이학박사 학위논문

Pinus densiflora root mycobiome: Diversity and community patterns according to spatial and temporal distance

소나무 뿌리 진균 군집의 공간적, 시간적 거리에 따른 다양성 및 군집 변화

2021 년 8 월

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Ph.D. Dissertation of Science

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Abstract

Pinus densiflora root mycobiome: Diversity and community patterns according to spatial and temporal distance

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Advance in sequencing technologies including NGS (next generation sequencing) enabled the researchers to study interactions between microbial communities and surrounding organisms from environmental samples using DNA metabarcoding. Fungi, one of the major parts of microbial community, are key components of forest ecosystems, with various ecological roles in plant roots and surrounding soil. Plant-associated fungal communities improve the fitness of host plant by the supply of essential nutrients from soil, improving the productivity, suppressing the activity of plant pathogens, and reducing biotic and abiotic stress, though some of them act as opportunistic pathogens. Several tree species have obligatory relationships with fungal communities, such as ectomycorrhizal tree species of Pinaceae. One of such species, *Pinus densiflora* Siebold & Zucc. (red pine) is the

dominant tree species in Korea with ecologically and economically importance, occupying the largest habitat area as a single tree species. Due to climate change and plant diseases, its habitat is constantly decreasing, and according to recent studies predicted that the habitat of *P. densiflora* will move to higher elevations and latitudes. Therefore, the investigation on spatial and temporal changes of *P. densiflora* associated fungal community is needed to maintain the fitness and minimized the habitat loss of *P. densiflora*. Further, differences in analysis results according to sequencing technique were identified to check precautions in the interpretation of microbiome analysis results.

In chapter 1, spatial change of fungal communities associated with *P. densiflora* was investigated at macro- and micro- scales. While the influences of spatial distances were reported in various organisms, only a few studies have been conducted in fungal communities. To understand their influence, root and soil samples of 80 *P. densiflora* trees were collected in 16 *P. densiflora* forests. The effect of altitude, geographic distance, and microhabitat was investigated for the composition and richness of mycobiome associated with *P. densiflora*. The altitude, geographic distance, and microhabitat all had a significant influence on fungal communities. The richness and composition of root-inhabiting fungi were influenced by both altitude and geographic distance, but only altitude had significant influence on soil-inhabiting fungal communities. This study revealed that root-inhabiting fungi were more sensitive to spatial change, and provided basis for understanding the relationships between fungal communities and other host plants in different microhabitats.

In chapter 2, the biases and differences of fungal community analysis were investigated between high-throughput sequencing methods. In contrast to previously used short-read NGS platforms with limited length of sequences, long-read NGS platforms are expected to improve the identification resolution by using the full ITS (internal transcribed spacer) barcode regions. However, the relatively low throughput and high error rate are drawbacks of long-read NGS platforms. To identify the influence of NGS platforms, *P. densiflora* root mycobiome datasets from PacBio Sequel (long-read NGS) platform and Illumina MiSeq (short-read NGS) were compared. The taxonomic identity of dominant taxa did not show significant differences, but their abundances were significantly different. The taxonomic resolution was similar at the family level, but the proportion of unidentified taxa was significantly higher at the genus level in the PacBio dataset. In addition, due to low throughput, PacBio datasets were more sensitive to differences in abundance between sampling sites than MiSeq datasets. Overall, significant influence of sequencing platforms was found in mycobiome analysis, which represent precautions in interpretation of NGS results. Meanwhile, the presence of economically important plant pathogen and gourmet mushrooms with low abundance and frequency were found, suggesting the possibility of monitoring fungal species with DNA metabarcoding from environmental samples.

In chapter 3, the influence of temporal distance was investigated in fungal communities associated with seedlings of *P. densiflora*. The growth of host plant changes the composition and function of fungal communities by replacing early-stage pioneer species by more competitive late colonizers. To understand the succession of fungal communities, *P. densiflora* seedlings were

cultivated for three years and their root mycobiome were investigated. Significant succession in the composition of root fungal communities was observed, with the increase in diversity following the growth of host plant. In addition, functional changes during the succession were predicted using PICRUST2 and keystone species with the important roles in the interaction of fungal species were identified using network analysis.

This study investigated the change of fungal communities according to the spatial and temporal distances to understand the structure of mycobiome associated with *P. densiflora*. This work can be applied to preservation and reforestation of *P. densiflora* habitats. Furthermore, NGS platform bias found in this mycobiome study should be considered in future study on fungal community associated with other tree species. Finally, many taxa found in this study were not reported in National List of species of Korea, which provides valuable data in excavation of new and undescribed species. The part of this thesis includes manuscripts that were submitted to peer-reviewed journals as a fulfilment of Ph.D. courses.

Keyword : *Pinus densiflora*, mycobiome, spatio-temporal distance, biodiversity, root fungi, soil fungi

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General Introduction

Plants harbor various microbial organisms with either positive or negative influences on the fitness of the host plants. Those plant-associated microbes, or plant microbiota form close interactions with their host plants. Fungi are one of the most diverse group in plant microbiota, and they perform diverse roles in plants, including root and surrounding soil (Johnson *et al.*, 1997; Baldrian, 2016). Plant-associated fungal community, or mycobiome is composed of a variety of symbiotrophic, saprotrophic, and pathogenic fungi (Fig. 1).

In root mycobiome, symbiotrophic mycorrhizal fungi, such as ectomycorrhizal fungi, arbuscular mycorrhizal fungi, ericoid mycorrhizal fungi, or orchid mycorrhizal fungi are one of the most studied fungal guilds. They form mutualistic symbioses with most of the plants, exchanging mineral nutrients for photosynthetic products (Smith & Read, 2008; Baldrian, 2016; Genre *et al.*, 2020). They often feature host specificity, especially in ectomycorrhizal and orchid mycorrhizal fungi (Tedersoo *et al.*, 2020). However, most of them were reported to improve the productivity and promote the tolerance to abiotic and biotic stresses by supporting water and nutrient absorption from soil or eliciting plant immune responses (Bahadur *et al.*, 2019; Genre *et al.*, 2020). Besides mycorrhizal fungi, endophytic fungi or saprotrophic endophytic fungi are inhabitants of root microhabitats, and several ericoid mycorrhizal species were reported to act as endophyte in non-ericoid mycorrhizal plants. Those fungi are commonly found in plant roots (Hiruma *et al.*, 2016; Almario *et al.*, 2017), and sometimes affect negative influence on their hosts in a form

of opportunistic pathogen. However, in most cases, they improve the stress tolerance of host plants by facilitating the nutrient acquisition and suppressing the activity of plant pathogens (Van Der Heijden *et al.*, 2008a; Hiruma *et al.*, 2016; Almario *et al.*, 2017; Bahadur *et al.*, 2019). In contrast to mycorrhizal fungi that are mostly composed of Ascomycota, Basidiomycota, or Mucormycota, endophytic fungi feature much wider taxonomic range (van der Heijden *et al.*, 2015).

Soil mycobiome are also composed of various species that redistribute carbon from plants to soil (Lindahl *et al.*, 2007; Baldrian, 2008). They have close relationship with adjacent roots, dead or alive. Mycorrhizal fungi extend from root and form mycelial network around soil. The mycelial network connects multiple trees and understory vegetations, transporting nutrients from soil or dead organic matter to plant (Högberg *et al.*, 1999; Lindahl & Tunlid, 2015). in temperate forests, ectomycorrhizal mycelial network occupy large quantity of biomass, that may take up to one third of total microbial biomass in soil (Högberg & Högberg, 2002). In addition, saprotrophic fungi are other major inhabitants of soil microhabitats. They decompose deadwood and other organic matter, contributing for nutrient cycle in soil ecosystem (Lindahl *et al.*, 2007; Baldrian, 2008). Because of their essential roles, plant-associated fungal communities can be applied for forest management, reforestation, bioremediation, and seedling production of conifer or broadleaf trees (Baldrian, 2008; Pietro-Souza *et al.*, 2020; Genre *et al.*, 2020).

Plant microbiome are also affected by various abiotic and biotic factors in addition to their habitats (Fig. 2A). Soil properties (pH, soil carbon, nitrogen), climate (temperature, precipitation), host identity, and plant genotypes are well known factors that affect plant

microbiome composition. Further, spatial distance coupled with altitude or geographic distances also limit the dispersal, causing mycobiome dissimilarity over broad areas (Talbot *et al.*, 2014; Cobian *et al.*, 2019; Bernard *et al.*, 2020). However, most of studies were conducted on bacterial communities (Bryant *et al.*, 2008; Fierer *et al.*, 2011; Sundqvist *et al.*, 2013), only a few studies were focused on fungal communities. Those studies also have their limitations. Most of those studies were conducted on specific guilds (ectomycorrhizal or arbuscular mycorrhizal fungi), and multiple plant species were targeted, thus resulting a greater effect of host identity than spatial distance. (Tedersoo *et al.*, 2012; Bahram *et al.*, 2013; Geml, 2017; Zhang *et al.*, 2017). Similarly, the fungal communities in soil and root microhabitats have distinct composition and guilds, the different influences of spatial distance on mycobiome in both microhabitats or different guilds have been seldom studied (Zhang *et al.*, 2017; Yao *et al.*, 2017; Bernard *et al.*, 2020). While those studies have provided valuable insights on the relationship between mycobiome and spatial distances, their relationship is still unclear.

The temporal change in community composition, or succession is generally reported in plant, characterized by soil development and structural change in vegetation. It naturally occurs when new substrates are exposed or deposited, such as mine spoils, glacier retreat, or lava flows. In contrast, the influence of temporal distances was less studied in plant-associated fungal communities (Fig. 2C). Mycobiome succession studies were mostly conducted on root decomposition (Kohout *et al.*, 2021), mine spoils (Krüger *et al.*, 2017; Kolaříková *et al.*, 2017), or retreat of glacier forefront (Mühlmann & Peintner, 2008). Similar studies were reported in woody plants in temperate forests,

which revealed temporal change in fungal community composition following the growth of mature trees (Twieg *et al.*, 2007) and young seedlings (Matsuda *et al.*, 2009). The majority of succession dynamics study in fungal communities have been focused on specified guilds, including ectomycorrhizal fungi, arbuscular mycorrhizal fungi, or saprotrophic fungi, according to target substrates. However, the study on inter-relationship between fungi are needed to understand the influence of fungal communities during the growth of plants, as mycobiome with intimate relationship with host plants (i.e. mycorrhizal fungi, endophytic fungi, pathogens) have complex interactions with each other (Brundrett, 2007; Almario *et al.*, 2017; Kolaříková *et al.*, 2017).

The advance in NGS (next generation sequencing), or HTS (high-throughput sequencing) techniques made those ecological studies (Fig. 2B). Previously used traditional methods (cultivation, sporocarp sampling, morphological identification) have limitations due to unculturable microbes and difficulties in precise identification of cryptic species. NGS-based DNA metabarcoding approach have revealed the presence of unculturable microbes and facilitated the ability to explore interactions between those microbes and surrounding organisms including human (Methé *et al.*, 2012). In ecological studies, the use of environmental DNA obtained from environmental samples (soil, water, air) enabled more efficient sampling approach in large-scale diversity monitoring (Thomsen & Willerslev, 2015). This is achieved by amplifying the conserved marker genes for taxonomic groups, such as 16S rRNA for bacteria and archaea, internal transcribed spacer (ITS) for fungi, or cytochrome oxidase c subunit I (COI) for

animals, and comparing those sequences to reference sequences databases.

The most commonly used NGS technique was second-generation sequencing platforms, such as Illumina, 454 pyrosequencing, or Ion Torrent. Those NGS platforms can generate much higher throughput of sequences compared to Sanger sequencing (Yang *et al.*, 2018), but they suffer from lower taxonomic resolution as they can sequence only short length sequences in marker genes. The third-generation sequencing platforms such as PacBio or nanopores can generate much longer sequences and are expected to improve the taxonomic resolutions (Nilsson *et al.*, 2019). However, they produce much fewer reads than second-generation platforms, and have suffered from low read quality until recently, while the read quality was improved to the level of Sanger sequencing in newer platforms (Walder *et al.*, 2017; Tedersoo *et al.*, 2018). In addition, regardless of NGS platforms, errors in sequencing and PCR, different fidelity of marker genes across taxonomic groups, false positive and negative results from primer bias, relic DNA, or chimeric sequences are challenges in the result interpretation and identification processes (Nilsson *et al.*, 2019; Vu *et al.*, 2019; Garlapati *et al.*, 2019).

In this dissertation, *Pinus densiflora*, or red pine was selected as target species. *P. densiflora* has obligatory relationship with ectomycorrhizal fungi, similar to other members of Pinaceae (Dickie *et al.*, 2010). While those plants can mature with fertilizer additions in the absence of ectomycorrhiza, the survival rate of nonmycorrhizal seedlings are extremely low (Mikola, 1970; Hayward *et al.*, 2015). In South Korea, *P. densiflora* is the most dominant tree species, covering about one fourth of forest area (KFRI, 2016). While this species is

culturally and economically important, its habitat is gradually declining due to climate warming and plant diseases such as pine wilt disease. According to recent studies, the *P. densiflora* habitat is predicted to move northward and to higher elevation (Hirata *et al.*, 2017; An *et al.*, 2019; Cho *et al.*, 2020).

The objective of this dissertation is overall investigation of plant mycobiome associated with *P. densiflora* using NGS-based DNA metabarcoding methods for management and protection of *P. densiflora* habitats. In order to accomplish this objective, the influence of spatial and temporal distances on mycobiome associated with *P. densiflora* was investigated in various directions (Figs. 2 and 3). In chapter 1, root and soil samples were collected from *P. densiflora* forests across South Korea to study the influence of spatial distance on the composition and diversity of fungal communities were investigated in macro- and microscale. Based on this results, the different effect of spatial distances on mycobiome was verified at microhabitat and guilds levels. In chapter 2, results from two different NGS platforms were compared to find the influence of NGS platform and target regions in DNA metabarcoding studies. In this comparison, I selected short-read Illumina MiSeq, the most commonly used NGS platform and long-read PacBio Sequel platform, that has recently begun to be used in microbiome study. In chapter 3, the temporal succession of root mycobiome was studied following the growth of *P. densiflora* seedlings over 3 years.

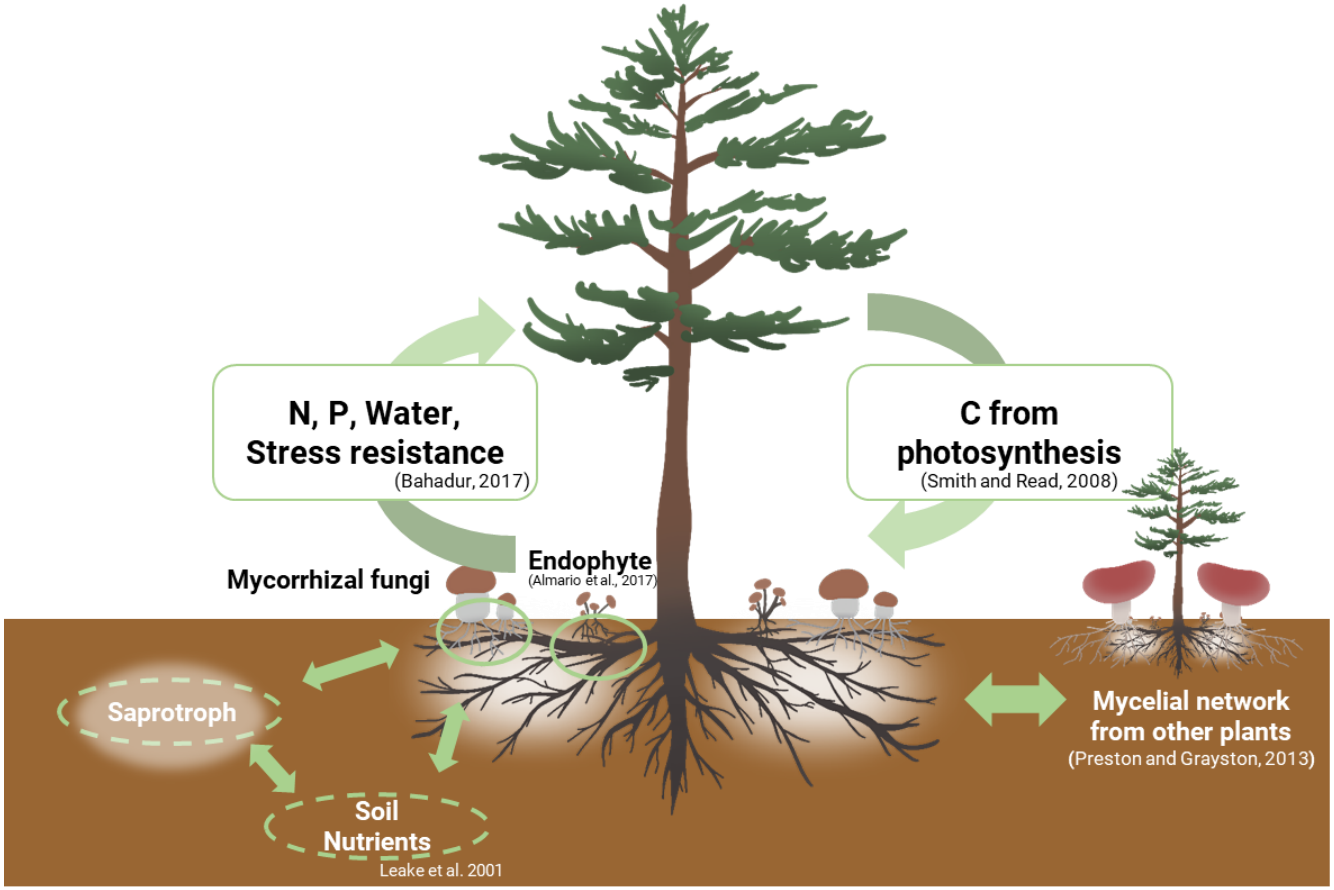

Figure 1. The major interactions between plant-associated fungi and host plants in belowground

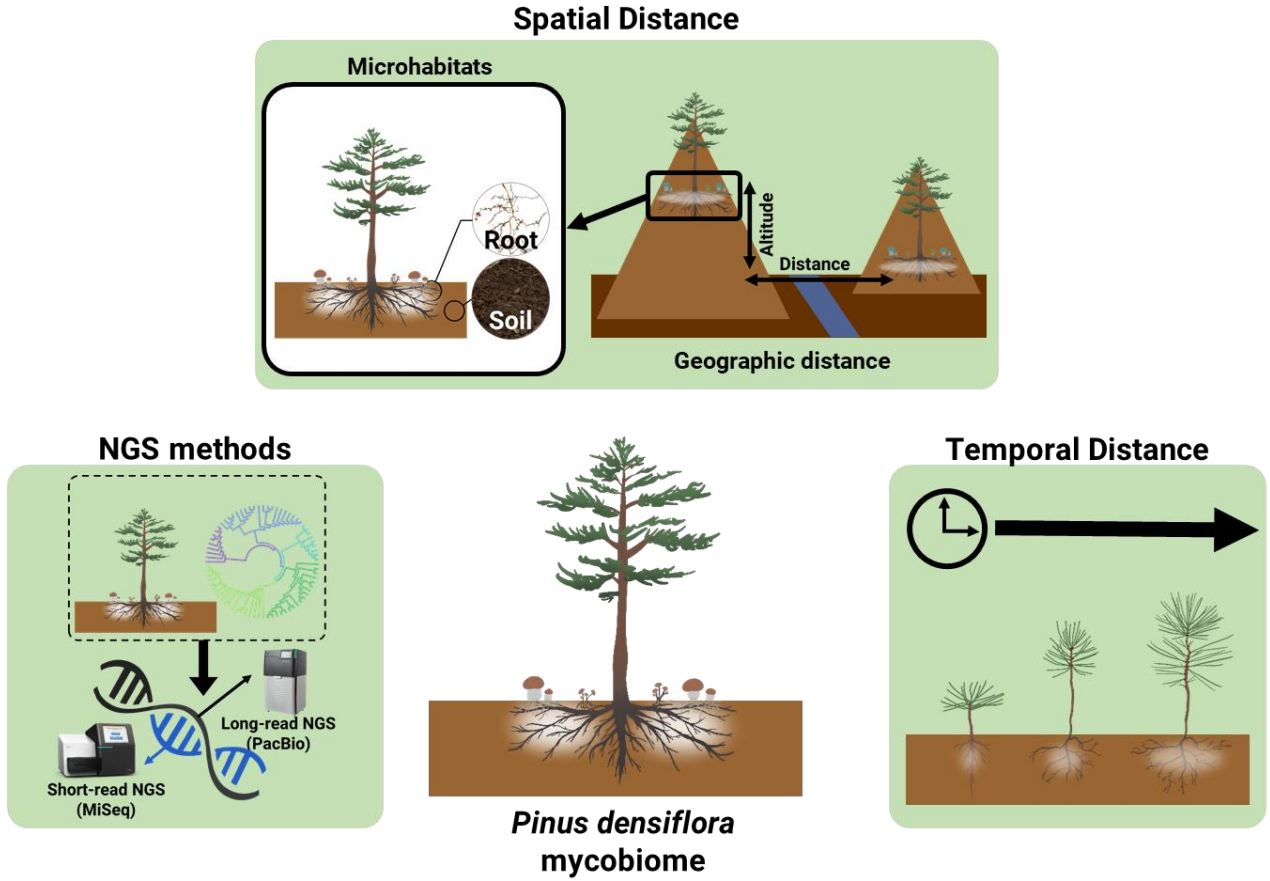

Figure 2. Proposed scheme for investigation of mycobiome associated with *Pinus densiflora*.

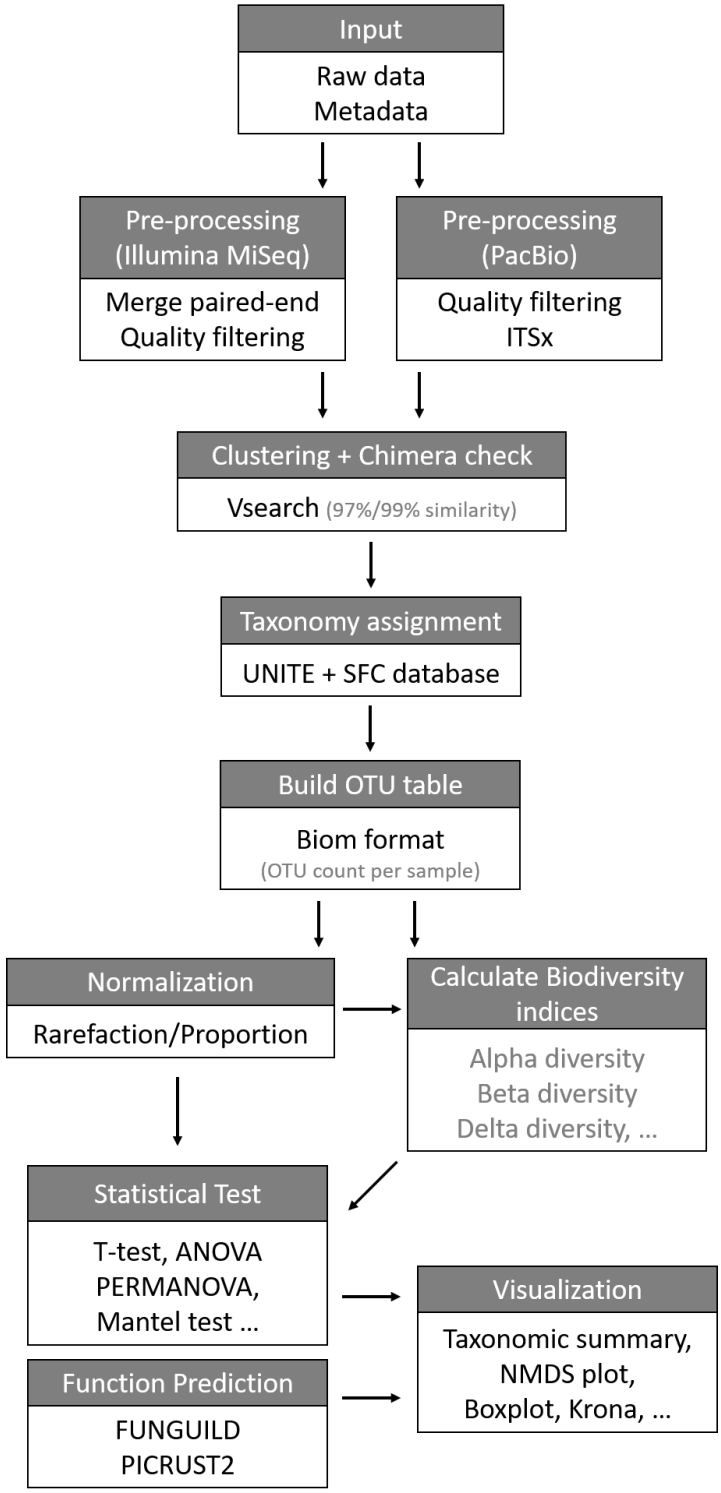

Figure 3. Workflow for processing metabarcoding data and statistical analysis.

Chapter 1. Different patterns of belowground fungal diversity along spatial distance with respect to microhabitat and guild types

Abstract

Fungi are key components of belowground ecosystems with various ecological roles in forests. Although the changes in the richness and composition of belowground fungi across altitudinal gradients have been widely reported, only a few studies have focused on the microhabitat types along altitudinal gradients. Here, I analyzed the effect of altitude on the ectomycorrhizal and non-ectomycorrhizal fungal communities in belowground microhabitats. The root and soil samples were collected from sixteen *Pinus densiflora* forests at various altitudes across Korea, and the soil properties were measured as potential factors. Fungal communities were analyzed by high-throughput sequencing of the internal transcribed spacer 2 (ITS2) region. The altitude negatively affected the species richness of root-inhabiting fungi but did not influence that of soil-inhabiting fungi. In addition, the composition of ectomycorrhizal (ECM) fungi was less influenced by altitude than non-ECM fungi. Most of the soil properties did not show a significant relationship with altitude, but the effect of soil properties was different across microhabitat types and ecological roles of fungi. This results reveal that microhabitat types and altitudinal gradients differently affect the richness and composition of fungal communities associated with *P. densiflora*, providing a better understanding of plant-associated fungal communities.

1.1. Introduction

Belowground fungi play an important role in nutrient cycle of forest ecosystems (Baldrian, 2016; Peay *et al.*, 2016). Distinct fungal communities widely interact along a continuum of mutualism to parasitism with vascular plants and other microbes in root and soil microhabitats (Johnson *et al.*, 1997; Baldrian, 2016). Previous studies have reported that species with different functions inhabit different microhabitats in reliant to exudates from plants (Ekblad *et al.*, 2013; Clemmensen *et al.*, 2013; Wang *et al.*, 2020). For example, in roots, mycorrhizal fungi establish associations with the majority of plants in the form of ectomycorrhizal fungi (ECM), arbuscular mycorrhizal fungi, and ericoid mycorrhizal fungi (Smith & Read, 2008; Baldrian, 2016). Fungal endophytes and potentially endophytic saprotrophs are also commonly observed in the roots (Hiruma *et al.*, 2016; Almario *et al.*, 2017), facilitating the nutrient acquisition of their plant host and improving their tolerance to stress (Van Der Heijden *et al.*, 2008a; Hiruma *et al.*, 2016; Almario *et al.*, 2017; Bahadur *et al.*, 2019). In addition, soils harbor saprotrophic fungi that decompose dead wood and redistribute nutrients into the soil (Lindahl *et al.*, 2007; Baldrian, 2008). Mycorrhizal fungi also spread mycelia around the soil (Prescott & Grayston, 2013) to mobilize limiting nutrients (Lindahl & Tunlid, 2015), link multiple trees and understory vegetation, and transport carbon through the mycelial network (Högberg *et al.*, 1999). Because of their essential roles in ecosystem processes, belowground fungi are important for forest management and ecosystem restoration (Mandyam & Jumpponen, 2005; Porras-Alfaro & Bayman, 2011; Mello *et al.*, 2015). However, the understanding of the belowground ecosystem of forests is

still limited due to the complexity of belowground fungal species (Porras-Alfaro & Bayman, 2011; Tedersoo *et al.*, 2014), diverse forms of plant–fungi interactions (Toju *et al.*, 2014b, 2018), and the variable spatial structure within belowground forest.

The distinct distribution patterns of organisms along altitudinal gradients have been studied by ecologists for long. Previous studies on altitudinal patterns have focused on various macroorganisms (Ohsawa & Ide, 2008; Rowe, 2009; Sundqvist *et al.*, 2013). Recently, owing to advances in molecular ecology techniques, altitudinal patterns in belowground bacterial communities have been revealed (Bryant *et al.*, 2008; Fierer *et al.*, 2011; Sundqvist *et al.*, 2013). However, only a few studies on the changes in fungal community composition along altitudinal gradients have been reported. Intriguingly, the altitudinal patterns of alpha diversity showed inconsistent or non-significant patterns (Bahram *et al.*, 2012; Coince *et al.*, 2014; Matsuoka *et al.*, 2016; Geml, 2017; Guo *et al.*, 2020). Furthermore, in some studies on multiple vegetation with different hosts, the identity of vegetation had a greater effect on the community than abiotic factors (Bahram *et al.*, 2012; Miyamoto *et al.*, 2014). Only a few studies have focused on a single host that minimize the effect of host identity on the altitudinal patterns of the fungal community (Scattolin *et al.*, 2014; Coince *et al.*, 2014; Rincón *et al.*, 2015; Jarvis *et al.*, 2015). However, while the composition changes in the fungal community were found, significant changes in species richness were not observed in in those studies (Coince *et al.*, 2014; Jarvis *et al.*, 2015).

There are only a few reports on how altitudinal gradients are related to fungi in different belowground microhabitats (Bernard *et al.*, 2020) or different guilds (Yao *et al.*, 2017). Although these studies

provided valuable insights of the relationship between altitudinal gradients and fungal communities, the relationship between altitudinal gradients and the fungal community of different microhabitats or guilds remain unknown.

To this end, I examined how altitudinal gradients affect belowground fungal communities in different microhabitats (root and soil) and different guilds (ECM and other non-ECM guilds). The widely distributed ectomycorrhizal tree *Pinus densiflora* Sieb. et Zucc. (red pine) were selected as a target species to minimize the host effect and focus on altitude and microhabitat (Kong *et al.*, 2014). In the present study, the composition and diversity of fungal communities were compared between microhabitats and altitudinal gradients, and the results were interpreted according to the fungal guilds and soil properties. I expected a significant change in community compositions across altitudinal gradients in both microhabitats and guilds, especially in the fungal communities that are not directly associated with *P. densiflora*. In species richness level, I hypothesized that plant-associated fungi (i.e. ECM or root-inhabiting fungi) would be more affected by altitudinal gradients due to change in host fitness along the altitude (Appendix 1). Those findings would offer a more comprehensive understanding of the fungal interactions between root and soil microhabitats. A clear understanding of these changes can help to predict the reactions of microbial diversity to changing environments and climate (Körner, 2007; Bryant *et al.*, 2008).

1.2. Materials and Methods

Sample Collection

Sampling was conducted in sixteen well-protected, *P. densiflora*-dominant South Korean forests from 2019 – 2020 (Figs. 4 and 5, Table 1). The sampling sites were sorted into three groups (high, mid, and low) according to their altitude with respect to the vegetation distribution patterns of the nearby area (Yu et al., 2003; Han et al., 2014). Five mature *P. densiflora* trees with similar diameter at breast height (DBH) were randomly selected per site that were at least 20 m apart (Bahram et al., 2013). The trees were estimated to be 30–40 years old with a DBH of 32.36 ± 12.17 cm. To avoid including the roots from other plants, roots were manually traced from the trunk of *P. densiflora* after removing the litter layers around the trees. In total, root and soil samples were collected from 80 *P. densiflora* trees. For each tree, at least two lateral roots with mycorrhizal root tips from different directions were sampled, and two soil samples were sampled from each root sample in different directions.

The root and soil samples were stored in plastic bags and transported in an icebox. In the laboratory, the soil samples were sieved, and those from the same tree were pooled into one sample and stored at −20 °C until DNA extraction. Root samples were stored at 4 °C prior to surface sterilization and DNA extraction. Half of soil samples were mixed according to their sampling sites, and sent to National Instrumentation Center for Environmental Management (Seoul National University, Seoul, South Korea) for soil property analysis within a week of sampling. The following soil properties were measured: pH, total organic carbon (TOC), total nitrogen (TN), total phosphorus (P), soil

moisture, replaceable potassium (K), and ratios C:N (based on TOC/TN). Climate data (mean annual temperature (MAT) and annual temperature (AP) were obtained from the database of Korea Meteorological Administration (KMA), by searching the nearest automatic weather station (AWS) from each location. Soil properties and climate data did not show a significant change across altitudinal gradients in linear regression test, except for pH (Table 1). Sampling was permitted and accompanied by the Korea National Arboretum Authority. Hereafter, the two sample types (root and soil) were referred as "microhabitats" or "belowground microhabitats," which will be treated as categorical variables.

DNA extraction

For the root samples, the soil and debris were removed from the surface by gently shaking them in distilled water to prevent damage to the root. After washing, all fine roots with fresh-looking root tips were separated using sterilized scissors and the surface of the separated roots were sterilized by submerging in 3% hydrogen peroxide for 1 min. Surface-sterilized root samples were rinsed three times with sterile distilled water to remove possible contaminants from the soil. Root sterilization was conducted within a week of sampling. After sterilization, the root samples from the same tree were pooled into one sample and air-dried them on sterilized filter paper placed on a clean bench overnight. Dried fine roots were finely ground in an autoclaved mortar using liquid nitrogen. Microbial genomic DNA was extracted from soil samples (0.25 g) and ground root samples (0.20 g) in triplicate using the MoBio Power Soil DNA Isolation kit (MoBio Laboratories, Carlsbad, CA, USA) following the manufacturer's guidelines. Each DNA extracted in

triplicate was then pooled and stored at −20 °C for further experiments. In total, 80 samples for each root DNA and soil DNA were obtained.

PCR amplification and sequencing

For MiSeq sequencing, the 5.8S and internal transcribed spacer 2 (ITS2) regions of the fungal rRNA gene were amplified with 5.8S-Fun and ITS4-Fun primers with adaptor sequences to avoid the amplification of plant DNA (Taylor et al., 2016). PCR amplification was performed in triplicate using an AccuPower PCR PreMix kit (Bioneer, Daejeon, South Korea). The first round of PCR was conducted using to the following protocol: 96 °C for 5 min; 30 cycles of 94 °C for 30 s, 58 °C for 40 s, and 72 °C for 2 min; and 72 °C for 10 min for the final extension. PCR products were separated on a 1% agarose gel and purified using an ExpinTM PCR SV kit (GeneAll Biotechnology, Seoul, South Korea). The second round of PCR was conducted using the same protocol, but cycles were reduced to 10, and the unique identifier sequences were attached following the Nextera XT index kit protocol (Illumina, San Diego, CA, USA). Products from the second PCR were checked and purified as described above. The concentration of each amplicon library was measured using NanoDrop2000 (Thermo Fisher Scientific, Waltham, MA, USA) and then pooled in equimolar quantities. Sequencing was performed on an Illumina MiSeq platform at Macrogen (Seoul, South Korea).

Sequence data analysis

Raw sequencing data were processed using QIIME v. 1.8.0 (Caporaso et al., 2010). After merging paired-end sequences and quality filtering ($Q \geq 20$), sequences were clustered into operational taxonomic units

(OTUs) with 97% similarity using Vsearch v. 2.6.2 (Rognes et al., 2016). Chimeras were identified and removed using the 'uchime_denovo' method in Vsearch (Rognes et al., 2016) with the reference database from UNITE 8.2 (Kõljalg et al., 2013) and Seoul National University Fungal Collection (SFC). Representative sequences of OTUs were assigned to the most abundant sequences. For taxonomic assignment, the BLAST algorithm and reference database from UNITE 8.2 (Kõljalg et al., 2013) and SFC were used following the criteria of Tedersoo et al. (Tedersoo et al., 2014). Non-fungal sequences and rare OTUs (≤10 reads or ≤5 samples) were filtered out to remove potential sequencing errors (Smith and Peay, 2014). The post-clustering curation method was performed in the default setting to merge co-occurring 'daughter' OTUs with more abundant 'parent' OTUs (Frøslev et al., 2017) in addition to a manual BLAST search in NCBI Genbank for better taxonomic resolution. Among the 160 samples, seven were discarded due to low sequence reads (two root samples and five soil samples; root: GY03P-4R, GW04P-3R; soil: CC01P-3S, GY01P-4S, GY03P-5S, GB02P-2S, and GW02P-3S).

For the trophic mode assignment, I used the FUNGuild database (Nguyen et al., 2016) and manually checked the guild classification of OTUs based on the literature and field experience. OTUs with proper reference or confidence rankings of 'probable' or 'highly probable' were selected for further analysis of the ecological guilds. Then, the OTUs were sorted into six guilds (ectomycorrhiza, ericoid mycorrhiza, endophytes, saprotrophs, saprotrophs + endophytes, and saprotrophs + plant pathogens) and rarefied the samples to a minimum number of sequences (total: 5,600; ECM: 1,100; non-ECM: 1,000). For further analysis, the sequences were averaged and pooled according to their

sampling sites and microhabitat types. In total, sixteen root and soil samples were acquired respectively (Table 1; Table S1). Alpha-diversity indices (the number of OTUs, chao1 richness, Shannon diversity, and good coverage) were calculated in QIIME for each functional group.

Statistical analysis

R v.3.6.1 was used for further statistical analysis (Team R Development Core, 2018). The relationship between alpha-diversity indices and altitude gradients was examined using the Pearson correlation method with the cor.test function and linear regression test with the lm function in R. The relative abundances (sequence reads of ECM OTUs/sequence reads of all OTUs in the sample) of the root and soil samples from each altitudinal range were compared using t-test. The differences between communities were analyzed using the Jaccard dissimilarity index and assessed using non-metric multidimensional scaling (NMDS) methods in the phyloseq R package (McMurdie and Holmes, 2013). Permutational multivariate analysis of variance (PERMANOVA) statistical tests were performed to determine the differences in community composition with sampling sites, altitude, and microhabitat (root and soil) using 'adonis' function in vegan R package with 999 permutations and Jaccard dissimilarity index (Oksanen et al., 2013). To incorporate the vectors of soil property factors in ordination plots, 'envfit' function in the vegan package was used. Variation partitioning analysis was performed to check the effects of sampling sites, altitude, microhabitats, soil property, and climate using varpart function in vegan R package with jaccard distance. UPGMA (average linkage clustering) dendrograms were constructed based on the Jaccard distance to identify the relationship between root and soil samples. To

determine the changes in the fungal community composition along altitudinal and geographic distances (pairwise distance between two sampling sites), Mantel correlations were calculated using Jaccard dissimilarity metrics with Spearman's rank correlation and 9,999 permutations in the vegan R package (Oksanen et al., 2013). As the sampling was conducted at multiple sites across various altitudes, geographic distance was added in the analysis, along with altitude, to compensate for the effect of geographic distance on community composition. Geographic distances between sampling sites were calculated using the GPS coordinates. The 'core' genera of fungi were defined if that genera are present in all sampling regions from both microhabitats. The relative abundances of core genera in each sampling sites were visualized by line plot. The R package ggplot2 was used to visualize the results (Valero-Mora, 2010).

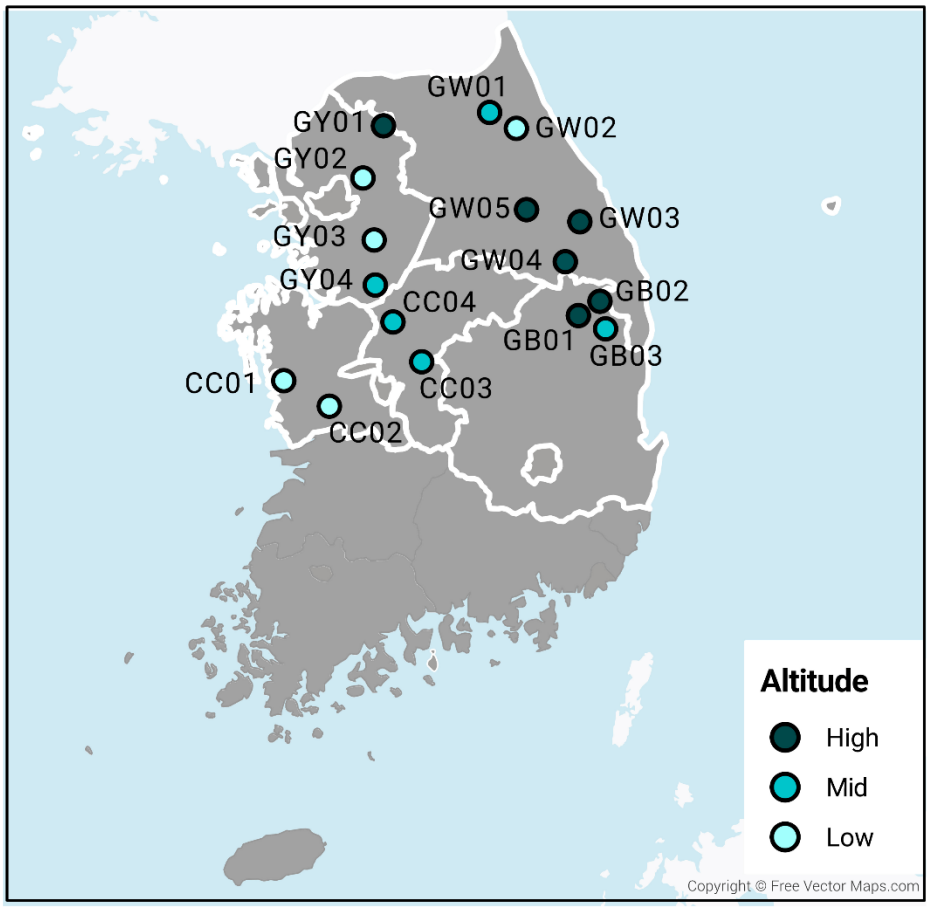

Figure 4. Geographic distribution of sampling sites in this study. Circles indicate the sampling locations, and the colors indicate the range of altitude. Altitude ranges: high 〉 650 m, mid 300–650 m, low 〈 300 m.

Table 1. Geographic information and soil chemical properties of sampling sites. Abbreviations: MAT, mean annual temperature; AP, annual precipitation; TOC, total organic matter; TN, total nitrogen; K, replaceable potassium; P, total phosphorus; Water, soil moisture; C:N, carbon to nitrogen ratio.

ID	Latitude (°)	Longitude (°)	Altitude (m)	Altitude classification	Sampling date	MAT (°C)	AP (mm)	pH	TOC (%)	TN (%)	K (mg/kg)	P (mg/kg)	Water (%)	C:N
CC01	36.422933	126.614875	205	Low	2019.06.17	11.4	1007.5	4.2	6.38	0.25	49.82	136.35	21.8	25.83
CC02	36.287900	126.968680	185	Low	2019.06.17	12.0	843	4.2	3.5	0.15	24.92	178.31	6.4	23.97
CC03	36.538444	127.763163	465	Mid	2019.06.18	11.0	1041.5	4.1	5.39	0.22	41.27	121.55	12.5	24.61
CC04	36.811423	127.533476	330	Mid	2019.06.18	12.7	841	4.3	5.39	0.22	60.69	102.53	15.6	24.50
GY01	37.980825	127.456962	649	Mid	2019.06.10	10.8	1032	4.3	4.66	0.25	38.39	143.93	22.6	18.64
GY02	37.682968	127.293288	220	Low	2019.06.10	11.6	1009	4	11.92	0.44	53.37	280.14	23.1	27.03
GY03	37.282817	127.371432	230	Low	2019.06.14	12.1	1057.5	4	8.97	0.48	33.26	275.36	22.4	18.69
GY04	37.02884	127.391773	425	Mid	2019.06.05	12.2	913	4	5.14	0.26	31.79	136.64	17.6	20.08

Table 1, Continued

ID	Latitude (°)	Longitude (°)	Altitude (m)	Altitude classification	Sampling date	MAT (°C)	AP (mm)	pH	TOC (%)	TN (%)	K (mg/kg)	P (mg/kg)	Water (%)	C:N
GW01	38.062439	128.306534	618	High	2020.06.12	10.8	1566	4.4	20.71	0.16	68.68	144.24	8.9	130.25
GW02	37.965	128.520828	229	Low	2020.06.11	12.5	2433.5	4.7	4.53	0.32	92.49	562.48	27.4	14.34
GW03	37.394307	129.028686	673	High	2020.05.20	13.3	1658.5	4.4	5.92	0.24	45.93	162.71	19.7	24.26
GW04	37.150021	128.912155	1333	High	2020.05.20	10.2	1366.8	4.9	4.97	0.29	65.06	562.69	14.7	17.20
GW05	37.468468	128.601944	742	High	2020.06.11	10.8	1258.5	4.6	5.28	0.30	80.99	233.72	23.1	17.78
GB01	36.820801	129.016922	657	High	2020.05.19	8.8	1438	4.4	3.41	0.13	68.30	103.05	8.6	26.23
GB02	36.9081	129.189484	825	High	2020.05.20	12.7	1763	4.8	6.64	0.54	133.21	818.42	34.6	12.41
GB03	36.739689	129.234329	618	Mid	2020.05.19	10.6	1529	4.4	4.34	0.19	30.83	200.33	14.4	23.21

1.3. Results

Overall characteristics of fungal communities

The fungal community structure associated with *P. densiflora* was determined for sixteen root and soil samples in South Korea (Fig. 4; Table 1). In total, 11,150,431 rRNA sequence reads were obtained after quality filtering, and 10,712,967 reads were further analyzed after filtering singletons and non-fungal sequences. The average length of sequence reads was 386.01 ± 48.18 base pairs. After post-clustering, filtering rare OTUs (≤ 10 reads or ≤ 5 samples) and rarefaction process, 2,206 OTUs were found in total dataset. From each microhabitat, 1,831 OTUs and 1,928 OTUs were found in the roots and soil, respectively. On average, 69.00 ± 15.30 genera and 72.41 ±19.15 genera were found in each root and soil sample. In the OTU level, 195.86 ± 53.13 OTUs and 222.76 ± 71.42 OTUs were found in each root and soil sample, respectively. Good's coverage ranged from 0.991 to 0.998 with an average of 0.996 ± 0.001, indicating that the sequencing depth was sufficient to represent most of the fungal OTUs (Table 2).

