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Fungal communities in a Korean red pine stand, Gwangneung Forest, Korea

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ABSTRACT

For the seasonal changes of fungi (Ascomycota and Basidiomycota) diversity, we performed a biweekly survey of macrofungi on defined plot in a Korean red pine (*Pinus densiflora*) stand (Gwangneung Forest, Pochen-si, Korea) from April 2014 to December 2014. The plot was surveyed 18 times. We also investigated the diversity of the soil fungi community during four seasons using pyrosequencing method. The collected macrofungi (25 specimens) were classified into one phylum, one class, four orders, 10 families, 13 genera, and 17 species; the soil fungal communities were classified into two phyla, 15 classes, 43 orders, 91 families, and 124 genera (49,937 sequence reads), designated as 124 genus-level operational taxonomic units. Using macrofungal collection data, environmental factor data ($n = 10$), and pyrosequencing data, we evaluated changes in fungal diversity with seasons and soil layers. Nonmetric multidimensional scaling ordination revealed distinct clusters of genus-level operational taxonomic units assemblage with season. Two environmental factors (exchangeable K and C/N ratio) were found to be significantly associated with soil fungi communities in the Korean red pine stand. This study will lead to a better understanding of relationships between Korean red pine stand stands and soil fungal communities.

Introduction

Gwangneung Forest (Pocheon-si, Gyeonggi-do) is one of the best natural forests in Korea, and also a precious global repository of biodiversity (Cho et al 2007; Kim and Han 2008). The Gwangneung Forest has been thoroughly protected and managed since its designation as a mausoleum forest of King Sejo in 1468 (Cho et al 2007). Various plants (over 1000 species), macrofungi (nearly 700 species), and animals (nearly 3000 species) have been recorded there; therefore, the United Nations Educational, Scientific, and Cultural Organization designated this place as the fourth Korean biosphere reserve, on June 2, 2010 (http://www.kna.go.kr) (Cho et al 2007; Kim and Han 2008).

Investigation of macrofungal diversity based solely on macrofungal sampling is limited by the number of collected specimens; further, the specimens might be difficult to discern. Fruit bodies of some macrofungi are small and difficult to see with the naked eye (e.g. Ascomycota species, such as *Chlorociboria*, *Scutellinia*, *Peziza*, etc.). In addition, some small-sized or fragile species (e.g. *Clitopilus*, *Crepidotus*, *Mycena*, etc.) generally quickly disappear (Andrew et al 2013). Accordingly, next-generation sequencing is an alternative approach of surveying the fungal diversity and discovering new biological groups (Jumpponen et al 2010; Lim et al 2010; Kim et al 2016). Lim *et al* (2010) and Kim *et al* (2016) attempted to detect soil fungal communities in Korea using one next-generation sequencing technique (pyrosequencing) generating *ca*. 400 bp for each operational taxonomic unit (OTU). They suggested that pyrosequencing is a reliable tool for surveying the fungal diversity in the soil, facilitating the understanding of ecological types (saprophytes, symbionts, and parasites) of fungi. Although fungal DNA barcoding is still unaccomplished and interpretation of the relationship between fungal communities and environmental factors is

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plagued, pyrosequencing is a useful tool for investigating soil fungal diversity (Jumpponen et al 2010; Kim et al 2016).

We previously investigated the seasonal dynamics of soil fungal communities in a Mongolian oak-dominated forest in Gwangneung Forest (Kim et al 2016). Here, we subsequently investigated a different forest stand, Korean red pine stand. The investigation was based on the sampling of macrofungi, analysis of soil chemistries, and pyrosequencing, to obtain ecological data. Further, the relationship between the soil fungal community and chemical properties of the soil was examined by multivariate statistical analyses.

Materials and methods

Study site

The study site was a Korean red pine stand (37°46′27.26″ N, 127°11′14.34″ E, elevation 379 m); *ca*. 85% of the tree layer coveraged by Korean red pine (*Pinus densiflora* Siebold & Zucc.), mixed with few *Rhododendron mucronulatum* Turcz. and *Disporum smilacinum* A. Gray, in the Gwangneung Forest, Pocheon-si, Gyeonggi Province, Korea (Figure 1).

Figure 1. A–D, Sampling sites during four seasons and E, sampling of soil-layers in the Korean red pine stand (Gwangneung Forest).

Macrofungi collection and identification

Defined plots were surveyed 18 times on a biweekly basis, for macrofungi collection, from April 2014 to December 2014. The collected specimens were identified based on the macroscopic characteristics (basidiomata; cap size, shape, color, surface texture, and surface moisture; gill color, attachment, spacing, lamellules; stem size, the presence or absence of partial and universal veils, etc.) and microscopic characteristics (the size and shape of basidiospores, basidia, cystidia, etc.). Microscopic observations were made using an Olympus BX53 microscope and Jenoptik ProgRes C14 Plus Camera (Jenoptik Corporation, Jena, Germany). Microscopic parameters were measured using ProgRes Capture Pro v.2.8.8. software (Jenoptik Corporation). All collected specimens ($n = 25$) are listed in Table 1; dried specimens were deposited in the herbarium of Korea National Arboretum, Pocheon, Korea.

Soil sampling and chemical analyses of the soil

The soil was sampled in the spring (April 24, 2014), summer (August 11, 2014), autumn (October 16, 2014), and winter (December 1, 2014) from a defined plot (20 m × 10 m). Three soil frames (*ca.* 25 cm × 25 cm) were collected at about 1-m distance from different dominant plants (*Pinus densiflora*); these frames were divided into an L-layer (litter layer; 0–1 cm), H-layer (humic layer; 1–3 cm), and Ah-layer (a layer between the humic and mineral matter layers; 3–5 cm; Figure 1). The soil samples were sieved using a 2-mm sieve. The resulting material was combined to yield a composite sample from each horizon (Figure 1). Subsamples for chemical analyses (soil) and DNA extraction were frozen and stored at −80°C. The chemical analyses were conducted in the National Instrumentation Center for Environmental Management (Seoul National University, Seoul, Korea). The following environment factors were measured: pH, exchangeable K, total phosphorus

Table 1. List of collected macrofungi at the defined plot of Korean red pine stand in Gwangneung Forest.

No.	Specimen	Collection date	Family	Morphological identification	Ecological living type
1	KA14-0161	Jun 10, 2014	Pluteaceae	*Pluteus* sp.	Saprophyte
2	KA14-0396	Jul 3, 2014	Fomitopsidaceae	*Postia tephroleuca*	Saprophyte
3	KA14-0397	Jul 3, 2014	Omphalotaceae	*Gymnopus confluens*	Saprophyte
4	KA14-0398	Jul 3, 2014	Marasmiaceae	*Xeromphalina* sp.	Saprophyte
5	KA14-0399	Jul 3, 2014	Tricholomataceae	*Leucopaxillus tricolor*	Saprophyte
6	KA14-0734	Jul 29, 2014	Omphalotaceae	*Gymnopus confluens*	Saprophyte
7	KA14-0784	Aug 11, 2014	Strophariaceae	*Pholiota multicingulata*	Saprophyte
8	KA14-0785	Aug 11, 2014	Amanitaceae	*Amanita flavipes*	Symbiont
9	KA14-0786	Aug 11, 2014	Mycenaceae	*Mycena pura*	Saprophyte
10	KA14-0787	Aug 11, 2014	Omphalotaceae	*Gymnopus confluens*	Saprophyte
11	KA14-0788	Aug 11, 2014	Boletaceae	*Xanthoconium* sp.	Symbiont
12	KA14-0789	Aug 11, 2014	Amanitaceae	*Amanita flavipes*	Symbiont
13	KA14-1355	Aug 29, 2014	Amanitaceae	*Amanita flavipes*	Symbiont
14	KA14-1356	Aug 29, 2014	Strophariaceae	*Pholiota multicingulata*	Saprophyte
15	KA14-1357	Aug 29, 2014	Marasmiaceae	*Marasmius* sp.	Saprophyte
16	KA14-1358	Aug 29, 2014	Suillaceae	*Suillus pictus*	Symbiont
17	KA14-1359	Aug 29, 2014	Marasmiaceae	*Marasmius* sp.	Saprophyte
18	KA14-1360	Aug 29, 2014	Russulaceae	*Russula cyanoxantha*	Symbiont
19	KA14-1361	Aug 29, 2014	Omphalotaceae	*Rhodocollybia butyracea*	Saprophyte
20	KA14-1423	Sept 12, 2014	Russulaceae	*Russula mariae*	Symbiont
21	KA14-1424	Sept 12, 2014	Boletaceae	*Tylopilus* sp.	Symbiont
22	KA14-1425	Sept 12, 2014	Amanitaceae	*Amanita rubescens*	Symbiont
23	KA14-1426	Sept 12, 2014	Omphalotaceae	*Gymnopus confluens*	Saprophyte
24	KA14-1427	Sept 12, 2014	Amanitaceae	*Amanita* sp.	Symbiont
25	KA14-1428	Sept 12, 2014	Amanitaceae	*Amanita alboflavescens*	Symbiont

Table 2. Soil-chemistry of Korean red pine stand by seasons and soil-layers.

