博士論文

Distribution patterns and composition of ectomycorrhizal fungi: evaluating the effects of spatial distance, environmental factors, and hosts

（外生菌根菌の分布と群集構造：距離、環境、および宿主樹木の影響）

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Supplementary material

List of ectomycorrhizal species and morphological images

Acronyms and abbreviations

BLAST	basic local alignment search tool
C/N	carbon/nitrogen
CTAB	cetyltrimethyl ammonium bromide
Cg	*Cenococcum geophilum*
EM	ectomycorrhizal
FDR	false discovery rate
INDS	international nucleotide sequence database
ITS	internal transcribed spacer
MAFFT	multiple alignment program for amino acid or nucleotide sequences
NMDS	nonmetric dimensional scaling
PCNM	principal coordinates of neighbor matrices
PCR	polymerase chain reaction
PCoA	principal coordinate analysis
RDA	redundancy analysis
RFLP	restriction fragment length polymorphism
SD	standard deviation
TBE	Tris/Borate/EDTA buffer

Abstract

1. Background

The distribution patterns of organisms provide fundamental knowledge of their ecology. Such information is essential in establishing conservation strategies under global environmental change. Although soil microorganisms play critical roles in carbon and nutrient cycles in ecosystems, their distribution patterns remain largely unknown mainly because of technical difficulties in examining microorganisms in the field. Ectomycorrhizal (EM) fungi are a functional group of soil fungi that establish obligate symbiotic associations with tree roots. EM fungi receive photosynthetically derived carbon from host trees while associated trees can improve water and nutrient uptake with the help of fungal mycelia that extend into soil. EM fungi are associated with many ecologically and economically important tree species that cover a large proportion of global forests, including Pinaceae, Fagaceae, Betulaceae, and Depterocarpaceae. These trees cannot survive without EM fungi under natural conditions. Thus, EM fungi are essential in forest establishment and functions. EM fungi are functionally and taxonomically diverse and >20,000 fungal species are estimated to form EM associations globally. Details of distribution patterns and community structures of EM fungi are essential to understand fungus-environment relationships and to predict how fungal communities respond to global environmental change.

The main objectives of this study are 1) to examine the distribution patterns of individual EM fungal species and community structures, and 2) to examine the relative importance of spatial distance, environmental factors, and host identity on EM fungal composition. These questions are addressed at various spatial scales, among forest types, and between fungal developmental stages (i.e., spores and existing EM roots).

Chapter 1 provides the background of this study. Chapter 2 examines EM fungal distributions and community structures at a stand scale (~1ha) and compares relative importance of predictor variables among seven forest types. Chapter 3 extends the analyses of distribution patterns and community structures to local to regional scales to infer how distance, environmental factors (climate and soil properties), and host identity affect the EM fungal composition. Chapter 4 investigates spore communities in soil which are compared with the community structures of EM fungi on existing tree roots. Chapter 5 provides a summary of this thesis and overall discussions. A supplementary material includes a list of all EM fungi recorded in this study and their morphological images.

2. Materials and Methods

Seven study sites were established in closed-canopy natural forests along elevation gradients on two mountains in Japan. Four sites were located on the northwestern slope of Mt. Fuji, Yamanashi (N35º E138º), and three were on the southern slope of Mt. Ishizuchi, Ehime (N33º

E133º). The highest sampling sites on Mt. Fuji (2250 m) and on Mt. Ishizuchi (1850 m) were located just below the treelines. The study sites were characterized by typical vegetation on the Pacific Ocean side of Japan. Fifty soil cores (5 × 5 cm to 10 cm depth) were collected from a 1-ha area at each site in 2011-2012. Litter depth and geographic coordinates were recorded at each sampling point. Soil pH, total carbon (C) and total nitrogen (N) of each soil sample were measured in the laboratory.

EM root tips were collected from each soil sample and classified based on morphological characteristics. Fungal DNA for each morphological type was extracted using the cetyltrimethyl ammonium bromide (CTAB) method. Polymerase chain reaction (PCR) was performed to amplify internal transcribed spacer (ITS) regions of the rDNA. PCR products were then subjected to direct sequencing. ITS sequences were aligned, manually edited, and clustered into molecular operational taxonomic units (hereafter 'species') at ≥97% similarities. Host genera of individual EM tips used for fungal identification were determined by using the trnL region of chloroplast DNA.

Fungal species in soil spore communities were investigated using bioassay experiments. Conifer (*Pinus densifolia*) and deciduous (*Salix reinii* or *Betula maximowicziana*) host seedlings were grown in 15 ml tubes containing soils collected in the field. Fifty bioassay seedlings per host per site were prepared (a total of 700 seedlings), and grown in a growth chamber for 5-6 months. EM fungi colonized on root tips were identified as described above.

The relative importance of predictor variables for fungal composition was examined using variation partitioning in redundancy analyses. Predictor variables included spatial distance, mean annual temperatures, mean annual precipitation, soil C/N, soil pH, litter depth, and host identity.

3. Results and discussion

In total, 454 EM fungal species were identified on existing root tips in 330 soil samples. This is among the highest EM fungal richness reported in a single study using similar identification approaches. The richness ranged from 55 to 113 with an average of 89 species per site. Estimated richness ± SD (using Chao 2 nonparametric estimator) was 475 ± 38.3 species on Mt. Fuji and 355 ± 40.6 species on Mt. Ishizuchi. Most fungal species belonged to lineages that typically dominate in temperate forests (/russula-lactarius, /tomentella-thelephora, and /cortinarius).

Overlaps of individual EM fungal species between sites mostly occurred at adjacent sites along elevation gradients (Figure 1). For example, 73 species occurred at multiple sites on Mt. Fuji, and 72 (99%) of them were shared between adjacent sites along the elevation. Similarly, 89% (33 of 38 species) of site-shared fungi occurred at adjacent sites on Mt. Ishizuchi. These results indicate that individual EM fungi have restricted distribution ranges, which may be determined by distance or environmental conditions associated with elevation. Furthermore,

analyses including both mountains revealed that most of the 47 mountain-shared species occurred in similar forest types of both mountains despite the geographic distance of 550 km. Therefore, EM fungal distributions may not be restricted by geographic distance at this spatial scale but constrained by contemporary environmental factors.

Host identity significantly separated EM fungal compositions in conifer-broadleaf mixed forests, and the strength of host effects increased with host phylogenetic diversity (fitted in a linear model: $R^2 = 0.97$, $P < 0.01$). EM fungal composition was positively correlated with tree composition (the Mantel test; $P = 0.04$) at the regional scale, suggesting that above- and belowground communities are closely interlinked. However, host identity alone did not explain variance in EM fungal composition while climate factors (temperature and precipitation; 13.3%), soil properties (5.2%), and geographic distance (4.7%) explained larger variance in EM fungal composition at the regional scale (Figure 2). These results imply that the host preferences among fungi may be detected at stand scales where climate and soil properties remain constant, but less likely at larger spatial scales at which other factors (i.e., climate) become more influential. Therefore, the observed correlation between forest tree and EM fungal compositions may not result from a causal relationship, but rather indicates both trees and EM fungi respond to climate factors synchronously but independently.

Twenty-nine EM fungal species were detected in spore communities on a total of 668 bioassay seedlings. The communities were composed of many pioneer fungal genera including *Rhizopogon*, *Laccaria* and *Scleroderma*, which were rarely found on the existing EM roots. Host identity significantly separated spore communities across sites, while site conditions (including both distance and environmental factors) were insignificant. Previous studies have suggested that germination of EM fungal spores may be triggered by compatible host roots. Thus, host identity may be critical for spore germination in pioneer fungi, which first establish symbioses with regenerating trees in post-disturbed habitats.

4. Conclusions

Microbial communities are usually composed of numerous rare species, which are inevitably overlooked with limited sampling efforts. Thus, demonstrating geographic distributions of microorganisms is especially challenging. In this study, I used an intensive and consistent sampling approach, which has rarely been applied in microbial studies, and obtained a relatively large EM fungal community dataset for each site. This approach effectively detected many species shared across sites, clearly confirming the existence of microbial species distribution ranges. The importance of climate factors in structuring EM fungal compositions implies that global climate change possibly affects the distributions and compositions of EM fungi, which play critical roles in forest establishment and functions.

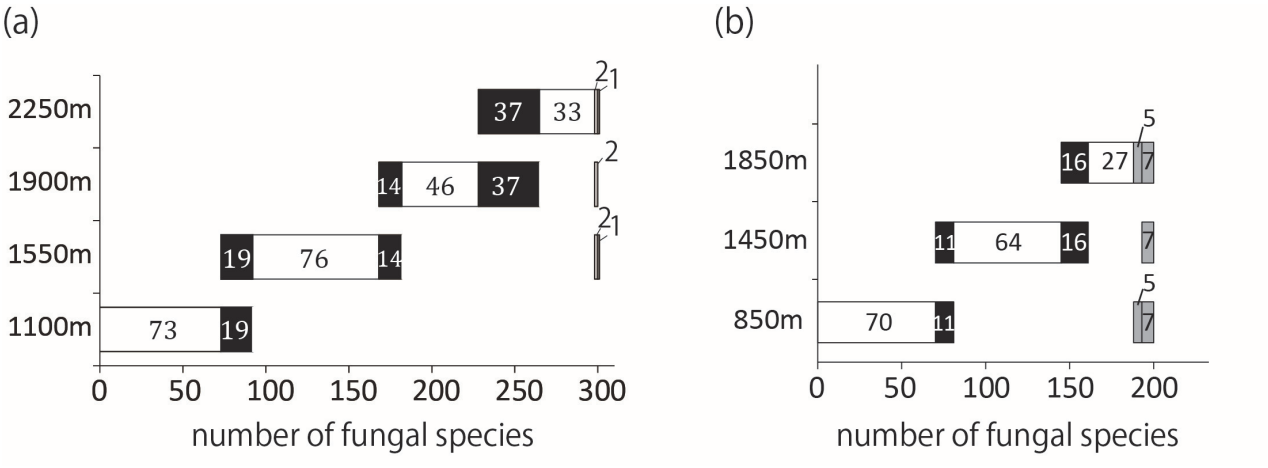


Figure 1 Overlaps of EM fungal occurrence along the elevation gradients on (a) Mt. Fuji and (b) Mt. Ishizuchi. Cumulative number of fungal species, from low to high elevation sites is shown. Values in the columns are the numbers of species. Open columns indicate site-specific species and closed columns indicate site-shared species between adjacent site pairs. Shaded columns at the right end indicate species that were found across multiple adjacent or two non-adjacent sites.

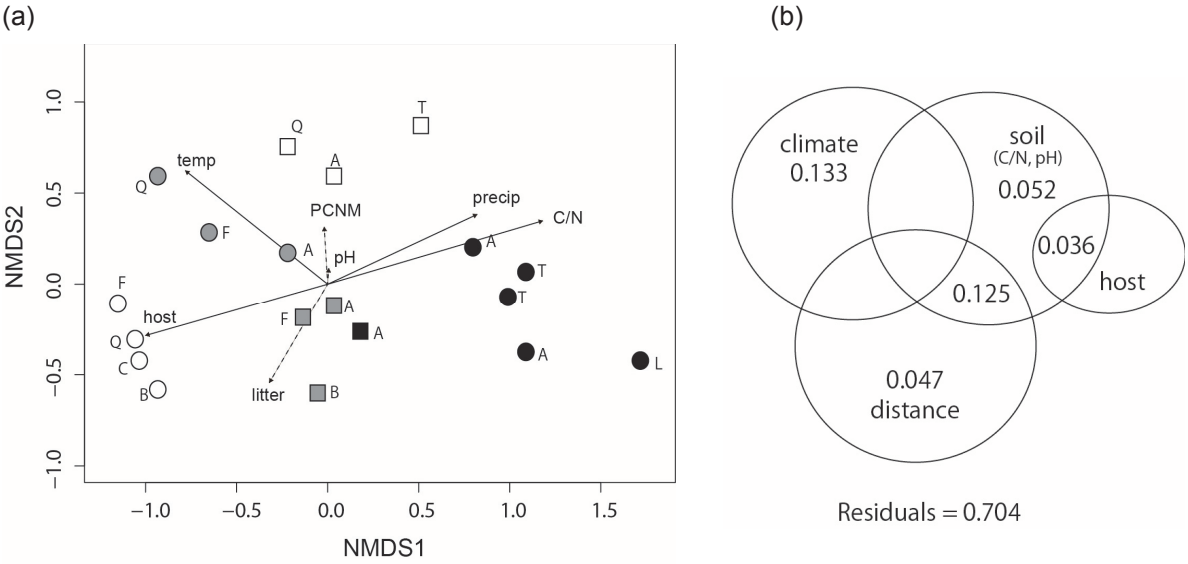


Figure 2 (a) Nonmetric multidimensional scaling (NMDS) graph of 19 ectomycorrhizal fungal communities. Circles and squares indicate sites on Mt. Fuji and Mt. Ishizuchi, respectively. Predictor variables are fitted to the NMDS ordination. The abbreviations are temp (temperature), precip (precipitation), host (host phylogenetic eigenvector), C/N (carbon/nitrogen), and PCNM (spatial principal coordinates of neighbor matrices eigenvector). Solid and dashed vectors are significant and insignificant variables, respectively. A letter near each symbol indicate host genus; *Fagus* (F), *Quercus* (Q), *Betula* (B), *Carpinus* (C), *Abies* (A), *Tsuga* (T), and *Larix* (L). (b) Individual and interaction effects of putative factors explaining fungal composition as revealed by variation partitioning in redundancy analysis.

Chapter 1 General introduction

1.1 Distribution patterns of organisms

The distribution patterns of terrestrial plants and animals have long been studied, and such information contributes to ecosystem management in the face of rapid biodiversity loss around the globe (Pimm *et al*. 2014). Knowledge of distribution patterns and species-environment relationships promotes development of management programs for endangered species, invasive species, and biological hotspots, and helps predict the responses of organisms to accelerated land use and environmental change (Guisan and Thuiller 2005). Although biological conservation of plants and animals is proceeding apace, natural communities of microorganisms have received much less attention largely because of technical difficulties and the lack of information on their ecology (Martiny *et al*. 2006). Details of distribution patterns and the mechanisms that regulate them are essential for the incorporation of microorganisms into conservation programs that are generally applied to plants and animals (Wardle *et al*. 2004). Soil microorganisms are among the most important groups of the biota in terrestrial ecosystems particularly because they play important roles in carbon, nutrient and water cycles. Microorganisms have traditionally been assumed to be cosmopolitan and exhibit no biogeographic patterns because of their small body masses, large population sizes, and long-distance dispersal abilities (the “Baas-Becking hypothesis”; de Wit and Bouvier 2006; O’Malley 2007). Advancement in molecular techniques has greatly improved identification and quantification of soil microorganisms in environmental samples. Recent studies suggest that soil microorganisms seem to exhibit biogeographic patterns that can be resulted from historical and contemporary environments (Martiny *et al*. 2006).

The distribution patterns of organisms and the factors that generate them often vary across spatial scales and ecosystems. Thus, relevant predictor variables should be selected before assessing organism-environment relationships at the scale of interests (Austin 2002).

Soil microorganisms probably respond directly to microhabitat conditions, such as water availability, nutrient concentrations, temperatures, pH, and soil textures (Lilleskov and Parrent 2007). These are called “proximal” variables, which may directly influence the physiological activities of organisms. Proximal variables likely operate as mechanisms regulating microbial distribution patterns at small spatial scales, but measuring these variables at larger spatial scales become challenging. In contrast, “distal” variables, such as latitude, longitude, elevation, and climate, are those that indirectly influence biological distribution patterns. Distal variables are more easily measured than proximal variables, and can therefore be useful predictors at scales of management interest, especially when they are highly correlated with proximal variables (Austin 2002).

1.2 Mycorrhizal symbioses

The mycorrhizal symbiosis is an obligate symbiotic association between plant roots and fungi that occurs ubiquitously in terrestrial ecosystems (Smith and Read 2008). About 85% of land plants form this association, which is among the most widespread mutualisms in nature (Brundrett 2009). Fungi receive photosynthetically fixed carbon and habitats from their plant hosts, while mycorrhizal plants can improve water and nutrient uptake (Smith and Read 2008), drought resistance (Alvarez *et al*. 2009), heavy metal tolerance (Adriaensen *et al*. 2004), and resistance to pathogen infections (Newsham *et al*. 1995). Fungi receive up to 40% of the carbon produced by host plants, while plants receive up to 90% of the soil phosphorous and nitrogen with the help of fungal hyphae that extend into soil (Horton and van der Heijden 2008).

An ectomycorrhiza is a form of mycorrhizal association in which fungal hyphae surround outside of plant cells and form a sheath-like structure, called “mantle”, around the root tips. Approximately 250 fungal genera (Tedersoo *et al*. 2010) and 20,000 species form

ectomycorrhizae (Brundrett 2009). Ecomycorrhizal (EM) fungi colonize on many ecologically and economically important trees that dominate forests worldwide, including Pinaceae, Fagaceae, Betulaceae, Salicaceae, Nothofagaceae, and Dipterocarpaceae. EM fungi contribute greatly to forest ecosystem functioning especially nutrient and carbon cycles (Hobbie 2006; Hobbie and Hobbie 2006). Indeed, trees cannot survive under natural conditions without EM fungi in most cases. Forests cover one third of the earth's land surface and provide many indispensable economic services to human society (e.g., timber products, fuels, and foods) and ecological benefits (e.g., carbon dioxide fixation, mitigation of global climate change, and provision of habitats to many organisms). Forests harbor hundreds of taxonomically and functionally diverse EM fungi (Read and Perez-Moreno 2003; Tedersoo *et al*. 2010), but the majority has not yet described (Hibbett *et al*. 2011). The basic ecology of EM fungi in natural ecosystems remain largely unknown, including distribution patterns of individual species, community structures, richness patterns and the adaptability of these fungi to environmental change.

1.3 Detecting fungal distributions

The advent of molecular techniques in the past two decades has enabled identification of fungi in field samples, revealing highly diverse EM fungal communities in forests. However, most EM fungi are observed rarely and sporadically, and fungal communities have often been incompletely described by inherently limited sampling efforts (Taylor 2002). A clear tradeoff exists between the number of samples collected and the number of sites surveyed. Extensive sampling methods (i.e., more sampling sites but fewer samples per site) tend to result in inventories that detect fewer species per site while the majority of species may remain undetected (Taylor 2002). These methods may be appropriate for broad observations of diversity patterns (Grytnes 2003; Bahram *et al*. 2012). Alternatively, intensive sampling

methods (i.e., fewer sampling sites but many samples per site) tend to provide more comprehensive inventories of EM fungi at a given site. This approach may be more suitable to detect species distribution patterns and community structures across study sites along environmental gradients.

"Gradient analysis" is used to characterize the strength of predictor variables among ecosystems along any environmental gradient, and has been mostly applied for terrestrial organisms. This analysis is particularly useful for sessile organisms that are adapted to the local conditions, and would be appropriate for studying microbe-environment relationships (Bryant *et al*. 2008; Wang *et al*. 2011). Elevation gradient provides a natural experimental setting to study biological responses to environments (Körner 2007). Biological patterns can be observed along wide environmental ranges within narrow geographical areas, without confounding effects of geographic locations and history. Elevation gradients have been widely used to study the distribution patterns of plants and animals, but equivalent studies on microorganisms are rare. For example, Bryant *et al*. (2008) have shown that richness patterns along an elevation gradient differ between trees and soil bacteria. Bahram *et al*. (2012) reported that EM fungal richness decreases with elevation. These studies clearly demonstrated richness-elevation relationships based on small sampling sizes per site, but species distribution and composition patterns remained less clear. EM fungal compositions have yet to be compared across a wide range of environments encompassing various forest types. Applying intensive samplings along elevation gradients may provide novel insights into the distribution patterns of EM fungi.

1.4 Objectives and outline of this thesis

In this study, I examined the distribution patterns and composition of EM fungi and determined predictor variables generating such patterns at various spatial scales, among forest

types, and between fungal developmental stages. I intensively and systematically sampled EM fungal communities from seven forest stands spanning a wide climate range along elevation gradients on two mountains. Each elevation gradient spanned most of the typical forest types in a temperate region of Japan, ranging from evergreen temperate to subalpine conifer forests within a short geographical distance (Figure 1.1). I evaluated the relative importance of spatial distance, soil properties, climate factors, and host identity.

Chapter 2 focuses on the distribution patterns of individual fungi at a stand scale. I visualized the shape and degree of the fungal distribution patterns within each stand and tested if individual fungi exhibited any aggregated distributions. Chapter 3 examines range overlaps of fungal species among sites along elevation gradients and between mountains. Chapter 3 further examines the relationships between tree and fungal compositions to address links between above- and belowground communities. Chapter 4 examines the community structure of spores, which have many ecologically important roles, including dispersal and enhancement of genetic variability. Chapter 5 summarizes key findings of this study and provides general discussions. A supplementary material provides a list of all EM fungi recorded in this study and their morphological images.

Mt. Fuji

Warm temperate deciduous forest
(1100 m)

Cool temperate mixed forest
(1550 m)

Subalpine conifer forest
(1900 m)

Subalpine conifer forest
(2250 m)

Mt. Ishizuchi

Warm temperate mixed forest
(850 m)

Cool temperate mixed forest
(1450 m)

Subalpine conifer forest
(1850 m)

Figure 1.1 Seven study sites in various forest types along elevation gradients on two mountains. Elevation is shown in the parentheses.

Chapter 2 Spatial structures of ectomycorrhizal fungi at the stand scale

2.1 Introduction

Spatial structures have been increasingly recognized as common phenomena in biological community at all spatial scales from micrometers to global. Spatial autocorrelation is a statistical property describing patches and gradients across geographic space (Legendre 1993). Spatial patterns may result from the responses of organisms to resource availability ('niche processes'; Cottenie 2005) or from biological processes such as dispersal limitation ('neutral processes'; Hubbell 2001). Detecting spatial structures helps to clarify the underlying mechanisms generating distribution patterns of individual species and a community as a whole. Spatially autocorrelated data also violate the assumption of independence in standard statistical procedures, which may mislead the interpretation of observed patterns when testing the effects of environmental factors (Legendre 1993).

The distribution patterns of belowground soil fungi are technically difficult to examine. Several studies have investigated the distribution patterns of ectomycorrhizal (EM) fungi through systematic core samplings, showing vertical and horizontal patchiness for some EM fungal species at centimeter to meter scales (Genney *et al*. 2006; Pickles *et al*. 2009). Molecular genetic methods have also been used to assess genet sizes of a few target EM fungal species in soil (Redecker *et al*. 2001; Lian *et al*. 2006; Carriconde *et al*. 2008). Genet sizes of EM fungi are highly variable among species and may range between 1-10m^2 for some typical late-stage EM fungal species in temperate forests (Redecker *et al*. 2001). However, evaluating the distribution patterns of many individual species concurrently at larger spatial scales is difficult.

Similarities in fungal composition tend to decrease with geographic distance ('distance-decay'; Bahram *et al*. 2013a). A global scale meta-analysis reported that nearly half

(7 of 16) of the EM fungal communities studied were spatially autocorrelated, although the degrees and rates of distance-decay varied considerably among ecosystems (Bahram *et al*. 2013a). For example, spatial autocorrelations occurred within 4-m distance scales in EM fungal communities of boreal forests (Lilleskov *et al*. 2004; Pickles *et al*. 2009, 2012), and extended to 150 m in various studies around the globe (Bahram *et al*. 2013a). Stand scale spatial structures of EM fungal communities are often investigated in homogeneous forests where other variables remain relatively constant (Lilleskov *et al*. 2004; Pickles *et al*. 2009, 2012). Conversely, many studies have investigated the effects of environmental factors, such as hosts (Ishida *et al*. 2007; Tedersoo *et al*. 2008), soil nutrients (Toljander *et al*. 2006; Twieg *et al*. 2009), climate (Bahram *et al*. 2012) and stand age (Tweig *et al*. 2007), on EM fungal compositions in more heterogeneous sites, often without explicitly considering spatial autocorrelations. Biotic and abiotic factors generally vary along spatial gradients; thus, spatial relationships must be explicitly and simultaneously tested to accurately predict the effects of environmental factors on community structures.

Both distance and environmental factors may influence soil microbial communities interactively, and the strengths of effects may differ (Dumbrell *et al*. 2010). Moreover, the relative strengths of predictor variables will likely differ among sites because of different resource limitations (Neilsen *et al*. 2012). For an extreme example, niche differentiation of EM fungi among hosts is possible in forests composed of ≥ 2 host taxa but not in monoculture forests, where other factors (e.g., dispersal limitation and soil properties) may become more influential on fungal composition. Few studies have compared the relative strengths of spatial distance and environmental factors across various forest types using consistent sampling methods. Gradient analyses may provide the shift in the strengths of various predictor variables influencing EM fungal communities.

This study aimed to examine the distribution patterns of individual EM fungal species

and compositions and to determine factors that generate them at the stand scale. I compared the relative importance of spatial distance, environmental factors and host among various forest types located along elevation gradients using a consistent sampling method. I tested a hypothesis that spatial distance is more important in environmentally homogeneous forests (Pickles *et al*., 2009) whereas niche differentiation is more important in environmentally and taxonomically diverse forests (Toljander *et al*. 2006; Ishida *et al*. 2007).

2.2 Materials and methods

Study sites

Field sampling was conducted on the northwestern slope of Mt. Fuji (Yamanashi Prefecture) in 2011, and on the southern slope of Mt. Ishizuchi (Ehime Prefecture) in 2012 (Table 2.1). The mountains were selected using the following criteria: 1) natural forests with minimum human disturbance along the elevation gradients, 2) the presence of distinct forest types, and 3) accessibility to study sites. The study region has a temperate climate with warm, wet summers and cool, moderately dry winters. Four and three study sites were established on Mt. Fuji and Mt. Ishizuchi, respectively (Table 2.1). All sites were located in closed-canopy forests. The highest sampling sites on Mt. Fuji (2250 m) and on Mt. Ishizuchi (1850 m) were located just below treelines.

The tree species compositions in the studied stands comprised typical vegetation on the Pacific Ocean side of Japan (Appendix A). Site F1 was characterized by a deciduous forest with *Quercus crispula*, *Fagus crenata*, *F. japonica*, *Carpinus tschonoskii* and *Betula grossa*. Sites F2 and I2 were characterized by mixed forests dominated by *F. crenata* and *Abies homolepis*. Site I1 was a mixed forests dominated by evergreen *Quercus salicina*, *A. homolepis* and *Tsuga sieboldii*. Sites F3, F4 and I3 were subalpine coniferous forests exclusively dominated by Pinaceae species. Dominant trees included *Abies veitchii* at Sites F3

and I3 and *Tsuga diversifolia* at Site F4.

Sampling

Fifty rectangular soil cores (5 × 5 cm to 10-cm depth) were collected from a 1-ha area at each site. Soils at Site I3 were collected from two forest patches (180 m apart) because the forest was fragmented at the treeline. A distance of 5–10 m was maintained between soil sampling locations to ensure independence of the samples (Lilleskov *et al*. 2004; Pickles *et al*. 2009). Soil cores were stored separately in plastic bags at 4 ºC until processing. A vegetation survey was conducted at every other soil sampling location. Tree species and diameter at breast height (1.3 m) were recorded for all living trees (>1.3 m tall) within a 5-m radius of the sampling point. Litter depth and geographic coordinates (recorded with a Garmin 62S, Garmin International, Olathe, KS, USA) were recorded at each sampling point.

Molecular analyses

All roots were carefully collected from each soil sample. EM root tips were classified by their morphological characteristics, including surface color, texture of the mantle surface, emanating hyphae, and rhizomorphs. Healthy EM root tips (n = 1–3) were sampled from each morphotype of each core for molecular analyses. Morphotyping was completed within 3 weeks after soil sampling.

Fungal DNA was extracted from root tips using the cetyltrimethyl ammonium bromide (CTAB) method. Polymerase chain reaction (PCR) was performed to amplify internal transcribed spacer (ITS) regions (ITS1, 5.8S, ITS2) of the rDNA mainly using the forward primer ITS1F and various reverse primers, i.e., ITS4, LR21, and LR22, depending on their amplification success with each morphotype (Appendix B). The amplified PCR products were checked on 1.2% agarose gels (0.5× TBE buffer) and visualized under UV light to examine

the quality and quantity of amplicons. Restriction fragment length polymorphism (RFLP) patterns were compared among replicates of each morphotype using *Hinf*I and *Alu*I restriction enzymes. All PCR products with unique RFLP types in each morphotype were purified and subjected to direct sequencing (3730xl DNA analyzer; Applied Biosystems, Foster City, CA, USA). Sanger sequencing was conducted primarily using primer ITS1. Another sequencing primer, ITS4, was additionally used for poorly sequenced samples.

High-quality ITS sequences >350 bp in length were aligned, manually edited, and clustered into molecular operational taxonomic units (hereafter 'species') at ≥97% similarity (Izzo *et al*. 2005) using ATGC ver. 7 software (Genetyx Corp., Tokyo, Japan). The sequences were compared with known sequences in the international nucleotide sequence database (INSD); species names were assigned based on the taxonomy of those with which they shared the highest homology in the Basic Local Alignment Search Tool (BLAST) searches. Sequences that showed high homology with saprophytic and parasitic fungi or with nonfungal sequences were excluded from subsequent analyses. *Cenococcum geophilum* (hereafter 'Cg') is a species complex that is not well classified solely by its variability in ITS regions (Douhan and Rizzo, 2005). Thus, Cg was identified primarily by its unique morphology, as in many previous studies (e.g. Twieg *et al*. 2007; Murata *et al*. 2013).

Host trees associated with EM fungal species were identified to genus based on RFLP patterns using *Hinf*I and *Alu*I restriction enzymes. The *trn*L intron of chloroplast rDNA was amplified using primer pairs *trnC*-*trnD* or *trnE*-*trnF* (Taberlet *et al*. 1991; Murata *et al*. 2013). RFLP patterns of root-tip samples were then compared with those obtained from leaves of host species identified in the field. Direct sequencing was applied to samples with unclear RFLP patterns. Although *Tilia* is a potential EM fungal host (Smith and Read 2008), I did not treat *Tilia* as an EM host species because *Tilia* was not detected in any EM roots examined.

Soil data collection

Soil samples were air dried and passed through a 1-mm sieve, and pH was measured from a 5-g air-dried soil sample in a 1:5 ratio with Milli-Q water (Millipore, Billerica, MA, USA) using a HM-25G glass electrode (DKK-TOA Corp., Tokyo, Japan). The soils were further sieved through a 250-μm screen and total carbon (C) and total nitrogen (N) were measured by dynamic flash combustion using a CN Analyzer (Flash EA 1112; AMCO Inc., Tokyo, Japan). C/N in each soil sample was then calculated.

Statistical analyses

Estimated species richness was computed for each site and for each mountain using Chao2 nonparametric estimator (Chao 1984) implemented in the EstimateS software ver. 8.20 (Colwell *et al*. 2012) with 1000 randomizations without replacement. Statistical analyses were conducted using R ver. 3.0.3 (R Development Core Team 2013). Statistical significance was set at $P = 0.05$ unless otherwise noted.

Species that occurred in > 3 soil cores were used to examine the distribution patterns of individual EM fungal species. Randomization procedures were applied to individual EM fungal species to test the statistical significance of spatial aggregation patterns. Euclidean distances between randomly selected sampling points (geographical coordinates) were computed 9999 times to generate reference distributions. The observed value for each species was computed as the Euclidean distance between points that contained the focal species. The probability of obtaining values that were lower than or equal to the observed value was subsequently estimated from the reference distributions. This procedure was repeated 10 times and an average probability was calculated for each species. Fisher's exact tests were conducted for individual EM fungal species to compare frequencies among host genera. A binomial logistic regression model (glm function in the stats package) was applied to examine

the effect of soil variables (pH, C/N, and litter depth) on the presence of fungal species that showed a spatial aggregation pattern. Soil variables were log-transformed prior to analyses.

The presence-absence data for EM fungal species per soil core per host root were treated as sample units in the analyses of community structure. A community matrix was built for each site. Principal coordinates of neighbor matrices (PCNM) analysis was applied to capture spatial structures of EM fungal composition at various scales (Borcard and Legendre 2002; Legendre and Legendre, 2012). The PCNM is a spatial modeling method that computes spatial eigenvectors from a modified geographic distance matrix using an ordination technique (a principal coordinate analysis [PCoA]). Extracted PCNM vectors are orthogonal and indicate positive spatial correlations between sampling points at a wide range of spatial scales. The PCNM vectors are used as spatial explanatory variables in multiple regression or redundancy analysis (RDA). I calculated a Euclidean distance matrix of geographical coordinates between sampling points to construct PCNM vectors for each site separately using the PCNM package. Truncated distance was determined as the longest distance between two points of the minimum spanning tree of the distance matrix. Forward selection (implemented in the packfor package) was subsequently used to identify significant PCNM vectors associated with EM fungal composition based on 999 permutations (Borcard *et al*. 2011). The explanatory variables were selected on the basis of R^2 values. Significant PCNM vectors were then regressed against log-transformed soil variables (pH, C/N and litter depth) to examine potential associations between soil variables and spatial structures of fungi. RDA (using the vegan package) was conducted to test whether EM fungal composition was associated with soil variables.

Host identity was measured as the eigenvectors computed from phylogenetic distance among the seven host genera, according to Tedersoo *et al*. (2013). Plastid matK and trnL gene sequences were downloaded from the INSD or generated during the molecular host

identification procedures (Appendix C). These sequence regions sufficiently distinguished host genera, whereas they were identical at the species level. The sequences for each region were aligned using MAFFT ver. 7.147 with the iterative refinement method (L-INS-i algorithm; Katoh and Toh 2008). A phylogenetic tree was constructed using trnL-matK by applying maximum likelihood algorithms with a general time reversible model and 500 bootstrap replicates using the MEGA ver. 6.0 software (Tamura *et al*. 2013). Pairwise patristic distances (pairwise sum of the branch length connecting two terminal taxa) were then calculated using the ape package of R (Paradis *et al*. 2004). The pairwise patristic distance matrix was converted to phylogenetic eigenvectors using PCoA. PCoA vectors are orthogonal and represent phylogenetic relations among host trees and were used in RDA. Significant eigenvectors were forward selected prior to analyses. Host phylogenetic distance of a site was calculated as the mean pairwise branch length between hosts occurring at each site. The strength of host effects was regressed on the phylogenetic distance of sites.

2.3 Results

General descriptions of EM fungi

Tree roots colonized by EM fungi were found in 330 of the 350 cores. In total, 4464 root tips were collected for species identification. Excluding Cg, 3030 of 3805 root tips were successfully identified to EM fungal species through RFLP and sequencing analyses. I identified 454 EM fungal species, including 225 singletons (49.6% of the total) and 89 doubletons (19.6%) (Table 2.2; Supplementary material). The mean length of sequences was 576 bp and 86.6% of the species (393 of 454) were longer than 500 bp. Rarefaction curves of observed richness did not reach an asymptote at any site, whereas estimated (Chao 2) richness became nearly asymptotic in most sites (Figure 2.1). Chao2-estimated richness ± SD were 475 ± 38.3 species on Mt. Fuji and 355 ± 40.6 species on Mt. Ishizuchi. The most frequently

observed “lineages” (monophyletic groups of the EM fungal genera defined by Tedersoo *et al*. 2010) included /russula–lactarius (85 species), /tomentella–thelephora (82), and /cortinarius (71). Most species were Basidiomycetes; Ascomycetes (28 species or 6.2%) were a minor component (Figure 2.2). The most frequently observed species was Cg (232 of 350 soil cores; 66.3%), followed by *Xerocomus* sp.2 (30 cores; 8.6%), *Clavulina castaneipes* (27 cores; 7.7%), and *Russula bicolor* sp.1 (20 cores; 5.7%). Pearson linear correlation analysis showed a positive relationship between EM fungal richness (Chao 2) and belowground host genus richness ($r = 0.83$, $t_5 = 3.34$, $P = 0.021$).

Cg occurred at all seven sites. Exceptionally abundant Cg, which likely contained some cryptic species (Douhan and Rizzo 2005), and singleton species were removed from further analyses to improve analytical accuracy.

Individual fungal taxa

Thirteen of 98 individual fungal species examined (13.4%) showed significantly aggregated distribution patterns more than expected by chance at <5% based on the randomization procedures (Figure 2.3). The binomial logistic regression model showed that Cg at I2 occurred preferentially in soil cores with low pH values ($z = –2.21$, $P = 0.03$). Fisher’s exact tests revealed that 10 of 122 tested EM fungal species exhibited significant host preferences (Table 2.3).