Table 2. Alpha diversity indices of fungal communities

Community	Sample ID	Type	# of OTU	Chao1 richness	Shannon's diversity	Good's coverage
Total Fungal Community	CC01R	Root	505	522	6.207	0.997
	CC02R	Root	423	463	5.402	0.997
	CC03R	Root	416	427	5.788	0.998
	CC04R	Root	483	633	5.571	0.996
	GY01R	Root	410	417	5.178	0.998
	GY02R	Root	617	642	6.524	0.996
	GY03R	Root	695	713	7.139	0.994
	GY04R	Root	466	484	4.586	0.997
	GB01R	Root	417	424	5.984	0.998
	GB02R	Root	428	441	5.709	0.998
	GB03R	Root	401	418	5.361	0.997
	GW01R	Root	426	453	6.04	0.996
	GW02R	Root	576	584	6.390	0.998
	GW03R	Root	489	497	5.911	0.998
	GW04R	Root	350	395	5.666	0.997
	GW05R	Root	430	453	5.773	0.998
	CC01S	Soil	509	520	6.877	0.996
	CC02S	Soil	597	645	6.269	0.994
	CC03S	Soil	531	552	6.482	0.996
	CC04S	Soil	849	902	7.302	0.991
	GY01S	Soil	529	573	5.997	0.993
	GY02S	Soil	670	684	6.746	0.996
	GY03S	Soil	675	725	6.554	0.993
	GY04S	Soil	633	675	6.623	0.994
	GB01S	Soil	388	394	5.139	0.998
	GB02S	Soil	417	468	4.845	0.996
	GB03S	Soil	406	413	5.154	0.998
	GW01S	Soil	466	504	5.245	0.996
	GW02S	Soil	560	604	6.221	0.993
	GW03S	Soil	605	653	5.814	0.996
	GW04S	Soil	525	554	6.409	0.995
	GW05S	Soil	537	552	6.035	0.997

Table 2, Continued

Community	Sample ID	Type	# of OTU	Chao1 richness	Shannon's diversity	Good's coverage
ECM Fungal Community	CC01R	Root	124	152	4.379	0.993
	CC02R	Root	97	100	4.516	0.996
	CC03R	Root	74	74	3.541	0.999
	CC04R	Root	103	104	4.234	0.998
	GY01R	Root	86	87	3.602	0.998
	GY02R	Root	142	145	4.796	0.994
	GY03R	Root	186	195	5.420	0.990
	GY04R	Root	74	75	2.325	0.998
	GB01R	Root	77	78	3.934	0.998
	GB02R	Root	59	59	3.255	0.998
	GB03R	Root	76	77	3.535	0.997
	GW01R	Root	70	70	4.069	1.000
	GW02R	Root	99	102	4.386	0.997
	GW03R	Root	107	110	4.471	0.995
	GW04R	Root	81	84	3.895	0.997
	GW05R	Root	64	64	3.282	0.999
	CC01S	Soil	128	133	5.048	0.993
	CC02S	Soil	142	147	5.342	0.995
	CC03S	Soil	99	101	4.794	0.997
	CC04S	Soil	202	228	5.559	0.988
	GY01S	Soil	138	140	4.344	0.996
	GY02S	Soil	122	127	4.834	0.995
	GY03S	Soil	195	212	5.329	0.985
	GY04S	Soil	175	179	5.141	0.994
	GB01S	Soil	66	68	3.867	0.995
	GB02S	Soil	63	66	3.443	0.996
	GB03S	Soil	73	73	3.997	0.998
	GW01S	Soil	84	84	4.159	0.998
	GW02S	Soil	84	89	4.497	0.995
	GW03S	Soil	122	129	4.475	0.994
	GW04S	Soil	105	106	5.021	0.998
	GW05S	Soil	74	75	4.535	0.997

Table 2, Continued

Community	Sample ID	Type	# of OTU	Chao1 richness	Shannon's diversity	Good's coverage
nonECM Fungal Community	CC01R	Root	262	268	6.004	0.991
	CC02R	Root	192	194	4.809	0.995
	CC03R	Root	244	253	6.040	0.988
	CC04R	Root	236	250	4.973	0.992
	GY01R	Root	246	303	5.656	0.981
	GY02R	Root	308	339	6.044	0.982
	GY03R	Root	341	356	6.509	0.983
	GY04R	Root	278	279	5.823	0.995
	GB01R	Root	233	239	5.703	0.990
	GB02R	Root	233	248	5.534	0.990
	GB03R	Root	249	270	5.885	0.985
	GW01R	Root	253	260	6.101	0.991
	GW02R	Root	302	308	6.443	0.993
	GW03R	Root	267	275	5.592	0.989
	GW04R	Root	169	189	5.126	0.984
	GW05R	Root	238	241	5.975	0.991
	CC01S	Soil	277	305	6.266	0.976
	CC02S	Soil	290	301	5.343	0.988
	CC03S	Soil	305	320	6.106	0.990
	CC04S	Soil	447	474	6.697	0.980
	GY01S	Soil	275	304	5.872	0.981
	GY02S	Soil	369	380	6.271	0.986
	GY03S	Soil	343	375	6.227	0.977
	GY04S	Soil	307	361	5.931	0.974
	GB01S	Soil	226	229	5.047	0.993
	GB02S	Soil	319	340	6.675	0.98
	GB03S	Soil	262	271	5.438	0.991
	GW01S	Soil	356	371	6.338	0.986
	GW02S	Soil	317	350	5.753	0.978
	GW03S	Soil	354	359	5.887	0.990
	GW04S	Soil	336	374	6.536	0.980
	GW05S	Soil	324	345	5.971	0.978

Fungal community composition

The composition of belowground fungi varied between the root and soil microhabitats. At the genus level, *Tomentella*, *Geminibasidium*, *Suillus*, *Oidiodendron*, and *Cortinarius* were the most abundant taxa (Fig. 5). Among the ectomycorrhizal genera, *Suillus* and *Phellodon* showed higher relative abundance in roots than in soil. In contrast, the relative abundance of *Tomentella* and *Cortinarius* was higher in the soil than in root. Meanwhile, non-ectomycorrhizal fungi also showed different relative abundances according to the microhabitats. The genera *Mycena*, *Trichoderma*, and *Basidiodendron* were more abundant in roots than in soil, whereas *Gemnibasidium*, *Umbelopsis*, and *Oidiodendron* were more abundant in soil. Among the plant pathogenic fungi, *Venturia* was mostly found in root microhabitats but in relatively low abundance compared to other guilds.

The composition of belowground fungal communities also changed across altitudinal gradients. *Suillus* was more abundant in root samples collected at high altitudes, whereas *Geminibasidium* was more abundant in soil samples from low altitudes. Several genera, such as *Mortierella* and *Tricholoma*, showed opposite altitudinal patterns between root and soil samples (Fig. 5). Across all the sampling sites and microhabitats, twenty genera were identified (Fig. 6). Despite the relatively low number, they accounted for 37.50% (root) and 46.10% (soil) of total sequence reads. Most of them were saprotrophic fungi, but ectomycorrhizal fungi (*Cenococcum*, *Cortinarius*, *Rhizopogon*, *Russula*, and *Tomentella*), ericoid mycorrhizal fungi (*Oidiodendron*), endophytic fungi (*Phialocephala*, *Mortierella*) were also included in the

list. However, their proportions were not stable across the sampling sites and altitudes.

In terms of ecological function, saprotrophs and ECM were the major guilds in both microhabitats. Saprotroph ($p < 0.01$), endophytes ($p < 0.001$), and saprotroph + plant pathogens ($p < 0.001$) were more abundant in roots, whereas the ECM ($p < 0.001$) and ericoid mycorrhiza ($p < 0.001$) were more abundant in the soil (Fig. 7). The relative abundance of saprotroph + endophytes was not significantly different between the microhabitats. Relative abundance of saprotroph + endophyte guild was higher in the soil than in the root at low altitudes, and the pattern was opposite at high altitudes ($p < 0.05$). Similarly, the relative abundance of ECM was significantly higher in the soil than in the roots only at high altitudes (Fig. 8, $p < 0.001$).

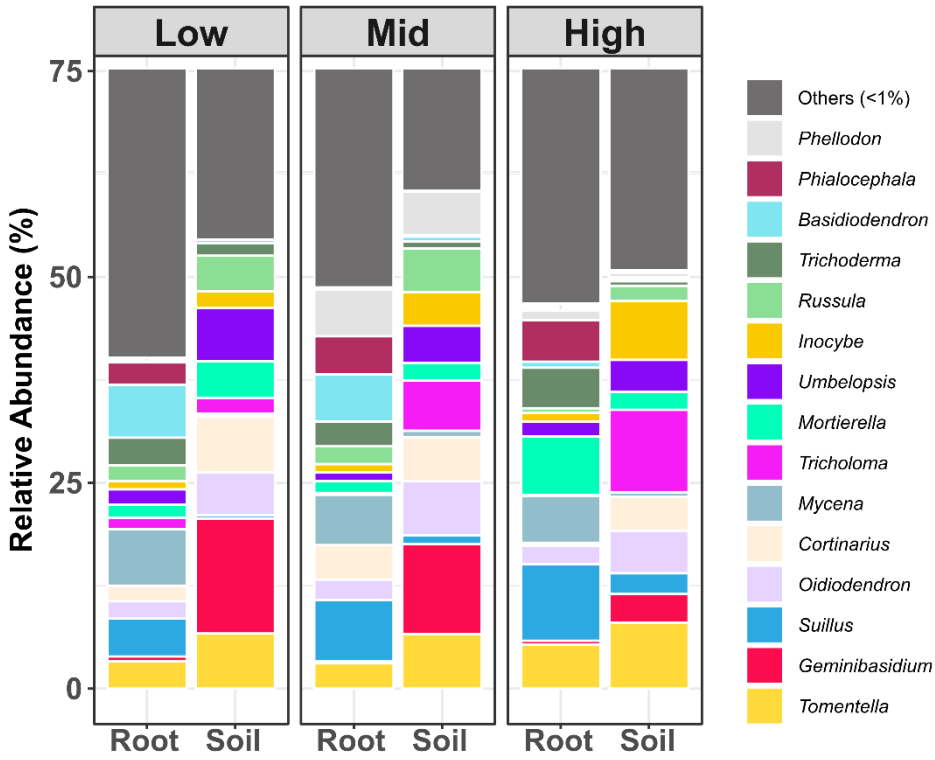

Figure 5. Taxonomic composition of fungal communities of the major genera (>1% in relative abundance). Altitude ranges: high > 650 m, mid 300–650 m, low < 300 m.

Figure 6. The relative abundance of core genera in each microhabitats.

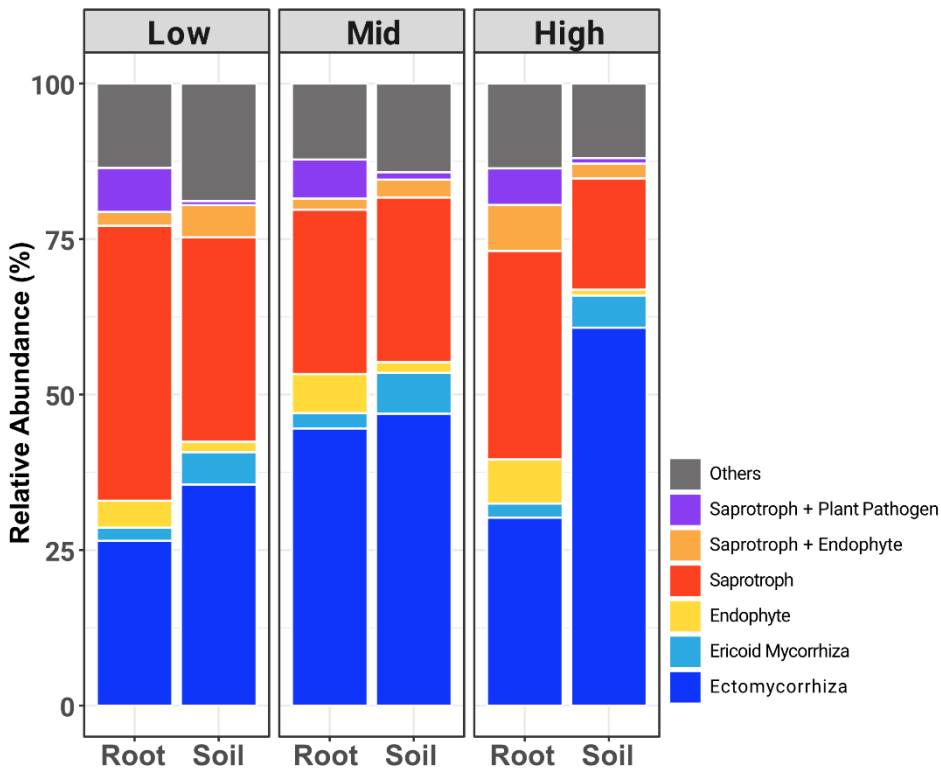

Figure 7. Composition and relative abundance of guild in fungal communities. Altitude ranges: high 〉 650 m, mid 300–650 m, low 〈 300 m.

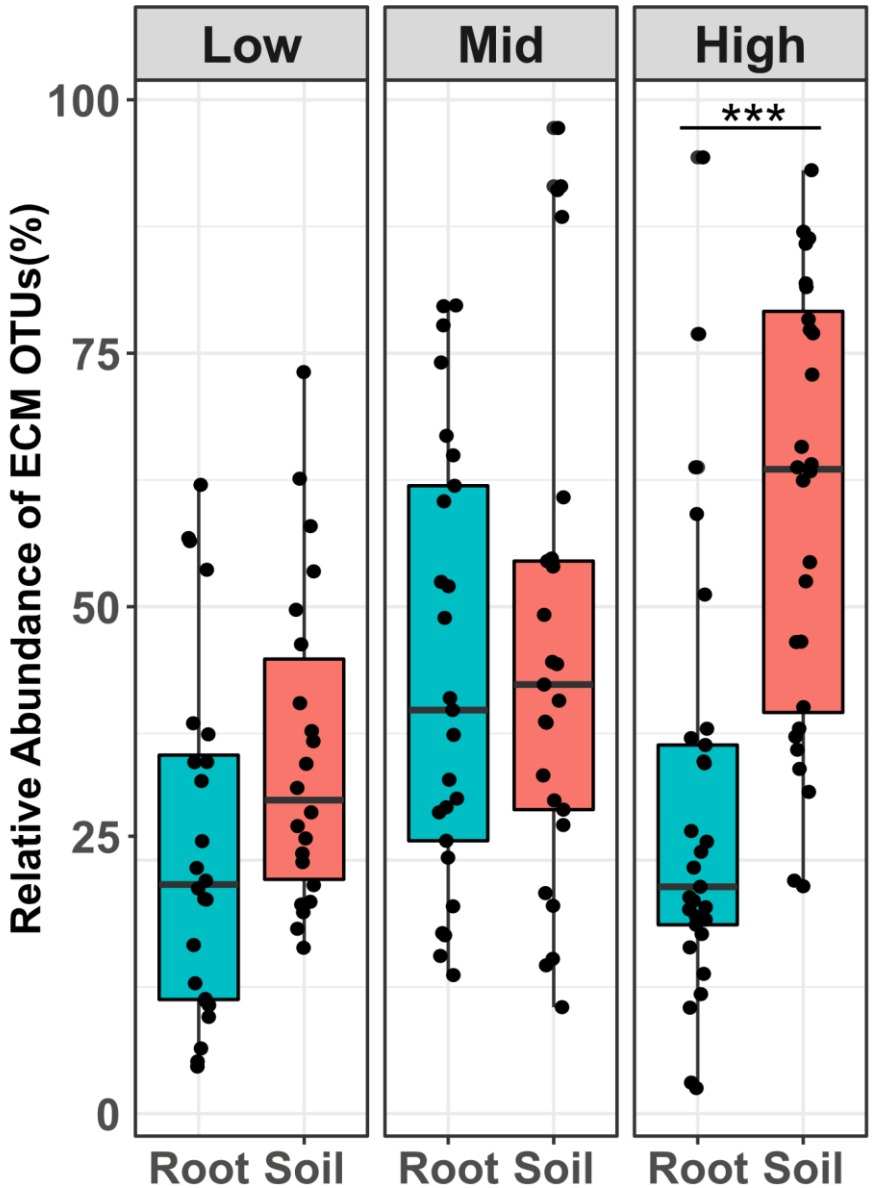

Figure 8. The proportion of ectomycorrhizal fungi in each microhabitats. (*$p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$).

Change in fungal diversity across microhabitats and altitudinal gradients

The microhabitat type had a significant effect on the community composition of belowground fungal communities (Table 11). However, their influence differed according to the ecological groups. According to the results of the PERMANOVA test, the effects of microhabitat were higher in non-ECM community (R^2 = 0.14) than both the ECM (R^2 = 0.053) and the entire fungal communities (R^2 = 0.097). UPGMA analysis showed that the composition of fungal communities between the two microhabitats was much more similar in ECM communities than in non-ECM communities (Fig. 9). In contrast, the influence of altitude was similar in both microhabitats and ecological groups (Table S4). Variation partitioning analysis showed that sampling sites, altitude, microhabitat, soil property and climate data explained about 44% (Total), 49% (ECM), and 38% (nonECM) in variation of fungal communities (Fig. 10). Similar to result of UPGMA analysis, microhabitat could explain 11% (Total), 5% (ECM), and 13% (nonECM) of fungal communities after excluding the effects of sampling sites and other factors.

PERMANOVA tests showed that various types of soil properties are related with changes in fungal communities in different microhabitats and ecological groups. In both microhabitats, altitude, pH, K, and P were significant factors in all ecological groups, while TN and soil moisture were significant factors in all ecological groups from soil microhabitats (Fig. 11). TOC and MAT were only significant factor in root ECM community, while AP was significant factor in root total fungal community, root nonECM fungal community and soil ECM

fungal community (Table 4, 5). Similar to the results of the PERMANOVA test, those of the Mantel test revealed that altitude exhibited distance-decay patterns with fungal communities, though the differences between roots and soil samples were varied to the ecological groups. The change of community composition across altitudinal gradients was much higher in nonECM groups than that of ECM groups (Fig. 12). In contrast, the similarities of fungal communities were significantly correlated with geographic distances only in roots (Fig. 13). Overall, microhabitat, soil properties, and altitude were significantly correlated with the fungal community composition. In most microhabitats, non-ectomycorrhizal fungal communities were more affected by altitudinal differences than the total fungal or ectomycorrhizal fungal communities.

The relationship of the alpha-diversity indices of fungal communities associated with *P. densiflora* and altitude varied according to microhabitats and ecological groups (Fig. 14). Both richness and diversity indices were higher in soil samples than in root samples. Species richness showed significant negative relationships with altitude in root samples, but not significant in soil samples. Diversity index was not significantly affected by altitudes in all groups, while non-ECM communities in soil exhibited different altitudinal pattern compared to other groups.

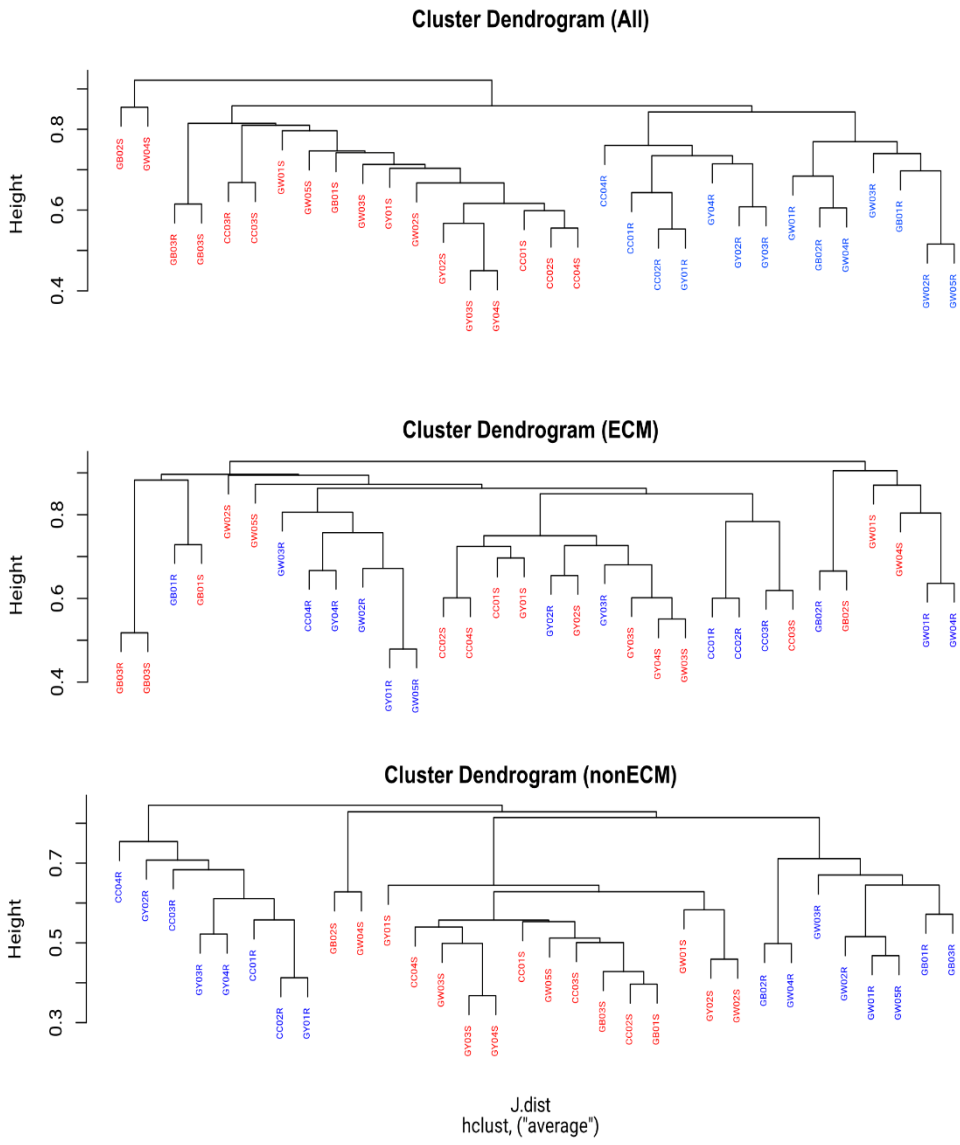

Figure 9. UPGMA dendrogram of fungal communities based on Jaccard dissimilarity index.

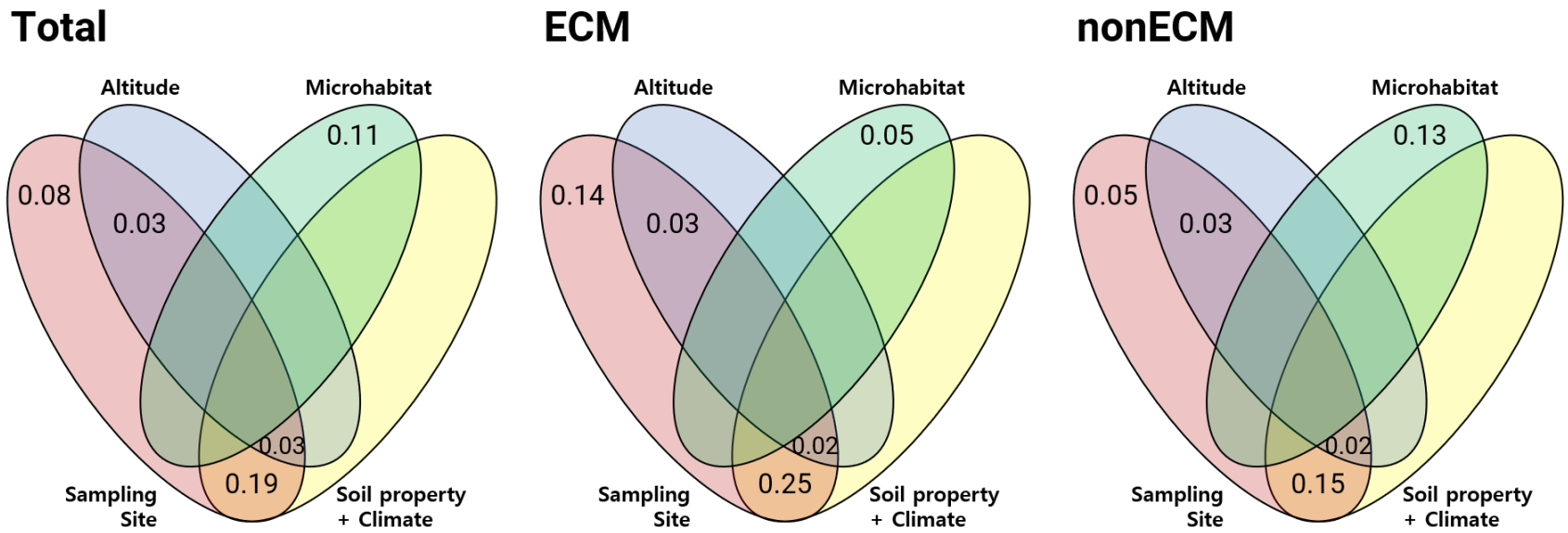

Figure 10. Venn diagram of variation-partitioning analysis showing the effect of sampling sites, altitude, microhabitat, soil property (pH, TOC, TN, K, P, Water, C:N) and climate data (MAT, AP). Abbreviations: MAT, mean annual temperature; AP, annual precipitation; TOC, total organic matter; TN, total nitrogen; K, replaceable potassium; P, total phosphorus; Water, soil moisture; C:N, carbon to nitrogen ratio.

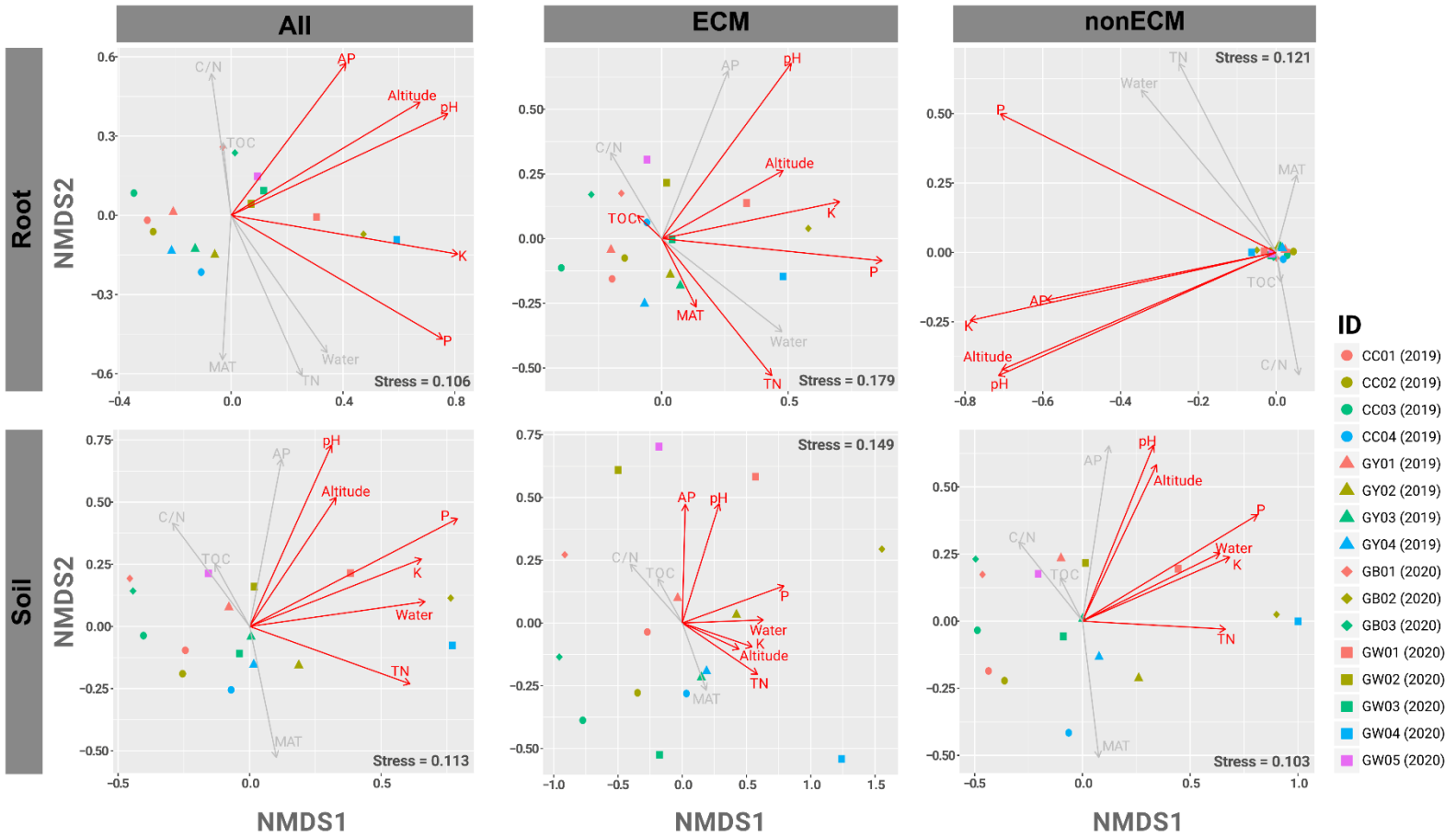
All
ECM
nonECM
Root
Soil
NMDS1
NMDS2
Stress = 0.106
Stress = 0.179
Stress = 0.121
Stress = 0.113
Stress = 0.149
Stress = 0.103
AP
Altitude
pH
C/N
TOC
K
P
MAT
Water
TN
ID
CC01 (2019)
CC02 (2019)
CC03 (2019)
CC04 (2019)
GY01 (2019)
GY02 (2019)
GY03 (2019)
GY04 (2019)
GB01 (2020)
GB02 (2020)
GB03 (2020)
GW01 (2020)
GW02 (2020)
GW03 (2020)
GW04 (2020)
GW05 (2020)

Figure 11. NMDS plots calculated using Jaccard dissimilarity index for each ecological group and microhabitat. The sampling sites are displayed in different colors and shapes. Soil and climate properties with significant relationships ($p < 0.05$) are indicated by red arrows. Abbreviations: TOC, total organic matter; TN, total nitrogen; K, replaceable potassium; P, total phosphorus; Water, soil moisture; C:N, carbon to nitrogen ratio; MAT, mean annual temperature; AP, annual precipitation.

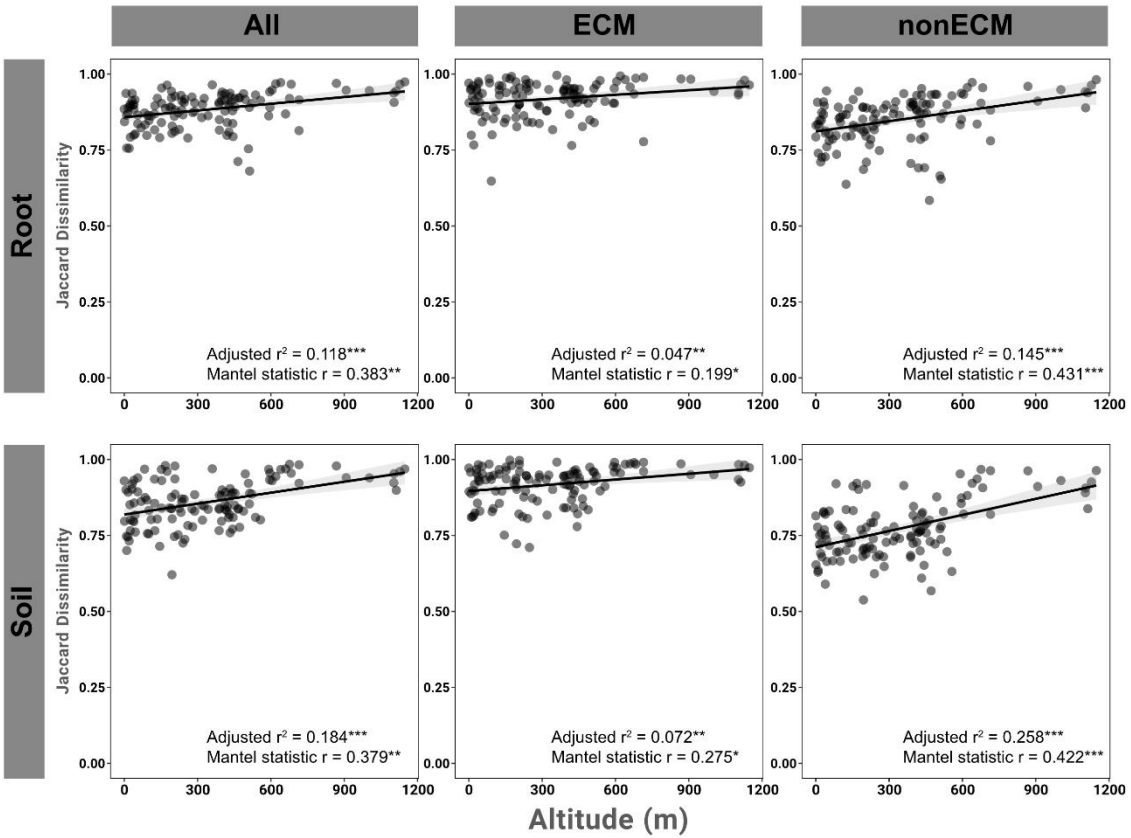

Figure 12. Mantel correlation plots calculated using Jaccard dissimilarity and the altitudinal differences between two microhabitats. (*$p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$).

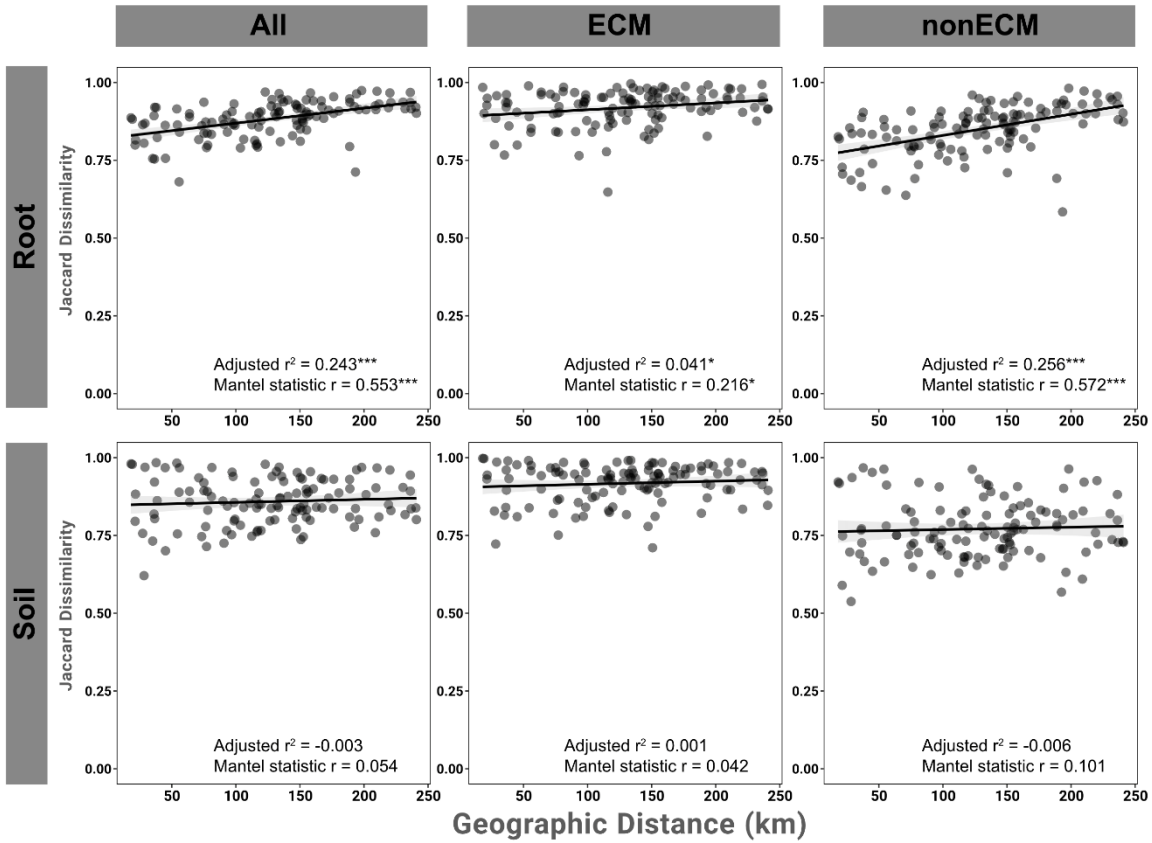

Figure 13. Mantel correlation plots calculated using Jaccard dissimilarity and the geographical distances between two microhabitats. (*$p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$).

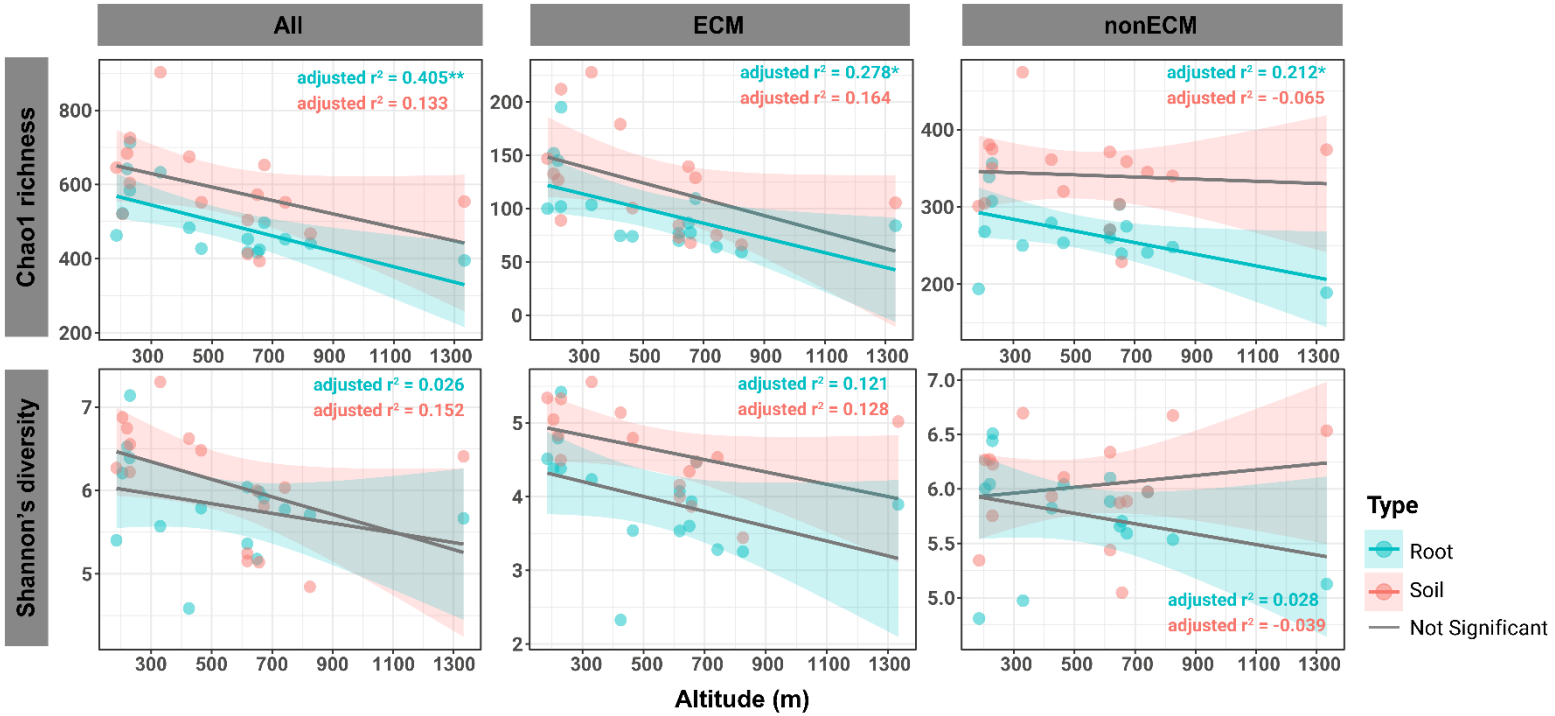

Figure 14. Relationship between altitude and alpha-diversity indices in root (blue) and soil (red) microhabitats. The line indicates linear regression fit, and the shaded band represents 95% confidence level. All: overall fungal communities; ECM: ectomycorrhizal fungal communities; non-ECM: non-ectomycorrhizal fungal communities. Non-significant relationships are marked with grey lines (*$p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$).

Table 3. Results of PERMANOVA tests. PERMANOVA tests were performed to identify the effects of environmental factors on fungal communities associated with the roots and soil of *P. densiflora*. The Jaccard dissimilarity index and abundance of OTUs were used for analysis with 999 permutations (*$p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$).

Community (Root + Soil)	Factor	Degree of freedoms	Sum of Squares	Mean Squares	F. Model	R^2	p value
Total Fungal Community	~Altitude	1	0.731	0.731	1.837	0.058	0.003**
	~ Microhabitat	1	1.223	1.223	3.205	0.097	0.001***
	~ Sampling Sites	15	6.642	0.443	1.176	0.524	0.006**
	~ pH	1	0.755	0.755	1.901	0.060	0.002**
	~ TOC	1	0.447	0.447	1.096	0.035	0.255
	~ TN	1	0.583	0.583	1.448	0.046	0.014*
	~ K	1	0.724	0.724	1.818	0.057	0.002**
	~ P	1	0.850	0.850	2.159	0.067	0.001***
	~ Soil Moisture	1	0.575	0.575	1.426	0.045	0.021*
	~ C:N Ratio	1	0.487	0.487	1.200	0.038	0.072
	~MAT	1	0.475	0.475	1.169	0.038	0.140
	~AP	1	0.552	0.552	1.368	0.044	0.024*
ECM Fungal Community	~Altitude	1	0.751	0.751	1.783	0.056	0.001***
	~ Microhabitat	1	0.709	0.709	1.676	0.053	0.001***
	~ Sampling Sites	15	7.872	0.525	1.520	0.588	0.001***
	~ pH	1	0.750	0.750	1.780	0.056	0.001***
	~ TOC	1	0.577	0.577	1.349	0.043	0.007**

Table 3, Continued

Community (Root + Soil)	Factor	Degree of freedoms	Sum of Squares	Mean Squares	F. Model	R^2	p value
ECM Fungal Community	~ TN	1	0.673	0.673	1.587	0.050	0.002**
	~ K	1	0.685	0.685	1.617	0.051	0.001***
	~ P	1	0.844	0.845	2.017	0.063	0.001***
	~ Soil Moisture	1	0.607	0.607	1.425	0.045	0.001***
	~ C:N Ratio	1	0.597	0.597	1.398	0.045	0.015*
	~MAT	1	0.642	0.642	1.510	0.048	0.002**
	~AP	1	0.588	0.588	1.378	0.044	0.008**
nonECM Fungal Community	~Altitude	1	0.734	0.734	2.020	0.063	0.006**
	~ Microhabitat	1	1.658	1.658	4.988	0.143	0.001***
	~ Sampling Sites	15	5.581	0.372	0.984	0.480	0.564
	~ pH	1	0.806	0.806	2.235	0.069	0.001***
	~ TOC	1	0.308	0.308	0.816	0.026	0.805
	~ TN	1	0.595	0.595	1.617	0.051	0.025*
	~ K	1	0.804	0.804	2.227	0.069	0.002**
	~ P	1	0.956	0.956	2.686	0.082	0.001***
	~ Soil Moisture	1	0.632	0.632	1.723	0.054	0.021*
	~ C:N Ratio	1	0.379	0.379	1.011	0.033	0.400
	~MAT	1	0.358	0.358	0.951	0.031	0.517
	~AP	1	0.549	0.549	1.486	0.047	0.044*

Abbreviations: TOC, total organic matter; TN, total nitrogen; K, replaceable potassium; P, total phosphorus; MAT, mean annual temperature; AP, annual precipitation.

Table 4. Results of PERMANOVA tests. PERMANOVA tests were performed to identify the effects of environmental factors on fungal communities associated with the roots of *P. densiflora*. The Jaccard dissimilarity index and abundance of OTUs were used for analysis with 999 permutations (*$p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$).

Community (Root)	Factor	Degree of freedoms	Sum of Squares	Mean Squares	F. Model	R^2	p value
Total Fungal Community	~Altitude	1	0.669	0.669	1.801	0.114	0.003**
	~ pH	1	0.766	0.766	2.103	0.131	0.001***
	~ TOC	1	0.406	0.406	1.041	0.069	0.336
	~ TN	1	0.431	0.431	1.110	0.073	0.227
	~ K	1	0.643	0.643	1.723	0.110	0.003**
	~ P	1	0.655	0.655	1.758	0.112	0.001***
	~ Soil Moisture	1	0.392	0.392	1.001	0.067	0.442
	~ C:N Ratio	1	0.404	0.404	1.034	0.069	0.295
	~MAT	1	0.400	0.400	1.024	0.068	0.379
	~AP	1	0.598	0.598	1.587	0.102	0.004**
ECM Fungal Community	~Altitude	1	0.591	0.591	1.437	0.093	0.002**
	~ pH	1	0.658	0.658	1.620	0.104	0.001***
	~ TOC	1	0.520	0.520	1.250	0.082	0.049*
	~ TN	1	0.525	0.525	1.261	0.083	0.074

Table 4, Continued

Community (Root)	Factor	Degree of freedoms	Sum of Squares	Mean Squares	F. Model	R^2	p value
ECM Fungal Community	~ K	1	0.577	0.577	1.400	0.091	0.011*
	~ P	1	0.663	0.663	1.633	0.104	0.001***
	~ Soil Moisture	1	0.410	0.410	0.965	0.065	0.569
	~ C:N Ratio	1	0.477	0.477	1.138	0.075	0.081
	~MAT	1	0.524	0.524	1.260	0.083	0.040*
	~AP	1	0.460	0.460	1.092	0.072	0.234
nonECM Fungal Community	~Altitude	1	0.727	0.727	2.157	0.133	0.002**
	~ pH	1	0.882	0.882	2.705	0.162	0.001***
	~ TOC	1	0.348	0.348	0.955	0.064	0.534
	~ TN	1	0.401	0.400	1.111	0.073	0.251
	~ K	1	0.687	0.687	2.020	0.126	0.005**
	~ P	1	0.682	0.682	2.003	0.125	0.003**
	~ Soil Moisture	1	0.382	0.381	1.054	0.070	0.323
	~ C:N Ratio	1	0.366	0.366	1.009	0.067	0.427
	~MAT	1	0.361	0.361	0.994	0.066	0.422
	~AP	1	0.692	0.692	2.036	0.127	0.003**

Abbreviations: TOC, total organic matter; TN, total nitrogen; K, replaceable potassium; P, total phosphorus; MAT, mean annual temperature; AP, annual precipitation.

Table 5. Results of PERMANOVA tests. PERMANOVA tests were performed to identify the effects of environmental factors on fungal communities associated with the soil of *P. densiflora*. The Jaccard dissimilarity index and abundance of OTUs were used for analysis with 999 permutations (*$p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$).