Season	Soil-layer	pH	Exch. K (mg/kg)	T-P (mg/kg)	NH_4^+ (mg/kg)	NO_3^-	OM (%)	WR (%)	TOC (%)	T-N (%)	C/N ratio
Apr.	L-layer	4.0	2.9	374.7	65.2	76.2	33.6	19.7	19.5	0.8	24.6
	H-layer	3.8	2.1	284.9	51.4	53.1	27.0	23.1	15.6	0.7	23.5
	Ah-layer	3.9	1.4	249.8	28.8	32.4	14.4	17.1	8.4	0.4	24.2
	avg. ± SE	3.9 ± 0.06	2.1 ± 0.45	303.1 ± 37.21	48.5 ± 10.61	53.9 ± 12.65	25.0 ± 5.62	20.0 ± 1.72	14.5 ± 3.26	0.6 ± 0.13	24.1 ± 0.33
Aug.	L-layer	3.8	83.2	416.6	62.0	0.0	40.4	44.0	23.5	0.8	28.7
	H-layer	3.9	53.1	373.1	47.1	0.0	32.0	31.8	18.6	0.7	28.1
	Ah-layer	4.1	31.1	276.6	26.0	0.0	15.5	22.8	9.0	0.4	22.9
	avg. ± SE	3.9 ± 0.09	55.8 ± 15.08	355.4 ± 41.37	45.0 ± 10.44	0.0 ± 0.00	29.3 ± 7.33	32.89 ± 6.14	17.0 ± 4.25	0.6 ± 0.12	26.6 ± 1.84
Oct.	L-layer	3.8	140.8	414.5	77.9	55.4	53.1	17.2	30.8	1.0	29.9
	H-layer	3.7	56.8	320.8	54.1	26.7	32.0	18.4	18.6	0.7	25.7
	Ah-layer	3.7	35.6	281.1	34.3	15.2	20.1	14.9	11.7	0.4	27.5
	avg. ± SE	3.7 ± 0.03	77.7 ± 32.12	338.8 ± 39.54	55.5 ± 12.60	32.4 ± 11.93	35.1 ± 9.64	16.8 ± 1.04	20.4 ± 5.59	0.7 ± 0.17	27.7 ± 1.22
Dec.	L-layer	3.8	106.3	417.8	70.2	74.4	30.9	34.8	17.9	1.1	16.5
	H-layer	3.7	40.8	259.7	21.0	22.9	13.7	19.7	7.9	0.5	16.1
	Ah-layer	3.8	36.0	199.1	13.9	11.4	5.9	15.1	3.4	0.3	12.8
	avg. ± SE	3.8 ± 0.03	61.0 ± 22.65	292.2 ± 65.20	35.0 ± 17.71	36.2 ± 19.37	16.8 ± 7.41	23.2 ± 5.93	9.8 ± 4.30	0.6 ± 0.25	15.1 ± 1.15

C/N ratio = TOC/T-N; OM = organic matter; SE = standard error; T-N = total nitrogen; TOC = total organic carbon; WR = water retention.

Table 3. One-way ANOVA ($n = 12$) tested the statistical differences among measured responses.

Response	Mean ± SE	Season		Soil-layer	
		F	p	F	p
Environmental factors					
pH	3.8 ± 0.0	2.889	0.102	0.672	0.534
Exch. K (%)	49.2 ± 10.7	2.419	0.141	2.518	0.135
T-P (mg/kg)	322.4 ± 18.7	0.395	0.760	17.063	0.001‡
NH_4^+ (mg/kg)	46.0 ± 5.2	0.417	0.745	15.847	0.001‡
NO_3^- (mg/kg)	30.6 ± 7.0	2.979	0.096	2.222	0.164
OM (%)	26.6 ± 3.3	1.015	0.435	9.396	0.006†
WR (%)	23.2 ± 2.3	2.506	0.133	1.861	0.211
TOC (%)	15.4 ± 1.9	1.016	0.435	9.388	0.006†
T-N (%)	0.6 ± 0.1	0.104	0.955	26.786	< 0.001‡
C/N ratio	23.4 ± 1.4	2.506	0.133	1.861	0.211
Sequences *	4161.4 ± 175.5	3.588	0.066	0.223	0.804
Richness (GOTU)	39.3 ± 1.4	5.808	0.021†	0.163	0.852
Evenness	0.7 ± 0.0	2.507	0.133	0.032	0.968
Diversity indices					
Simpon's D	0.9 ± 0.0	29.357	< 0.001‡	0.018	0.982
Shannon's H′	2.6 ± 0.0	30.514	< 0.001‡	0.136	0.875

ANOVA = analysis of variance; C/N ratio = TOC/T-N; GOTU = genus-level operational taxonomic unit; OM = organic matter; SE = standard error; T-N = total nitrogen; TOC = total organic carbon; WR = water retention.

* Sequences: using reads of soil-higher fungi which belong to Ascomycota and Basidiomycota only; † $p < 0.05$.‡ $p < 0.005$.

(T-P), NH_4^+, NO_3^-, organic matter (OM), water retention, total organic carbon (TOC), total nitrogen (T-N), and C/N ratio (based on TOC/T-N; Table 2).

DNA extraction, 454-pyrosequencing, and taxonomic assignment

DNA of the soil microorganisms was extracted using a PowerSoil DNA isolation kit (MoBio Laboratories Inc., Carlsbad, CA, USA). DNA library was prepared using PCR products, according to the GS FLX plus library prep guide (Roche, Basel, Switzerland). The DNA libraries were quantified using Picogreen assay (using Victor 3 machine). Clonal amplification of a purified library, emPCR, was carried out using the GS-FLX plus emPCR kit (Roche, Basel, Switzerland). A 20-ng aliquot of each DNA sample was used for a 50-μL PCR reaction. Primers ITS1F/ITS4 (White et al 1990) were used to amplify the ITS region of fungal rRNA gene. FastStart High Fidelity PCR system (Roche) was used for PCR amplification, with the following conditions: 94°C for 3 minutes; followed by 35 cycles of: 94°C for 15 seconds, 55°C for 45 seconds, and 72°C for 1 minute; and a final elongation step at 72°C for 8 minutes. The PCR products were purified using AMPure beads (Beckman Coulter, Inc., Brea, CA, USA). Sequencing was performed at Macrogen Ltd. (Seoul, Korea).

All sequence reads were compared with the sequences deposited in the Silva rRNA database (https://www.arb-silva.de/). Sequence reads with sequence similarity with E value < 0.01 were treated as partial ITS sequence. Less than 1% of all sequences were non-ITS sequences. Taxonomic assignment of the sequenced reads was carried out using National Center for Biotechnology Information (NCBI) Taxonomy Databases (http://www.ncbi.nlm.nih.gov/taxonomy); for each sequence, the five most similar sequences were identified based on the bit scores and E-values of the Basic Local Alignment Search Tool (BLAST) program. The Needleman–Wunsch global alignment algorithm was used to find the optimum alignment of two sequences along their entire length. Pairwise global alignment was performed with the selected candidate hits to find the best-aligned hit. The taxonomy of a sequence with the highest similarity was assigned to the sequence read. Similarly, taxonomy was assigned down to the following taxonomical hierarchies: species, > 97% similarity; genus, > 94%; family, > 90%; order, > 85%; class, > 80%; and phylum, > 75%.

Diversity and statistical analyses

Genus-level OTUs (GOTUs) and groups were surveyed in each seasonal layer using the analysis of variance (ANOVA; Table 3). The responses were analyzed using repeated-measures ANOVA (Table 3). The Duncan procedure was used to compare treatment means (Kim et al 2016). Environmental factors were visualized by principal component analysis (PCA) plots. Relationships between fungal communities and environmental factors (including the sampling season and soil layer) were visualized using nonmetric multidimensional scaling (NMDS; an unconstrained ordination) on the basis of Bray–Curtis distance matrix using the metaMDS function in the vegan package in R (Oksanen 2015). The envfit function in the vegan package in R was then used to fit vectors of environmental factors that were significantly related to soil fungal community structure in the Mantel tests to the NMDS ordination (Oksanen 2015). The p-values of regressions were obtained from 999 permutations.

Results and discussion

Macrofungal diversity

Twenty-five specimens were collected from a defined plot from April 2014 to December 2014 (Table 1). Based on morphological characteristics, they were classified to 18 species that belong to 14 genera in 10 families, four orders in Agariocomycetes. The genus *Amanita* (6 specimens; 24%) was most prevalent, followed by the genera *Gymnopus* (4 specimens; 16%), *Marasmius* (12%), etc. (Table 1 and Figure 2). According to the ecological type, the collected macrofungi were divided into two groups, saprophytes (56%) and symbionts (44%; Table 1). The generated list of macrofungi provides baseline information required for the assessment of changes of biological diversity in the Korean red pine stand and is an important first step toward producing a checklist of macrofungi at this site.