Driving forces of composition

RDA showed that spatial eigenvectors (PCNM vectors) were correlated with EM fungal compositions in five of seven stands (Figure 2.4). Host phylogeny was significant in all conifer-broadleaf mixed forests (Sites F2, I1 and I2). Soil C/N was also correlated with EM fungal compositions at Sites F2 and I3. The significant PCNM vector at Site F2 was also

linearly correlated with soil C/N. Spatial distance generally had a higher explanatory power (measures as adjusted R^2) than host identities or soil variables (Figure 2.4). The strength of host effects increased with phylogenetic distance of hosts within stands (Figure 2.5).

2.4 Discussion

General descriptions of EM fungal species

I recorded 454 EM fungal species in 330 soil samples from seven sites along two elevation gradients in the temperate region. This is among the highest EM fungal richness reported in a single study. Using similar identification approaches, Tedersoo *et al*. (2011) found 326 species from four sites in Africa, Bahram *et al*. (2012) detected 367 species from 261 soil samples in Iran, and Murata *et al*. (2013) reported 136 species from 100 samples in Japan. Most fungal species recorded in this study belonged to lineages that typically dominate in temperate forests (/russula-lactarius, /tomentella-thelephora, and /cortinarius; Tedersoo and Nara 2010).

Individual fungal species

I applied randomization procedures to examine whether EM fungal species within a stand (~1ha) exhibited aggregated distribution patterns in various forests. I found that 13 of 98 species (13.4%) showed aggregated distribution patterns from the entire study sites, ranging from 5.5% (F2) to 18.7% (F4) of tested fungal species. The shape and degree of aggregation highly varied among species (Figure 2.3). Previous stand scale studies have indicated that the scale of aggregation for most individual species is < 4 m (Lilleskov *et al*. 2004; Pickles *et al*. 2012). My results suggest that EM fungi exhibit spatial aggregations more commonly and at greater extent than previously anticipated.

The aggregated distribution patterns in some fungal species may be explained by either niche-related processes or by fungal internal factors. The occurrence of Cg (I2) was related to

soil pH, and *Piloderma fallax* sp.2 showed host preferences (Table 2.3). Thus, soil factors and host identity may explain some spatial aggregation patterns. However, most species (11 of 13 species) exhibiting aggregated patterns were not associated with hosts or soil environmental factors. Therefore, spatial structures may be partly explained by fungal internal processes (i.e., dispersal limitations via spores or genet expansion; Redecker *et al*. 2001; Peay *et al*. 2010a, 2012). Unmeasured variables, such as microclimate, interspecific competition, and root availability, may also influence fungal distribution patterns (Pickles *et al*. 2012).

The randomization procedure was effective to assess the aggregation patterns of individual fungi gathered with a relatively simple soil collection method. However, accurate detection of fungal distribution patterns remains challenging because sampling all of the soil in a forest remains intractable. Moreover, it is unclear whether the fungal aggregates detected comprised single or multiple genotypes because genets were not identified in this study.

Compositions

Spatial eigenvector analyses (PCNM method) detected significant spatial structures in EM fungal composition at five of seven sites, suggesting that the compositions were spatially structured at the stand scale in many forest types, including highly diverse mixed-forests and low diverse Pinaceae-dominated forests. A previous global meta-analysis reported the distance-decay in EM fungal community structures in homogeneous forests dominated by either one family or two closely related families of trees (Fagaceae and Betulaceae; Bahram *et al*. 2013a). I provided further evidence of spatial structures in more heterogeneous forests. Because spatial distance had greater effects than either host identities or soil properties at many sites, EM fungal community structures were more likely determined by internal than external factors. It is still unclear the mechanism driving spatial structures because large variances remained unexplained. Many studies on EM fungal communities have attempted to

avoid spatial autocorrelations by increasing distances between sampling locations. However, the effects of environmental factors can be overemphasized if spatial distance is not incorporated in explaining community structures (e.g., Toljander *et al*. 2006).

Niche differentiation among host trees may be more pronounced in heterogeneous forests than in forests that are more homogeneous. A previous study in a mixed forest (Ishida *et al*. 2007) and a meta-analysis (Dickie 2007) predicted that EM fungal composition would separate among phylogenetically distant hosts. I compared host effects along a gradient of host phylogenetic distance using consistent sampling methods, and provide data that explicitly support this prediction. Significant host effects were found in all three stands that were composed of angiosperm and gymnosperm hosts, and the strength of the effect increased with increasing host phylogenetic distance (Figures 2.4, 2.5). Most EM fungi are thought to be generalists that occur on multiple hosts (Bruns *et al*. 2002), whereas some EM fungal genera are restricted to distinct host groups (Molina *et al*. 1992). Although not specifically host specialists, some EM fungal species exhibit strong host preferences (Smith *et al*. 2009). High host taxonomic diversity likely promotes habitat complexity by increasing diversities in root morphology (e.g. densities and structures; Burton *et al*. 2000), root exudates (Duddridge 1987), litter types and qualities (Aponte *et al*. 2010) and phenology (Gange *et al*. 2007), allowing many fungal taxa to coexist within a stand (Hooper *et al*. 2000, Waldrop *et al* 2006).

Soil C/N appeared to separate fungal compositions at Sites F2 and I3 (Figure 2.4). Soil characteristics would be important at sites where the effects of other factors (hosts and distance) were minor; e.g., at Site I3, which was dominated by a single host. However, the effects of soil variables on EM fungal composition were relatively minor at most sites, probably because soil factors were not strongly variable within the stands. Previous studies have reported significant soil effects along steep nutrient gradients (Lilleskov *et al*. 2002; Toljander *et al*. 2006; Cox *et al*. 2010) and in habitats with distinctly different moisture levels

(Walker *et al*. 2005) and soil types (Peay *et al*. 2010b). However, my results agree with previous reports indicating that soil factors (i.e., C/N, pH, nutrients) had little impact on EM fungal communities in natural forests with moderate range of soil variability (Twieg *et al*. 2009).

My results supported the hypothesis that host effects on EM fungal composition became stronger in taxonomically diverse forests. However, I found that spatial distance was important in both environmentally homogeneous and heterogeneous sites. Moreover, the influence of spatial distance on fungal compositions was stronger than that of host identity or soil properties at most sites. Thus, spatial autocorrelations should be examined before we attempt to accurately predict the effects of environmental factors on fungal compositions in various forests.

Table 2.1 Description of study sites.

Location	Mt. Fuji				Mt. Ishizuchi		
Site	F1	F2	F3	F4	I1	I2	I3
Elevation (m)	1100	1550	1900	2250	850	1450	1850
Coordinates	N35°27′ E138°38′	N35°25′ E138°41′	N35°23′ E138°41′	N35°23′ E138°42′	N33°44′ E133°07′	N33°44′ E133°09′	N33°46′ E133°07′
slope (°)	5	5	19	14	36	26	27
Temperature (°C) [a]	9.2	6.3	5.5	3.6	9.4	6.2	4.6
Precipitation (mm) [a]	1883	2315	2734	2737	2823	3137	2806
Tree density (stems per ha)	1636	886	1462	1477	2363	2450	963
Tree basal area (m^2 per ha)	55.8	72.0	49.5	93.7	46.9	39.1	33.2
Number of tree species	25	25	5	6	34	25	10
Number of host tree species	9	8	5	5	4	4	3
pH (H_2O) [b]	4.1-5.1 (4.6)	4.4-5.8 (5.1)	3.9-5.5 (4.6)	4.0-5.5 (4.9)	3.5-5.5 (4.1)	3.4-4.7 (3.8)	3.4-4.8 (3.8)
C/N [b]	10.7-20.2 (13.6)	11.3-21.1 (15.2)	5.4-34.3 (22.7)	12.6-30.5 (22.1)	11.9-36.9 (18.3)	9.0-20.8 (15.6)	6.8-21.2 (17.3)
Litter (cm) [b]	2.0-8.0 (4.6)	1.5-5.0 (3.1)	0.5-5.5 (2.8)	2.0-6.0 (3.5)	0-5.0 (2.4)	0.5-5.0 (1.9)	0.5-4.0 (1.7)

[a] Site-specific mean annual temperature and mean annual precipitation obtained from the interpolated mesh data (1×1 km^2; 30-year averages from 1981 to 2010) provided by the Japan Meteorological Agency (2014).
[b] Values are minimum–maximum with the mean in parentheses.
Stand characteristics are based on the aboveground tree survey.
Abbreviation: C/N, carbon/nitrogen.

Table 2.2 Summary of ectomycorrhizal fungal diversity.

Site	F1	F2	F3	F4	I1	I2	I3
Mean richness per soil core	3.9	5.2	5.3	4.4	4.0	5.6	3.5
Observed richness	93	113	100	74	93	98	55
β-diversity[a]	23.8	21.7	18.9	16.8	23.3	17.5	15.9
Proportion of singleton species	0.62	0.62	0.54	0.49	0.71	0.57	0.49
Estimated richness (Chao2)	177	236	186	155	221	203	96
Shannon's diversity index	4.1	4.2	4.0	3.7	4.2	4.0	3.5
Simpson's diversity index (1/D)	44.7	28.2	24.0	19.5	61.7	32.6	22.1

[a] β-diversity was calculated as observed richness divided by the mean richness per soil core.

Table 2.3 List of fungal species that showed significant host preferences revealed by Fisher's exact test ($P < 0.05$) and their frequency of occurrence (number of cores).

Site	Species	Host (occurrence)	*p* -value
F1	*Lactarius* sp.2	*Fagus* (6)	0.010
F2	*Entoloma* sp.1	*Abies* (1), *Betula* (1), *Fagus* (6), *Quercus* (4)	0.032
	Piloderma fallax sp.2	*Abies* (3), *Betula* (2)	0.024
	Tomentella stuposa sp.1	*Abies* (3), *Picea* (1), *Betula* (1)	0.026
F3	*Amanita* sp.1	*Tsuga* (3)	0.015
	Russula densifolia	*Abies* (1), *Tsuga* (3)	0.039
F4	*Lactarius vietus*	*Abies* (3)	0.030
I1	Boletaceae sp.1	*Abies* (6)	0.030
I2	*Lactarius tabidus*	*Abies* (3), *Betula* (7), *Fagus* (5)	0.001
	Sebacina sp.1	*Abies* (2), *Betula* (2), *Fagus* (1)	0.028

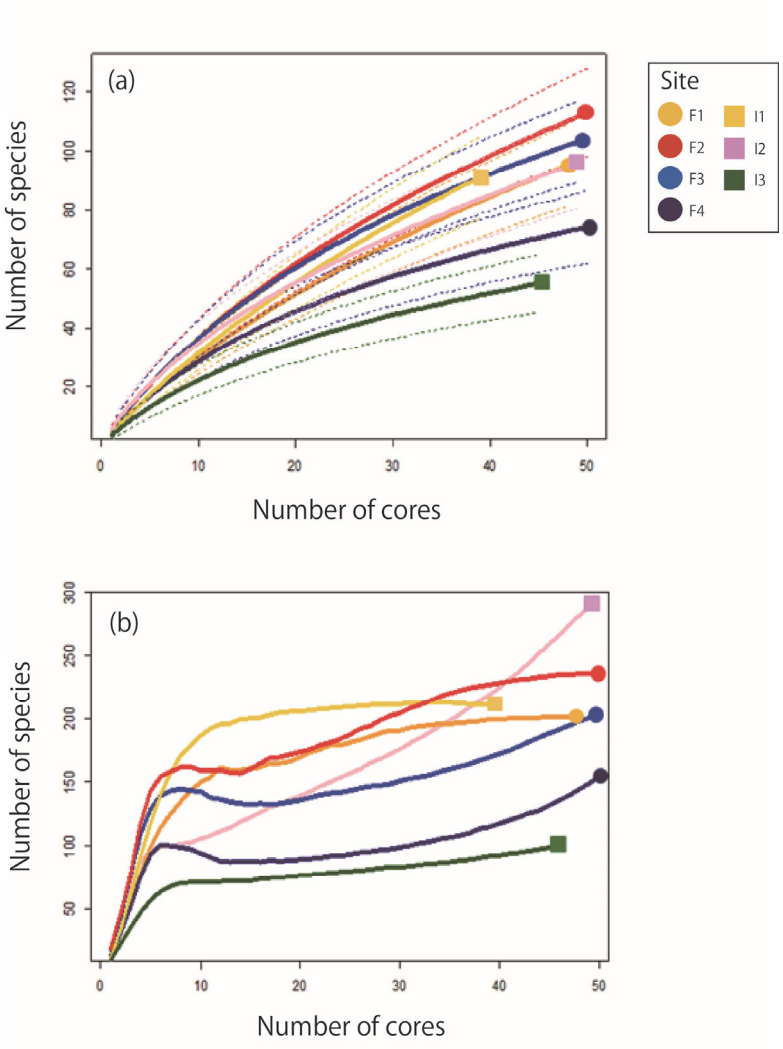


Figure 2.1 Rarefaction curves. (a) Observed richness (solid lines) and standard deviation (dotted lines). (b) Chao 2 nonparametric estimated richness.

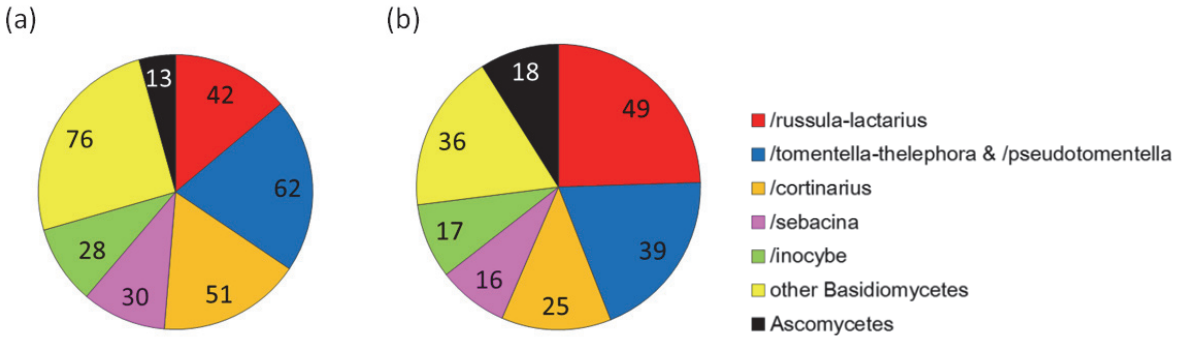


Figure 2.2 Number of fungal species assigned to ectomycorrhizal fungal lineages on (a) Mt. Fuji and (b) Mt. Ishizuchi. Fungal lineages are based on Tedersoo *et al*. (2010).

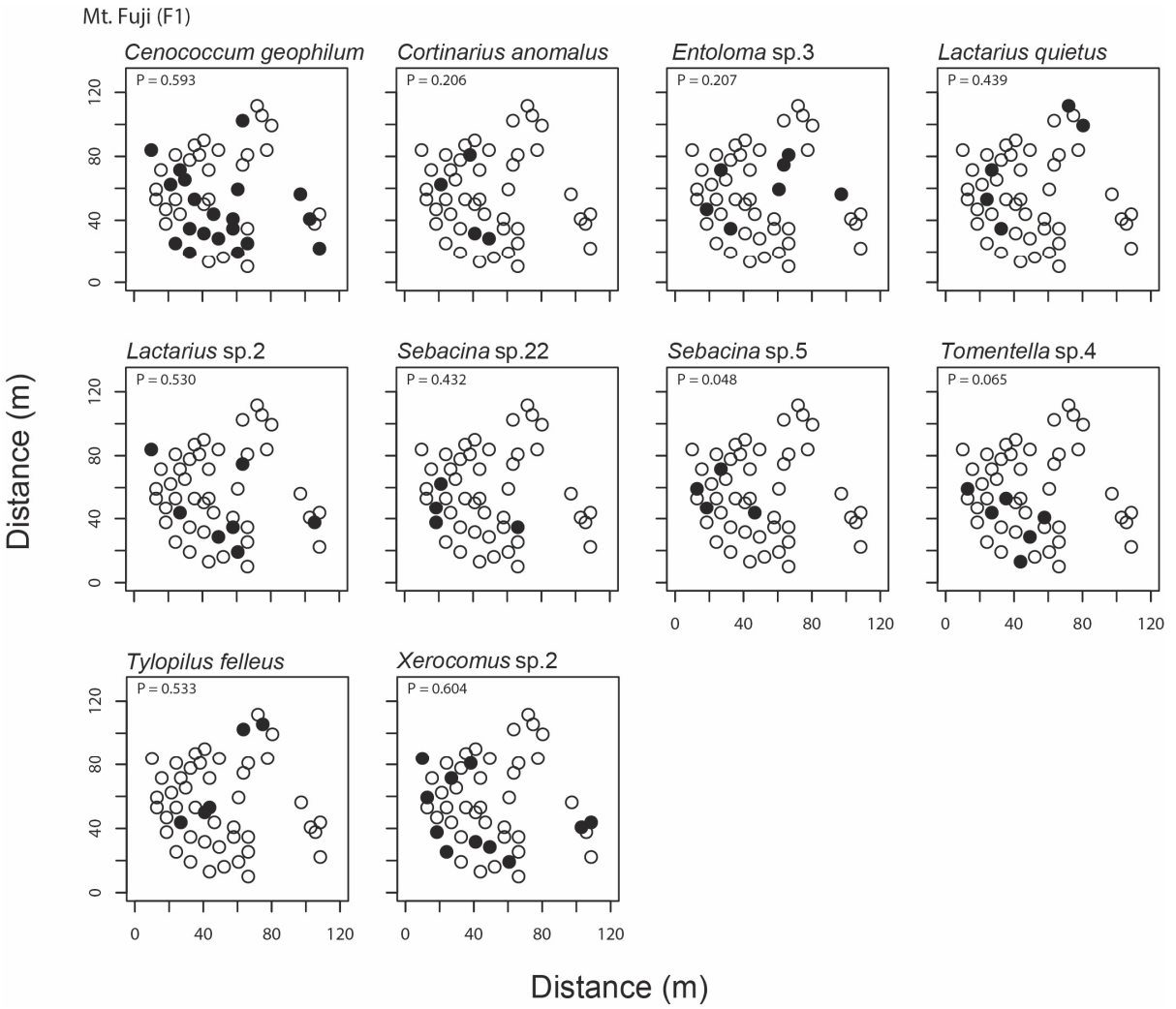
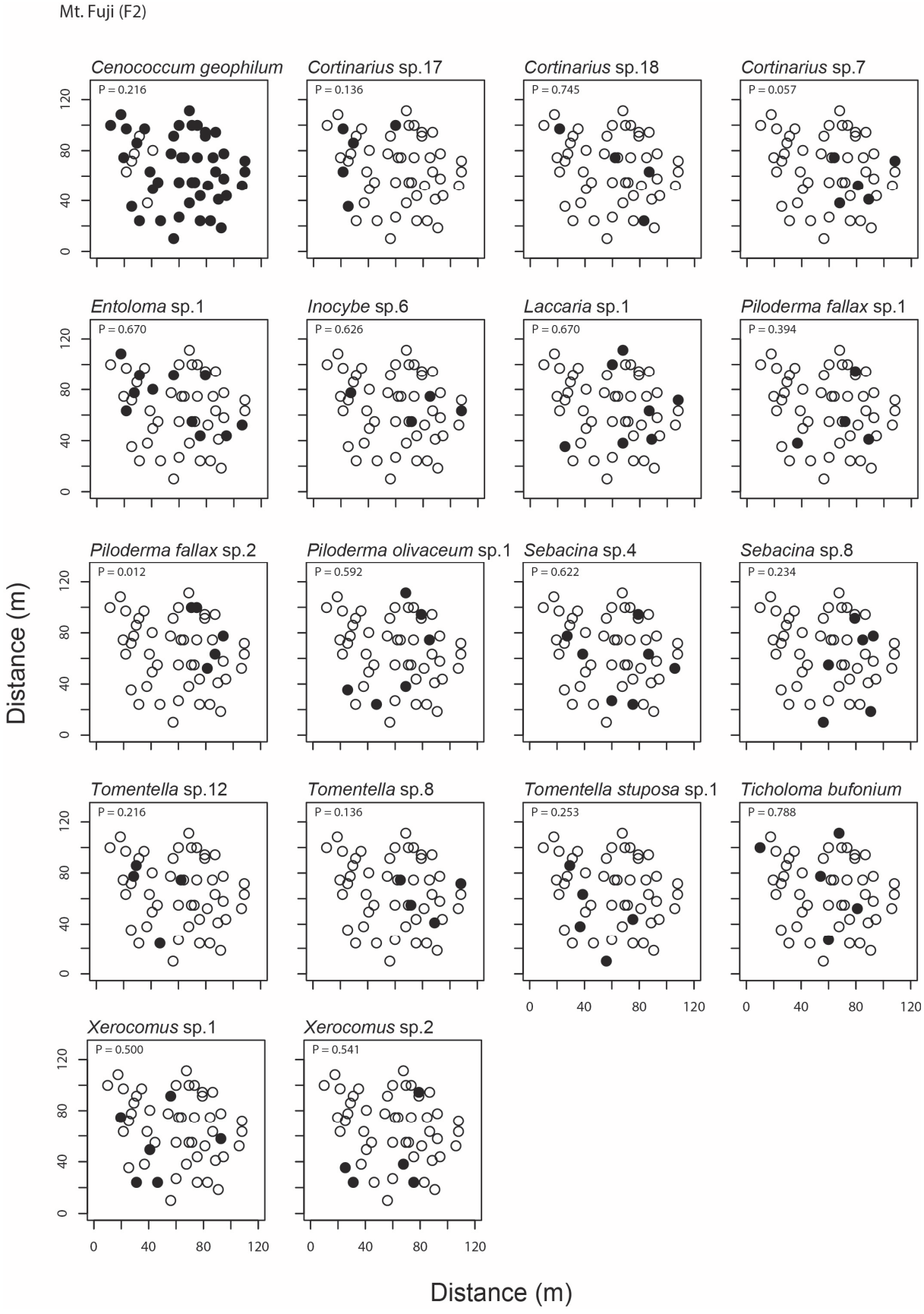


Figure 2.3 Distribution patterns of individual ectomycorrhizal fungal species within sites. *P* is the probability of obtaining aggregated distribution patterns more than expected by chance in the randomization procedures. Circles are sampling points. Filled circles are the points where the species was recorded. The gap between the two forest patches at Site I3 is indicated by a dotted line. Randomization procedures for the entire site at I3 were not performed for species occurring in both patches; in these cases, randomizations were performed separately for each patch.



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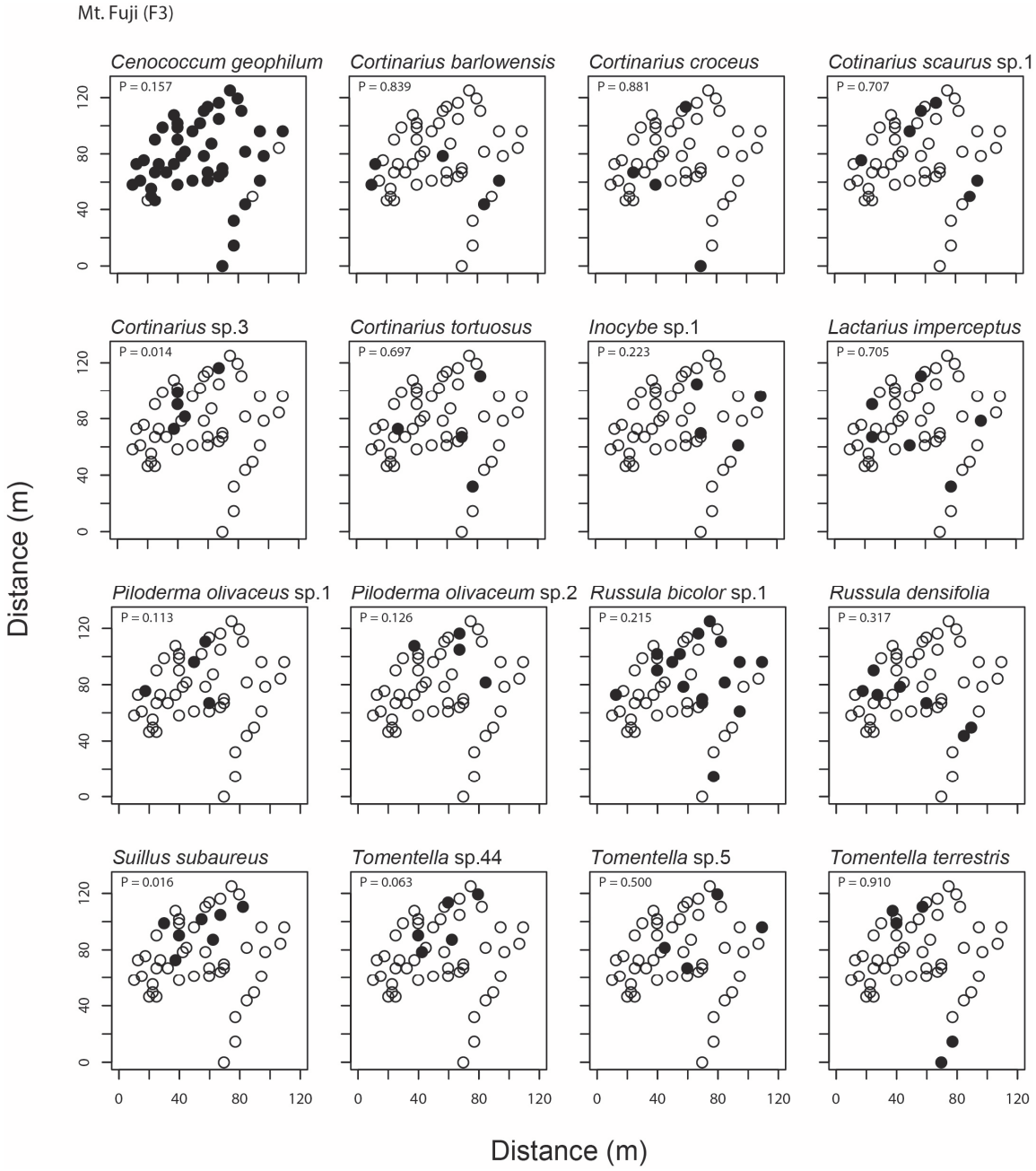

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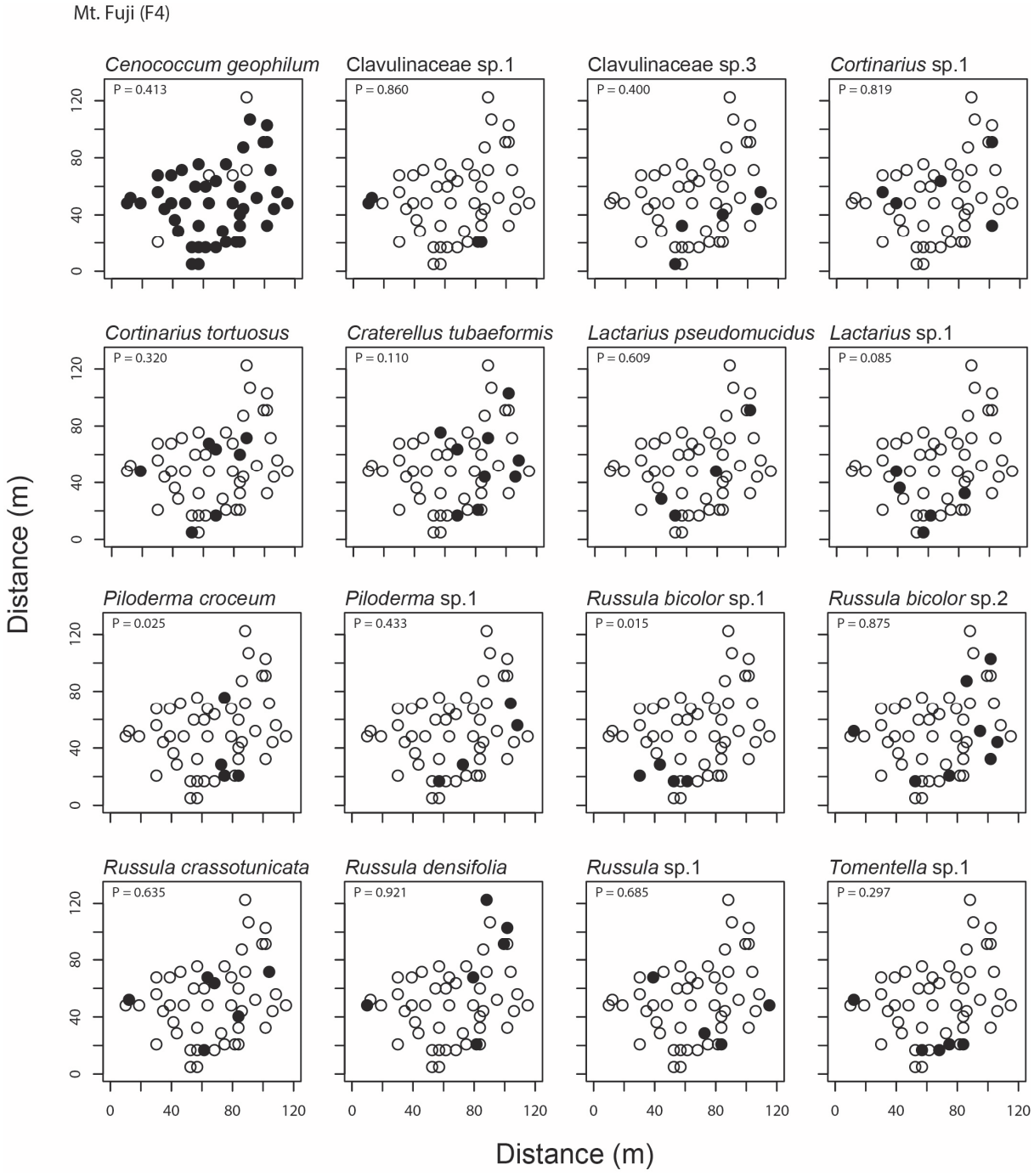

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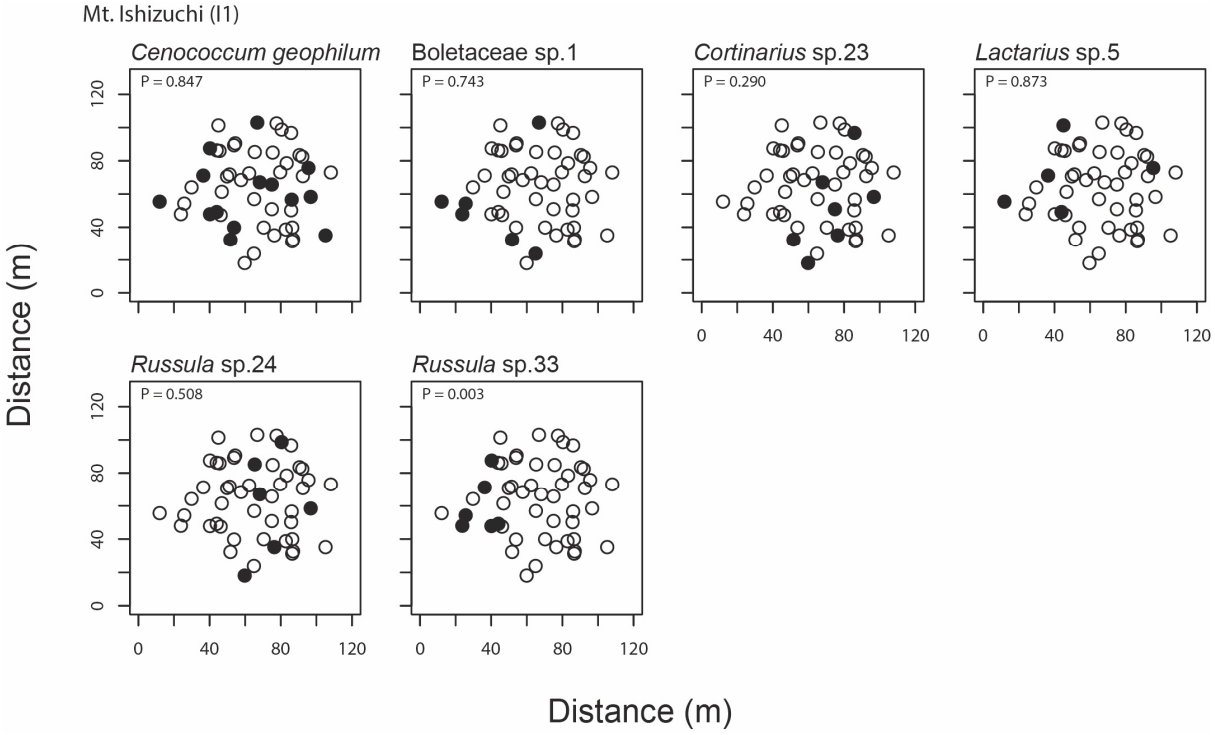

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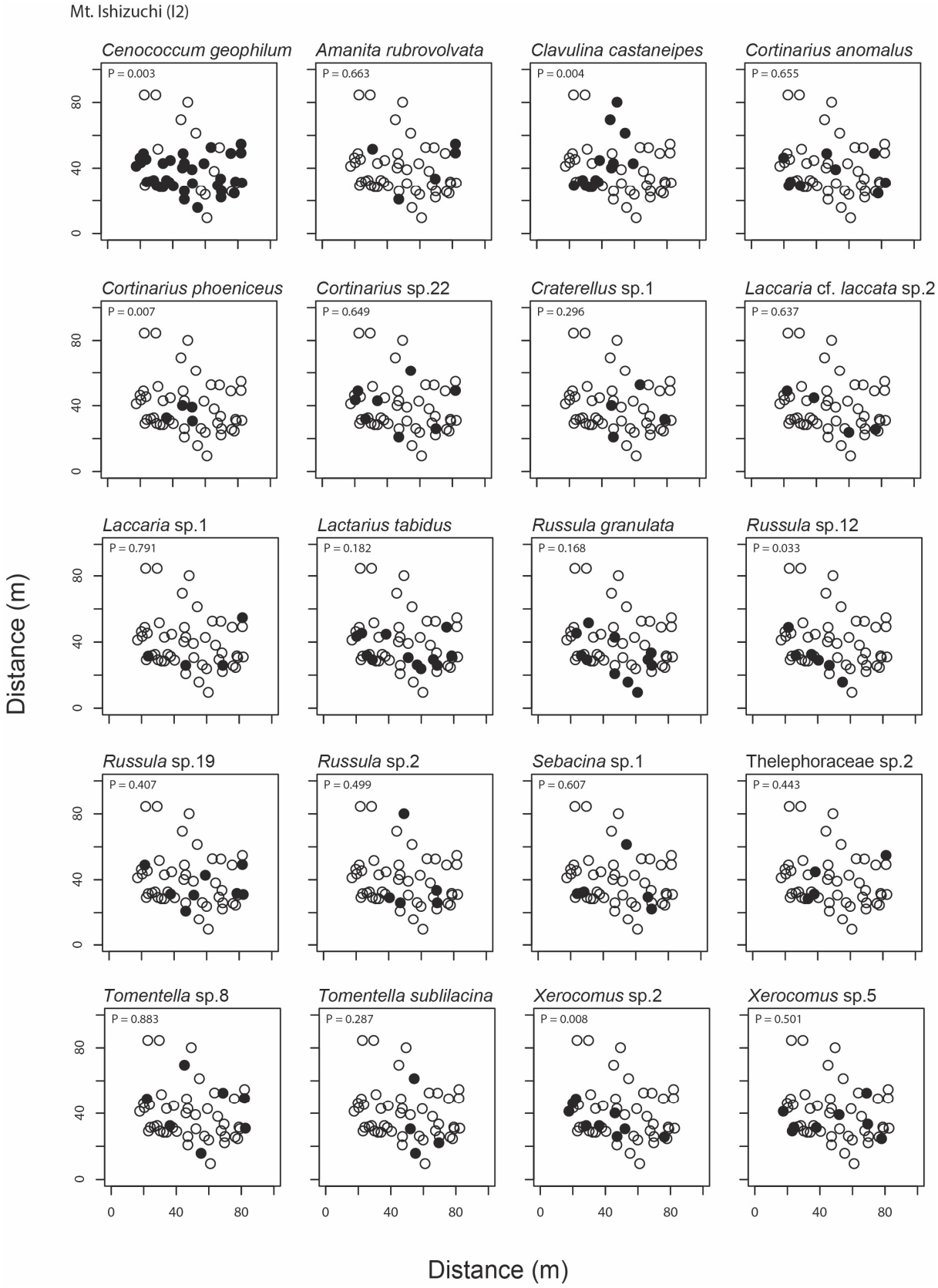