Community (Soil)	Factor	Degree of freedoms	Sum of Squares	Mean Squares	F. Model	R^2	p value
Total Fungal Community	~Altitude	1	0.576	0.576	1.612	0.103	0.005**
	~ pH	1	0.514	0.514	1.421	0.092	0.009**
	~ TOC	1	0.358	0.358	0.959	0.064	0.548
	~ TN	1	0.528	0.528	1.464	0.095	0.007**
	~ K	1	0.577	0.577	1.615	0.103	0.004**
	~ P	1	0.694	0.694	1.989	0.124	0.001***
	~ Soil Moisture	1	0.537	0.537	1.492	0.096	0.002**
	~ C:N Ratio	1	0.407	0.407	1.101	0.073	0.171
	~MAT	1	0.419	0.419	1.139	0.075	0.147
	~AP	1	0.398	0.398	1.075	0.071	0.272
ECM Fungal Community	~Altitude	1	0.546	0.546	1.319	0.086	0.004**
	~ pH	1	0.504	0.504	1.208	0.079	0.045*
	~ TOC	1	0.421	0.421	0.996	0.066	0.509
	~ TN	1	0.514	0.514	1.237	0.081	0.034*

Table 5, Continued

Community (Soil)	Factor	Degree of freedoms	Sum of Squares	Mean Squares	F. Model	R^2	p value
ECM Fungal Community	~ K	1	0.531	0.531	1.280	0.084	0.013*
	~ P	1	0.616	0.616	1.507	0.097	0.001***
	~ Soil Moisture	1	0.526	0.526	1.267	0.083	0.014*
	~ C:N Ratio	1	0.469	0.469	1.120	0.074	0.214
	~MAT	1	0.484	0.484	1.158	0.076	0.081
	~AP	1	0.508	0.508	1.219	0.080	0.036*
nonECM Fungal Community	~Altitude	1	0.533	0.533	1.868	0.118	0.013**
	~ pH	1	0.489	0.489	1.696	0.108	0.024*
	~ TOC	1	0.216	0.216	0.701	0.048	0.938
	~ TN	1	0.591	0.591	2.104	0.131	0.006**
	~ K	1	0.624	0.624	2.240	0.138	0.001***
	~ P	1	0.789	0.789	2.960	0.175	0.001***
	~ Soil Moisture	1	0.639	0.639	2.302	0.141	0.001***
	~ C:N Ratio	1	0.313	0.313	1.040	0.069	0.247
	~MAT	1	0.299	0.299	0.992	0.066	0.422
	~AP	1	0.289	0.289	0.954	0.064	0.499

Abbreviations: TOC, total organic matter; TN, total nitrogen; K, replaceable potassium; P, total phosphorus; MAT, mean annual temperature; AP, annual precipitation.

1.4. Discussion

The altitudinal gradients and microhabitats were both significant factors affecting the richness and composition of fungal communities without host vegetation change. However, their influence and pattern differed between both of microhabitats and ECM / non-ECM communities. Most of the soil properties did not significantly changed across altitudinal gradients, but ordination suggested that the effect of soil properties also influenced community variations in all microhabitats and ecological groups. This is one of the few studies that covered the altitudinal changes in fungal communities across different microhabitats and guilds. It is important to note that in contrast to previous studies, ECM fungal richness showed significant change along altitudinal gradients.

The effect of microhabitat on fungal communities

Similar to the findings of previous studies (Coleman-Derr *et al.*, 2016; Bernard *et al.*, 2020), microhabitat significantly affects belowground fungal community composition. However, in this study, the difference between microhabitats was much smaller in ECM communities than in non-ectomycorrhizal communities. The similar composition of ECM communities is likely due to the ECM mycelia in the soil as most ectomycorrhizal fungi form extraradical hyphae in the soil environment around colonized roots (Baldrian, 2016). Although ectomycorrhizal fungi in both microhabitats mostly overlapped, species richness and diversity were consistently higher in the soil than in the root. In addition, altitudinal gradients were significantly related

with ECM community in soil, while not in root microhabitats. Several factors may explain the differences between microhabitats. First, inactive spore banks or relic DNA from dead mycelia may influence these differences in alpha diversity. Spore banks are formed by spores from nearby fruiting bodies that are spread by wind. Dormant spores stored in the soil can initiate associations if the right host plant is present (Taylor & Bruns, 1999) and can survive in soil for several years even in the absence of the right host (Miyamoto & Nara, 2016). Similarly, DNA fragments from dead fungi have been detected for several years (Carini *et al.*, 2016). Because the active and inactive fungi cannot be separated using NGS, DNA from inactive fungi might have contributed to higher ectomycorrhizal fungal richness in soil in this data. Second, ECM fungi colonizing non-ectomycorrhizal hosts (Harrington & Mitchell, 2002; Murata *et al.*, 2014; Schneider-Maunoury *et al.*, 2020) or herbaceous plants (Smith & Read, 2008) have been reported in previous studies. Mycelia from other plant hosts could have also influenced ECM richness in soil.

On the other hand, the composition of non-ECM fungi exhibited higher variation than ECM fungi between microhabitats. The clear functional separation may explain this difference. Most non-ECM genera that were abundant in roots were endophytes or plant pathogens, such as *Phialocephala*, *Mycnea*, and *Venturia*. *Phialocephala* is a well-known endophyte found in the roots of various host trees, including *P. densiflora* (Grünig *et al.*, 2008). On the other hand, several *in vitro* studies suggested that *Mycena* can colonize ECM plant roots and promote growth of host plant (Grelet *et al.*, 2017; Thoen *et al.*, 2020). *Venturia* is a plant pathogenic fungus that is widely

distributed in the Northern temperate area (González-Domínguez *et al.*, 2017). However, the presence of *Venturia* in the roots of *P. densiflora* has not been reported previously; therefore, further studies are needed to understand their relationship. In the soil, ericoid mycorrhizal fungi from *Oidiodendron* were more abundant than in the root. The presence of *Oidiodendron* in soil around the roots of ectomycorrhizal trees may be attributed to nearby understory vegetation or their saprobic activity in soil (Rice & Currah, 2007).

The effect of altitudinal gradient on fungal communities

In addition, compositional changes were observed along the altitudinal gradients. Decrease in similarity of ECM communities following altitudinal gradients has been observed in most studies, irrespective of host identity control (Talbot *et al.*, 2014; Geml, 2017; Wang *et al.*, 2019; Yang *et al.*, 2019). Interestingly, the effect of altitudinal gradients was weaker in the ECM than in the non-ECM groups of both microhabitats in this study. As this study focused on a single host species, the ECM community may have been less affected at the regional scale. Bahram et al. (Bahram *et al.*, 2012) have reported that host tree species was the most important factors that affected the ECM community composition. In contrast, free-living fungi, such as saprotrophs, in soil are expected to be more affected by geographic distance than host-dependent fungi because the former are relatively more functionally overlapped (Talbot *et al.*, 2014) and sensitive to species pools in the area (Gao *et al.*, 2016; Erlandson *et al.*, 2018; Yang *et al.*, 2019). Similarly, belowground endophytes in roots have been reported to be relatively host-independent (Glynou *et al.*, 2016) and

dispersal-limited, which may account for the differences in altitudinal and geographic patterns. However, other environmental factors such as annual temperature or soil chemical properties around individual trees could have also resulted in these differences (Bahram *et al.*, 2012; Jarvis *et al.*, 2015). In variation partitioning and PERMANOVA test, the sampling site explained the biggest variation in fungal communities (Matsuoka *et al.*, 2016). As sampling was conducted in multiple areas, these results could be due to the combined effect of altitude, soil property, and climate data that are directly associated with sampling site. Though results from mantel tests showed that the influence of geographic distance does not correspond to that of altitudinal gradients, further studies would be required to separate the effect of spatial distance from the overall variations.

Significant decrease in richness was found along altitudinal gradients in the root fungal communities of *P. densiflora*, and diversity indices showed a similar pattern. Whereas, altitudinal patterns of alpha diversity did not show significant change in the soil fungal communities. In previous studies, a decline in ECM species richness has been observed along altitudinal gradients in both microhabitats (Kernaghan & Harper, 2001; Bahram *et al.*, 2012). However, studies with controlled host species have reported no relationship between ECM richness and the altitude in the roots of *Fagus sylvatica* (Coince *et al.*, 2014). This could be due to the characteristics of the host tree (*P. densiflora*) in this study. The nutrient input from leaves and roots are different between conifers and broadleaves (Awad *et al.*, 2019), as well as hyphal production and nutrient demands of ECM fungi across tree host species (Bakker *et al.*, 2015; Rosinger *et al.*, 2020); however, further studies are

needed to clarify the effect of altitude on fungal richness associated with *P. densiflora*. Although the alpha diversity of the non-ECM group decreased at high altitudes in the root microhabitat, there was no significant change in soil. The lowered productivity of host trees might have affected the richness and diversity of associated fungi, i.e., ECM and root-associated non-ECM groups (Hiiesalu *et al.*, 2017), as decreased growth and photosynthetic productivity of *P. densiflora* has been reported at high altitudes (Kim *et al.*, 2020). The non-significant relationship between altitude and alpha diversity of soil-inhabiting fungi may suggest that the decomposition of organic matter and/or productivity of understory vegetation is less affected by altitude (Guo *et al.*, 2013).

The effect of soil properties and climate on fungal communities

In case of soil properties and climate, pH, phosphorus, and potassium were associated with fungal community composition regardless of their microhabitats or ecological roles. However, it is hard to explain these results with altitude as most of soil properties were not affected by altitude, except for pH, in contrast to previous studies (Coince *et al.*, 2014; Jarvis *et al.*, 2015). Soil pH is an important factor in fungal communities, but the significance of pH's influence was varied by sampling sites and host identities (Rousk *et al.*, 2010; Coince *et al.*, 2014; Jarvis *et al.*, 2015; McGee *et al.*, 2019; Vasco-Palacios *et al.*, 2020). While significant relationship was found between pH and fungal community composition in this study, it could be an indirect relationship as fungi have high tolerance to pH change (Rousk *et al.*, 2010). Similarly, role of phosphorus on fungal community structure was

already reported in several studies, but potassium's role in still unclear (Lauber *et al.*, 2013; Coince *et al.*, 2014; Rosenstock *et al.*, 2016; González-Domínguez *et al.*, 2017). TN and soil moisture were significantly correlated with soil fungal communities, which could be important factors in soil fungal community due to fungi's high demand for nitrogen (Cox *et al.*, 2010) and its narrow optimal range of soil moisture contents (Kaisermann *et al.*, 2015; Erlandson *et al.*, 2016). On the other hand, only ECM communities in root had correlation with MAT and the organic carbon in addition to other soil properties. Soil carbon contents (Coince *et al.*, 2014; Mrak *et al.*, 2020) and temperature (Cox *et al.*, 2010; Coince *et al.*, 2014) were both reported to have relationship with root ECM communities. Temperature can influence ECM communities by affecting the host plant, soil nutrients or physical tolerances. For organic carbon, the accumulation of photosynthetic products in soil from ectomycorrhizal fungi would be associated with this result (Smith & Read, 2008; Clemmensen *et al.*, 2013; Erlandson *et al.*, 2016).

Overall, this study provides a basis for understanding belowground fungal community compositions associated with *P. densiflora* in temperate forests of South Korea along altitudinal gradients. These results indicate strong correlations between richness and altitude in root-inhabiting fungal communities, but not in soil-inhabiting fungal communities. Microhabitat types and guilds of fungal taxa are important factors in determining fungal community structures along altitudinal gradients, which highlights the need to study various ranges of microhabitat types to understand the factors that influence belowground fungal communities. By extending the targets to diverse

types of plant hosts and microhabitats, these findings can provide a solid foundation for plant microbiome management and highlight the importance of small-scale variations in preservation of fungal diversity.

Chapter 2. Structure of root mycobiomes in *Pinus densiflora* accessed by long-read and short-read NGS technique

Abstract

Root associated fungal communities (mycobiome) play a critical role in plant health, by providing nutrients and suppressing possible pathogens. Recent studies uncovered a high diversity within root associated mycobiome using next-generation sequencing (NGS), but short read length of previous NGS platforms might cause limitations in taxonomic assignment. Here, DNA was extracted from root samples of *Pinus densiflroa*, and the full-length fungal ITS region of *P. densiflroa* root mycobiome was generated using long-read PacBio Sequel platform. Across sixteen *P. densiflora* forests across South Korea, core taxa that were detected in all of the sampling sites were found in root mycobiome. In a genus level, fourteen core genera were found, including *Cladophialaphora*, *Oidiodendron*, *Mortierella*, *Penicillium*, and *Russula*, representing various functions (saprotrophs, endophytes, and mycorrhizal fungi). When compared to results of short-read NGS platform (Illumina MiSeq), presence/absence patterns were mostly similar, but sequences of rare taxa were sometimes absent in each datasets. In addition. the proportion of each taxa differed between datasets. Overall, these results identified a number of fungi in root mycobiome in *P. densiflora* by long-read sequencing and highlighted the importance of non-mycorrhizal fungi in root mycobiome structure.

2.1. Introduction

Root-associated fungi exhibit various influences on their host and nearby forest environments. Their ecological functions are categorized by trophic groups such as symbiotrophic, saprotrophic, or pathotrophic fungi (Nguyen *et al.*, 2016). Symbiotrophic fungi include mycorrhizal and endophytic fungi, that improve the nutrient uptake of plants and protect from pathogen and other environmental stresses (Borowicz, 2001; Smith & Read, 2008; Segaran & Sathiavelu, 2019). Saprotrophic fungi contribute to the nutrient cycle by decomposing dead wood and litter in soil (Högberg *et al.*, 2003), but recent studies revealed root colonization by saprotrophic fungi (Vasiliauskas *et al.*, 2007; Smith *et al.*, 2017). Soil-borne plant pathogens infect plant roots and cause inhibition of root growth, wilt diseases, or plant death. Because of the relationship between plant and root-associated fungi, it is important to understand the structure and roles of root mycobiome. Recent studies revealed that a group of microbial groups is widely found at various locations, or 'core' microbiota, while the compositions of microbial communities are different across sampling sites (Yeoh *et al.*, 2017; Delgado-Baquerizo *et al.*, 2018; Jiao *et al.*, 2019; Xiong *et al.*, 2020). In contrast, at the other end of this spectrum, rare taxa represent the majority of mycobiome diversity and improve the resiliency of the ecosystem (Lynch & Neufeld, 2015; Xiong *et al.*, 2020).

Recent studies have revealed a wide taxonomic and functional range of root-associated fungi by next-generation sequencing (NGS) (Toju *et al.*, 2014a; Toju & Sato, 2018). Using environmental DNA and NGS is more efficient sampling method for studying root-associated

fungi compared to traditional methods such as sporocarp sampling, root tip observation, or culture (Thomsen & Willerslev, 2015; Nilsson *et al.*, 2019). However, there are several issues with NGS-based method in understanding the diversity of fungal communities. While the internal transcribed spacers (ITS) region of rRNA is commonly used for the identification of fungi (Schoch *et al.*, 2012), only ITS1 or ITS2 sub-regions of ITS has been utilized for taxonomic identification in commonly used short-read NGS platforms due to limitations in technology (Nilsson *et al.*, 2019). The long-read sequencing technology like Pacific Biosciences (PacBio) is an alternative approach to acquire full-length ITS rRNA sequences and higher taxonomic resolution (Walder *et al.*, 2017; Tedersoo *et al.*, 2018). Still, the PacBio platform has own its drawbacks such as low sequencing yields compared to previous NGS platforms and consequent susceptibility to primer choice and normalization (Tedersoo *et al.*, 2018).

In this study, *Pinus densiflora* Sieb. et Zucc. was selected as target species. *P. densiflora* is a widely distributed ectomycorrhizal tree that covers a third of forest in South Korea (Korea Forest Service, 2020), but their habitat suffers from plant diseases, drought, and extreme temperature due to climate change(Hirata *et al.*, 2017; An *et al.*, 2019; Cho *et al.*, 2020). Furthermore, overall fungal diversity associated with Korean *P. densiflora* was not studied until recently, except for seedlings in special environments (Sim & Eom, 2009; Lee *et al.*, 2012). To minimize the influence of underground vegetation, I targeted root, instead of soil, for investigation of mycobiome associated with *P. densiflora*. Root samples were collected from *Pinus densiflora* in 16 temperate forests in South Korea, then root mycobiome composition

and ecological roles were investigated with full-ITS region obtained by long-read sequencing from Pacbio platform. In addition, to cover the potential biases from sequencing methods, the data from the PacBio platform were compared with previous data of 5.8S and ITS2 region obtained from the Illumina MiSeq platform. As the sequence reads are not the best representative of the fungal community due to variable number of rRNA varies across taxonomic phylogenetic distance (Lofgren *et al.*, 2019), I aim to focus on the reliable representation of the fungal diversity and change of abundance in lower taxonomic levels. The core taxa of root mycobiome were defined by the dataset, then presence/absence and abundance patterns were tested on whether they are well represented between different platforms. Finally, the rare taxa with notable traits (i.e. plant pathogens or economically important mushrooms) were investigated (Appendix 2).

2.2. Materials and Methods

Sample collection and DNA extraction

P. densiflora root samples were sampled from sixteen *P. densiflora*-dominant forests in Korea from 2019 to 2020 (Table 1). For each sampling site, five mature *P. densiflora* trees with similar diameter at breast height (DBH) were randomly chosen at each sampling site. For each tree, the lateral roots were traced from the trunk and two lateral root samples with ectomycorrhizal root tips were collected from different directions using garden scissors sterilized by ethanol. Sampling was permitted and accompanied by the Korea National Arboretum Authority.

For DNA extraction, soil debris was gently removed by carefully shaking root samples in distilled water to prevent mechanical root damage. After washing, fine roots were separated with sterilized scissors and surgical gloves and disinfected with 3% hydrogen peroxide and sterile distilled water to remove possible contaminants from soil. The root samples were air-dried on sterilized filter paper, then finely ground using a mortar, pestle, and liquid nitrogen. Genomic DNA was extracted from ground root samples in triplicate using the MoBio Power Soil DNA Isolation kit (MoBio Laboratories, Carlsbad, CA, USA) following the manufacturer's guidelines. Additional details about sampling and DNA extraction methods are described in chapter 1.

Data generation

For PacBio sequencing, PCR products were generated following the PacBio Barcoded Universal Primers for Multiplexing Amplicons protocol (https://www.pacb.com/wp-content/uploads/Procedure-Checklist-Preparing-SMRTbell-Libraries-using-PacBio-Barcoded-Universal-Primers-for-Multiplexing-Amplicons.pdf). The ITS and LSU regions of fungal rRNA gene were amplified by ITSOF-T (Taylor & McCormick, 2008) and LR5F primers (Tedersoo *et al.*, 2008) containing adaptor sequences for PacBio sequencing. As suggested in a previous study (Nilsson *et al.*, 2019), ITSOF-T and LR5F primers were selected to avoid amplifying plant DNA. PCR reactions were performed three times for each sample using the AccuPower PCR PreMix kit (Bioneer, Daejeon, South Korea). The first round of PCR was conducted with the following protocols: 95℃ for 10 min, 20 cycles of 95℃ for 1 min, 55℃ for 1 min, and 72℃ for 2 min, and 72℃ for 10 min as a final extension. PCR products were checked on 1% agarose gel and purified using ExpinTM PCR SV kit (GeneAll Biotechnology, Seoul, South Korea). In the second round of PCR, PacBio barcodes were attached using PacBio barcode primers (Pacific Biosciences, Menlo Park, CA) with the same protocol. After purification, concentrations of amplicon libraries were measured using a NanoDrop2000 (Thermo Fisher Scientific, Waltham, MA, USA) and pooled in equimolar quantities. Amplicon libraries were sequenced in the Sequel platform (Pacific Biosciences, Menlo Park, CA) at Macrogen (Seoul, Korea).

For comparison, the dataset from Illumina MiSeq sequencing produced in Chapter 1 was used. In this dataset, the 5.8S and ITS2 regions of the fungal rRNA gene were amplified using 5.8S-Fun and

ITS4-Fun primers to reduce the amplification of plant DNA (Taylor *et al.*, 2016).

Data processing

Demultiplexing and Circular Consensus Sequences (CCS) generation were conducted in Macrogen (Seoul, Korea). CCS sequences were processed using QIIME v.1.8.0 (Caporaso *et al.*, 2010). For samples collected in 2019, the sequence data was provided in fasta format after quality filtering (Q ≥ 30). For samples collected in 2020, sequence data was provided in fastq format and gone through quality filtering (Q ≥ 30). the full ITS sequences were extracted using ITSx v.1.1b (Bengtsson-Palme *et al.*, 2013) and filtered sequences shorter than 400 bp. Operational taxonomic units (OTUs) were clustered with a 99% similarity model with the open-source sequence search tool Vserach v. 2.6.2 (Rognes *et al.*, 2016). Chimeric sequences were filtered using 'uchime_denovo' methods and reference database of UNITE 8.2 (Kõljalg *et al.*, 2013) and Seoul National University Fungal Collection (SFC) in Vserach v. 2.6.2 (Rognes *et al.*, 2016). The most abundant sequence was selected as a representative sequence of each OTU. Sequences from UNITE v. 7.2 (Kõljalg *et al.*, 2013) and sequences from Seoul National University Fungal Collection (SFC) were used as a reference database for taxonomic assignment with NCBI BLAST algorithm following the criteria of Tedersoo et al. (Tedersoo *et al.*, 2014). In addition, the post-clustering curation method LULU (Frøslev *et al.*, 2017) was performed (minimum_match = 99) to merge co-occurring 'daughter' OTUs with similar, but more abundant 'parent' OTUs. Non-

fungal sequences and rare OTUs (<0.001% of the total number of sequences) were filtered for quality control (Bokulich *et al.*, 2013). Among 80 samples, 1 sample was discarded due to low sequence reads (GY01P-4). For Illumina MiSeq data, the OTU table produced in Chapter 1 was used. Post-clustering and removal of rare OTUs were conducted in the same methods as PacBio sequencing data. two samples were discarded due to low sequence reads (GY03P-4R, GW04P-3R). The samples from the same region were pooled before further analysis.

For trophic mode assignment, FUNGuild was used as a database (Nguyen *et al.*, 2016). OTUs with confidence ranking of 'possible' or 'unassigned' were classified based on literature. In analysis related to the guild, only OTUs that were identified at the genus level were categorized to guilds to improve credibility. OTU tables were examined to find rare OTUs with unique characteristics (pathogen with reports of plant disease in *Pinus* spp. or valued edible mushrooms) based on literature search and FUNGuild data. Before further analysis, samples were normalized using the proportions to cover all of the OTUs discovered. In addition, the list of fungal species was compared to the National List of species of Korea (2020).

Statistical analysis

To visualize the quantitative and hierarchical composition of fungal communities, an interactive sunburst chart was generated for each dataset and major guilds (ectomycorrhizal fungi, saprotroph, endophyte, plant pathogens) with Krona (Ondov *et al.*, 2011). All

statistical analysis was performed in R v.4.0.4. (R Core Team, 2021). The relative abundances (sequence reads of specific OTU/total sequence reads) of each OTUs were log-transformed for statistical analyses. To compare the community composition from two datasets, non-metric multidimensional scaling (NMDS) analysis was conducted at genus level with Jaccard diversity metrics (presence/absence) and 999 permutations using phyloseq R package (McMurdie & Holmes, 2013). Permutational multivariate analysis of variance (PERMANOVA) statistical test implemented as 'adonis' in vegan R package (Oksanen *et al.*, 2013) was used to analyze the difference in community compositions between datasets. The proportion of unidentified OTUs were calculated at phylum, class, order, family, and genus level for each dataset, and compared the difference using a t-test. Venn diagrams were produced to visualize the number and proportion of shared and exclusive taxa for each dataset at phylum, class, order, family, and genus level. Here, the 'core' taxon of fungi was defined if that taxon is present in all sampling regions. The number of core taxa and their proportion in the relative abundances were visualized at the genus (PacBio, MiSeq) and OTU (PacBio) levels. The correlation analysis was performed with cor.test function (based on Pearson correlation) in R to test the relationship between the relative abundance of core taxa obtained from two sequencing methods. The R package ggplot2 was used to visualize the results (Valero-Mora, 2010).

2.3. Results

Sequencing analysis and OTU classification

A total of 522,121 sequences were obtained from 80 samples (PacBio). After post-clustering and filtering of non-fungal OTUs, rare OTUs (<0.001% of the total number of sequence reads) and sample with the low number of sequence reads, 494,227 sequences, and 2,266 OTUs remained. A total of 5,216,632 sequences were found in the MiSeq dataset. After the same filtering procedure with the PacBio dataset, 4,894,141 sequences and 2,306 OTUs remained. The rarefaction curve reached saturation in most of the samples from both datasets (Fig. 15). On average, 57.87 ± 11.88 genera and 227.54 OTUs ± 48.70 were found from single tree sample in PacBio dataset. In MiSeq dataset, 90.82 ± 18.53 genera and 488.00 ±145.49 OTUs were found from each single tree sample.

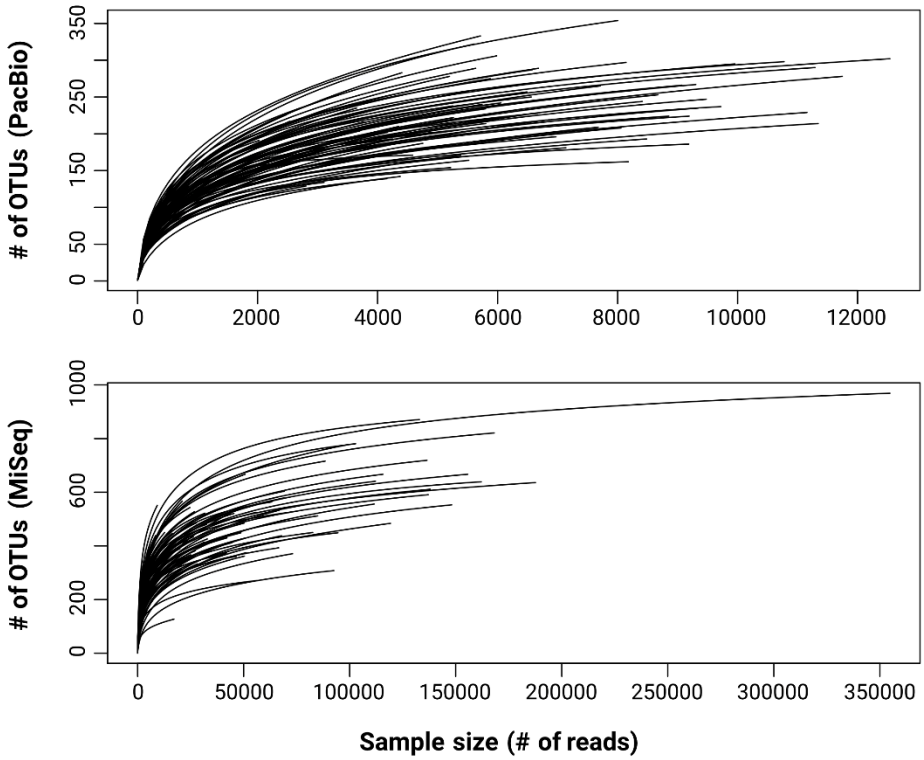

Figure 15. Rarefaction curves of rRNA gens of fungal communities from PacBio and Illumina MiSeq datasets. Rarefaction curves were generated based on 79 (PacBio) and 78 samples (Illumina MiSeq) with 100 iteration.

Taxonomic and functional composition of fungi

In the PacBio dataset, 8 phyla, 30 classes, 73 orders, 142 families, and 267 genera were found (Fig. 16). The most dominant and diverse phylum was Ascomycota (54.96% / 1,117 OTUs), followed by Basidiomycota (38.74% / 897 OTUs) and Mortierellomycota (74 OTUs). 9 OTUs (0.019%) were not identified at the phylum level. At the genus level, the most abundant genus was *Cladophialphora*, followed by *Archaeorhizomyces*, *Oidiodendron*, and *Mortierella*, but their abundance was greatly differed by sampling sites (Fig. 17). Including overlapping OTUs, saprotrophic fungi (41.47% / 892 OTUs) was the most common guild, followed by endophyte (27.66% / 263 OTUs), ectomycorrhizal fungi (20.02% / 425 OTUs), and plant pathogen (11.67% / 251 OTUs) group. Saprotrophic fungi were mostly composed of Ascomycota, Basidiomycota, and Mortierellomycota, and major genera were *Cladophialphora*, *Mortierella*, and *Penicillium*. Basidiomycota was the most dominant phylum in the ectomycorrhiza guild, followed by Ascomycota. *Lactarius*, *Russula*, and *Tomentella* were the most dominant genera. The composition of endophyte and plant pathogen guilds were overlapped with each other. The most dominant genera were *Mortierella*, *Penicillium*, and *Trichoderma* in endophyte, while *Penicillium*, *Venturia*, and *Mycena* were the most dominant genera in the plant pathogen guild.

Figure 16. Krona plot visualizing taxonomic distribution of all fungi in PacBio dataset based on relative abundance of OTUs.

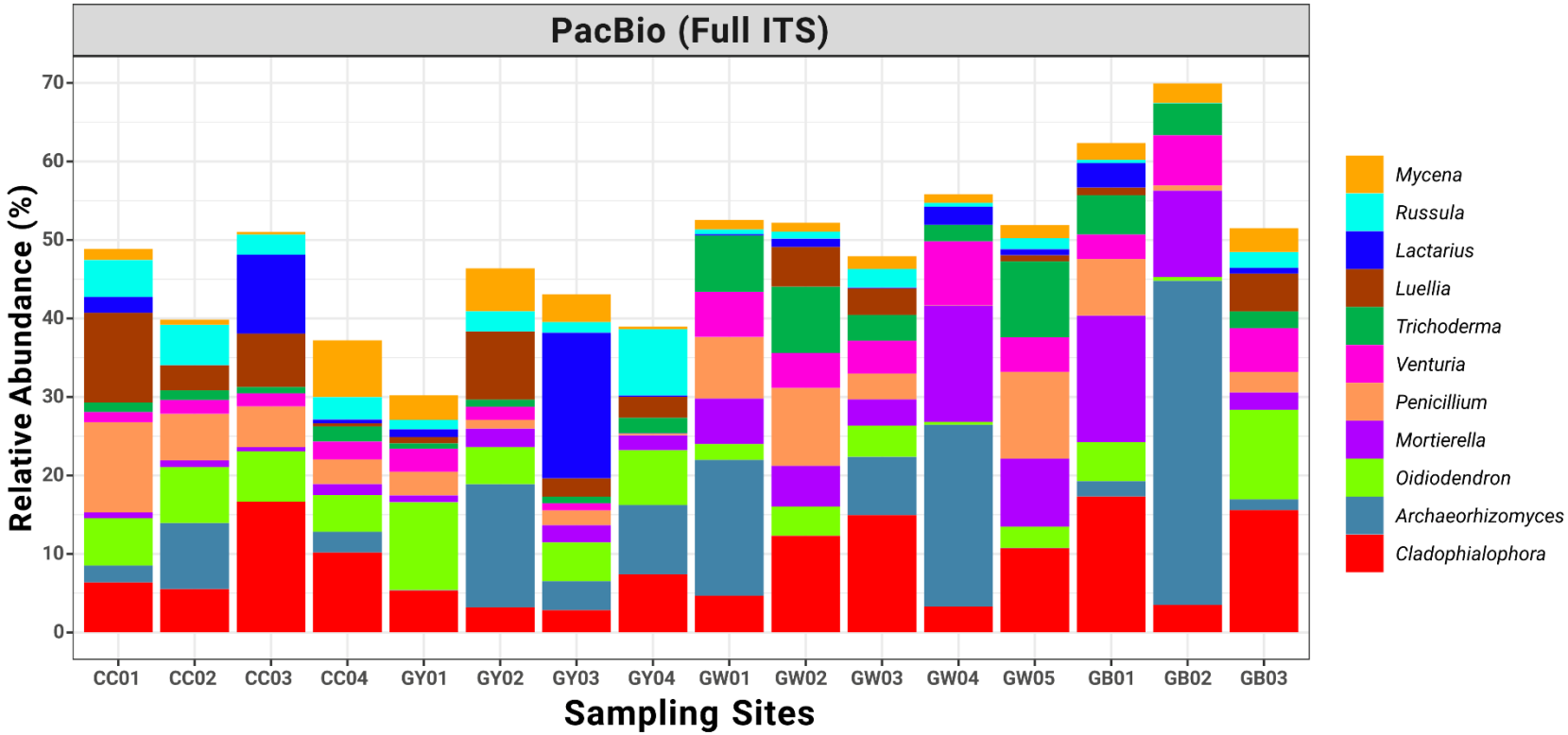

Figure 17. Taxonomic assignment and composition of the major genera (〉1% in relative abundance) at the genus level in PacBio dataset.

Compared with the MiSeq dataset, most of the taxa were shared between datasets, and the proportion of dataset-specific taxa was less than 2% except for genus level (Fig. 18). However, relative abundances were greatly different between datasets in several taxa (Figs. 19 and 20). For example, the proportion of Ascomycota was much smaller (28.41% / 955 OTUs), and that of Basidiomycota was higher in MiSeq dataset (64.61% / 1,110 OTUs). The proportion of each genera and guilds were different from that of PacBio dataset. The most abundant genus was *Suillus*, followed by *Mycena*, *Tomentella*, and *Phialocephala*. Interestingly, the proportion of *Archaeorhizomyces* was lower than 0.1 % in MiSeq data, while it was 8.38 % in PacBio data. Saprotrophic fungi were still the most common guild (45.07%), but proportion of ectomycorrhizal fungi (which were mostly belonged to Basidiomycota) was increased to 30.27%. Further, major genera were changed in MiSeq dataset. For example, *Mycena*, *Basidiodendron*, and *Trichoderma* were the most dominant saprotrophic genera, while *Suillus*, *Tomentella*, and *Pseudotomentella* were the most dominant ectomycorrhizal genera.

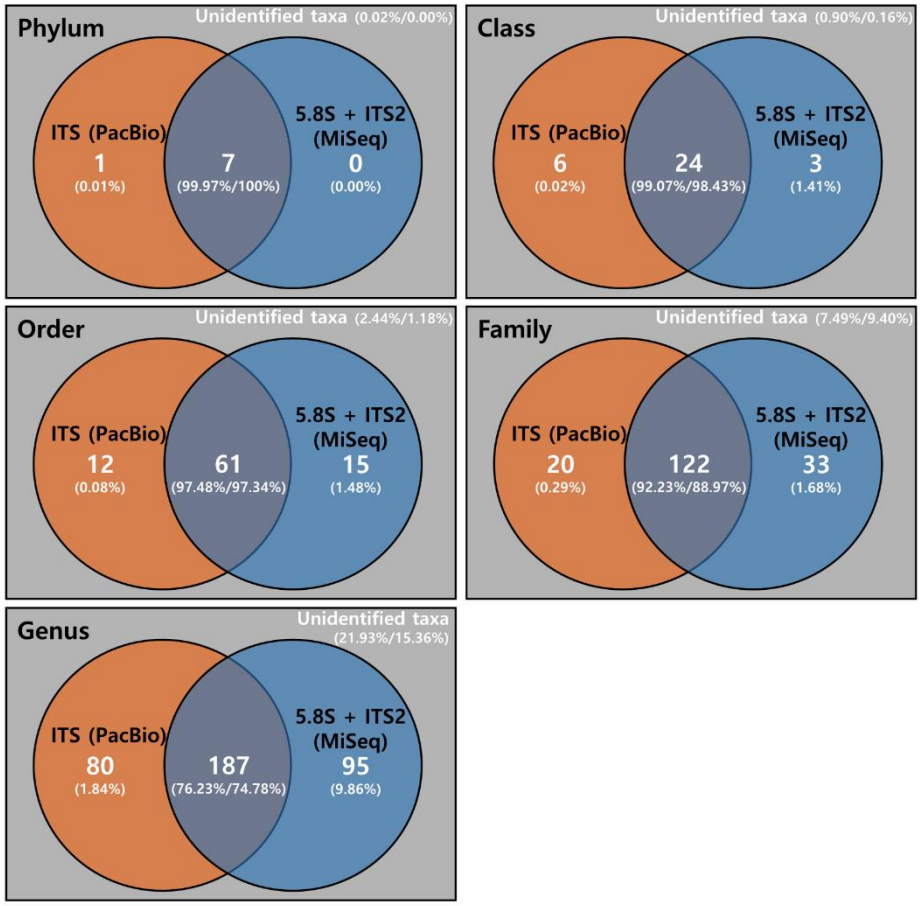

Figure 18. The number and relative abundance of shared and dataset-specific taxa.

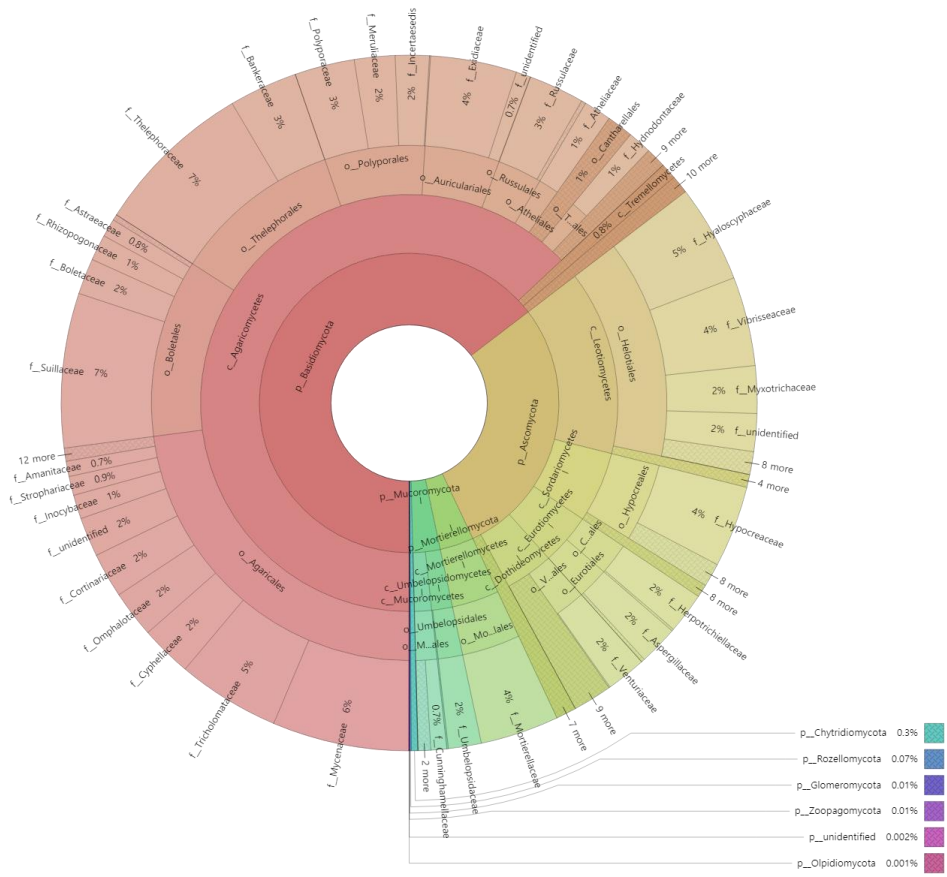

Figure 19. Krona plot visualizing taxonomic distribution of all fungi in MiSeq dataset based on relative abundance of OTUs.

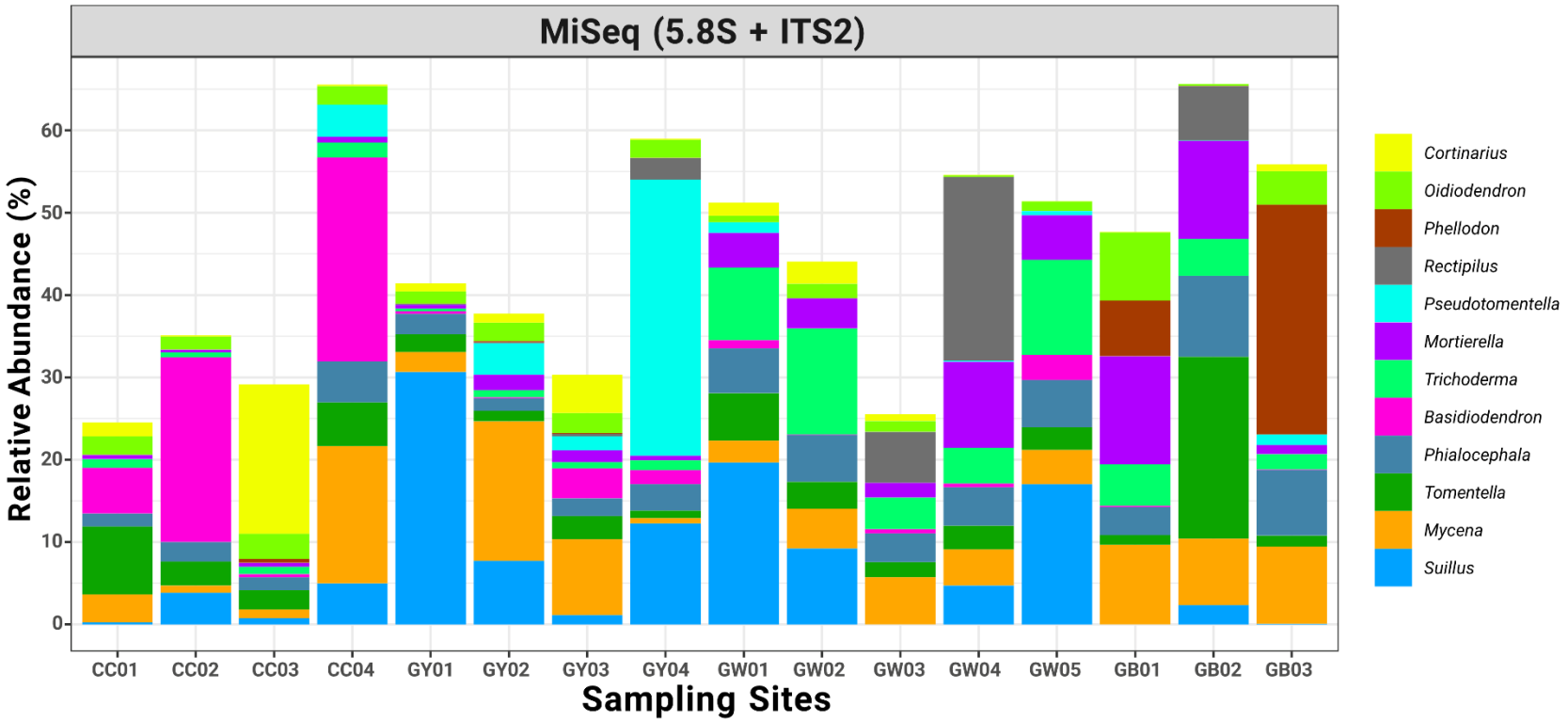

Figure 20. Taxonomic assignment and composition of the major genera (>1% in relative abundance) at the genus level in MiSeq dataset.

The relative abundances of unidentified taxa were significantly higher in PacBio datasets class, order, and genus level, though they were similar in phylum and family level (Fig. 21). When compared at the genus level, both sequencing methods and sampling sites had a significant influence on the root mycobiome. But the effect of sampling sites (R^2 = 58.72%) was much higher than that of sequencing methods (R^2 = 13.17%). In addition, albeit at low abundance and/or frequency, rare OTUs with unique characteristics were found in root mycobiome (Table 6). In both datasets, valued gourmet mushrooms (*Tricholoma matsutake*) and plant pathogenic fungi (*Dactylonectria, Fusarium*, *Ilyonectria, Phacidium*) were found with slightly different distributions between datasets. In contrast, other plant pathogenic fungi (*Armillaria*, *Phoma*) were detected only in the PacBio dataset.

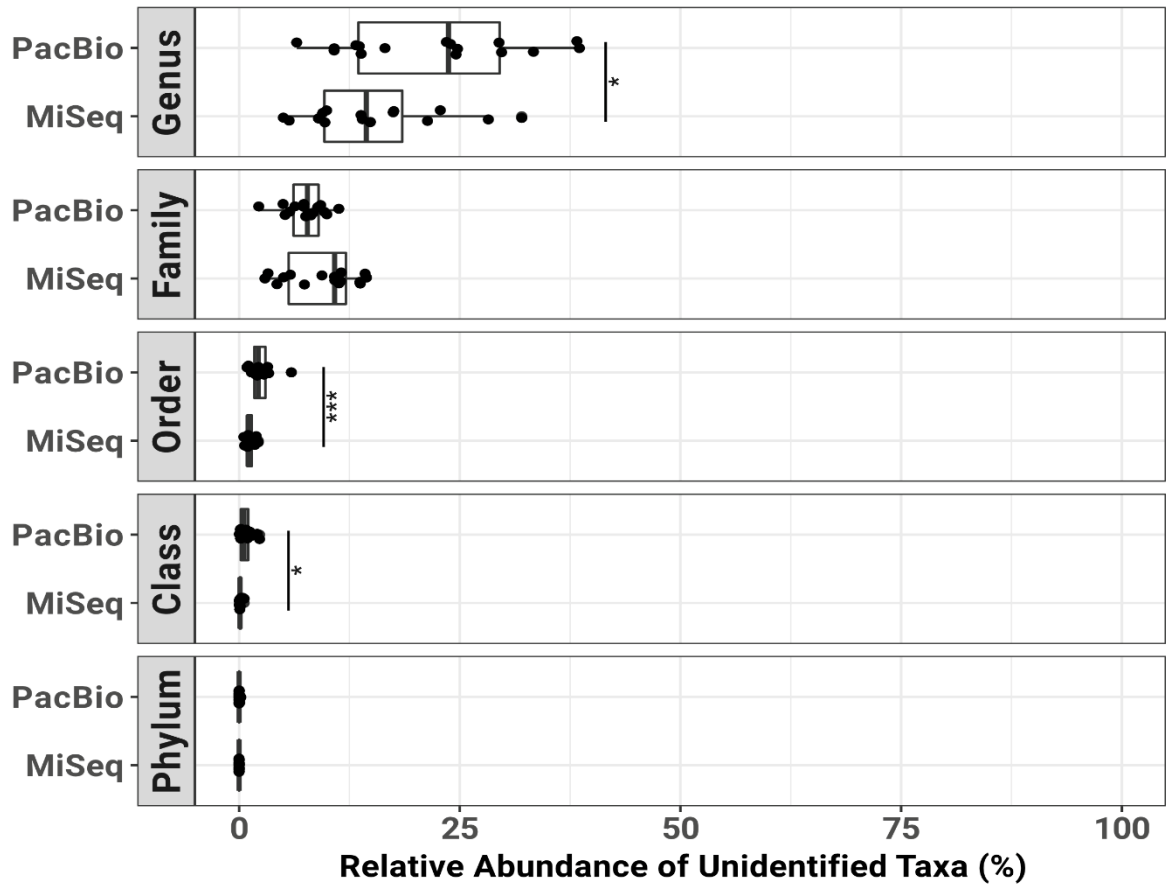

Figure 21. The relative abundance of unidentified taxa in each datasets (* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$).