Analysis of Korean red pine stand soil chemistry by ANOVA and PCA plot

Previous studies have shown that the chemical properties of the soil determine various environmental factors, such as pH, carbon, and nitrogen availability (Bissett et al 2011; Kim et al 2016). In addition, correlation studies have shown that plant species and the soil type are associated with the variation of microbial communities (Fierer et al 2007; Bissett et al 2011; Garbisu et al 2011; De Vries et al 2012; Kuramae et al 2012; Schulz et al 2012; Shange et al 2012; Kim et al 2016). In the current study, according to the ANOVA test, T-N, T-P, NH_4^+, OM, and TOC were significantly correlated with the soil layer ($p < 0.05$); all environmental factors were weakly correlated with the season (Tables 2 and 3). As shown in Figure 3, Dim1 explained 59.4% of variation in the data with a very small p-value reflected by the significance analysis (correlated factors: OM, TOC, NH_4^+, T-P, T-N, and exchangeable K; $p < 0.01$). Based on these results, most environmental factors changed substantially with the soil layer. Some chemical factors were influenced by seasonal variability (e.g. NO_3^- and pH; Table 3 and Figure 3), but seasonal trends were not readily apparent (Table 3 and Figure 3). By contrast, the GOTU richness and diversity indices correlated with the seasons (Table 3).

Soil fungal community composition and diversity in the Korean red pine stand

To characterize the soil fungi communities in the Korean red pine stand, we used pyrosequencing and sequenced 94,236 fungal

Figure 2. Collected macrofungi in the Korean red pine stand. A, *Amanita flavipes* KA14-0789; B, *Amanita rubescens* KA14-1425; C, *Amanita* sp. 2 KA14-1427; D, *Amanita vaginata* KA14-1428; E, *Tylopilus* sp. KA14-1424; F, *Clitocybe* sp. 3 KA14-1359; G, *Gymnopus confluens* KA14-0397; H, *Leucopaxillus tricolor* KA14-0399; I, *Mycena pura* KA14-0786; J, *Pholiota multicingulata* KA14-0784; K, *Pluteus* sp. KA14-0161; L, *Postia tephroleuca* KA14-0396; M, *Marasmius butyracea* KA14-1357; N, *Russula cyanoxantha* KA14-1360; O, *Russula mariae* KA14-1423; P, *Suillus pictus* KA14-1358; Q, *Xanthoconium affine* KA14-0788; and R, *Xeromphalina cauticinalis* KA14-0398. <scale bars: 3 cm>

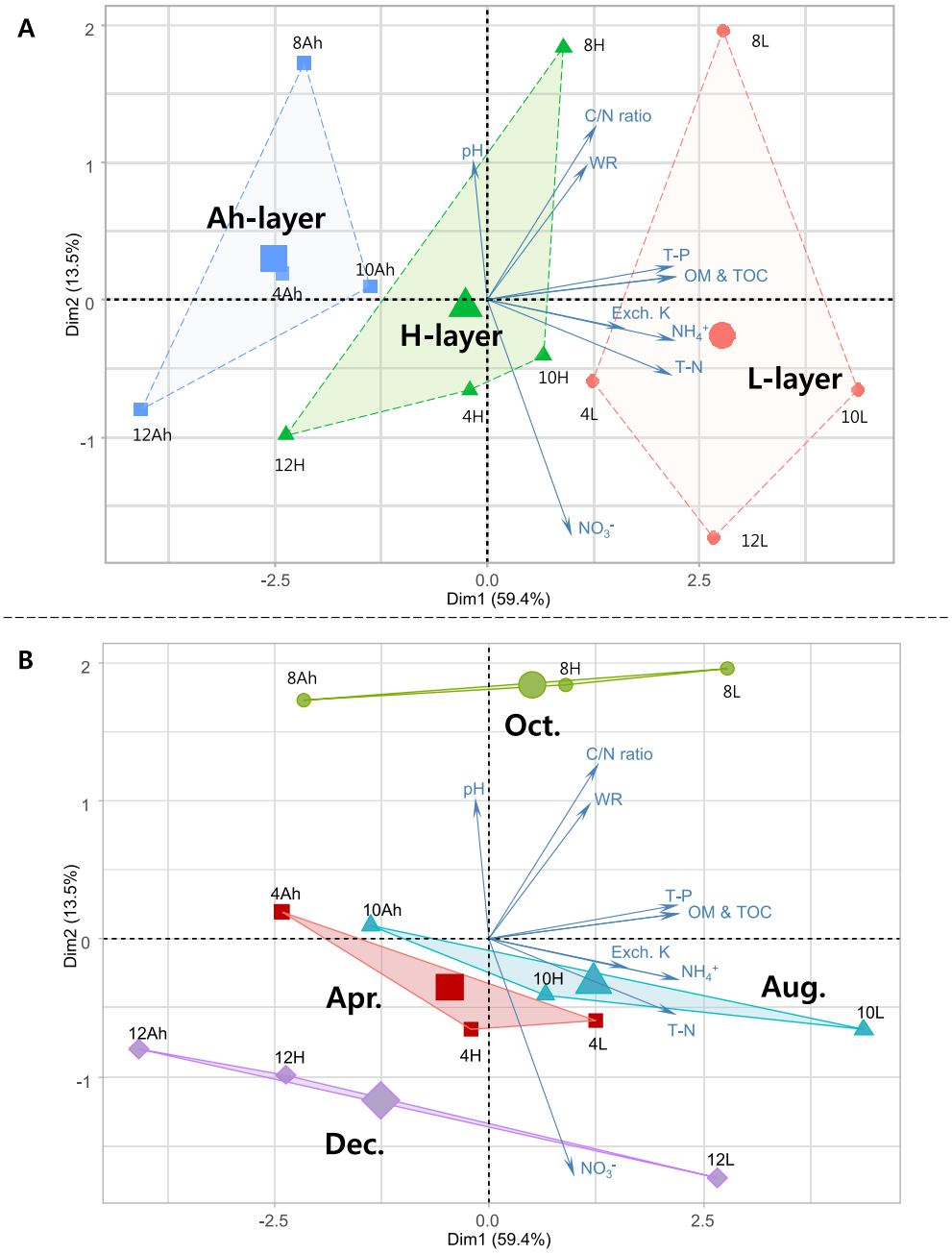

Figure 3. Principal component analysis based on environmental factors. The axis scores are calculated based on the soil-chemistry of each sampling sites during four seasons. A, grouping by soil-layer and B, grouping by season.

amplicons (excluding short and poor-quality reads) from samples collected during four seasons (Table 4). The overall fungal community was dominated by phylum-level sequences assigned as unidentified (43.8%), Basidiomycota (27.2%), Ascomycota (27.2%), Zygomycota (1.7%), etc. (Table 4). Interestingly, the highest number of unidentified fungi was detected in August (66.2%) and April (48.8%; Table 4). To evaluate the soil fungi diversity (i.e. Ascomycota and Basidiomycota fungi), we excluded Zygomycota,

Table 4. Total sequence reads of obtained soil-fungi by seasons in phylum-level.

Month	Unidentified	Ascomycota	Basidiomycota	Chytridiomycota	Glomeromycota	Zygomycota	Total
Apr.	13,111 (48.8)	8281 (30.8)	5320 (19.8)	0 (0.0)	0 (0.0)	162 (0.6)	26,874 (100)
Aug.	14,880 (66.2)	3821 (17.0)	2922 (13.0)	0 (0.0)	41 (0.2)	813 (3.6)	22,477 (100)
Oct.	8088 (28.6)	7068 (25.0)	12,713 (44.9)	3 (0.0)	13 (0.0)	442 (1.6)	28,327 (100)
Dec.	4525 (30.1)	6072 (40.4)	4304 (28.6)	0 (0.0)	0 (0.0)	125 (0.8)	15,026 (100)
Sum	40604 (43.8)	25,242 (27.2)	25,259 (27.2)	3 (0.0)	54 (0.1)	1542 (1.7)	94,236 (100)

Data are presented as *n* (%).

Chytridiomycota, Glomeromycota, and unidentified fungi from analysis. Interestingly, the rate of detected Ascomycota sequence reads in winter (December) was nearly 1.4 times higher than that of Ascomycota detected rates during the spring, summer, and autumn (April, August, and October); the rate of detected Basidiomycota sequence reads was 1.8 times higher than that of Ascomycota in the autumn (Table 4). It appears that temperature is an important factor that affects the fungal community at phylum level; however, we were unable to draw clear conclusions on account of the lack of soil-temperature data. This issue should be further investigated in the future. In August, the rate of unidentified sequence reads (66.2%) is higher than other seasons (Table 4). Therefore, summer season is probably the best time to collect and discover the new fungal groups.

Since many Ascomycota and Basidiomycota sequence reads could not be classified to the species level, GOTUs were introduced to evaluate the soil fungal communities. Sequences without valid primer sequences or DNA tags, those containing ambiguous bases, or ones that were shorter than the length threshold (200 bp) were removed for quality control. According to one-way ANOVA analysis, the GOTU richness and diversity indices (Simpon: Shannon = D′: H′) were significantly correlated with seasonal changes ($p < 0.05$); however, no correlation was observed with the soil layer (Table 3). With regard to the seasons, the GOTU richness in April (53.0) was higher than in other seasons (August, 46.7; October, 39.7; December, 17.7), while the evenness in August (0.80) was higher than other seasons (April, 0.71; October, 0.73; December, 0.67; Figure 4). The patterns of two diversity indices were similar: the numbers for August (D′: H′ = 0.93: 3.05) were the highest among all seasons (April, D′: H′ = 0.90: 2.79; October, D′: H′ = 0.89: 2.64; December, D′: H′ = 0.77: 1.91; Figure 4).