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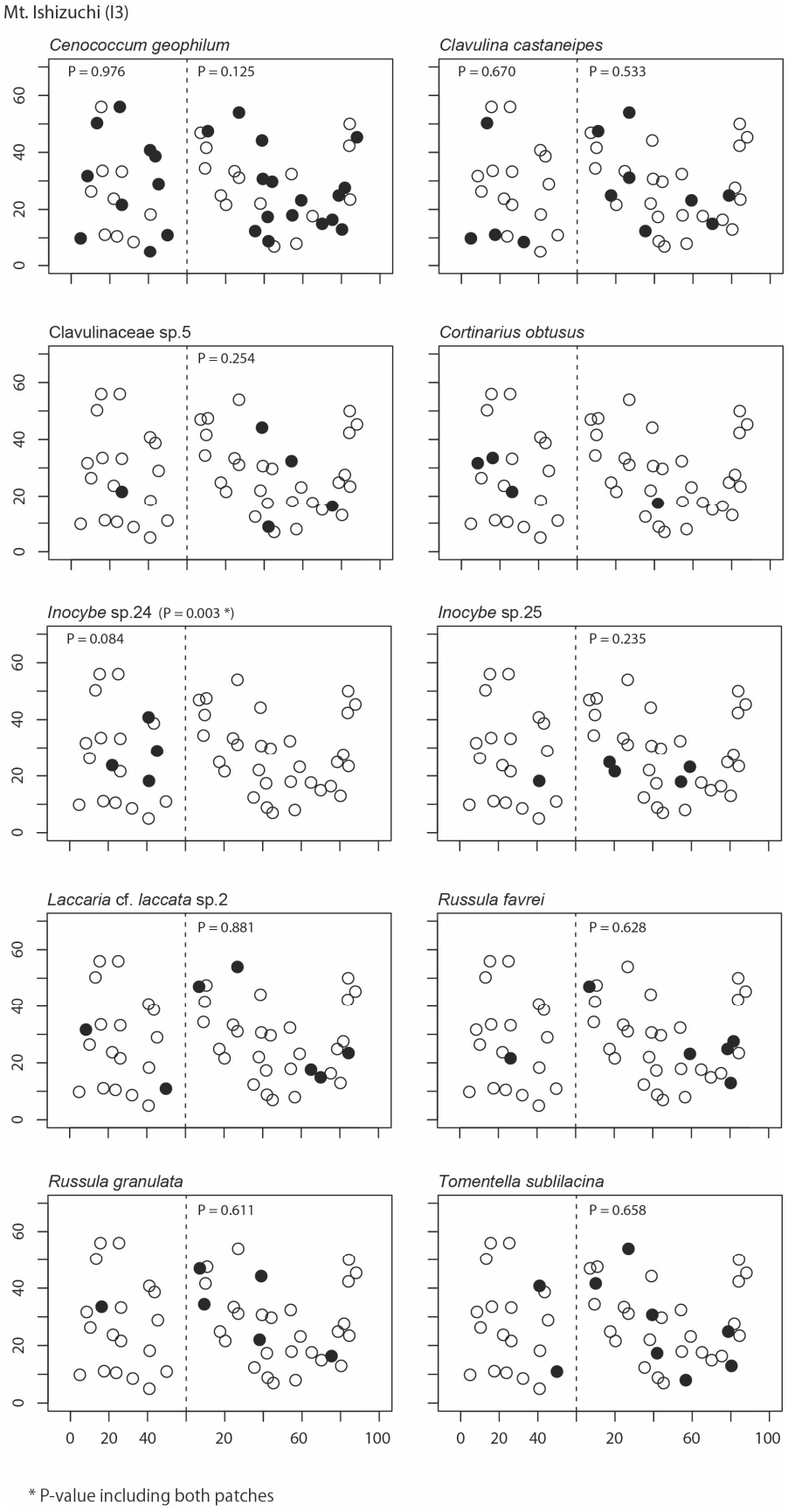

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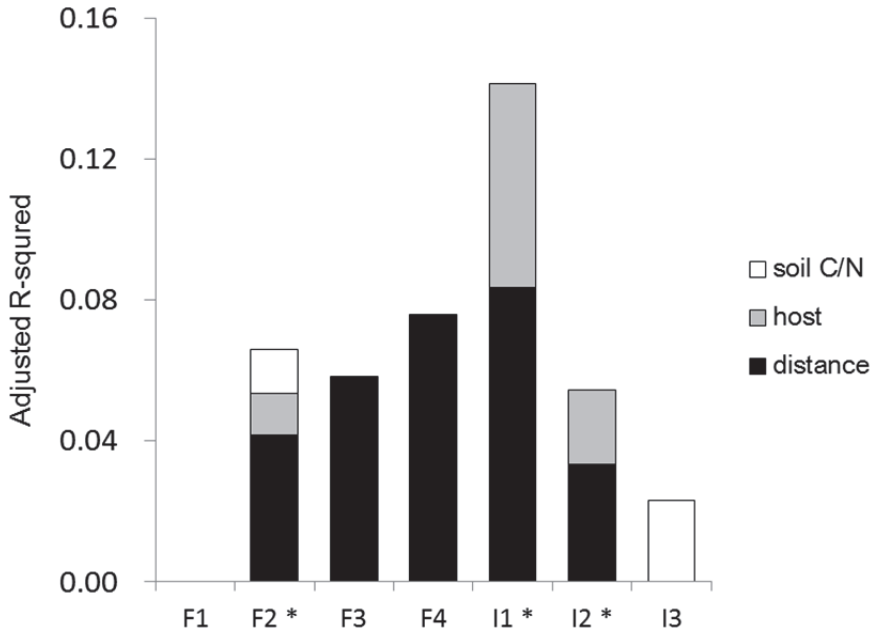


Figure 2.4 Proportions (adjusted R^2) of predictor variables explaining variation in ectomycorrhizal fungal composition at each site revealed by redundancy analysis. Distance refers to the significant principal coordinates of neighbor matrices (PCNM) vectors. One PCNM vector was significant at each of Sites F2, F3, F4, and I2; two PCNM vectors were significant at Site I1. Asterisks indicate conifer-broadleaf mixed forests. See Table 2.1 for the site codes. Abbreviation: C/N, carbon/nitrogen.

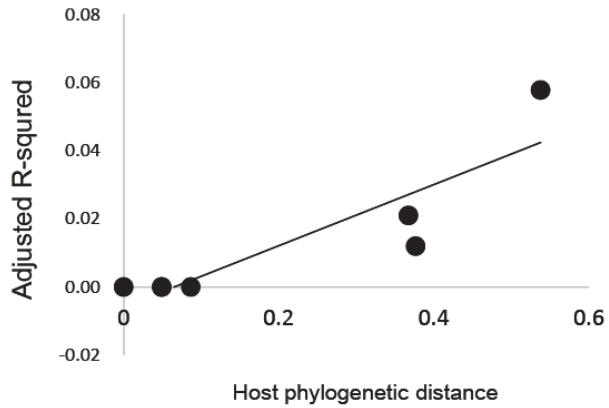


Figure 2.5 Host effects on ectomycorrhizal composition along a host phylogenetic distance gradient. Adjusted R^2 values were computed by redundancy analysis (Figure 2.4). Host phylogenetic distance is the average pairwise distance of host trees occurring at each site. A linear regression model was fitted ($F_{1,5} = 19.1$, $R^2 = 0.79$, $P = 0.007$). Site I3 was composed of a single host and was not included in the model.

Chapter 3 Fungal distributions and community structures at the local to regional scales

3.1 Introduction

Many studies have attempted to clarify the drivers of ectomycorrhizal (EM) fungal community compositions at various scales and ecosystems. Numerous studies have reported that EM fungal communities are structured by both biotic and abiotic factors, including host plants (Ishida *et al*. 2007; Tedersoo *et al*. 2008; Murata *et al*. 2013), soil properties (e.g., pH, nutrients, and litter characteristics; Aponte *et al*. 2010; Cox *et al*. 2010; Peay *et al*. 2010b), climate (Bahram *et al*. 2012), succession (Visser 1995; Nara *et al*. 2003; Twieg *et al*. 2007), and competition (Pickles *et al*. 2012). The host plant is regarded as one of the most important factors that influences EM fungal composition, especially at the stand scale, where hosts coexist in relatively narrow spatial and environmental ranges (Kennedy *et al*. 2003; Richard *et al*. 2005; Ishida *et al*. 2007; Tedersoo *et al*. 2008; Smith *et al*. 2009; Murata *et al*. 2013). However, isolating the host effect at larger spatial scales becomes difficult because the host composition changes with climate conditions and geological history (e.g., Tedersoo *et al*. 2012). Moreover, EM fungal communities often exhibit spatial structures (Lilleskov *et al*. 2004; Bahram *et al*. 2013a), which may be driven by autocorrelations in environmental factors (Toljander *et al*. 2006; Tedersoo *et al*. 2012) or independently by fungal internal factors (e.g., dispersal; Peay *et al*. 2010a). Thus, at larger regional and global scales, whether EM fungal communities are differentiated by hosts, environmental factors, or geographical distance is less clear. Distinguishing the effects of these factors is critical in examining if fungal communities are primarily constrained by contemporary environmental variations or by past dispersal events (Martiny *et al*. 2006). Systematic sampling strategies are needed to clarify the relative importance of these factors (Lilleskov and Parrent 2007).

Species distributions should be accurately determined before assessing fungal community-environment relationships. Centimeter to meter scale distribution patterns of EM fungi have been well studied for both horizontal and vertical directions (Guidot *et al*. 2004; Genney *et al*. 2006; Pickles *et al*. 2012). On the other hand, it has been increasingly recognized the importance of larger-scale microbial distribution patterns, which would provide fundamental implications on the potential impacts of human-induced environmental change on fungal communities. The global pattern of EM fungal communities was first reported by Tedersoo *et al*. (2012) based on meta-analyses. However, it is still immature to discuss global EM fungal patterns because data are sparse and many environmental variables are spatially autocorrelated. The regional scale studies will likely fill the knowledge gap between the well-studied micro-scale studies and currently emerging global scale studies.

Although species distributions have been widely studied in plants and animals, examining such patterns in soil microorganisms remains a major challenge. This is mainly because soil fungi are difficult to detect and quantify accurately in the field without applying molecular analyses. Limited sampling efforts tend to result in missing many fungi existing at a site and incompletely describing fungal communities because most EM fungi are inherently rare and patchily distributed (Taylor 2002; Chapter 2). Intensive sampling (many samples from each community) may be required for recording a relatively large number of fungal taxa in a community and for identifying species range overlaps among study sites. Consistent sampling across study sites also helps to remove the variations imposed by methodological discrepancies found in many global scale meta-analyses (Nakagawa and Santos 2012). A detailed examination of species overlaps among communities would improve the chances of detecting distribution patterns, community structures, and community turnover along environmental gradients (Leibold and Mikkelson 2002; Presley *et al*. 2010; Thébault 2013).

This chapter aims to identify the degree of species range overlap among the seven forest

sites on the two mountains (Table 2.1), and to evaluate the relative importance of geographical distance, environmental factors, and hosts in structuring EM fungal composition at local- to regional scales. Relationship between forest tree and fungal compositions were examined to address potential links between above- and belowground organisms.

3.2 Materials and methods

I used the data obtained in Chapter 2. These were 454 EM fungal species including 225 singletons and 89 doubletons. R version 3.0.3 (R Development Core Team, 2013) was used for statistical analyses, with the significance level set at $P < 0.05$ (unless otherwise noted).

The forest types at the seven sites were defined based on the composition of all tree species using a single linkage agglomerative clustering of tree compositions (stats package of R; Figure 3.1). Four forest types were identified: subalpine conifer (*Abies–Tsuga*-dominated; F3, F4, I3), cool temperate conifer-deciduous mixed (*Abies–Fagus*-dominated; F2, I2), warm temperate deciduous (*Quercus*-dominated; F1), and warm temperate conifer-broadleaf mixed (*Abies–Quercus*-dominated; I1).

The χ^2 tests with Yates correction were used to examine if site-shared fungal species occurred continuously along the elevation. The degree of species overlaps between EM fungal communities was calculated as the number of shared species divided by the total number of species recorded at each pair of sites. Fisher's exact tests were conducted to test whether the fungal species found on both mountains ("mountain shared species") that were recorded at a particular site occurred randomly at sites on the other mountain (e.g., the null hypothesis was that the mountain shared species that were recorded at F1 on Mt. Fuji were randomly distributed at I1, I2, and I3 on Mt. Ishizuchi). The mountain shared species were tested to determine whether the frequency of the species was biased toward particular forest types using Fisher's exact test. The Benjamini and Hochberg false discovery rate (FDR) correction

(Verhoeven *et al*. 2005) was performed to adjust type I error for multiple comparisons using the fmsb package.

The occurrence of fungal species per site was treated as a sample unit (n = 7) for community analyses. The relationship between EM fungal and tree species composition similarities was tested using a Mantel test with Bray–Curtis distance matrices. Spatial autocorrelation among the fungal composition was tested using a Mantel test with fungal composition against geographical Euclidean distance. Significance was tested with 9999 permutations. Separation of EM fungal composition by location (Mt. Fuji vs. Mt. Ishizuchi) and forest type was tested using Adonis (permutation-based multivariate analysis of variance) in the vegan package of R. Adonis partitions a distance matrix among categorical or continuous variables, and computes the strength and significance of the explanatory variables (Anderson 2001). The significance was tested with 999 permutations. Community dissimilarities were visualized using nonmetric multidimensional scaling (NMDS) with 999 permutations. Bray–Curtis distances were calculated prior to Adonis and NMDS visualization. The effect of individual environmental variables on NMDS ordination was examined using environmental fitting tests in the envfit function in vegan, and the significance of vectors was tested with 999 permutations. The variables included were climate (mean annual temperature and mean annual precipitation), soil (C/N, pH, and litter depth) and geographical distance. Monthly temperatures, mean growing season (May-September) temperatures and precipitation, annual and summer heat-moisture index, and continentally index (defined in Hamann and Wang 2006) were used in preliminary analyses, but these variables were highly correlated; thus, only mean annual values were included in the final analyses. Stand characteristics (basal area and stem density) and slope inclination were tested in the preliminary analyses, but removed from the final analyses because no correlations were detected. Soil variables were log-transformed prior to analyses. Geographical distance

(latitude and longitude) was transformed to the principal coordinates of neighbor matrices (PCNM) vectors that represented the geographical distances at various spatial scales (Borcard *et al*. 2004; Dray *et al*. 2006). PCNM vectors were calculated from the pairwise Euclidean distance of geographical coordinates between study sites (Chapter 2). Host identity was defined as phylogenetic distance among genera computed from phylogenetic eigenvectors described in Chapter 2.

Furthermore, the EM fungal community per site per host was treated as a sample unit (n = 19) in an analysis to evaluate the effect of host identity. Hosts represented by ≤5 cores were excluded from the analysis. The relative effects of categories (i.e. climate, soil, host identity, and geographical distance) were analyzed using variation partitioning in redundancy analysis (RDA; the varpart function in vegan). Forward selection by RDA was used prior to variation partitioning to identify significant variables within each category associated with EM fungal compositions based on 999 permutations (Borcard *et al*. 2011). Only significant variables were used to compute the total variance of the EM fungal composition explained by these variables.

3.3 Results

Species overlaps among communities

On Mt. Fuji, 73 species (24.2%) occurred in two or more sites. *Piloderma fallax* sp.2 and *Sebacina* sp.1 occurred in three sites (1550–2250 m). Most species that were present in >1 site (72 species) occurred in adjacent sites, and only one (*Amphinema byssoides*) appeared in non-adjacent sites (Figure 3.2). The lowest- and highest-elevation sites did not share any species in common. The proportion of species shared among sites was significantly higher in adjacent site pairs than in non-adjacent site pairs ($P < 0.001$) on Mt. Fuji. On Mt. Ishizuchi, 38 species occurred in more than one sites of which 33 species occurred in adjacent site pairs.

The χ^2 test showed that the proportion of species shared among sites was not significantly different between adjacent site pairs and non-adjacent site pairs ($P = 0.12$).

A total of 47 species were shared between the mountains (Table 3.1; Figure 3.3). Sites F2 and I2 (cool temperate mixed forest pairs) shared 20 species (18.4% of the total number of species recorded at these sites without singletons). Sites F3 and I3 (*Abies*-dominated subalpine conifer forest pairs) shared 14 species (14.1%). These shared fungi appeared to occur across multiple host genera (Figure 3.4). Fisher's exact test with FDR correction showed that the mountain shared species recorded in the subalpine conifer forests on Mt. Fuji (Sites F3 and F4) occurred at significantly higher frequencies in the subalpine conifer forest (Site I3) than in the other forest types (Sites I1 or I2) on Mt. Ishizuchi ($P < 0.043$). Similarly, the mountain shared species recorded at Site I3 on Mt. Ishizuchi occurred at a significantly higher frequency at Site F3 on Mt. Fuji ($P = 0.019$). The mountain shared species in the mixed forest on Mt. Ishizuchi (Site I2) also occurred at higher frequency in the mixed forest on Mt. Fuji (Site F2) at $P = 0.085$. About 66% (31 of 47) of mountain shared species appeared to occur in particular forest types and Fisher's exact test showed significantly biased occurrence in some fungal species (Table 3.1).

Composition and underlying mechanisms

Singleton species that were found in one core across the entire data set were removed to improve community analyses, leaving 228 species for community analyses.

A Mantel test showed that the similarities in EM fungal composition were significantly correlated with those of forest tree composition ($r_M = 0.47$, $P = 0.042$), but not with geographical distance ($P = 0.146$) at the regional scale. A partial Mantel test revealed that geographical distance was significant when the effect of tree composition was excluded ($r_M = 0.45$, $P = 0.033$). Tree composition remained significant when the distance effect was

excluded (r_M = 0.55, P = 0.024). Adonis tests showed that the larger variance was explained by forest type than location (Table 3.2, Figure 3.5a).

Furthermore, the EM fungal composition on *Abies*, which occurred at six of seven studied sites, was analyzed separately to remove host effects. The results were similar to those of the entire community (Figure 3.5b). An Adonis test showed that forest type explained the larger variance in fungal composition ($F_{2,2}$ = 1.75, R^2 = 0.47, P = 0.02) than location ($F_{1,2}$ = 1.96, R^2 = 0.26, P = 0.03).

EM fungal communities on individual hosts per site were separated by forest type and environmental factors (Figure 3.5c). Variation partitioning showed that climate (temperature and precipitation) alone explained the largest variance (R^2_{adj} = 13.3%) in the EM fungal composition, followed by soil (C/N and pH; R^2_{adj} = 5.2%) and geographical distance (R^2_{adj} = 4.7%) at the regional scale (Figure 3.6). No significant effect of host identity alone was detected in variation partitioning. Temperature, precipitation, C/N, and host identity were significantly correlated with NMDS vectors based on the environmental fitting test (Table 3.3).

3.4 Discussion

Differentiating the effects of hosts, environmental factors, and geographical distance on EM fungal communities is difficult when these factors covary at large spatial scales (Toljander *et al*. 2006; Tedersoo *et al*. 2012). I applied intensive and consistent sampling methods to identify the degree of species overlaps between similar habitats on geographically distant mountains. I clearly demonstrated that 20.6% of the EM fungal species (without singletons) occurred on the two mountains and that they tended to inhabit similar forest types (Figure 3.3, Table 3.1). The species overlaps were particularly notable between the cool temperate mixed forests on both mountains, where the highest proportion (18.4%) of EM fungi overlapped

despite the geographical distance of ~550 km. In contrast, only one and five EM fungal species were shared between non-adjacent sites within Mt. Fuji (F2 and F4) or Mt. Ishizuchi (I1 and I3), respectively, despite the close geographical distances of <10 km, indicating a weak effect of geographical distance *per se*. These results suggest that some EM fungi are widely distributed across the region and their presence is constrained by contemporary environmental factors. To support this, many mountain shared species (31 of 47 species, or 66.0%) displayed niche preferences to particular forest types (Table 3.1). Those EM fungal species restricted to particular habitats are likely more sensitive to environmental change than habitat generalists (Carignan and Villard 2002), such as *Tomentella sublilacina* and *Laccaria laccata* which were recorded across wide environmental ranges.

Variation partitioning further indicated that climate alone had a stronger effect on the EM fungal composition than geographical distance or host identity (Figure 3.6). Previous studies have reported the importance of climate (Bahram *et al*. 2012; Tedersoo *et al*. 2012) and soils (Cox *et al*. 2010; Jarvis *et al*. 2013) on EM fungal composition at regional to global scales. However, the potential effects of geographical distance remain unclear in most studies because environmental factors covary along spatial gradients. Alternatively, Põlme *et al*. (2013) and Roy *et al*. (2013) examined a single host genus (i.e., *Alnus*) and identified significant spatial structures in the EM fungal compositions, but environmental factors (i.e., soil properties) had a stronger influence on the community structures. *Alnus* is associated with N-fixing bacteria and usually form unique EM fungal communities with low diversity (Kennedy and Hill 2010). My results indicated that a stronger effect of environmental factors than geographical distance could be applicable to the more species-rich EM fungal communities that are associated with many typical EM host genera. This was further confirmed in my analysis using a single host genus, *Abies* (Figure 3.5b).

The host family has been reported to influence EM fungal composition at the global scale

(Tedersoo *et al*. 2012). However, whether EM fungal composition is driven by host identity *per se* or other confounding environmental factors is unknown because climate and geological history inherently affect host distributions at the global scale. In contrast, I clearly showed that host identity has a minor role in explaining EM fungal composition at the regional scale. First, different host genera coexisting at the same site tended to harbor similar EM fungi (Figure 3.5c). Second, although I detected significant host effects in the environmental fitting test (Table 3.3), the hosts alone were insignificant when environmental factors were excluded in the variation partitioning analysis (Figure 3.6). Finally, mountain shared species tended to occur in similar forests on different mountains, but not strictly on the same hosts (Figure 3.4). Thus, any host effect at the global scale (Tedersoo *et al*. 2012) would be a consequence of climate and geological history, and not derived from phylogenetic constraints between the symbiotic partners.

The lack of large host effects at the regional scale may be related to my studied system that was dominated by generalist EM partners. The forests were composed of typical EM host trees (e.g., *Fagus*, *Quercus*, *Abies*) that harbored generalist EM fungal groups such as Russulaceae and Thelephoraceae (Smith *et al*. 2009). In contrast, host effects would be more prominent for specialist symbionts, such as *Alnus*- *Alpova* and *Pinus*-*Rhizopogon* (Molina *et al*. 1992; Kennedy and Hill 2010). Indeed, I detected some host specific fungal genera such as *Suillus* and *Tylospora*, which occurred only on conifer hosts. But their occurrence appeared to be too low to separate the community among hosts. Moreover, the significance of host effects would depend on spatial scales and host composition within a site. The stand scale analyses detected significant host effects at some sites, especially in conifer–broadleaf mixed forests (Chapter 2), as in many previous studies (Ishida *et al*. 2007; Tedersoo *et al*. 2008; Smith *et al*. 2009). However, most EM fungi are assumed to be host generalists (Bruns *et al*. 2002), which is supported by numerous studies (Kennedy *et al*. 2003; Roy *et al*. 2008; Trocha *et al*. 2012;

Bahram *et al*. 2013b). Therefore, although the host may influence the EM fungal community within a narrow range of climate conditions at stand scales, the effect could be masked by more dominant determinants (e.g., climate) at larger spatial scales. Plant communities are well known to be primarily determined by climate, and to some extent by soil conditions, within a biogeographic region (Kira 1991; Morin *et al*. 2007). This is largely because climate conditions (e.g., temperature and precipitation) and nutrients are the major growth limiting resources for autotrophic plants. Both this work and previous studies (e.g., Bahram *et al*. 2012) have indicated that temperature and precipitation could restrict EM fungal distributions.

I found a positive correlation between the compositional similarities of EM fungi and trees in the Mantel test, which agreed with previous studies on plant and fungal communities at regional scales (Edwards and Zak 2010; Peay *et al*. 2013). However, these correlations do not necessarily represent the direct links between plants and microorganisms, and the empirical evidence of direct links appears to be inconclusive (Hedlund *et al*. 2003; Waldrop *et al*. 2006; Bryant *et al*. 2008; Queloz *et al*. 2011; van der Putten *et al*. 2013). In fact, hosts *per se* had little predictive power with regard to the EM fungal composition in my analysis. Thus, EM fungi and trees may synchronously, but independently, respond to the same environmental factors, particularly climate conditions. Global climate change is becoming a serious issue in forest ecosystems (Bonan 2008; Allen *et al*. 2010). Given the short generation turnover (e.g., Wadud *et al*. 2014), fungi may exhibit a higher adaptive capacity to environmental change than trees. My data provide important insights that host plants and mutualistic fungi may respond to climate change independently, potentially altering carbon and nutrient cycles in relation to the plant-fungus associations.

Table 3.1 List of mountain shared ectomycorrhizal fungal species. Symbols indicate the presence of a species. Circles represent sites on Mt. Fuji, and squares represent sites on Mt. Ishizuchi. Forest types are subalpine conifer (black), cool temperate mixed (grey), and warm temperate mixed and deciduous (white).

		Fuji				Ishizuchi			
Forest type	Species	F1	F2	F3	F4	I1	I2	I3	*P*-value
Subalpine conifer	Clavulinaceae sp.5			●	●			■	*
	Clavulina castaneipes			●	●		■	■	*
	Cortinarius scaurus sp.1			●	●			■	*
	Sebacina sp.3			●	●			■	
	Lactarius imperceptus			●				■	*
	Amanita sp.1			●				■	
	Piloderma sp.3			●				■	
	Sebacina sp.29			●				■	
Cool temperate mixed	*Genea hispidula*		●				■		
	Russula sp.2		●				■		
	Russula sp.12		●				■		
	Tomentella sp.6		●				■		
	Tomentella sp.12		●				■		
	Tomentella sp.50		●				■		
Cool temperate mixed and warm temperate (deciduous and mixed)	*Cortinarius* sp.17		●			□	■		
	Tuber sp.2		●			□	■		
	Inocybe sp.15		●			□			
	Tomentella sp.11		●			□			
	Tomentella stuposa sp.1		●			□			
	Entoloma sp.1	○	●			□	■		*
	Laccaria sp.1	○	●			□	■		*
	Sebacina sp.4	○	●			□	■		*
	Xerocomus sp.2	○	●			□	■	■	*
	Cortinarius anomalus	○				□	■		*
	Cortinarius sp.14	○					■		
	Cortinarius umbrinolens	○					■		
	Lactarius quietus	○					■		
Warm temperate (deciduous and mixed)	*Entoloma* sp.3	○				□			*
	Tomentella sp.41	○				□			
	Tomentella sp.48	○				□			
	Xerocomus sp.3	○				□			
Various forests	Clavulinaceae sp.1			●	●		■		
	Inocybe sp.11			●	●		■	■	
	Laccaria cf. *laccata* sp.2		●				■	■	
	Russula peckii		●	●				■	
	Russula turci				●	□		■	
	Sebacina sp.1		●	●	●		■	■	
	Sebacina sp.2			●	●	□		■	
	Sebacina sp.20		●				■	■	
	Thelephoraceae sp.2		●			□	■	■	
	Tomentella sp.5		●	●		□	■		
	Tomentella sp.7		●	●		□	■		
	Tomentella sp.8		●	●		□	■	■	
	Tomentella sp.18		●	●			■		
	Tomentella sp.21			●	●	□			
	Tomentella sp.31			●		□		■	
	Tomentella sublilacina	○					■	■	

Asterisks indicate species whose presence is significantly biased by forest type based on Fisher's exact tests after false discovery rate (FDR) correction (Verhoeven *et al*. 2005).

Table 3.2 Effect of forest type and location in separating the ectomycorrhizal fungal composition (Bray–Curtis distance) as revealed by the Adonis test

	DF	SS	MS	F-statistic	R^2	P-value
Forest type	3	1.41	0.47	2.22	0.59	0.003
Location	1	0.56	0.56	2.66	0.24	0.006
Residuals	2	0.42	0.42			
Total	6	2.4	2.39			

Abbreviations: DF, degree of freedom; SS, sum of squares; MS, mean square.

Table 3.3 Effects of environmental variables on fungal composition fitted to the nonmetric multidimensional scaling ordination. *P*–values in bold indicate the significance level < 0.05.

Variables	R^2	P-value
(a) 7 sites		
Annual temperature	0.795	**0.043**
Annual precipitation	0.703	0.057
PCNM vector	0.799	0.067
pH	0.828	0.055
C/N	0.754	0.069
Litter	0.601	0.144
(b) 6 communities on *Abies*		
Annual temperature	0.805	0.052
Annual precipitation	0.602	0.211
PCNM vector	0.848	0.170
pH	0.659	0.237
C/N	0.582	0.274
Litter	0.560	0.282
(c) 19 host communities		
Annual temperature	0.579	**0.003**
Annual precipitation	0.476	**0.006**
PCNM vector	0.058	0.640
pH	0.005	0.973
C/N	0.903	**0.001**
Litter	0.141	0.318
Host	0.628	**0.002**

Abbreviations: PCNM, spatial principal coordinates of neighbor matrices; C/N, carbon/nitrogen; Host, host phylogenetic eigenvector.

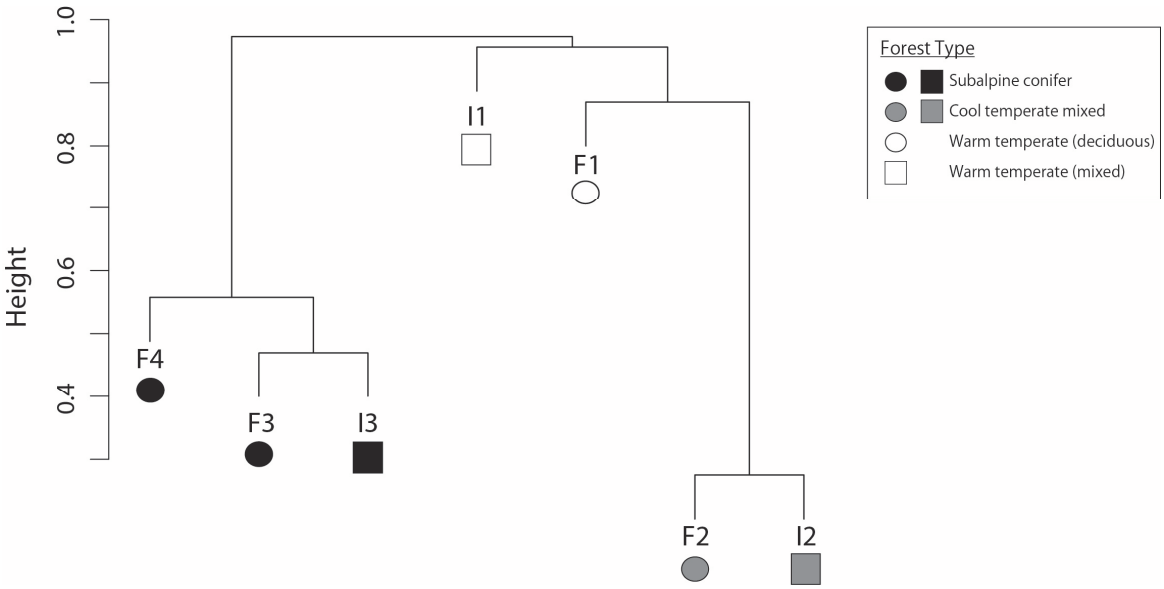


Figure 3.1 A single linkage agglomerative clustering of a tree composition matrix of Bray–Curtis distance among sites. Circles and squares represent sites on Mt. Fuji and Mt. Ishizuchi, respectively. Tree composition is based on the basal area (m^2/ha) of all species recorded at each site.

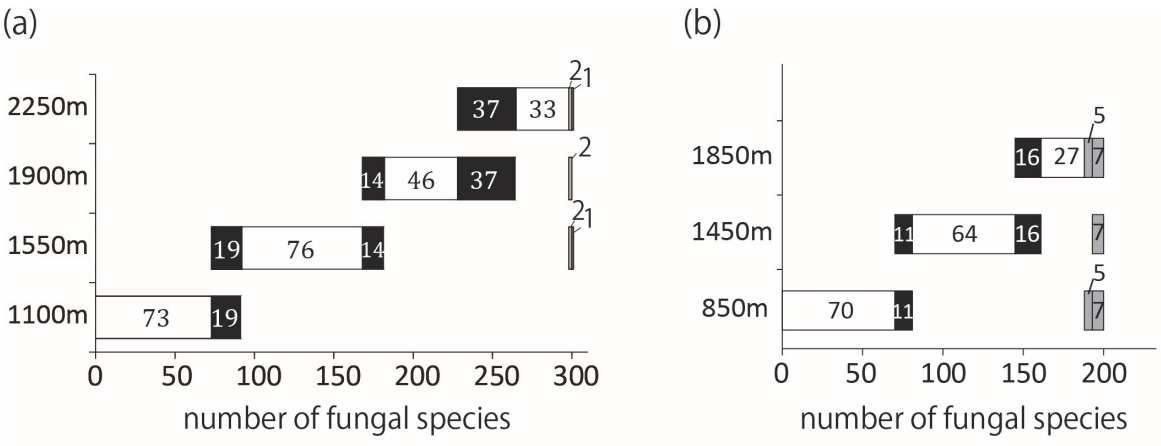


Figure 3.2 The species overlaps along the elevation gradient on (a) Mt. Fuji and (b) Mt. Ishizuchi. Cumulative number of fungal species, from low to high elevation sites, is shown excluding Cg. Values in the columns indicate the numbers of species. Open columns indicate site-specific species and closed columns indicate site-shared species between adjacent site pairs. Shaded columns at the right end indicate species that were found across multiple adjacent or two non-adjacent sites.

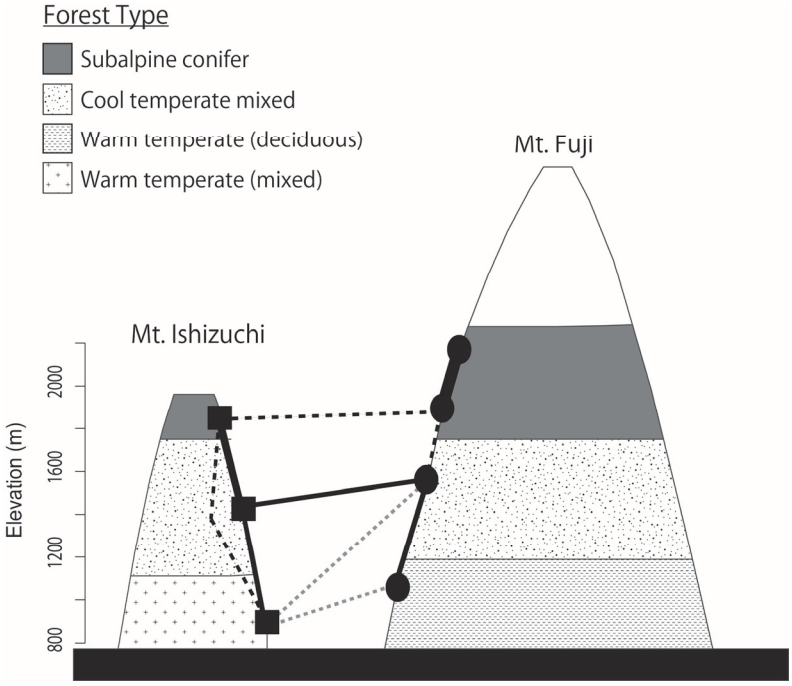


Figure 3.3 The degree of species overlaps among study sites. Circles indicate the study sites on Mt. Fuji, and squares indicate those on Mt. Ishizuchi along the elevation gradients. The thickness of the connecting line represents the pairwise proportion of shared fungal species between sites (i.e., the number of shared species divided by the total number of species at paired sites). Solid lines indicate a proportion of >15%, dashed black lines indicate a proportion of 13–15%, and dotted gray lines indicate a proportion of 10–13%. The proportion <10% is not shown for clarity.