Table 6. List of rare taxa with unique characteristics

Dataset	Frequency	Average relative abundance (%)	BLAST result				Characteristics
			OTU ID	Identification	Per. Identity	Accession No.	
PacBio	1/16	0.017	OTU 696	*Armillaria ostoyae*	100%	KT822292	Armillaria root rot
MiSeq				NA			
PacBio	1/16	0.002	OTU 21794	*Dactylonectria macrodidyma*	100%	HM036602	Root rot in seedlings of *Pinus* spp.
PacBio	1/16	0.009	OTU 559	*D. macrodidyma*	99%	MK841907	
MiSeq	3/16	0.005	OTU 881	*D. macrodidyma*	99%	MK841907	
PacBio	8/16	0.086	OTU 151	*Ilyonectria destructans*	100%	KY322656	Root rot in seedlings of *Pinus* spp.
PacBio	9/16	0.035	OTU 15185	*Ilyonectria radicicola* (=*I. destructans*)	100%	JQ272460	
MiSeq	13/16	0.097	OTU 142	*I. destructans*	99%	KC180706	
MiSeq	8/16	0.119	OTU 1593	*I. destructans*	99%	FJ430732	
MiSeq	1/16	0.003	OTU 383144	*I. destructans*	99%	KC989076	
MiSeq	3/16	0.002	OTU 405595	*I. destructans*	99%	KC989075	

Table 6, Continued

Dataset	Frequency	Average relative abundance (%)	BLAST result				Characteristics
			OTU ID	Identification	Per. Identity	Accession No.	
PacBio	3/16	0.002	OTU 1276	*Phacidium lacerum*	99%	MH859847	Snow-blight disease in *Pinus* spp.
PacBio	9/16	0.079	OTU 152	*P. lacerum*	99%	MH859847	
PacBio	1/16	0.001	OTU 1860	*P. lacerum*	99%	MH859847	
PacBio	4/16	0.006	OTU 21129	*P. lacerum*	100%	MH859847	
PacBio	5/16	0.016	OTU 9985	*P. lacerum*	100%	MH856297	
MiSeq	10/16	0.008	OTU 224297	*P. lacerum*	99%	FR837911	
MiSeq	12/16	0.007	OTU 324763	*P. lacerum*	99%	FR837911	
MiSeq	12/16	0.005	OTU 328932	*P. lacerum*	99%	FR837911	
MiSeq	11/16	0.005	OTU 354335	*P. lacerum*	99%	FR837911	
PacBio	4/16	0.046	OTU 277	*Phoma sp.*	100%	KC928322	Phoma blight in *Pinus* spp.
MiSeq				NA			
PacBio	1/16	0.022	OTU 501	*Tricholoma matsutake*	100%	KJ874166	Gourmet mushroom
MiSeq	2/16	0.001	OTU 218714	*T. matsutake*	100%	JF908729	
MiSeq	2/16	0.009	OTU 218841	*T. matsutake*	100%	JF908729	
MiSeq	2/16	0.005	OTU 219219	*T. matsutake*	99%	JF908729	
MiSeq	2/16	0.003	OTU 220141	*T. matsutake*	100%	U62964	

Core taxa of *P. densiflora* root mycobiome

The small group of ubiquitous taxa and a large fraction of taxa related to the small number of samples were found across sampling sites in both genus and OTU level (Figs. 22 and 23). Among 267 genera, only 14 genera were present in all sampling sites, while 50 genera were found in only one site. Similarly, within 2,266 OTUs, only 14 OTUs were ubiquitous, and 577 OTUs were found only in a single sampling site. Those core genera and OTUs account for 38.60% and 11.19% of total sequence read, respectively. Similar results were found in the MiSeq dataset, but the number of core taxa and their proportion were much higher than those of the PacBio dataset, 52.91% and 11.19% in genus and OTU level, respectively (Figs. 24 and 25). Core genera of the PacBio dataset were also included in the core genera of the MiSeq dataset (Fig. 26). In addition, in the MiSeq dataset, the proportion of ectomycorrhizal fungi was much higher in both the number and relative abundance of genera (Fig. 27).

The components of core genera were mostly similar to major genera, but *Luellia* and *Lactarius* were not included in core genera, while less abundant genera were found in all sampling sites, such as *Lachnum*, *Cenococcum*, *Metapochonia*, and *Sagenomella* (Fig. 26). Ecological roles of core taxa were various, but the relative abundance of core genera was greatly different between sampling sites regardless of their guilds. When compared to that of the Miseq dataset, the proportions of core genera were mostly similar between datasets and were significantly correlated across the sampling sites (Fig. 26). Much more ectomycorrhizal genera were found in the MiSeq dataset-only core genera (Fig. 27). 15 genera were also found in both datasets, but

Helicoma was not detected in the PacBio dataset. In contrast to core genera that were found in both datasets, MiSeq dataset-only core genera exhibited a much lower abundance in PacBio datasets. At the OTU level, OTUs belonging to *Cladophialaphora*, *Mortierella*, *Oidiodendron*, *Penicillium*, and *Venturia* were found in the core OTU group. Most of them were saprotroph, endophytes, or ericoid mycorrhizal fungi and there was no ectomycorrhizal OTU that were found in all of the sampling sites.

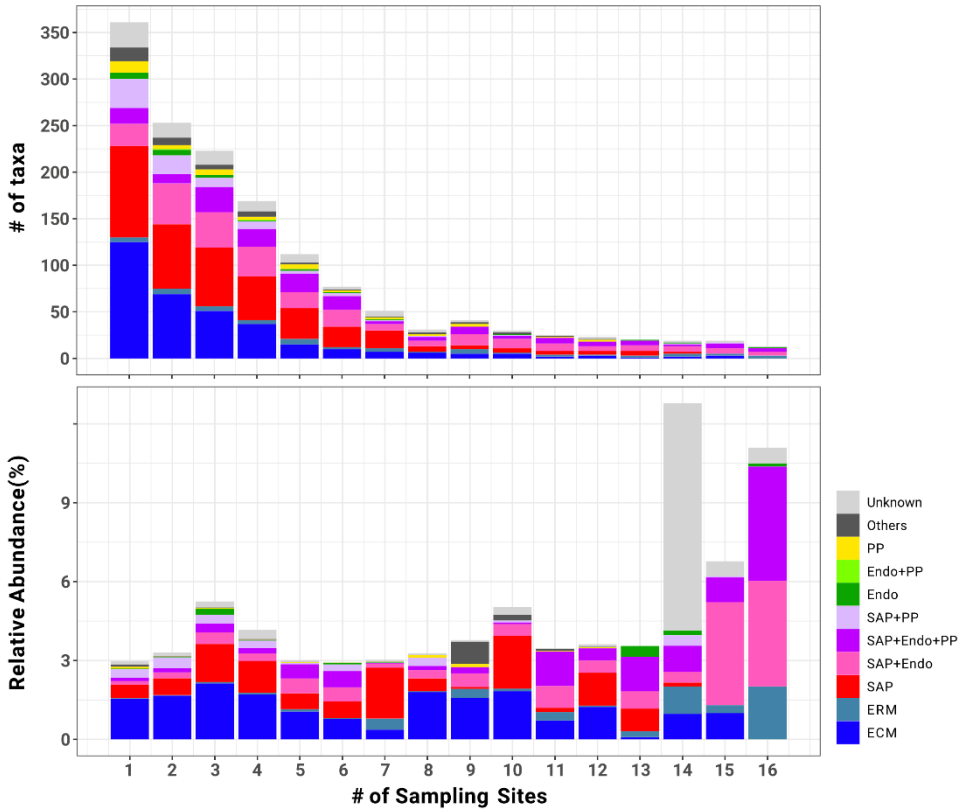

Figure 22. Core and site-specific fungal microbiome at genus level in PacBio dataset. Distribution and relative abundance of the fungal taxa that were found in a given number of sampling sites, classified by guild level. Abbreviations: ECM, ectomycorrhiza; ERM, ericoid mycorrhiza; SAP, saprotroph; Endo, endophyte; PP, plant pathogen.

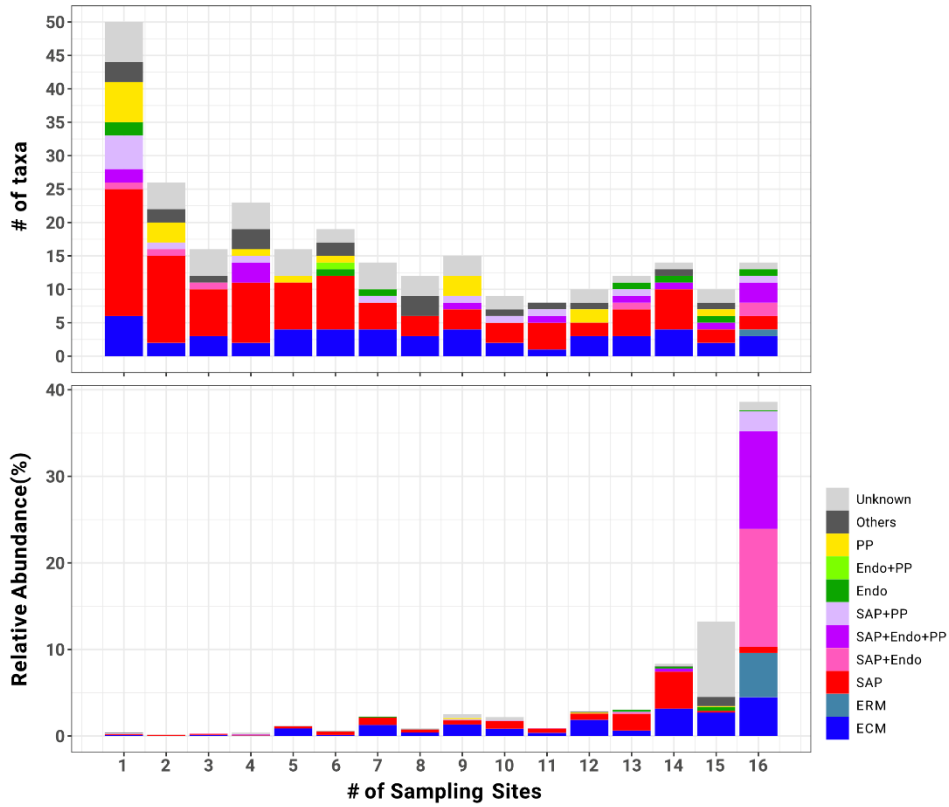

Figure 23. Core and site-specific fungal microbiome at OTU level in PacBio dataset. Distribution and relative abundance of the fungal taxa that were found in a given number of sampling sites, classified by guild level. Abbreviations: ECM, ectomycorrhiza; ERM, ericoid mycorrhiza; SAP, saprotroph; Endo, endophyte; PP, plant pathogen.

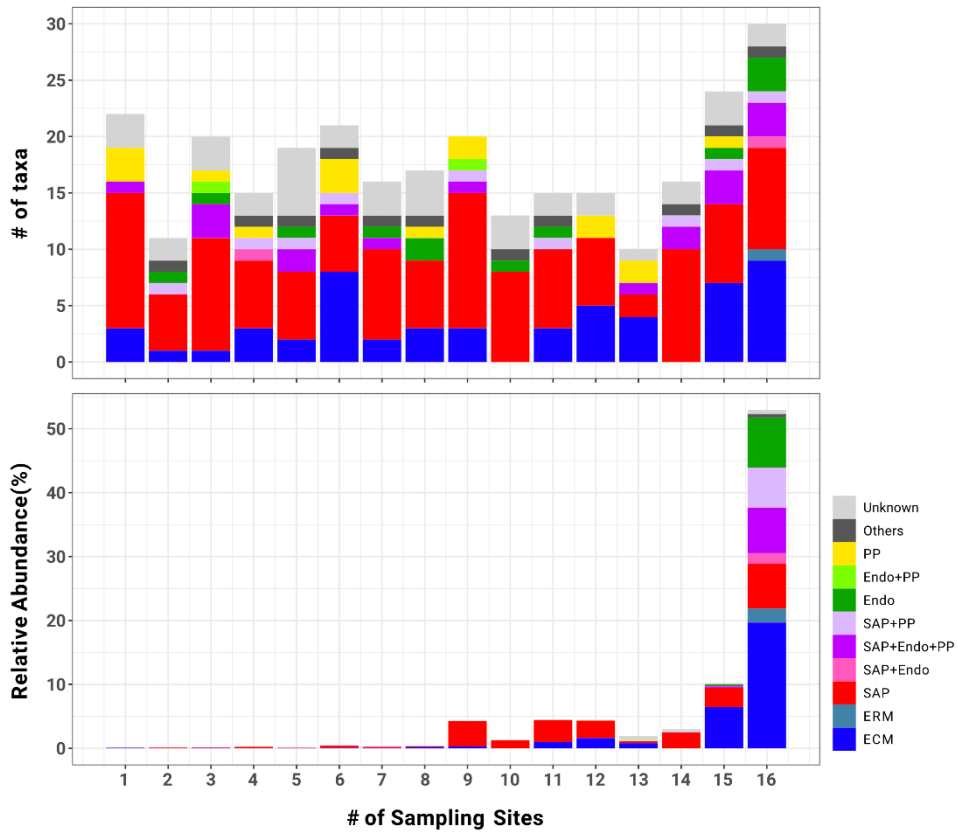

Figure 24. Core and site-specific fungal microbiome at genus level in MiSeq dataset. Distribution and relative abundance of the fungal taxa that were found in a given number of sampling sites, classified by guild level. Abbreviations: ECM, ectomycorrhiza; ERM, ericoid mycorrhiza; SAP, saprotroph; Endo, endophyte; PP, plant pathogen.

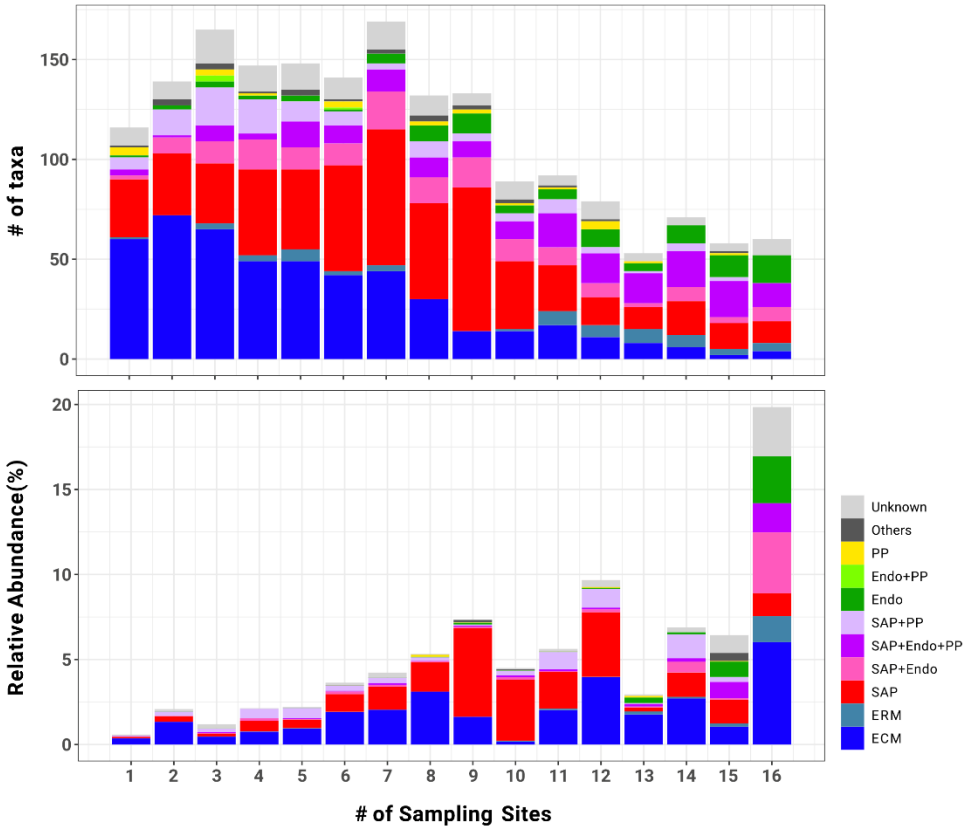

Figure 25. Core and site-specific fungal microbiome at OTU level in MiSeq dataset. Distribution and relative abundance of the fungal taxa that were found in a given number of sampling sites, classified by guild level. Abbreviations: ECM, ectomycorrhiza; ERM, ericoid mycorrhiza; SAP, saprotroph; Endo, endophyte; PP, plant pathogen.

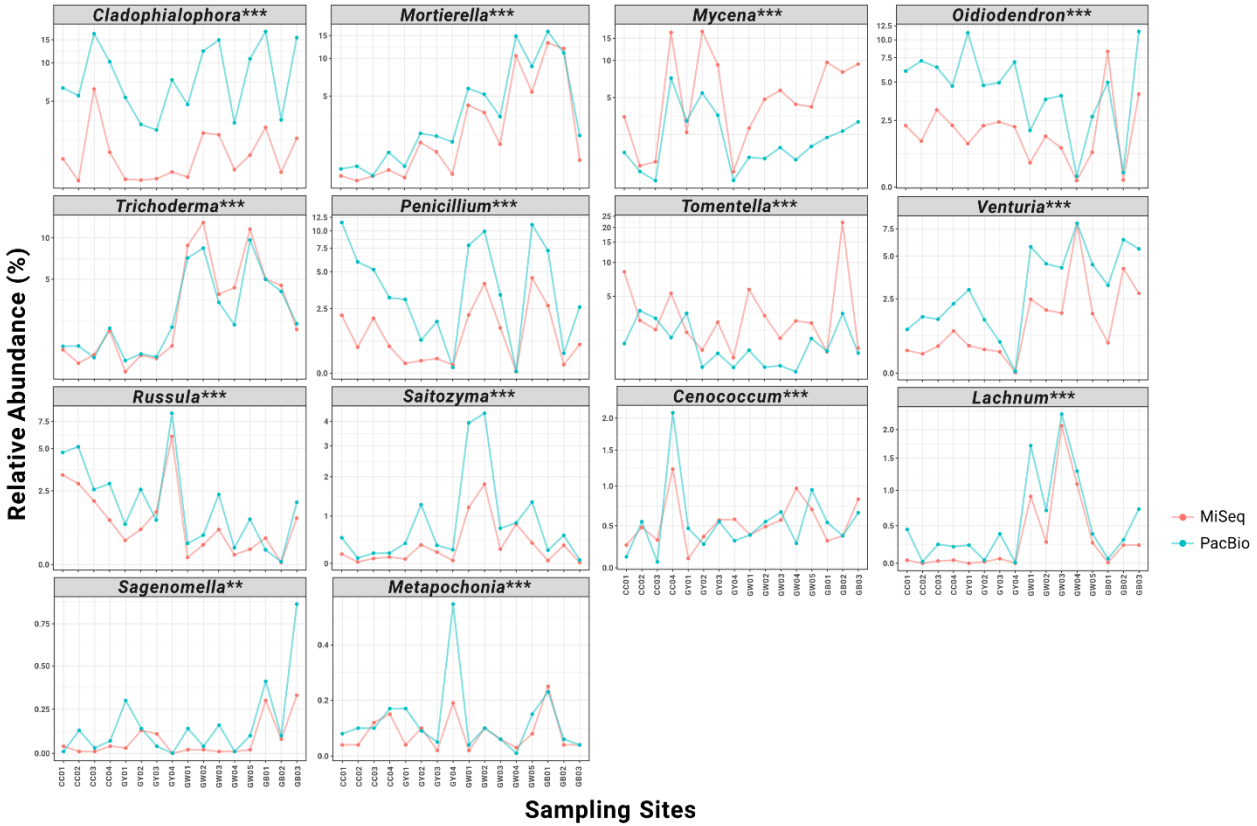

Figure 26. Relative abundance of core genera (in both PacBio and MiSeq datasets) in the different sampling sites. A base-10 log scale was used for y axis (*$p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$).

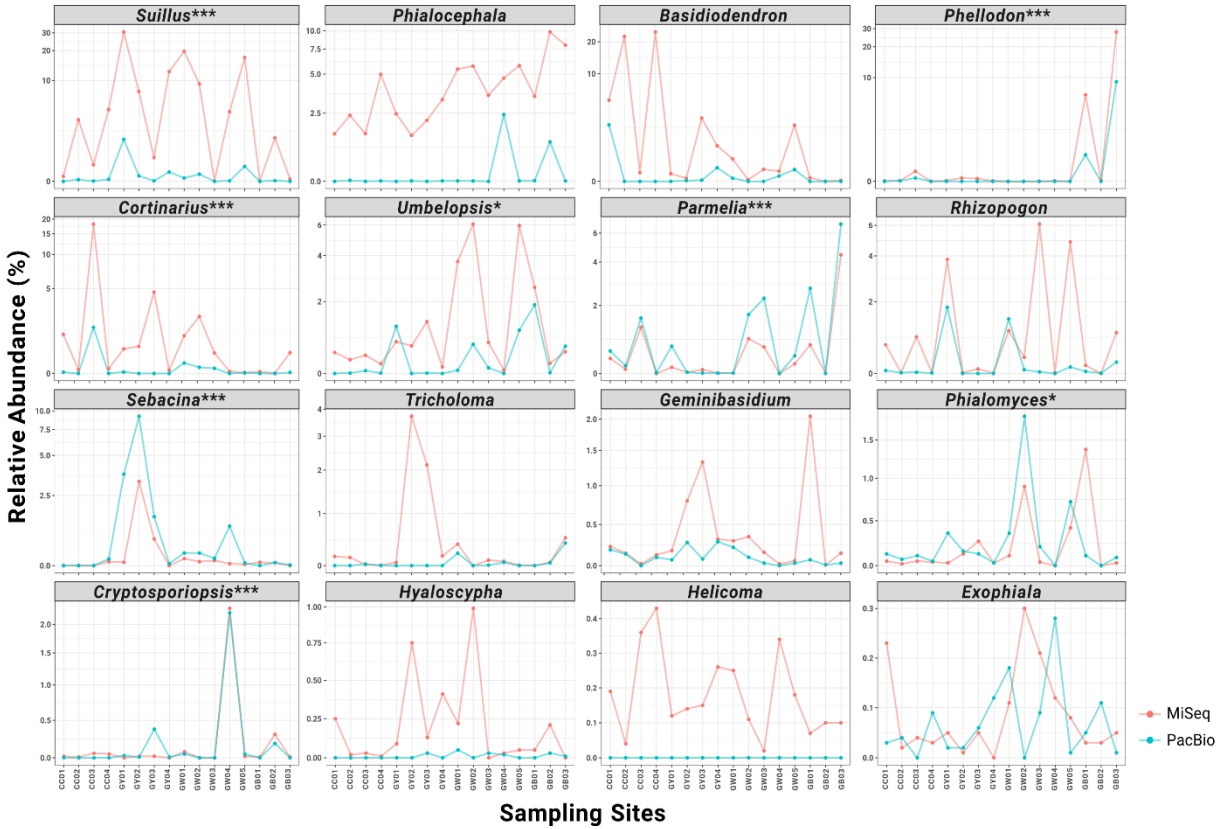

Figure 27. Relative abundance of core genera (MiSeq dataset only) in the different sampling sites. A base-10 log scale was used for y axis (*$p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$).

2.4. Discussion

My study provided a detailed information on the structure and compositions of mycobiome associated with root of *P. densiflora* by both PacBio and Illumina MiSeq sequencing. By directly sampling root samples from tree, roots from understory vegetations or other allied species of host trees could be minimized. Previous studies revealed that plants interact with diverse fungi in terms of their taxonomy and functions (Newsham, 2011; Toju & Sato, 2018). In these results, the groups of commonly found fungi were composed of various guilds: ectomycorrhizal fungi, saprotrophic fungi, and endophytic fungi.

The structure of root-associated mycobiome in *P. densiflora*

In both datasets, most of the core genera were belonged to saprotrophic fungi or endophytic fungi. Among them, the most abundant genera in PacBio datasets were *Cladophialaphora*, *Mortierella*, and *Trichoderma*. Species in those genera have been reported to have saprotrophic, pathogenic, and endophytic abilities (Usuki & Narisawa, 2007; Melo *et al.*, 2014; Rim *et al.*, 2021). In addition, *Cladophialophora* and *Mortierella* were reported to have close relationship with other fungi in network of root fungal communities (Toju *et al.*, 2018). One of those species, *Cladophialaphora chaetospira* has been known to enhance the growth of host plant by providing nitrogen to its host (Usuki & Narisawa, 2007). *Mortierella* and *Trichoderma* were also abundant in MiSeq dataset, but *Phialocephala*

and *Basidiodendron* were belonged to core genera only in MiSeq dataset. Presence of *Phialocephala* was commonly reported in root of pine seedlings (Sim & Eom, 2009; Lee *et al.*, 2012; Park *et al.*, 2020). In contrast, *Basidiodendron* was rarely reported in living root of *P. densiflora* and often found in soil or deadwood (Mäkipää *et al.*, 2017). While their status is unclear, *Basidiodendron* might have formed mycorrhiza-like structure in root tips (Ning *et al.*, 2018).

Within the mycorrhizal fungi, *Oidiodendron*, *Russula*, *Tomentella*, and *Cenococcum* were found to be core genera in both datasets. *Oidiodendron* is known as ericoid mycorrhiza, and often found in soil environment. However, species in this genera were also found in non-ericaceous trees including *P. densiflora* (Toju & Sato, 2018; Park *et al.*, 2020; Zhao *et al.*, 2020). Other core mycorrhizal genera (*Russula*, *Tomentella*, *Cenococcum*) are commonly found ectomycorrhizal fungi in *P. densiflora*. In contrast, other ectomycorrhizal basidiomycota that are frequently found in *Pinus* spp. (*Suillus*, *Cortinarius*, *Tricholoma*, *Rhizopogon*, and *Sebacina*) were detected in all sampling sites only in MiSeq datasets (Akiyoshi & Keizo, 2001; Dickie *et al.*, 2010; Koizumi *et al.*, 2018). In contrast to those core genera, *Archaeorhizomycetes* was not found in some sampling sites despite high abundance in PacBio datasets. The ecological role of *Archaeorhizomycetes* is still uncertain, but current studies revealed that their distributions are influenced by elevation-associated environmental (Pinto-Figueroa *et al.*, 2019).

In a plant pathogen group, core genera such as *Venturia* and *Mycena* did not have reports of plant diseases in mature pine trees and had saprotrophic or endophytic abilities (Schlegel *et al.*, 2018; Thoen

et al., 2020). Similarly, most of other taxa in plant pathogen group had saprotrophic or endophytic abilities. I expect their roles in root of *P. densiflora* are closer to endophyte or decomposers of dead mycelia/plant tissue as any visual sign of plant diseases were detected in the samples. As root samples were collected from healthy trees without visible symptoms, low frequency of severe plant pathogens was expected. However, among the rare taxa, several genera did cause plant disease in *Pinus* spp. (*Dactylonectria*, *Fusarium*, *Ilyonectria*, *Phacidium*, *Phoma*), but those reports were restricted to leaf blight disease or root rot death of conifer seedlings (Unestam *et al.*, 1989; Menkis & Burokienė, 2012; Stenström *et al.*, 2014). Those taxa might have invaded from dead roots and act as dormant reservoir in living roots (Unestam *et al.*, 1989; Menkis & Burokienė, 2012). Though the proportion was small (0.27%), *Armillaria ostoyae* was detected in one of the sampling sites (GW05P; Jeongseon-gun), which is known as the cause of Armillaria root rot in conifer trees. Intriguingly, the report of Armillaria root rot in *Pinus strobus* trees was found in vicinity of sampling sites from pest monitoring reports (KFRI, 2016).

Comparison between PacBio (full ITS) and Illmina MiSeq (5.8S + ITS2) datasets

Due to lower sequence throughput compared to Illumina MiSeq platform, PacBio sequencing was reported to have several concerns with characterization of fungal community from environmental samples (Tedersoo *et al.*, 2018; Kennedy *et al.*, 2018). In this study, PacBio datasets had disagreements in abundances of fungi compared

to MiSeq datasets. In presence/absence comparison of core taxa across sampling sites, dozens of taxa displayed such differences due to low sequence throughput of PacBio datasets or primer bias. When compared within same genera, proportion patterns were similar across sampling sites, and their relative abundances were similar between datasets in core taxa in PacBio datasets. However, in core genera of MiSeq datasets, many genera were absent in several sampling sites, and their sequences were not amplified enough in PacBio datasets than that of MiSeq datasets. As described earlier, some of ectomycorrhizal genera showed stark proportion across sampling sites in PacBio datasets. Due to low sequencing depth or primer bias, it seems that the sequences were not amplified in some regions with fewer relative abundance of specific taxa (Kennedy *et al.*, 2018). These results emphasize the need for caution in comparison of fungal communities with different sequencing platforms and primer sets.

Previous studies reported higher identification rate of full ITS compared to partial ITS region (ITS1 or ITS2) (Walder *et al.*, 2017; Tedersoo *et al.*, 2018; Purahong *et al.*, 2019). However, improvements of identification resolution were not found in PacBio datasets. Combing 5.8S region with ITS2 region may have improved the resolution of MiSeq datasets (Heeger *et al.*, 2019), but use of different pipelines (i.e. QIIME2, Mothur, Pipecraft) may improve the resolution of PacBio datasets. Interestingly, in PacBio dataset, the relative abundances of Rozellomycota and some of Ascomycota (Saccharomycetes and Archaeorhizomycetes) were significantly higher in PacBio datasets than that of MiSeq datasets ($p < 0.05$). Similar result was reported in previous studies, where full ITS datasets exhibited higher proportions of those

taxa compared to ITS1 and ITS2 datasets (Tedersoo *et al.*, 2018). While the direct comparison is not possible as primer set and sequencing platforms are different, this result may indicate the usefulness of full ITS sequences in detection of certain taxonomic groups in environmental DNA.

This work presents the composition of root mycobiome in *P. densiflora* of South Korea using PacBio sequencing of the full ITS region. This result revealed that fungal communities associated with *P. densiflora* are taxonomically and functionally diverse, and core fungal genera were found across various sampling sites. High proportion of non-mycorrhizal fungi (i.e. saprotrophic and endophytic fungi) in core genera suggests that they play vital roles in plant root. In addition, DNA metabarcoding method was able to detect economically important plant pathogens and gourmet mushrooms with low abundance and frequency, revealing its possibility in monitoring fungi from environmental samples. But the results should be interpreted with caution due to possible bias in methods. When compared to the results of Illumina MiSeq platform targeting 5.8S + ITS2 region, their taxonomic compositions were mostly similar with PacBio datasets in terms of presence/absence. Nonetheless, lower throughput of PacBio platform and primer bias caused difference in composition of rare taxa, emphasizing caution in primer selection and result interpretation of NGS results.

Chapter 3. Successional change of the fungal microbiome in pine seedling roots

Abstract

Temporal succession of fungal communities associated with plant was reported in both mature trees and young seedlings. During development of plant, early-stage fungal communities are replaced by later colonizers. Pine mushroom (*Tricholoma matsutake*) is a late-stage ectomycorrhizal fungus that produces a commercially-valuable and edible mushrooms, but artificial cultivation of *T. matsutake* is still unsuccessful. Few studies have been done to test whether *T. matsutake* persists on pine seedling roots in the wild or gets replaced by other fungi. Here, the composition and the interaction of the root fungal microbiome of *P. densiflora* seedlings inoculated with *T. matsutake* were investigated over 3 years after field transplantation using high-throughput sequencing. A decline of *T. matsutake* colonization was found on pine roots and succession of mycorrhizal fungi as *P. densiflora* seedlings grew. Early root microbiome was colonized by fast-growing saprotrophic Ascomycota, then was replaced by early stage ectomycorrhiza such as *Wilcoxina*. At the end, more competitive *Suillus* species dominated the host roots. Four keystone species were identified during succession: *Fusarium oxysporum*, *F. trincintum*, *Suillus granulatus*, and *Cylindrocarpon pauciseptatum*. These findings have important implications for further studies on the succession of fungal communities associated with tree seedlings.

3.1. Introduction

Ectomycorrhizal fungi are one of the most common forms of plant-fungal root symbioses in woody plants (Brundrett, 2009; van der Heijden *et al.*, 2015), and improve nutrition and stress resistance of the host plant (Berendsen *et al.*, 2012; Smith & Read, 2013; van der Heijden *et al.*, 2015). Ectomycorrhizal fungi compete with each other to colonize root tips (Koide *et al.*, 2005; Kennedy *et al.*, 2009; Bakker *et al.*, 2014) or co-exist within the root tips (Perry *et al.*, 1989; Yamamoto *et al.*, 2014). Succession of the mycorrhizal community was reported in several host plants (Twieg *et al.*, 2007). This phenomenon not only occurs in mature trees, but also in seedlings, where the dominant ectomycorrhizal taxa can change (Obase *et al.*, 2009). Early stage ectomycorrhizal fungi (e.g. members of *Inocybe*, *Rhizopogon*, or *Suillus*) require small amount of carbon from hosts and are usually found in pine seedling in disturbed area (Colpaert *et al.*, 1996; Sim & Eom, 2009). Arrival sequence of ectomycorrhizal fungi often influences colonization at early stages, with negative consequences for later colonizers (Alford & Wilbur, 1985; Shorrocks & Bingley, 1994). This phenomenon is called the priority effect, and has been reported in the early stage of interaction between ectomycorrhizal fungi and pine seedlings (Kennedy & Bruns, 2005; Fukami, 2015).

The pine mushroom (*Tricholoma matsutake*; Agaricales, Tricholomataceae) produces edible fruiting body during symbiosis with members of Pinaceae, especially *Pinus densiflora* (Yamada *et al.*, 2010). Due to its commercial value, artificial cultivation of *T. matsutake* have

been attempted, but thus far been unsuccessful. An alternative method unsuccessfully tried was to induce fruiting bodies of *T. matsutake* in the wild through inoculating cultured *T. matsutake* hyphae in soil (Lee *et al.*, 2007), spraying of *T. matsutake* spores from fruiting body (Eto, 2000), and transplanting of 'shiro' (aggregate of *T. matsutake* mycorrhiza) to uninfected pine trees (Kareki & Kawakami, 1985). The last approach of transplanting shiro to uninfected pine trees has been tried extensively in Korea. In vitro ectomycorrhization of *T. matsutake* was reliably successful (Yamada *et al.*, 1999, 2006; Saito *et al.*, 2018), however, field trials showed limited production of fruiting bodies (Ka *et al.*, 2018). In order to make this method efficient, *T. matsutake* must persist the pine seedling roots. Currently, it is unclear after pine seedlings are transplanted to the wild, whether *T. matsutake* are maintained in the roots or are replaced with other fungi.

Advances in high-throughput sequencing have greatly contributed to understanding the diversity and function of fungi in various environments (Nilsson *et al.*, 2019), and have been used to study the succession of fungal communities (Dickie *et al.*, 2013, 2017; Voříškova *et al.*, 2014; Hannula *et al.*, 2017). In this study, high-throughput sequencing method was used to examine the change in root microbiome after transplantation of *T. mastuake* inoculated pine seedlings, focusing on the succession of mycorrhiza and interaction between root associated fungi (Appendix 4).

3.2. Materials and Methods

Study design and sample collection

This experiment was conducted at Gyeongsangbuk-do Forest & Environment Research Institute in Gyeonju, South Korea. *Tricholoma matsutake* strain KBFERI 20T05 (GenBank accession no. AF367417) was cultured in K-liquid media, and transferred to autoclaved culture vessel filled with mixed soil (perlite: peat moss = 80:1) as described by patent of Park *et al.* (U.S. Patent No. 7,269,923.). For surface sterilization, *P. densiflora* seeds were placed in 70% ethanol for 60 seconds, and transferred to 2% NaClO solution for 4 minutes. Cleaned seeds were washed 3 times with sterile water then germinated in nutrient broth agar plates (Scharalu). Uncontaminated seedlings were transferred to culture vessels inoculated with *T. matsutake* in a sterilized culture room, then co-cultured for 3 months in a clean room illuminated with a fluorescent lamp (20℃; 25,000 lux; 24 hours). Then, *P. densiflora* seedlings were moved to a greenhouse filled with autoclaved soil from a nearby pine forest. Sixteen pine seedlings were sampled at 6 different post *T. matsutake*-inoculation periods: 3 months (M03; in a sterilized culture room), 10, 17, 24, 31, and 38 months (M10, M17, M24, M31, M38; in a greenhouse). In total, ninety-six seedling roots were harvested.

DNA extraction

Harvested seedlings were placed on ice, transported to the laboratory in Seoul National University (Seoul, South Korea), and stored at −80℃ prior to DNA extraction. Seedling roots were gently washed with running water to cleanse debris and sterilized with 3% sodium hypochlorite for 2 min. Then samples were washed with distilled water for 5 min. Roots were separated from surface-sterilized roots, cut into 5cm fragments, and air-dried. For each sample, three root fragments were wet with 500 μl of cetyltrimethylammonium bromide buffer (Biosesang, Seongnam, South Korea) and ground with a mortar. For each sample, genomic DNA was extracted from seedling root using modified CTAB methods (Rogers & Bendich, 1994).

PCR amplification and high throughput sequencing

Fungal internal transcribed spacer 2 (ITS2) of rRNA region was amplified with primers ITS3 and ITS4 (White *et al.*, 1990) with Illumina sequencing adaptors attached. PCR was conducted 3 times for each samples using AccuPower PCR PreMix kit (Bioneer, Daejeon, South Korea). PCR conditions were as follows: 94℃ for 5 min, 30 cycles of 94℃ for 30 sec, 55℃ for 30 sec, and 72℃ for 40 sec, and 72℃ for 10 min as final extension. PCR products were confirmed on 1% agarose gel (BIOFACT, Daejeon, South Korea) with gel electrophoresis. After purification using the Expin™ PCR SV kit (GeneAll Biotechnology, Seoul, South Korea), a unique identifier sequence was attached to each PCR products with a second round PCR following the Nextera XT index kit protocol (Illumina, San Diego, CA, USA). Second PCR products were

purified as above. Concentration of each amplicon library were measured using a NanoDrop2000 (Thermo Fisher Scientific, Waltham, MA, USA). Amplicon libraries were pooled in equimolar quantities and sequenced using Illumina MiSeq platform at Macrogen (Seoul, South Korea).

Bioinformatics and statistical analysis

After sequencing, the raw data were processed using the Quantitative Insights Into Microbial Ecology v.1.8.0. (QIIME) pipeline (Caporaso *et al.*, 2010). Fastq-join was used for merging paired-end sequences. After filtering low-quality sequences (Q 〈 20, length 〈 200 bp), 9,513,644 reads were retained for later analyses. Clustering of operational taxonomic units (OTUs) was performed with the open-source sequence search tool Vsearch v. 2.6.2 (Rognes *et al.*, 2016) with 97% similarity level. For taxonomic identification, the most abundant sequence was selected as an OTU's representative sequence. The UNITE v. 8.0 (Kõljalg *et al.*, 2013) database was used to determine OTU's taxonomic identity with NCBI BLAST, following the criteria of Tedersoo *et al.* (Tedersoo *et al.*, 2014). Chimeric sequences were removed based on the reference database of UCHIME (Edgar *et al.*, 2011). Singleton OTUs and non-fungal sequences were removed, and all samples were rarefied to minimum number of sequences before further analysis. Taxonomic identity of major OTUs (OTUs with total relative abundance 〉 0.5%) were checked manually with NCBI and other databases. FUNGuild was used as a database for fungal trophic mode assignment (Nguyen *et al.*, 2016).

Alpha diversity indices (chao1 richness, Shannon's diversity, and Good's coverage) were calculated in QIIME. Statistical analysis was performed in R v.3.6.1. software (R Core Team, 2021). One-way ANOVA tests were performed to compare the diversity indices between sampling times with Tukey post-hoc test. Ordination analysis was performed by non-metric multidimensional scaling (NMDS) based on Jaccard dissimilarity index (presence/absence data) using the phyloseq package (McMurdie & Holmes, 2013). Difference of community compositions among sampling times were tested with permutational multivariate analysis of variance (PERMANOVA) with 999 permutations, implemented as 'adonis' in the vegan package (Oksanen *et al.*, 2013), and pairwise post-hoc tests were done using the Adonis package with Bonferroni correction of the Jaccard dissimilarity matrix (Arbizu, 2017).

Two diversity indices, Average taxonomic distinctness ($\Delta+$) and variation in taxonomic distinctness ($\Lambda+$) were calculated with 'taxondive' function in vegan package. These indices indicate the average taxonomic distance between individual species within each sample, and unevenness of taxonomic distance within each sample, respectively (Clarke & Warwick, 1998). Statistical significance of sampling time was tested using one-way ANOVA and Tukey post-hoc test for each diversity indices. Functional profiles of the fungal communities were predicted using PICURST2 (Douglas *et al.*, 2020). OTU tables and representative sequences were imported into PICRUST2 pipeline with default setting, except for fungal database references. Then, MetaCYC pathway abundances were predicted based on Enzyme Commission (EC) Numbers. Indicator species analysis (ISA) and Linear discriminant analysis effect size (LEfSe) analysis were conducted to

identify OTUs and pathways that have significant associations with each sampling period. ISA was conducted using 'multipatt' in the indicspecies package (De Cáceres & Legendre, 2009). LefSe analysis was conducted using Huttenhower lab galaxy site with default options (LDA 〉 2.0, p 〈 0.05; https://huttenhower.sph.harvard.edu/galaxy/) (Segata *et al.*, 2011).

To test for correlation between each species, Sparse Correlations for Compositional data (SparCC) (Friedman & Alm, 2012) network analysis was performed at the OTU level (OTUs with total relative abundance 〉 0.5%) with the Galaxy-based analysis pipeline, IDENAP (Feng *et al.*, 2019). The significance of correlation was calculated by comparing the shuffled data from 100 permutations. Following previous studies, correlations with SparCC 〉0.3 and p 〈0.05 were included (Kurtz *et al.*, 2015). The network was visualized with Cytoscape version 3.7.2 (Shannon *et al.*, 2003). Clusters were detected with Markov Clustering algorithms (Van Dongen & Abreu-Goodger, 2012). For the overall network, species with degree greater than 19, betweenness centrality higher than 0.038, and closeness centrality higher than 0.69 were selected as the keystone taxa. NMDS ordination and network analysis were performed without M03 samples as they were distinctly different from other samples due to high abundance of *T. matsutake* (〉94% in average).

3.3. Results

Sequencing results and alpha diversity indices

A total of 7,697,559 sequence reads were obtained from 96 samples through Illumina MiSeq sequencing with 25,244–228,456 sequence reads per sample. After rarefaction to 25,000 reads, 826 OTUs (range: 4–191) remained with a Good's coverage of 0.998–0.999. Based on taxonomic level, the OTUs covered 8 phyla, 28 classes, 89 orders, 188 families, and 327 genera. The number of OTUs significantly increased with the age of *P. densiflora* seedlings, from 63 OTUs found in M03 (7.88 OTUs per sample) to 487 OTUs of M38 with 155.88 OTUs per sample (Fig. 28). chao1 richness and Shannon's diversity also showed significant increase following growth of *P. densiflora* seedlings especially between M03 and other sampling periods (Fig. 28; Table 7, 8).

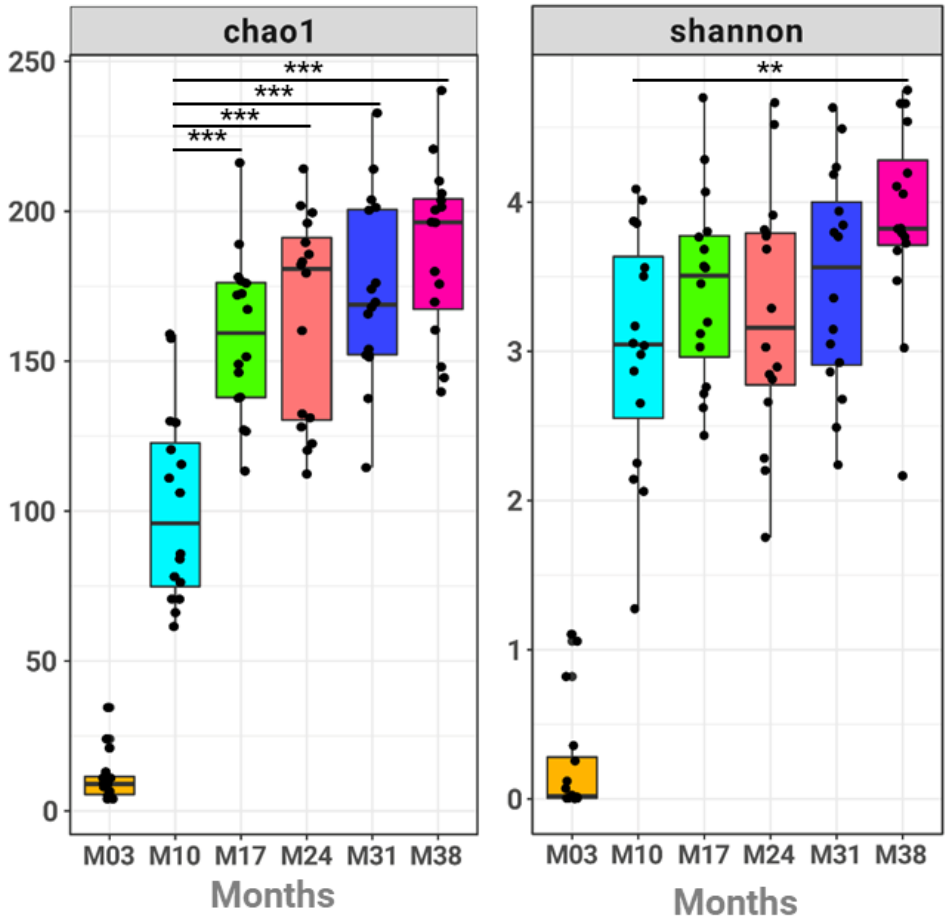

Figure 28. chao1 richness and Shannon diversity indices of fungal communities of *Pinus densiflora* seedlings.

Table 7. Results from one-way ANOVA and Tukey post-hoc test corresponding to chao1 richness index (*$p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$).

ANOVA

Source	DF	Sum Sq	Mean Sq	F-Value	p value
Month	5	352096	70419	88.23	<2e-16***
Residuals	90	71835	798		

Tukey test

Pairs	Differences	Lower	Upper	p.adjusted	
M10-M03	90.11	61.03	119.20	0.00	***
M17-M03	147.30	118.21	176.38	0.00	***
M24-M03	153.62	124.54	182.71	0.00	***
M31-M03	161.69	132.60	190.77	0.00	***
M38-M03	175.78	146.70	204.87	0.00	***
M17-M10	57.18	28.09	86.27	0.00	***
M24-M10	63.51	34.42	92.60	0.00	***
M31-M10	71.57	42.49	100.66	0.00	***
M38-M10	85.67	56.58	114.75	0.00	***
M24-M17	6.33	-22.76	35.41	0.99	
M31-M17	14.39	-14.70	43.48	0.70	
M38-M17	28.49	-0.60	57.57	0.06	
M31-M24	8.06	-21.02	37.15	0.97	
M38-M24	22.16	-6.93	51.25	0.24	
M38-M31	14.10	-14.99	43.18	0.72	

Table 8. Results from one-way ANOVA and Tukey post-hoc test corresponding to shannon diversity index (*$p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$).

ANOVA

Source	DF	Sum Sq	Mean Sq	F-Value	p value
Month	5	140.60	28.11	59.12	<2e-16***
Residuals	90	42.80	0.48		

Tukey test

Pairs	Differences	Lower	Upper	p.adjusted	
M10-M03	2.78	2.07	3.49	0.00	***
M17-M03	3.18	2.47	3.89	0.00	***
M24-M03	3.00	2.29	3.71	0.00	***
M31-M03	3.24	2.53	3.95	0.00	***
M38-M03	3.65	2.94	4.356	0.00	***
M17-M10	0.40	-0.31	1.11	0.58	
M24-M10	0.22	-0.49	0.93	0.94	
M31-M10	0.45	-0.26	1.16	0.43	
M38-M10	0.87	0.16	1.58	0.008	***
M24-M17	-0.18	-0.89	0.53	0.98	
M31-M17	0.055	-0.66	0.76	1.00	
M38-M17	0.47	-0.24	1.18	0.40	
M31-M24	0.23	-0.48	0.94	0.93	
M38-M24	0.64	-0.066	1.35	0.098	
M38-M31	0.41	-0.30	1.12	0.54	
M38-M24	0.64	-0.066	1.35	0.098	
M38-M31	0.41	-0.230	1.12	0.54	

The NMDS ordination of Jaccard dissimilarity based on OTU-level presence/absence revealed clear separation of fungal communities between most groups, except M24 and M31 (Fig. 28). This result was supported by pairwise adonis tests, where all but the M24-M31 comparison were statistically significant (Table 9). The significant shift of overall fungal community was observed in *P. densiflora* seedlings as seedling grew, based on the PERMANOVA analysis (R^2=39.4%, p = 0.001; Fig. 29, Table 9).