Compared with traditional macrofungi sampling, pyrosequencing has notably improved the resolution of fungal community studies concerned with forest ecology by increasing the number of sequences per samples (Jumpponen et al 2010; Voříšková et al 2014; Kim et al 2016). Nevertheless, many fungal groups within samples remain unidentified (see Table 4). Discovery and procurement of useful microorganisms or new species from such unidentified fungal groups may be accomplished using some soil fungi isolation methods, although it remains time- and effort-consuming.

Comparison of the fungal diversity in soil layers collected during different seasons

The obtained soil fungi were classified into 124 genera (49,937 sequence reads) that belong to 91 families in 43 orders, 15 classes in Ascomycota or Basidiomycota (Table 5). From these, 124 GOTUs were obtained (75 Ascomycota GOTUs and 49 Basidiomycota GOTUs; Table 5); 75 GOTUs were obtained in April, with 36 in the L-layer, 67 in the H-layer, and 56 in the Ah-layer; 69 GOTUs in August, with 56 in the L-layer, 44 in the H-layer, and 40 in the Ah-layer; 54 GOTUs in October, with 62 in the L-layer, 36 in the H-layer, and 29 in the Ah-layer; and 20 GOTUs in December, with 20 in the L-layer, 18 in the H-layer, and 15 in the Ah-layer (Table 5 and Figure 5).

In April, Unknown24 (from the phylum Ascomycota) was the most abundant GOTU (17.2%), followed by the genera *Oidiodendron* (16.8%), Unknown30 (from the order Boletales; 7.1%), *Gerhardtia* (6.5%), Unknown09 (from the order Helotiales; 5.9%), and *Geminibasidium* (5.0%; Table 5 and Figure 6). In August, Unknown24 was the most abundant GOTU (11.9%), followed by the genera *Oidiodendron* (11.6%), *Fusarium* (6.4%), Unknown37 (from the order Trechisporales; 6.3%), *Trichoderma* (6.1%), and Unknown30 (5.0%; Table 5 and Figure 6). In October, the genus *Mycena* was most abundant GOTU (13.2%), followed by the genera *Cryptococcus* (12.6%), Unknown24 (11.8%), Unknown33 (from the family Russulaceae; 9.2%), *Trichoderma* (6.2%), and *Gymnopus* (5.0%; Table 5 and Figure 6). In December, the genus *Cryptococcus* was the most abundant GOTU (29.6%), followed by the genera *Oidiodendron* (16.7%), *Trichoderma* (14.4%), Unknown19 (from the family Ophiocordycipitaceae; 11.1%), Unknown33 (6.1%), and Unknown09 (from the order Helotiales; 4.5%). Collectively, the genus *Cryptococcus* was the most abundant GOTU (11.2%) in the Korean red pine stand, followed by the genera *Oidiodendron* (11.0%), Unknown24 (10.8%), *Trichoderma* (7.5%), Unknown33 (6.5%), and *Mycena* (5.4%; Table 5 and Figure 6).

The seasonal differences in relative abundances of fungal genera (GOTUs) in the soil were significant ($p = 0.021$; Table 3). For

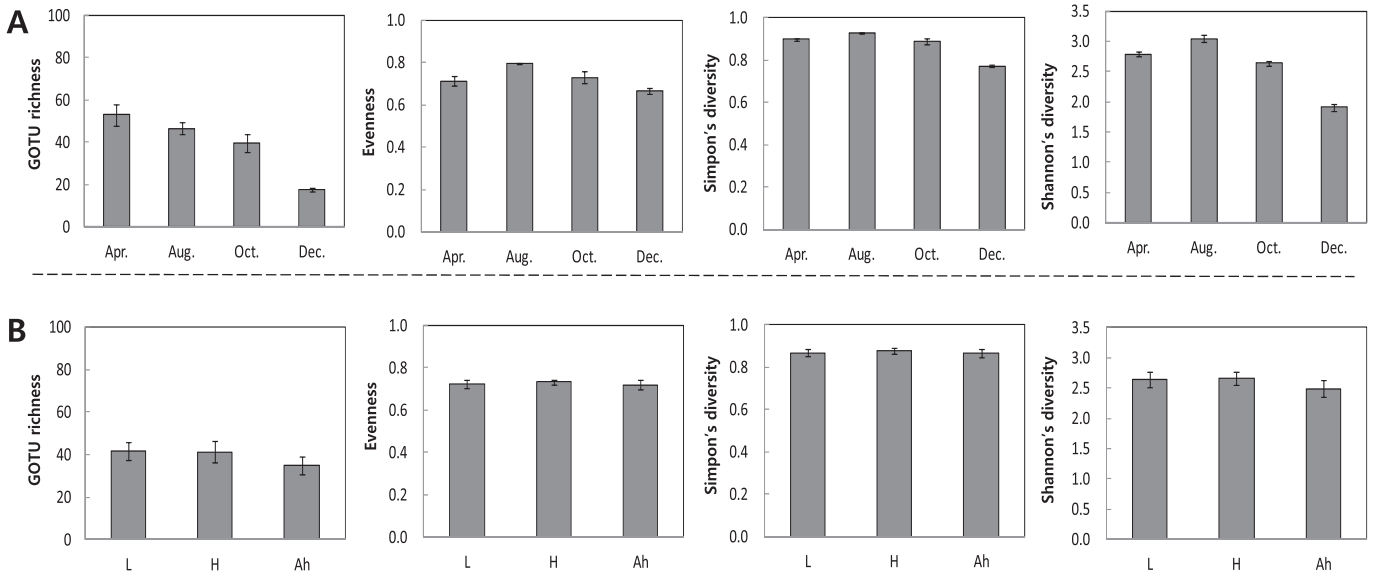

Figure 4. Genus-level operational taxonomic unit (GOTU) richness, evenness, and their diversity indices (Simpon and Shannon) according to A, season and B, soil layer.

Table 5. List of classified soil fungi and their reads of GOTU.