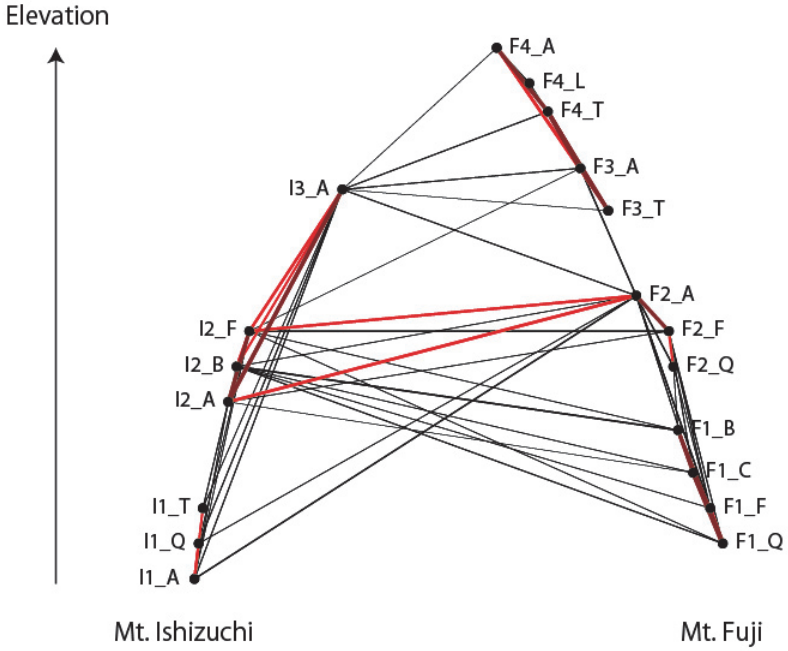


Figure 3.4 The degree of species overlaps among host communities. The line thickness represents the pairwise proportion of shared ectomycorrhizal fungal species between communities (i.e., the number of shared species divided by the total number of species at paired communities). Red lines indicate a proportion of >15% and black lines indicate a proportion of 5-15%. The proportion of <5% is not shown for clarity. Site codes are given for Mt. Fuji sites (F1-F4 from low to high elevation) and Mt. Ishizuchi sites (I1–I3 from low to high elevation). The letters after the site code indicate the host genus: A (*Abies*), T (*Tsuga*), L (*Larix*), B (*Betula*), C (*Carpinus*), F (*Fagus*), and Q (*Quercus*). Host communities that contained ectomycorrhizal fungi in >5 soil samples are shown. Elevation is not an exact scale. The communities are arbitrarily positioned to show species overlaps between sites clearly.

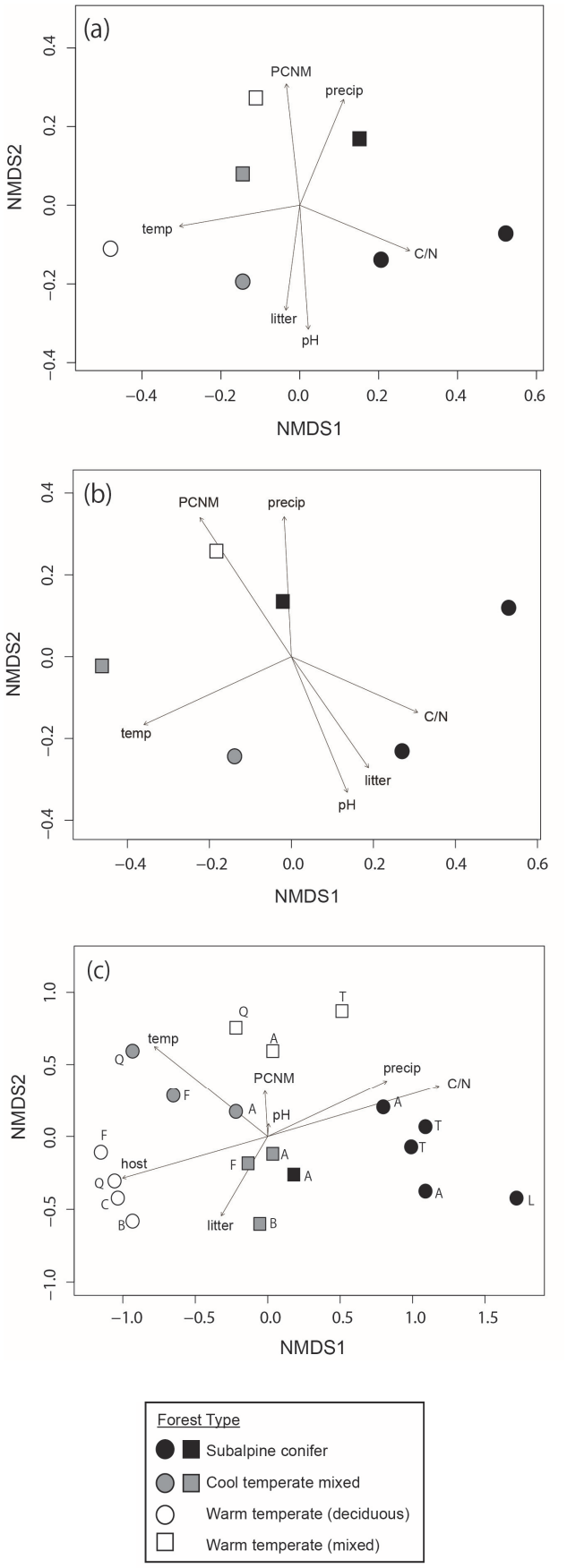


Figure 3.5 Nonmetric multidimensional scaling (NMDS) graphs of the ectomycorrhizal fungal communities. Environmental variables are fitted to the NMDS ordination. The abbreviations are temp (temperature), precip (precipitation), C/N (carbon/nitrogen) host (host phylogenetic eigenvector), and PCNM (spatial principal coordinates of neighbor matrices eigenvector). The significance of the vectors is shown in Table 3.3. Circles and squares indicate sites on Mt. Fuji and Mt. Ishizuchi, respectively. (a) Seven sites (stress = 0.08), (b) Six fungal communities on *Abies* (stress = 0.05), and (c) 19 host communities (stress = 0.10). The letters besides symbols indicate the host genus: A (*Abies*), T (*Tsuga*), L (*Larix*), B (*Betula*), C (*Carpinus*), F (*Fagus*), and Q (*Quercus*). Host communities that contained ectomycorrhizal fungi in >5 soil samples are shown.

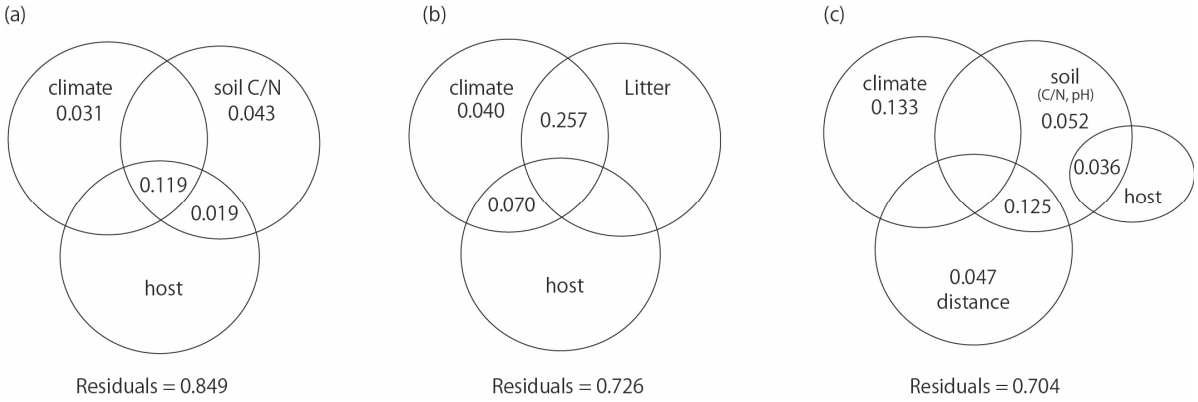


Figure 3.6 Individual and interaction effects of putative factors explaining fungal compositions as revealed by variation partitioning in redundancy analysis. (a) Mt. Fuji (n=12 communities), (b) Mt. Ishizuchi (n=7), and (c) both mountains (n=19). Values are adjusted R^2 (those <0.01 are not shown). Climate (mean annual temperature and mean annual precipitation), distance (spatial principal coordinates of neighbor matrices eigenvector), and host (host phylogenetic eigenvector). Abbreviation: C/N, carbon/nitrogen.

Chapter 4 Spore community structures

4.1 Introduction

Spores promote survival and fitness of fungal species by enhancing genetic variability, dispersing into new environments, and escaping unfavorable conditions through dormancy. Some fungi produce spores that can persist for years to germinate, forming spore communities (Taylor and Bruns 1999; Nguyen *et al*. 2012). Despite the ecological significance of fungal spores, the community structures of spores receive less attention compared with the communities of existing ectomycorrhizal (EM) fungi on root tips (hereafter 'existing root communities').

Spore communities are mostly studied in post-disturbed and extreme habitats such as post-wildfire forests, heathlands, and barren (Barr *et al*. 1999; Bidartondo *et al*. 2001; Collier and Bidartondo 2009). Only a few studies documented spore communities of EM fungi in mature forests (Taylor and Bruns 1999; Izzo *et al*. 2006b). These studies report striking compositional differences between spore and existing root communities of EM fungi. In general, spore communities are low diverse and composed of pioneer fungal species, whereas existing root communities are highly diverse and composed of many late-stage species. These patterns are based on studies conducted in *Pinus* forests in North America where stand-replacing wildfires occur frequently (Taylor and Bruns 1999; Izzo *et al*. 2006b). In such ecosystems, tree regeneration may heavily rely on resistant spores to establish fungal symbiosis in response to disturbance events. In contrast, few studies examined spore communities in more stable, old-growth forests in temperate regions. EM fungi reproduce mainly by spores and vegetative growth of mycelia in mature forests, but spore propagation probably occurs frequently based on relatively small genet sizes and high genetic variability within populations in EM fungi (Fiore-Donno and Martin 2001; Redecker *et al*. 2001). It is

highly possible that fungi exhibiting different colonization strategies (existing as spores or on root tips) coexist in mature forests, resulting in higher ecosystem resilience to disturbances.

Underlying mechanisms of community structures of EM fungi have been extensively studied for existing roots at various scales and in various ecosystems across wide environmental ranges (Chapter 3). Existing root communities are highly heterogeneous across sites and may be influenced by environmental factors. On the other hand, studies on spore communities are restricted at stand scales and thus driving forces and species turnover of spore community structures are poorly understood. Spore communities seem to be homogeneous among previous studies, which reported some common pioneer fungi such as *Tomentella sublilacina*, *Suillus bovinus* and *Laccaria* spp. (e.g., Barr *et al*. 1999; Taylor and Bruns 1999; Collier and Bidartondo 2009; Buscardo *et al*. 2010). These observations suggest that pioneer fungi are widespread (Deacon and Fleming, 1992), leading to a hypothesis that spore communities are homogeneous among different forest types covering wide climate ranges.

In this study, I conducted bioassay experiments to examine spore communities in old-growth forests. Early-successional host seedlings were used to detect fungi that likely colonize first after disturbance events. I focused on two aspects of the structures of spore communities. First, spore communities were compared across a various forest types along elevation gradients. I tested a hypothesis that spore communities are homogeneous and predictable across the study sites along wide climate ranges. Second, I examined the effect of soil organic matter on fungal occurrence in spore communities. Soil organic matter relates to forest development and there is some evidence of soil layer (i.e., organic and mineral soil layers) preferences among EM fungi (Taylor and Bruns 1999). However, the effects have not yet been evaluated quantitatively. I tested a hypothesis that fungal spores show niche preferences to microhabitats with different levels of soil carbon contents.

4.2 Materials and methods

Soil samples

Rectangular soil cores (5 × 5 cm to 10 cm depth) were collected at seven sites on Mt. Fuji in 2011 and Mt. Ishizuchi in 2012. Fifty soil cores were collected right beside the soil samples collected for the existing root community study (Chapter 2). Roots, twigs and small stones were removed and soils were air dried for >6 months. A cotton ball was placed to the bottom of a 15 ml tube on which drainage holes were made, and each soil sample was contained in the tube.

Bioassay experiments

A total of 100 tubes per site were prepared, half of which were used for a conifer host and the other half were used for a deciduous host. Selected host trees were early successional species that would likely predominant right after disturbance in the study ecosystems. *Pinus densifolia* was selected as a conifer host because it occurs many disturbed sites in a wide range of temperate region in Japan. *Salix* and *Betula* occur after disturbance on Mt. Fuji and on Mt. Ishizuchi, respectively, and used as deciduous hosts. *Salix reinii* seeds were collected approximately at 1600 m on Mt. Fuji in July 2012, air-dried for three days after collection, and sawed within 10 days. Commercially available *P. densifolia* and *Betula maximowicziana* seeds (Hikari Ryokuchi Ink.) were used. *Pinus* and *Betula* seeds were soaked in deionized water for 1-hour and surface-sterilized with 5% Antiformin (sodium hypochlorite solution) for >10 min and rinsed before planting. Four to five seeds were planted on each tube and later removed to leave one seedling per tube. New seeds were planted on subsequent days on tubes when any seeds did not germinate. For the Mt. Fuji samples, bioassay was set for *Pinus* in March and for *Salix* in July, 2012. Bioassay was set between February and March, 2013 for the Mt. Ishizuchi samples. Seedlings were grown for 5-6 months in a growth chamber, where

no contamination has been observed in any previous experiments in my laboratory. The growth chamber was set at 20 ºC under light for 16 hours and 18 ºC in dark for 8 hours. Seedlings were watered every three to five days. EM fungi colonized on root tips were morphologically classified and identified to species by molecular identification as described in Chapter 2.

I also conducted preliminary bioassay experiments using *Quercus crispula* and *Abies veitchii* seeds collected in the field on Mt. Fuji. However, the number of germinated seeds was small and only ≤25 seedlings were examined. The methods and results are presented in Appendix D.

Statistical analyses

Randomization procedures as described in Chapter 2 were conducted for individual fungal species found on >3 seedlings separately for each host to test aggregated distribution patterns.

Community compositional similarity was visualized using a nonmetric dimensional scaling (NMDS) graph with Bray-Curtis distance of a community matrix and 999 permutations. Fungal composition per host per site was treated as a sample unit. The Adonis function (permutation-based multivariate analysis of variance) in the vegan package was used to test whether community structures were different between existing roots and spores, and to examine the effects of host and site on spore composition. Bray-Curtis distances were calculated from a community matrix and significance was computed based on 999 permutations.

The effect of soil carbon contents on the presence of fungal species was tested for species that occurred in >2 seedlings per host per site using a binomial logistic regression model (glm function in the stats package). Total carbon contents were log transformed prior to analyses.

Simple linear regression was used to examine relationships between spore fungal richness

and environmental factors at each site. Environmental factors tested were as described in Chapter 3.

4.3 Results

General descriptions of EM fungi

In total, 668 of 700 seedlings (96%) survived and were examined for the spore communities. EM fungal colonization was observed in 310 seedlings, and 192 seedlings were colonized solely by *Cenococcum geophilum* (hereafter "Cg"; Figure 4.1). Twenty two seedlings were colonized concurrently by Cg and other EM fungi, and 96 seedlings were colonized by EM fungi other than Cg. In total, 128 of 134 morphotypes (95.5%) and 275 of 315 sequenced tips (87.3 %) were identified to species, and 29 species were recorded (Table 4.1). Frequently observed fungal species in the bioassay experiments except Cg were *Paxillus involutus* sp.2 (29 seedlings), *Tomentella sublilacina* (18 seedlings), *Tomentella* sp.75 (12 seedlings) and *Scleroderma citrinum* (12 seedlings). Ten species were recorded both in the field and bioassay experiments. Eleven species were recorded on Mt. Fuji and 23 species were recorded on Mt. Ishizuchi. Richness of spores was positively correlated with slope inclination ($F_{1,5} = 15.19$, $R^2 = 0.70$, $P = 0.01$) and growing season precipitation ($F_{1,5} = 15.05$, $R^2 = 0.70$, $P = 0.01$), and negatively correlated with soil pH ($F_{1,5} = 8.68$, $R^2 = 0.56$, $P = 0.03$). Only data from Mt. Ishizuchi were used in further analyses because of the small number of fungi detected on Mt. Fuji.

The distribution patterns of individual fungi found on >3 cores, including spore and existing root communities, were visualized in Figure 4.2. Six species (found on > 3 seedlings) were tested for aggregated distribution patterns and only *P. involutus* sp.2 at Site I2 showed an aggregated distribution pattern.

Compositional patterns

Cg does not produce sexual spores and reproduces asexually via sclerotia, mitotically derived propagules (Douhan *et al*. 2007); thus, this species was excluded from the analyses of spore composition in the following analyses. Species occurrence on Mt. Ishizuchi showed contrasting patterns between existing roots and spores along the elevation gradient. Existing root community was composed of 110 (55.3%) singletons and 50 (25.1%) common species (i.e., recorded in >1 samples), of which only six species (3%) occurred at the all three sites. In contrast, spore community was composed of six (26.1%) singletons and 15 common species (65.2%), of which seven species (30.4%) occurred at the all three sites (Figure 4.3). Adonis showed that composition similarity was significantly separated by colonization strategies (existing root vs. spores) ($F_{1,13} = 3.22$, $R^2 = 0.20$, $P < 0.01$; Figure 4.4). Spore community composition was separated by hosts but not by sites (Table 4.2).

Effects of soil carbon content

Tomentella species generally occurred in soil cores with higher soil carbon contents (Figure 4.5). Statistical significance was detected for *Tomentella sublilacina* on *Betula* at I3 ($P = 0.041$), and marginally for *Tomentella* sp.75 ($P = 0.069$) and *T. sublilacina* ($P = 0.065$) on Betula at I1. *Rhizopogon* sp.1 at I1 ($P = 0.048$) and Cg at I2 ($P = 0.040$) on *Pinus* occurred in soil cores with lower soil carbon contents.

4.4 Discussion

Community compositions between spores and existing roots

EM fungal communities on existing roots have been extensively studied, revealing high species diversity and compositional heterogeneities in many ecosystems around the world (Chapter 3). In contrast, spore communities have received less attention despite the ecological

significance in fungal life cycles and tree regeneration after disturbances. Moreover, most studies are based on stand scale studies in *Pinus* dominated forests in North America (Baar *et al*. 1999; Izzo *et al*. 2006b). This is the first systematic study of EM fungal spore communities in old-growth temperate forests along wide environmental gradients. Existing root communities showed high species turnover along the elevation gradients and species occurrence appeared to be largely determined by environmental factors (Chapter 3). In contrast, spore communities showed low species turnover along the gradient and occurrence of some fungi were less likely restricted by distance or environmental factors (Figure 4.3). For example, the number of species that occurred at all three sites on Mt. Ishizuchi was only six (3.0% of the total species) on the existing root communities whereas seven (30.4%) in the spore communities. My results indicate that spores of some fungal species are widespread across distinct forest types located within 10 km. These results support the hypothesis that spore communities are relatively homogeneous and predictable regardless of forest types in contrast to existing root tip communities. Although dispersal capability of fungal spores are largely unknown, some pioneer EM fungal spores were reported to travel long distances ~10 km (Peay *et al*. 2012; Hynson *et al*. 2013).

The differences in the compositions between spores and existing roots may represent a colonization-competition trade-off (Tilman 1994) in fungal strategies. Many fungi found as spores were pioneer species, which would colonize quickly on available roots before competitive fungi come into the habitats after disturbance (Deacon and Fleming 1992). These fungi may be weak competitors in later succession stages but strong colonizers by producing long-lived and high drought-resistant spores that are distributed widely across climate ranges (Nguyen *et al*. 2012). In contrast, fungi found frequently on existing roots may maximize survival and propagation in specific habitats. Reproduction may occur via asexual mycelia and spores that germinate under conditions specific to late-successional stages, e.g., require

the presence of mycelia of the same species for spore germination (Fries 1987; Miller *et al*. 1993). The variation in colonization strategies among fungi may enhance ecosystem resilience to disturbance events because pioneer fungi existing as spores in mature forests could help tree regeneration upon disturbance when competitor fungi are removed.

Host effects

I found contrasting host effects on community structures between spores and existing roots. Hosts was a key factor separating spore communities, while played a minor role on the existing root communities (Chapter 3). Many studies have reportd the importance of host identity in spore gemination (e.g., Fries 1987; Ali and Jackson 1988). For example, spores of *Suillus* require root exudates of *Pinus* to germinate (Fries 1987; Kikuchi *et al*. 2007). In this study, I detected *Rhizopogon* and *Suillus* on *Pinus* seedlings, which are well known Pinaceae-specific EM fungi (Mollina *et al*. 1992). Similarily, *Paxillus involutus* sp.2 was detected only on *Betula* with the highest infection rates (28 seedlings) among the observed fungi. Although this species occurs on various hosts on mature tree roots (Hedh *et al*. 2008; Jargeat *et al*. 2014), germination rates of this species were reported to be much higher on roots of *Betula* than *Picea* (Ali and Jackson 1988). These results suggest that host identity may be critical especially during spore germination, possibly because fungi need to ensure the presence of proper hosts to colonize in early succesional habitats.

Soil carbon

Taylor and Bruns (1999) demonstrated that EM fungi may exhibit niche preferences to organic or mineral soil layers. These observations are based on soil depth, but the effect of soil carbon content has yet to be evaluated quantitatively. In this study, Cg and *Rhizopogon* sp.1 occurred in soils with lower carbon contents (Figure 4.5). *Rhizopon* species are pioneer

fungi whose spores are highly resistant to heat as an adaption to wildfires that dramatically remove organic matters in soil (Izzo *et al*. 2006a; Peay *et al*. 2009). These spores may exist for years in deeper mineral soils before germinate in response to disturbance events. In contrast, *Tomentella sublilacina* existed in cores with significantly higher carbon content in this study. *T. sublilacina* is suggested to be abundant in organic layers and is often detected both in spore and existing root communities (Taylor and Bruns 1999; Lilleskov and Bruns 2003). This species also produce long-lived spores that remain viable after animal ingestion (Lilleskov and Bruns 2005). This species may be competitive in both pioneer and mature habitats by reproducing frequently by spores. Although statistical significance was not detected in many fungi in this study, it is probably because of small sampling sizes rather than the lack of biological patterns. Other *Tomentella* species that found in soil with high carbon content (Figure 4.5) may possibly be competitive in both pioneer and mature forests.

Richness patterns

I detected 23 fungal species on Mt. Ishizuchi whereas only 11 species on Mt. Fuji. The reasons of the observations are less clear. One potential reason is soil pH that was negatively correlated with spore fungal richness. Mt. Ishizuchi had lower soil pH (pH 3.8-4.1) than Mt. Fuji (pH 4.6-5.1). Low soil pH may reduce the activities of soil animals and microorganisms (Rousk *et al*. 2009) that could increase the chances of spore survival. Another possibility is disturbance frequencies. Disturbances including windfalls and landslides may occur more frequently on Mt. Ishizuchi than Mt. Fuji. For examples, slopes of Mt. Ishizuchi are steeper (Table 2.1) and this area receives higher summer precipitation. To support this, spore richness was positively correlated with slope and summer precipitation. Moreover, the highest richness was found at I1 site on Mt. Ishizuchi, which was characterized by rock exposed forest floor and the steepest slopes (Table 2.1). *Rhizopogon* species are thought to be disturbance-adapted

and only recorded at this site, suggesting the potential roles of disturbances on the richness of spores. Although richness was relatively low on Mt. Fuji, spores may exist in deeper soils because some EM fungi tend to favor mineral soil layers (Taylor and Bruns 1999; Murata and Nara 2014).

Methodological considerations

Although richness was much lower in the spore community than existing root community, the result does not necessarily indicate that propagules of other species are absent. Previous studies on population genetics have reported that late-staged EM fungi reproduce frequently by spores (Redecker *et al*. 2001; Carriconde *et al*. 2008). But their spores may be short-lived or germinate under specific conditions that represent late successional stages, such as the presence of micro-organisms (Ali and Jakson 1989), mycelia of the same species (Fries 1987; Miller *et al*. 1993) or roots of older plants (Theodorou and Bowen 1987). Bioassay experiments likely underestimate the richness of resistant spores in soils because the experiments only detect fungal propagules that germinate in the presence of young roots of tested hosts (6 month-old *Pinus* and *Salix* or *Betula* seedlings in this study). Still, bioassay experiments are valuable in examine spore communities because pioneer hosts tend to regenerate right after disturbances. Bioassay experiments sufficiently represent disturbed conditions, and thus provide insight into colonization patterns of EM fungi after disturbance. My data imply that forests harbor diverse fungi exhibiting different colonization strategies that enhance resilience of the ecosystem to disturbances, even in relatively stable old-growth temperate forests.

Table 4.1 List of ectomycorrhizal fungal species. Numbers show the count of seedlings for spore communities and number of cores for existing root communities.

	Mt. Fuji								Mt. Ishizuchi						Root com.	
	Salix				*Pinus*				*Betula*			*Pinus*			F	I
Species	F1	F2	F3	F4	F1	F2	F3	F4	I1	I2	I3	I1	I2	I3		
Astraeus hygrometricus		1							2			1				
Cenococcum geophilum	21	31	11	15	14	12	21	8	5	34	5	7	23	7	156	76
*Hebeloma crustuliniforme**				1												
*Hebeloma rivulosuma**									1							
Hydnotrya sp.2										2						2
Hydnotrya sp.4										2						
Laccaria cf. *laccata* sp.1*				1						1					2	
Laccaria cf. *laccata* sp.2*											2				1	11
*Laccaria laccata**		1	1												2	
Laccaria sp.1*										1					10	5
Melanogaster sp.													1			
Paxillus involutus sp.1*				1												
Paxillus involutus sp.2*	1								8	15	5					
Rhizopogon sp.1*												3				
Rhizopogon sp.2*												6				
Russula velenovskyi								1							2	
*Scleroderma bovista**		2	1													
*Scleroderma citrinum**									1	1	3	2	2	3		4
*Suillus bovinus**												1	2	1		
Suillus granulatus														1		
Suillus luteus												1				
Tomentella sp.49		1													2	
Tomentella sp.74									1	2	3	1		1		
Tomentella sp.75									7	3	2					
Tomentella sp.76											1					
Tomentella sp.77									1	2	2					
Tomentella sp.78									1							
Tomentella sp.79									7							
*Tomentella sublilacina**				1					6	3	8				2	13
No. seedlings	49	46	41	44	47	47	46	49	50	50	49	50	50	48		
Infection rate	0.45	0.72	0.29	0.41	0.26	0.45	0.17	0.31	0.68	0.96	0.59	0.38	0.54	0.25		
Richness	2	5	3	5	1	1	1	2	11	11	9	8	4	5		

Abbreviations: Root com, existing root communities; F, Mt. Fuji; I, Mt. Ishizuchi.
Asterisks indicate pioneer fungal species (Deacon and Fleming 1992; Cairney and Chambers 1999; Nara 2009). Pioneer fungi are defined as those that are often reported in early successional habitats (i.e., young forests and disturbed habitats) as mushrooms and ectomycorrhizas. Note that pioneer fungi first appear on early stages but may also occur at later stages. *Russula velenovskyi* is assumed to be a late-stage fungus. Successional status of other fungi is less clear.

Table 4.2 The effect of host and site on the spore composition on Mt. Ishizuchi, revealed by the Adonis test.

	DF	SS	MS	*F*-statistic	R^2	*P*-value
Host	1	0.89	0.89	7.83	0.63	0.02
Site	2	0.29	0.15	1.27	0.21	0.59
Residuals	2	0.23	0.11		0.16	
Total	5	1.41			1	

Abbreviations: DF, degree of freedom; SS, sum of squares; MS, mean square.

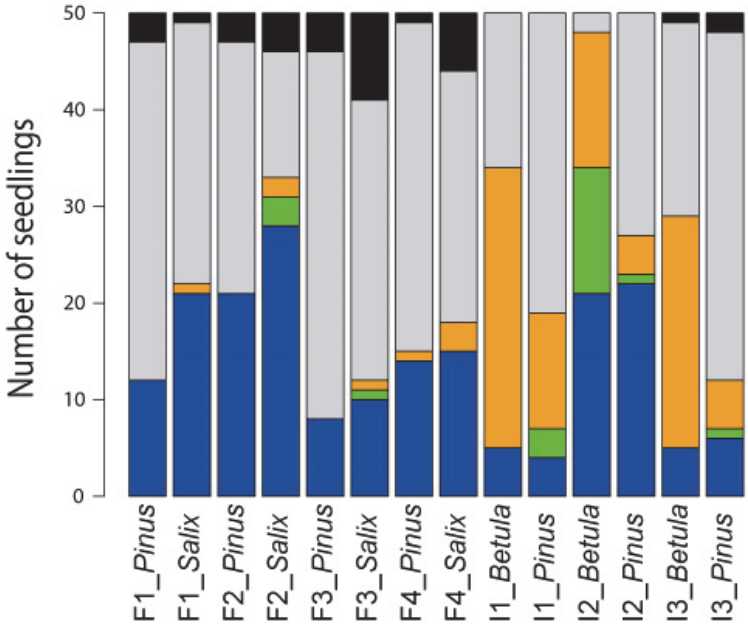


Figure 4.1 Number of seedlings colonized by ectomycorrhizal (EM) fungal species per site per host. Blue indicates seedlings colonized sololy by *Cenococcum geophilum* (Cg), green by Cg and other EM fungi, and orange by EM fungi other than Cg. Grey indicates no EM formation and black represents dead seedlings. See site codes for Table 2.1.

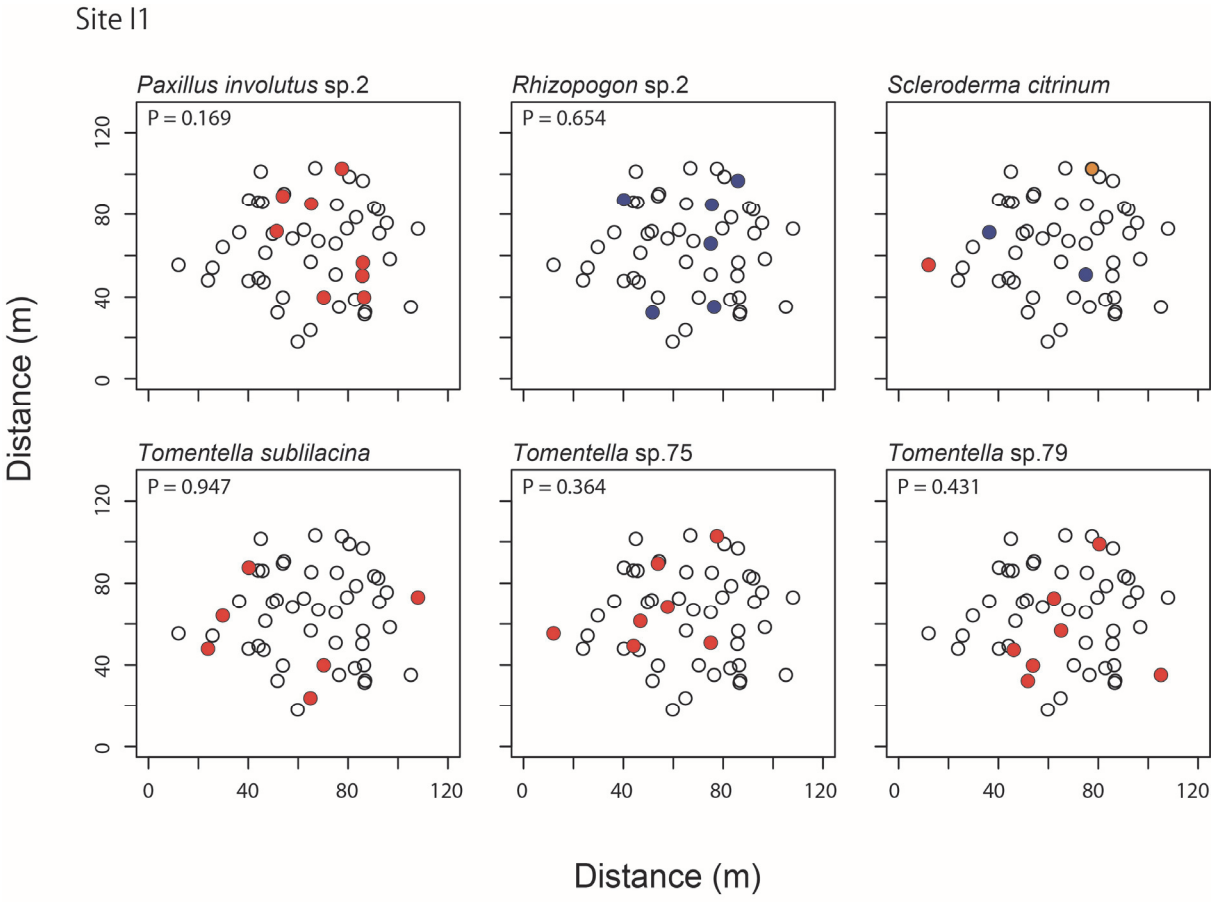


Figure 4.2 The distribution patterns of individual ectomycorrhizal fungal species within sites. Circles are sampling points. Color indicates points where species was recorded: *Betula* seedlings (red), *Pinus* seedlings (blue), existing roots of broadleaf host(s) (orange), existing roots of conifer host(s) (black). Green indicates points where the species was found on multiple categories and abbreviations above the point show the categories: P-B (*Pinus* and *Betula* seedlings) and B-Ec (*Betula* seedlings and existing roots of conifer host). The size of the circles is not an exact scale. *P* is the probability of obtaining aggregated distribution patterns than expected by chance in the randomization procedures as described in Chapter 2. *P*-values were computed for species found on >3 seedlings separately per host (samples from existing roots were not combined or tested). A gap (180 m) between the two forest patches at Site I3 is indicated by a dotted line. Randomization procedures were not applied for species that were recorded in the two patches at Site I3.

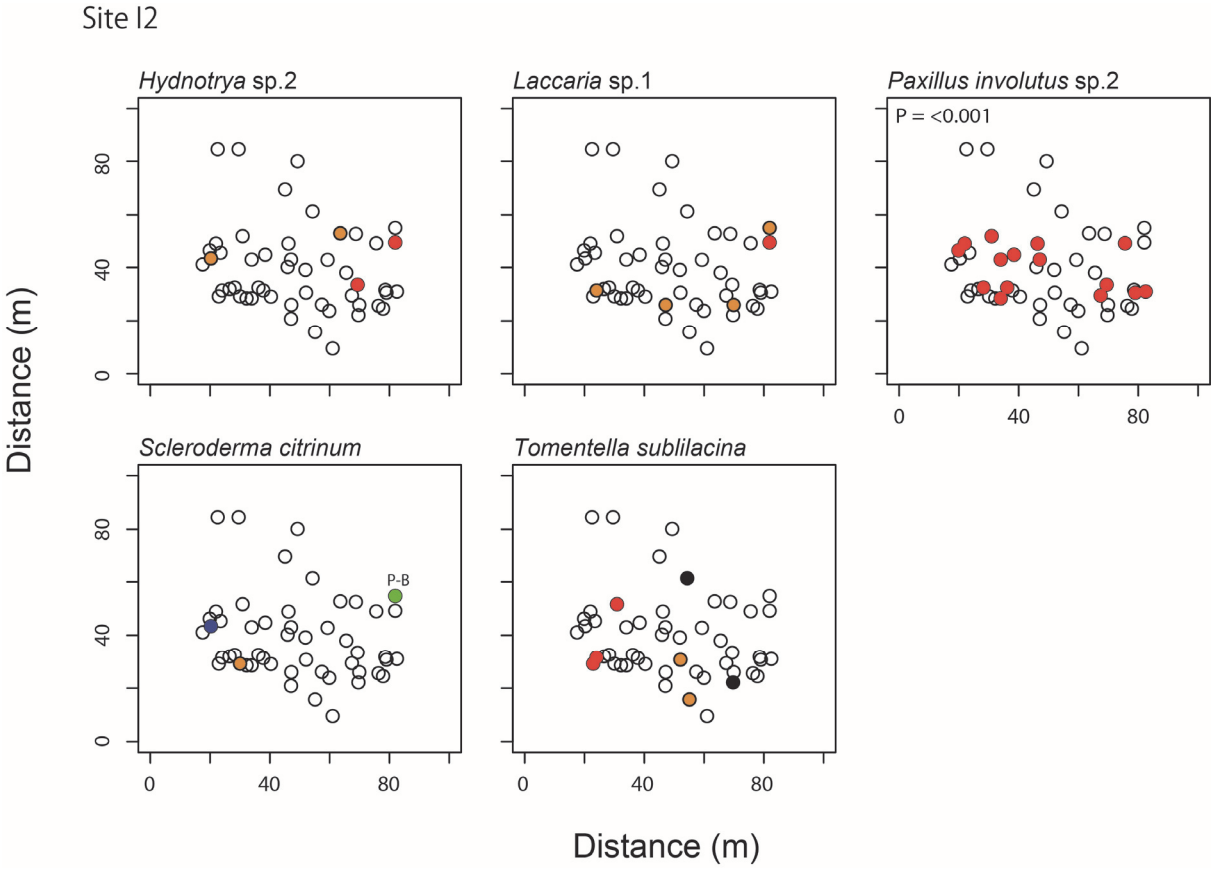


Figure 4.2 (continued.)