Fungal community composition in *P. densiflora* seedlings

The total abundances of major fungal phyla were relatively high: Ascomycota (64.353%) and Basidiomycota (35.516%). Abundance of the next most abundant phylum, Mortierellomycota, was low at less than 0.1%. The abundance of Basidiomycota was high during the inoculation stage (M03, 94.1%), but drastically decreased after transplantation (4.0% in M10; 1.7% in M17), replaced by Ascomycota. The abundance of Basidiomycota increased in M24 (42.5%) and M31 (44.1%), but decreased again in M38 (26.7%). The pattern of relative abundance at the species level was similar to that at the genus level (Fig. 30A). The most abundant OTUs of each sampling period were *T. matsutake* (OTU 1, 94.0%) and *Cladosporium* sp. (OTU 7, 2.81%) in M03, *Pseudogymnoascus pannorum* (OTU 6, 28.5%) and *Oidiodendron echinulatum* (OTU 5, 21.5%) in M10, and *Wilcoxina mikolae* (OTU 2, 35.7%) in M17. After M24, the most abundant OTU was *Suillus granulatus* (OTU 3, 33.11% in M24; 25.19% in M31; 20.39% in M38) followed by *W. mikolae* (OTU 2, 11.84%) in M24, *S. luteus* in M31 (OTU

13, 7.37%), and *W. mikolae* (10.22%) in M38 (Fig. 30B). The results of ISA followed the patterns relative abundance data (Table 10).

Both average taxonomic distinctness and variation in taxonomic distinctness showed significant differences for sampling groups (Fig. 31; Table 11 and 12). M03 group had the highest average taxonomic distinctness and variation in taxonomic distinctness, with low number of OTUs, indicating low homogeneity in taxonomic structure. In contrast, other groups (M10 – M38) did not have significant differences in average taxonomic distinctness and variation in taxonomic distinctness, while the number of OTUs increased following the growth of *P. densiflora* seedlings. The only significant differences within the other groups were found in the pair of M24 and M31 in terms of variation in taxonomic distinctness (Table 12).

Functional abundance prediction by PICRUST2

The changes in function of microbial communities were predicted using PICRUST2 software (Fig. 32, Table 13). However, only three pathways were predicted to be significantly up-regulated both in ISA and LefSe analysis. Pathways related with fatty acid biosynthesis (PWY-7663, PWY-5989) and amino acid degradation (LEU-DEG2-PWY) were significant indicator pathways of M17 (Table 13). In addition, the proportion of pathway related with fatty acid synthesis (FASYN-ELONG-PWY) was greatly increased after M10, but its change was significant only in ISA.

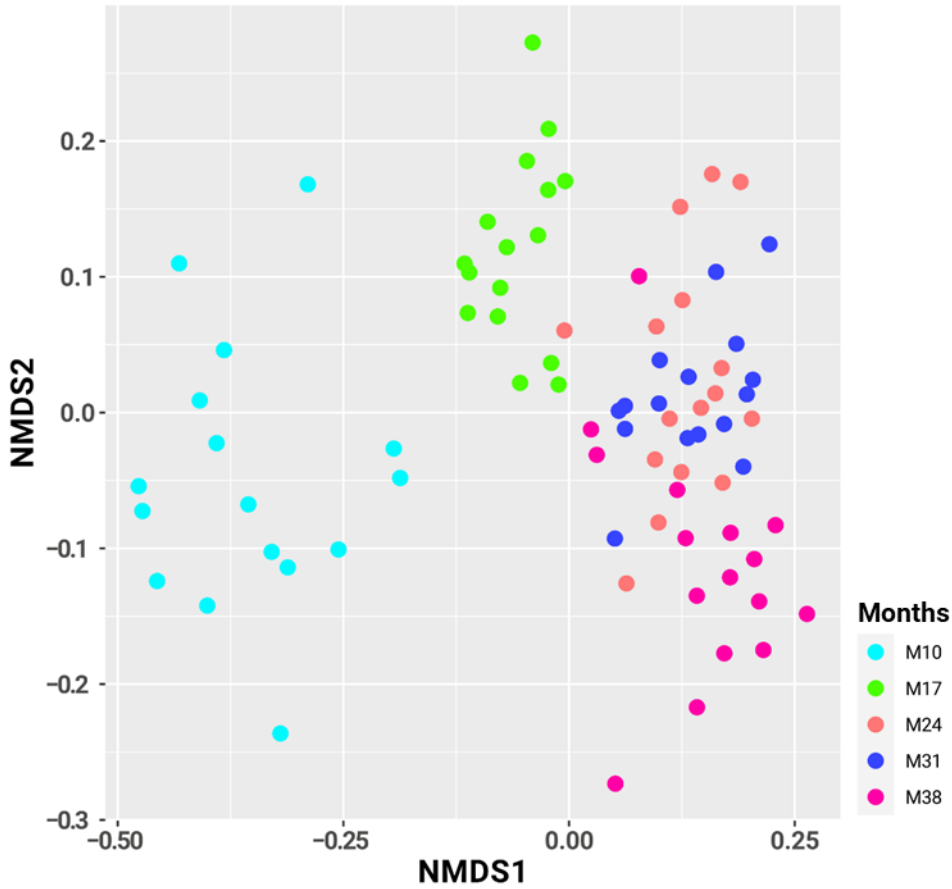

Figure 29. NMDS plot of fungal community structures of *Pinus densiflora* seedlings based on Jaccard dissimilarity index.

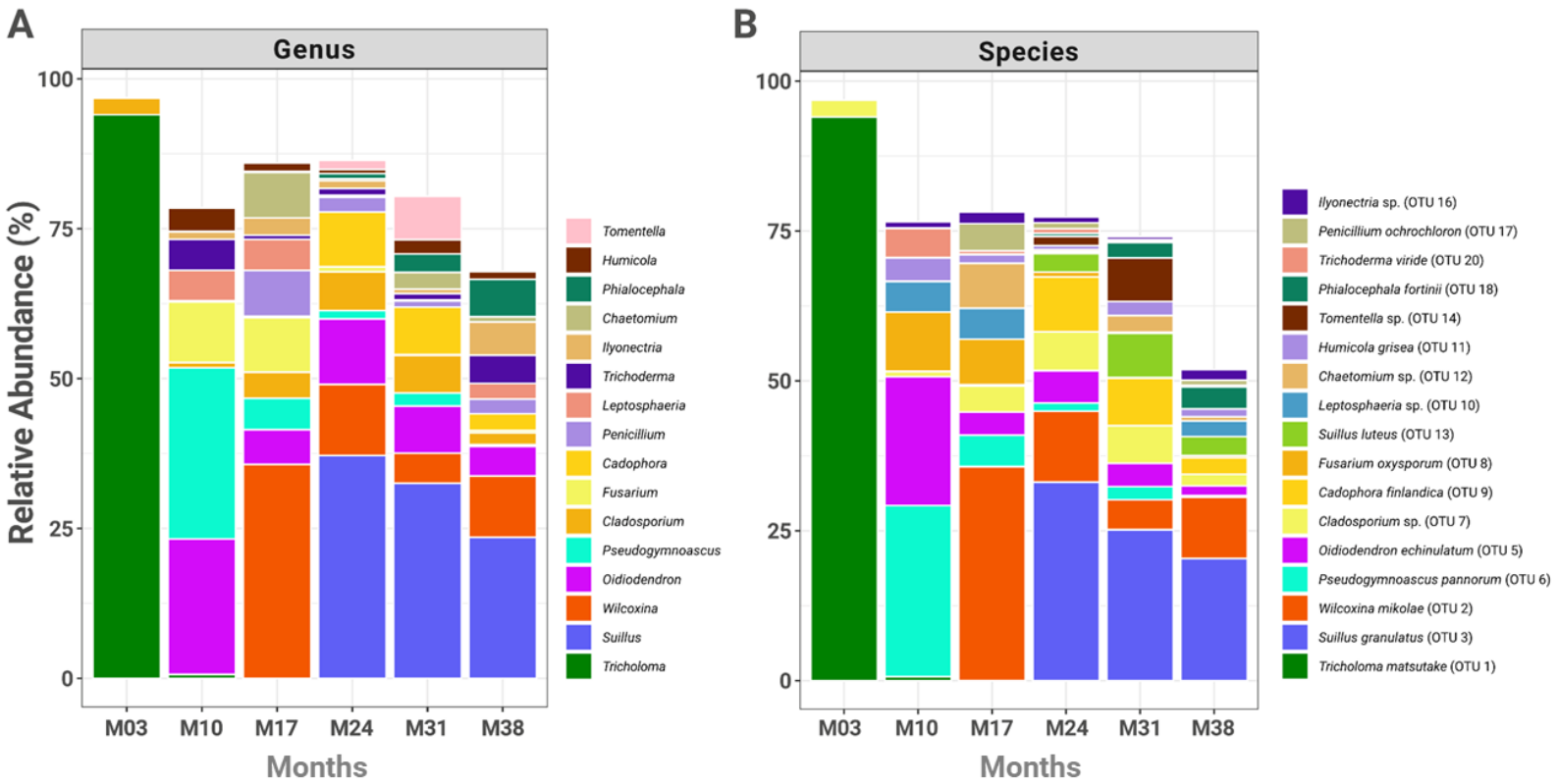

Figure 30. Taxonomic assignment and composition of the major genera (A) and major OTUs (B). Taxa with total relative abundance higher than 1% were chosen as major taxa

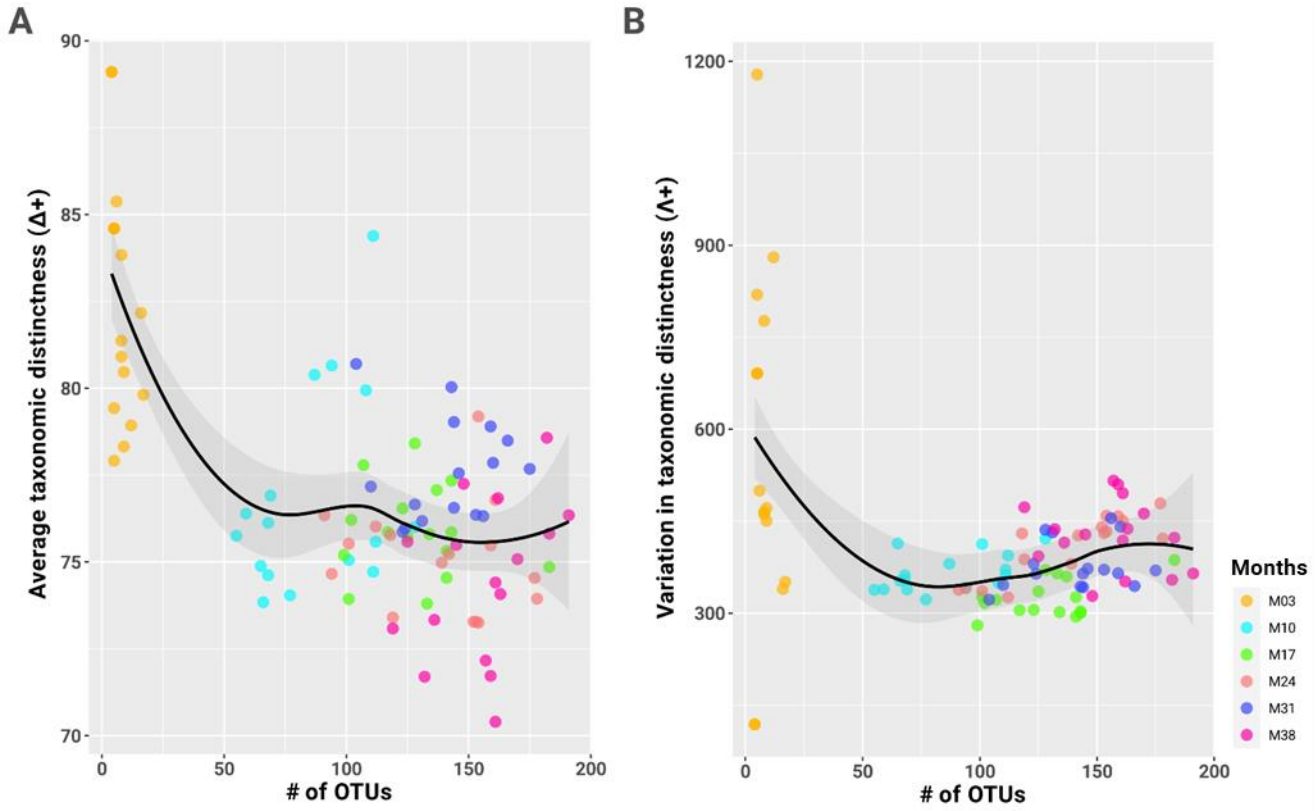

Figure 31. Scatter plot of average taxonomic distinctness (A, Δ^+) and variation in taxonomic distinctness (B, Λ^+) versus number of OTUs found in each *P. densiflora* seedlings. Grey areas indicate 95% confidence interval for the regression line.

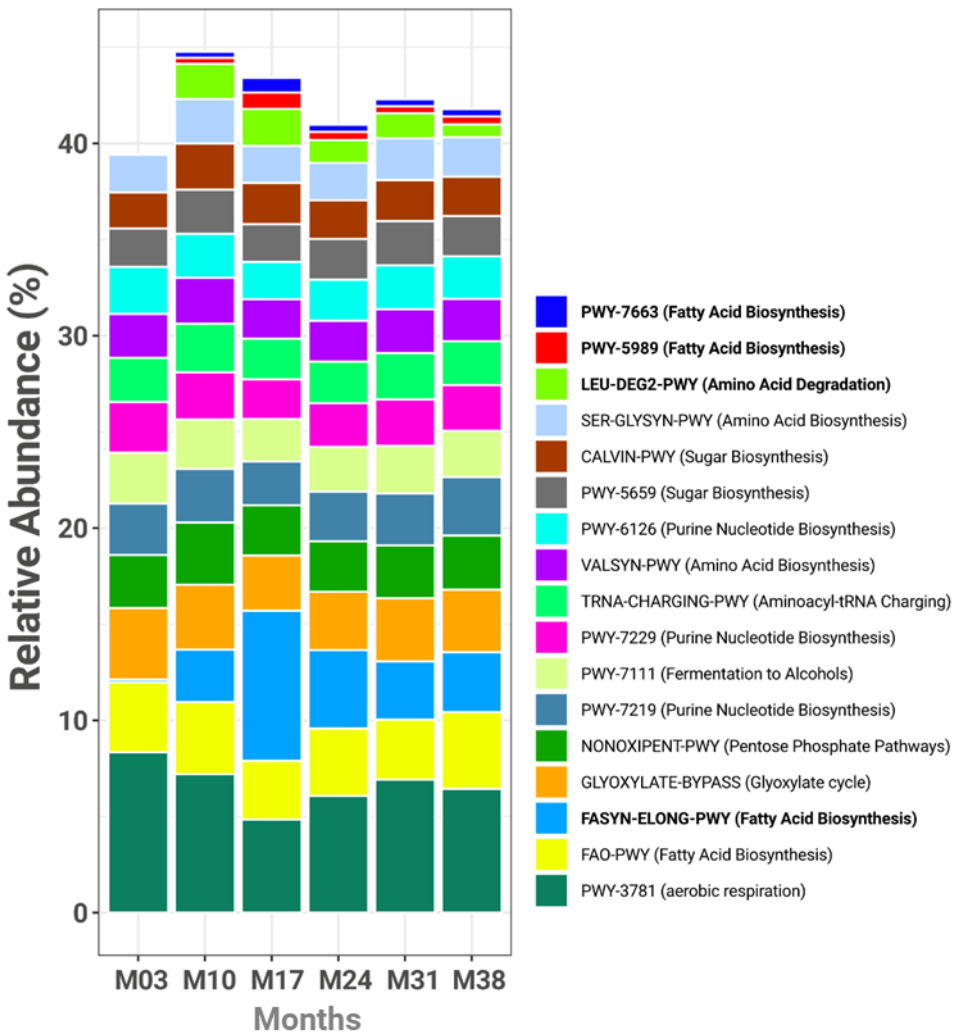

Figure 32. Predicted functional profiles of fungal communities in *P. densiflora* seedlings assessed by PICRUST2.

Table 9. Results from the post-hoc PERMANOVA (Adonis) among sampling groups (* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$).

Pairs	Df	SumsOfSqs	F.Model	R^2	p.value	p.adjusted
M10 vs M17	1	3.01	19.81	0.40	0.001***	0.01**
M10 vs M24	1	3.83	20.87	0.41	0.001***	0.01**
M10 vs M31	1	3.47	16.45	0.35	0.001***	0.01**
M10 vs M38	1	3.53	15.71	0.34	0.001***	0.01**
M17 vs M24	1	2.60	16.83	0.36	0.001***	0.01**
M17 vs M31	1	3.14	17.27	0.37	0.001***	0.01**
M17 vs M38	1	2.46	12.56	0.30	0.001***	0.01**
M24 vs M31	1	0.61	2.85	0.087	0.008***	0.08
M24 vs M38	1	0.82	3.62	0.11	0.004***	0.04*
M31 vs M38	1	0.88	3.46	0.10	0.001***	0.01**

Table 10. Taxonomic identification and average relative abundance of top indicator OTUs selected in ISA

Group	OTU ID	Identification	Indicator Value Score	p value	Relative abundance (%)
M03	OTU 1	*Tricholoma matsutake*	0.99	0.000	15.78
M10	OTU 6	*Pseudogymnoascus pannorum*	0.78	0.000	5.52
M10	OTU 8	*Fusarium oxysporum*	0.53	0.000	3.13
M10	OTU 37	*Fusarium* sp.	0.90	0.000	0.52
M17	OTU 2	*Wilcoxina mikolae*	0.57	0.000	10.46
M17	OTU 12	*Chaetomium* sp.	0.66	0.001	1.90
M17	OTU 17	*Penicillium ochrochloron*	0.68	0.000	1.11
M24	OTU 3	*Suillus granulatus*	0.39	0.000	13.11
M24	OTU 9	*Cadophora finlandica*	0.50	0.000	2.26
M24	OTU 27	*Oidiodendron rhodogenum*	0.54	0.004	0.58
M31	OTU 13	*Suillus luteus*	0.30	0.015	2.28
M31	OTU 14	*Tomentella* sp.	1.00	0.000	1.21
M31	OTU 3530	*Cadophora finlandica*	0.45	0.001	1.04
M38	OTU 19	*Dactylonectria* sp.	0.68	0.000	0.89
M38	OTU 23	Hyaloscyphaceae sp.	0.87	0.000	0.88
M38	OTU_15	*Cylindrocarpon pauciseptatum*	0.41	0.002	0.87

Table 11. Results from one-way ANOVA and Tukey post-hoc test corresponding to average taxonomic distinctness (*$p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$).

ANOVA

Source	DF	Sum Sq	Mean Sq	F-Value	p value
Month	5	540131	108026	7.399	7.5E-06***
Residuals	90	1314039	14600		

Tukey test

Pairs	Differences	Lower	Upper	p.adjusted	
M10-M03	-196.82	-321.22	-72.41	0.00	***
M17-M03	-237.78	-362.19	-113.37	0.00	***
M24-M03	-154.02	-278.43	-29.62	0.007	***
M31-M03	-184.63	-309.03	-60.22	0.001	***
M38-M03	-137.20	-261.60	-12.80	0.022	*
M17-M10	-40.97	-165.37	83.44	0.93	
M24-M10	42.79	-81.61	167.20	0.92	
M31-M10	12.19	-112.21	136.59	1.00	
M38-M10	59.62	-64.79	184.02	0.73	
M24-M17	83.76	-40.65	208.16	0.37	
M31-M17	53.15	-71.25	177.56	0.81	
M38-M17	100.58	-23.82	224.99	0.18	
M31-M24	-30.60	-155.01	93.80	0.98	
M38-M24	16.82	-107.58	141.23	1.00	
M38-M31	47.43	-76.98	171.83	0.88	

Table 12. Results from one-way ANOVA and Tukey post-hoc test corresponding to variation in taxonomic distinctness (*$p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$).

ANOVA

Source	DF	Sum Sq	Mean Sq	F-Value	p value
Month	5	672.90	134.57	24.58	1.58E-15***
Residuals	90	492.70	5.47		

Tukey test

Pairs	Differences	Lower	Upper	p.adjusted	
M10-M03	-5.70	-8.11	-3.29	0.00	***
M17-M03	-6.64	-9.05	-4.23	0.00	***
M24-M03	-7.43	-9.84	-5.02	0.00	***
M31-M03	-4.95	-7.36	-2.54	0.00	***
M38-M03	-8.04	-10.45	-5.63	0.00	***
M17-M10	-0.94	-3.35	1.47	0.87	
M24-M10	-1.72	-4.13	0.69	0.31	
M31-M10	0.75	-1.66	3.16	0.94	
M38-M10	-2.34	-4.75	0.071	0.062	
M24-M17	-0.79	-3.19	1.62	0.93	
M31-M17	1.69	-0.72	4.10	0.33	
M38-M17	-1.40	-3.81	1.01	0.54	
M31-M24	2.48	0.067	4.89	0.040	*
M38-M24	-0.61	-3.02	1.80	0.98	
M38-M31	-3.09	-5.50	-0.68	0.004	

Table 13. Predicted function and relative abundance of indicator pathways selected both in ISA and LefSe

Group	BioCyc ID	ISA		LefSe		Pathway	Superclass
		Indicator Value Score	p value	LDA Score	p value		
M17	LEU-DEG2-PWY	0.27	0.0001	4.05	6.17E-12	L-leucine degradation I	Amino Acid Degradation
M17	PWY-7663	1.00	0.0001	3.73	1.15E-09	gondoate biosynthesis (anaerobic)	Fatty Acid and Lipid Biosynthesis
M17	PWY-5989	1.00	0.0001	3.75	4.41E-10	stearate biosynthesis II (bacteria and plants)	Fatty Acid and Lipid Biosynthesis
M17	FASYN-ELONG-PWY	0.34	0.0001			fatty acid elongation -- saturated	Fatty Acid and Lipid Biosynthesis

Relative abundance (%)

Group	BioCyc ID	M03	M10	M17	M24	M31	M38
M17	LEU-DEG2-PWY	0.0001	1.83	1.92	1.19	1.31	0.66
M17	PWY-7663	0.0002	0.32	0.86	0.42	0.36	0.42
M17	PWY-5989	0.15	2.74	7.81	4.06	3.03	3.13
M17	LEU-DEG2-PWY	0.0001	1.83	1.92	1.19	1.31	0.66

Network features and correlation within fungal community *P. densiflora* seedlings

To identify the potential interaction among fungal species in *P. densiflora* seedlings, SparCC analysis was performed. *T. matsutake* (OTU 1) and 35 major OTUs with relative abundances 〉 0.5%, accounting for 83.4% of total sequence reads, were clustered into 4 groups, with 1 isolated OTU (Table 14). The network had a clustering coefficient of 0.589 and network centralization of 0.308 (Fig. 33). Ten fungal OTUs showed significant positive correlations with *T. matsutake*. Most of those OTUs were saprotrophs or plant pathogens, with the exception of *O. echinulatum* (ericoid mycorrhiza). Based on the selection criterion (closeness centrality higher than 0.69) four OTUs were identified as keystone species during fungal succession of pine seedling roots: *Cylindrocarpon pauciseptatum* (OTU 15), *Suillus granulatus* (OTU 3), *Fusarium oxysporum* (OTU 8), and *Fusarium* sp. (OTU 37). All of the keystone taxa were belonged to the same cluster (Table 15).

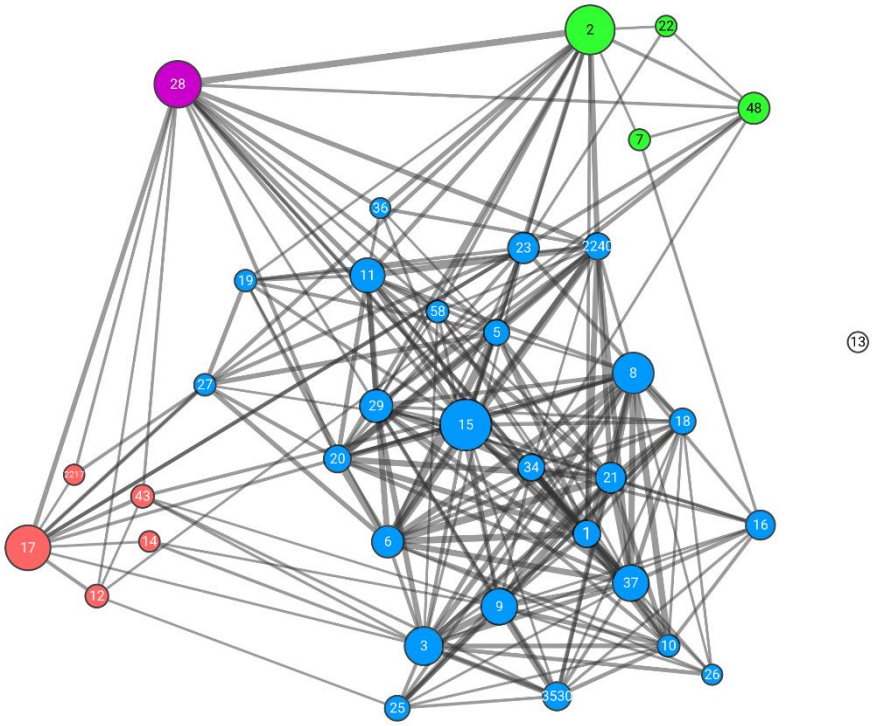

Figure 33. SparCC network map in OTU level in microbiome of *Pinus densiflora* seedlings. Each point represents a fungal species; edges indicate the relationship between species. Each clusters are shown in different colors, with isolated species are marked with white. The size of the nodes follows betweenness centrality scores. The width of the edges follows SparCC correlation coefficients. Species with total relative abundance higher than 0.5% were chosen. Only statistically significant edges corresponding to correlations with a magnitude higher than 0.3 ($p < 0.05$) were drawn. Identity of each OTU is described in Table 3-8.

Table 14. Identity and node properties of the OTUs (relative abundance 〉0.5%) in *Pinus densiflora* seedling root microbiome network. OTUs in bold font indicate keystone taxa.

ID	Species	Cluster	Betweenness Centrality	Closeness Centrality	Clustering Coefficient	Degree	NCBI Blast Result	Accession No.	Identity
OTU 1	*Tricholoma matsutake*	1	0.017	0.67	0.67	17	*Tricholoma matsutake*	MN088515.1	100.00%
OTU 2	*Wilcoxina mikolae*	3	0.068	0.63	0.44	15	*Wilcoxina mikolae*	LC029799.1	100.00%
OTU 3	***Suillus granulatus***	1	0.043	0.69	0.54	19	*Suillus granulatus*	MF421107.1	100.00%
OTU 5	*Oidiodendron echinulatum*	1	0.013	0.62	0.69	14	*Oidiodendron echinulatum*	AF062791.1	100.00%
OTU 6	*Pseudogymnoascus pannorum*	1	0.027	0.63	0.60	17	*Geomyces pannorum* (=*Pseudogymnoascus pannorum*)	JX131373.1	100.00%
OTU 7	*Cladosporium* sp.	3	0.002	0.46	0.33	3	*Cladosporium cladosporioides*	MT598826.1	100.00%
OTU 8	***Fusarium oxysporum***	1	0.049	0.71	0.54	21	*Fusarium oxysporum*	MT610978.1	100.00%
OTU 9	*Cadophora finlandica*	1	0.038	0.64	0.58	17	*Cadophora finlandica*	KF850368.1	98.80%
OTU 10	*Leptosphaeria sclerotioides*	1	0.004	0.59	0.82	13	*Leptosphaeria sclerotioides*	MK764998.1	100.00%
OTU 11	*Trichocladium* sp.	1	0.034	0.64	0.62	16	*Trichocladium* sp./ *Humicola grisea* (=*Trichocladium griseum*)	MT348608.1/ MH860993.1	100.00%/ 100.00%
OTU 12	*Chaetomium* sp.	2	0.006	0.51	0.50	5	*Chaetomium angustispirale* / *Chaetomium globosum*	MT453288.1/ MN960568.1	100.00%/ 100.00%
OTU 13	*Suillus luteus*		0.00	0.00	0.00	0	*Suillus luteus*	KX213740.1	100.00%
OTU 14	*Tomentella* sp.	2	0.001	0.46	0.33	3	uncultured *Tomentella* clone	KY684656.1	98.45%
OTU 15	***Cylindrocarpon pauciseptatum***	1	0.073	0.74	0.52	22	*Dactylonectria pauciseptata* (=*Cylindrocarpon pauciseptatum*)	MK602783.1	100.00%

Table 14, Continued

ID	Species	Cluster	Betweenness Centrality	Closeness Centrality	Clustering Coefficient	Degree	NCBI Blast Result	Accession No.	Identity
OTU 16	*Ilyonectria* sp.	1	0.021	0.531	0.78	10	*Ilyonectria liriodendri* / *Ilyonectria destructans*	MK602788.1/ KY910190.1	100.00%
OTU 17	*Penicillium ochrochloron*	2	0.058	0.596	0.24	11	*Penicillium ochrochloron*	MK450704.1	100.00%
OTU 18	*Phialocephala fortinii*	1	0.013	0.618	0.71	15	*Phialocephala fortinii*	KF313097.1	100.00%
OTU 19	*Dactylonectria* sp.	1	0.004	0.531	0.78	9	*Dactylonectria novozelandica* / *Dactylonectria torresensis*	MN817697.1/ MN988721.1	100.00%/ 100.00%
OTU 20	*Trichoderma* sp.	1	0.016	0.63	0.66	15	*Trichoderma viride*	MH794191.1	100.00%
OTU 21	*Paraphaeosphaeria sporulosa*	1	0.024	0.68	0.64	19	*Paraphaeosphaeria sporulosa*	MT576023.1	100.00%
OTU 22	Xenochalara juniperi	3	0.002	0.44	0.33	3	*Xenochalara juniperi*	MK952344.1	100.00%
OTU 23	Hyaloscyphaceae sp.	1	0.025	0.57	0.47	9	Hyaloscyphaceae sp.	AB986450.1	97.61%
OTU 25	Helotiales sp.	1	0.011	0.52	0.78	9	Helotiales sp.	JX243904.1	100.00%
OTU 26	*Trichoderma* sp.	1	0.00	0.52	0.95	7	*Trichoderma* sp.	MT557542.1	100.00%
OTU 27	*Oidiodendron rhodogenum*	1	0.004	0.53	0.71	7	*Oidiodendron rhodogenum*	AF062803.1	100.00%
OTU 28	*Penicillium* sp.	4	0.062	0.65	0.44	16	*Penicillium* sp.	MT482617.1	100.00%
OTU 29	*Oidiodendron* sp.	1	0.03	0.65	0.60	16	*Oidiodendron tenuissimum*/ *Oidiodendron griseum*	MH864345.1 / AF062797.1	99.69%/ 99.69%
OTU 34	*Knufia* sp.	1	0.015	0.62	0.73	14	*Knufia* sp.	KX610444.1	98.79%
OTU 36	*Entrophospora* sp.	1	0.00	0.52	1.00	6	*Entrophospora* sp.	HM208740.1	99.41%
OTU 37	***Fusarium* sp.**	1	0.038	0.71	0.59	21	*Fusarium acuminatum*/ *Fusarium tricinctum*	MT649858.1/ MT453281.1	100.00%/ 100.00%

Table 14, Continued

ID	Species	Cluster	Betweenness Centrality	Closeness Centrality	Clustering Coefficient	Degree	NCBI Blast Result	Accession No.	Identity
OTU 43	*Talaromyces* sp.	2	0.007	0.53	0.52	7	*Talaromyces amestolkiae*	MN511323.1	100.00%
OTU 48	*Exophiala* sp.	3	0.026	0.56	0.36	8	*Exophiala* sp.	MF619956.1	100.00%
OTU 58	*Sebacina* sp.	1	0.005	0.59	0.71	10	*Sebacina* sp.	KY271862.1	98.99%
OTU 2217	*Corynascella inaequalis*	2	0.001	0.45	0.67	3	*Corynascella inaequalis*	MT453282.1	99.41%
OTU 2240	*Pseudogymnoascus pannorum*	1	0.015	0.64	0.67	16	*Pseudogymnoascus pannorum*	MN013920.1	99.70%
OTU 3530	*Cadophora finlandica*	1	0.019	0.60	0.68	13	*Cadophora finlandica*	KF850368.1	98.80%

Table 15. Edge properties of the *Pinus densiflora* seedling root microbiome network. Identity of OTUs are available in Table 14.

OTU(A)	cluster A	OTU(B)	cluster B	SparCC correlation (A-B)	p-value	Relationship
OTU 1	1	OTU 2	3	-0.366	0	negative
OTU 1	1	OTU 3	1	-0.341	0	negative
OTU 1	1	OTU 5	1	0.516	0	positive
OTU 1	1	OTU 6	1	0.690	0	positive
OTU 1	1	OTU 8	1	0.474	0	positive
OTU 1	1	OTU 9	1	-0.364	0	negative
OTU 1	1	OTU 10	1	0.332	0	positive
OTU 1	1	OTU 11	1	0.470	0	positive
OTU 1	1	OTU 15	1	-0.585	0	negative
OTU 1	1	OTU 18	1	-0.410	0	negative
OTU 1	1	OTU 20	1	0.596	0	positive
OTU 1	1	OTU 21	1	0.643	0	positive
OTU 1	1	OTU 28	4	-0.447	0	negative
OTU 1	1	OTU 34	1	0.384	0	positive
OTU 1	1	OTU 37	1	0.660	0	positive
OTU 1	1	OTU 58	1	-0.364	0	negative
OTU 1	1	OTU 2240	1	0.599	0	positive
OTU 2	3	OTU 5	1	-0.345	0	negative
OTU 2	3	OTU 7	3	0.312	0	positive
OTU 2	3	OTU 11	1	-0.484	0	negative
OTU 2	3	OTU 15	1	0.524	0	positive
OTU 2	3	OTU 17	2	0.452	0	positive
OTU 2	3	OTU 19	1	0.324	0	positive
OTU 2	3	OTU 20	1	-0.436	0	negative
OTU 2	3	OTU 22	3	-0.312	0	negative
OTU 2	3	OTU 28	4	0.673	0	positive
OTU 2	3	OTU 29	1	0.388	0	positive
OTU 2	3	OTU 36	1	0.507	0	positive
OTU 2	3	OTU 48	3	-0.400	0	negative
OTU 2	3	OTU 58	1	0.379	0	positive
OTU 2	3	OTU 2240	1	-0.571	0	negative
OTU 3	1	OTU 6	1	-0.404	0	negative
OTU 3	1	OTU 8	1	-0.582	0	negative
OTU 3	1	OTU 9	1	0.678	0	positive
OTU 3	1	OTU 10	1	-0.499	0	negative

Table 15, Continued

OTU(A)	cluster A	OTU(B)	cluster B	SparCC correlation (A-B)	p-value	Relationship
OTU 3	1	OTU 15	1	0.416	0	positive
OTU 3	1	OTU 16	1	-0.301	0	negative
OTU 3	1	OTU 17	2	-0.313	0	negative
OTU 3	1	OTU 18	1	0.515	0	positive
OTU 3	1	OTU 21	1	-0.432	0	negative
OTU 3	1	OTU 23	1	0.324	0	positive
OTU 3	1	OTU 25	1	0.407	0	positive
OTU 3	1	OTU 26	1	0.455	0	positive
OTU 3	1	OTU 29	1	0.580	0	positive
OTU 3	1	OTU 34	1	-0.482	0	negative
OTU 3	1	OTU 37	1	-0.489	0	negative
OTU 3	1	OTU 43	2	-0.339	0.01	negative
OTU 3	1	OTU 58	1	0.314	0	positive
OTU 3	1	OTU 3530	1	0.580	0	positive
OTU 5	1	OTU 6	1	0.769	0	positive
OTU 5	1	OTU 8	1	0.312	0.01	positive
OTU 5	1	OTU 11	1	0.404	0	positive
OTU 5	1	OTU 15	1	-0.455	0	negative
OTU 5	1	OTU 19	1	-0.415	0	negative
OTU 5	1	OTU 20	1	0.751	0	positive
OTU 5	1	OTU 21	1	0.501	0	positive
OTU 5	1	OTU 27	1	0.531	0	positive
OTU 5	1	OTU 28	4	-0.449	0	negative
OTU 5	1	OTU 36	1	-0.301	0	negative
OTU 5	1	OTU 37	1	0.333	0	positive
OTU 5	1	OTU 2240	1	0.638	0	positive
OTU 6	1	OTU 8	1	0.644	0	positive
OTU 6	1	OTU 10	1	0.401	0	positive
OTU 6	1	OTU 11	1	0.611	0	positive
OTU 6	1	OTU 15	1	-0.510	0	negative
OTU 6	1	OTU 18	1	-0.404	0	negative
OTU 6	1	OTU 19	1	-0.301	0	negative
OTU 6	1	OTU 20	1	0.783	0	positive
OTU 6	1	OTU 21	1	0.743	0	positive
OTU 6	1	OTU 23	1	-0.355	0	negative
OTU 6	1	OTU 26	1	0.416	0	negative
OTU 6	1	OTU 27	1	-0.301	0	positive

Table 15, Continued

OTU(A)	cluster A	OTU(B)	cluster B	SparCC correlation (A-B)	p-value	Relationship
OTU 6	1	OTU 34	1	0.402	0	positive
OTU 6	1	OTU 37	1	0.625	0	positive
OTU 6	1	OTU 2240	1	0.783	0	positive
OTU 7	3	OTU 16	1	-0.354	0.01	negative
OTU 7	3	OTU 48	3	-0.343	0	negative
OTU 8	1	OTU 9	1	-0.497	0	negative
OTU 8	1	OTU 10	1	0.676	0	positive
OTU 8	1	OTU 11	1	0.423	0	positive
OTU 8	1	OTU 15	1	-0.384	0	negative
OTU 8	1	OTU 16	1	0.401	0	positive
OTU 8	1	OTU 17	2	0.378	0	positive
OTU 8	1	OTU 18	1	-0.493	0	negative
OTU 8	1	OTU 20	1	0.415	0	positive
OTU 8	1	OTU 21	1	0.719	0	positive
OTU 8	1	OTU 25	1	-0.408	0	negative
OTU 8	1	OTU 26	1	-0.310	0	negative
OTU 8	1	OTU 29	1	-0.466	0	negative
OTU 8	1	OTU 34	1	0.581	0	positive
OTU 8	1	OTU 37	1	0.638	0	positive
OTU 8	1	OTU 43	2	0.397	0	positive
OTU 8	1	OTU 2240	1	0.402	0	positive
OTU 8	1	OTU 3530	1	-0.473	0	negative
OTU 9	1	OTU 10	1	-0.483	0	negative
OTU 9	1	OTU 14	2	0.311	0.01	positive
OTU 9	1	OTU 15	1	0.416	0	positive
OTU 9	1	OTU 16	1	-0.313	0.02	negative
OTU 9	1	OTU 18	1	0.452	0	positive
OTU 9	1	OTU 21	1	-0.406	0	negative
OTU 9	1	OTU 25	1	0.514	0	positive
OTU 9	1	OTU 26	1	0.334	0	positive
OTU 9	1	OTU 29	1	0.447	0	positive
OTU 9	1	OTU 34	1	-0.365	0	negative
OTU 9	1	OTU 37	1	-0.516	0	negative
OTU 9	1	OTU 43	2	-0.301	0	negative
OTU 9	1	OTU 58	1	0.359	0	positive
OTU 9	1	OTU 3530	1	0.402	0	positive
OTU 10	1	OTU 16	1	0.625	0	positive

Table 15, Continued

OTU(A)	cluster A	OTU(B)	cluster B	SparCC correlation (A-B)	p-value	Relationship
OTU 10	1	OTU 18	1	-0.322	0	negative
OTU 10	1	OTU 21	1	0.517	0	positive
OTU 10	1	OTU 25	1	-0.326	0	negative
OTU 10	1	OTU 29	1	-0.423	0	negative
OTU 10	1	OTU 34	1	0.667	0	positive
OTU 10	1	OTU 37	1	0.560	0	positive
OTU 10	1	OTU 3530	1	-0.447	0	negative
OTU 11	1	OTU 15	1	-0.496	0	negative
OTU 11	1	OTU 19	1	-0.370	0.01	negative
OTU 11	1	OTU 20	1	0.504	0	positive
OTU 11	1	OTU 21	1	0.482	0	positive
OTU 11	1	OTU 28	4	-0.516	0	negative
OTU 11	1	OTU 29	1	-0.517	0	negative
OTU 11	1	OTU 36	1	-0.361	0.01	negative
OTU 11	1	OTU 37	1	0.410	0.01	positive
OTU 11	1	OTU 58	1	-0.362	0	negative
OTU 11	1	OTU 2217	2	0.385	0.01	positive
OTU 11	1	OTU 2240	1	0.638	0	positive
OTU 12	2	OTU 17	2	0.402	0	positive
OTU 12	2	OTU 25	1	-0.306	0	negative
OTU 12	2	OTU 28	4	0.322	0	positive
OTU 12	2	OTU 43	2	0.304	0	positive
OTU 12	2	OTU 48	3	-0.315	0.01	negative
OTU 13	0	OTU 13	0	0.000	0	none
OTU 14	2	OTU 17	2	-0.320	0	negative
OTU 14	2	OTU 3530	1	0.363	0	positive
OTU 15	1	OTU 18	1	0.438	0	positive
OTU 15	1	OTU 19	1	0.403	0	positive
OTU 15	1	OTU 20	1	-0.610	0	negative
OTU 15	1	OTU 21	1	-0.437	0	negative
OTU 15	1	OTU 23	1	0.326	0.01	positive
OTU 15	1	OTU 26	1	0.340	0	positive
OTU 15	1	OTU 27	1	-0.307	0.03	negative
OTU 15	1	OTU 28	4	0.437	0	positive
OTU 15	1	OTU 29	1	0.313	0	positive
OTU 15	1	OTU 34	1	-0.322	0	negative
OTU 15	1	OTU 36	1	0.517	0	positive

Table 15, Continued

OTU(A)	cluster A	OTU(B)	cluster B	SparCC correlation (A-B)	p-value	Relationship
OTU 15	1	OTU 37	1	-0.510	0	negative
OTU 15	1	OTU 2240	1	-0.557	0	negative
OTU 15	1	OTU 3530	1	0.312	0.01	positive
OTU 16	1	OTU 21	1	0.390	0	positive
OTU 16	1	OTU 29	1	-0.345	0.03	negative
OTU 16	1	OTU 34	1	0.370	0.01	positive
OTU 16	1	OTU 37	1	0.440	0	positive
OTU 16	1	OTU 3530	1	-0.349	0	negative
OTU 17	2	OTU 23	1	-0.393	0	negative
OTU 17	2	OTU 27	1	0.304	0.01	positive
OTU 17	2	OTU 28	4	0.513	0	positive
OTU 17	2	OTU 43	2	0.441	0	positive
OTU 17	2	OTU 48	3	-0.445	0	negative
OTU 17	2	OTU 2217	2	-0.309	0	negative
OTU 18	1	OTU 21	1	-0.470	0	negative
OTU 18	1	OTU 23	1	0.449	0	positive
OTU 18	1	OTU 25	1	0.440	0	positive
OTU 18	1	OTU 26	1	0.327	0	positive
OTU 18	1	OTU 29	1	0.348	0	positive
OTU 18	1	OTU 34	1	-0.329	0	negative
OTU 18	1	OTU 37	1	-0.479	0	negative
OTU 18	1	OTU 3530	1	0.320	0.01	positive
OTU 19	1	OTU 20	1	-0.363	0.01	negative
OTU 19	1	OTU 23	1	0.380	0	positive
OTU 19	1	OTU 27	1	-0.483	0	negative
OTU 19	1	OTU 2240	1	-0.339	0	negative
OTU 20	1	OTU 21	1	0.639	0	positive
OTU 20	1	OTU 23	1	-0.425	0	negative
OTU 20	1	OTU 27	1	0.437	0	positive
OTU 20	1	OTU 28	4	-0.434	0	negative
OTU 20	1	OTU 37	1	0.444	0	positive
OTU 20	1	OTU 58	1	-0.301	0	negative
OTU 20	1	OTU 2240	1	0.649	0	positive
OTU 21	1	OTU 25	1	-0.420	0	negative
OTU 21	1	OTU 29	1	-0.305	0.01	negative
OTU 21	1	OTU 34	1	-0.510	0	positive
OTU 21	1	OTU 37	1	-0.557	0	positive

Table 15, Continued

OTU(A)	cluster A	OTU(B)	cluster B	SparCC correlation (A-B)	p-value	Relationship
OTU 21	1	OTU 58	1	-0.322	0	negative
OTU 21	1	OTU 2240	1	0.542	0	positive
OTU 21	1	OTU 3530	1	-0.382	0	negative
OTU 22	3	OTU 23	1	0.343	0	positive
OTU 22	3	OTU 48	3	0.317	0	positive
OTU 23	1	OTU 27	1	-0.439	0	negative
OTU 25	1	OTU 37	1	-0.315	0	negative
OTU 25	1	OTU 3530	1	0.360	0	positive
OTU 26	1	OTU 37	1	-0.320	0	negative
OTU 28	4	OTU 29	1	0.337	0	positive
OTU 28	4	OTU 36	1	0.477	0	positive
OTU 28	4	OTU 37	1	-0.321	0.01	negative
OTU 28	4	OTU 43	2	0.349	0	positive
OTU 28	4	OTU 48	3	-0.364	0.01	negative
OTU 28	4	OTU 58	1	0.351	0	positive
OTU 28	4	OTU 2217	2	-0.352	0	negative
OTU 28	4	OTU 2240	1	-0.524	0	negative
OTU 29	1	OTU 34	1	-0.495	0	negative
OTU 29	1	OTU 37	1	-0.494	0	negative
OTU 29	1	OTU 48	3	-0.467	0	negative
OTU 29	1	OTU 2240	1	-0.393	0	negative
OTU 29	1	OTU 3530	1	0.354	0	positive
OTU 34	1	OTU 37	1	0.564	0	positive
OTU 34	1	OTU 48	3	0.333	0.01	positive
OTU 34	1	OTU 2240	1	0.309	0	positive
OTU 36	1	OTU 2240	1	-0.411	0.01	negative
OTU 37	1	OTU 58	1	-0.311	0	negative
OTU 37	1	OTU 2240	1	0.540	0	positive
OTU 37	1	OTU 3530	1	-0.398	0	negative
OTU 43	2	OTU 3530	1	-0.353	0	negative
OTU 58	3	OTU 2240	1	-0.324	0	negative

3.4. Discussion

Change of Fungal Communities in Pine Seedlings After Transplantation

The root fungal communities significantly changed through seedling development. These results showed that root colonization of *T. matsutake* dramatically decreased after being transplanted to the greenhouse, and they were replaced by other fungi. Increase in alpha diversity indices was expected as seedlings were transplanted from controlled environment to relatively open environment. After transplantation, fast-growing Ascomycota dominated, and then were replaced by early-stage ectomycorrhizal fungi. Previous studies looked at the mycorrhizal succession in pine seedlings (Peay *et al.*, 2011; Rudawska *et al.*, 2019; Herzog *et al.*, 2019), and the shift of root associated fungi follows the general trend, despite being inoculated with *T. matsutake*.