No.	Phylum	Class	Order	Family	Genus	GOTU	Ecol. type*	Reads of GOTU												
								4L	4H	4Ah	8L	8H	8Ah	10L	10H	10Ah	12L	12H	12Ah	Total
1	Ascomycota	Dothideomycetes	Botryosphaeriales	Botryosphaeriaceae	Guignardia	Guignardia	PAR	0	0	0	0	0	0	0	0	0	109	96	25	230
2	Ascomycota	Dothideomycetes	Capnodiales	Davidiellaceae	Cladosporium	Cladosporium	SAP	11	0	0	6	5	0	0	0	0	0	0	0	22
3	Ascomycota	Dothideomycetes	Capnodiales	Mycosphaerellaceae	unidentified	Unknown01	PAR	0	0	0	8	0	0	0	0	0	0	0	0	8
4	Ascomycota	Dothideomycetes	Capnodiales	Teratosphaeriaceae	Devriesia	Devriesia	PAR	0	0	0	10	0	0	0	0	0	0	0	0	10
5	Ascomycota	Dothideomycetes	Capnodiales	unidentified	unidentified	Unknown02	PAR	0	4	0	16	12	2	7	9	0	0	0	0	50
6	Ascomycota	Dothideomycetes	Hysteriales	Gloniaceae	Cenococcum	Cenococcum	SYM	0	0	0	39	52	2	0	0	0	0	0	0	93
7	Ascomycota	Dothideomycetes	Incertae sedis	Myxotrichaceae	Oidiodendron	Oidiodendron	SAP	62	664	1554	121	268	394	46	196	466	81	222	1432	5506
8	Ascomycota	Dothideomycetes	Pleosporales	Montagnulaceae	Paraphaeosphaeria	Paraphaeosphaeria	SAP	0	0	0	14	1	3	0	0	0	0	0	0	18
9	Ascomycota	Dothideomycetes	Pleosporales	Mytilinidiaceae	Lophium	Lophium	SAP	0	5	1	6	0	0	0	0	0	0	0	0	12
10	Ascomycota	Dothideomycetes	Pleosporales	Venturiaceae	Cylindrosympodium	Cylindrosympodium	SAP	0	15	0	0	0	0	0	0	0	0	0	0	15
11	Ascomycota	Dothideomycetes	Venturiales	unidentified	unidentified	Unknown03	SAP	2	8	4	20	0	0	29	3	0	0	0	0	66
12	Ascomycota	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	Capronia	Capronia	SYM	0	0	0	0	0	0	7	0	0	0	0	0	7
13	Ascomycota	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	Cladophialophora	Cladophialophora	PAR	1	21	5	0	0	0	0	0	0	0	0	0	27
14	Ascomycota	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	Exophiala	Exophiala	PAR	0	0	0	9	0	0	0	0	0	0	0	0	9
15	Ascomycota	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	Phialophora	Phialophora	SAP	0	53	10	0	0	0	0	0	0	0	0	0	63
16	Ascomycota	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	unidentified	Unknown04	SAP	0	7	8	19	14	15	3	11	0	0	0	0	77
17	Ascomycota	Eurotiomycetes	Chaetothyriales	unidentified	unidentified	Unknown05	SAP	0	0	0	0	0	0	4	2	0	0	0	0	6
18	Ascomycota	Eurotiomycetes	Eurotiales	Elaphomycetaceae	unidentified	Unknown06	SAP	0	0	0	0	0	8	0	0	0	0	0	0	8
19	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	Aspergillus	Aspergillus	SAP	6	0	0	0	0	0	0	0	0	0	0	0	6
20	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	Paecilomyces	Paecilomyces	PAR	5	6	1	0	0	0	9	0	0	0	0	0	21
21	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	Penicillium	Penicillium	SAP	2	43	36	6	3	17	42	13	0	85	96	23	366
22	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	Sagenomella	Sagenomella	PAR	31	51	89	36	17	35	8	16	246	0	0	0	529
23	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	unidentified	Unknown07	SAP	0	12	4	0	0	0	0	0	0	0	0	0	16
24	Ascomycota	Eurotiomycetes	Eurotiales	unidentified	unidentified	Unknown08	SAP	0	9	4	5	0	0	15	201	0	0	0	0	234
25	Ascomycota	Incertae sedis	Incertae sedis	Incertae sedis	Chalara	Chalara	PAR	0	12	7	37	0	0	34	4	0	0	0	0	94
26	Ascomycota	Incertae sedis	Incertae sedis	Incertae sedis	Ochroconis	Ochroconis	SAP	0	0	0	7	0	0	0	0	0	0	0	0	7
27	Ascomycota	Incertae sedis	Incertae sedis	Incertae sedis	Sympodiella	Sympodiella	SAP	0	16	0	0	0	0	0	0	0	0	0	0	16
28	Ascomycota	Lecanoromycetes	Lecanorales	Byssolomataceae	Micarea	Micarea	SYM	0	0	0	1	0	0	0	0	0	0	0	0	1
29	Ascomycota	Leotiomycetes	Helotiales	Helotiaceae	Crocicreas	Crocicreas	SAP	0	18	3	0	0	0	0	0	0	0	0	0	21
30	Ascomycota	Leotiomycetes	Helotiales	Helotiaceae	Rhizoscyphus	Rhizoscyphus	SYM	0	0	0	0	0	11	0	0	0	0	0	0	11
31	Ascomycota	Leotiomycetes	Helotiales	Hyaloscyphaceae	Arachnopeziza	Arachnopeziza	SAP	0	0	6	0	0	0	0	0	0	0	0	0	6
32	Ascomycota	Leotiomycetes	Helotiales	Hyaloscyphaceae	Polydesmia	Polydesmia	PAR	0	8	0	0	0	0	0	0	0	0	0	0	8
33	Ascomycota	Leotiomycetes	Helotiales	Incertae sedis	Cadophora	Cadophora	PAR	19	7	4	2	15	0	0	0	0	0	0	0	47
34	Ascomycota	Leotiomycetes	Helotiales	Phacidiaceae	Allantophomopsis	Allantophomopsis	PAR	0	2	2	0	0	0	0	0	0	0	0	0	4
35	Ascomycota	Leotiomycetes	Helotiales	unidentified	unidentified	Unknown09	SAP	86	226	486	23	71	140	140	372	163	199	252	16	2174
36	Ascomycota	Leotiomycetes	Helotiales	Vibrisseaceae	Phialocephala	Phialocephala	SYM	0	0	0	55	22	116	0	0	0	0	0	0	193
37	Ascomycota	Leotiomycetes	Incertae sedis	Incertae sedis	Geomyces	Geomyces	SAP	1	9	68	9	13	28	0	0	0	0	0	0	128
38	Ascomycota	Leotiomycetes	Incertae sedis	Incertae sedis	Sarea	Sarea	SAP	0	0	0	12	0	0	0	0	0	0	0	0	12
39	Ascomycota	Leotiomycetes	unidentified	unidentified	unidentified	Unknown10	PAR	0	17	0	0	0	0	0	0	0	0	0	0	17
40	Ascomycota	Orbiliomycetes	Orbiliales	Orbiliaceae	Monacrosporium	Monacrosporium	SAP	0	0	6	0	0	0	0	0	0	0	0	0	6
41	Ascomycota	Orbiliomycetes	Orbiliales	Orbiliaceae	unidentified	Unknown11	PAR	0	0	0	0	9	0	0	0	0	0	0	0	9
42	Ascomycota	Pezizomycetes	Pezizales	Pyronemataceae	Scutellinia	Scutellinia	SAP	0	5	0	0	0	0	0	0	0	0	0	0	5
43	Ascomycota	Pezizomycetes	Pezizales	Pyronemataceae	Sphaerosporella	Sphaerosporella	SAP	0	0	0	0	0	6	0	0	0	0	0	0	6
44	Ascomycota	Pezizomycetes	Pezizales	Tuberaceae	Tuber	Tuber	SYM	0	2	3	0	0	0	0	0	0	0	0	0	5
45	Ascomycota	Pezizomycetes	Pezizales	unidentified	unidentified	Unknown12	SAP	0	0	0	1	0	0	0	0	0	0	0	0	1
46	Ascomycota	Saccharomycetes	Saccharomycetales	unidentified	unidentified	Unknown13	U	0	0	10	0	0	0	0	0	0	0	0	0	10
47	Ascomycota	Saccharomycetes	unidentified	unidentified	unidentified	Unknown14	SAP	89	61	337	8	11	6	0	0	0	0	0	0	512
48	Ascomycota	Sordariomycetes	Chaetosphaeriales	Chaetosphaeriaceae	Chaetosphaeria	Chaetosphaeria	SAP	0	70	5	0	0	4	11	24	0	10	0	3	127
49	Ascomycota	Sordariomycetes	Chaetosphaeriales	unidentified	unidentified	Unknown15	SAP	0	5	14	0	0	0	0	0	0	0	0	0	19