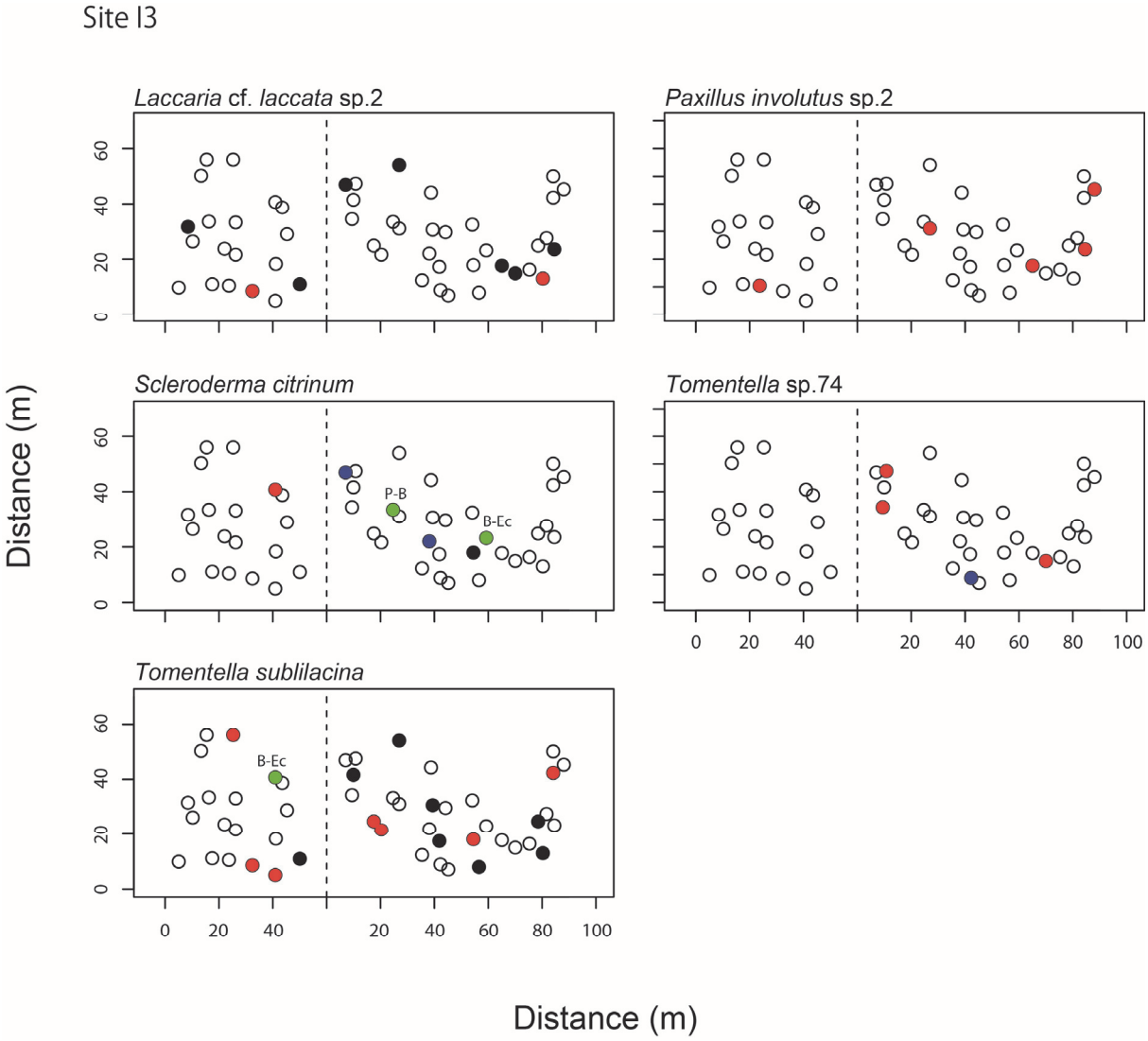


Figure 4.2 (continued.)

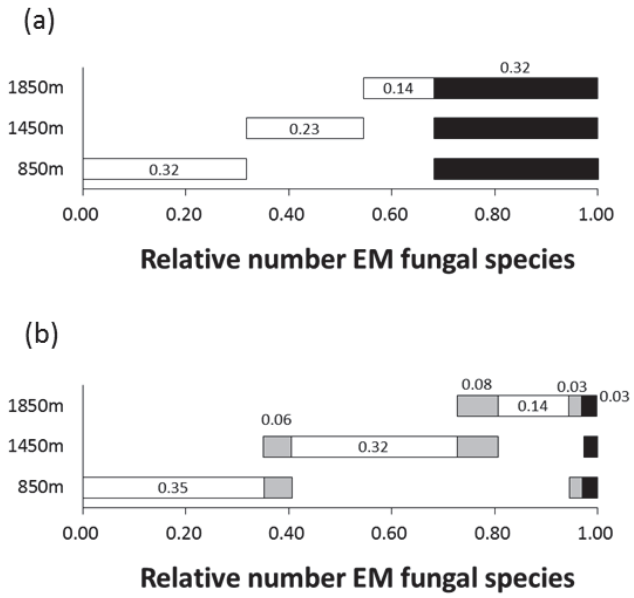


Figure 4.3 Relative number of ectomycorrhizal (EM) fungal species along an elevation gradient on Mt. Ishizuchi. (a) Spore (23 species) and (b) existing root communities (200 species). Black bars indicate the proportion of species that were shared at all sites. White and grey bars indicate the proportion of species occurred at one sites and two sites, respectively.

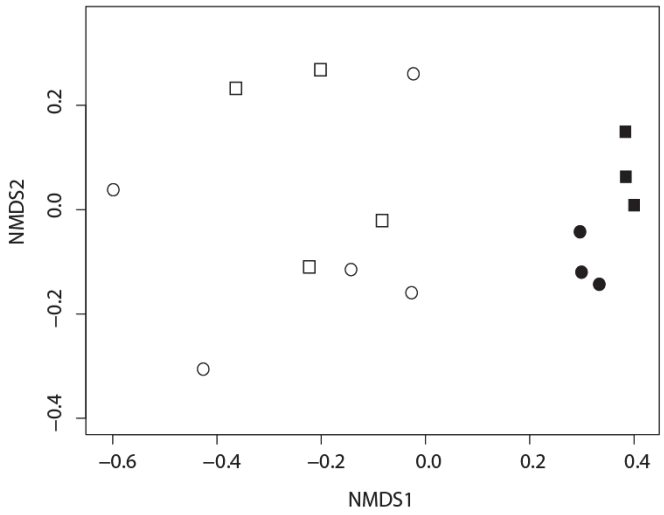


Figure 4.4 Similarity of fungal compositions revealed by the nonmetric dimentional scaling (NMDS) graph. Fungal composition per host per site is treated as a unit. Fungal compositions of existing roots represented by >4 cores are included in the analyses. Black and white symbols indicate spore and existing root communities, respectively. Circles and squres are broadleaf and conifer hosts, respectively. Stress = 0.078.

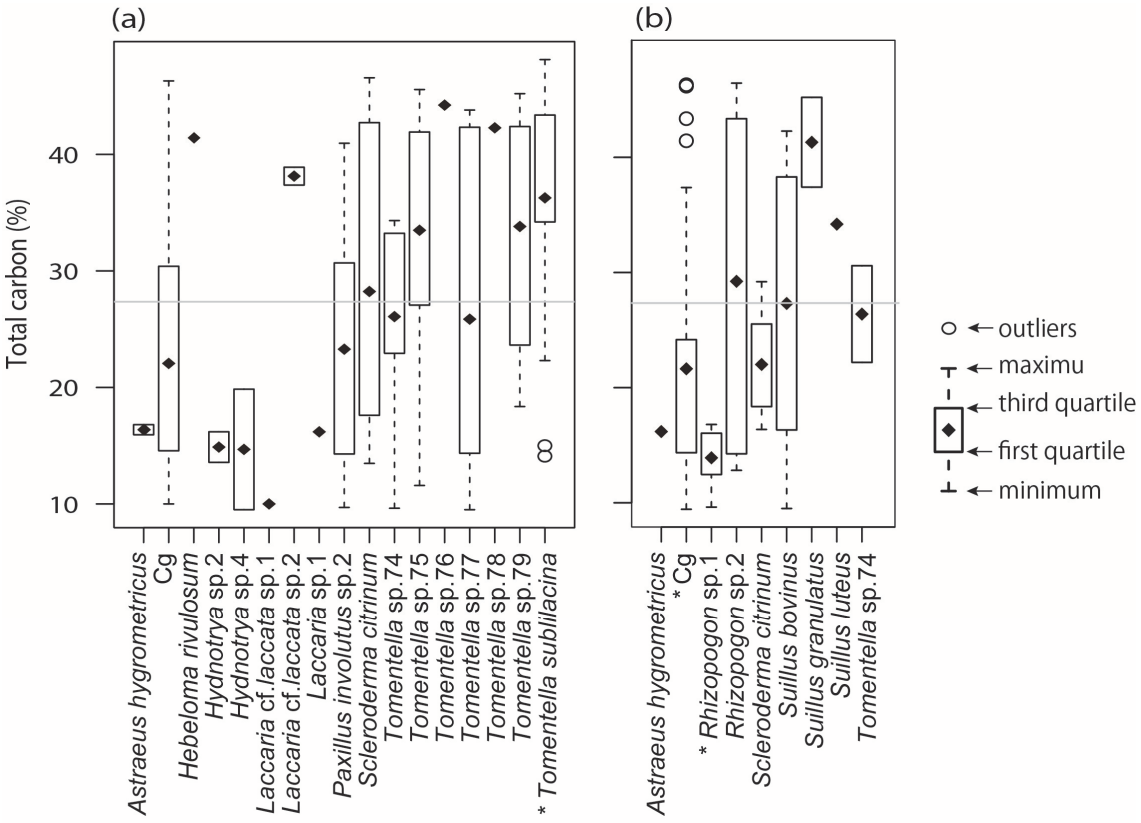


Figure 4.5 The occurrence of ectomycorrhizal fungal species in relation to soil carbon content. Closed diamonds represent the mean values. (a) *Betula* and (b) *Pinus* on Mt. Ishizuchi. Grey horizontal lines show the average carbon content from the all soil cores. Data from all sites are pooled in the graph, but statistical significance was tested per host per site and shown in the text (asterisk indicates species that showed significant differences). Cg is *Cenococcum geophilum*.

Chapter 5 Summary and conclusions

5.1 Summary of the thesis

I examined the distribution patterns and community structures of ectomycorrhizal (EM) fungi at various spatial scales, with the overall goal of determining effective predictor variables for EM fungal composition at a regional scale. Field survey was conducted in seven forest stands along elevation gradients on two mountains in a temperate region of Japan. I recorded the largest EM fungal richness from a single study among the previous studies (e.g., Tedersoo *et al*. 2011; Bahram *et al*. 2012; Murata *et al*. 2013). This is the first study to explicitly demonstrate the existence of EM fungal species range overlaps among sites. The key findings for the EM fungal communities on existing tree roots were 1) fungal species range overlaps occurred continuously along elevation gradients, and 2) range overlaps were especially notable between similar forest types on different mountains. These results imply that many fungi exhibiting wide distribution ranges are constrained by contemporary environmental factors. Furthermore, host tree genera associated with EM fungi were identified through molecular analyses, which provided critical information for explicitly assessing host-fungus relationships across sites. Regional scale analyses showed that EM fungal compositions were more strongly influenced by climate factors (temperature and precipitation) than by geographic distance, soil properties, or host identity, without considering interaction effects.

Overall, the distribution patterns and predictive variables for fungal communities were context dependent that varied by spatial scales, forest compositions, and fungal developmental stages (Table 5.1). Several important findings from this study are as follows.

1) At the stand scale (~1ha), I detected aggregated distribution patterns in some fungal species and spatial structures in fungal compositions at most sites (Chapter 2). Spatial distance generally predicted EM fungal composition better than host identity or soil properties at

the stand scale. These results suggest that spatial autocorrelations in fungal distributions, possibly derived from internal processes, should be considered when accurately evaluating the effects of environmental factors and hosts (Pickles *et al*. 2009; Bahram *et al.* 2013a).

2) Host effects on fungal composition highly varied by spatial scales and forest types (Chapters 2 and 3). Host trees played an important role in differentiating EM fungal compositions in each of the conifer-broadleaf mixed forests, as reported previously (Ishida *et al*. 2007, Tedersoo *et al*. 2008). On the other hand, host effects were minor in phylogenetically low diverse forests or at larger spatial scales across the sites.
3) I demonstrated a positive correlation between tree and EM fungal compositions at the regional scale, but the relatively weak host effect imply the possibility of individualistic responses of the symbiotic partners to climate (Chapter 3).
4) Spore communities were, for the first time, examined in detail in several old-growth forests. Spore communities comprised many pioneer fungi, which differed dramatically from the fungal communities of existing tree roots (Chapter 4).
5) Spore community was mainly differentiated by host identity. Host identity may be critical for spore germination in pioneer fungi, which establish EM associations with regenerating trees in post-disturbance habitats (Chapter 4).

5.2 Detection of spatial structures

Microbial communities are usually composed of numerous rare species, which are inevitably overlooked with limited sampling efforts (Taylor 2002). Thus, sampled microbial communities may not represent the actual microbial communities in the field, and the application of ecological models developed for macroorganisms to microorganisms is therefore inappropriate. Demonstrating geographical distributions of microorganisms is especially challenging. In this study, I used an intensive and consistent sampling approach and

obtained a relatively large EM fungal community dataset for each site. This approach effectively detected many species shared across sites, clearly confirming the existence of microbial species distribution ranges. These findings indicate that intensive sampling approaches likely contribute to ecological studies of microorganisms at large spatial scales.

Spatial distance has not generally been considered as a predictor variable for community structures despite its biological relevance that is unrelated to environmental factors (i.e., dispersal limitation; Legendre 1993). In this study, spatial eigenvectors were included as potential predictors to partition the variation explained by abiotic or biotic variables. I demonstrated that stand scale fungal communities were explained mainly by distance but less by host identity or soil properties (Figure 2.4), suggesting that distance alone could be an important predictor of stand scale fungal structures in many forests. In contrast, the regional scale analyses clearly showed that the distributions of some fungi were not restricted within a 550-km range and geographic distance *per se* played a minor role in explaining fungal composition (Figure 3.6). Note that accurate detection of fungal distributions remains challenging because limited soil sampling provides no information for a large proportion of site or area where the soil was not collected. At larger scales, the intensive sampling approach inherently limits the ability to sample many sites. Although these limitations are currently inherent to all sampling approaches, future research may provide solutions to the difficulties. This study is a critical step toward detecting the distribution patterns of microorganisms belowground.

5.3 Effects of climate factors

Climate is the main driver of biodiversity and geographical distributions of plants and animals (Currie *et al*. 2004). Moreover, recent studies have shown that temperature influences community composition of EM (Bahram *et al*. 2012) and saprophytic (Meier *et al*. 2010)

fungi. In this study, climate factors (13%) had relatively large influences on EM fungal composition, suggesting that EM fungal communities are sensitive to climate change. Climate change may alter fungal communities directly by affecting fungal physiological activities, and indirectly by changing plant community structures, host performance (i.e., carbon allocation to fungi), and competitive interactions among fungi. I found a positive association between tree and EM fungal compositions, suggesting that above- and belowground communities are closely interlinked. However, the weak host effect on EM fungal composition at the regional scale implies that the symbiotic partners are correlated but respond to climate factors independently. Thus, climate change may affect the two trophic groups at different rates and to different degrees, which may alter the carbon and nutrient cycling processes between the symbiotic partners (Pickles *et al*. 2012; Johnson *et al*. 2013).

5.4 Host effects

Host trees have been suggested to influence EM fungal composition (Ishida *et al*. 2007). However, empirical evidence for significant host effects is inconsistent, and effects have been detected in some cases (e.g., Tedersoo *et al*. 2008; Morris *et al*. 2009; Smith *et al*. 2009) but not in others (e.g., Kennedy *et al*. 2003; Smith *et al*. 2011; Tedersoo *et al*. 2011) depending on studied ecosystems, host species, and statistical methods applied. Host phylogenetic distance is now widely considered as a key factor explaining host preferences among fungi (Ishida *et al*. 2007). However, the majority of the discussion on this topic relies on stand scale studies across narrow environmental range. Although a meta-analysis suggested an important effect of host family on EM fungal composition at a global scale (Tedersoo *et al*. 2012), cautious interpretation is required because data on EM fungal communities were scarce and patchily distributed, different methodologies were applied among studies, and host distributions were correlated with climate regions and geographic history at a global scale. More detailed local to

regional scale investigations are needed before extending the debate to a global scale.

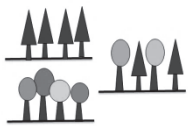
This study provided critical insights into host effects on EM fungal composition by examining various forest types with similar geographic history. I found significant host effects in conifer-broadleaf mixed forests, and the strength of the effects increased with the host phylogenetic diversity (Figure 2.5). These results supported a previous hypothesis that EM fungal composition is influenced by host phylogeny within stands (Ishida *et al*. 2007). However, I found that host effects were relatively weak at the larger spatial scales (Chapter 3). These results imply that host preferences among fungi may be detected at stand scales but less likely at larger spatial scales, at which other factors (i.e., climate) become more influential. In contrast, hosts played a prominent role in separating spore communities along an elevation gradient encompassing a wide climate range. Spore communities were mainly composed of pioneer species, which may require specific hosts for spore germination to ensure colonization of appropriate trees in disturbed habitats. Thus, host effects may be critical only during spore germination or in disturbed habitats. For example, a previous study showed that germination rates of spores of *Paxillus involutus* were much higher in the presence of *Betula* roots than *Picea* roots (Ali and Jackson 1988), although this species occurs on existing roots of various hosts in field (Hedh *et al*. 2008; Jargeat *et al*. 2014).

The minor role of host identity detected at the regional scale may be related to the studied system that was mainly composed of generalist EM partners, such as *Fagus*, *Betula*, and *Abies* and fungi belonging to Russulaceae and Thelephoraceae (Smith *et al*. 2009). Bruns *et al*. (2002) also suggested that most EM fungi are generalists and fungal host specificity (e.g., *Alpova*, *Rhizopogon*) may be exceptional. Furthermore, many empirical studies have demonstrated strong compatibilities between EM fungi and a range of hosts in temperate regions (Twieg *et al*. 2007; Trocha *et al*. 2012; Bahram *et al*. 2013b), suggesting that host-sharing among EM fungi may occur frequently in typical temperate forests.

5.5 Future directions

Although knowledge of global distribution ranges of organisms is fundamental to promote biological conservation programs, the distribution ranges of soil microbes are very limited, mainly because they are not easily observed by ordinary ground surveys or by remote sensing. I examined two transects in a region, representing a small contribution toward overall understanding of the distribution patterns of soil fungi. Ultimately, collecting more samples at larger geographical scales would be desirable to better detect the biogeography of EM fungi. However, collecting large quantities of samples over wide geographic ranges for molecular identification is difficult especially for soil fungi. Therefore, systematic data collection is needed to effectively detect any biogeographic patterns. Ideally, additional data would be collected across wide environmental and spatial gradients at regular intervals using consistent sampling methods. Unstudied or undersampled areas and environmental conditions should be prioritized in future surveys. Combining data collected by systematic sampling strategies may enable effective development of current distribution maps for soil fungi, which would provide a useful tool for predicting the future status of soil fungi responding to environmental change.

Table 5.1 Summary of ectomycorrhizal fungal distributions and the strong predictor variables on fungal composition.

Developmental stages	Ectomycorrhizal fungi on existing tree roots			Spores
Scale	Stand 	Local (elevation) 	Regional 	Local (elevation) 
Distribution patterns of individual species	Aggregation in some taxa	Range overlaps along gradients	Range overlaps between similar forest types on different mountains	Wide distribution range
Strong predictor variables	Spatial distance Hosts in mixed-forests	Climate (soil)	Climate (soil and distance)	Host

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Appendix

Appendix A. List of tree species recorded at study sites. The first 19 tree species are ectomycorrhizal host trees.

	Basal area (m^2 per hectare)							Density (stems per hectare)						
Tree species	F1	F2	F3	F4	I1	I2	I3	F1	F2	F3	F4	I1	I2	I3
Abies firma	0.83				10.11			29.4				147.7		
Abies homolepis		20.80				6.37			156.7				147.7	
Abies veitchii (including *A.mariesii*)			24.94	3.29			31.98			560.2	269.9			855.6
Tsuga diversifolia			21.65	68.79						876.0	993.1			
Tsuga sieboldii	0.06				17.24	0.41		19.6				96.8	5.1	
Picea jezoensis var. *hondoensis*		2.87	0.91						4.9	10.2				
Pinus parviflora			1.99							15.3				
Larix kaempferi				21.37							183.3			
Fagus crenata	4.30	33.90				19.21		53.9	102.8				142.6	
Fagus japonica	6.96							195.9						
Quercus crispula	20.12	2.13					0.10	166.5	49.0					10.2
Quercus salicina					9.79							275.0		
Betula ermanii		0.46		0.16			0.34		14.7		5.1			25.5
Betula grossa	4.63	0.26				2.28		53.9	14.7				25.5	
Betula maximowicziana	0.48							4.9						
Carpinus cordata	1.47							367.3	4.9					
Carpinus japonica		0.01							4.9					
Carpinus laxiflora					0.15							10.2		
Carpinus tschonoskii	6.03							146.9						
Acanthopanax sciadophylloides		0.13				0.06			14.7				10.2	5.1
Acer argutum						0.42							81.5	
Acer micranthum		0.05							4.9					
Acer mono var. *marmoratum*	3.38	0.02			2.79	0.27		137.1	19.6			40.7	10.2	
Acer nikoense	0.36							49.0						
Acer nipponicum						0.15							50.9	
Acer palmatum					1.09							10.2		
Acer palmatum subsp. *amoenum*	1.25	0.19						102.8	49.0				5.1	
Acer rufinerve	1.30	0.35						9.8	39.2					
Acer shirasawanum		1.04						4.9	53.9					
Acer sieboldianum		0.45			0.01	0.73	0.49	4.9	24.5			5.1	91.7	15.3
Acer tenuifolium	0.27	0.03						68.6	24.5					
Acer tschonoskii var. *australe*							0.02						10.2	10.2
Alangium platanifolium var.*trilobum*					0.01							15.3		
Callicarpa japonica					0.07							264.8		
Callicarpa mollis												15.3		
Celtis sinensis												5.1		
Cercidiphyllum japonicum		0.05							4.9					
Chamaecyparis obtusa					0.05							30.6		
Chamaecyparis pisifera	2.09							78.4						
Clethra barbinervis					0.01	0.10						15.3	20.4	

Appendix A. (continued)

	Basal area (m^2 per hectare)							Density (stems per hectare)						
Tree species	F1	F2	F3	F4	I1	I2	I3	F1	F2	F3	F4	I1	I2	I3
Cleyera japonica					0.49							30.6		
Cornus controversa	0.32	0.15			0.02			4.9	4.9			15.3		
Cornus kousa	0.17							68.6						
Daphniphyllum macropodum												5.1		
Euonymus oxyphyllus						0.05							20.4	
Eurya japonica					0.07							142.6		
Fraxinus langinosa f. *serrata*	1.31	0.02			0.01	0.03		14.7	14.7			5.1	5.1	
Helwingia japonica													5.1	
Hydrangea scandens					0.06							117.1		
Ilex crenata												5.1		
Ilex macropoda	0.03							14.7					10.2	
Illicium anisatum					2.30							682.5		
Kalopanax pictus						4.33							5.1	
Lindera erythrocarpa					0.02							10.2		
Lindera sericea												5.1		
Lindera umbellata					0.02							117.1		
Magnolia praecocissima	0.15							9.8						
Morus australis					0.04							10.2		
Neolitsea sericea													5.1	
Parabenzoin trilobum					0.13	3.18						56.0	1619.6	
Phyllanthus flexuosus					0.01							25.5		
Pieris japonica	0.02							9.8						
Prunus incisa									4.9					
Prunus jamasakura		0.05			0.01				4.9			5.1		
Prunus maximowiczii		0.09							4.9					
Prunus shikokuensis							0.01							10.2
Prunus sp.	0.22							10.2						
Pterostyrax corymbosa					0.04							5.1	5.1	
Rhododendron brachycarpum				0.05							25.5			
Rhododendron dilatatum												5.1		
Sorbus alnifolia		0.11							19.6					
Sorbus commixta		0.02					0.22		14.7					5.1
Stewartia monadelpha					1.80	0.49						86.6	50.9	
Stewartia pseudocamellia						0.90							61.1	
Symplocos coreana						0.11							40.7	
Taxus cuspidata							0.04							20.4
Tilia japonica		8.85							230.2					
Torreya nucifera	0.01				0.08			9.8				25.5		
Viburnum furcatum													5.1	
Viburnum wrghtii						0.01						10.2	15.3	
Weigela decora														5.1
Zelkova serrata					0.46							66.2		
total	55.76	72.03	49.48	93.66	46.87	39.09	33.19	1636.0	886.4	1461.7	1477.0	2363.1	2449.7	962.6

Appendix B. List of primers used in the molecular analyses.

taxa	region	primer name	sequence >5'---3'
fungi	ITS	ITS1	TCC GTA GGT GAA CCT GCG G
		ITS1F	CTT GGT CAT TTA GAG GAA GTA A
		ITS3	GCA TCG ATG AAG AAC GCA GC
		ITS4	TCC TCC GCT TAT TGA TAT GC
		ITS4B	CAG GAG ACT TGT ACA CGG TCC AG
		ITS5	GGA AGT AAA AGT CGT AAC AAG G
	LSU	LR3	CCG TGT TTC AAG ACG GG
		LR5	TCC TGA GGG AAA CTT CG
		LR21	ACT TCA AGC GTT TCC CTT T
		LR22	CCT CAC GGT ACT TGT TCG CT
plants	trnL	trnC	CGA AAT CGG TAG ACG CTA CG
		trnD	GGG GAT AGA GGG ACT TGA AC
		trnE	GGT TCA AGT CCC TCT ATC CC
		trnF	ATT TGA ACT GGT GAC ACG AG

Abbreviations: ITS, internal transcribed spacer; LSU, large subunit.

Appendix C. List of host species and International Nucleotide Sequence Database accession numbers used to compute the pairwise host phylogenetic distances.

Species	matK	trnL
Abies firma	JQ512383	JF276155
Tsuga sieboldii	JQ512505	AB979731†
Larix kaempferi	JQ512435	AB045064
Betula maximowicziana	AY372020	AB979732†
Carpinus tschonoskii	AY211998	AY211414
Fagus crenata	AB060058	AB046513
Quercus mongolica subsp. *crispula*	AB727882	AB979733†

† The gene sequences generated in this study

Appendix D. Results for preliminary bioassay experiments using *Quercus crispula* and *Abies veitchii* on Mt. Fuji. *Quercus* and *Abies* seeds were collected at Sites F1 and F3, respectively, in September 2011. Seeds were surface sterilized and grown in autoclaved soil before transplanted in tubes containing field collected soils. Bioassay experiments were conducted using 50 ml tubes for *Quercus* and 15 ml tubes for *Abies*.

Table D1. List of ectomycorrhizal fungal species. The numbers show the count of seedlings.

	Quercus				*Abies*
Species	F1	F2	F3	F4	F3
Astraeus hygrometricus			1		
Cenococcum geophilum	9	10	6	11	7
Entoloma sp.3	1				
Laccaria laccata		1			
Paxillus involutus sp.1			1		
Paxillus involutus sp.2	1				
Russula velenovskyi				1	
No. seedlings	20	20	21	21	25
Infection rate	0.55	0.55	0.38	0.57	0.28
Richness	3	2	3	2	1

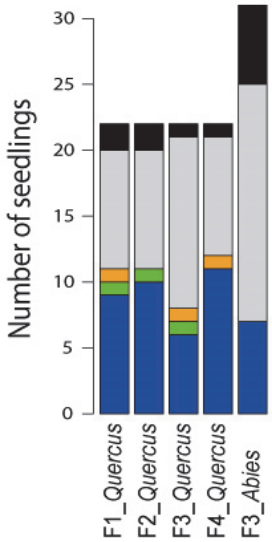

Figure D1. The number of seedlings colonized by ectomycorrhizal (EM) fungal species per site per host. Blue indicates seedlings colonized sololy by *Cenococcum geophilum* (Cg), green by Cg and other EM fungi, and orange by EM fungi other than Cg. Grey indicates seedlings without EM formation and black represents dead seedlings.

Acknowledgements

I would like to express the deepest appreciation to Associate Professor Kazuhide Nara for supervising all of my work presented here. Without his guidance and persistent help this dissertation would not have been possible.

I would like to thank my committee members, Professor Kenji Fukuda, Professor Shigeaki Kojima, Associate Professor Maki Suzuki, and Associate Professor Norihisa Matsushita for all of their guidance through this process, constructive discussions and insightful comments on this thesis.

I would like to thank Dr. Takashi Nakano at Mount Fuji Research Institute and Dr. Atsushi Sakai at Forestry and Forest Products Research Institute for cooperation in this research. I thank Dr. Akihiko Kinoshita, Dr. Masao Murata and Dr. Megumi Tanaka for technical supports in the lab and helpful discussions throughout this research; Katsuaki Jimbo, Sho Ohashi, Yasunori Matsuo, Kenshiro Minami, Ai Shimizu and Takuya Yamazaki for field assistance; Dr. Masahira Hattori, Dr. Kenshiro Oshima, Emi Omori, and Yukiko Takayama in the Center for Omics and Bioinformatics at the University of Tokyo, for assistance of sequencing; Dr. Ayumi Oda, Masanori Miyata, and Dr. Mariko Norisada for help with soil analyses; all members of the Biosphere Function Lab and Evaluation of Natural Environment Lab for enjoyable discussions throughout the seminars.

This project was funded by the Grants-in-Aid for JSPS Fellows to Y.M. and Grants-in-Aid for Scientific Research to K.N. I thank financial supports from Mount Fuji Research Institute and Academic Research Grant for GSFS Doctor Course Students, the University of Tokyo.

Supplementary material

List of ectomycorrhizal species and morphological images

Organism	Species ID	DDBJ Accession Number	Length	Best BLAST match			Occurrence			No
				Accession	Query coverage	Max ident	F	I	B	
Amanita franchetii	Amanita S3	AB922859	631	JQ396480.1	100%	99%		■		1
Amanita fritillaria	Amanita S2	AB922858	645	KF245913.1	96%	99%		■		2
Amanita imazekii	Amanita Y2	AB848401	535	AB038768.1	100%	99%	■			3
Amanita novinupta	Amanita S5	AB922860	522	KC152067.1	100%	97%		■		4
Amanita rubrovolvata	Amanita S1	AB922857	632	AB015689.1	100%	100%		■		5
Amanita subjunquillea	Amanita Y3	AB848402	616	FJ176733.1	100%	99%	■			6
Amanita virosa	Amanita S6	AB922861	597	GU373492.1	100%	100%		■		7
Amanita sp.1	Amanita Y1	AB848400	642	AB218141.1	99%	99%	■	■		8
Amanita sp.2	Amanita Y4	AB848403	594	FJ176736.1	99%	99%	■			9
Amphinema byssoides	Amphinema Y1	AB848404	476	JN943921.1	100%	99%	■			10
Amphinema sp.1	Amphinema Y2	AB848405	461	JN943905.1	97%	95%	■			11
Astraeus hygrometricus	Astraeus B1	AB923012	597	AB507396.1	100%	100%			■	12
Atheliaceae sp.1	Atheliaceae Y1	AB848406	496	JQ666661.1	100%	97%	■			13
Boletaceae sp.1	Boletus S1	AB922862	604	AB807902.1	100%	99%		■		14
Boletaceae sp.2	Boletus S2	AB922863	608	JQ991917.1	95%	87%		■		15
Boletaceae sp.3	Boletus S3	AB922864	603	EU837225.1	88%	92%		■		16
Boletaceae sp.4	Boletus S4	AB922865	455	HM190078.1	92%	90%		■		17
Boletaceae sp.5	Tylopilus S1	AB923009	609	AB218092.1	100%	99%		■		18
Boletus edulis	Boletus Y4	AB848409	694	JF899550.1	99%	99%	■			19
Boletus sp.1	Boletus Y1	AB848407	678	AB218129.1	100%	99%	■			20
Boletus sp.2	Boletus Y2	AB848408	564	HM347653.1	100%	93%	■			21
Boletus sp.3	Boletus Y9	AB848414	652	AB211279.1	100%	96%	■			22
Boletus sp.4	Boletus Y8	AB848413	568	FR731377.1	83%	85%	■			23
Boletus sp.5	Boletus Y5	AB848410	691	HQ022035.1	90%	94%	■			24
Boletus sp.6	Boletus Y6	AB848411	545	AY656926.1	99%	99%	■			25
Boletus sp.7	Boletus Y7	AB848412	506	EU569236.1	99%	91%	■			26
Byssocorticium sp.1	Byssocorticium Y1	AB848415	426	FM992893.1	99%	95%	■			27
Byssoporia terrestris	Byssoporia Y1	AB848416	523	EU118608.1	100%	98%	■			28
Cantharellales sp.1	ECM Y4	AB848706	635	AB605641.1	100%	99%	■			29
Cenococcum geophilum	Cenococcum geophilum	AB848417	472	JN943885.1	100%	99%	■	■	■	30
Clavulina castaneipes	Clavulina Y1	AB848419	613	DQ424944.1	100%	99%	■	■		31
Clavulina cristata	Clavulina S5	AB922867	529	AY292292.1	100%	98%		■		32
Clavulina sp.1	Clavulina Y3	AB848421	602	FR852048.1	94%	96%	■			33
Clavulina sp.2	Clavulina Y2	AB848420	585	EF434094.1	100%	97%	■			34
Clavulinaceae sp.1	Clavulinaceae Y1	AB848422	713	FN565253.1	56%	92%	■	■		35
Clavulinaceae sp.2	Clavulinaceae Y2	AB848423	534	FJ348368.1	84%	98%	■			36
Clavulinaceae sp.3	Clavulinaceae Y3	AB848424	617	HQ021761.1	81%	88%	■			37
Clavulinaceae sp.4	Clavulinaceae Y4	AB848425	539	HM105503.1	99%	95%	■			38
Clavulinaceae sp.5	Clavulinaceae Y5	AB848426	639	AY641465.1	100%	85%	■	■		39
Clavulinaceae sp.6	Clavulinaceae Y6	AB848427	607	JQ393122.1	97%	86%	■			40
Clavulinaceae sp.7	Clavulinaceae Y7	AB848428	644	FJ196907.1	99%	88%	■			41
Clavulinaceae sp.8	Clavulina S8	AB922869	578	FJ236849.1	99%	88%		■		42
Clavulinaceae sp.9	Clavulina S4	AB922866	578	EU816652.1	100%	97%		■		43
Clavulinaceae sp.10	Clavulina S7	AB922868	512	JF960632.1	99%	87%		■		44
Cortinarius acutovelatus	Cortinarius Y39	AB848466	411	AY669655.1	99%	99%	■			45
Cortinarius acutus	Cortinarius Y32	AB848459	645	HQ604674.1	100%	98%	■			46
Cortinarius albovariegatus	Cortinarius Y40	AB848467	431	JF907914.1	100%	100%	■			47
Cortinarius angelesianus	Cortinarius Y42	AB848469	538	JF907945.1	99%	100%	■			48
Cortinarius anomalus	Cortinarius Y11	AB848438	624	AY669645.1	100%	97%	■	■		49
Cortinarius anthracinus	Cortinarius Y16	AB848443	539	AY669670.1	100%	99%	■			50
Cortinarius aprinus	Cortinarius Y17	AB848444	500	HQ115587.1	100%	99%	■			51
Cortinarius azureus	Cortinarius Y28	AB848455	638	JQ393043.1	100%	99%	■			52
Cortinarius barlowensis	Cortinarius Y9	AB848436	620	FJ039658.1	100%	99%	■			53
Cortinarius biformis	Cortinarius Y46	AB848473	437	DQ233767.1	100%	99%	■			54
Cortinarius camphoratus	Cortinarius Y20	AB848447	631	FJ717505.1	100%	99%	■			55
Cortinarius caninus	Cortinarius Y36	AB848463	619	EU313201.1	100%	99%	■			56
Cortinarius caperatus	Cortinarius Y27	AB848454	611	JF899568.1	100%	100%	■			57
Cortinarius croceus	Cortinarius Y21	AB848448	623	FJ039700.1	100%	99%	■			58
Cortinarius delibutus	Cortinarius S21	AB922887	532	JX436868.1	100%	96%		■		59
Cortinarius ectypus	Cortinarius Y15	AB848442	543	EU266688.1	100%	100%	■			60
Cortinarius flexipes sp.1	Cortinarius Y29	AB848456	539	FM992903.1	99%	99%	■			61
Cortinarius flexipes sp.2	Cortinarius Y22	AB848449	537	AM087248.1	100%	100%	■			62
Cortinarius fulvescens	Cortinarius Y41	AB848468	537	HQ446012.1	98%	98%	■			63
Cortinarius hemitrichus	Cortinarius Y14	AB848441	539	FJ039543.1	100%	99%	■			64
Cortinarius herpeticus	Cortinarius Y12	AB848439	614	AF478586.1	99%	99%	■			65