After transplantation to greenhouse, Ascomycota species became dominant in seedling roots In M10, most of the dominant OTUs were saprotrophs or pathotrophs, such as *Pseudogymnoascus* and *Fusarium*, with one exception being *Oidiodendron echinulatum*, an ericoid mycorrhiza. In M17, the abundance of symbiotrophs (e.g. *Wilcoxina mikolae*) increased, while pathotrophs decreased. Among these saprotroph species, *Pseudogymnoascus pannorum* is widely distributed in the soil and adapted to nutrient poor environments (Minnis & Lindner, 2013; Chaturvedi *et al.*, 2018). Previous studies of pine seedling roots also discovered the presence of *Pseudogymnoascus* species (Menkis & Vasaitis, 2011; Moler & Aho, 2018). Other taxa, like

Oidiodendron and *Wilcoxina*, are well known species that are common in early successional or disturbed ecosystems (Berch *et al.*, 2006; Lee *et al.*, 2012; Lee & Eom, 2013; Rudawska *et al.*, 2019). As these species were absent in samples from M03, and both taxa found in the study are expected to have been dispersed by wind (Horton, 2017). A noteworthy result is the high abundance of *Fusarium* in M10–M17 samples. Usually, *Fusarium* is considered a plant pathogen (Gordon, 2017), but *Fusarium* species have also been found as endophytes of a wide range of wild plants (Kuldau & Yates, 2000; Min *et al.*, 2014). For example, growth-enhancement or pathogen-resistance conferred by non-pathogenic *Fusarium* species were widely reported (Forsyth *et al.*, 2006; Waweru *et al.*, 2014). Their role is uncertain in the study, and further study would be needed to understand *Fusarium* 's role in roots of pine seedlings.

The proportion of ectomycorrhizal (*Suillus* and *Tomentella*) and endophytic fungi (*Cadophora* and *Phialocephala*) increased after M24, which are considered as common fungi in early successional stage (Colpaert *et al.*, 1996; Berch *et al.*, 2006; Sim & Eom, 2009; Lee *et al.*, 2012; Lee & Eom, 2013; Lee & Koo, 2016). In particular, *Suillus* species are known to be important in the establishment of pine seedlings (Hayward *et al.*, 2015). *Suillus* species might be more competitive than other mycorrhizal fungi found in first year, such as *Wilcoxina*. *Wilcoxina* is known as a weak competitor ectomycorrhizal fungi that prospers only in absence of competitor ectomycorrhizal fungi (Danielson & Prudel, 1990). *Suillus* species are known to form ectomycorrhiza with pine trees that span a large area, thanks to long distance dispersal of spores combined with high volume of spore production and large sporocarps (Peay *et al.*, 2012; Horton, 2017).

Other species, such as *Cadophora* and *Tomentella* are considered common fungi of pine seedlings in early successional stage or disturbed areas (Colpaert *et al.*, 1996; Berch *et al.*, 2006; Sim & Eom, 2009; Lee *et al.*, 2012; Lee & Eom, 2013; Lee & Koo, 2016).

While *T. matsutake* was still found in several *P. densiflora* seedlings, their frequency and abundance steadily decreased after transplantation. Although the priority effect in ectomycorrhiza was reported in previous studies (Kennedy & Bruns, 2005; Kennedy *et al.*, 2009; Fukami, 2015), it did not apply to *T. matsutake* in the study. As *T. matsutake* is usually known to form symbiotic relationship with mature pine trees in the field (Wang *et al.*, 2018), the results suggest that the symbiosis between *T. matsutake* and young seedlings is not sustainable outside of sterile environment without proper support. I suggest that this is due to a slow growth rate and higher carbon demand of *T. matsutake* as a late-stage ectomycorhizal fungus (Smith & Read, 2013).

In PICRUST2 data, both ISA and LefSe showed that pathways related to fatty acid synthesis are predicted to be up-regulated in M17. During mycorrhizal colonization, large amounts of fatty acids are required for membrane synthesis and storage lipids (Sancholle *et al.*, 2001). In arbuscular mycorrhizal fungi, upregulation of fungal fatty acid biosynthesis pathways were reported during mycorrhization (Wewer *et al.*, 2014). Though similar results were not discovered in ectomycorrhizal fungi, gene related with fatty acid synthesis were discovered in ectomycorrhizal fungus *Laccaria bicolor* (Reich *et al.*, 2009).

Only few studies have applied taxonomic distinctness indices in diversity study of fungi (Vieira *et al.*, 2018; Cox *et al.*, 2019). Those indices provide information for taxonomic relatedness between species in community (Clarke & Warwick, 1998). In both indices, little differences were found between groups except for M03 group. M03 group showed higher average taxonomic distinctness and variation in taxonomic distinctness than other groups. This result could be explained with high proportion of single species and taxonomically less related composition of M03 group (*T. matsutake* + *Cladosporium* species), even though the number of OTUs was much lower. After exposure to wild mycobiome, taxonomic distances between OTUs did not changed significantly though the proportion and compositions were highly different and the number of OTUs were gradually increased.

Network Analysis and Keystone Taxa

Microbial network analysis has been used to visualize taxa with a strong effect on network structure, or highly connected taxa in various environments (Barberán *et al.*, 2012; Gilbert *et al.*, 2012; Agler *et al.*, 2016). A network of 35 fungal OTUs that were abundant during pine seedling growth was constructed with SparCC correlations. Interaction and network formation between functionally diverse fungi were previously reported (Toju *et al.*, 2016), and the results were similar; a combination of functionally different OTUs were observed in each cluster in the network.

Four OTUs were identified as keystone taxa: *S. granulatus*, *C. pauciseptatum*, *Fusarium* sp. (OTU 37), and *Fusarium oxysporum*

(Table 2). Keystone taxa are taxa highly connected to other network members, play important roles in the microbiome (Banerjee *et al.*, 2018), and they are required to understand ecosystem's response to disturbance (Stinson *et al.*, 2006). *Suillus granulatus* was expected as a keystone species as it was reported as crucial species in establishment of pine seedlings and strong competitor (Dickie *et al.*, 2010; Kohout *et al.*, 2011; Hayward *et al.*, 2015; Urcelay *et al.*, 2017; Policelli *et al.*, 2019). However, *C. pauciseptatum* or *Fusarium* species were not expected as keystone species, as *C. pauciseptatum* and *Fusarium* species are known as soil saprotrophs or plant pathogens. The presence of *C. pauciseptatum* were reported in *Pinus sylvestris* (Menkis & Vasaitis, 2011), the relationship between *C. pauciseptatum* and *P. densiflora* is still unknown. However, it is possible that *C. pauciseptatum* indirectly influenced microbiome by affecting quality of pine seedlings (Agler *et al.*, 2016). *Fusarium oxysporum* and *F. trincintum* are known as plant pathogens or mutualistic endophytes (Kuldau & Yates, 2000; Forsyth *et al.*, 2006; Vu *et al.*, 2006; Michielse & Rep, 2009; Min *et al.*, 2014; Waweru *et al.*, 2014; Vasundhara *et al.*, 2016). While their exact function is not certain in this study, both endophyte and plant pathogen might influence on root microbiome by positive or negative effects (Van Der Heijden *et al.*, 2008b).

In summary, I have documented the change in fungal community composition in pine seedlings after the *T. matsutake* inoculation, and introduced a SparCC analysis to predict the cross-fungi associations from NGS data. The root microbiome drastically changed at alpha- and beta-diversity levels after transplantation. Temporal succession of the mycorrhizal community suggests a weak

priority effect as *T. matsutake* was rapidly replaced by *W. mikolae*, *S. granulatus*, and other fungi. In addition, four keystone species were found during microbiome succession that might play an important role in microbiome composition in pine seedlings.

General Conclusions

In this thesis, the influence of geographic and temporal distances on fungal communities associated with *P. densiflora* were studied with DNA metabarcoding. The effect of geographic distance on mycobiome has been reported in previous studies (Talbot *et al.*, 2014; Geml, 2017; Wang *et al.*, 2019; Yang *et al.*, 2019), but its influence on fungal communities in different microhabitats and guilds were rarely studied. Furthermore, it is much harder to find such studies with host identity controls studies (Coince *et al.*, 2014; Jarvis *et al.*, 2015). In terms of temporal distance, the succession of plant-associated fungal communities was reported in both mature plants and seedlings (Obase *et al.*, 2009). However, the succession of non-ectomycorrhizal fungi in plants were less studied as most of previous studies were conducted with root tip observation, thus the results were mostly focused on ectomycorrhizal fungal communities.

To study the influence of spatial distance on plant-associated fungal communities in macro- and micro- scales, the root and soil samples were collected from 80 *P. densiflora* trees in 16 forests. In chapter 1, altitude was the significant factor in determination of fungal community composition in both microhabitats (Fig. 12). However, the influence of geographic distance was significant only in root fungal communities (Fig. 13). Similarly, significant correlations between richness of fungal community and altitude were only found in root fungal communities (Fig. 14). In addition, the strength of altitude and geographic distance was lower in ectomycorrhizal fungal communities than non-ectomycorrhizal fungal communities (i.e. endophyte,

saprotroph), indicating host-independent nature of endophyte (Glynou *et al.*, 2016) and free-living saprotroph (Talbot *et al.*, 2014). In contrast, host species identity has been reported as the most important factor in ectomycorrhizal fungal community composition (Bahram *et al.*, 2012). Guild also influenced similarity of fungal community between microhabitats (Fig. 11). The ectomycorrhizal fungal communities shared more species between microhabitats than non-ectomycorrhizal fungal communities due to functional separation. These findings suggest that fungal communities in different microhabitats/guilds respond dissimilarly, and root fungal communities are more sensitive to geographic distances.

As a next step, geographic distribution of root fungal communities was investigated with long-read PacBio sequencing platforms and the results were compared with data from short-read Illumina MiSeq platform (chapter 2; Appendix 3). In both datasets, a core of conserved taxa could be found across fungal communities of sampling sites. Due to relatively low throughput of PacBio platform (Tedersoo *et al.*, 2018), PacBio datasets were more sensitive to variation of proportion between sampling sites (Figs. 26 and 27). In addition, when data from two platforms were compared, taxonomic resolution was similar at phylum to family level, but the proportion of unidentified taxa was significantly lower in PacBio dataset at genus level (Fig. 21). The most abundant components of core taxa were belonged to saprotrophic, or endophytic fungi (*Cladophialaphora*, *Mortierella*, *Trichoderma*), though mycorrhizal fungi were also found in core group (*Oidiodendron*, *Russula*, *Tomentella*). In addition, plant pathogens (*Armillaria*) and commercially important ectomycorrhizal mushrooms

(*Tricholoma matsutake*) could be detected in both datasets, albeit at low abundances. These results suggest that DNA metabarcoding can detect both essential taxa with possible mutual relationship with host plants and pathogenic taxa that may harm the fitness of plants. In addition, the possibility of DNA metabarcoding was suggested for monitoring of economically important plant pathogens and gourmet mushrooms. But the results should be interpreted with caution due to possible bias in methods.

In addition, the index of discovered taxa in root of *P. densiflroa* was provided in Appendix 3. In total, 11 phylum, 38 class, 88 order, 174 family, and 363 genera were found. Among those taxa, 4 phylum, 21 class, 52 order, 123 family, and 199 genera were also found in the National List of Species of Korea, representing 50% of phylum, 75% of class, 54.74% of order, 45.22% of family, and 19.41% of genera in the list. However, 7 phylum, 17 class, 36 order, 51 family, and 164 genera were not found in the list. Meanwhile, 463 species were identified at the species level, with only 164 species matching the National List of Species of Korea. The list of taxa in *P. densiflora* root mycobiome will provide the valuable backbone data for fungal diversity study in South Korea.

The effect of temporal distance on fungal communities were evaluated using seedlings of *P. densiflora* (chapter 3). At first, fast-growing saprotrophic Ascomycota species were dominant, but those species were replaced with ericoid mycorrhiza and pioneer ectomycorrhizal species (Fig. 30). Root mycobiome became relatively stabilized after colonization by early stage ectomycorrhizal (i.e. *Suillus*) and endophytic fungal species (*Cadophora* and *Phialocephala*).

Indicator species analysis revealed that upregulation of pathways related with fatty acid synthesis were predicted during succession in PICRUST2 analysis, indicating increased membrane synthesis during root colonization (Sancholle *et al.*, 2001). Using network analysis, four keystone species that are highly connected to other species were found: *S. granulatus*, *C. pauciseptatum*, *Fusarium* sp., and *Fusarium oxysporum*. Those species are expected to play important roles during construction of fungal community in *P. densiflora* seedlings.

In conclusion, data shown in this thesis suggested strength and cautions for microbiome studies using DNA metabarcoding (Appendix 5). The approach used in chapter 1 unveiled the different responses of fungal communities to geographic distance according to their sample type and guilds. In chapter 3, succession of fungal communities could be found across temporal distance. The results provided the backbone data for sample selection to study the effect of geographic and temporal distance on fungal communities. The results from chapter 2 revealed the possibility of DNA metabarcoding to detect dangerous pathogens from environmental samples, but also exposed its weakness in interpretation of datasets from different methods.

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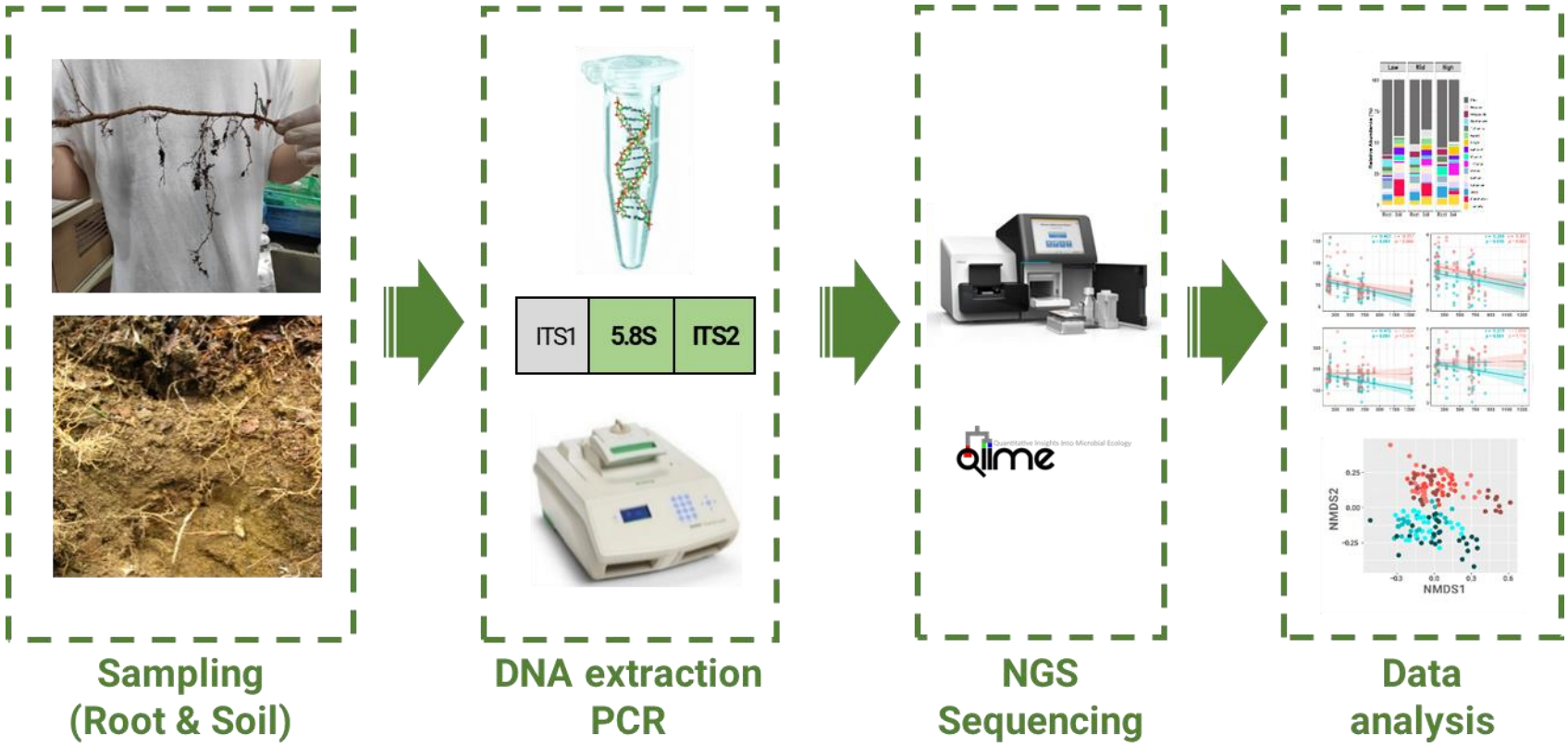
Appendix

Appendix 1. Proposed workflow for chapter 1



Appendix 2. Proposed workflow for chapter 2

Appendix 3. List of fungal species found in root of *P. densiflora.*

Phylum	Class	Order	Family	Genus	Species	PacBio	MiSeq	NLSK (Genus)	NLSK (Species)
Ascomycota	Archaeorhizomycetes	Archaeorhizomycetales	Archaeorhizomycetaceae	*Archaeorhizomyces*	*Archaeorhizomyces borealis*	O	O		
Ascomycota	Archaeorhizomycetes	Archaeorhizomycetales	Archaeorhizomycetaceae	*Archaeorhizomyces*	*Archaeorhizomyces sp.*	O			
Ascomycota	Archaeorhizomycetes	Archaeorhizomycetales	Archaeorhizomycetaceae	Unidentified		O	O		
Ascomycota	Archaeorhizomycetes	Archaeorhizomycetales	Unidentified	Unidentified		O			
Ascomycota	Arthoniomycetes	Lichenostigmatales	Phaeococcomycetaceae	*Phaeococcomyces*	*Phaeococcomyces sp.*	O	O		
Ascomycota	Dothideomycetes	Asterinales	Melaspileaceae	Unidentified		O			
Ascomycota	Dothideomycetes	Botryosphaeriales	Botryosphaeriaceae	*Botryosphaeria*	*Botryosphaeria sp.*	O		O	
Ascomycota	Dothideomycetes	Botryosphaeriales	Botryosphaeriaceae	Unidentified		O			
Ascomycota	Dothideomycetes	Capnodiales	Capnodiaceae	*Antennariella*	*Antennariella placitae*	O			
Ascomycota	Dothideomycetes	Capnodiales	Incertae sedis	*Hispidoconidioma*	*Hispidoconidioma alpinum*		O		
Ascomycota	Dothideomycetes	Capnodiales	Cladosporiaceae	*Cladosporium*	*Cladosporium sphaerospermum*		O	O	O
Ascomycota	Dothideomycetes	Capnodiales	Mycosphaerellaceae	*Geastrumia*	*Geastrumia sp.*	O			
Ascomycota	Dothideomycetes	Capnodiales	Mycosphaerellaceae	*Mycosphaerella*	*Mycosphaerella tassiana*	O	O	O	
Ascomycota	Dothideomycetes	Capnodiales	Mycosphaerellaceae	*Sphaerulina*	*Sphaerulina socia*		O	O	
Ascomycota	Dothideomycetes	Capnodiales	Mycosphaerellaceae	Unidentified		O	O		
Ascomycota	Dothideomycetes	Capnodiales	Teratosphaeriaceae	*Capnobotryella*	*Capnobotryella renispora*		O		
Ascomycota	Dothideomycetes	Capnodiales	Teratosphaeriaceae	*Catenulostroma*	*Catenulostroma hermanusense*	O			

NLSK=National List of species of Korea (국가생물종목록)

Appendix 3, Continued

Phylum	Class	Order	Family	Genus	Species	PacBio	MiSeq	NLSK (Genus)	NLSK (Species)
Ascomycota	Dothideomycetes	Capnodiales	Teratosphaeriaceae	*Devriesia*	*Devriesia sp.*	O	O		
Ascomycota	Dothideomycetes	Capnodiales	Teratosphaeriaceae	*Neocatenulostroma*	*Neocatenulostroma sp.*	O			
Ascomycota	Dothideomycetes	Capnodiales	Teratosphaeriaceae	*Teratosphaeria*	*Teratosphaeria sp.*	O	O		
Ascomycota	Dothideomycetes	Capnodiales	Teratosphaeriaceae	Unidentified		O	O		
Ascomycota	Dothideomycetes	Capnodiales	Unidentified	Unidentified		O	O		
Ascomycota	Dothideomycetes	Dothideales	Aureobasidiaceae	*Aureobasidium*	*Aureobasidium pullulans*		O	O	
Ascomycota	Dothideomycetes	Dothideales	Dothideaceae	*Dothidea*	*Dothidea sambuci*		O		
Ascomycota	Dothideomycetes	Dothideales	Dothideaceae	*Scleroconidioma*	*Scleroconidioma sphagnicola*		O	O	
Ascomycota	Dothideomycetes	Dothideales	Incertae sedis	*Hortaea*	*Hortaea sp.*		O		
Ascomycota	Dothideomycetes	Dothideales	Dothioraceae	*Hormonema*	*Hormonema macrosporum*	O	O		
Ascomycota	Dothideomycetes	Dothideales	Dothioraceae	*Perusta*	*Perusta inaequalis*	O	O		
Ascomycota	Dothideomycetes	Dothideales	Unidentified	Unidentified		O			
Ascomycota	Dothideomycetes	Incertae sedis	Incertae sedis	*Septonema*	*Septonema sp.*		O		
Ascomycota	Dothideomycetes	Mytilinidales	Gloniaceae	*Cenococcum*	*Cenococcum geophilum*	O	O		
Ascomycota	Dothideomycetes	Mytilinidales	Gloniaceae	*Cenococcum*	*Cenococcum sp.*	O	O		
Ascomycota	Dothideomycetes	Mytilinidiales	Mytilinidiaceae	*Lophium*	*Lophium arboricola*	O	O		
Ascomycota	Dothideomycetes	Mytilinidiales	Mytilinidiaceae	*Pseudocamaropycnis*	*Pseudocamaropycnis pini*	O	O		

NLSK=National List of species of Korea (국가생물종목록)

Appendix 3, Continued

Phylum	Class	Order	Family	Genus	Species	PacBio	MiSeq	NLSK (Genus)	NLSK (Species)
Ascomycota	Dothideomycetes	Mytilinidiales	Mytilinidiaceae	*Pseudocamaropycnis*	*Pseudocamaropycnis sp.*		O		
Ascomycota	Dothideomycetes	Mytilinidiales	Mytilinidiaceae	Unidentified		O	O		
Ascomycota	Dothideomycetes	Mytilinidiales	Unidentified	Unidentified		O			
Ascomycota	Dothideomycetes	Patellariales	Patellariaceae	*Banhegyia*	*Banhegyia sp.*		O		
Ascomycota	Dothideomycetes	Pleosporales	Didymellaceae	*Didymella*	*Didymella exigua*		O	O	
Ascomycota	Dothideomycetes	Pleosporales	Didymellaceae	*Phoma*	*Phoma sp.*	O		O	
Ascomycota	Dothideomycetes	Pleosporales	Didymosphaeriaceae	*Cylindroaseptospora*	*Cylindroaseptospora leucaenae*		O		
Ascomycota	Dothideomycetes	Pleosporales	Didymosphaeriaceae	*Didymosphaeria*	*Didymosphaeria variabile*		O	O	
Ascomycota	Dothideomycetes	Pleosporales	Didymosphaeriaceae	*Paraphaeosphaeria*	*Paraphaeosphaeria sporulosa*	O		O	
Ascomycota	Dothideomycetes	Pleosporales	Didymosphaeriaceae	*Paraphaeosphaeria*	*Paraphaeosphaeria viridescens*		O	O	
Ascomycota	Dothideomycetes	Pleosporales	Didymosphaeriaceae	Unidentified		O			
Ascomycota	Dothideomycetes	Pleosporales	Leptosphaeriaceae	*Acicuseptoria*	*Acicuseptoria rumicis*	O	O		
Ascomycota	Dothideomycetes	Pleosporales	Leptosphaeriaceae	*Plenodomus*	*Plenodomus biglobosus*		O	O	
Ascomycota	Dothideomycetes	Pleosporales	Leptosphaeriaceae	*Plenodomus*	*Plenodomus sp.*	O		O	
Ascomycota	Dothideomycetes	Pleosporales	Lophiostomataceae	*Lophiostoma*	*Lophiostoma corticola*	O		O	
Ascomycota	Dothideomycetes	Pleosporales	Melanommataceae	*Herpotrichia*	*Herpotrichia juniperi*	O	O		
Ascomycota	Dothideomycetes	Pleosporales	Phaeosphaeriaceae	*Paraphoma*	*Paraphoma rhaphiolepidis*	O	O		

NLSK=National List of species of Korea (국가생물종목록)

Appendix 3, Continued

Phylum	Class	Order	Family	Genus	Species	PacBio	MiSeq	NLSK (Genus)	NLSK (Species)
Ascomycota	Dothideomycetes	Pleosporales	Phaeosphaeriaceae	Unidentified		O	O		
Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	*Alternaria*	*Alternaria alternata*		O	O	O
Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	*Alternaria*	*Alternaria senecionicola*	O		O	
Ascomycota	Dothideomycetes	Pleosporales	Sporormiaceae	*Preussia*	*Preussia minipascua*	O			
Ascomycota	Dothideomycetes	Pleosporales	Sporormiaceae	*Preussia*	*Preussia sp.*	O			
Ascomycota	Dothideomycetes	Pleosporales	Sporormiaceae	*Sporormiella*	*Sporormiella leporina*		O		
Ascomycota	Dothideomycetes	Pleosporales	Unidentified	Unidentified		O	O		
Ascomycota	Dothideomycetes	Tubeufiales	Tubeufiaceae	*Helicoma*	*Helicoma sp.*		O		
Ascomycota	Dothideomycetes	Tubeufiales	Tubeufiaceae	*Podonectria*	*Podonectria sp.*	O			
Ascomycota	Dothideomycetes	Unidentified	Unidentified	Unidentified		O	O		
Ascomycota	Dothideomycetes	Venturiales	Sympoventuriaceae	*Ochroconis*	*Ochroconis sp.*	O	O	O	
Ascomycota	Dothideomycetes	Venturiales	Unidentified	Unidentified		O	O		
Ascomycota	Dothideomycetes	Venturiales	Venturiaceae	*Cylindrosympodium*	*Cylindrosympodium variabile*	O			
Ascomycota	Dothideomycetes	Venturiales	Venturiaceae	*Sympodiella*	*Sympodiella quercina*	O	O		
Ascomycota	Dothideomycetes	Venturiales	Venturiaceae	Unidentified		O	O		
Ascomycota	Dothideomycetes	Venturiales	Venturiaceae	*Venturia*	*Venturia sp.*	O	O	O	
Ascomycota	Eurotiomycetes	Chaetothyriales	Cyphellophoraceae	*Cyphellophora*	*Cyphellophora sp.*	O	O		

NLSK=National List of species of Korea (국가생물종목록)

Appendix 3, Continued

Phylum	Class	Order	Family	Genus	Species	PacBio	MiSeq	NLSK (Genus)	NLSK (Species)
Ascomycota	Eurotiomycetes	Chaetothyriales	Cyphellophoraceae	Unidentified		O			
Ascomycota	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	*Capronia*	*Capronia sp.*	O	O		
Ascomycota	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	*Cladophialophora*	*Cladophialophora chaetospira*	O	O	O	
Ascomycota	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	*Cladophialophora*	*Cladophialophora minutissima*	O	O	O	
Ascomycota	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	*Cladophialophora*	*Cladophialophora sylvestris*	O	O	O	
Ascomycota	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	*Cladophialophora*	*Cladophialophora sp.*	O	O	O	
Ascomycota	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	*Exophiala*	*Exophiala alcalophila*	O	O	O	
Ascomycota	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	*Exophiala*	*Exophiala equina*	O		O	
Ascomycota	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	*Exophiala*	*Exophiala opportunistica*	O		O	
Ascomycota	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	*Exophiala*	*Exophiala salmonis*	O	O	O	
Ascomycota	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	*Exophiala*	*Exophiala sp.*	O	O	O	
Ascomycota	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	*Rhinocladiella*	*Rhinocladiella sp.*		O	O	
Ascomycota	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	Unidentified		O	O		
Ascomycota	Eurotiomycetes	Chaetothyriales	Trichomeriaceae	*Knufia*	*Knufia peltigerae*	O	O		
Ascomycota	Eurotiomycetes	Chaetothyriales	Trichomeriaceae	*Knufia*	*Knufia sp.*	O			
Ascomycota	Eurotiomycetes	Chaetothyriales	Trichomeriaceae	Unidentified			O		
Ascomycota	Eurotiomycetes	Chaetothyriales	Unidentified	Unidentified		O	O		

NLSK=National List of species of Korea (국가생물종목록)

Appendix 3, Continued

Phylum	Class	Order	Family	Genus	Species	PacBio	MiSeq	NLSK (Genus)	NLSK (Species)
Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae	*Aspergillus*	*Aspergillus fumigatus*		O	O	O
Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae	*Aspergillus*	*Aspergillus tubingensis*		O	O	O
Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae	*Aspergillus*	*Aspergillus sp.*	O	O	O	
Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae	*Penicillium*	*Penicillium adametzii*	O	O	O	
Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae	*Penicillium*	*Penicillium arenicola*	O	O	O	
Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae	*Penicillium*	*Penicillium arianeae*	O	O	O	
Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae	*Penicillium*	*Penicillium atrosanguineum*	O	O	O	
Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae	*Penicillium*	*Penicillium austricola*		O	O	
Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae	*Penicillium*	*Penicillium bialowiezense*	O	O	O	O
Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae	*Penicillium*	*Penicillium canescens*	O	O	O	
Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae	*Penicillium*	*Penicillium daejeonium*	O		O	O
Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae	*Penicillium*	*Penicillium desertorum*		O	O	
Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae	*Penicillium*	*Penicillium dierckxii*	O		O	O
Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae	*Penicillium*	*Penicillium herquei*		O	O	O
Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae	*Penicillium*	*Penicillium infrapurpureum*	O		O	
Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae	*Penicillium*	*Penicillium marthae-christensenae*		O	O	
Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae	*Penicillium*	*Penicillium melinii*	O	O	O	O

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Appendix 3, Continued

Phylum	Class	Order	Family	Genus	Species	PacBio	MiSeq	NLSK (Genus)	NLSK (Species)
Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae	*Penicillium*	*Penicillium nodositatum*	O	O	O	
Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae	*Penicillium*	*Penicillium parviverrucosum*		O	O	
Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae	*Penicillium*	*Penicillium paxilli*		O	O	O
Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae	*Penicillium*	*Penicillium penicillioides*	O	O	O	
Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae	*Penicillium*	*Penicillium polonicum*	O	O	O	O
Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae	*Penicillium*	*Penicillium porphyreum*	O		O	
Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae	*Penicillium*	*Penicillium restingae*		O	O	
Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae	*Penicillium*	*Penicillium riverlandense*	O	O	O	
Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae	*Penicillium*	*Penicillium sclerotiorum*	O		O	O
Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae	*Penicillium*	*Penicillium spinulosum*	O	O	O	O
Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae	*Penicillium*	*Penicillium sumatraense*	O		O	O
Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae	*Penicillium*	*Penicillium toxicarium*	O	O	O	
Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae	*Penicillium*	*Penicillium wisconsinense*	O		O	
Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae	*Penicillium*	*Penicillium sp.*	O	O	O	
Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae	*Phialomyces*	*Phialomyces sp.*	O	O		
Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae	Unidentified		O	O		
Ascomycota	Eurotiomycetes	Eurotiales	Elaphomycetaceae	*Elaphomyces*	*Elaphomyces sp.*	O	O		

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Appendix 3, Continued

Phylum	Class	Order	Family	Genus	Species	PacBio	MiSeq	NLSK (Genus)	NLSK (Species)
Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	*Rasamsonia*	*Rasamsonia sp.*	O	O		
Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	*Sagenomella*	*Sagenomella diversispora*	O	O		
Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	*Sagenomella*	*Sagenomella verticillata*	O	O		
Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	*Talaromyces*	*Talaromyces erythromellis*		O	O	
Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	*Talaromyces*	*Talaromyces marneffei*	O	O	O	
Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	*Talaromyces*	*Talaromyces purpureus*	O		O	
Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	*Talaromyces*	*Talaromyces rugulosus*	O	O	O	O
Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	*Talaromyces*	*Talaromyces sp.*	O	O	O	
Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	Unidentified		O			
Ascomycota	Eurotiomycetes	Eurotiales	Unidentified	Unidentified		O	O		
Ascomycota	Eurotiomycetes	Onygenales	Ajellomycetaceae	*Emergomyces*	*Emergomyces sp.*	O			
Ascomycota	Eurotiomycetes	Onygenales	Onygenaceae	Unidentified		O			
Ascomycota	Eurotiomycetes	Onygenales	Unidentified	Unidentified			O		
Ascomycota	Eurotiomycetes	Phaeomoniellales	Phaeomoniellaceae	*Aequabiliella*	*Aequabiliella sp.*		O		
Ascomycota	Eurotiomycetes	Sclerococcales	Sclerococcaceae	Unidentified			O		
Ascomycota	Eurotiomycetes	Unidentified	Unidentified	Unidentified		O			
Ascomycota	Eurotiomycetes	Verrucariales	Verrucariaceae	Unidentified		O			

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Appendix 3, Continued

Phylum	Class	Order	Family	Genus	Species	PacBio	MiSeq	NLSK (Genus)	NLSK (Species)
Ascomycota	Geoglossomycetes	Geoglossales	Unidentified	Unidentified		O			
Ascomycota	Laboulbeniomycetes	Pyxidiophorales	Unidentified	Unidentified		O			
Ascomycota	Lecanoromycetes	Lecanorales	Lecanoraceae	*Lecidella*	*Lecidella sp.*	O			
Ascomycota	Lecanoromycetes	Lecanorales	Parmeliaceae	*Parmelia*	*Parmelia sp.*	O	O		
Ascomycota	Lecanoromycetes	Lecanorales	Unidentified	Unidentified		O	O		
Ascomycota	Lecanoromycetes	Incertae sedis	Incertae sedis	*Sarea*	*Sarea resinae*	O			
Ascomycota	Lecanoromycetes	Incertae sedis	Incertae sedis	*Sarea*	*Sarea sp.*	O			
Ascomycota	Lecanoromycetes	Ostropales	Stictidaceae	*Cryptodiscus*	*Cryptodiscus sp.*	O			
Ascomycota	Lecanoromycetes	Ostropales	Stictidaceae	Unidentified		O	O		
Ascomycota	Lecanoromycetes	Trapeliales	Trapeliaceae	Unidentified			O		
Ascomycota	Lecanoromycetes	Umbilicariales	Ophioparmaceae	Unidentified			O		
Ascomycota	Lecanoromycetes	Umbilicariales	Umbilicariaceae	Unidentified		O			
Ascomycota	Lecanoromycetes	Unidentified	Unidentified	Unidentified		O	O		
Ascomycota	Leotiomycetes	Helotiales	Ascocorticiaceae	*Ascocorticium*	*Ascocorticium sp.*	O	O		
Ascomycota	Leotiomycetes	Helotiales	Cenangiaceae	*Cenangiopsis*	*Cenangiopsis sp.*		O		
Ascomycota	Leotiomycetes	Helotiales	Dermateaceae	*Cryptosporiopsis*	*Cryptosporiopsis sp.*	O	O	O	
Ascomycota	Leotiomycetes	Helotiales	Dermateaceae	*Mollisia*	*Mollisia cinerea*	O	O	O	O

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Appendix 3, Continued

Phylum	Class	Order	Family	Genus	Species	PacBio	MiSeq	NLSK (Genus)	NLSK (Species)
Ascomycota	Leotiomycetes	Helotiales	Dermateaceae	*Mollisia*	*Mollisia sp.*	O	O	O	
Ascomycota	Leotiomycetes	Helotiales	Dermateaceae	*Neofabraea*	*Neofabraea vagabunda*	O	O		
Ascomycota	Leotiomycetes	Helotiales	Dermateaceae	*Pezicula*	*Pezicula brunnea*	O	O	O	
Ascomycota	Leotiomycetes	Helotiales	Dermateaceae	*Rhizodermea*	*Rhizodermea veluwensis*	O	O		
Ascomycota	Leotiomycetes	Helotiales	Dermateaceae	Unidentified		O	O		
Ascomycota	Leotiomycetes	Helotiales	Helotiaceae	*Articulospora*	*Articulospora sp.*	O		O	
Ascomycota	Leotiomycetes	Helotiales	Helotiaceae	*Collophora*	*Collophora sp.*	O	O		
Ascomycota	Leotiomycetes	Helotiales	Helotiaceae	*Infundichalara*	*Infundichalara minuta*	O	O		
Ascomycota	Leotiomycetes	Helotiales	Helotiaceae	*Meliniomyces*	*Meliniomyces sp.*	O			
Ascomycota	Leotiomycetes	Helotiales	Helotiaceae	*Mycofalcella*	*Mycofalcella calcarata*	O			
Ascomycota	Leotiomycetes	Helotiales	Helotiaceae	*Mycosymbioces*	*Mycosymbioces sp.*	O	O		
Ascomycota	Leotiomycetes	Helotiales	Helotiaceae	*Pseudoclathrosphaerina*	*Pseudoclathrosphaerina sp.*	O			
Ascomycota	Leotiomycetes	Helotiales	Helotiaceae	*Scytalidium*	*Scytalidium album*	O		O	
Ascomycota	Leotiomycetes	Helotiales	Helotiaceae	*Scytalidium*	*Scytalidium sp.*		O	O	
Ascomycota	Leotiomycetes	Helotiales	Helotiaceae	*Tetracladium*	*Tetracladium sp.*	O	O		
Ascomycota	Leotiomycetes	Helotiales	Helotiaceae	Unidentified		O	O		
Ascomycota	Leotiomycetes	Helotiales	Incertae sedis	*Acephala*	*Acephala sp.*		O		

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Appendix 3, Continued

Phylum	Class	Order	Family	Genus	Species	PacBio	MiSeq	NLSK (Genus)	NLSK (Species)
Ascomycota	Leotiomycetes	Helotiales	Incertae sedis	*Cadophora*	*Cadophora sp.*	O	O		
Ascomycota	Leotiomycetes	Helotiales	Incertae sedis	*Chalara*	*Chalara angustata*	O	O		
Ascomycota	Leotiomycetes	Helotiales	Incertae sedis	*Chalara*	*Chalara holubovae*	O	O		
Ascomycota	Leotiomycetes	Helotiales	Incertae sedis	*Chalara*	*Chalara pseudoaffinis*		O		
Ascomycota	Leotiomycetes	Helotiales	Incertae sedis	*Chalara*	*Chalara sp.*	O	O		
Ascomycota	Leotiomycetes	Helotiales	Incertae sedis	*Lauriomyces*	*Lauriomyces bellulus*	O			
Ascomycota	Leotiomycetes	Helotiales	Incertae sedis	*Leohumicola*	*Leohumicola sp.*	O	O		
Ascomycota	Leotiomycetes	Helotiales	Incertae sedis	*Leohumicola*	*Leohumicola verrucosa*	O	O		
Ascomycota	Leotiomycetes	Helotiales	Incertae sedis	*Leptodontidium*	*Leptodontidium sp.*	O	O		
Ascomycota	Leotiomycetes	Helotiales	Incertae sedis	*Leptodontidium*	*Leptodontidium trabinellum*	O	O		
Ascomycota	Leotiomycetes	Helotiales	Incertae sedis	*Mycoarthris*	*Mycoarthris corallina*	O	O		
Ascomycota	Leotiomycetes	Helotiales	Incertae sedis	*Phaeomollisia*	*Phaeomollisia piceae*		O		
Ascomycota	Leotiomycetes	Helotiales	Incertae sedis	*Spirosphaera*	*Spirosphaera sp.*	O	O		
Ascomycota	Leotiomycetes	Helotiales	Incertae sedis	*Xenopolyscytalum*	*Xenopolyscytalum pinea*	O	O		
Ascomycota	Leotiomycetes	Helotiales	Incertae sedis	*Xenopolyscytalum*	*Xenopolyscytalum sp.*	O			
Ascomycota	Leotiomycetes	Helotiales	Hyaloscyphaceae	*Arachnopeziza*	*Arachnopeziza sp.*	O		O	
Ascomycota	Leotiomycetes	Helotiales	Hyaloscyphaceae	*Clathrosphaerina*	*Clathrosphaerina sp.*		O		

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Appendix 3, Continued

Phylum	Class	Order	Family	Genus	Species	PacBio	MiSeq	NLSK (Genus)	NLSK (Species)
Ascomycota	Leotiomycetes	Helotiales	Hyaloscyphaceae	*Glutinomyces*	*Glutinomyces sp.*	O			
Ascomycota	Leotiomycetes	Helotiales	Hyaloscyphaceae	*Hyalopeziza*	*Hyalopeziza sp.*	O			
Ascomycota	Leotiomycetes	Helotiales	Hyaloscyphaceae	*Hyaloscypha*	*Hyaloscypha finlandica*	O	O		
Ascomycota	Leotiomycetes	Helotiales	Hyaloscyphaceae	*Hyphodiscus*	*Hyphodiscus sp.*	O			
Ascomycota	Leotiomycetes	Helotiales	Hyaloscyphaceae	*Lachnellula*	*Lachnellula sp.*	O		O	
Ascomycota	Leotiomycetes	Helotiales	Hyaloscyphaceae	*Lachnum*	*Lachnum pulverulentum*	O		O	
Ascomycota	Leotiomycetes	Helotiales	Hyaloscyphaceae	*Lachnum*	*Lachnum pygmaeum*	O	O	O	O
Ascomycota	Leotiomycetes	Helotiales	Hyaloscyphaceae	*Lachnum*	*Lachnum sp.*	O		O	
Ascomycota	Leotiomycetes	Helotiales	Hyaloscyphaceae	*Phialea*	*Phialea sp.*		O		
Ascomycota	Leotiomycetes	Helotiales	Hyaloscyphaceae	*Phialea*	*Phialea strobilina*	O			
Ascomycota	Leotiomycetes	Helotiales	Hyaloscyphaceae	*Polydesmia*	*Polydesmia pruinosa*	O	O		
Ascomycota	Leotiomycetes	Helotiales	Hyaloscyphaceae	Unidentified		O	O		
Ascomycota	Leotiomycetes	Helotiales	Leotiaceae	*Gorgomyces*	*Gorgomyces sp.*	O	O		
Ascomycota	Leotiomycetes	Helotiales	Leotiaceae	*Leotia*	*Leotia lubrica*		O	O	
Ascomycota	Leotiomycetes	Helotiales	Leotiaceae	*Neobulgaria*	*Neobulgaria sp.*	O		O	
Ascomycota	Leotiomycetes	Helotiales	Leotiaceae	*Pezoloma*	*Pezoloma ciliifera*		O		
Ascomycota	Leotiomycetes	Helotiales	Myxotrichaceae	*Oidiodendron*	*Oidiodendron cereale*		O	O	

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Appendix 3, Continued

Phylum	Class	Order	Family	Genus	Species	PacBio	MiSeq	NLSK (Genus)	NLSK (Species)
Ascomycota	Leotiomycetes	Helotiales	Myxotrichaceae	*Oidiodendron*	*Oidiodendron chlamydosporicum*	O	O	O	
Ascomycota	Leotiomycetes	Helotiales	Myxotrichaceae	*Oidiodendron*	*Oidiodendron echinulatum*	O	O	O	
Ascomycota	Leotiomycetes	Helotiales	Myxotrichaceae	*Oidiodendron*	*Oidiodendron flavum*	O	O	O	
Ascomycota	Leotiomycetes	Helotiales	Myxotrichaceae	*Oidiodendron*	*Oidiodendron maius*	O	O	O	
Ascomycota	Leotiomycetes	Helotiales	Myxotrichaceae	*Oidiodendron*	*Oidiodendron periconioides*	O	O	O	
Ascomycota	Leotiomycetes	Helotiales	Myxotrichaceae	*Oidiodendron*	*Oidiodendron pilicola*	O	O	O	
Ascomycota	Leotiomycetes	Helotiales	Myxotrichaceae	*Oidiodendron*	*Oidiodendron rhodogenum*	O	O	O	
Ascomycota	Leotiomycetes	Helotiales	Myxotrichaceae	*Oidiodendron*	*Oidiodendron sp.*	O	O	O	
Ascomycota	Leotiomycetes	Helotiales	Myxotrichaceae	Unidentified			O		
Ascomycota	Leotiomycetes	Helotiales	Rutstroemiaceae	*Lanzia*	*Lanzia sp.*		O		
Ascomycota	Leotiomycetes	Helotiales	Rutstroemiaceae	Unidentified		O			
Ascomycota	Leotiomycetes	Helotiales	Sclerotiniaceae	*Botrytis*	*Botrytis caroliniana*	O	O	O	
Ascomycota	Leotiomycetes	Helotiales	Sclerotiniaceae	*Martininia*	*Martininia sp.*	O			
Ascomycota	Leotiomycetes	Helotiales	Sclerotiniaceae	*Moellerodiscus*	*Moellerodiscus sp.*		O		
Ascomycota	Leotiomycetes	Helotiales	Sclerotiniaceae	Unidentified		O			
Ascomycota	Leotiomycetes	Helotiales	Unidentified	Unidentified		O	O		
Ascomycota	Leotiomycetes	Helotiales	Vibrisseaceae	*Phialocephala*	*Phialocephala humicola*	O	O	O	O

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Appendix 3, Continued

Phylum	Class	Order	Family	Genus	Species	PacBio	MiSeq	NLSK (Genus)	NLSK (Species)
Ascomycota	Leotiomycetes	Helotiales	Vibrisseaceae	*Phialocephala*	*Phialocephala sp.*	O	O	O	
Ascomycota	Leotiomycetes	Phacidiales	Phacidiaceae	*Phacidium*	*Phacidium lacerum*	O	O	O	O
Ascomycota	Leotiomycetes	Rhytismatales	Rhytismataceae	*Coccomyces*	*Coccomyces sp.*	O			
Ascomycota	Leotiomycetes	Rhytismatales	Rhytismataceae	Unidentified			O		
Ascomycota	Leotiomycetes	Thelebolales	Pseudeurotiaceae	*Leuconeurospora*	*Leuconeurospora sp.*	O	O		
Ascomycota	Leotiomycetes	Thelebolales	Pseudeurotiaceae	*Pseudogymnoascus*	*Pseudogymnoascus roseus*	O	O	O	
Ascomycota	Leotiomycetes	Thelebolales	Pseudeurotiaceae	*Pseudogymnoascus*	*Pseudogymnoascus sp.*	O		O	
Ascomycota	Leotiomycetes	Unidentified	Unidentified	Unidentified		O	O		
Ascomycota	Orbiliomycetes	Orbiliales	Orbiliaceae	*Drechslerella*	*Drechslerella sp.*	O	O	O	
Ascomycota	Orbiliomycetes	Orbiliales	Orbiliaceae	Unidentified		O	O		
Ascomycota	Orbiliomycetes	Orbiliales	Unidentified	Unidentified		O	O		
Ascomycota	Pezizomycetes	Pezizales	Ascodesmidaceae	Unidentified		O			
Ascomycota	Pezizomycetes	Pezizales	Pezizaceae	*Delastria*	*Delastria sp.*		O		
Ascomycota	Pezizomycetes	Pezizales	Pezizaceae	*Peziza*	*Peziza*		O	O	
Ascomycota	Pezizomycetes	Pezizales	Pezizaceae	*Peziza*	*Peziza sp.*	O		O	
Ascomycota	Pezizomycetes	Pezizales	Pyronemataceae	*Trichophaea*	*Trichophaea sp.*		O	O	
Ascomycota	Pezizomycetes	Pezizales	Pyronemataceae	Unidentified		O	O		

