47], which suggests that SPF can influence the host directly. Thus, our results suggest that SPF have a significant impact on resource acquisition, growth and disease resistance in *Q. mongolica*.

#### 4.2. Guild related community patterns and implications on sampling effort

There were clear differences in community structure between EMF and SPF. EMF communities were highly affected by geography, while SPF communities were more dependent on source (e.g. soil versus root) (Figure 4). The spatial distribution of EMF

may be associated with environmental conditions such as soil properties [7–9]. Another explanation for the geographic distinction of EMF communities is the limited dispersal ability of EMF spores. Dispersal distance of EMF spores is limited to approximately 1 m from the fruit body [49], a trait that may be favored by selection due to proximity to the host. Therefore, spore dispersal may constrain the distribution of the EMF community, which may explain the distinct EMF community even within the same host species. On the other hand, EMF showed similar community structure between roots and soils in same site (Figure 4(A)). EMF in soil may be present as extramatrical hyphae extended from the ectomycorrhiza. If a high proportion of EMF indicates a high abundance of EMF hyphae in soil, then overall soil microbial activity may be largely affected by the hyphal activity of EMF. In contrast, EMF detected in the soil may be not be restricted to extramatrical hyphae from ectomycorrhiza, but also represent spores or non-interactive EMF mycelia (e.g. dead mycelium and non-host fungi) [50–52]. This assumption is supported by the higher alpha diversity of EMF in soil compared to roots (Figure 2). Therefore, the abundant EMF detected in soil surrounding plant roots may represent a diverse set of EMF life stages (extramatrical hyphae, spore, and free-living mycelia).

Compared to the EMF community, the SPF community was more dependent on source than geography (Figure 4(B)). In SPF communities, *Mycena* was dominant in roots and *Cryptococcus* was dominant in soil, which suggests habitat differentiation between root and soil. *Mycena* and *Cryptococcus* may compete with each other for substrates, and



such competition can induce such habitat differentiation. On the other hand, during the rinsing process *Cryptococcus* may be washed away because in its yeast form it is more easily separated from the root surface than in its hyphal form. Therefore, distinct root and soil SPF community structure in *Q. mongolica* may be associated with the lifestyle form of the dominant SPF genera.

Fruiting bodies are usually used to determine forest fungal communities [53,54]; however, recent advances in molecular based methods (e.g. DGGE, RFLP, next generation sequencing) have improved our understanding of fungal communities in forest soils immensely [40,54,55] and an enormous diversity of fungal communities associated with roots or as mycelium in the soil has been described [56]. EMF community studies comparing root tip and soil samples have revealed significant differences in EMF abundance between sample types [54]. Our results show that fungal communities can have different distributional patterns depending on the guild and sample type. The EMF community was relatively similar between root and soil in same individual, and largely influenced by spatial differences (Figure 5). In contrast, the SPF community was more influenced by the source, with low correspondence between root and soil samples.

Because sampling strategy is largely connected to community distribution patterns, the guild pattern of community structure may provide insights toward improved research design. The high dissimilarity of EMF communities found in our study suggests a patchy distribution of EMF (Figure 5), consistent with other studies [57–59]. In addition, SPF community dissimilarity was high between root and soil samples, irrespective of spatial distance (Figure 5). Because this study was conducted on restrict area in Mt. Jeombong, it is possible that SPF community might be different depending on distance in larger geographical distance scales. Therefore, it is likely that both root and soil samples are needed to adequately sample SPF and EMF communities of *Q. mongolica*.

## 5. Conclusion

We surveyed the basidiomycetes associated with *Q. mongolica* and found different community patterns depending on the fungal guild. Among 53 genera in basidiomycete detected, 15 genera were closely associated with *Q. mongolica*. *Sebacina*, *Russula*, *Tomentella*, and *Amanita* were major EMF, while *Cryptococcus*, *Mycena*, *Marasmiellus*, and *Marasmius* were major SPF. The proportion and alpha diversity of EMF were higher in soil than those of SPF. In addition, geography is likely a major factor determining EMF communities, while source was more

important for SPF communities. These finding suggest that the determining factor for community structure is dependent on the fungal guild. Many fungi–tree association studies, especially those of oak trees, focused on the EMF community. Our results, however, show that various SPF genera are also closely associated with *Q. mongolica*, relatively independent of geography, which suggests that SPF are an essential component of the core fungal community associated with oak species.


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## Disclosure statement

No potential conflict of interest was reported by the authors.

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