Organism	Species ID	DDBJ Accession Number	Length	Best BLAST match			Occurrence			No
				Accession	Query coverage	Max ident	F	I	B	
Cortinarius illuminus	Cortinarius Y13	AB848440	615	FJ039602.1	100%	99%	■			66
Cortinarius junghuhnii sp.1	Cortinarius Y31	AB848458	533	HQ604725.1	100%	98%	■			67
Cortinarius junghuhnii sp.2	Cortinarius Y37	AB848464	627	HQ604666.1	100%	97%	■			68
Cortinarius keralensis	Cortinarius Y45	AB848472	532	AY083188.1	98%	97%	■			69
Cortinarius laetissimus	Cortinarius Y30	AB848457	540	GQ159898.1	100%	99%	■			70
Cortinarius obtusus	Cortinarius S8	AB922875	636	AJ438981.2	99%	97%		■		71
Cortinarius olivaceopictus	Cortinarius S26	AB922890	502	FR851993.1	99%	99%		■		72
Cortinarius phoeniceus	Cortinarius S4	AB922872	642	HQ604652.1	100%	98%		■		73
Cortinarius saniosus	Cortinarius Y52	AB848479	431	AB669642.1	99%	99%	■			74
Cortinarius saturninus	Cortinarius S27	AB922879	563	FN669181.1	100%	98%		■		75
Cortinarius scaurus sp.1	Cortinarius Y10	AB848437	603	JF300799.1	97%	99%	■	■		76
Cortinarius scaurus sp.2	Cortinarius Y7	AB848434	452	JF907876.1	100%	98%	■			77
Cortinarius tortuosus	Cortinarius Y8	AB848435	530	AY669669.1	100%	99%	■			78
Cortinarius turibulosus	Cortinarius S14	AB922881	608	GQ159774.1	99%	96%		■		79
Cortinarius umbrinolens	Cortinarius Y35	AB848462	535	HQ604701.1	100%	98%	■	■		80
Cortinarius venetus	Cortinarius Y25	AB848452	626	EF600897.1	100%	99%	■			81
Cortinarius vibratilis	Cortinarius Y33	AB848460	436	HQ604684.1	100%	99%	■			82
Cortinarius violaceus	Cortinarius S22	AB922888	626	AY669579.1	100%	94%		■		83
Cortinarius sp.1	Cortinarius Y1	AB848429	545	EU057110.2	100%	99%	■			84
Cortinarius sp.2	Cortinarius Y2	AB848430	520	HQ285383.1	100%	100%	■			85
Cortinarius sp.3	Cortinarius Y3	AB848431	590	GQ159858.1	100%	98%	■			86
Cortinarius sp.4	Cortinarius Y4	AB848432	528	DQ481814.1	100%	100%	■			87
Cortinarius sp.5	Cortinarius Y5	AB848433	623	FR852019.1	100%	97%	■			88
Cortinarius sp.6	Cortinarius Y51	AB848478	573	FN669185.1	71%	94%	■			89
Cortinarius sp.7	Cortinarius Y50	AB848477	552	JQ711860.1	100%	94%	■			90
Cortinarius sp.8	Cortinarius Y47	AB848474	565	HQ604699.1	99%	95%	■			91
Cortinarius sp.9	Cortinarius Y34	AB848461	527	AY669668.1	100%	96%	■			92
Cortinarius sp.10	Cortinarius Y24	AB848451	569	AY669592.1	99%	92%	■			93
Cortinarius sp.11	Cortinarius Y26	AB848453	655	HQ604671.1	100%	94%	■			94
Cortinarius sp.12	Cortinarius Y44	AB848471	414	GU256200.1	100%	96%	■			95
Cortinarius sp.13	Cortinarius Y48	AB848475	494	HE814098.1	99%	94%	■			96
Cortinarius sp.14	Cortinarius Y49	AB848476	567	EF433964.1	95%	93%	■	■		97
Cortinarius sp.15	Cortinarius Y23	AB848450	517	GU998417.1	100%	96%	■			98
Cortinarius sp.16	Cortinarius Y38	AB848465	614	FJ769528.1	100%	95%	■			99
Cortinarius sp.17	Cortinarius Y19	AB848446	587	DQ388822.1	99%	91%	■	■		100
Cortinarius sp.18	Cortinarius Y18	AB848445	623	FJ039657.1	100%	96%	■			101
Cortinarius sp.19	Cortinarius S11	AB922878	581	EU292639.1	100%	99%		■		102
Cortinarius sp.20	Cortinarius S20	AB922886	595	GQ159887.1	100%	99%		■		103
Cortinarius sp.21	Cortinarius S18	AB922885	637	HQ604685.1	100%	96%		■		104
Cortinarius sp.22	Cortinarius S2	AB922870	684	FN669181.1	100%	96%		■		105
Cortinarius sp.23	Cortinarius S3	AB922871	540	FJ827154.1	99%	93%		■		106
Cortinarius sp.24	Cortinarius S5	AB922873	635	GQ159887.1	100%	96%		■		107
Cortinarius sp.25	Cortinarius S25	AB922889	524	GQ159780.1	100%	95%		■		108
Cortinarius sp.26	Cortinarius S6	AB922874	601	DQ328086.1	100%	91%		■		109
Cortinarius sp.27	Cortinarius S9	AB922876	641	AF430288.1	100%	95%		■		110
Cortinarius sp.28	Cortinarius S10	AB922877	571	AB828023.1	93%	99%		■		111
Cortinarius sp.29	Cortinarius S15	AB922882	558	FJ717605.1	98%	95%		■		112
Cortinarius sp.30	Cortinarius S16	AB922883	421	AB807929.1	100%	99%		■		113
Cortinarius sp.31	Cortinarius S17	AB922884	653	EU057110.2	99%	93%		■		114
Craterellus sp.1	Craterellus S1	AB922891	610	JQ991715.1	63%	89%		■		115
Craterellus sp.2	Craterellus S2	AB922892	536	GQ268596.1	97%	86%		■		116
Craterellus tubaeformis	Craterellus Y1	AB848480	612	AF385633.1	97%	96%	■			117
Dermocybe malicoria	Dermocybe Y1	AB848481	489	U56045.1	100%	99%	■			118
Elaphomyces sp.1	Elaphomyces Y1	AB848482	593	GU550112.1	99%	99%	■			119
Elaphomyces sp.2	Elaphomyces S1	AB922893	372	JQ991721.1	97%	89%		■		120
Entoloma rhodopolium	Entoloma Y2	AB848484	402	AB301602.1	100%	99%	■			121
Entoloma sp.1	Entoloma Y1	AB848483	687	FR852295.1	99%	96%	■	■		122
Entoloma sp.2	Entoloma Y4	AB848486	760	AB301602.1	99%	91%	■			123
Entoloma sp.3	Entoloma Y3	AB848485	586	HM057203.1	99%	92%	■	■	■	124
Entoloma sp.4	Entoloma S2	AB922894	631	FR852294.1	100%	97%		■		125
Genea hispidula	Pezizales Y1	AB848544	615	AJ534926.2	100%	99%	■	■		126
Hebeloma crustuliniforme	Hebeloma B2	AB923014	544	KC984861.1	100%	99%			■	127
Hebeloma incarnatulum	Hebeloma Y1	AB848487	653	FN669203.1	100%	99%	■			128
Hebeloma mesophaeum	Hebeloma Y2	AB848488	637	EU292525.1	100%	98%	■			129
Hebeloma rivulosum	Hebeloma B1	AB923013	382	HE687052.1	100%	99%			■	130

Organism	Species ID	DDBJ Accession Number	Length	Best BLAST match			Occurrence			No
				Accession	Query coverage	Max ident	F	I	B	
Helotiales sp.1	Helotiales S1	AB922897	362	KC876172.1	100%	99%		■		131
Helotiales sp.1	Helotiales Y1	AB848489	492	HM044552.1	98%	99%	■			132
Helotiales sp.2	Helotiales S2	AB922898	496	FN393125.1	98%	99%		■		133
Helotiales sp.2	Helotiales Y2	AB848490	485	HM146841.1	92%	92%	■			134
Helotiales sp.3	Helotiales S3	AB922899	473	FN298686.1	100%	98%		■		135
Helotiales sp.5	Helotiales S5	AB922901	496	HQ021751.1	100%	99%		■		136
Helvella sp.1	Helvella Y1	AB848491	654	FJ897187.1	99%	88%	■			137
Humaria hemisphaerica	Humaria Y1	AB848492	419	DQ200832.1	100%	98%	■			138
Hydnotrya sp.1	Hydnotrya S1	AB922903	573	AB428790.1	93%	92%		■		139
Hydnotrya sp.2	Hydnotrya S2	AB922904	614	AB428790.1	100%	99%		■	■	140
Hydnotrya sp.3	Hydnotrya S3	AB922905	575	KC702645.1	99%	97%		■		141
Hydnotrya sp.4	Hydnotrya B2	AB923015	559	AB218071.1	100%	97%			■	142
Hydnum sp.1	Hydnum S1	AB922902	536	FJ596769.1	100%	99%		■		143
Inocybe flocculosa	Inocybe Y23	AB848508	631	HQ604361.1	100%	99%	■			144
Inocybe lilacina	Inocybe Y12	AB848496	439	JF908236.1	99%	98%	■			145
Inocybe napipes	Inocybe S4	AB922908	738	AM882927.2	100%	99%		■		146
Inocybe nitidiuscula	Inocybe Y4	AB848515	437	HQ604245.1	99%	97%	■			147
Inocybe phaeocomis	Inocybe Y13	AB848497	600	AM882848.1	100%	98%	■			148
Inocybe praetervisa	Inocybe Y15	AB848499	561	HQ604492.1	100%	99%	■			149
Inocybe sp.1	Inocybe Y1	AB848493	563	AM882711.2	98%	95%	■			150
Inocybe sp.2	Inocybe Y2	AB848504	625	JF899559.1	100%	91%	■			151
Inocybe sp.3	Inocybe Y3	AB848514	620	AM882789.2	100%	93%	■			152
Inocybe sp.4	Inocybe Y27	AB848512	583	DQ493548.1	98%	98%	■			153
Inocybe sp.5	Inocybe Y5	AB848516	656	FR750632.1	98%	87%	■			154
Inocybe sp.6	Inocybe Y6	AB848517	592	AB218072.1	100%	99%	■			155
Inocybe sp.7	Inocybe Y7	AB848518	629	JF908111.1	100%	96%	■			156
Inocybe sp.8	Inocybe Y8	AB848519	545	FJ196927.1	100%	93%	■			157
Inocybe sp.9	Inocybe Y9	AB848520	484	AB218093.1	100%	99%	■			158
Inocybe sp.10	Inocybe Y10	AB848494	398	JQ801414.1	78%	93%	■			159
Inocybe sp.11	Inocybe Y11	AB848495	510	AB218065.1	99%	99%	■	■		160
Inocybe sp.12	Inocybe Y26	AB848511	611	DQ493570.1	83%	95%	■			161
Inocybe sp.13	Inocybe Y25	AB848510	459	AM882752.1	99%	91%	■			162
Inocybe sp.14	Inocybe Y14	AB848498	664	EF218781.1	100%	100%	■			163
Inocybe sp.15	Inocybe Y28	AB848513	516	AB218065.1	93%	95%	■	■		164
Inocybe sp.16	Inocybe Y16	AB848500	574	FJ389455.1	100%	91%	■			165
Inocybe sp.17	Inocybe Y17	AB848501	586	GU233318.1	99%	84%	■			166
Inocybe sp.18	Inocybe Y18	AB848502	605	JQ975958.1	100%	83%	■			167
Inocybe sp.19	Inocybe Y19	AB848503	551	FN550906.1	99%	92%	■			168
Inocybe sp.20	Inocybe Y20	AB848505	593	HQ604215.1	100%	92%	■			169
Inocybe sp.21	Inocybe Y21	AB848506	589	HQ604626.1	100%	86%	■			170
Inocybe sp.22	Inocybe Y22	AB848507	533	AB701390.1	85%	87%	■			171
Inocybe sp.23	Inocybe Y24	AB848509	517	FJ904152.1	100%	96%	■			172
Inocybe sp.24	Inocybe S1	AB922906	657	FN550881.1	99%	92%		■		173
Inocybe sp.25	Inocybe S3	AB922907	803	HQ604561.1	88%	92%		■		174
Inocybe sp.26	Inocybe S5	AB922909	500	JQ085933.1	80%	84%		■		175
Inocybe sp.27	Inocybe S7	AB922910	736	HQ604546.1	100%	91%		■		176
Inocybe sp.28	Inocybe S8	AB922911	684	FN550885.1	87%	89%		■		177
Inocybe sp.29	Inocybe S9	AB922912	616	HQ604561.1	98%	90%		■		178
Inocybe sp.30	Inocybe S10	AB922913	754	HQ604525.1	97%	91%		■		179
Inocybe sp.31	Inocybe S12	AB922914	633	FN550881.1	98%	90%		■		180
Inocybe sp.32	Inocybe S13	AB922915	793	JF273524.1	78%	90%		■		181
Inocybe sp.33	Inocybe S14	AB922916	595	AB218126.1	100%	99%		■		182
Inocybe sp.34	Inocybe S15	AB922917	594	HQ604553.1	94%	90%		■		183
Inocybe sp.35	Inocybe S16	AB922918	547	EU523557.1	98%	92%		■		184
Inocybe sp.36	Inocybe S17	AB922919	559	KC818316.1	100%	88%		■		185
Inocybe sp.37	Inocybe S18	AB922920	542	HQ604084.1	100%	95%		■		186
Laccaria cf. *laccata* sp.1	Laccaria Y5	AB848525	566	JN942782.1	100%	99%	■		■	187
Laccaria cf. *laccata* sp.2	Laccaria Y4	AB848524	606	JN942813.1	95%	99%	■	■	■	188
Laccaria laccata	Laccaria Y2	AB848522	593	AB211273.1	100%	99%	■		■	189
Laccaria sp.1	Laccaria Y1	AB848521	614	GQ205354.1	99%	99%	■	■	■	190
Laccaria sp.2	Laccaria Y3	AB848523	559	AB218107.1	100%	99%	■			191
Laccaria sp.3	Laccaria S5	AB922921	633	AB218097.1	93%	97%		■		192
Lactarius badiosanguineus	Lactarius Y11	AB848528	668	JF908284.1	99%	99%	■			193
Lactarius caespitosus	Lactarius Y14	AB848531	611	FJ845421.1	100%	99%	■			194
Lactarius chichuensis	Lactarius S12	AB922931	490	AB636105.1	100%	100%		■		195

Organism	Species ID	DDBJ Accession Number	Length	Best BLAST match			Occurrence			No
				Accession	Query coverage	Max ident	F	I	B	
Lactarius chrysorrheu	Lactarius S8	AB922928	593	AB807948.1	100%	100%		■		196
Lactarius deliciosus	Lactarius Y10	AB848527	594	EF685091.1	99%	99%	■			197
Lactarius deterrimus	Lactarius Y9	AB848539	674	AF249286.1	100%	99%	■			198
Lactarius flexuosus	Lactarius S11	AB922930	383	FR852038.1	100%	98%		■		199
Lactarius gracilis	Lactarius S4	AB922924	590	KF433017.1	99%	98%		■		200
Lactarius imperceptus	Lactarius Y4	AB848534	650	DQ777991.1	100%	97%	■	■		201
Lactarius lignyotus	Lactarius S7	AB922927	625	JQ446117.1	100%	100%		■		202
Lactarius picinus	Lactarius Y12	AB848529	592	GU258279.1	99%	97%	■			203
Lactarius pseudomucidus	Lactarius Y2	AB848532	602	DQ474613.1	100%	99%	■			204
Lactarius quietus	Lactarius Y7	AB848537	664	AB597656.1	100%	99%	■	■		205
Lactarius tabidus	Lactarius S1	AB922922	663	HM189833.1	100%	99%		■		206
Lactarius uvidus sp.1	Lactarius Y5	AB848535	634	JN197640.1	100%	99%	■			207
Lactarius uvidus sp.2	Lactarius Y13	AB848530	469	FJ596848.1	97%	98%	■			208
Lactarius vietus	Lactarius Y1	AB848526	658	EF218784.1	100%	99%	■			209
Lactarius yazooensis	Lactarius Y8	AB848538	637	EU598169.1	100%	99%	■			210
Lactarius sp.1	Lactarius Y3	AB848533	652	FJ607371.1	100%	100%	■			211
Lactarius sp.2	Lactarius Y6	AB848536	735	AB218153.1	100%	99%	■			212
Lactarius sp.3	Lactarius S3	AB922923	611	AB597677.1	100%	100%		■		213
Lactarius sp.4	Lactarius S13	AB922932	603	FJ454900.1	99%	93%		■		214
Lactarius sp.5	Lactarius S5	AB922925	593	JQ991758.1	99%	98%		■		215
Lactarius sp.6	Lactarius S6	AB922926	639	EU711589.1	99%	99%		■		216
Lactarius sp.7	Lactarius S10	AB922929	579	KF879461.1	100%	97%		■		217
Leccinum sp.1	Leccinum Y2	AB848541	704	AF454588.1	97%	87%	■			218
Leccinum versipelle	Leccinum Y1	AB848540	714	AF454574.1	100%	100%	■			219
Leucogastraceae sp.1	Leucogastraceae Y1	AB848542	448	EU057102.2	98%	95%	■			220
Melanogaster sp.1	Melanogaster B1	AB923016	400	AJ555513.1	100%	88%			■	221
Meliniomyces sp.1	Meliniomyces S1	AB922933	421	KC876251.1	100%	96%		■		222
Octaviania japonimontana	Octaviania S1	AB922934	607	JQ619174.1	100%	99%		■		223
Pachyphloeus sp.1	Pachyphloeus Y1	AB848543	612	FN669232.1	100%	97%	■			224
Pachyphloeus sp.2	Peziza S3	AB922936	497	HM189838.1	100%	98%		■		225
Paxillus involutus sp.1	Paxillus B3	AB923018	621	AB828028	100%	99%			■	226
Paxillus involutus sp.2	Paxillus B1	AB923017	570	JN661726.1	100%	99%			■	227
Peziza sp.1	Pezizales Y2	AB848545	467	FR852089.1	100%	97%	■			228
Pezizales (*Genea* sp.1)	Genea S1	AB922895	606	AB218168.1	100%	99%		■		229
Pezizales (*Genea* sp.2)	Genea S2	AB922896	609	DQ974835.1	41%	91%		■		230
Pezizales (*Helvella*)	Pezizales Y3	AB848546	723	AJ969435.1	94%	87%	■			231
Pezizales sp.1	Peziza S1	AB922935	534	AB571493.1	98%	97%		■		232
Phylloporus maculatus	Phylloporus S1	AB922937	604	JQ678696.1	100%	99%		■		233
Phylloporus sp.1	Phylloporus S2	AB922938	609	JQ003649.1	88%	94%		■		234
Piloderma croceum	Piloderma Y2	AB848553	567	AJ438982.1	100%	99%	■			235
Piloderma fallax sp.1	Piloderma Y4	AB848554	506	EF619737.1	100%	99%	■			236
Piloderma fallax sp.2	Piloderma Y5	AB848555	562	EF040872.1	100%	100%	■			237
Piloderma lanatum	Piloderma Y7	AB848556	573	EU880221.1	99%	98%	■			238
Piloderma olivaceum sp.1	Piloderma Y1	AB848547	563	EF611138.1	100%	99%	■			239
Piloderma olivaceum sp.2	Piloderma Y8	AB848557	569	JQ711944.1	100%	99%	■			240
Piloderma sp.1	Piloderma Y11	AB848549	575	EF619739.1	66%	97%	■			241
Piloderma sp.2	Piloderma Y12	AB848550	574	HQ021998.1	100%	97%	■			242
Piloderma sp.3	Piloderma Y13	AB848551	575	FR877519.1	100%	98%	■	■		243
Piloderma sp.4	Piloderma Y9	AB848558	562	AF476983.1	100%	99%	■			244
Piloderma sp.5	Piloderma Y10	AB848548	564	FN669237.1	93%	99%	■			245
Pseudotomentella humicola	Pseudotomentella F1	AB848559	646	AM490945.1	99%	99%	■			246
Pseudotomentella sp.1	Pseudotomentella F3	AB848561	591	EU057113.2	93%	88%	■			247
Pseudotomentella sp.2	Pseudotomentella F2	AB848560	662	FM993212.1	92%	94%	■			248
Pseudotomentella sp.3	Pseudotomentella S1	AB922939	569	EU292570.1	98%	94%		■		249
Rhizopogon sp.1	Rhizopogon B1	AB923019	594	JQ991778.1	100%	96%			■	250
Rhizopogon sp.2	Rhizopogon B2	AB923020	616	AB253521.1	100%	99%			■	251
Russula abietina	Russula Y13	AB848566	615	EU598179.1	100%	99%	■			252
Russula aquosa	Russula Y27	AB848581	470	AY061657.1	99%	99%	■			253
Russula bicolor sp.1	Russula Y5	AB848586	582	AB476543.1	100%	99%	■			254
Russula bicolor sp.2	Russula Y2	AB848573	633	EU597058.1	100%	99%	■			255
Russula brevipes	Russula S24	AB922958	539	JX630807.1	99%	98%		■		256
Russula cerolens	Russula S19	AB922953	617	JX434674.1	100%	98%		■		257
Russula crassotunicata	Russula Y4	AB848585	605	EU057119.2	100%	99%	■			258
Russula cremeirosea	Russula S42	AB922971	553	KF879471.1	100%	97%		■		259
Russula densifolia	Russula Y3	AB848584	619	FJ946973.1	99%	99%	■			260

Organism	Species ID	DDBJ Accession Number	Length	Best BLAST match			Occurrence			No
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Russula emetica	Russula Y29	AB848583	598	JQ888196.1	99%	97%	■			261
Russula favrei	Russula S3	AB922942	620	KC581298.1	100%	99%		■		262
Russula granulata	Russula S1	AB922940	567	AB828032.1	100%	99%		■		263
Russula lepida sp.1	Russula S36	AB922967	566	AF418641.1	99%	98%		■		264
Russula lepida sp.2	Russula S39	AB922969	628	AF418641.1	98%	97%		■		265
Russula mairei	Russula Y17	AB848570	626	AF418620.1	100%	98%	■			266
Russula ochroleuca	Russula S22	AB922956	552	AB831853.1	100%	100%		■		267
Russula peckii	Russula Y16	AB848569	547	EU598173.1	99%	98%	■	■		268
Russula puellaris	Russula Y19	AB848572	633	HM189941.1	100%	99%	■			269
Russula puellula	Russula Y12	AB848565	588	JF908706.1	99%	97%	■			270
Russula rosea	Russula S33	AB922965	543	AB597642.1	100%	99%		■		271
Russula solaris	Russula Y22	AB848576	589	JN944007.1	100%	97%	■			272
Russula turci	Russula Y6	AB848587	521	AB597704.1	98%	97%	■	■		273
Russula velenovskyi	Russula Y7	AB848588	621	AY061721.1	99%	99%	■		■	274
Russula vesca	Russula Y14	AB848567	616	HM189956.1	100%	98%	■			275
Russula sp.1	Russula Y1	AB848562	643	DQ777980.1	100%	95%	■			276
Russula sp.2	Russula Y23	AB848577	555	AB218078.1	100%	99%	■	■		277
Russula sp.3	Russula Y25	AB848579	592	GQ219888.1	100%	97%	■			278
Russula sp.4	Russula Y30	AB904791	457	DQ377401.1	100%	93%	■			279
Russula sp.5	Russula Y28	AB848582	553	AB218161.1	96%	99%	■			280
Russula sp.6	Russula Y20	AB848574	610	JF519253.1	100%	98%	■			281
Russula sp.7	Russula Y21	AB848575	584	HQ021944.1	99%	95%	■			282
Russula sp.8	Russula Y8	AB848589	620	FJ152483.1	100%	99%	■			283
Russula sp.9	Russula Y9	AB848590	467	GU327497.1	98%	96%	■			284
Russula sp.10	Russula Y10	AB848563	368	JQ975976.1	100%	93%	■			285
Russula sp.11	Russula Y11	AB848564	641	JN944002.1	97%	96%	■			286
Russula sp.12	Russula Y15	AB848568	681	FJ876171.1	100%	94%	■	■		287
Russula sp.13	Russula Y18	AB848571	548	AB587767.1	100%	99%	■			288
Russula sp.14	Russula S14	AB922948	619	AB807983.1	100%	96%		■		289
Russula sp.15	Russula S15	AB922949	646	AJ893219.1	100%	96%		■		290
Russula sp.16	Russula S16	AB922950	653	HE814239.1	92%	99%		■		291
Russula sp.17	Russula S17	AB922951	620	AB218100.1	100%	97%		■		292
Russula sp.18	Russula S18	AB922952	639	KF245517.1	100%	97%		■		293
Russula sp.19	Russula S2	AB922941	635	JQ991802.1	94%	94%		■		294
Russula sp.20	Russula S20	AB922954	509	AF418609.1	94%	93%		■		295
Russula sp.21	Russula S21	AB922955	590	AY061689.1	100%	95%		■		296
Russula sp.22	Russula S12	AB922947	639	KC581346.1	100%	96%		■		297
Russula sp.23	Russula S23	AB922957	626	AB600187.1	100%	100%		■		298
Russula sp.24	Russula S4	AB922943	618	AB831843.1	100%	99%		■		299
Russula sp.25	Russula S25	AB922959	585	JQ991820.1	100%	100%		■		300
Russula sp.26	Russula S6	AB922944	609	AB218194.1	99%	97%		■		301
Russula sp.27	Russula S8	AB922945	632	GQ359820.1	100%	95%		■		302
Russula sp.28	Russula S28	AB922960	546	AF350057.1	85%	99%		■		303
Russula sp.29	Russula S29	AB922961	568	EU248593.1	99%	96%		■		304
Russula sp.30	Russula S30	AB922962	576	JX857275.1	98%	93%		■		305
Russula sp.31	Russula S31	AB922963	542	HM105560.1	100%	99%		■		306
Russula sp.32	Russula S32	AB922964	402	JX425398.1	98%	96%		■		307
Russula sp.33	Russula S11	AB922946	530	AB218203.1	100%	99%		■		308
Russula sp.34	Russula S40	AB922970	559	EU819424.1	97%	93%		■		309
Russula sp.35	Russula S35	AB922966	612	AB218154.1	100%	99%		■		310
Russula sp.36	Russula S38	AB922968	608	KC679827.1	100%	100%		■		311
Scleroderma bovista	Scleroderma B2	AB923021	604	JX030277.1	100%	99%			■	312
Scleroderma citrinum	Scleroderma S2	AB922972	516	HE820314.1	100%	97%		■	■	313
Sebacina incrustans	Sebacina S9	AB922978	672	JQ665548.1	100%	99%		■		314
Sebacina sp.1	Sebacina Y1	AB848591	605	HQ022151.1	92%	99%	■	■		315
Sebacina sp.2	Sebacina Y2	AB848602	566	HM488519.1	99%	98%	■	■		316
Sebacina sp.3	Sebacina Y3	AB848613	556	HM146865.1	100%	99%	■	■		317
Sebacina sp.4	Sebacina Y4	AB848616	548	JF519117.1	100%	98%	■	■		318
Sebacina sp.5	Sebacina Y5	AB848617	575	FJ803936.1	100%	96%	■			319
Sebacina sp.6	Sebacina Y6	AB848618	553	AM161532.1	100%	96%	■			320
Sebacina sp.7	Sebacina Y7	AB848619	567	AB218091.1	100%	99%	■			321
Sebacina sp.8	Sebacina Y8	AB848620	471	HQ154273.1	99%	95%	■			322
Sebacina sp.9	Sebacina Y9	AB848621	408	FR852356.1	88%	96%	■			323
Sebacina sp.10	Sebacina Y10	AB848592	553	FN610948.1	100%	99%	■			324
Sebacina sp.11	Sebacina Y11	AB848593	542	HQ154286.1	100%	98%	■			325

Organism	Species ID	DDBJ Accession Number	Length	Best BLAST match			Occurrence			No
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Sebacina sp.12	Sebacina Y12	AB848594	487	DQ974767.1	99%	96%	■			326
Sebacina sp.14	Sebacina Y14	AB848596	412	HQ154377.1	100%	96%	■			327
Sebacina sp.15	Sebacina Y15	AB848597	529	AB218068.1	100%	99%	■			328
Sebacina sp.16	Sebacina Y16	AB848598	444	FR852370.1	100%	98%	■			329
Sebacina sp.17	Sebacina Y17	AB848599	585	GU327499.1	99%	96%	■			330
Sebacina sp.18	Sebacina Y18	AB848600	547	AF465191.1	99%	98%	■			331
Sebacina sp.19	Sebacina Y19	AB848601	559	AB218113.1	100%	97%	■			332
Sebacina sp.20	Sebacina Y20	AB848603	536	AB218122.1	100%	99%	■	■		333
Sebacina sp.21	Sebacina Y21	AB848604	566	AB218081.1	100%	97%	■			334
Sebacina sp.22	Sebacina Y22	AB848605	537	AB568450.2	100%	99%	■			335
Sebacina sp.23	Sebacina Y23	AB848606	562	FR852369.1	100%	98%	■			336
Sebacina sp.24	Sebacina Y24	AB848607	533	GU327499.1	100%	97%	■			337
Sebacina sp.25	Sebacina Y25	AB848608	545	EU645626.1	100%	95%	■			338
Sebacina sp.26	Sebacina Y26	AB848609	456	HQ154316.1	100%	98%	■			339
Sebacina sp.27	Sebacina Y27	AB848610	556	JQ666564.1	100%	99%	■			340
Sebacina sp.28	Sebacina Y28	AB848611	565	HQ667893.1	99%	98%	■			341
Sebacina sp.29	Sebacina Y29	AB848612	549	HQ154286.1	100%	97%	■	■		342
Sebacina sp.30	Sebacina Y30	AB848614	567	AB506992.1	100%	99%	■			343
Sebacina sp.31	Sebacina Y31	AB848615	559	AB506989.1	100%	98%	■			344
Sebacina sp.32	Sebacina S4	AB922973	673	AF440651.1	100%	96%		■		345
Sebacina sp.33	Sebacina S5	AB922974	666	AF440651.1	100%	97%		■		346
Sebacina sp.34	Sebacina S6	AB922975	684	HQ154346.1	100%	99%		■		347
Sebacina sp.35	Sebacina S7	AB922976	619	HQ154314.1	100%	96%		■		348
Sebacina sp.36	Sebacina S8	AB922977	629	FR852338.1	99%	98%		■		349
Sebacina sp.37	Sebacina S10	AB922979	599	AB506935.1	100%	99%		■		350
Sebacina sp.38	Sebacina S11	AB922980	494	KF419120.1	92%	95%		■		351
Sebacina sp.39	Sebacina S14	AB922981	466	AB506948.1	100%	99%		■		352
Sebacina sp.40	Sebacina S16	AB922982	772	FR852342.1	100%	98%		■		353
Sistotrema sp.1	Sistotrema Y1	AB848622	364	JN889865.1	100%	97%	■			354
Sistotrema sp.2	Sistotrema Y2	AB848623	621	FN610983.1	99%	93%	■			355
Sordariomycetes sp.1	Sordariomycete Y1	AB848624	512	GQ900524.1	94%	93%	■			356
Suillus bovinus	Suillus B1	AB923022	555	AB354281.1	100%	100%			■	357
Suillus cavipes	Suillus Y3	AB848627	659	AF166505.2	100%	99%	■			358
Suillus granulatus	Suillus B3	AB923024	512	AB587774.1	100%	99%			■	359
Suillus intermedius	Suillus Y2	AB848626	450	AB284473.1	100%	98%	■			360
Suillus luteus	Suillus B2	AB923023	503	KC185411.1	100%	100%			■	361
Suillus subaureus	Suillus Y1	AB848625	605	L54109.1	99%	98%	■			362
Suillus variegatus	Suillus Y4	AB848628	597	JF300822.1	100%	98%	■			363
Thelephora sp.1	Thelephora F1	AB848629	581	DQ482000.1	99%	97%	■			364
Thelephora sp.2	Thelephora F2	AB848630	573	GU184075.1	100%	97%	■			365
Thelephoraceae sp.1	Thelephoraceae F3	AB848633	573	GU328629.1	100%	97%	■			366
Thelephoraceae sp.2	Thelephoraceae F2	AB848632	533	GU328629.1	100%	98%	■	■		367
Tomentella badia	Tomentella S16	AB922993	518	AF430259.1	100%	98%		■		368
Tomentella ramosissima	Tomentella F39	AB848669	605	FJ196988.1	100%	99%	■			369
Tomentella stuposa sp.1	Tomentella F7	AB848685	592	AB218147.1	100%	100%	■	■		370
Tomentella stuposa sp.2	Tomentella F40	AB848671	617	FJ389456.1	100%	99%	■			371
Tomentella sublilacina	Tomentella F45	AB848676	637	HM189984.1	100%	99%	■	■	■	372
Tomentella terrestris	Tomentella F17	AB848645	608	FJ236853.1	100%	99%	■			373
Tomentella sp.1	Tomentella F1	AB848636	598	EF218840.1	100%	99%	■			374
Tomentella sp.2	Tomentella F2	AB848648	585	FR852130.1	100%	98%	■			375
Tomentella sp.3	Tomentella F3	AB848659	551	FJ196983.1	100%	96%	■			376
Tomentella sp.4	Tomentella F4	AB848670	464	AB688993.1	99%	100%	■			377
Tomentella sp.5	Tomentella F5	AB848681	595	EU526855.1	100%	98%	■	■		378
Tomentella sp.6	Tomentella F6	AB848684	513	FJ013063.1	100%	97%	■	■		379
Tomentella sp.7	Tomentella F8(2)	AB848687	605	EU529972.1	100%	98%	■	■		380
Tomentella sp.8	Tomentella F8	AB848686	602	FN669278.1	100%	99%	■	■		381
Tomentella sp.9	Tomentella F9	AB848688	699	FJ196997.1	100%	98%	■			382
Tomentella sp.10	Tomentella F10	AB848638	596	HQ021875.1	99%	98%	■			383
Tomentella sp.11	Tomentella F11	AB848639	651	FJ196977.1	100%	96%	■	■		384
Tomentella sp.12	Tomentella F12	AB848640	685	AJ893343.1	100%	96%	■	■		385
Tomentella sp.13	Tomentella F13	AB848641	553	FR852141.1	100%	99%	■			386
Tomentella sp.14	Tomentella F14	AB848642	596	HQ271388.1	100%	99%	■			387
Tomentella sp.15	Tomentella F15	AB848643	614	FN669257.1	100%	99%	■			388
Tomentella sp.16	Tomentella F16	AB848644	606	FN565377.1	100%	99%	■			389
Tomentella sp.17	Tomentella F52	AB848683	575	FR852197.1	100%	93%	■			390