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Appendix 3, Continued

Phylum	Class	Order	Family	Genus	Species	PacBio	MiSeq	NLSK (Genus)	NLSK (Species)
Ascomycota	Pezizomycetes	Pezizales	Pyronemataceae	*Wilcoxina*	*Wilcoxina mikolae*	O			
Ascomycota	Pezizomycetes	Pezizales	Sarcoscyphaceae	*Desmazierella*	*Desmazierella acicola*	O			
Ascomycota	Pezizomycetes	Pezizales	Sarcosomataceae	*Plectania*	*Plectania melastoma*	O	O		
Ascomycota	Pezizomycetes	Pezizales	Sarcosomataceae	*Pseudoplectania*	*Pseudoplectania sp.*	O	O	O	
Ascomycota	Pezizomycetes	Pezizales	Tuberaceae	*Tuber*	*Tuber sp.*	O	O	O	
Ascomycota	Saccharomycetes	Saccharomycetales	Debaryomycetaceae	*Schwanniomyces*	*Schwanniomyces yamadae*	O	O	O	
Ascomycota	Saccharomycetes	Saccharomycetales	Debaryomycetaceae	Unidentified		O			
Ascomycota	Saccharomycetes	Saccharomycetales	Incertae sedis	*Candida*	*Candida palmioleophila*		O	O	
Ascomycota	Saccharomycetes	Saccharomycetales	Incertae sedis	*Candida*	*Candida tropicalis*		O	O	
Ascomycota	Saccharomycetes	Saccharomycetales	Incertae sedis	*Candida*	*Candida zeylanoides*		O	O	
Ascomycota	Saccharomycetes	Saccharomycetales	Incertae sedis	*Teunomyces*	*Teunomyces tritomae*		O		
Ascomycota	Saccharomycetes	Saccharomycetales	Trichomonascaceae	*Blastobotrys*	*Blastobotrys robertii*	O			
Ascomycota	Saccharomycetes	Saccharomycetales	Trichomonascaceae	*Spencermartinsiella*	*Spencermartinsiella sp.*		O		
Ascomycota	Saccharomycetes	Saccharomycetales	Trichomonascaceae	*Sugiyamaella*	*Sugiyamaella novakii*	O			
Ascomycota	Saccharomycetes	Saccharomycetales	Trichomonascaceae	Unidentified		O	O		
Ascomycota	Sordariomycetes	Chaetosphaeriales	Chaetosphaeriaceae	*Chaetosphaeria*	*Chaetosphaeria sp.*	O	O		
Ascomycota	Sordariomycetes	Chaetosphaeriales	Chaetosphaeriaceae	*Chloridium*	*Chloridium aseptatum*		O		

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Appendix 3, Continued

Phylum	Class	Order	Family	Genus	Species	PacBio	MiSeq	NLSK (Genus)	NLSK (Species)
Ascomycota	Sordariomycetes	Chaetosphaeriales	Chaetosphaeriaceae	*Chloridium*	*Chloridium sp.*	O			
Ascomycota	Sordariomycetes	Chaetosphaeriales	Chaetosphaeriaceae	*Codinaea*	*Codinaea sp.*	O			
Ascomycota	Sordariomycetes	Chaetosphaeriales	Chaetosphaeriaceae	*Porosphaerella*	*Porosphaerella cordanophora*	O	O		
Ascomycota	Sordariomycetes	Chaetosphaeriales	Chaetosphaeriaceae	Unidentified		O	O		
Ascomycota	Sordariomycetes	Chaetosphaeriales	Chaetosphaeriaceae	*Zignoella*	*Zignoella sp.*	O			
Ascomycota	Sordariomycetes	Coniochaetales	Coniochaetaceae	*Lecythophora*	*Lecythophora sp.*		O		
Ascomycota	Sordariomycetes	Coniochaetales	Coniochaetaceae	Unidentified		O	O		
Ascomycota	Sordariomycetes	Coniochaetales	Unidentified	Unidentified			O		
Ascomycota	Sordariomycetes	Diaporthales	Diaporthaceae	*Diaporthe*	*Diaporthe citrichinensis*		O	O	
Ascomycota	Sordariomycetes	Diaporthales	Incertae sedis	*Tubakia*	*Tubakia seoraksanensis*		O	O	
Ascomycota	Sordariomycetes	Diaporthales	Schizoparmaceae	*Coniella*	*Coniella tibouchinae*		O	O	
Ascomycota	Sordariomycetes	Diaporthales	Sydowiellaceae	Unidentified			O		
Ascomycota	Sordariomycetes	Diaporthales	Unidentified	Unidentified		O	O		
Ascomycota	Sordariomycetes	Hypocreales	Bionectriaceae	*Clonostachys*	*Clonostachys rosea*		O	O	
Ascomycota	Sordariomycetes	Hypocreales	Bionectriaceae	*Clonostachys*	*Clonostachys sp.*	O		O	
Ascomycota	Sordariomycetes	Hypocreales	Bionectriaceae	*Nectriopsis*	*Nectriopsis fuliginicola*		O		
Ascomycota	Sordariomycetes	Hypocreales	Clavicipitaceae	*Metapochonia*	*Metapochonia bulbillosa*	O	O		

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Appendix 3, Continued

Phylum	Class	Order	Family	Genus	Species	PacBio	MiSeq	NLSK (Genus)	NLSK (Species)
Ascomycota	Sordariomycetes	Hypocreales	Clavicipitaceae	*Metapochonia*	*Metapochonia suchlasporia*		O		
Ascomycota	Sordariomycetes	Hypocreales	Clavicipitaceae	*Metarhizium*	*Metarhizium carneum*	O	O	O	
Ascomycota	Sordariomycetes	Hypocreales	Clavicipitaceae	*Metarhizium*	*Metarhizium sp.*	O		O	
Ascomycota	Sordariomycetes	Hypocreales	Clavicipitaceae	*Paecilomyces*	*Paecilomyces sp.*		O	O	
Ascomycota	Sordariomycetes	Hypocreales	Clavicipitaceae	Unidentified		O	O		
Ascomycota	Sordariomycetes	Hypocreales	Cordycipitaceae	*Beauveria*	*Beauveria sp.*	O	O	O	
Ascomycota	Sordariomycetes	Hypocreales	Cordycipitaceae	*Cordyceps*	*Cordyceps brongniartii*		O	O	O
Ascomycota	Sordariomycetes	Hypocreales	Cordycipitaceae	*Lecanicillium*	*Lecanicillium fungicola*		O	O	O
Ascomycota	Sordariomycetes	Hypocreales	Cordycipitaceae	*Lecanicillium*	*Lecanicillium fusisporum*	O		O	
Ascomycota	Sordariomycetes	Hypocreales	Cordycipitaceae	*Leptobacillium*	*Leptobacillium leptobactrum*		O		
Ascomycota	Sordariomycetes	Hypocreales	Cordycipitaceae	Unidentified		O	O		
Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	*Hypomyces*	*Hypomyces microspermus*	O		O	
Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	*Hypomyces*	*Hypomyces ochraceus*	O	O	O	
Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	*Hypomyces*	*Hypomyces samuelsii*	O	O	O	
Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	*Hypomyces*	*Hypomyces sp.*	O	O	O	
Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	*Trichoderma*	*Trichoderma asperellum*	O	O	O	
Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	*Trichoderma*	*Trichoderma delicatulum*	O		O	

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Appendix 3, Continued

Phylum	Class	Order	Family	Genus	Species	PacBio	MiSeq	NLSK (Genus)	NLSK (Species)
Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	*Trichoderma*	*Trichoderma harzianum*	O	O	O	O
Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	*Trichoderma*	*Trichoderma lixii*	O		O	
Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	*Trichoderma*	*Trichoderma sp.*	O	O	O	
Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	*Trichoderma*	*Trichoderma spirale*	O	O	O	O
Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	*Trichoderma*	*Trichoderma tawa*		O	O	
Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	*Trichoderma*	*Trichoderma virens*		O	O	
Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	*Trichoderma*	*Trichoderma viride*	O	O	O	O
Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Unidentified		O	O		
Ascomycota	Sordariomycetes	Hypocreales	Incertae sedis	*Chlorocillium*	*Chlorocillium sp.*	O			
Ascomycota	Sordariomycetes	Hypocreales	Incertae sedis	*Myxocephala*	*Myxocephala albida*	O	O		
Ascomycota	Sordariomycetes	Hypocreales	Incertae sedis	*Sarocladium*	*Sarocladium strictum*		O		
Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	*Calcarisporium*	*Calcarisporium sp.*	O	O		
Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	*Cylindrocladiella*	*Cylindrocladiella variabilis*	O			
Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	*Cylindrocladium*	*Cylindrocladium buxicola*	O		O	
Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	*Dactylonectria*	*Dactylonectria anthuriicola*	O		O	
Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	*Dactylonectria*	*Dactylonectria macrodidyma*	O	O	O	
Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	*Dialonectria*	*Dialonectria sp.*	O		O	

NLSK=National List of species of Korea (국가생물종목록)

Appendix 3, Continued

Phylum	Class	Order	Family	Genus	Species	PacBio	MiSeq	NLSK (Genus)	NLSK (Species)
Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	*Fusarium*	*Fusarium oxysporum*		O	O	O
Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	*Fusarium*	*Fusarium solani*		O	O	O
Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	*Fusarium*	*Fusarium sp.*	O		O	
Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	*Fusarium*	*Fusarium tricinctum*	O		O	O
Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	*Fusicolla*	*Fusicolla sp.*		O		
Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	*Gibberella*	*Gibberella tricincta*		O	O	
Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	*Ilyonectria*	*Ilyonectria destructans*	O	O		
Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	*Mariannaea*	*Mariannaea samuelsii*	O	O	O	O
Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	*Microcera*	*Microcera rubra*	O			
Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	*Nectria*	*Nectria ramulariae*	O	O	O	
Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	*Penicillifer*	*Penicillifer diparietisporus*		O		
Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	*Thelonectria*	*Thelonectria sp.*		O	O	
Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	Unidentified		O	O		
Ascomycota	Sordariomycetes	Hypocreales	Ophiocordycipitaceae	*Purpureocillium*	*Purpureocillium lilacinum*		O	O	O
Ascomycota	Sordariomycetes	Hypocreales	Ophiocordycipitaceae	*Tolypocladium*	*Tolypocladium album*		O		
Ascomycota	Sordariomycetes	Hypocreales	Ophiocordycipitaceae	*Tolypocladium*	*Tolypocladium inflatum*	O	O		
Ascomycota	Sordariomycetes	Hypocreales	Ophiocordycipitaceae	*Tolypocladium*	*Tolypocladium sp.*	O	O		

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Appendix 3, Continued

Phylum	Class	Order	Family	Genus	Species	PacBio	MiSeq	NLSK (Genus)	NLSK (Species)
Ascomycota	Sordariomycetes	Hypocreales	Ophiocordycipitaceae	*Tolypocladium*	*Tolypocladium tropicale*	O			
Ascomycota	Sordariomycetes	Hypocreales	Stachybotryaceae	*Myrothecium*	*Myrothecium roridum*		O	O	
Ascomycota	Sordariomycetes	Hypocreales	Stachybotryaceae	*Striatibotrys*	*Striatibotrys eucylindrospora*	O			
Ascomycota	Sordariomycetes	Hypocreales	Unidentified	Unidentified		O	O		
Ascomycota	Sordariomycetes	Myrmecridiales	Myrmecridiaceae	Unidentified		O			
Ascomycota	Sordariomycetes	Ophiostomatales	Ophiostomataceae	*Hawksworthiomyces*	*Hawksworthiomyces sp.*	O			
Ascomycota	Sordariomycetes	Ophiostomatales	Ophiostomataceae	*Sporothrix*	*Sporothrix guttuliformis*	O		O	
Ascomycota	Sordariomycetes	Ophiostomatales	Ophiostomataceae	*Sporothrix*	*Sporothrix sp.*	O		O	
Ascomycota	Sordariomycetes	Sordariales	Cephalothecaceae	*Cephalotheca*	*Cephalotheca sp.*	O	O		
Ascomycota	Sordariomycetes	Sordariales	Cephalothecaceae	*Phialemonium*	*Phialemonium sp.*	O	O	O	
Ascomycota	Sordariomycetes	Sordariales	Chaetomiaceae	*Chaetomium*	*Chaetomium grande*		O	O	
Ascomycota	Sordariomycetes	Sordariales	Chaetomiaceae	*Chaetomium*	*Chaetomium homopilatum*		O	O	
Ascomycota	Sordariomycetes	Sordariales	Chaetomiaceae	*Chaetomium*	*Chaetomium olivicolor*		O	O	
Ascomycota	Sordariomycetes	Sordariales	Chaetomiaceae	*Chaetomium*	*Chaetomium sp.*	O		O	
Ascomycota	Sordariomycetes	Sordariales	Chaetomiaceae	*Humicola*	*Humicola nigrescens*	O	O	O	
Ascomycota	Sordariomycetes	Sordariales	Chaetomiaceae	*Thielavia*	*Thielavia inaequalis*		O	O	
Ascomycota	Sordariomycetes	Sordariales	Chaetomiaceae	*Trichocladium*	*Trichocladium opacum*	O	O	O	

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Appendix 3, Continued

Phylum	Class	Order	Family	Genus	Species	PacBio	MiSeq	NLSK (Genus)	NLSK (Species)
Ascomycota	Sordariomycetes	Sordariales	Chaetomiaceae	Unidentified		O			
Ascomycota	Sordariomycetes	Sordariales	Chaetomiaceae	*Zopfiella*	*Zopfiella pleuropora*		O	O	
Ascomycota	Sordariomycetes	Sordariales	Lasiosphaeriaceae	*Fimetariella*	*Fimetariella sp.*	O	O		
Ascomycota	Sordariomycetes	Sordariales	Lasiosphaeriaceae	*Podospora*	*Podospora longicollis*	O			
Ascomycota	Sordariomycetes	Sordariales	Lasiosphaeriaceae	*Podospora*	*Podospora sp.*		O		
Ascomycota	Sordariomycetes	Sordariales	Lasiosphaeriaceae	Unidentified		O			
Ascomycota	Sordariomycetes	Sordariales	Sordariaceae	*Neurospora*	*Neurospora terricola*	O	O	O	
Ascomycota	Sordariomycetes	Sordariales	Incertae sedis	*Rhexodenticula*	*Rhexodenticula sp.*	O			
Ascomycota	Sordariomycetes	Sordariales	Unidentified	Unidentified		O	O		
Ascomycota	Sordariomycetes	Incertae sedis	Incertae sedis	*Xylolentia*	*Xylolentia sp.*		O		
Ascomycota	Sordariomycetes	Togniniales	Togniniaceae	Unidentified			O		
Ascomycota	Sordariomycetes	Unidentified	Unidentified	Unidentified		O	O		
Ascomycota	Sordariomycetes	Xylariales	Apiosporaceae	*Arthrinium*	*Arthrinium phaeospermum*		O	O	
Ascomycota	Sordariomycetes	Xylariales	Apiosporaceae	*Arthrinium*	*Arthrinium sp.*		O	O	
Ascomycota	Sordariomycetes	Xylariales	Diatrypaceae	*Eutypa*	*Eutypa sp.*		O	O	
Ascomycota	Sordariomycetes	Xylariales	Sporocadaceae	*Adisciso*	*Adisciso kaki*		O		
Ascomycota	Sordariomycetes	Xylariales	Sporocadaceae	*Neopestalotiopsis*	*Neopestalotiopsis foedans*		O	O	

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Appendix 3, Continued

Phylum	Class	Order	Family	Genus	Species	PacBio	MiSeq	NLSK (Genus)	NLSK (Species)
Ascomycota	Sordariomycetes	Xylariales	Sporocadaceae	*Pestalotiopsis*	*Pestalotiopsis microspora*	O		O	
Ascomycota	Sordariomycetes	Xylariales	Sporocadaceae	*Pestalotiopsis*	*Pestalotiopsis rhododendri*	O		O	
Ascomycota	Sordariomycetes	Xylariales	Sporocadaceae	*Pestalotiopsis*	*Pestalotiopsis vismiae*		O	O	
Ascomycota	Sordariomycetes	Xylariales	Sporocadaceae	Unidentified		O			
Ascomycota	Sordariomycetes	Xylariales	Unidentified	Unidentified		O	O		
Ascomycota	Sordariomycetes	Xylariales	Xylariaceae	*Biscogniauxia*	*Biscogniauxia atropunctata*	O	O	O	
Ascomycota	Sordariomycetes	Xylariales	Xylariaceae	*Xylaria*	*Xylaria multiplex*		O	O	
Ascomycota	Sordariomycetes	Xylariales	Xylariaceae	*Xylaria*	*Xylaria sp.*	O		O	
Ascomycota	Sordariomycetes	Xylariales	Incertae sedis	*Fusidium*	*Fusidium sp.*		O		
Ascomycota	Taphrinomycetes	Taphrinales	Taphrinaceae	*Taphrina*	*Taphrina sp.*	O		O	
Ascomycota	Taphrinomycetes	Taphrinales	Taphrinaceae	*Taphrina*	*Taphrina tormentillae*	O		O	
Ascomycota	Unidentified	Unidentified	Unidentified	Unidentified		O	O		
Basidiomycota	Agaricomycetes	Agaricales	Agaricaceae	*Agaricus*	*Agaricus parasubrutilescens*	O	O	O	
Basidiomycota	Agaricomycetes	Agaricales	Agaricaceae	*Agaricus*	*Agaricus sinoplacomyces*		O	O	
Basidiomycota	Agaricomycetes	Agaricales	Agaricaceae	*Agaricus*	*Agaricus sp.*		O	O	
Basidiomycota	Agaricomycetes	Agaricales	Agaricaceae	*Coprinus*	*Coprinus sp.*		O	O	
Basidiomycota	Agaricomycetes	Agaricales	Agaricaceae	*Lycoperdon*	*Lycoperdon spadiceum*	O		O	

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Appendix 3, Continued

Phylum	Class	Order	Family	Genus	Species	PacBio	MiSeq	NLSK (Genus)	NLSK (Species)
Basidiomycota	Agaricomycetes	Agaricales	Agaricaceae	*Lycoperdon*	*Lycoperdon ericaeum*	O		O	O
Basidiomycota	Agaricomycetes	Agaricales	Agaricaceae	*Lycoperdon*	*Lycoperdon excipuliforme*	O	O	O	
Basidiomycota	Agaricomycetes	Agaricales	Agaricaceae	*Lycoperdon*	*Lycoperdon mammiforme*		O	O	O
Basidiomycota	Agaricomycetes	Agaricales	Agaricaceae	*Lycoperdon*	*Lycoperdon perlatum*	O		O	O
Basidiomycota	Agaricomycetes	Agaricales	Agaricaceae	*Lycoperdon*	*Lycoperdon sosinii*		O	O	
Basidiomycota	Agaricomycetes	Agaricales	Agaricaceae	*Lycoperdon*	*Lycoperdon subperlatum*	O	O	O	O
Basidiomycota	Agaricomycetes	Agaricales	Agaricaceae	Unidentified			O		
Basidiomycota	Agaricomycetes	Agaricales	Amanitaceae	*Amanita*	*Amanita aff orientifulva*	O	O	O	
Basidiomycota	Agaricomycetes	Agaricales	Amanitaceae	*Amanita*	*Amanita aff rimosa*	O	O	O	
Basidiomycota	Agaricomycetes	Agaricales	Amanitaceae	*Amanita*	*Amanita citrina*	O	O	O	O
Basidiomycota	Agaricomycetes	Agaricales	Amanitaceae	*Amanita*	*Amanita excelsa*		O	O	O
Basidiomycota	Agaricomycetes	Agaricales	Amanitaceae	*Amanita*	*Amanita flavipes*	O	O	O	O
Basidiomycota	Agaricomycetes	Agaricales	Amanitaceae	*Amanita*	*Amanita fritillaria*	O	O	O	O
Basidiomycota	Agaricomycetes	Agaricales	Amanitaceae	*Amanita*	*Amanita pallidorosea*	O	O	O	O
Basidiomycota	Agaricomycetes	Agaricales	Amanitaceae	*Amanita*	*Amanita pallidorosea*		O	O	
Basidiomycota	Agaricomycetes	Agaricales	Amanitaceae	*Amanita*	*Amanita pantherina*	O	O	O	O
Basidiomycota	Agaricomycetes	Agaricales	Amanitaceae	*Amanita*	*Amanita rubescens*	O	O	O	O

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Appendix 3, Continued

Phylum	Class	Order	Family	Genus	Species	PacBio	MiSeq	NLSK (Genus)	NLSK (Species)
Basidiomycota	Agaricomycetes	Agaricales	Amanitaceae	*Amanita*	*Amanita sp.*		O	O	
Basidiomycota	Agaricomycetes	Agaricales	Amanitaceae	*Amanita*	*Amanita spissacea*	O	O	O	O
Basidiomycota	Agaricomycetes	Agaricales	Bolbitiaceae	*Agrocybe*	*Agrocybe erebia*	O	O	O	
Basidiomycota	Agaricomycetes	Agaricales	Bolbitiaceae	Unidentified		O	O		
Basidiomycota	Agaricomycetes	Agaricales	Clavariaceae	*Clavulinopsis*	*Clavulinopsis sp.*	O		O	
Basidiomycota	Agaricomycetes	Agaricales	Clavariaceae	Unidentified		O			
Basidiomycota	Agaricomycetes	Agaricales	Cortinariaceae	*Cortinarius*	*Cortinarius alboviolaceus*		O	O	O
Basidiomycota	Agaricomycetes	Agaricales	Cortinariaceae	*Cortinarius*	*Cortinarius heterodepressus*	O	O	O	
Basidiomycota	Agaricomycetes	Agaricales	Cortinariaceae	*Cortinarius*	*Cortinarius hinnuleus*	O	O	O	O
Basidiomycota	Agaricomycetes	Agaricales	Cortinariaceae	*Cortinarius*	*Cortinarius pinophilus*		O	O	
Basidiomycota	Agaricomycetes	Agaricales	Cortinariaceae	*Cortinarius*	*Cortinarius purpurascens*		O	O	O
Basidiomycota	Agaricomycetes	Agaricales	Cortinariaceae	*Cortinarius*	*Cortinarius rubellus*		O	O	
Basidiomycota	Agaricomycetes	Agaricales	Cortinariaceae	*Cortinarius*	*Cortinarius sp.*	O	O	O	
Basidiomycota	Agaricomycetes	Agaricales	Cortinariaceae	*Cortinarius*	*Cortinarius spilomeus*		O	O	
Basidiomycota	Agaricomycetes	Agaricales	Cortinariaceae	*Gymnopilus*	*Gymnopilus decipiens*		O	O	
Basidiomycota	Agaricomycetes	Agaricales	Cortinariaceae	Unidentified			O		
Basidiomycota	Agaricomycetes	Agaricales	Cyphellaceae	*Rectipilus*	*Rectipilus sp.*	O	O	O	

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Appendix 3, Continued

Phylum	Class	Order	Family	Genus	Species	PacBio	MiSeq	NLSK (Genus)	NLSK (Species)
Basidiomycota	Agaricomycetes	Agaricales	Cyphellaceae	Unidentified		O			
Basidiomycota	Agaricomycetes	Agaricales	Entolomataceae	*Entocybe*	*Entocybe sp.*	O			
Basidiomycota	Agaricomycetes	Agaricales	Entolomataceae	*Entoloma*	*Entoloma aff juncinum*	O		O	
Basidiomycota	Agaricomycetes	Agaricales	Entolomataceae	*Entoloma*	*Entoloma luteofuscum*	O		O	
Basidiomycota	Agaricomycetes	Agaricales	Entolomataceae	*Entoloma*	*Entoloma rhodocylix*	O	O	O	
Basidiomycota	Agaricomycetes	Agaricales	Entolomataceae	*Entoloma*	*Entoloma rhodopolium*	O		O	O
Basidiomycota	Agaricomycetes	Agaricales	Entolomataceae	*Entoloma*	*Entoloma rhodopolium*	O		O	
Basidiomycota	Agaricomycetes	Agaricales	Entolomataceae	*Entoloma*	*Entoloma sp.*	O	O	O	
Basidiomycota	Agaricomycetes	Agaricales	Entolomataceae	*Entoloma*	*Entoloma zuccherellii*	O		O	
Basidiomycota	Agaricomycetes	Agaricales	Entolomataceae	Unidentified		O			
Basidiomycota	Agaricomycetes	Agaricales	Hydnangiaceae	*Laccaria*	*Laccaria alba*	O	O	O	O
Basidiomycota	Agaricomycetes	Agaricales	Hydnangiaceae	*Laccaria*	*Laccaria araneosa*	O	O	O	O
Basidiomycota	Agaricomycetes	Agaricales	Hydnangiaceae	*Laccaria*	*Laccaria japonica*	O	O	O	O
Basidiomycota	Agaricomycetes	Agaricales	Hydnangiaceae	*Laccaria*	*Laccaria proxima*		O	O	
Basidiomycota	Agaricomycetes	Agaricales	Hydnangiaceae	*Laccaria*	*Laccaria sp.*	O		O	
Basidiomycota	Agaricomycetes	Agaricales	Hydnangiaceae	*Laccaria*	*Laccaria vinaceoavellanea*	O	O	O	O
Basidiomycota	Agaricomycetes	Agaricales	Hygrophoraceae	*Hygrophorus*	*Hygrophorus sp.*		O	O	

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Appendix 3, Continued

Phylum	Class	Order	Family	Genus	Species	PacBio	MiSeq	NLSK (Genus)	NLSK (Species)
Basidiomycota	Agaricomycetes	Agaricales	Hymenogastraceae	*Hymenogaster*	*Hymenogaster sp.*		O		
Basidiomycota	Agaricomycetes	Agaricales	Hymenogastraceae	Unidentified			O		
Basidiomycota	Agaricomycetes	Agaricales	Inocybaceae	*Inocybe*	*Inocybe cincinnata*	O		O	O
Basidiomycota	Agaricomycetes	Agaricales	Inocybaceae	*Inocybe*	*Inocybe maculata*	O		O	O
Basidiomycota	Agaricomycetes	Agaricales	Inocybaceae	*Inocybe*	*Inocybe sp.*	O	O	O	
Basidiomycota	Agaricomycetes	Agaricales	Inocybaceae	*Inocybe*	*Inocybe tigrina*		O	O	
Basidiomycota	Agaricomycetes	Agaricales	Inocybaceae	*Simocybe*	*Simocybe serrulata*		O	O	
Basidiomycota	Agaricomycetes	Agaricales	Inocybaceae	Unidentified		O	O		
Basidiomycota	Agaricomycetes	Agaricales	Lycoperdaceae	*Lycoperdon*	*Lycoperdon pyriforme*		O	O	
Basidiomycota	Agaricomycetes	Agaricales	Lycoperdaceae	*Lycoperdon*	*Lycoperdon subincarnatum*		O	O	
Basidiomycota	Agaricomycetes	Agaricales	Lycoperdaceae	*Lycoperdon*	*Lycoperdon utriforme*	O		O	
Basidiomycota	Agaricomycetes	Agaricales	Lyophyllaceae	*Gerhardtia*	*Gerhardtia sp.*		O		
Basidiomycota	Agaricomycetes	Agaricales	Mycenaceae	*Mycena*	*Mycena abramsii*		O	O	
Basidiomycota	Agaricomycetes	Agaricales	Mycenaceae	*Mycena*	*Mycena amicta*	O	O	O	
Basidiomycota	Agaricomycetes	Agaricales	Mycenaceae	*Mycena*	*Mycena cf maculata*	O		O	
Basidiomycota	Agaricomycetes	Agaricales	Mycenaceae	*Mycena*	*Mycena corynephora*		O	O	
Basidiomycota	Agaricomycetes	Agaricales	Mycenaceae	*Mycena*	*Mycena flavoalba*	O	O	O	

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Appendix 3, Continued

Phylum	Class	Order	Family	Genus	Species	PacBio	MiSeq	NLSK (Genus)	NLSK (Species)
Basidiomycota	Agaricomycetes	Agaricales	Mycenaceae	*Mycena*	*Mycena leptocephala*		O	O	
Basidiomycota	Agaricomycetes	Agaricales	Mycenaceae	*Mycena*	*Mycena metata*	O	O	O	
Basidiomycota	Agaricomycetes	Agaricales	Mycenaceae	*Mycena*	*Mycena monticola*	O		O	
Basidiomycota	Agaricomycetes	Agaricales	Mycenaceae	*Mycena*	*Mycena olivaceomarginata*		O	O	
Basidiomycota	Agaricomycetes	Agaricales	Mycenaceae	*Mycena*	*Mycena pearsoniana*		O	O	O
Basidiomycota	Agaricomycetes	Agaricales	Mycenaceae	*Mycena*	*Mycena plumipes*		O	O	
Basidiomycota	Agaricomycetes	Agaricales	Mycenaceae	*Mycena*	*Mycena pura*	O	O	O	O
Basidiomycota	Agaricomycetes	Agaricales	Mycenaceae	*Mycena*	*Mycena rosea*		O	O	
Basidiomycota	Agaricomycetes	Agaricales	Mycenaceae	*Mycena*	*Mycena silvae-nigrae*		O	O	
Basidiomycota	Agaricomycetes	Agaricales	Mycenaceae	*Mycena*	*Mycena sinar var. tangkaisinar*	O	O	O	
Basidiomycota	Agaricomycetes	Agaricales	Mycenaceae	*Mycena*	*Mycena zephirus*	O	O	O	O
Basidiomycota	Agaricomycetes	Agaricales	Mycenaceae	*Mycena*	*Mycena sp.*	O	O	O	
Basidiomycota	Agaricomycetes	Agaricales	Mycenaceae	Unidentified		O	O		
Basidiomycota	Agaricomycetes	Agaricales	Omphalotaceae	*Gymnopus*	*Gymnopus aff dichrous*	O	O	O	
Basidiomycota	Agaricomycetes	Agaricales	Omphalotaceae	*Gymnopus*	*Gymnopus aff omphalodes*	O	O	O	
Basidiomycota	Agaricomycetes	Agaricales	Omphalotaceae	*Gymnopus*	*Gymnopus sp.*	O	O	O	
Basidiomycota	Agaricomycetes	Agaricales	Omphalotaceae	*Marasmiellus*	*Marasmiellus candidus*		O	O	

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Appendix 3, Continued

Phylum	Class	Order	Family	Genus	Species	PacBio	MiSeq	NLSK (Genus)	NLSK (Species)
Basidiomycota	Agaricomycetes	Agaricales	Omphalotaceae	*Marasmiellus*	*Marasmiellus ramealis*	O	O	O	
Basidiomycota	Agaricomycetes	Agaricales	Omphalotaceae	*Mycetinis*	*Mycetinis scorodonius*	O	O	O	
Basidiomycota	Agaricomycetes	Agaricales	Omphalotaceae	*Rhodocollybia*	*Rhodocollybia butyracea*	O	O	O	
Basidiomycota	Agaricomycetes	Agaricales	Omphalotaceae	Unidentified		O			
Basidiomycota	Agaricomycetes	Agaricales	Physalacriaceae	*Armillaria*	*Armillaria ostoyae*	O		O	O
Basidiomycota	Agaricomycetes	Agaricales	Psathyrellaceae	*Coprinellus*	*Coprinellus disseminatus*		O	O	O
Basidiomycota	Agaricomycetes	Agaricales	Psathyrellaceae	*Coprinellus*	*Coprinellus domesticus*		O	O	O
Basidiomycota	Agaricomycetes	Agaricales	Psathyrellaceae	*Psathyrella*	*Psathyrella candolleana*		O	O	O
Basidiomycota	Agaricomycetes	Agaricales	Psathyrellaceae	*Psathyrella*	*Psathyrella phegophila*		O	O	O
Basidiomycota	Agaricomycetes	Agaricales	Pterulaceae	*Radulomyces*	*Radulomyces sp.*	O	O	O	
Basidiomycota	Agaricomycetes	Agaricales	Schizophyllaceae	*Schizophyllum*	*Schizophyllum commune*		O	O	O
Basidiomycota	Agaricomycetes	Agaricales	Stephanosporaceae	*Cristinia*	*Cristinia sp.*		O	O	
Basidiomycota	Agaricomycetes	Agaricales	Strophariaceae	*Hypholoma*	*Hypholoma fasciculare*		O	O	O
Basidiomycota	Agaricomycetes	Agaricales	Strophariaceae	*Hypholoma*	*Hypholoma lateritium*		O	O	O
Basidiomycota	Agaricomycetes	Agaricales	Strophariaceae	*Pholiota*	*Pholiota alnicola*		O	O	O
Basidiomycota	Agaricomycetes	Agaricales	Strophariaceae	*Pholiota*	*Pholiota multicingulata*	O	O	O	O
Basidiomycota	Agaricomycetes	Agaricales	Strophariaceae	*Stropharia*	*Agaricus guizhouensis*	O		O	

NLSK=National List of species of Korea (국가생물종목록)

Appendix 3, Continued

Phylum	Class	Order	Family	Genus	Species	PacBio	MiSeq	NLSK (Genus)	NLSK (Species)
Basidiomycota	Agaricomycetes	Agaricales	Strophariaceae	*Stropharia*	*Stropharia sp.*	O		O	
Basidiomycota	Agaricomycetes	Agaricales	Strophariaceae	Unidentified			O		
Basidiomycota	Agaricomycetes	Agaricales	Tricholomataceae	*Clitocybula*	*Clitocybula sp.*		O	O	
Basidiomycota	Agaricomycetes	Agaricales	Tricholomataceae	*Delicatula*	*Delicatula integrella*	O	O	O	O
Basidiomycota	Agaricomycetes	Agaricales	Tricholomataceae	*Hydropus*	*Hydropus sp.*	O	O	O	
Basidiomycota	Agaricomycetes	Agaricales	Tricholomataceae	*Resinomycena*	*Resinomycena rhododendri*	O		O	
Basidiomycota	Agaricomycetes	Agaricales	Tricholomataceae	*Roridomyces*	*Roridomyces roridus*		O	O	
Basidiomycota	Agaricomycetes	Agaricales	Tricholomataceae	*Tricholoma*	*Tricholoma imbricatum*	O	O	O	O
Basidiomycota	Agaricomycetes	Agaricales	Tricholomataceae	*Tricholoma*	*Tricholoma joachimii*	O		O	
Basidiomycota	Agaricomycetes	Agaricales	Tricholomataceae	*Tricholoma*	*Tricholoma matsutake*	O	O	O	O
Basidiomycota	Agaricomycetes	Agaricales	Tricholomataceae	*Tricholoma*	*Tricholoma saponaceum*	O	O	O	O
Basidiomycota	Agaricomycetes	Agaricales	Tricholomataceae	*Tricholoma*	*Tricholoma sp.*	O	O	O	
Basidiomycota	Agaricomycetes	Agaricales	Tricholomataceae	Unidentified		O	O		
Basidiomycota	Agaricomycetes	Agaricales	Tricholomataceae	*Xeromphalina*	*Xeromphalina cauticinalis*	O	O	O	
Basidiomycota	Agaricomycetes	Agaricales	Tricholomataceae	*Xeromphalina*	*Xeromphalina setulipes*	O		O	
Basidiomycota	Agaricomycetes	Agaricales	Unidentified	Unidentified		O	O		
Basidiomycota	Agaricomycetes	Incertae sedis	Incertae sedis	*Xenasmatella*	*Xenasmatella sp.*	O	O		

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Appendix 3, Continued

Phylum	Class	Order	Family	Genus	Species	PacBio	MiSeq	NLSK (Genus)	NLSK (Species)
Basidiomycota	Agaricomycetes	Atheliales	Atheliaceae	*Amphinema*	*Amphinema sp.*	O	O	O	
Basidiomycota	Agaricomycetes	Atheliales	Atheliaceae	*Athelia*	*Athelia epiphylla*		O	O	O
Basidiomycota	Agaricomycetes	Atheliales	Atheliaceae	*Athelia*	*Athelia sp.*	O	O	O	
Basidiomycota	Agaricomycetes	Atheliales	Atheliaceae	*Athelopsis*	*Athelopsis lembospora*	O	O		
Basidiomycota	Agaricomycetes	Atheliales	Atheliaceae	*Athelopsis*	*Athelopsis sp.*	O	O		
Basidiomycota	Agaricomycetes	Atheliales	Atheliaceae	*Piloderma*	*Piloderma bicolor*	O	O	O	
Basidiomycota	Agaricomycetes	Atheliales	Atheliaceae	*Piloderma*	*Piloderma byssinum*	O	O	O	O
Basidiomycota	Agaricomycetes	Atheliales	Atheliaceae	*Piloderma*	*Piloderma sp.*	O	O	O	
Basidiomycota	Agaricomycetes	Atheliales	Atheliaceae	*Tylospora*	*Tylospora sp.*	O		O	
Basidiomycota	Agaricomycetes	Atheliales	Atheliaceae	Unidentified			O		
Basidiomycota	Agaricomycetes	Auriculariales	Auriculariaceae	*Auricularia*	*Auricularia sp.*	O	O	O	
Basidiomycota	Agaricomycetes	Auriculariales	Auriculariaceae	Unidentified		O	O		
Basidiomycota	Agaricomycetes	Auriculariales	Exidiaceae	*Basidiodendron*	*Basidiodendron caesiocinereum*		O		
Basidiomycota	Agaricomycetes	Auriculariales	Exidiaceae	*Basidiodendron*	*Basidiodendron sp.*	O	O		
Basidiomycota	Agaricomycetes	Auriculariales	Exidiaceae	Unidentified		O	O		
Basidiomycota	Agaricomycetes	Auriculariales	Hyaloriaceae	Unidentified		O	O		
Basidiomycota	Agaricomycetes	Auriculariales	Unidentified	Unidentified		O	O		

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Appendix 3, Continued

Phylum	Class	Order	Family	Genus	Species	PacBio	MiSeq	NLSK (Genus)	NLSK (Species)
Basidiomycota	Agaricomycetes	Boletales	Astraeaceae	*Astraeus*	*Astraeus smithii*		O	O	
Basidiomycota	Agaricomycetes	Boletales	Astraeaceae	*Astraeus*	*Astraeus sp.*	O	O	O	
Basidiomycota	Agaricomycetes	Boletales	Astraeaceae	*Astraeus*	*Astraeus telleriae*	O		O	
Basidiomycota	Agaricomycetes	Boletales	Boletaceae	*Boletellus*	*Boletellus chrysenteroides*	O		O	O
Basidiomycota	Agaricomycetes	Boletales	Boletaceae	*Boletellus*	*Boletellus sp.*	O	O	O	
Basidiomycota	Agaricomycetes	Boletales	Boletaceae	*Boletus*	*Boletus sp.*	O	O	O	
Basidiomycota	Agaricomycetes	Boletales	Boletaceae	*Leccinum*	*Leccinum rugosiceps*	O	O	O	O
Basidiomycota	Agaricomycetes	Boletales	Boletaceae	*Leccinum*	*Leccinum sp.*	O	O	O	
Basidiomycota	Agaricomycetes	Boletales	Boletaceae	*Retiboletus*	*Retiboletus sp.*		O	O	
Basidiomycota	Agaricomycetes	Boletales	Boletaceae	*Strobilomyces*	*Strobilomyces sp.*		O	O	
Basidiomycota	Agaricomycetes	Boletales	Boletaceae	*Tylopilus*	*Tylopilus felleus*	O		O	O
Basidiomycota	Agaricomycetes	Boletales	Boletaceae	*Tylopilus*	*Tylopilus neofelleus*	O	O	O	O
Basidiomycota	Agaricomycetes	Boletales	Boletaceae	*Tylopilus*	*Tylopilus virens*		O	O	O
Basidiomycota	Agaricomycetes	Boletales	Boletaceae	Unidentified		O	O		
Basidiomycota	Agaricomycetes	Boletales	Boletaceae	*Xanthoconium*	*Xanthoconium affine*	O	O	O	O
Basidiomycota	Agaricomycetes	Boletales	Boletaceae	*Xerocomellus*	*Xerocomellus sp.*	O		O	
Basidiomycota	Agaricomycetes	Boletales	Boletaceae	*Xerocomus*	*Xerocomus sp.*	O		O	

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Appendix 3, Continued

Phylum	Class	Order	Family	Genus	Species	PacBio	MiSeq	NLSK (Genus)	NLSK (Species)
Basidiomycota	Agaricomycetes	Boletales	Gomphidiaceae	*Chroogomphus*	*Chroogomphus confusus*	O	O	O	
Basidiomycota	Agaricomycetes	Boletales	Gomphidiaceae	*Chroogomphus*	*Chroogomphus rutilus*	O	O	O	O
Basidiomycota	Agaricomycetes	Boletales	Gomphidiaceae	*Gomphidius*	*Gomphidius roseus*	O	O	O	O
Basidiomycota	Agaricomycetes	Boletales	Rhizopogonaceae	*Rhizopogon*	*Rhizopogon luteolus*	O	O	O	
Basidiomycota	Agaricomycetes	Boletales	Rhizopogonaceae	*Rhizopogon*	*Rhizopogon sp.*	O	O	O	
Basidiomycota	Agaricomycetes	Boletales	Sclerodermataceae	*Scleroderma*	*Scleroderma sp.*	O	O	O	
Basidiomycota	Agaricomycetes	Boletales	Suillaceae	*Suillus*	*Suillus americanus*		O	O	O
Basidiomycota	Agaricomycetes	Boletales	Suillaceae	*Suillus*	*Suillus bovinus*	O	O	O	O
Basidiomycota	Agaricomycetes	Boletales	Suillaceae	*Suillus*	*Suillus granulatus*	O	O	O	O
Basidiomycota	Agaricomycetes	Boletales	Suillaceae	*Suillus*	*Suillus luteus*		O	O	O
Basidiomycota	Agaricomycetes	Boletales	Suillaceae	*Suillus*	*Suillus pictus*	O	O	O	O
Basidiomycota	Agaricomycetes	Boletales	Suillaceae	*Suillus*	*Suillus sp.*		O	O	
Basidiomycota	Agaricomycetes	Boletales	Unidentified	Unidentified			O		
Basidiomycota	Agaricomycetes	Cantharellales	Botryobasidiaceae	*Botryobasidium*	*Botryobasidium medium*	O		O	O
Basidiomycota	Agaricomycetes	Cantharellales	Botryobasidiaceae	*Botryobasidium*	*Botryobasidium sp.*	O		O	
Basidiomycota	Agaricomycetes	Cantharellales	Cantharellaceae	*Craterellus*	*Craterellus aureus*		O	O	O
Basidiomycota	Agaricomycetes	Cantharellales	Cantharellaceae	*Craterellus*	*Craterellus fallax*		O	O	

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Appendix 3, Continued

Phylum	Class	Order	Family	Genus	Species	PacBio	MiSeq	NLSK (Genus)	NLSK (Species)
Basidiomycota	Agaricomycetes	Cantharellales	Cantharellaceae	*Craterellus*	*Craterellus lutescens*	O	O	O	
Basidiomycota	Agaricomycetes	Cantharellales	Incertae sedis	*Sistotrema*	*Sistotrema sp.*	O	O	O	
Basidiomycota	Agaricomycetes	Cantharellales	Ceratobasidiaceae	*Ceratobasidium*	*Ceratobasidium sp.*	O	O		
Basidiomycota	Agaricomycetes	Cantharellales	Ceratobasidiaceae	*Rhizoctonia*	*Rhizoctonia sp.*	O		O	
Basidiomycota	Agaricomycetes	Cantharellales	Ceratobasidiaceae	Unidentified		O			
Basidiomycota	Agaricomycetes	Cantharellales	Clavulinaceae	*Clavulina*	*Clavulina amethystina*	O	O	O	O
Basidiomycota	Agaricomycetes	Cantharellales	Clavulinaceae	*Clavulina*	*Clavulina coralloides*		O	O	O
Basidiomycota	Agaricomycetes	Cantharellales	Clavulinaceae	*Clavulina*	*Clavulina rugosa*	O	O	O	O
Basidiomycota	Agaricomycetes	Cantharellales	Clavulinaceae	*Clavulina*	*Clavulina sp.*	O	O	O	
Basidiomycota	Agaricomycetes	Cantharellales	Clavulinaceae	*Membranomyces*	*Membranomyces sp.*	O	O		
Basidiomycota	Agaricomycetes	Cantharellales	Clavulinaceae	Unidentified		O	O		
Basidiomycota	Agaricomycetes	Cantharellales	Hydnaceae	*Hydnum*	*Hydnum minum*	O	O	O	
Basidiomycota	Agaricomycetes	Cantharellales	Hydnaceae	*Hydnum*	*Hydnum sp.*	O	O	O	
Basidiomycota	Agaricomycetes	Cantharellales	Hydnaceae	Unidentified		O	O		
Basidiomycota	Agaricomycetes	Cantharellales	Unidentified	Unidentified			O		
Basidiomycota	Agaricomycetes	Corticiales	Corticiaceae	Unidentified			O		
Basidiomycota	Agaricomycetes	Geastrales	Geastraceae	*Nidulariopsis*	*Nidulariopsis iowensis*		O		

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Appendix 3, Continued

Phylum	Class	Order	Family	Genus	Species	PacBio	MiSeq	NLSK (Genus)	NLSK (Species)
Basidiomycota	Agaricomycetes	Gomphales	Gomphaceae	*Ramaria*	*Ramaria gracilis*		O	O	
Basidiomycota	Agaricomycetes	Gomphales	Gomphaceae	*Ramaria*	*Ramaria sp.*		O	O	
Basidiomycota	Agaricomycetes	Hymenochaetales	Hymenochaetaceae	*Coltricia*	*Coltricia sp.*	O		O	
Basidiomycota	Agaricomycetes	Hymenochaetales	Hymenochaetaceae	*Coltricia*	*Coltricia weii*	O		O	
Basidiomycota	Agaricomycetes	Hymenochaetales	Hymenochaetaceae	*Coltriciella*	*Coltriciella dependens*	O	O	O	O
Basidiomycota	Agaricomycetes	Hymenochaetales	Hymenochaetaceae	*Coltriciella*	*Coltriciella pusilla*	O	O	O	O
Basidiomycota	Agaricomycetes	Hymenochaetales	Hymenochaetaceae	*Coltriciella*	*Coltriciella subglobosa*	O	O	O	
Basidiomycota	Agaricomycetes	Hymenochaetales	Hymenochaetaceae	*Hymenochaete*	*Hymenochaete subferruginea*		O	O	
Basidiomycota	Agaricomycetes	Hymenochaetales	Incertae sedis	*Peniophorella*	*Peniophorella pallida*	O		O	
Basidiomycota	Agaricomycetes	Hymenochaetales	Incertae sedis	*Resinicium*	*Resinicium bicolor*	O		O	
Basidiomycota	Agaricomycetes	Hymenochaetales	Schizoporaceae	*Hyphodontia*	*Hyphodontia pallidula*	O		O	O
Basidiomycota	Agaricomycetes	Hymenochaetales	Schizoporaceae	Unidentified			O		
Basidiomycota	Agaricomycetes	Hymenochaetales	Schizoporaceae	*Xylodon*	*Xylodon sp.*		O	O	
Basidiomycota	Agaricomycetes	Hymenochaetales	Unidentified	Unidentified		O			
Basidiomycota	Agaricomycetes	Phallales	Clathraceae	*Clathrus*	*Clathrus sp.*	O		O	
Basidiomycota	Agaricomycetes	Phallales	Clathraceae	Unidentified			O		
Basidiomycota	Agaricomycetes	Phallales	Phallaceae	*Kobayasia*	*Kobayasia nipponica*	O	O	O	O