50	Ascomycota	Sordariomycetes	Hypocreales	Bionectriaceae	Bionectria	Bionectria	PAR	0	7	0	0	0	0	0	0	0	0	0	0	7
51	Ascomycota	Sordariomycetes	Hypocreales	Clavicipitaceae	unidentified	Unknown16	SAP	0	46	25	25	13	8	46	21	16	0	0	0	200
52	Ascomycota	Sordariomycetes	Hypocreales	Cordycipitaceae	Isaria	Isaria	PAR	0	0	0	1	0	0	0	0	0	0	0	0	1
53	Ascomycota	Sordariomycetes	Hypocreales	Cordycipitaceae	Lecanicillium	Lecanicillium	PAR	0	0	9	0	0	7	16	0	0	0	0	0	32
54	Ascomycota	Sordariomycetes	Hypocreales	Cordycipitaceae	unidentified	Unknown17	PAR	0	0	0	0	0	0	0	0	0	57	49	44	150
55	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Hypomyces	Hypomyces	PAR	0	0	0	1	14	11	0	0	0	0	0	0	26
56	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Sepedonium	Sepedonium	PAR	0	6	0	0	0	0	0	0	0	0	0	0	6
57	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Trichoderma	Trichoderma	SAP	66	260	317	106	172	130	296	344	558	489	592	414	3744
58	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	unidentified	Unknown18	SAP	0	0	0	0	0	0	0	0	0	52	47	33	132
59	Ascomycota	Sordariomycetes	Hypocreales	Incertae sedis	Calcarisporium	Calcarisporium	PAR	5	8	4	0	0	0	0	0	0	0	0	0	17
60	Ascomycota	Sordariomycetes	Hypocreales	Incertae sedis	Ilyonectria	Ilyonectria	PAR	0	6	16	2	17	48	7	2	31	0	0	0	129
61	Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	Cylindrium	Cylindrium	SAP	0	0	0	0	0	0	8	0	0	0	0	0	8
62	Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	Fusarium	Fusarium	SAP	6	73	7	340	57	32	37	37	40	33	61	2	725
63	Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	Mariannaea	Mariannaea	SAP	10	12	2	0	0	0	14	0	0	0	0	0	38
64	Ascomycota	Sordariomycetes	Hypocreales	Ophiocordycipitaceae	Chaunopycnis	Chaunopycnis	PAR	0	0	0	0	0	0	12	0	7	8	12	0	39
65	Ascomycota	Sordariomycetes	Hypocreales	Ophiocordycipitaceae	unidentified	Unknown19	PAR	0	0	0	0	0	0	0	0	0	38	38	1077	1153
66	Ascomycota	Sordariomycetes	Hypocreales	unidentified	unidentified	Unknown20	SAP	28	479	116	56	89	20	74	562	171	73	119	35	1822
67	Ascomycota	Sordariomycetes	Incertae sedis	Glomerellaceae	Colletotrichum	Colletotrichum	PAR	4	19	22	22	10	10	10	5	0	0	0	0	102
68	Ascomycota	Sordariomycetes	Incertae sedis	Plectosphaerellaceae	unidentified	Unknown21	SAP	0	0	0	49	2	0	0	0	0	0	0	0	51
69	Ascomycota	Sordariomycetes	Incertae sedis	Plectosphaerellaceae	Verticillium	Verticillium	SAP	0	0	0	0	0	0	0	0	0	1	108	41	150
70	Ascomycota	Sordariomycetes	Sordariales	Cephalothecaceae	Cephalotheca	Cephalotheca	SAP	0	9	0	0	0	5	0	0	0	0	0	0	14
71	Ascomycota	Sordariomycetes	Sordariales	Chaetomiaceae	Chaetomium	Chaetomium	SAP	0	12	0	0	0	0	0	0	0	0	0	0	12
72	Ascomycota	Sordariomycetes	Sordariales	unidentified	unidentified	Unknown22	SAP	0	10	0	0	0	0	0	0	0	0	0	0	10
73	Ascomycota	Sordariomycetes	unidentified	unidentified	unidentified	Unknown23	SAP	0	7	0	0	0	0	1	0	0	0	0	0	8
74	Ascomycota	Sordariomycetes	Xylariales	Xylariaceae	Xylaria	Xylaria	SAP	0	16	0	0	0	0	22	0	0	0	0	0	38
75	Ascomycota	unidentified	unidentified	unidentified	unidentified	Unknown24	SAP	149	1771	416	374	301	129	297	990	980	0	0	0	5407
76	Basidiomycota	Agaricomycetes	Agaricales	Agaricaceae	Agaricus	Agaricus	SAP	0	0	0	0	2	0	0	0	0	0	0	0	2
77	Basidiomycota	Agaricomycetes	Agaricales	Agaricaceae	Cystolepiota	Cystolepiota	SAP	0	0	0	0	0	0	23	0	0	0	0	0	23
78	Basidiomycota	Agaricomycetes	Agaricales	Agaricaceae	Melanophyllum	Melanophyllum	SAP	0	6	0	0	0	0	73	0	0	0	0	0	79
79	Basidiomycota	Agaricomycetes	Agaricales	Amanitaceae	Amanita	Amanita	SYM	16	42	0	0	1	8	28	354	281	5	21	0	756
80	Basidiomycota	Agaricomycetes	Agaricales	Clavariaceae	unidentified	Unknown25	SAP	0	0	0	0	0	0	23	0	0	0	0	0	23
81	Basidiomycota	Agaricomycetes	Agaricales	Entolomataceae	Entoloma	Entoloma	SYM	0	1	3	0	5	0	0	0	0	0	0	0	9
82	Basidiomycota	Agaricomycetes	Agaricales	Hygrophoraceae	Hygrophorus	Hygrophorus	SYM	0	0	0	0	2	4	0	0	0	0	0	0	6
83	Basidiomycota	Agaricomycetes	Agaricales	Lyophyllaceae	Gerhardtia	Gerhardtia	SAP	114	605	166	8	0	0	0	0	0	0	0	0	893
84	Basidiomycota	Agaricomycetes	Agaricales	Marasmiaceae	Gymnopus	Gymnopus	SAP	0	284	1	135	10	0	858	103	3	214	7	0	1615
85	Basidiomycota	Agaricomycetes	Agaricales	Mycenaceae	Mycena	Mycena	SAP	0	177	0	0	0	0	2294	244	4	0	0	0	2719
86	Basidiomycota	Agaricomycetes	Agaricales	Mycenaceae	unidentified	Unknown26	SAP	0	0	0	0	0	0	435	65	6	0	0	0	506
87	Basidiomycota	Agaricomycetes	Agaricales	Pluteaceae	Pluteus	Pluteus	SAP	0	0	0	17	0	0	0	0	0	0	0	0	17
88	Basidiomycota	Agaricomycetes	Agaricales	Tricholomataceae	Gamundia	Gamundia	SAP	7	85	9	205	34	11	77	35	81	16	0	0	560
89	Basidiomycota	Agaricomycetes	Agaricales	Tricholomataceae	unidentified	Unknown27	SAP	0	0	0	0	0	0	1	0	23	0	0	0	24
90	Basidiomycota	Agaricomycetes	Atheliales	unidentified	unidentified	Unknown28	SAP	0	0	0	0	0	0	0	0	0	310	2	0	312
91	Basidiomycota	Agaricomycetes	Auriculariales	unidentified	unidentified	Unknown29	SAP	0	21	0	18	1	22	27	2	15	0	0	0	106
92	Basidiomycota	Agaricomycetes	Boletales	Boletaceae	Tylopilus	Tylopilus	SYM	0	0	0	95	83	27	0	0	0	0	0	0	205
93	Basidiomycota	Agaricomycetes	Boletales	Rhizopogonaceae	Rhizopogon	Rhizopogon	SYM	0	0	7	0	0	0	0	0	0	0	0	0	7
94	Basidiomycota	Agaricomycetes	Boletales	Suillaceae	Suillus	Suillus	SYM	0	0	0	0	0	0	1	15	0	0	0	0	16
95	Basidiomycota	Agaricomycetes	Boletales	unidentified	unidentified	Unknown30	SYM	130	59	779	31	35	271	66	1	760	0	0	0	2132
96	Basidiomycota	Agaricomycetes	Cantharellales	Ceratobasidiaceae	Ceratobasidium	Ceratobasidium	SAP	0	0	0	0	0	0	10	0	0	0	0	0	10
97	Basidiomycota	Agaricomycetes	Cantharellales	Clavulinaceae	Clavulina	Clavulina	SYM	0	0	0	104	86	83	66	69	189	0	0	0	597
98	Basidiomycota	Agaricomycetes	Cantharellales	Hydnaceae	Sistotrema	Sistotrema	SAP	0	0	0	0	15	27	0	0	0	0	0	0	42
99	Basidiomycota	Agaricomycetes	Cantharellales	unidentified	unidentified	Unknown31	SYM	0	0	0	0	0	0	7	0	0	0	0	0	7
100	Basidiomycota	Agaricomycetes	Geastrales	Geastraceae	Geastrum	Geastrum	SAP	0	0	0	0	0	0	41	0	0	0	0	0	41
101	Basidiomycota	Agaricomycetes	Hymenochaetales	Hymenochaetaceae	Inonotus	Inonotus	SAP	1	1	4	0	0	0	0	0	0	0	0	0	6

(*continued on next page*)

Table 5 (*continued*)

No.	Phylum	Class	Order	Family	Genus	GOTU	Ecol. type*	Reads of GOTU												
								4L	4H	4Ah	8L	8H	8Ah	10L	10H	10Ah	12L	12H	12Ah	Total
102	Basidiomycota	Agaricomycetes	Polyporales	Polyporaceae	Abundisporus	Abundisporus	SAP	0	0	0	3	1	0	0	0	0	0	0	0	4
103	Basidiomycota	Agaricomycetes	Polyporales	unidentified	unidentified	Unknown32	U	4	66	1	0	0	0	0	0	0	0	0	0	71
104	Basidiomycota	Agaricomycetes	Polyporales	Xenasmataceae	Xenasmatella	Xenasmatella	SAP	0	2	5	0	0	0	59	305	275	0	0	0	646
105	Basidiomycota	Agaricomycetes	Russulales	Lachnocladiaceae	Scytinostroma	Scytinostroma	SAP	0	1	44	0	0	0	0	0	0	0	0	0	45
106	Basidiomycota	Agaricomycetes	Russulales	Russulaceae	Russula	Russula	SYM	12	109	58	10	0	0	0	0	0	0	0	0	189
107	Basidiomycota	Agaricomycetes	Russulales	Russulaceae	unidentified	Unknown33	SYM	24	323	279	78	63	82	1076	207	482	143	145	349	3251
108	Basidiomycota	Agaricomycetes	Russulales	unidentified	unidentified	Unknown34	SYM	0	0	0	0	0	0	27	15	28	0	0	0	70
109	Basidiomycota	Agaricomycetes	Sebacinales	Sebacinaceae	Sebacina	Sebacina	SAP	0	0	0	28	8	0	10	0	0	0	0	0	46
110	Basidiomycota	Agaricomycetes	Sebacinales	Sebacinaceae	unidentified	Unknown35	SYM	1	1	3	0	0	0	101	126	264	0	0	0	496
111	Basidiomycota	Agaricomycetes	Thelephorales	Thelephoraceae	Tomentella	Tomentella	SAP	0	0	0	0	0	0	28	29	47	0	0	0	104
112	Basidiomycota	Agaricomycetes	Thelephorales	Thelephoraceae	unidentified	Unknown36	SYM	18	228	41	0	0	0	0	0	0	0	0	0	287
113	Basidiomycota	Agaricomycetes	Trechisporales	Hydnodontaceae	Trechispora	Trechispora	SAP	0	0	0	0	6	10	67	69	30	0	0	0	182
114	Basidiomycota	Agaricomycetes	Trechisporales	unidentified	unidentified	Unknown37	SAP	9	208	13	192	236	0	52	47	45	0	0	0	802
115	Basidiomycota	Microbotryomycetes	Sporidiobolales	Incertae sedis	Rhodotorula	Rhodotorula	PAR	0	0	0	21	0	0	0	0	0	0	0	0	21
116	Basidiomycota	Microbotryomycetes	unidentified	unidentified	unidentified	Unknown38	PAR	12	21	86	62	69	19	0	0	0	0	0	0	269
117	Basidiomycota	Tremellomycetes	Cystofilobasidiales	unidentified	unidentified	Unknown39	SAP	0	0	0	6	0	0	8	0	0	0	0	0	14
118	Basidiomycota	Tremellomycetes	Tremellales	Incertae sedis	Fellomyces	Fellomyces	SYM	0	0	9	0	0	0	0	0	0	0	0	0	9
119	Basidiomycota	Tremellomycetes	Tremellales	Incertae sedis	Trimorphomyces	Trimorphomyces	SAP	0	0	0	11	0	0	0	0	0	0	0	0	11
120	Basidiomycota	Tremellomycetes	Tremellales	Tremellaceae	Cryptococcus	Cryptococcus	SAP	4	79	9	7	0	0	477	1078	868	1452	1239	383	5596
121	Basidiomycota	Tremellomycetes	Tremellales	unidentified	unidentified	Unknown40	SAP	9	83	204	15	36	51	0	0	0	1	7	10	416
122	Basidiomycota	Tremellomycetes	unidentified	unidentified	unidentified	Unknown41	SAP	4	7	2	54	16	19	0	0	0	0	0	0	102
123	Basidiomycota	unidentified	unidentified	unidentified	unidentified	Unknown42	SAP	1	40	107	76	62	147	11	0	42	0	0	0	486
124	Basidiomycota	Wallemiomycetes	Geminibasidiales	Geminibasidiaceae	Geminibasidium	Geminibasidium	SAP	41	140	494	73	29	92	6	110	263	0	0	0	1248
							Sum	990	6686	5925	2695	1988	2060	7147	5686	6384	3376	3113	3887	49,937

* Ecological living type: U = unknown; SAP = saprophyte; SYM = symbiont; PAR = parasite.