Organism	Species ID	DDBJ Accession Number	Length	Best BLAST match			Occurrence			No
				Accession	Query coverage	Max ident	F	I	B	
Tomentella sp.18	Tomentella F18	AB848646	608	FJ807975.1	100%	96%	■	■		391
Tomentella sp.19	Tomentella F19	AB848647	572	AY751562.1	100%	98%	■			392
Tomentella sp.20	Tomentella F20	AB848649	600	EU625823.1	100%	95%	■			393
Tomentella sp.21	Tomentella F21	AB848650	609	AB218148.1	100%	99%	■	■		394
Tomentella sp.22	Tomentella F22	AB848651	606	FN669277.1	99%	97%	■			395
Tomentella sp.23	Tomentella F23	AB848652	607	JN593361.1	100%	98%	■			396
Tomentella sp.24	Tomentella F24	AB848653	584	FM999527.1	100%	98%	■			397
Tomentella sp.25	Tomentella F25	AB848654	610	AJ534911.1	100%	98%	■			398
Tomentella sp.26	Tomentella F26	AB848655	596	EU625870.1	100%	98%	■			399
Tomentella sp.27	Tomentella F27	AB848656	614	HQ285397.1	99%	99%	■			400
Tomentella sp.28	Tomentella F28	AB848657	608	FJ196988.1	100%	98%	■			401
Tomentella sp.29	Tomentella F29	AB848658	628	JF748084.1	100%	96%	■			402
Tomentella sp.30	Tomentella F30	AB848660	604	FR852134.1	99%	99%	■			403
Tomentella sp.31	Tomentella F1(2)	AB848637	604	EF218840.1	100%	97%	■	■		404
Tomentella sp.32	Tomentella F32	AB848662	610	EU625911.1	100%	97%	■			405
Tomentella sp.33	Tomentella F33	AB848663	624	GQ469532.1	98%	97%	■			406
Tomentella sp.34	Tomentella F34	AB848664	607	FJ440944.1	100%	95%	■			407
Tomentella sp.35	Tomentella F35	AB848665	582	AJ893330.1	99%	98%	■			408
Tomentella sp.36	Tomentella F36	AB848666	547	EU516674.1	100%	97%	■			409
Tomentella sp.37	Tomentella F37	AB848667	587	AB251836.1	88%	99%	■			410
Tomentella sp.38	Tomentella F38	AB848668	583	JN969393.1	100%	95%	■			411
Tomentella sp.39	Tomentella F51	AB848682	521	JF304366.1	100%	97%	■			412
Tomentella sp.40	Tomentella F49	AB848680	554	HQ445522.1	100%	99%	■			413
Tomentella sp.41	Tomentella F41	AB848672	600	AM159594.1	99%	90%	■	■		414
Tomentella sp.42	Tomentella F42	AB848673	512	FJ827245.1	100%	96%	■			415
Tomentella sp.43	Tomentella F43	AB848674	602	EU625902.1	99%	98%	■			416
Tomentella sp.44	Tomentella F44	AB848675	630	HQ204744.1	100%	97%	■			417
Tomentella sp.45	Tomentella F48	AB848679	602	AJ534913.1	100%	99%	■			418
Tomentella sp.46	Tomentella F46	AB848677	501	AB605659.1	99%	97%	■			419
Tomentella sp.47	Tomentella F47	AB848678	526	EU625865.1	100%	94%	■			420
Tomentella sp.48	Thelephoraceae F5	AB848635	630	DQ150126.1	96%	97%	■	■		421
Tomentella sp.49	Thelephoraceae F4	AB848634	588	JN569357.1	92%	96%	■		■	422
Tomentella sp.50	Thelephoraceae F1	AB848631	550	JF519275.1	100%	96%	■	■		423
Tomentella sp.51	Tomentella S38	AB923005	615	EF411101.1	100%	97%		■		424
Tomentella sp.52	Tomentella S40	AB923006	569	AB218124.1	100%	98%		■		425
Tomentella sp.53	Tomentella S34	AB923002	630	EU645597.1	100%	97%		■		426
Tomentella sp.54	Tomentella S36	AB923004	616	FN610979.1	100%	99%		■		427
Tomentella sp.55	Thelephoraceae S2	AB922983	533	AB769921.1	100%	93%		■		428
Tomentella sp.56	Tomentella S35	AB923003	489	HE979401.1	100%	95%		■		429
Tomentella sp.57	Tomentella S33	AB923001	445	FJ807967.1	98%	97%		■		430
Tomentella sp.58	Tomentella S12	AB922990	597	HM146876.1	100%	96%		■		431
Tomentella sp.59	Tomentella S13	AB922991	668	EU563490.1	100%	97%		■		432
Tomentella sp.60	Tomentella S14	AB922992	614	EF218830.1	100%	97%		■		433
Tomentella sp.61	Tomentella S17	AB922994	475	AB259150.1	100%	99%		■		434
Tomentella sp.62	Tomentella S19	AB922995	598	FM993119.1	100%	97%		■		435
Tomentella sp.63	Tomentella S24	AB922996	584	EU625823.1	100%	97%		■		436
Tomentella sp.64	Tomentella S26	AB922997	599	AB701384.1	97%	99%		■		437
Tomentella sp.66	Tomentella S3	AB922984	617	AB218062.1	100%	99%		■		438
Tomentella sp.67	Tomentella S30	AB922998	495	HE820574.1	99%	95%		■		439
Tomentella sp.68	Tomentella S31	AB922999	524	AB587791.1	100%	99%		■		440
Tomentella sp.69	Tomentella S5	AB922985	614	FR877525.1	98%	99%		■		441
Tomentella sp.70	Tomentella S6	AB922986	610	HE978991.1	100%	97%		■		442
Tomentella sp.71	Tomentella S7	AB922987	570	AB828042.1	100%	97%		■		443
Tomentella sp.72	Tomentella S8	AB922988	606	AB839401.1	98%	99%		■		444
Tomentella sp.73	Tomentella S11	AB922989	583	EU625804.1	99%	96%		■		445
Tomentella sp.*74*	Tomentella B3	AB923027	563	AB769918.1	100%	99%			■	446
Tomentella sp.*75*	Tomentella B4	AB923028	386	JQ991873.1	100%	99%			■	447
Tomentella sp.*76*	Tomentella B13	AB923026	400	JQ991890.1	99%	99%			■	448
Tomentella sp.*77*	Tomentella B7	AB923030	483	JQ991887.1	100%	100%			■	449
Tomentella sp.*78*	Tomentella B11	AB923025	539	JN858076.1	100%	99%			■	450
Tomentella sp.*79*	Tomentella B5	AB923029	584	GQ240903.1	100%	97%			■	451
Tricholoma bufonium	Tricholoma Y1	AB848689	633	AY462029.1	100%	98%	■			452
Tricholoma flavovirens	Tricholoma Y5	AB848693	462	EU186309.1	100%	98%	■			453
Tricholoma sejunctum sp.1	Tricholoma Y7	AB848695	685	AB036899.1	100%	99%	■			454
Tricholoma sejunctum sp.2	Tricholoma Y3	AB848691	402	FJ807976.1	100%	99%	■			455

Organism	Species ID	DDBJ Accession Number	Length	Best BLAST match			Occurrence			No
				Accession	Query coverage	Max ident	F	I	B	
Tricholoma sp.1	Tricholoma Y4	AB848692	613	DQ658855.1	99%	98%	■			456
Tricholoma sp.2	Tricholoma Y2	AB848690	541	AF477002.1	97%	91%	■			457
Tricholoma sp.3	Tricholoma Y6	AB848694	634	FJ197006.1	100%	97%	■			458
Tricholomataceae sp.1	Tricholomataceae Y1	AB848696	551	AB218075.1	100%	98%	■			459
Tuber sp.1	Tuber Y1	AB848697	510	AB553511.1	100%	99%	■			460
Tuber sp.2	Tuber Y2	AB848698	597	AB553483.1	95%	99%	■	■		461
Tuber sp.3	Tuber S3	AB923008	509	AB553465.1	100%	97%		■		462
Tuber sp.4	Tuber S2	AB923007	511	AB218103.1	100%	96%		■		463
Tylopilus felleus	Tylopilus Y1	AB848699	406	AB218185.1	100%	99%	■			464
Tylospora fibrillosa sp.1	Tylospora Y1	AB848700	467	AF052561.1	99%	99%	■			465
Tylospora fibrillosa sp.2	Tylospora Y2	AB848701	509	AB254394.1	100%	99%	■			466
Tylospora sp.1	Tylospora Y3	AB848702	538	FJ152492.1	100%	98%	■			467
Tylospora sp.2	Tylospora Y5	AB904792	563	EU597067.1	100%	99%	■			468
Xerocomus sp.1	Xerocomus Y1	AB848703	671	AB218175.1	100%	99%	■			469
Xerocomus sp.2	Xerocomus Y2	AB848704	629	JN020975.1	64%	92%	■	■		470
Xerocomus sp.3	Xerocomus Y3	AB848705	616	GU220375.1	93%	92%	■	■		471
Xerocomus sp.4	Xerocomus S4	AB923011	565	JF273511.1	100%	99%		■		472
Xerocomus sp.5	Xerocomus S2	AB923010	593	AB218099.1	100%	99%		■		473

Black columns indicate species occurrence.

Abbreviatios: DNA Data Bank of Japan (DDBJ), Basic Local Alignment Search Tool (BLAST), Mt. Fuji (F), Mt. Ishizuch (I), Bioassay (B), Maximum identity (Max ident), and image number (No).