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Appendix 3, Continued

Phylum	Class	Order	Family	Genus	Species	PacBio	MiSeq	NLSK (Genus)	NLSK (Species)
Basidiomycota	Agaricomycetes	Phallales	Phallaceae	*Phallus*	*Phallus impudicus*		O	O	O
Basidiomycota	Agaricomycetes	Polyporales	Ganodermataceae	*Ganoderma*	*Ganoderma applanatum*	O	O	O	O
Basidiomycota	Agaricomycetes	Polyporales	Ganodermataceae	*Ganoderma*	*Ganoderma lingzhi*		O	O	
Basidiomycota	Agaricomycetes	Polyporales	Ganodermataceae	*Ganoderma*	*Ganoderma sichuanense*	O		O	
Basidiomycota	Agaricomycetes	Polyporales	Incertaesedis	*Crustodontia*	*Crustodontia chrysocreas*		O	O	
Basidiomycota	Agaricomycetes	Polyporales	Irpicaceae	*Ceriporia*	*Ceriporia reticulata*		O	O	
Basidiomycota	Agaricomycetes	Polyporales	Meruliaceae	*Gloeoporus*	*Gloeoporus dichrous*		O	O	O
Basidiomycota	Agaricomycetes	Polyporales	Meruliaceae	*Irpex*	*Irpex lacteus*		O	O	
Basidiomycota	Agaricomycetes	Polyporales	Meruliaceae	*Scopuloides*	*Scopuloides hydnoides*	O	O	O	
Basidiomycota	Agaricomycetes	Polyporales	Meruliaceae	*Steccherinum*	*Steccherinum murashkinskyi*		O	O	O
Basidiomycota	Agaricomycetes	Polyporales	Meruliaceae	*Stereopsis*	*Stereopsis burtiana*	O		O	O
Basidiomycota	Agaricomycetes	Polyporales	Phanerochaetaceae	*Ceriporia*	*Ceriporia sp.*	O	O	O	
Basidiomycota	Agaricomycetes	Polyporales	Phanerochaetaceae	*Hapalopilus*	*Hapalopilus nidulans*		O	O	
Basidiomycota	Agaricomycetes	Polyporales	Phanerochaetaceae	*Phanerochaete*	*Phanerochaete sp.*		O	O	
Basidiomycota	Agaricomycetes	Polyporales	Polyporaceae	*Coriolopsis*	*Coriolopsis strumosa*		O	O	O
Basidiomycota	Agaricomycetes	Polyporales	Polyporaceae	*Favolus*	*Favolus acervatus*		O	O	O
Basidiomycota	Agaricomycetes	Polyporales	Polyporaceae	*Favolus*	*Favolus subtropicus*		O	O	

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Appendix 3, Continued

Phylum	Class	Order	Family	Genus	Species	PacBio	MiSeq	NLSK (Genus)	NLSK (Species)
Basidiomycota	Agaricomycetes	Polyporales	Polyporaceae	*Perenniporia*	*Perenniporia fraxinea*	O		O	O
Basidiomycota	Agaricomycetes	Polyporales	Polyporaceae	*Perenniporia*	*Perenniporia subacida*		O	O	O
Basidiomycota	Agaricomycetes	Polyporales	Polyporaceae	*Polyporus*	*Polyporus orientivarius*		O	O	O
Basidiomycota	Agaricomycetes	Polyporales	Polyporaceae	*Polyporus*	*Polyporus subdictyopus*		O	O	
Basidiomycota	Agaricomycetes	Polyporales	Polyporaceae	*Polyporus*	*Polyporus tuberaster*		O	O	O
Basidiomycota	Agaricomycetes	Polyporales	Polyporaceae	*Polyporus*	*Polyporus varius*		O	O	O
Basidiomycota	Agaricomycetes	Polyporales	Polyporaceae	*Pycnoporus*	*Pycnoporus coccineus*		O	O	O
Basidiomycota	Agaricomycetes	Polyporales	Polyporaceae	*Trametes*	*Trametes orientalis*		O	O	O
Basidiomycota	Agaricomycetes	Polyporales	Polyporaceae	*Trametes*	*Trametes suaveolens*		O	O	O
Basidiomycota	Agaricomycetes	Polyporales	Polyporaceae	*Tyromyces*	*Tyromyces chioneus*		O	O	O
Basidiomycota	Agaricomycetes	Polyporales	Polyporaceae	Unidentified			O		
Basidiomycota	Agaricomycetes	Polyporales	Unidentified	Unidentified		O	O		
Basidiomycota	Agaricomycetes	Russulales	Albatrellaceae	*Albatrellus*	*Albatrellus sp.*	O		O	
Basidiomycota	Agaricomycetes	Russulales	Albatrellaceae	Unidentified			O		
Basidiomycota	Agaricomycetes	Russulales	Incertaesedis	*Pseudowrightoporia*	*Pseudowrightoporia crassihypha*		O		
Basidiomycota	Agaricomycetes	Russulales	Lachnocladiaceae	*Dichostereum*	*Dichostereum boidinii*	O	O		
Basidiomycota	Agaricomycetes	Russulales	Lachnocladiaceae	Unidentified			O		

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Appendix 3, Continued

Phylum	Class	Order	Family	Genus	Species	PacBio	MiSeq	NLSK (Genus)	NLSK (Species)
Basidiomycota	Agaricomycetes	Russulales	Russulaceae	*Boidinia*	*Boidinia sp.*	O			
Basidiomycota	Agaricomycetes	Russulales	Russulaceae	*Lactarius*	*Lactarius citrinus*	O	O	O	O
Basidiomycota	Agaricomycetes	Russulales	Russulaceae	*Lactarius*	*Lactarius curvatus*	O	O	O	
Basidiomycota	Agaricomycetes	Russulales	Russulaceae	*Lactarius*	*Lactarius hatsudake*	O	O	O	O
Basidiomycota	Agaricomycetes	Russulales	Russulaceae	*Lactarius*	*Lactarius lutescens*	O	O	O	O
Basidiomycota	Agaricomycetes	Russulales	Russulaceae	*Lactarius*	*Lactarius subquietus*	O	O	O	
Basidiomycota	Agaricomycetes	Russulales	Russulaceae	*Lactarius*	*Lactarius subzonarius*	O	O	O	O
Basidiomycota	Agaricomycetes	Russulales	Russulaceae	*Lactarius*	*Lactarius yazooensis*	O	O	O	
Basidiomycota	Agaricomycetes	Russulales	Russulaceae	*Lactarius*	*Lactarius sp.*	O	O	O	
Basidiomycota	Agaricomycetes	Russulales	Russulaceae	*Russula*	*Russula foetens*		O	O	O
Basidiomycota	Agaricomycetes	Russulales	Russulaceae	*Russula*	*Russula alboareolata*	O		O	O
Basidiomycota	Agaricomycetes	Russulales	Russulaceae	*Russula*	*Russula brevipes*	O	O	O	O
Basidiomycota	Agaricomycetes	Russulales	Russulaceae	*Russula*	*Russula catillus*	O	O	O	O
Basidiomycota	Agaricomycetes	Russulales	Russulaceae	*Russula*	*Russula cerolens*	O	O	O	O
Basidiomycota	Agaricomycetes	Russulales	Russulaceae	*Russula*	*Russula compacta*		O	O	
Basidiomycota	Agaricomycetes	Russulales	Russulaceae	*Russula*	*Russula earlei*	O		O	
Basidiomycota	Agaricomycetes	Russulales	Russulaceae	*Russula*	*Russula romelii*	O	O	O	

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Appendix 3, Continued

Phylum	Class	Order	Family	Genus	Species	PacBio	MiSeq	NLSK (Genus)	NLSK (Species)
Basidiomycota	Agaricomycetes	Russulales	Russulaceae	*Russula*	*Russula sanguinea*	O	O	O	O
Basidiomycota	Agaricomycetes	Russulales	Russulaceae	*Russula*	*Russula chloroides*	O	O	O	O
Basidiomycota	Agaricomycetes	Russulales	Russulaceae	*Russula*	*Russula cremoricolor*		O	O	
Basidiomycota	Agaricomycetes	Russulales	Russulaceae	*Russula*	*Russula cyanoxantha*	O		O	O
Basidiomycota	Agaricomycetes	Russulales	Russulaceae	*Russula*	*Russula delica*	O	O	O	O
Basidiomycota	Agaricomycetes	Russulales	Russulaceae	*Russula*	*Russula eccentrica*	O		O	
Basidiomycota	Agaricomycetes	Russulales	Russulaceae	*Russula*	*Russula nigricans*	O	O	O	O
Basidiomycota	Agaricomycetes	Russulales	Russulaceae	*Russula*	*Russula ochroleuca*	O	O	O	O
Basidiomycota	Agaricomycetes	Russulales	Russulaceae	*Russula*	*Russula pectinatoides*		O	O	
Basidiomycota	Agaricomycetes	Russulales	Russulaceae	*Russula*	*Russula poichilochroa*	O		O	
Basidiomycota	Agaricomycetes	Russulales	Russulaceae	*Russula*	*Russula pseudointegra*	O	O	O	
Basidiomycota	Agaricomycetes	Russulales	Russulaceae	*Russula*	*Russula romellii*	O	O	O	
Basidiomycota	Agaricomycetes	Russulales	Russulaceae	*Russula*	*Russula sanguinea*	O		O	O
Basidiomycota	Agaricomycetes	Russulales	Russulaceae	*Russula*	*Russula senecis*	O		O	O
Basidiomycota	Agaricomycetes	Russulales	Russulaceae	*Russula*	*Russula sp.*	O	O	O	
Basidiomycota	Agaricomycetes	Russulales	Russulaceae	*Russula*	*Russula violeipes*	O	O	O	O
Basidiomycota	Agaricomycetes	Russulales	Russulaceae	*Russula*	*Russula virescens*		O	O	O

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Appendix 3, Continued

Phylum	Class	Order	Family	Genus	Species	PacBio	MiSeq	NLSK (Genus)	NLSK (Species)
Basidiomycota	Agaricomycetes	Sebacinales	Sebacinaceae	*Sebacina*	*Sebacina sp.*	O	O	O	
Basidiomycota	Agaricomycetes	Sebacinales	Serendipitaceae	*Serendipita*	*Serendipita sp.*	O	O		
Basidiomycota	Agaricomycetes	Sebacinales	Serendipitaceae	Unidentified		O	O		
Basidiomycota	Agaricomycetes	Thelephorales	Bankeraceae	*Boletopsis*	*Boletopsis sp.*		O	O	
Basidiomycota	Agaricomycetes	Thelephorales	Bankeraceae	*Hydnellum*	*Hydnellum sp.*	O	O	O	
Basidiomycota	Agaricomycetes	Thelephorales	Bankeraceae	*Phellodon*	*Phellodon melaleucus*	O	O	O	O
Basidiomycota	Agaricomycetes	Thelephorales	Bankeraceae	*Phellodon*	*Phellodon sp.*	O	O	O	
Basidiomycota	Agaricomycetes	Thelephorales	Bankeraceae	*Sarcodon*	*Sarcodon glaucopus*	O	O	O	
Basidiomycota	Agaricomycetes	Thelephorales	Bankeraceae	*Sarcodon*	*Sarcodon sp.*	O	O	O	
Basidiomycota	Agaricomycetes	Thelephorales	Bankeraceae	Unidentified		O			
Basidiomycota	Agaricomycetes	Thelephorales	Thelephoraceae	*Odontia*	*Odontia fibrosa*		O		
Basidiomycota	Agaricomycetes	Thelephorales	Thelephoraceae	*Odontia*	*Odontia sp.*	O	O		
Basidiomycota	Agaricomycetes	Thelephorales	Thelephoraceae	*Pseudotomentella*	*Pseudotomentella griseopergamacea*	O	O		
Basidiomycota	Agaricomycetes	Thelephorales	Thelephoraceae	*Pseudotomentella*	*Pseudotomentella pinophila*	O	O		
Basidiomycota	Agaricomycetes	Thelephorales	Thelephoraceae	*Pseudotomentella*	*Pseudotomentella rhizopunctata*	O			
Basidiomycota	Agaricomycetes	Thelephorales	Thelephoraceae	*Pseudotomentella*	*Pseudotomentella sciastra*	O	O		
Basidiomycota	Agaricomycetes	Thelephorales	Thelephoraceae	*Pseudotomentella*	*Pseudotomentella sp.*	O	O		

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Appendix 3, Continued

Phylum	Class	Order	Family	Genus	Species	PacBio	MiSeq	NLSK (Genus)	NLSK (Species)
Basidiomycota	Agaricomycetes	Thelephorales	Thelephoraceae	*Thelephora*	*Thelephora atra*	O	O	O	
Basidiomycota	Agaricomycetes	Thelephorales	Thelephoraceae	*Thelephora*	*Thelephora sp.*	O	O	O	
Basidiomycota	Agaricomycetes	Thelephorales	Thelephoraceae	*Tomentella*	*Tomentella cinerascens*	O	O	O	
Basidiomycota	Agaricomycetes	Thelephorales	Thelephoraceae	*Tomentella*	*Tomentella coerulea*	O	O	O	
Basidiomycota	Agaricomycetes	Thelephorales	Thelephoraceae	*Tomentella*	*Tomentella ellisii*	O	O	O	
Basidiomycota	Agaricomycetes	Thelephorales	Thelephoraceae	*Tomentella*	*Tomentella fuscocinerea*		O	O	
Basidiomycota	Agaricomycetes	Thelephorales	Thelephoraceae	*Tomentella*	*Tomentella galzinii*		O	O	
Basidiomycota	Agaricomycetes	Thelephorales	Thelephoraceae	*Tomentella*	*Tomentella lapida*	O	O	O	
Basidiomycota	Agaricomycetes	Thelephorales	Thelephoraceae	*Tomentella*	*Tomentella lateritia*	O	O	O	
Basidiomycota	Agaricomycetes	Thelephorales	Thelephoraceae	*Tomentella*	*Tomentella muricata*		O	O	
Basidiomycota	Agaricomycetes	Thelephorales	Thelephoraceae	*Tomentella*	*Tomentella papuae*	O	O	O	
Basidiomycota	Agaricomycetes	Thelephorales	Thelephoraceae	*Tomentella*	*Tomentella sp.*	O	O	O	
Basidiomycota	Agaricomycetes	Thelephorales	Thelephoraceae	*Tomentella*	*Tomentella stuposa*	O	O	O	
Basidiomycota	Agaricomycetes	Thelephorales	Thelephoraceae	*Tomentella*	*Tomentella subclavigera*		O	O	
Basidiomycota	Agaricomycetes	Thelephorales	Thelephoraceae	*Tomentella*	*Tomentella terrestris*	O	O	O	
Basidiomycota	Agaricomycetes	Thelephorales	Thelephoraceae	*Tomentellopsis*	*Tomentellopsis sp.*		O		
Basidiomycota	Agaricomycetes	Thelephorales	Thelephoraceae	*Tomentellopsis*	*Tomentellopsis zygodesmoides*	O			

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Appendix 3, Continued

Phylum	Class	Order	Family	Genus	Species	PacBio	MiSeq	NLSK (Genus)	NLSK (Species)
Basidiomycota	Agaricomycetes	Thelephorales	Thelephoraceae	Unidentified		O	O		
Basidiomycota	Agaricomycetes	Thelephorales	Unidentified	Unidentified		O	O		
Basidiomycota	Agaricomycetes	Trechisporales	Hydnodontaceae	*Luellia*	*Luellia recondita*	O	O		
Basidiomycota	Agaricomycetes	Trechisporales	Hydnodontaceae	*Luellia*	*Luellia sp.*	O	O		
Basidiomycota	Agaricomycetes	Trechisporales	Hydnodontaceae	*Subulicystidium*	*Subulicystidium sp.*	O	O		
Basidiomycota	Agaricomycetes	Trechisporales	Hydnodontaceae	*Trechispora*	*Trechispora caucasica*		O	O	
Basidiomycota	Agaricomycetes	Trechisporales	Hydnodontaceae	*Trechispora*	*Trechispora invisitata*	O	O	O	
Basidiomycota	Agaricomycetes	Trechisporales	Hydnodontaceae	*Trechispora*	*Trechispora sp.*	O	O	O	
Basidiomycota	Agaricomycetes	Trechisporales	Hydnodontaceae	Unidentified		O	O		
Basidiomycota	Agaricomycetes	Trechisporales	Unidentified	Unidentified		O			
Basidiomycota	Agaricomycetes	Tremellodendropsidales	Unidentified	Unidentified		O	O		
Basidiomycota	Agaricomycetes	Unidentified	Unidentified	Unidentified		O	O		
Basidiomycota	Cystobasidiomycetes	Incertae sedis	Microsporomycetaceae	Unidentified			O		
Basidiomycota	Cystobasidiomycetes	Erythrobasidiales	Erythrobasidiaceae	*Erythrobasidium*	*Erythrobasidium sp.*		O	O	
Basidiomycota	Cystobasidiomycetes	Erythrobasidiales	Erythrobasidiaceae	Unidentified			O		
Basidiomycota	Cystobasidiomycetes	Erythrobasidiales	Incertae sedis	*Sakaguchia*	*Sakaguchia sp.*		O	O	
Basidiomycota	Cystobasidiomycetes	Unidentified	Unidentified	Unidentified		O	O		

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Appendix 3, Continued

Phylum	Class	Order	Family	Genus	Species	PacBio	MiSeq	NLSK (Genus)	NLSK (Species)
Basidiomycota	Exobasidiomycetes	Entylomatales	Incertae sedis	*Tilletiopsis*	*Tilletiopsis lilacina*		O	O	
Basidiomycota	Geminibasidiomycetes	Geminibasidiales	Geminibasidiaceae	*Geminibasidium*	*Geminibasidium sp.*	O	O		
Basidiomycota	GS25	GS25	Unidentified	Unidentified		O	O		
Basidiomycota	GS27	GS27	Unidentified	Unidentified			O		
Basidiomycota	Malasseziomycetes	Malasseziales	Malasseziaceae	*Malassezia*	*Malassezia restricta*	O	O		
Basidiomycota	Microbotryomycetes	Kriegeriales	Kriegeriaceae	*Libkindia*	*Libkindia masarykiana*		O		
Basidiomycota	Microbotryomycetes	Kriegeriales	Kriegeriaceae	*Libkindia*	*Libkindia sp.*	O			
Basidiomycota	Microbotryomycetes	Kriegeriales	Kriegeriaceae	*Phenoliferia*	*Phenoliferia psychrophenolica*	O			
Basidiomycota	Microbotryomycetes	Kriegeriales	Kriegeriaceae	*Yamadamyces*	*Yamadamyces rosulatus*	O			
Basidiomycota	Microbotryomycetes	Leucosporidiales	Leucosporidiaceae	*Leucosporidium*	*Leucosporidium creatinivorum*	O	O	O	
Basidiomycota	Microbotryomycetes	Leucosporidiales	Leucosporidiaceae	*Leucosporidium*	*Leucosporidium krtinense*		O	O	
Basidiomycota	Microbotryomycetes	Leucosporidiales	Unidentified	Unidentified		O			
Basidiomycota	Microbotryomycetes	Incertae sedis	Chrysozymaceae	*Bannozyma*	*Bannozyma arctica*	O			
Basidiomycota	Microbotryomycetes	Incertae sedis	Chrysozymaceae	*Fellozyma*	*Fellozyma inositophila*	O	O		
Basidiomycota	Microbotryomycetes	Incertae sedis	Chrysozymaceae	*Oberwinklerozyma*	*Oberwinklerozyma silvestris*	O	O		
Basidiomycota	Microbotryomycetes	Incertae sedis	Chrysozymaceae	*Oberwinklerozyma*	*Oberwinklerozyma yarrowii*	O			
Basidiomycota	Microbotryomycetes	Incertae sedis	Chrysozymaceae	*Pseudohyphozyma*	*Pseudohyphozyma sp.*	O			

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Appendix 3, Continued

Phylum	Class	Order	Family	Genus	Species	PacBio	MiSeq	NLSK (Genus)	NLSK (Species)
Basidiomycota	Microbotryomycetes	Incertae sedis	Chrysozymaceae	Unidentified			O		
Basidiomycota	Microbotryomycetes	Incertae sedis	Chrysozymaceae	*Yurkovia*	*Yurkovia mendeliana*	O	O		
Basidiomycota	Microbotryomycetes	Incertae sedis	Incertae sedis	*Colacogloea*	*Colacogloea falcata*	O			
Basidiomycota	Microbotryomycetes	Incertae sedis	Incertae sedis	*Curvibasidium*	*Curvibasidium cygneicollum*	O	O	O	
Basidiomycota	Microbotryomycetes	Sporidiobolales	Sporidiobolaceae	*Rhodosporidiobolus*	*Rhodosporidiobolus colostri*		O	O	
Basidiomycota	Microbotryomycetes	Sporidiobolales	Sporidiobolaceae	*Rhodotorula*	*Rhodotorula mucilaginosa*	O		O	O
Basidiomycota	Microbotryomycetes	Sporidiobolales	Sporidiobolaceae	*Rhodotorula*	*Rhodotorula sp.*		O	O	
Basidiomycota	Microbotryomycetes	Sporidiobolales	Sporidiobolaceae	*Sporobolomyces*	*Sporobolomyces sp.*	O	O	O	
Basidiomycota	Microbotryomycetes	Sporidiobolales	Sporidiobolaceae	Unidentified		O			
Basidiomycota	Microbotryomycetes	Sporidiobolales	Unidentified	Unidentified		O	O		
Basidiomycota	Microbotryomycetes	Unidentified	Unidentified	Unidentified		O	O		
Basidiomycota	Pucciniomycetes	Platygloeales	Unidentified	Unidentified		O			
Basidiomycota	Spiculogloeomycetes	Spiculogloeales	Unidentified	Unidentified			O		
Basidiomycota	Tremellomycetes	Cystofilobasidiales	Mrakiaceae	*Krasilnikovozyma*	*Krasilnikovozyma huempii*	O	O		
Basidiomycota	Tremellomycetes	Cystofilobasidiales	Mrakiaceae	*Mrakia*	*Mrakia sp.*	O		O	
Basidiomycota	Tremellomycetes	Cystofilobasidiales	Mrakiaceae	*Tausonia*	*Tausonia pullulans*	O			
Basidiomycota	Tremellomycetes	Cystofilobasidiales	Mrakiaceae	*Udeniomyces*	*Udeniomyces pyricola*	O			

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Appendix 3, Continued

Phylum	Class	Order	Family	Genus	Species	PacBio	MiSeq	NLSK (Genus)	NLSK (Species)
Basidiomycota	Tremellomycetes	Filobasidiales	Filobasidiaceae	*Goffeauzyma*	*Goffeauzyma gastrica*	O			
Basidiomycota	Tremellomycetes	Filobasidiales	Filobasidiaceae	*Heterocephalacria*	*Heterocephalacria sp.*	O	O		
Basidiomycota	Tremellomycetes	Filobasidiales	Filobasidiaceae	Unidentified		O			
Basidiomycota	Tremellomycetes	Filobasidiales	Piskurozymaceae	*Piskurozyma*	*Piskurozyma cylindrica*	O	O	O	
Basidiomycota	Tremellomycetes	Filobasidiales	Piskurozymaceae	*Piskurozyma*	*Piskurozyma sp.*	O	O	O	
Basidiomycota	Tremellomycetes	Filobasidiales	Piskurozymaceae	*Piskurozyma*	*Piskurozyma taiwanensis*	O		O	O
Basidiomycota	Tremellomycetes	Filobasidiales	Piskurozymaceae	*Solicoccozyma*	*Solicoccozyma sp.*	O			
Basidiomycota	Tremellomycetes	Filobasidiales	Piskurozymaceae	*Solicoccozyma*	*Solicoccozyma terrea*	O	O		
Basidiomycota	Tremellomycetes	Filobasidiales	Piskurozymaceae	*Solicoccozyma*	*Solicoccozyma terricola*	O	O		
Basidiomycota	Tremellomycetes	Filobasidiales	Unidentified	Unidentified			O		
Basidiomycota	Tremellomycetes	Tremellales	Bulleraceae	*Genolevuria*	*Genolevuria sp.*	O			
Basidiomycota	Tremellomycetes	Tremellales	Bulleribasidiaceae	*Hannaella*	*Hannaella sp.*		O	O	
Basidiomycota	Tremellomycetes	Tremellales	Cryptococcaceae	*Cryptococcus*	*Cryptococcus longus*	O	O	O	
Basidiomycota	Tremellomycetes	Tremellales	Cuniculitremaceae	*Fellomyces*	*Fellomyces sp.*	O			
Basidiomycota	Tremellomycetes	Tremellales	Cuniculitremaceae	*Kockovaella*	*Kockovaella sp.*	O			
Basidiomycota	Tremellomycetes	Tremellales	Phaeotremellaceae	*Phaeotremella*	*Phaeotremella sp.*	O	O		
Basidiomycota	Tremellomycetes	Filobasidiales	Filobasidiaceae	*Goffeauzyma*	*Goffeauzyma gastrica*	O			

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Appendix 3, Continued

Phylum	Class	Order	Family	Genus	Species	PacBio	MiSeq	NLSK (Genus)	NLSK (Species)
Basidiomycota	Tremellomycetes	Tremellales	Tremellaceae	*Cryptococcus*	*Cryptococcus musci*	O	O	O	
Basidiomycota	Tremellomycetes	Tremellales	Tremellaceae	*Cryptococcus*	*Cryptococcus pseudolongus*	O	O	O	
Basidiomycota	Tremellomycetes	Tremellales	Tremellaceae	*Cryptococcus*	*Cryptococcus sp.*	O	O	O	
Basidiomycota	Tremellomycetes	Tremellales	Tremellaceae	*Tremella*	*Tremella encephala*	O		O	
Basidiomycota	Tremellomycetes	Tremellales	Tremellaceae	*Tremella*	*Tremella sp.*	O	O	O	
Basidiomycota	Tremellomycetes	Tremellales	Tremellaceae	Unidentified			O		
Basidiomycota	Tremellomycetes	Tremellales	Trimorphomycetaceae	*Saitozyma*	*Saitozyma podzolica*	O	O		
Basidiomycota	Tremellomycetes	Tremellales	Unidentified	Unidentified		O	O		
Basidiomycota	Tremellomycetes	Trichosporonales	Trichosporonaceae	*Apiotrichum*	*Apiotrichum gracile*	O	O		
Basidiomycota	Tremellomycetes	Trichosporonales	Trichosporonaceae	*Apiotrichum*	*Apiotrichum porosum*	O	O		
Basidiomycota	Tremellomycetes	Trichosporonales	Trichosporonaceae	*Effuseotrichosporon*	*Effuseotrichosporon vanderwaltii*	O			
Basidiomycota	Tremellomycetes	Trichosporonales	Trichosporonaceae	*Vanrija*	*Vanrija humicola*	O			
Basidiomycota	Tremellomycetes	Trichosporonales	Unidentified	Unidentified			O		
Basidiomycota	Tremellomycetes	Unidentified	Unidentified	Unidentified		O	O		
Basidiomycota	Tritirachiomycetes	Tritirachiales	Tritirachiaceae	*Paratritirachium*	*Paratritirachium sp.*	O	O		
Basidiomycota	Tritirachiomycetes	Tritirachiales	Tritirachiaceae	*Tritirachium*	*Tritirachium cinnamomeum*	O			
Basidiomycota	Tritirachiomycetes	Tritirachiales	Tritirachiaceae	*Tritirachium*	*Tritirachium sp.*	O			

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Appendix 3, Continued

Phylum	Class	Order	Family	Genus	Species	PacBio	MiSeq	NLSK (Genus)	NLSK (Species)
Basidiomycota	Unidentified	Unidentified	Unidentified	Unidentified		O	O		
Blastocladiomycota	Blastocladiomycetes	GS15	Unidentified	Unidentified		O			
Chytridiomycota	Chytridiomycetes	Chytridiales	Chytriomycetaceae	*Rhizidium*	*Rhizidium phycophilum*	O			
Chytridiomycota	Chytridiomycetes	Chytridiales	Unidentified	Unidentified		O	O		
Chytridiomycota	Chytridiomycetes	Unidentified	Unidentified	Unidentified			O		
Chytridiomycota	Rhizophydiomycetes	Rhizophydiales	Rhizophydiaceae	Unidentified			O		
Chytridiomycota	Spizellomycetes	Spizellomycetales	Powellomycetaceae	*Powellomyces*	*Powellomyces sp.*		O		
Chytridiomycota	Spizellomycetes	Spizellomycetales	Unidentified	Unidentified			O		
Chytridiomycota	Unidentified	Unidentified	Unidentified	Unidentified		O	O		
Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	*Glomus*	*Glomus sp.*	O		O	
Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	Unidentified		O	O		
Kickxellomycota	Kickxellomycetes	Kickxellales	Unidentified	Unidentified		O			
Mortierellomycota	Mortierellomycetes	Mortierellales	Mortierellaceae	*Dissophora*	*Dissophora sp.*	O			
Mortierellomycota	Mortierellomycetes	Mortierellales	Mortierellaceae	*Mortierella*	*Mortierella amoeboidea*	O	O	O	
Mortierellomycota	Mortierellomycetes	Mortierellales	Mortierellaceae	*Mortierella*	*Mortierella angusta*		O	O	
Mortierellomycota	Mortierellomycetes	Mortierellales	Mortierellaceae	*Mortierella*	*Mortierella bainieri*	O	O	O	
Mortierellomycota	Mortierellomycetes	Mortierellales	Mortierellaceae	*Mortierella*	*Mortierella basiparvispora*	O	O	O	

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Appendix 3, Continued

Phylum	Class	Order	Family	Genus	Species	PacBio	MiSeq	NLSK (Genus)	NLSK (Species)
Mortierellomycota	Mortierellomycetes	Mortierellales	Mortierellaceae	*Mortierella*	*Mortierella cystojenkinii*	O	O	O	
Mortierellomycota	Mortierellomycetes	Mortierellales	Mortierellaceae	*Mortierella*	*Mortierella echinula*	O		O	
Mortierellomycota	Mortierellomycetes	Mortierellales	Mortierellaceae	*Mortierella*	*Mortierella elongata*	O	O	O	
Mortierellomycota	Mortierellomycetes	Mortierellales	Mortierellaceae	*Mortierella*	*Mortierella gemmifera*	O	O	O	
Mortierellomycota	Mortierellomycetes	Mortierellales	Mortierellaceae	*Mortierella*	*Mortierella globulifera*	O	O	O	
Mortierellomycota	Mortierellomycetes	Mortierellales	Mortierellaceae	*Mortierella*	*Mortierella horticola*	O		O	
Mortierellomycota	Mortierellomycetes	Mortierellales	Mortierellaceae	*Mortierella*	*Mortierella humilis*	O	O	O	
Mortierellomycota	Mortierellomycetes	Mortierellales	Mortierellaceae	*Mortierella*	*Mortierella nantahalensis*	O	O	O	
Mortierellomycota	Mortierellomycetes	Mortierellales	Mortierellaceae	*Mortierella*	*Mortierella parvispora*		O	O	
Mortierellomycota	Mortierellomycetes	Mortierellales	Mortierellaceae	*Mortierella*	*Mortierella pseudozygospora*	O	O	O	
Mortierellomycota	Mortierellomycetes	Mortierellales	Mortierellaceae	*Mortierella*	*Mortierella pulchella*	O	O	O	
Mortierellomycota	Mortierellomycetes	Mortierellales	Mortierellaceae	*Mortierella*	*Mortierella turficola*		O	O	
Mortierellomycota	Mortierellomycetes	Mortierellales	Mortierellaceae	*Mortierella*	*Mortierella sp.*	O	O	O	
Mortierellomycota	Mortierellomycetes	Mortierellales	Mortierellaceae	Unidentified		O	O		
Mucoromycota	Endogonomycetes	GS21	Unidentified	Unidentified		O	O		
Mucoromycota	Endogonomycetes	Unidentified	Unidentified	Unidentified		O			
Mucoromycota	Mucoromycetes	Mucorales	Cunninghamellaceae	*Absidia*	*Absidia glauca*		O	O	

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Appendix 3, Continued

Phylum	Class	Order	Family	Genus	Species	PacBio	MiSeq	NLSK (Genus)	NLSK (Species)
Mucoromycota	Mucoromycetes	Mucorales	Cunninghamellaceae	*Absidia*	*Absidia sp.*		O	O	
Mucoromycota	Mucoromycetes	Mucorales	Cunninghamellaceae	*Cunninghamella*	*Cunninghamella elegans*		O	O	
Mucoromycota	Mucoromycetes	Mucorales	Cunninghamellaceae	*Gongronella*	*Gongronella sp.*		O	O	
Mucoromycota	Mucoromycetes	Mucorales	Cunninghamellaceae	Unidentified			O		
Mucoromycota	Mucoromycetes	Mucorales	Mucoraceae	*Mucor*	*Mucor durus*		O	O	
Mucoromycota	Mucoromycetes	Mucorales	Mucoraceae	*Mucor*	*Mucor irregularis*		O	O	
Mucoromycota	Mucoromycetes	Mucorales	Mucoraceae	*Mucor*	*Mucor moelleri*		O	O	
Mucoromycota	Mucoromycetes	Mucorales	Mucoraceae	*Mucor*	*Mucor racemosus*		O	O	
Mucoromycota	Mucoromycetes	Mucorales	Mucoraceae	*Mucor*	*Mucor silvaticus*		O	O	
Mucoromycota	Mucoromycetes	Mucorales	Mucoraceae	*Mucor*	*Mucor zonatus*		O	O	
Mucoromycota	Mucoromycetes	Mucorales	Mucoraceae	*Mucor*	*Mucor sp.*		O	O	
Mucoromycota	Mucoromycetes	Mucorales	Unidentified	Unidentified			O		
Mucoromycota	Incertae sedis	Incertae sedis	Incertae sedis	*Bifiguratus*	*Bifiguratus adelaidae*	O	O		
Mucoromycota	Incertae sedis	Incertae sedis	Incertae sedis	*Bifiguratus*	*Bifiguratus sp.*	O			
Mucoromycota	Umbelopsidomycetes	GS23	Unidentified	Unidentified		O	O		
Mucoromycota	Umbelopsidomycetes	Umbelopsidales	Umbelopsidaceae	*Umbelopsis*	*Umbelopsis dimorpha*	O	O	O	
Mucoromycota	Umbelopsidomycetes	Umbelopsidales	Umbelopsidaceae	*Umbelopsis*	*Umbelopsis gibberispora*		O	O	

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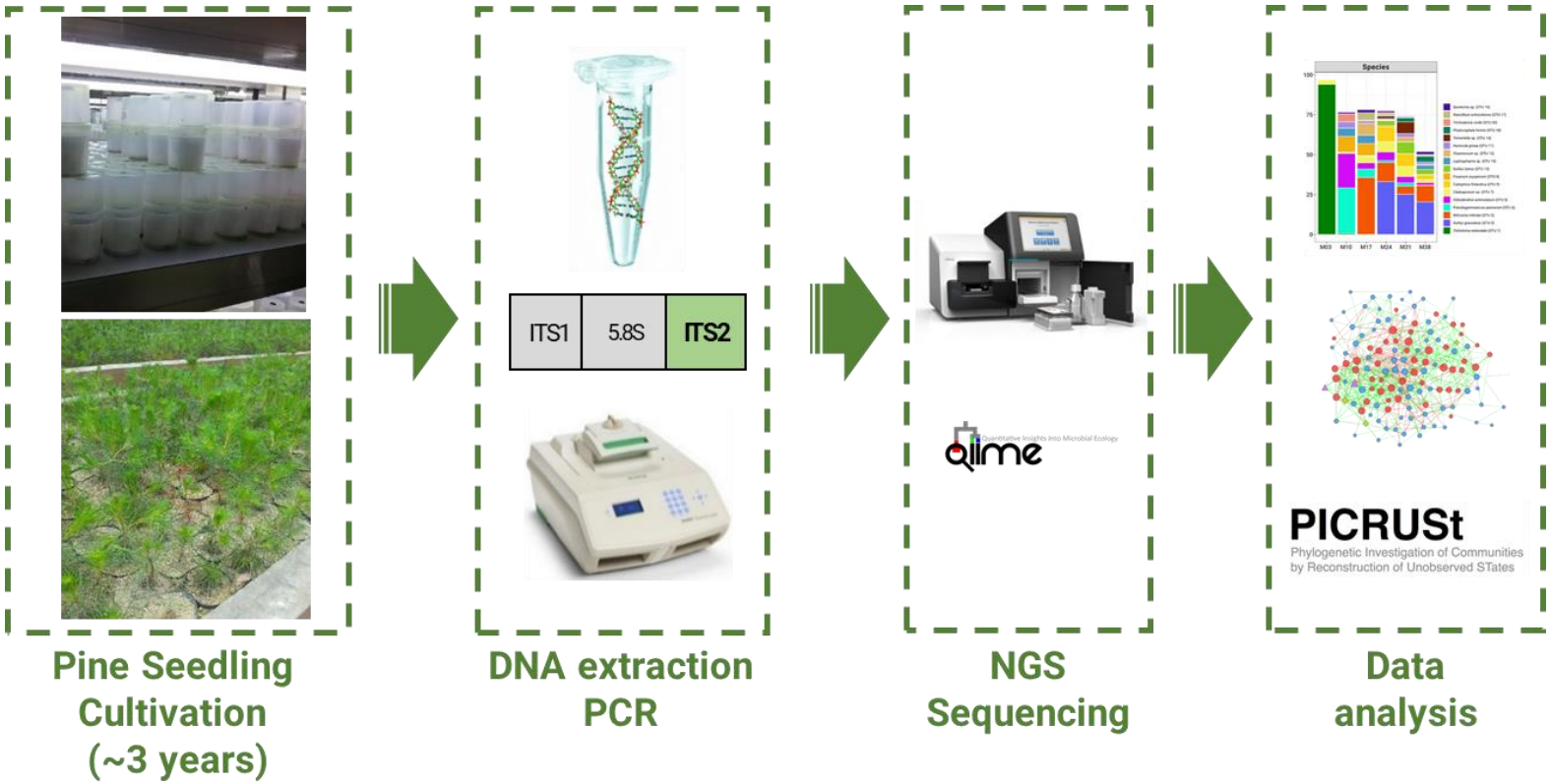
Appendix 3, Continued

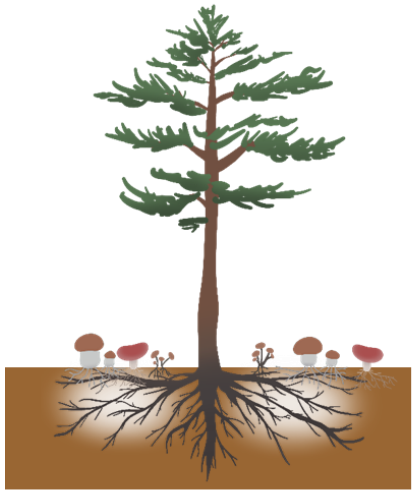
Phylum	Class	Order	Family	Genus	Species	PacBio	MiSeq	NLSK (Genus)	NLSK (Species)
Mucoromycota	Umbelopsidomycetes	Umbelopsidales	Umbelopsidaceae	*Umbelopsis*	*Umbelopsis isabellina*	O	O	O	
Mucoromycota	Umbelopsidomycetes	Umbelopsidales	Umbelopsidaceae	*Umbelopsis*	*Umbelopsis ramanniana*	O	O	O	
Mucoromycota	Umbelopsidomycetes	Umbelopsidales	Umbelopsidaceae	*Umbelopsis*	*Umbelopsis vinacea*	O	O	O	
Mucoromycota	Umbelopsidomycetes	Umbelopsidales	Umbelopsidaceae	*Umbelopsis*	*Umbelopsis sp.*		O	O	
Olpidiomycota	GS18	GS18	Unidentified	Unidentified		O	O		
Rozellomycota	Incertae sedis	Unidentified	Unidentified	Unidentified		O			
Rozellomycota	Incertae sedis	GS11	Unidentified	Unidentified		O	O		
Rozellomycota	Unidentified	Unidentified	Unidentified	Unidentified		O	O		
Zoopagomycota	Zoopagomycetes	Zoopagales	Piptocephalidaceae	*Piptocephalis*	*Piptocephalis fimbriata*	O			
Zoopagomycota	Zoopagomycetes	Zoopagales	Piptocephalidaceae	*Syncephalis*	*Syncephalis sp.*	O	O		

NLSK=National List of species of Korea (국가생물종목록)

Appendix 4. Proposed workflow for chapter 3



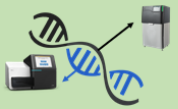
Appendix 5. General conclusion



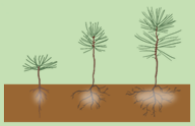
Pinus densiflora **mycobiome**

Different influence of spatial distance on microhabitats/guilds

Cautions in interpretation of DNA metabarcoding



Succession of root mycobiome in *P. densiflora* seedlings



Backbone data for understanding spatial/temporal distance on plant mycobiome

Construction of *P. densiflora* mycobiome map

Abstract in Korean

NGS (next generation sequencing)를 비롯한 염기서열 분석 기술의 발전으로 DNA 메타바코딩 기법을 이용하여 환경 샘플로부터 미생물 군집과 주변 생물체와의 상호작용을 연구할 수 있게 되었다. 주요 미생물 중 하나인 진균은 숲 생태계의 주요 구성원으로 식물의 뿌리 및 토양에서 다양한 역할을 수행하고 있다. 이들은 종종 기회성 병원균으로 작용하기도 하지만 많은 경우 식물의 토양 내 수분과 영양분 흡수를 도와 식물의 생물학적, 비생물학적 스트레스 저항성 및 생산성을 향상시키며 병원균의 활성을 억제하여 식물의 적합도 (fitness)를 높여주는 것으로 알려져 있다. 진균과 특히 밀접한 관계를 가지고 있는 외생균근성 나무인 소나무는 단일수종으로는 가장 넓은 면적을 차지하고 있는 한국의 주요 수종으로 경제적, 생태적으로 중요한 위치를 가지고 있다. 그러나 기후온난화와 수목병 등으로 인해 지속적으로 서식지가 감소하고 있으며 연구에 따르면 소나무 서식지는 점점 더 고지대, 고위도로 이동할 것으로 여겨진다. 따라서 본 연구에서는 소나무의 적합도를 유지하고 서식지 감소를 최소화하고자 DNA 메타바코딩 방법을 이용, 소나무 진균 군집의 시간적, 공간적 변화를 밝히고자 하였다. 또한, DNA 메타바코딩 기법에 따른 분석 결과의 차이를 확인하여 마이크로바이옴 분석 결과 해석에서 주의해야 할 부분을 확인하였다.

첫 번째 장에서는 공간적 변화에 따른 소나무 진균 군집의 변화를 관찰하였다. 공간적 거리 변화는 생태계 구성에 중요한 영향을 미치는 것으로 알려져 있으나 진균에서는 많이 연구된 바가 없다. 이를 확인하기 위해 소나무림 16 지역에서 80 개체의 소나무의 뿌리 및 토양 시료를 확보하여 지리적 거리, 고도, 미소 서식환경 (Microhabitat)의 차이에 따른 소나무와 연관된 진균 군집의 공간적 변화를 관찰하였다. 그 결과, 지리적 거리, 고도, 미소 서식환경 모두 진균 군집의 구조 차이에 유의한 영향을

주었다. 뿌리에 서식하는 진균의 종 풍부도와 군집구조는 고도 및 지리적 거리에 의한 변화를 유의하게 받았으나 토양에 서식하는 진균 군집 구조에는 고도만이 유의한 변화를 주었다. 이 연구를 통해 뿌리에 서식하는 진균 군집이 공간적 변화에 더 민감하게 반응함을 보았고 다른 미소 시식지에 서식하는 진균 군집과 기주 식물의 관계 이해에 대한 기틀을 제공하였다.

두 번째 장에서는 염기서열 분석 방법에 따른 진균 군집 분석 결과의 차이를 확인하였다. 짧은 염기서열만을 읽을 수 있는 기존 NGS 플랫폼과는 달리 긴 염기서열 (long-read) NGS 플랫폼은 전체 ITS (internal transcribed spacers)영역을 읽을 수 있다는 장점으로 동정 해상도를 높일 수 있을 것으로 기대되고 있으나 기존의 NGS 방법에 비해 확보할 수 있는 염기서열 숫자가 적고 오류 확률이 높다는 단점이 있다. NGS 플랫폼과 ITS 영역의 차이가 마이크로바이옴 분석에 미치는 영향을 보기 위해 PacBio Sequel (long-read NGS) 플랫폼과 Illumina MiSeq (short-read NGS) 플랫폼에서 나온 소나무 뿌리 내 진균 군집 분석 결과를 비교하였다. 그 결과, 주요 분류군의 경우 그 구성에는 큰 차이가 없지만 그 풍부도에는 상당한 차이가 나타남을 확인하였다. 동정 정확도는 과 수준까지는 비슷하였으나 속 수준에서는 PacBio 에서 미동정된 염기서열의 비율이 유의하게 높게 나왔다. 또한, MiSeq 데이터에 비해 PacBio 데이터는 낮은 염기서열 숫자 때문에 지역간 풍부도 차이에 더 민감하게 반응하였다. 이를 통해 분석 방법이 마이크로바이옴 분석에 큰 영향을 미침을 확인하였고 결과 해석에 주의해야 함을 보였다. 한편, 비록 그 비중 및 빈도는 낮았지만 경제적으로 중요한 식물 병원균과 식용버섯의 존재를 확인할 수 있었고 이를 통해 DNA 메타바코딩을 통한 모니터링의 가능성을 제시하였다.

세 번째 장에서는 시간적 변화가 진균 군집에 미치는 영향을 확인하였다. 기주 식물의 성장은 초기의 개척자 종들이 더 경쟁력 있는 후기 개척종으로 대체되면서 진균 군집의 구성과 기능에 영향을 준다. 진균 군집의 천이를 이해하기 위해 소나무 묘목을 3 년간 재배하여 진균 군집의

시간적 변화를 관찰하였다. 그 결과, 소나무 묘목의 연령이 진균 군집의 구조에 유의한 영향을 주었으며 묘목이 성장하면서 진균 군집의 다양성이 크게 증가하였다. 이 과정에서 PICRUST2 를 이용해 진균 군집의 기능 변화를 예측하였고 네트워크 분석을 통해 진균 군집의 상호작용에서 중요한 역할을 하는 핵심 종 (keystone species)을 확인하였다.

본 연구는 소나무와 연관된 진균 군집을 이해하기 위해 시간적, 공간적 거리에 따른 진균 군집의 변화를 관찰하였다. 이러한 연구 결과는 소나무 서식지의 보존과 조림 활동에 응용할 수 있을 것이다. 또한, 이 연구에서 확인된 NGS 분석 방법에 따른 진균 군집 분석 결과의 차이는 다른 목본 식물의 진균 군집 분석에서도 고려될 수 있을 것이다. 마지막으로, 이번 연구에서 국가생물종 목록에 포함되지 않은 다수의 미기록 분류군들을 확인하였으며 이는 추후 신종 및 미기록종 발굴에 참고할 수 있다. 본 논문에는 학위 과정 중 투고한 논문의 원고를 포함하였다.

주요어: 소나무, 마이코바이옴, 시공간적 거리, 생물다양성, 뿌리 내 진균, 토양 내 진균

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