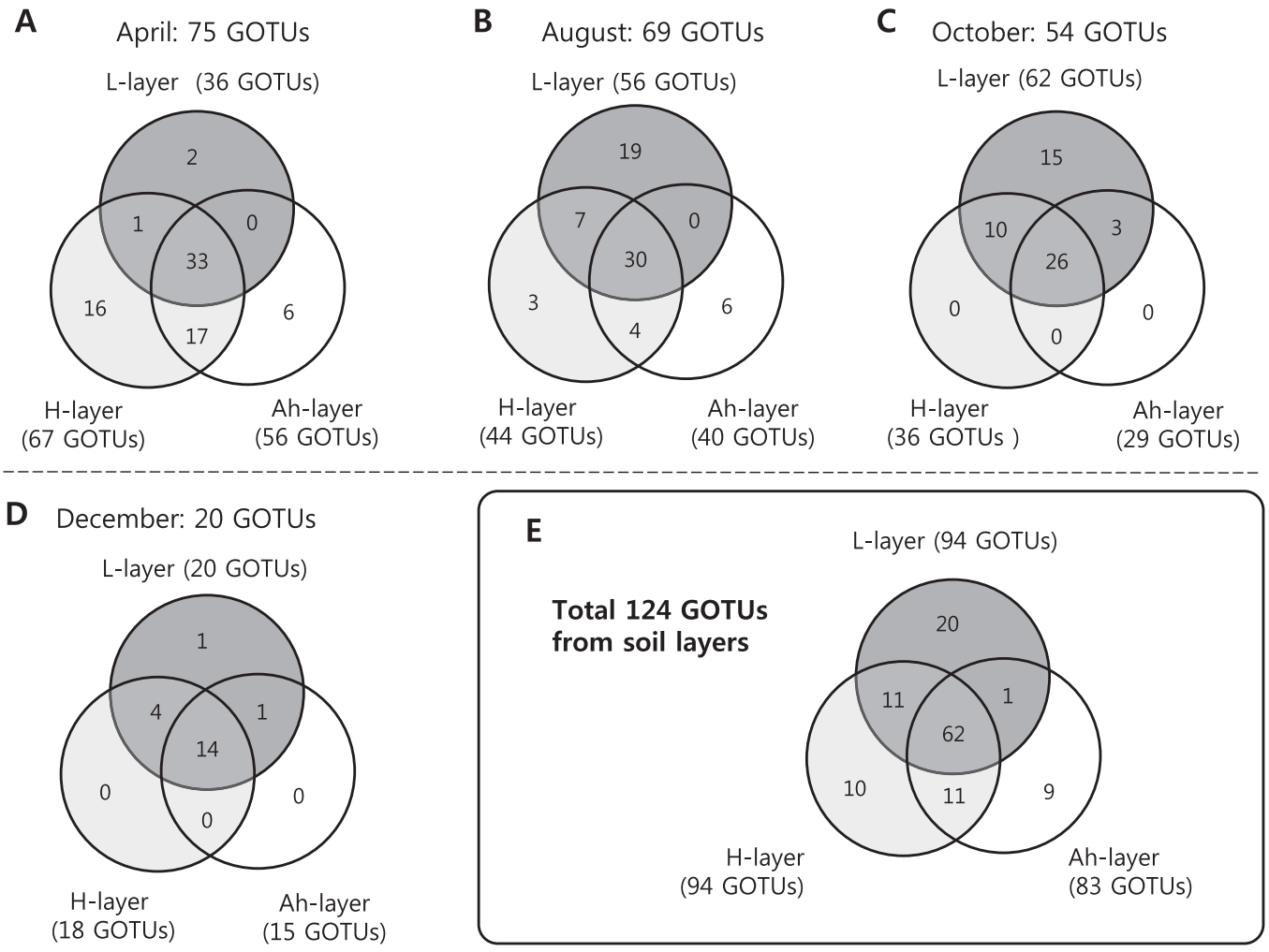

Figure 5. Comparison between soil fungi of each soil-layers based on genus-level operational taxonomic units (GOTUs): Venn diagrams show overlapping and nonoverlapping GOTUs a recovered. A, GOTUs in April; B, GOTUs in August; C, GOTUs in October; D, GOTUs in December; and E, total GOTUs.

example, the genus *Cryptococcus* represented 0.68% of the sequence reads in April and 29.6% in December, while Unknown24 represented 17.2% of all reads in April and 0.0% in December (Table 5). These seasonal changes were also clearly demonstrated by the NMDS ordination plot (Figure 7). The results of NMDS analysis revealed the relationship between fungal community structure and environmental factors. Each point on the ordination plot represents sampling sites during four seasons. The distances between samples in the ordination plot indicate the similarity level, as characterized by the Bray–Curtis distance. To investigate the validity of the NMDS ordination analysis, we performed a stress plot analysis (data not shown); this analysis indicated that the Bray–Curtis distance and the ordination distances were highly correlated ($R^2 = 0.995$). The direction and position of two environmental factors (exchangeable K and C/N ratio) on the ordination plot, calculated with the envfit function in the vegan package (Oksanen 2015), represented the gradient and the strength of the correlation between the environmental factor and the ordination. The vectors provided a good graphical representation ($p < 0.05$) to identify relationships between environmental gradients and patterns in fungal community structure (Figure 7). Our previous study of a Mongolian oak-dominated forest revealed that the composition of soil fungal communities was influenced by water retention and pH, and the ecological type was affected by seasonal change (Kim et al 2016). By contrast, the soil fungal communities in the Korean red pine stand investigated in the current study were significantly affected by the C/N ratio and exchangeable K; the ecological type was only slightly affected by seasonal change (see Figures 7 and 8). Generally, C, N, and K are important components of the soil, supporting the plant growth (O'Brien et al 2005; Lindahl et al 2007; Kaiser et al 2016). Apparently, the forest stands maybe impact the composition of soil fungal communities—the soil fungal diversity in a Mongolian oak-dominated forest (Kim et al 2016) and in the Korean red pine stand were achieved at same times. Although our ecological data were still insufficient to interpret between these two forest stands, they could be used for comparison studies of various forest stands through sustainably ecological data accumulation.

Composition of the soil fungi communities according to the ecological types

According to season, the following ecological types were represented: in April, unknown (2.7%), saprophyte (62.7%), symbiont (13.3%), and parasite (21.3%); in August, saprophyte (60.9%),

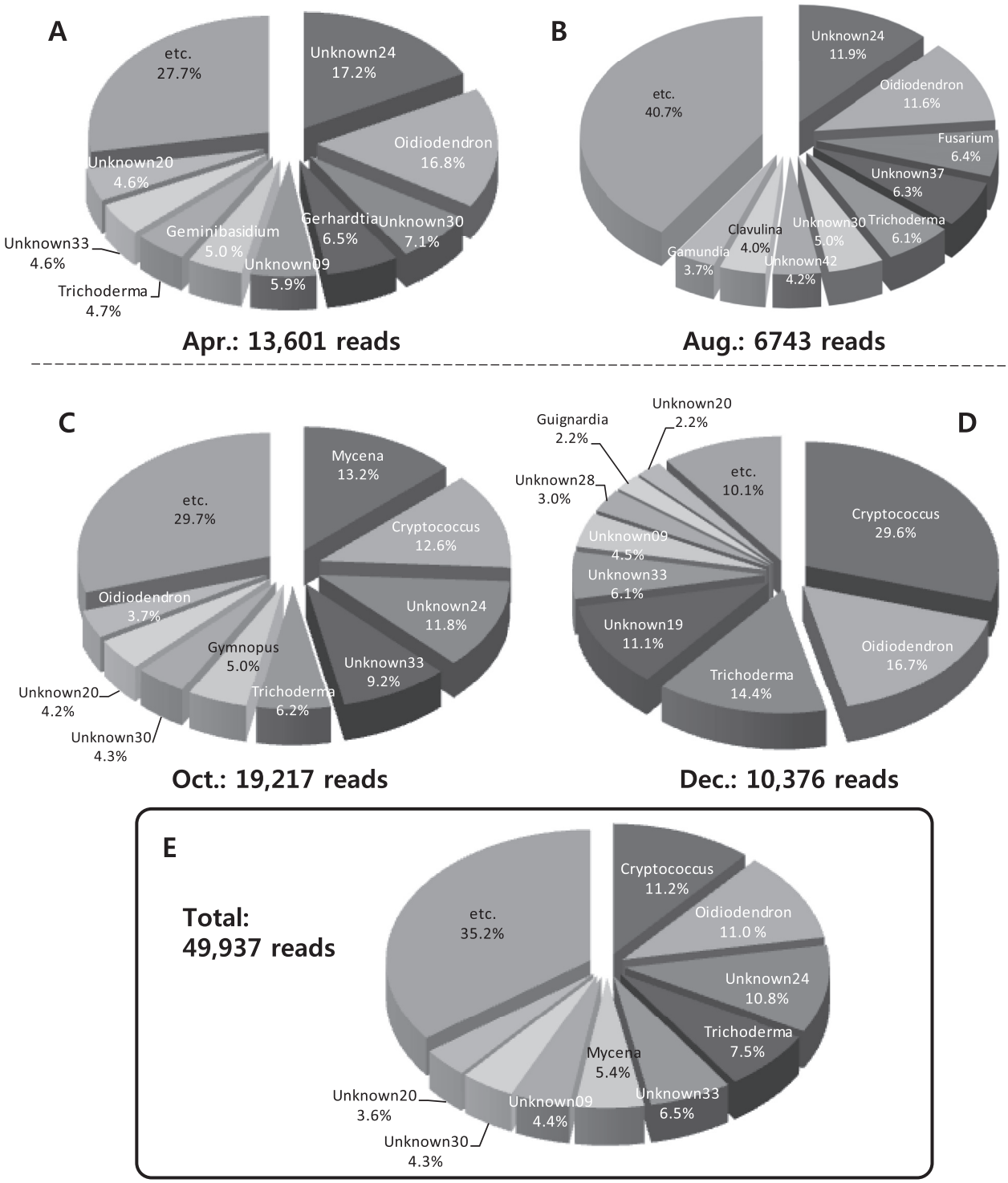

Figure 6. Composition rates of soil fungi in the Korean red pine stand. A, Genus-level operational taxonomic unit (GOTU) composition rate in April; B, GOTU composition rate in August; C, GOTU composition rate in October; D, GOTU composition rate in December; and E, total GOTU composition rate. Only the nine most dominant GOTUs are presented.

symbiont (17.4%), and parasite (21.7%); in October, saprophyte (68.5%), symbiont (16.7%), and parasite (14.8%); and in December, saprophyte (70.0%), symbionts (10.0%), and parasite (20.0%; Figure 8). According to the soil layer, the ecological types were represented as follows: in the L-layer, unknown (1.1%), saprophyte (60.6%), symbiont (16.0%), and parasite (22.3%); in the H-layer, unknown (1.1%), saprophyte (60.6%), symbiont (16.0%) and parasite (22.3%); in the Ah-layer, unknown (2.4%), saprophyte (56.6%), symbiont (20.5%), and parasite (20.5%); and collectively, unknown (1.6%), saprophyte (59.7%), symbiont (16.9%), and parasite (21.8%; Figure 8). The incidence of parasites in the Korean red pine stand was higher than that observed in a previous study of a Mongolian oak-dominated forest (Kim et al 2016). Although the overall forest health condition was not established in the current study, these basic data might nonetheless be used as evaluation indices of the forest health condition.

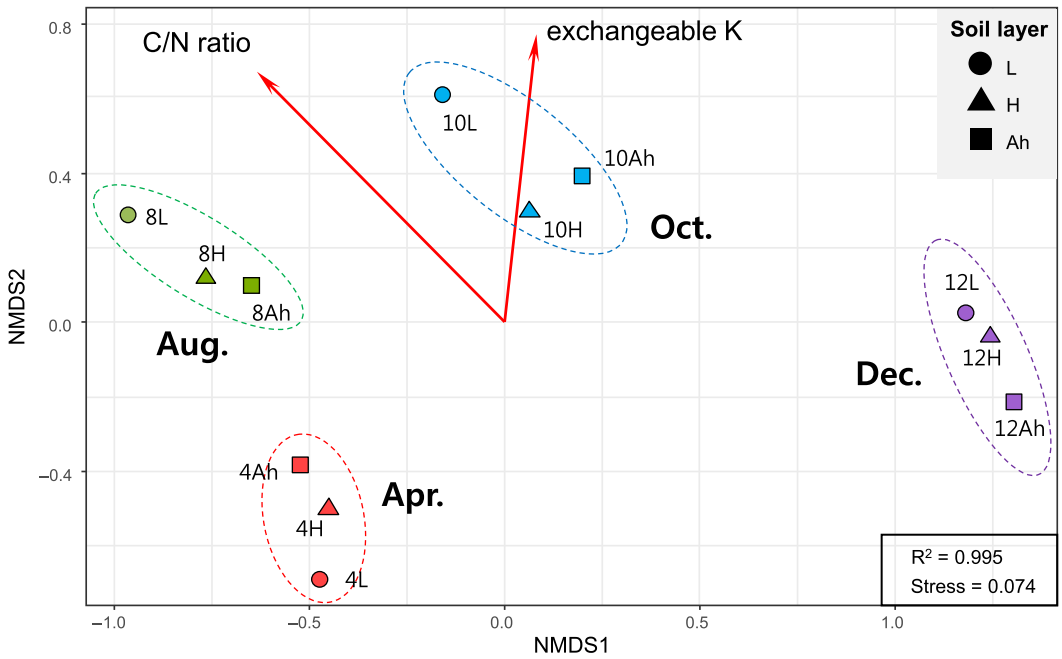

Figure 7. Nonmetric multidimensional scaling (NMDS) ordination plot of Bray–Curtis community dissimilarities based on sequence reads of genus-level operational taxonomic units. It shows that the fungal communities cluster base on well sampling seasons. Significant correlations ($p < 0.05$) of two environmental factors (exchangeable K and C/N ratio) were represented as vectors (exchangeable K, $p = 0.013$; C/N ratio, $p = 0.001$).

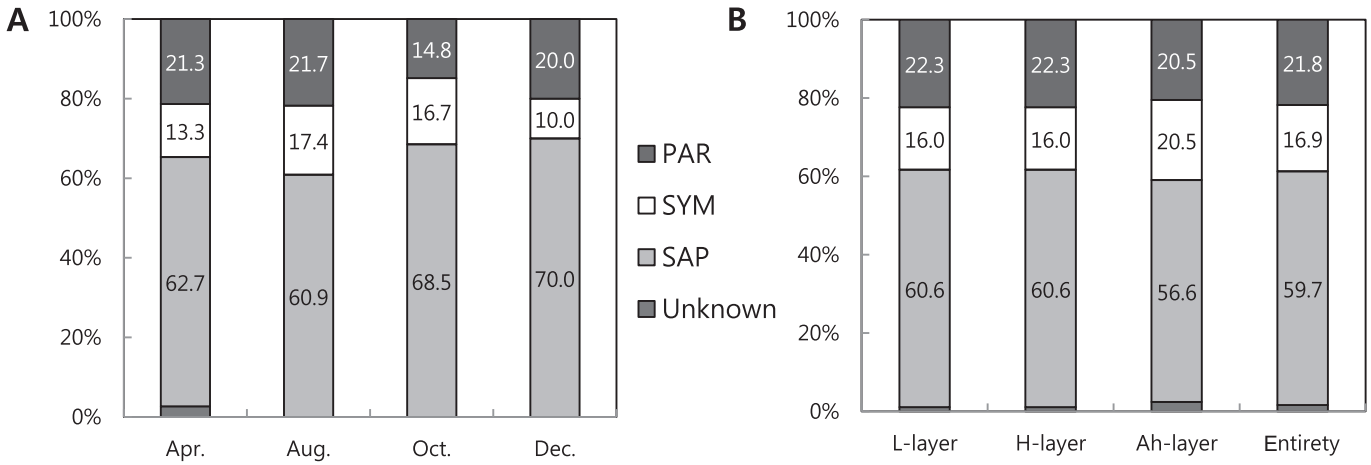

Figure 8. Composition of soil fungi based on ecological type (unknown, saprophytes, symbiont, and parasites) based on pyrosequencing data. A, Composition according to seasons and B, composition according to soil-layers.

Conclusion

In this study, we report the ecological data for a Korean red pine stand, the Gwangneung Forest; these data comprise observations of a macrofungal collection, soil fungi community sequencing, and environmental factor measurements. We anticipate that our study will facilitate the understanding of the relationship between Korean red pine and soil fungal communities during seasonal change. In addition, these data may constitute basic reference informing forest conservation efforts. The soil fungal communities are important for the ecosystem and for the organic matter cycle, but large gaps in our knowledge of their diversity and ecology still exist. Hence, it is necessary to continuously study fungal diversity in the soils of various forest stands to facilitate biodiversity conservation in the forest and forest management policies in Korea and elsewhere. This approach will also lead to a better understanding of the Gwangneung Forest ecosystems.

Conflicts of interest

The authors declare that there is no conflicts of interest.

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