Amanita franchetii

Host : *Fagus* 1

Amanita fritillaria

Host : *Abies* 2

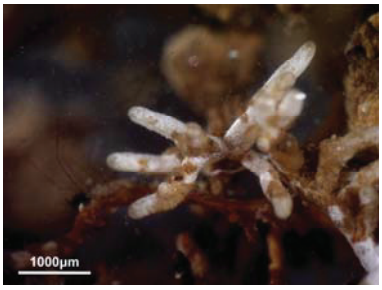
Amanita imazekii

Host : *Fagus* 3

Amanita novinupta

Host : *Fagus* 4

Amanita rubrovolvata



Host : *Fagus* 5

Amanita subjunquillea

Host : *Carpinus* 6

Amanita virosa

Host : *Fagus* 7

Amanita sp.1

Host : *Tsuga* 8

Amanita sp.2

Host : *Fagus* 9

Amphinema byssoides

Host : *Larix* 10

Amphinema sp.1

Host : *Abies* 11

Astraeus hygrometricus

Host : *Betula* 12

Atheliaceae sp.1

Host : unknown 13

Boletaceae sp.1

Host : *Abies* 14

Boletaceae sp.2

Host : *Quercus* 15

Boletaceae sp.3

Host : *Abies* 16

Boletaceae sp.4

Host : unknown 17

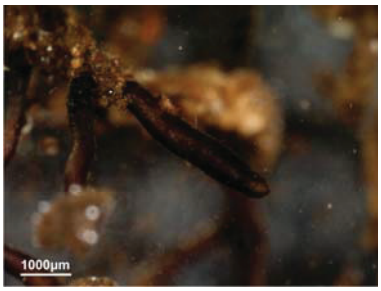
Boletaceae sp.5

Host : *Quercus* 18

Boletus edulis

Host : unknown 19

Boletus sp.1



Host : *Abies* 20

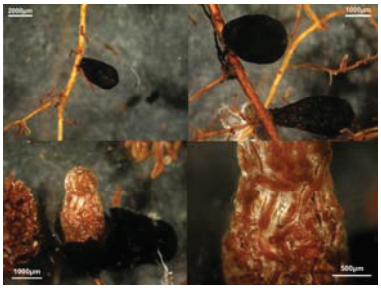
Boletus sp.2

Host : *Abies* 21

Boletus sp.3

Host : *Picea* 22

Boletus sp.4

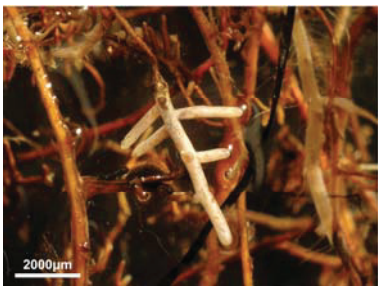


Host : *Carpinus* 23

Boletus sp.5

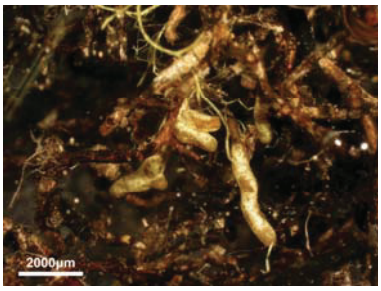
Host : unknown 24

Boletus sp.6



Host : *Carpinus* 25

Boletus sp.7



Host : *Abies* 26

Byssocorticium sp.1

Host : *Abies* 27

Byssoporia terrestris

Host : unknown 28

Cantharellales sp.1

Host : *Fagus* 29

Cenococcum geophilum

Host : *Quercus* 30

Clavulina castaneipes

Host : *Abies* 31

Clavulina cristata

Host : *Abies* 32

Clavulina sp.1

Host : *Abies* 33

Clavulina sp.2

Host : *Tsuga* 34

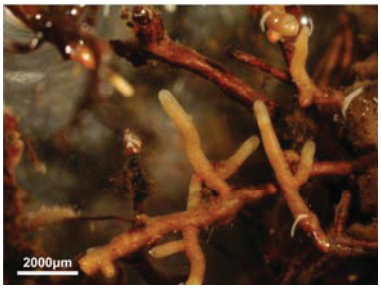
Clavulinaceae sp.1

Host : *Fagus* 35

Clavulinaceae sp.2

Host : *Carpinus* 36

Clavulinaceae sp.3



Host : *Tsuga* 37

Clavulinaceae sp.4

Host : *Fagus* 38

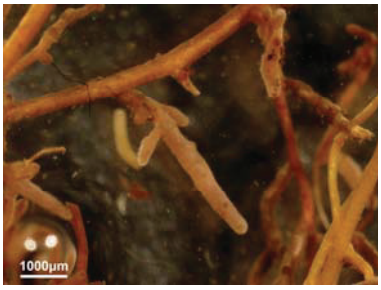
Clavulinaceae sp.5

Host : *Tsuga* 39

Clavulinaceae sp.6

Host : *Tsuga* 40

Clavulinaceae sp.7



Host : *Carpinus* 41

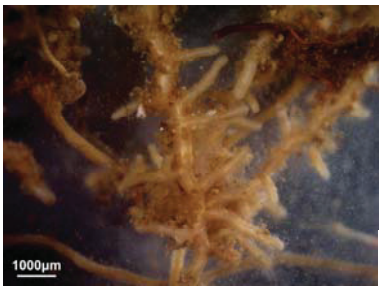
Clavulinaceae sp.8

Host : *Abies* 42

Clavulinaceae sp.9

Host : *Fagus* 43

Clavulinaceae sp.10



Host : *Betula* 44

Cortinarius acutovelatus

Host : unknown 45

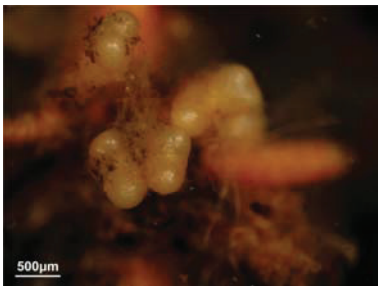
Cortinarius acutus

Host : *Abies* 46

Cortinarius albovariegatus

Host : *Tsuga* 47

Cortinarius angelesianus

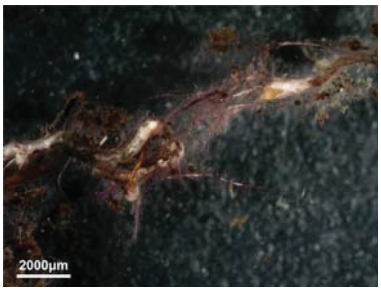


Host : *Pinus* 48

Cortinarius anomalus

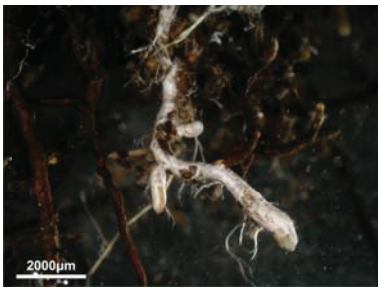
Host : *Betula* 49

Cortinarius anthracinus



Host : *Tsuga* 50

Cortinarius aprinus

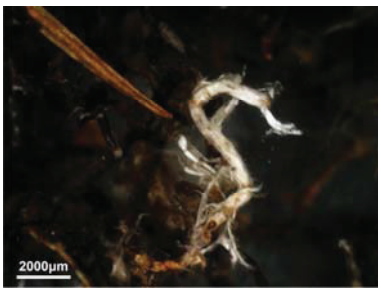


Host : *Tsuga* 51

Cortinarius azureus

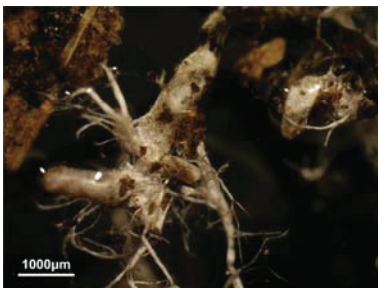
Host : unknown 52

Cortinarius barlowensis



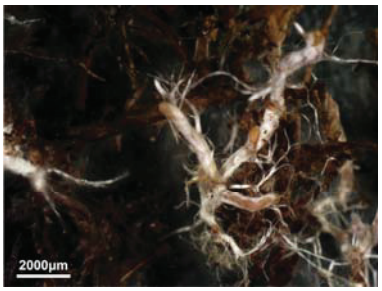
Host : *Tsuga* 53

Cortinarius biformis



Host : *Abies* 54

Cortinarius camphoratus



Host : *Tsuga* 55

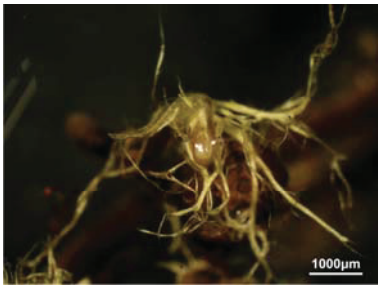
Cortinarius caninus

Host : *Abies* 56

Cortinarius caperatus

Host : *Tsuga* 57

Cortinarius croceus

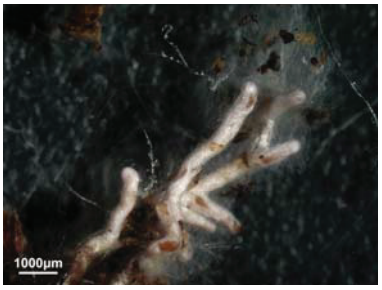


Host : *Abies* 58

Cortinarius delibutus

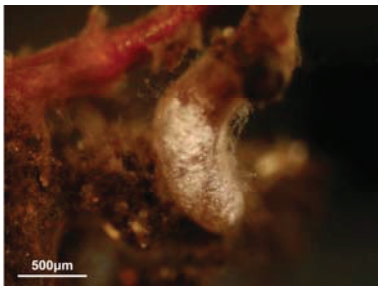
Host : *Fagus* 59

Cortinarius ectypus



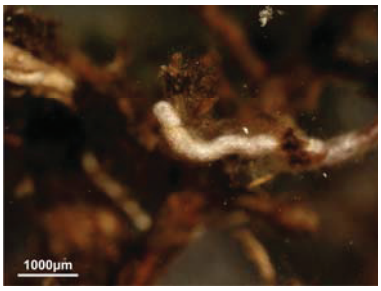
Host : *Tsuga* 60

Cortinarius flexipes sp.1



Host : unknown 61

Cortinarius flexipes sp.2

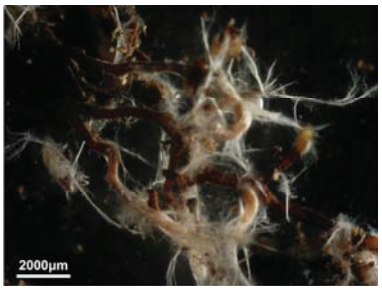


Host : unknown 62

Cortinarius fulvescens

Host : *Tsuga* 63

Cortinarius hemitrichus

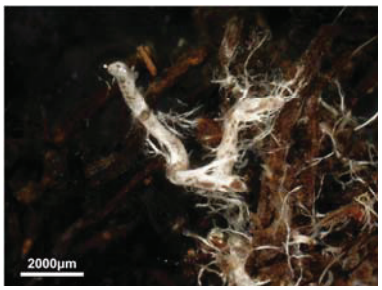


Host : *Larix* 64

Cortinarius herpeticus

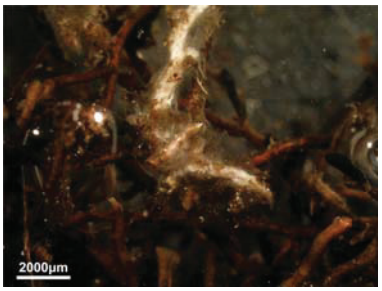
Host : *Tsuga* 65

Cortinarius illuminus



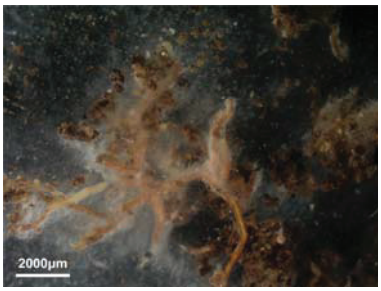
Host : *Tsuga* 66

Cortinarius junghuhnii sp.1



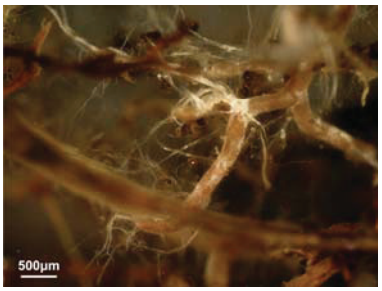
Host : *Abies* 67

Cortinarius junghuhnii sp.2



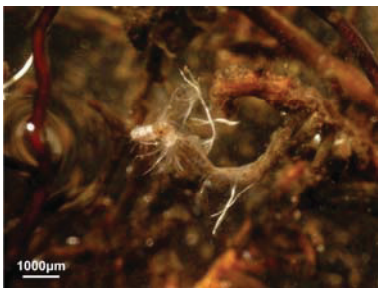
Host : unknown 68

Cortinarius keralensis



Host : unknown 69

Cortinarius laetissimus



Host : *Abies* 70

Cortinarius obtusus

Host : *Abies* 71

Cortinarius olivaceopictus

Host : *Abies* 72

Cortinarius phoeniceus

Host : *Fagus* 73

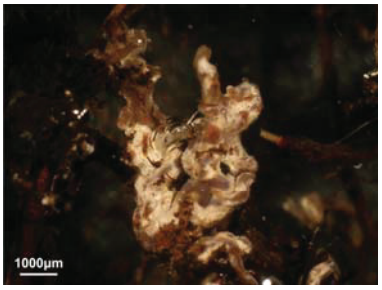
Cortinarius saniosus

Host : *Fagus* 74

Cortinarius saturninus

Host : *Betula* 75

Cortinarius scaurus sp.1



Host : *Abies* 76

Cortinarius scaurus sp.2

Host : unknown 77

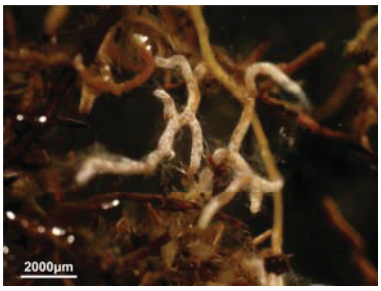
Cortinarius tortuosus

Host : *Tsuga* 78

Cortinarius turibulosus

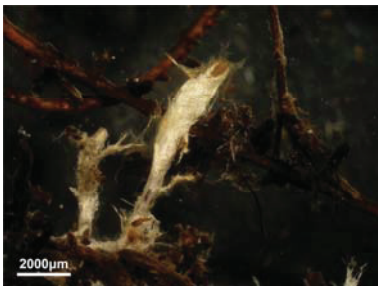
Host : *Fagus* 79

Cortinarius umbrinolens



Host : *Betula* 80

Cortinarius venetus

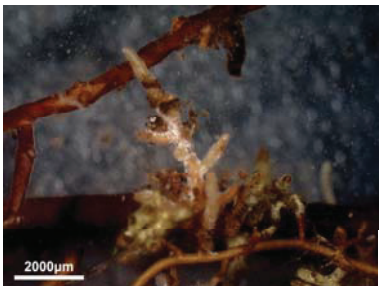


Host : *Tsuga* 81

Cortinarius vibratilis

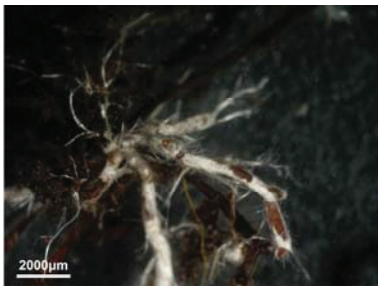
Host : *Abies* 82

Cortinarius violaceus



Host : *Fagus* 83

Cortinarius sp.1

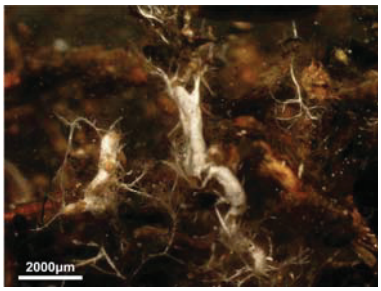


Host : *Tsuga* 84

Cortinarius sp.2

Host : *Tsuga* 85

Cortinarius sp.3



Host : *Abies* 86

Cortinarius sp.4

Host : *Tsuga* 87

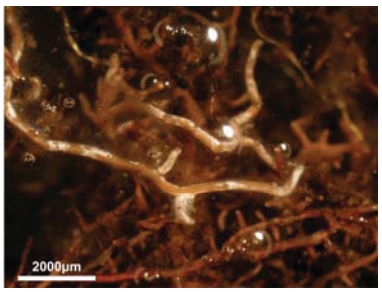
Cortinarius sp.5

Host : unknown 88

Cortinarius sp.6

Host : *Fagus* 89

Cortinarius sp.7



Host : *Fagus* 90

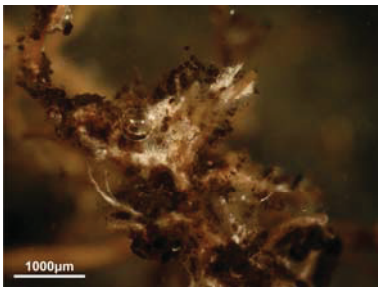
Cortinarius sp.8

Host : *Fagus* 91

Cortinarius sp.9

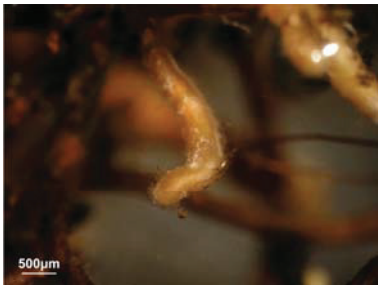
Host : unknown 92

Cortinarius sp.10



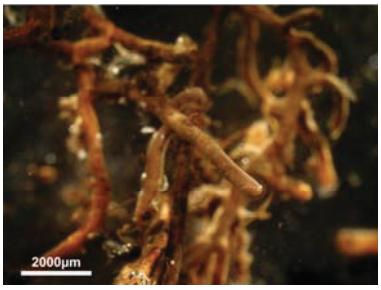
Host : *Fagus* 93

Cortinarius sp.11



Host : unknown 94

Cortinarius sp.12



Host : *Abies* 95

Cortinarius sp.13

Host : *Abies* 96

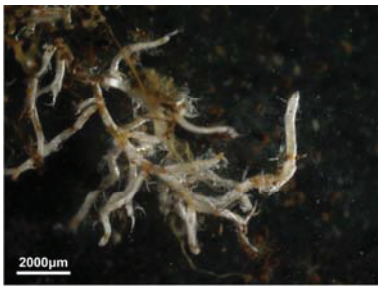
Cortinarius sp.14

Host : *Quercus* 97

Cortinarius sp.15

Host : *Fagus* 98

Cortinarius sp.16

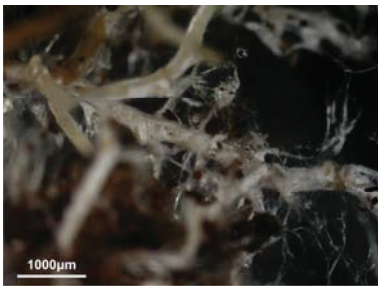


Host : *Fagus* 99

Cortinarius sp.17

Host : unknown 100

Cortinarius sp.18



Host : *Fagus* 101

Cortinarius sp.19

Host : *Abies* 102

Cortinarius sp.20

Host : *Abies* 103

Cortinarius sp.21

Host : *Abies*, *Fagus* 104

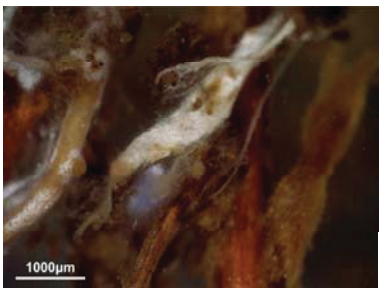
Cortinarius sp.22

Host : *Fagus* 105

Cortinarius sp.23

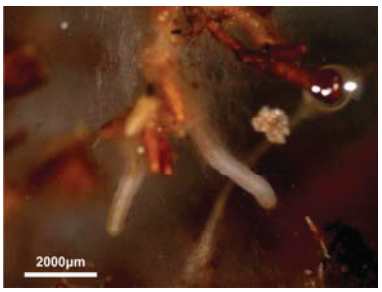
Host : *Quercus* 106

Cortinarius sp.24



Host : *Fagus* 107

Cortinarius sp.25



Host : *Fagus* 108

Cortinarius sp.26

Host : *Abies* 109

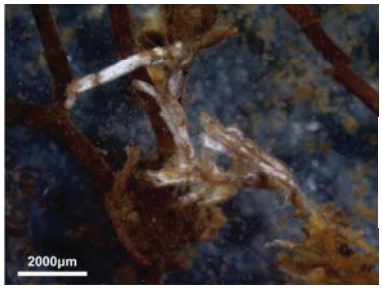
Cortinarius sp.27

Host : *Fagus* 110

Cortinarius sp.28

Host : *Abies* 111

Cortinarius sp.29

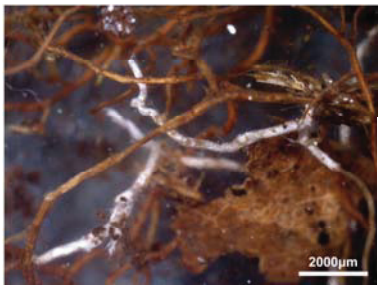


Host : *Fagus* 112

Cortinarius sp.30

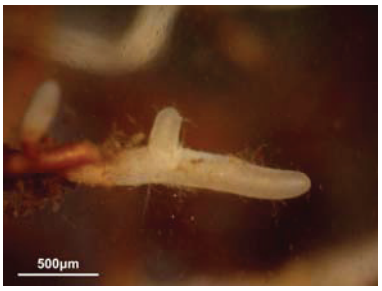
Host : *Quercus* 113

Cortinarius sp.31



Host : *Quercus* 114

Craterellus sp.1



Host : *Fagus* 115

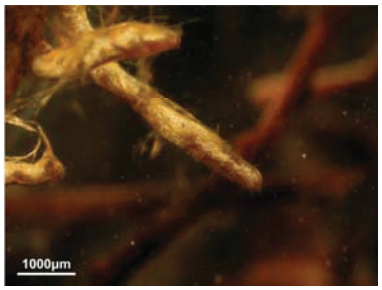
Craterellus sp.2

Host : *Fagus* 116

Craterellus tubaeformis

Host : *Abies* 117

Dermocybe malicoria

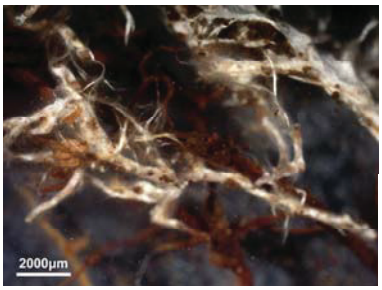


Host : *Tsuga* 118

Elaphomyces sp.1

Host : *Abies* 119

Elaphomyces sp.2

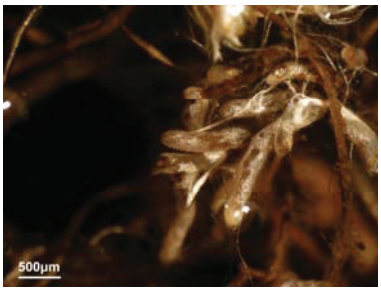


Host : *Quercus* 120

Entoloma rhodopolium

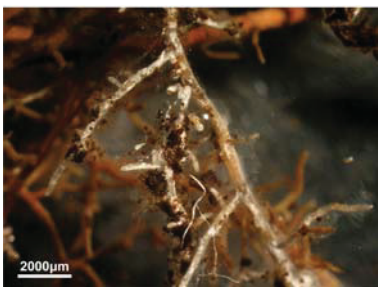
Host : unknown 121

Entoloma sp.1



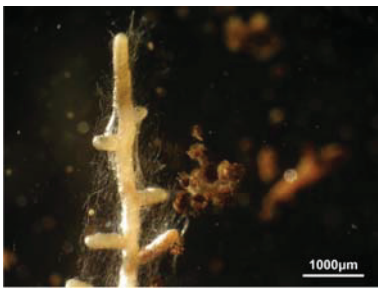
Host : *Betula* 122

Entoloma sp.2



Host : *Quercus* 123

Entoloma sp.3

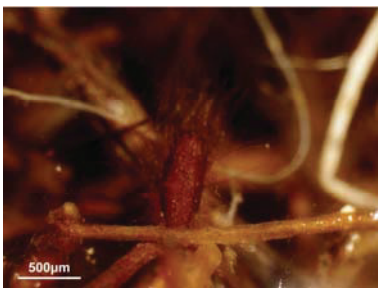


Host : *Quercus* 124

Entoloma sp.4

Host : unknown 125

Genea hispidula

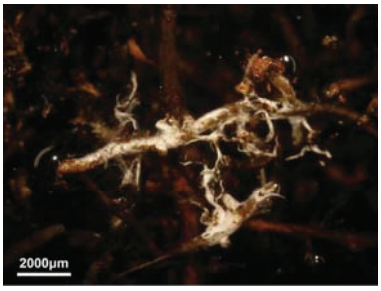


Host : *Betula* 126

Hebeloma crustuliniforme

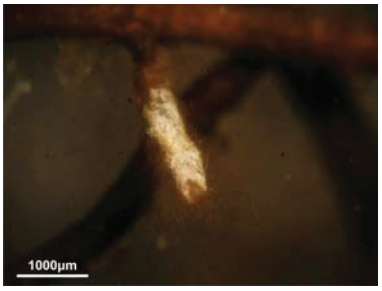
Host : *Salix* 127

Hebeloma incarnatulum



Host : *Abies* 128

Hebeloma mesophaeum



Host : unknown 129

Hebeloma rivulosum

Host : *Betula* 130

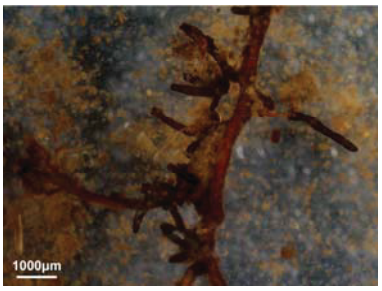
Helotiales sp.1

Host : *Abies* 131

Helotiales sp.1

Host : *Larix* 132

Helotiales sp.2

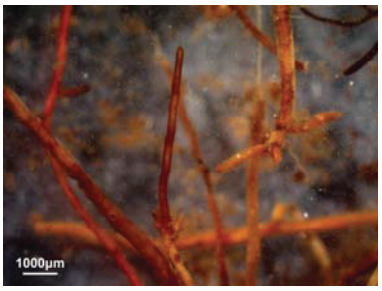


Host : *Fagus* 133

Helotiales sp.2

Host : *Abies* 134

Helotiales sp.3

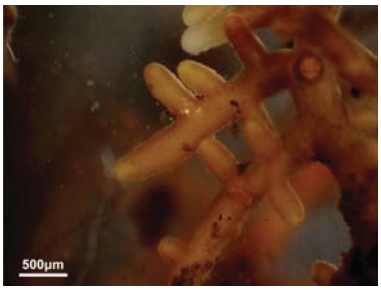


Host : unknown 135

Helotiales sp.5

Host : *Abies* 136

Helvella sp.1

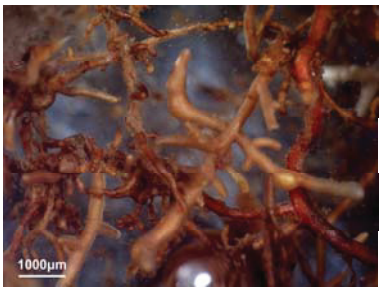


Host : *Fagus* 137

Humaria hemisphaerica

Host : *Betula* 138

Hydnotrya sp.1



Host : *Fagus* 139

Hydnotrya sp.2

Host : *Fagus* 140

Hydnotrya sp.3

Host : *Abies* 141

Hydnotrya sp.4

Host : *Betula* 142

Hydnum sp.1

Host : *Quercus* 143

Inocybe flocculosa

Host : *Quercus* 144

Inocybe lilacina

Host : *Quercus* 145

Inocybe napipes

Host : *Betula* 146

Inocybe nitidiuscula

Host : *Abies* 147

Inocybe phaeocomis

Host : *Abies* 148

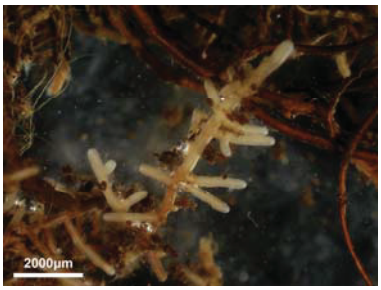
Inocybe praetervisa

Host : *Abies* 149

Inocybe sp.1

Host : *Tsuga* 150

Inocybe sp.2

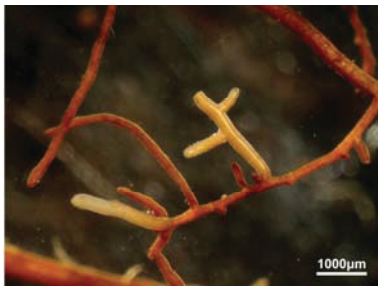


Host : *Fagus* 151

Inocybe sp.3

Host : *Larix* 152

Inocybe sp.4

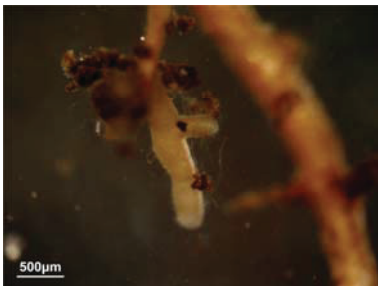


Host : *Quercus* 153

Inocybe sp.5

Host : *Fagus* 154

Inocybe sp.6



Host : *Quercus* 155

Inocybe sp.7

Host : *Fagus* 156

Inocybe sp.8

Host : *Carpinus* 157

Inocybe sp.9

Host : *Fagus* 158

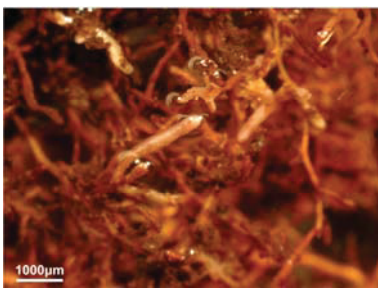
Inocybe sp.10

Host : *Fagus* 159

Inocybe sp.11

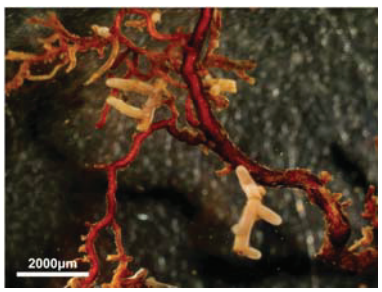
Host : *Abies* 160

Inocybe sp.12



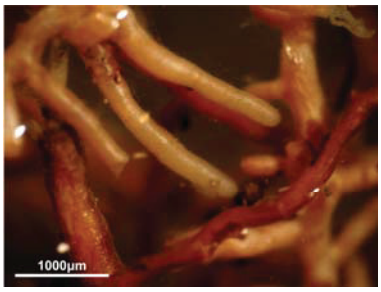
Host : *Fagus* 161

Inocybe sp.13



Host : *Betula* 162

Inocybe sp.14



Host : *Betula* 163

Inocybe sp.15

Host : *Betula* 164

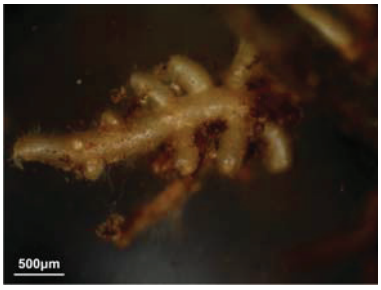
Inocybe sp.16

Host : *Fagus* 165

Inocybe sp.17

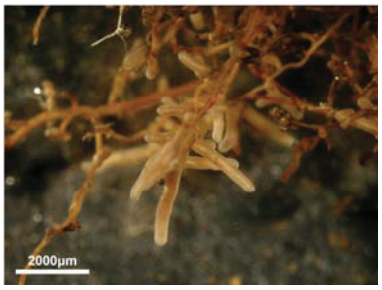
Host : *Fagus* 166

Inocybe sp.18



Host : *Fagus* 167

Inocybe sp.19

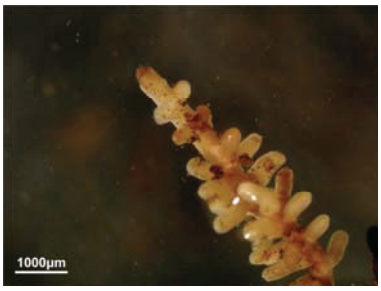


Host : *Fagus* 168

Inocybe sp.20

Host : *Fagus* 169

Inocybe sp.21

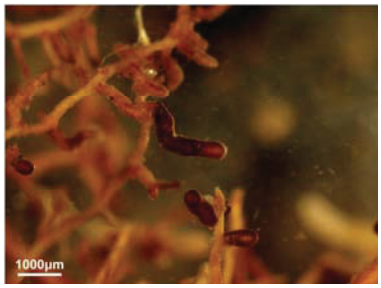


Host : *Fagus* 170

Inocybe sp.22

Host : *Fagus* 171

Inocybe sp.23



Host : *Fagus* 172

Inocybe sp.24

Host : *Quercus* 173

Inocybe sp.25

Host : *Abies* 174

Inocybe sp.26

Host : *Quercus* 175

Inocybe sp.27

Host : *Fagus* 176

Inocybe sp.28

Host : *Abies* 177

Inocybe sp.29

Host : *Abies* 178

Inocybe sp.30

Host : *Abies* 179

Inocybe sp.31

Host : *Fagus* 180

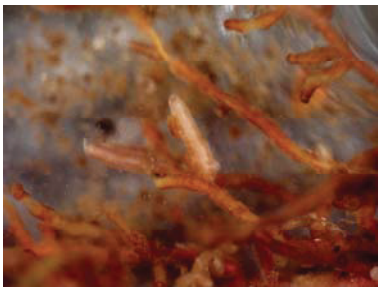
Inocybe sp.32

Host : *Abies* 181

Inocybe sp.33

Host : *Carpinus* 182

Inocybe sp.34

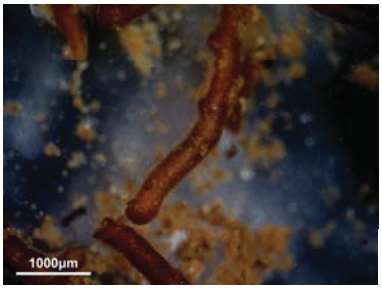


Host : *Betula* 183

Inocybe sp.35

Host : *Quercus* 184

Inocybe sp.36



Host : *Fagus* 185

Inocybe sp.37

Host : *Tsuga* 186

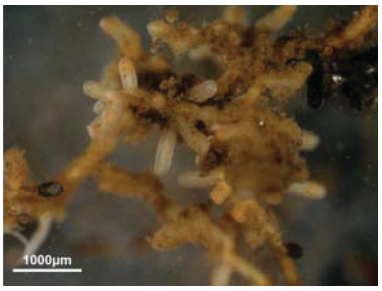
Laccaria cf. *laccata* sp.1

Host : unknown 187

Laccaria cf. *laccata* sp.2

Host : *Abies* 188

Laccaria laccata



Host : *Fagus* 189

Laccaria sp.1

Host : unknown 190

Laccaria sp.2

Host : *Fagus* 191

Laccaria sp.3

Host : *Abies* 192

Lactarius badiosanguineus

Host : *Abies* 193

Lactarius caespitosus

Host : *Abies* 194

Lactarius chichuensis

Host : *Tsuga* 195

Lactarius chrysorrheu

Host : *Tsuga* 196

Lactarius deliciosus

Host : *Abies* 197

Lactarius deterrimus

Host : unknown 198

Lactarius flexuosus

Host : unknown 199

Lactarius gracilis

Host : *Quercus* 200

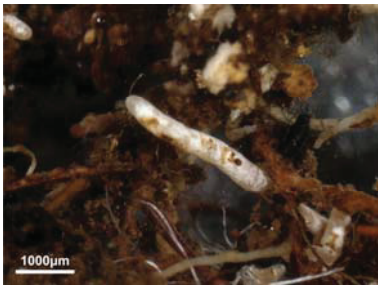
Lactarius imperceptus

Host : *Abies* 201

Lactarius lignyotus

Host : *Abies* 202

Lactarius picinus

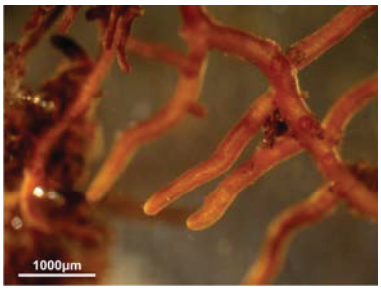


Host : *Fagus* 203

Lactarius pseudomucidus

Host : *Tsuga* 204

Lactarius quietus

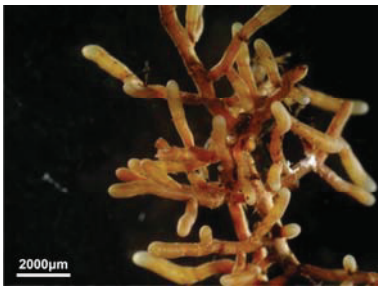


Host : *Fagus* 205

Lactarius tabidus

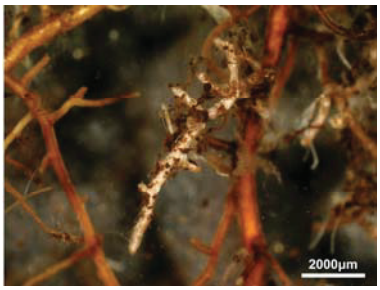
Host : *Betula* 206

Lactarius uvidus sp.1



Host : *Tsuga* 207

Lactarius uvidus sp.2



Host : *Carpinus* 208

Lactarius vietus

Host : *Abies* 209

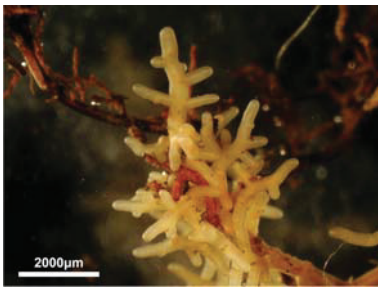
Lactarius yazooensis

Host : *Quercus* 210

Lactarius sp.1

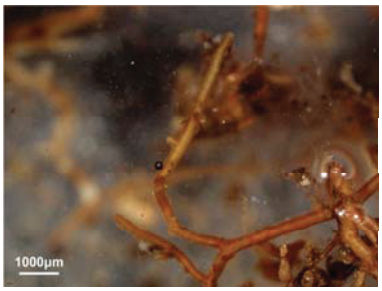
Host : *Abies* 211

Lactarius sp.2



Host : *Fagus* 212

Lactarius sp.3



Host : unknown 213

Lactarius sp.4

Host : *Quercus* 214

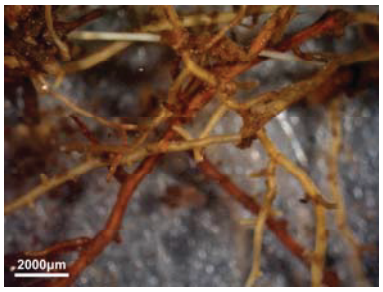
Lactarius sp.5

Host : *Quercus* 215

Lactarius sp.6

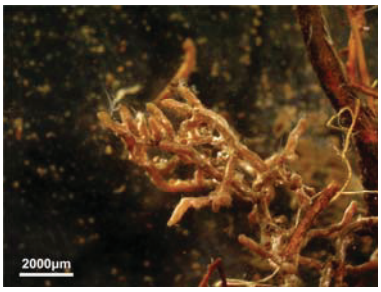
Host : *Tsuga* 216

Lactarius sp.7



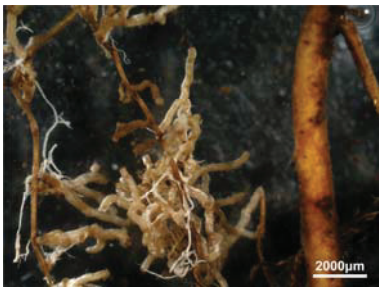
Host : *Quercus* 217

Leccinum sp.1



Host : *Carpinus* 218

Leccinum versipelle

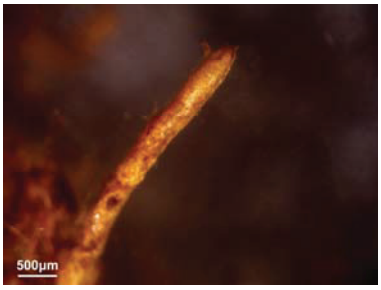


Host : *Betula* 219

Leucogastraceae sp.1

Host : *Abies* 220

Melanogaster sp.1



Host : *Pinus* 221

Meliniomyces sp.1

Host : *Betula* 222

Octaviania japonimontana

Host : *Fagus* 223

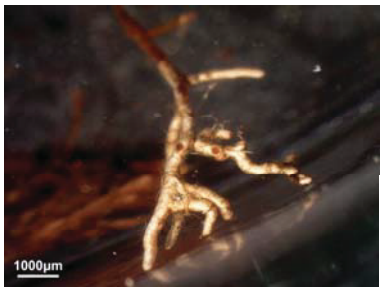
Pachyphloeus sp.1

Host : *Fagus* 224

Pachyphloeus sp.2

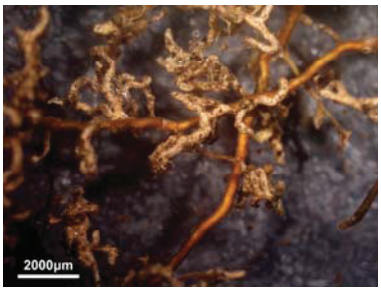
Host : *Fagus* 225

Paxillus involutus sp.1



Host : *Quercus* 226

Paxillus involutus sp.2

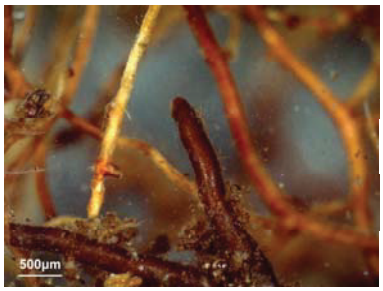


Host : *Betula* 227

Peziza sp.1

Host : *Fagus* 228

Pezizales (*Genea* sp.1)



Host : *Quercus* 229

Pezizales (*Genea* sp.2)

Host : *Abies* 230

Pezizales (*Helvella*)

Host : *Fagus* 231

Pezizales sp.1

Host : unknown 232

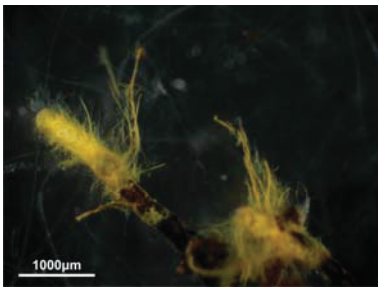
Phylloporus maculatus

Host : *Quercus* 233

Phylloporus sp.1

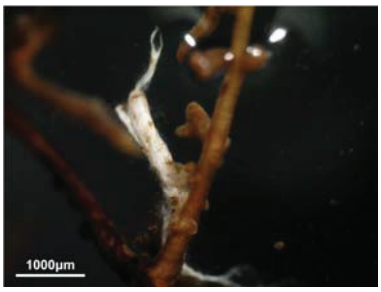
Host : *Abies* 234

Piloderma croceum



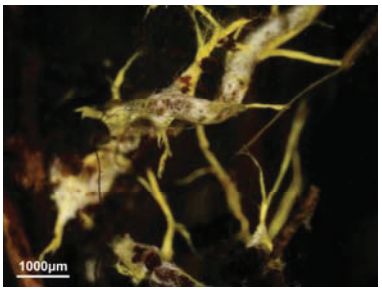
Host : *Tsuga* 235

Piloderma fallax sp.1



Host : *Fagus* 236

Piloderma fallax sp.2



Host : *Abies* 237

Piloderma lanatum

Host : *Quercus* 238

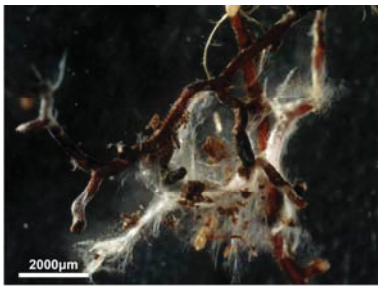
Piloderma olivaceum sp.1

Host : *Abies* 239

Piloderma olivaceum sp.2

Host : *Abies* 240

Piloderma sp.1



Host : *Tsuga* 241

Piloderma sp.2

Host : *Abies* 242

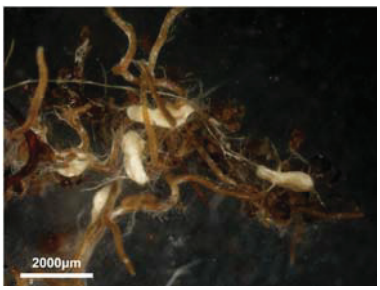
Piloderma sp.3

Host : *Abies* 243

Piloderma sp.4

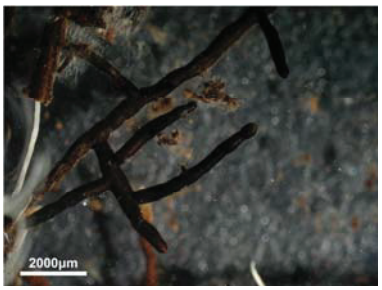
Host : *Tsuga* 244

Piloderma sp.5



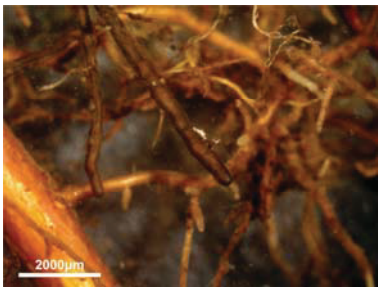
Host : unknown 245

Pseudotomentella humicola



Host : *Abies* 246

Pseudotomentella sp.1



Host : *Abies* 247

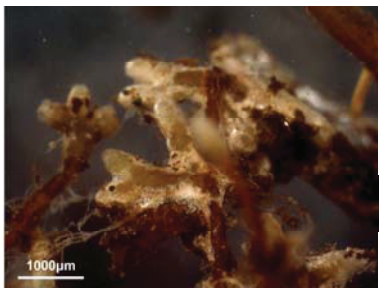
Pseudotomentella sp.2

Host : *Fagus* 248

Pseudotomentella sp.3

Host : *Abies* 249

Rhizopogon sp.1



Host : *Pinus* 250

Rhizopogon sp.2

Host : *Pinus* 251

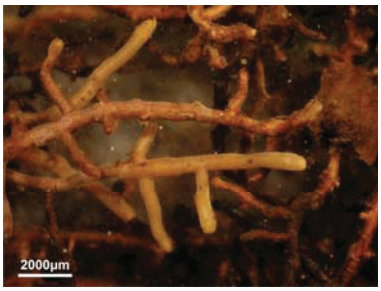
Russula abietina

Host : *Tsuga* 252

Russula aquosa

Host : *Tsuga* 253

Russula bicolor sp.1



Host : *Abies* 254

Russula bicolor sp.2

Host : *Tsuga* 255

Russula brevipes

Host : unknown 256

Russula cerolens

Host : *Abies* 257

Russula crassotunicata

Host : *Tsuga* 258

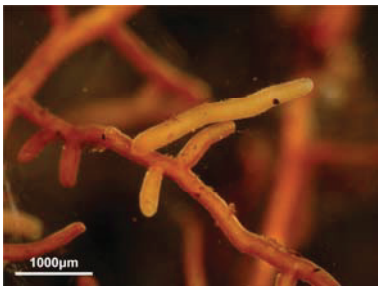
Russula cremeirosea

Host : *Abies* 259

Russula densifolia

Host : *Abies* 260

Russula emetica



Host : *Quercus* 261

Russula favrei

Host : *Abies* 262

Russula granulata

Host : *Fagus* 263

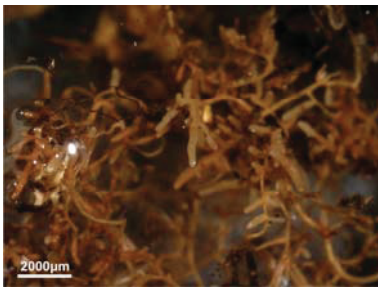
Russula lepida sp.1

Host : *Abies* 264

Russula lepida sp.2

Host : *Abies* 265

Russula mairei



Host : *Fagus* 266

Russula ochroleuca

Host : *Abies* 267

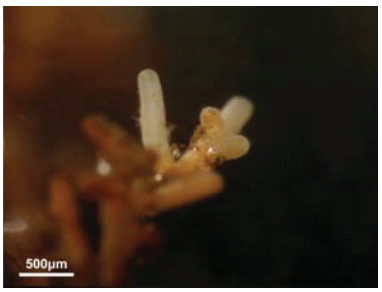
Russula peckii

Host : *Betula* 268

Russula puellaris

Host : unknown 269

Russula puellula

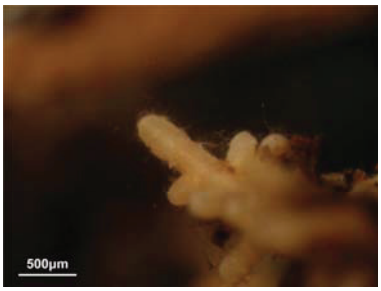


Host : *Fagus* 270

Russula rosea

Host : *Quercus* 271

Russula solaris



Host : unknown 272

Russula turci

Host : *Abies* 273

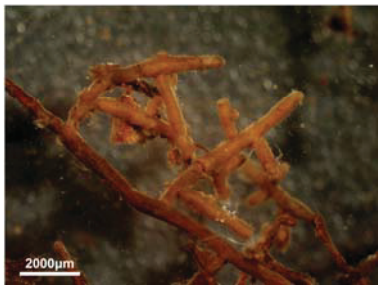
Russula velenovskyi

Host : unknown 274

Russula vesca

Host : *Abies* 275

Russula sp.1

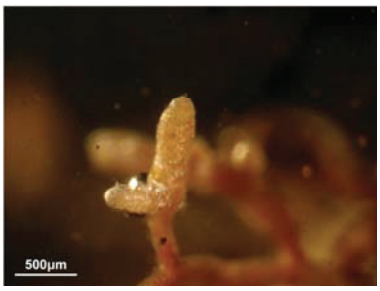


Host : *Tsuga* 276

Russula sp.2

Host : *Fagus* 277

Russula sp.3



Host : *Fagus* 278

Russula sp.4

Host : *Fagus* 279

Russula sp.5

Host : *Quercus* 280

Russula sp.6

Host : *Carpinus* 281

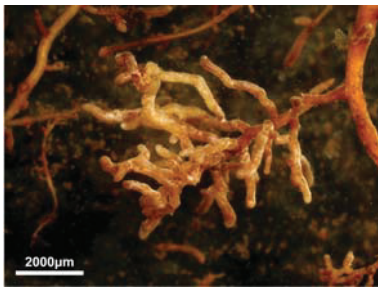
Russula sp.7

Host : *Fagus* 282

Russula sp.8

Host : *Tsuga* 283

Russula sp.9



Host : *Fagus* 284

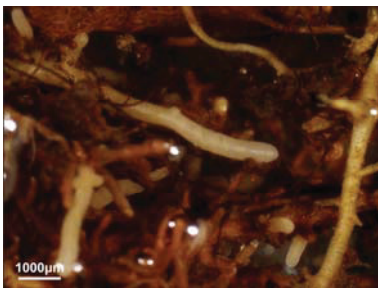
Russula sp.10

Host : unknown 285

Russula sp.11

Host : *Abies* 286

Russula sp.12



Host : *Fagus* 287

Russula sp.13

Host : *Abies* 288

Russula sp.14

Host : unknown 289

Russula sp.15

Host : *Abies* 290

Russula sp.16

Host : *Quercus* 291

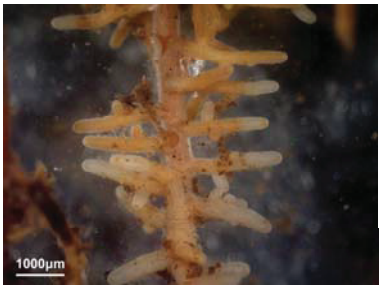
Russula sp.17

Host : *Fagus* 292

Russula sp.18

Host : *Abies* 293

Russula sp.19



Host : *Fagus* 294

Russula sp.20

Host : *Carpinus* 295

Russula sp.21

Host : *Quercus* 296

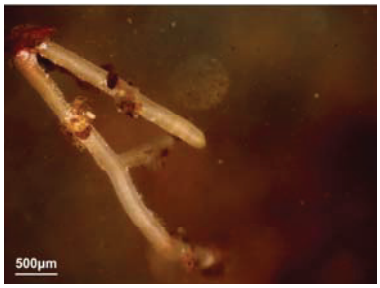
Russula sp.22

Host : *Quercus* 297

Russula sp.23

Host : *Abies* 298

Russula sp.24



Host : *Quercus* 299

Russula sp.25

Host : *Quercus* 300

Russula sp.26

Host : *Abies* 301

Russula sp.27

Host : *Fagus* 302

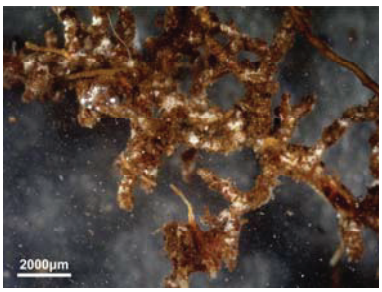
Russula sp.28

Host : *Quercus* 303

Russula sp.29

Host : *Fagus* 304

Russula sp.30



Host : *Carpinus* 305

Russula sp.31

Host : *Fagus* 306

Russula sp.32

Host : *Quercus* 307

Russula sp.33

Host : *Abies* 308

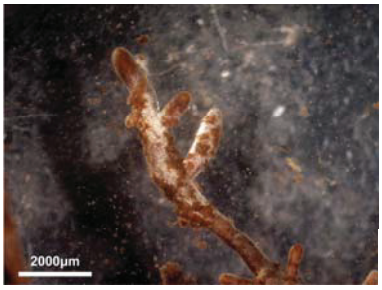
Russula sp.34

Host : *Quercus* 309

Russula sp.35

Host : *Fagus* 310

Russula sp.36

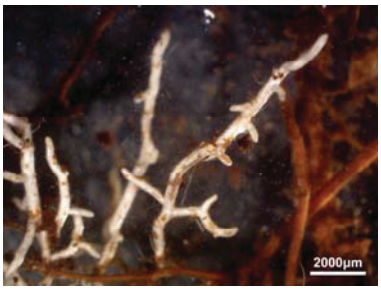


Host : *Abies* 311

Scleroderma bovista

Host : *Salix* 312

Scleroderma citrinum



Host : *Quercus* 313

Sebacina incrustans

Host : *Fagus* 314

Sebacina sp.1

Host : *Abies* 315

Sebacina sp.2

Host : *Abies* 316

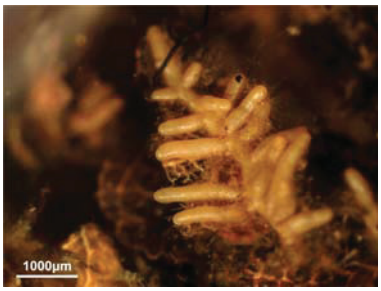
Sebacina sp.3

Host : *Tsuga* 317

Sebacina sp.4

Host : *Carpinus* 318

Sebacina sp.5



Host : *Carpinus* 319

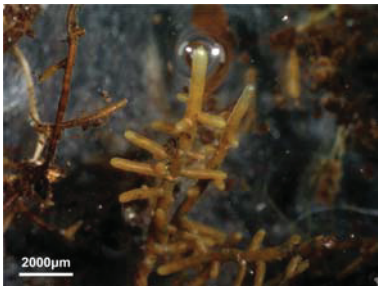
Sebacina sp.6

Host : unknown 320

Sebacina sp.7

Host : unknown 321

Sebacina sp.8



Host : *Picea* 322

Sebacina sp.9

Host : *Fagus* 323

Sebacina sp.10

Host : *Abies* 324

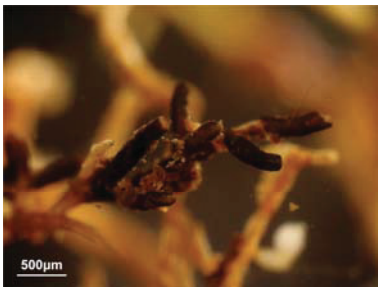
Sebacina sp.11

Host : *Abies* 325

Sebacina sp.12

Host : *Fagus* 326

Sebacina sp.14

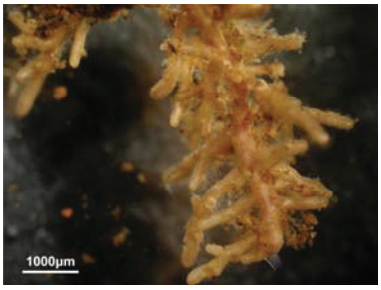


Host : *Quercus* 327

Sebacina sp.15

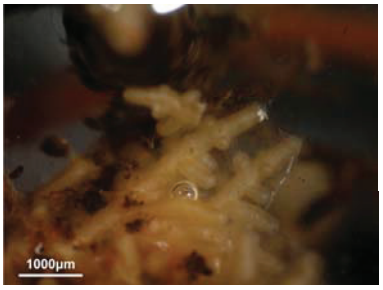
Host : unknown 328

Sebacina sp.16



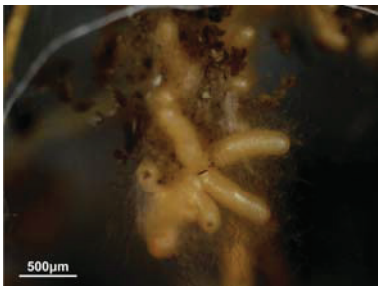
Host : *Carpinus* 329

Sebacina sp.17



Host : unknown 330

Sebacina sp.18



Host : *Fagus* 331

Sebacina sp.19

Host : unknown 332

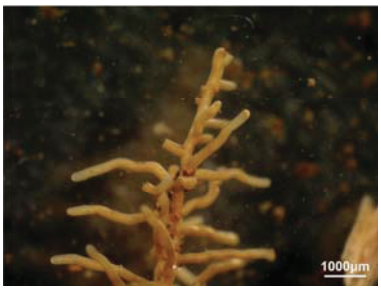
Sebacina sp.20

Host : *Fagus* 333

Sebacina sp.21

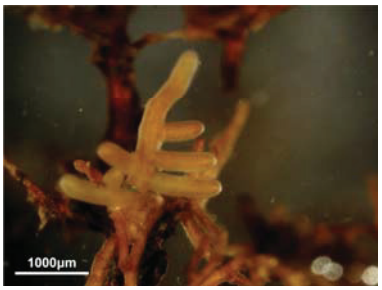
Host : *Carpinus* 334

Sebacina sp.22



Host : *Carpinus* 335

Sebacina sp.23



Host : *Fagus* 336

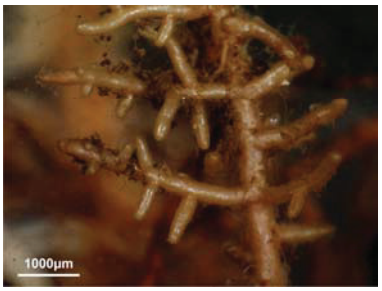
Sebacina sp.24

Host : unknown 337

Sebacina sp.25

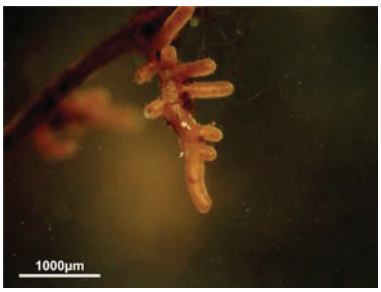
Host : *Fagus* 338

Sebacina sp.26



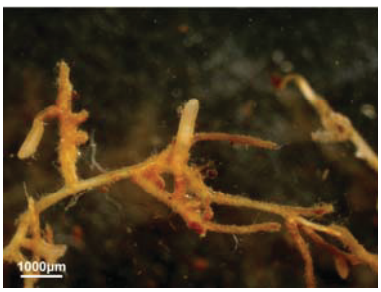
Host : *Fagus* 339

Sebacina sp.27



Host : unknown 340

Sebacina sp.28

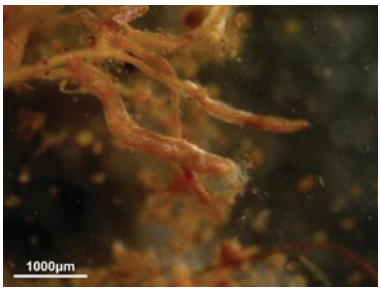


Host : *Betula* 341

Sebacina sp.29

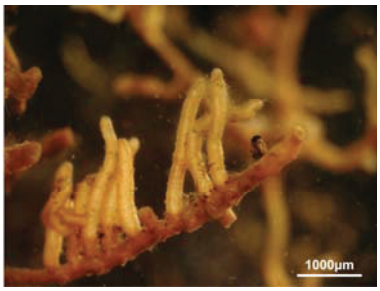
Host : unknown 342

Sebacina sp.30



Host : *Fagus* 343

Sebacina sp.31



Host : *Quercus* 344

Sebacina sp.32

Host : *Abies* 345

Sebacina sp.33

Host : *Fagus* 346

Sebacina sp.34

Host : *Tsuga* 347

Sebacina sp.35

Host : *Quercus* 348

Sebacina sp.36

Host : *Fagus* 349

Sebacina sp.37

Host : *Fagus* 350

Sebacina sp.38

Host : *Quercus* 351

Sebacina sp.39

Host : *Abies* 352

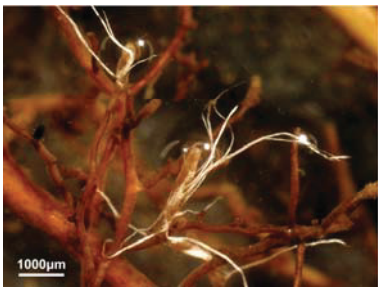
Sebacina sp.40

Host : *Betula* 353

Sistotrema sp.1

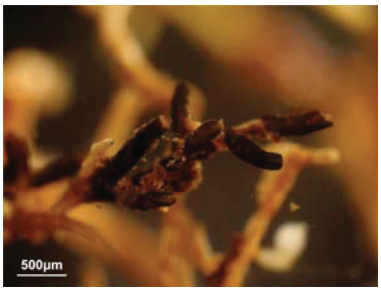
Host : *Abies* 354

Sistotrema sp.2



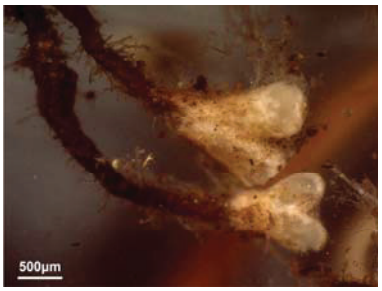
Host : unknown 355

Sordariomycetes sp.1



Host : *Quercus* 356

Suillus bovinus

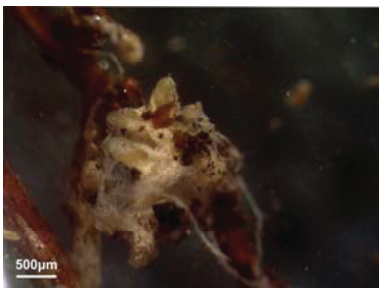


Host : *Pinus* 357

Suillus cavipes

Host : *Larix* 358

Suillus granulatus



Host : *Pinus* 359

Suillus intermedius

Host : *Pinus* 360

Suillus luteus

Host : *Pinus* 361

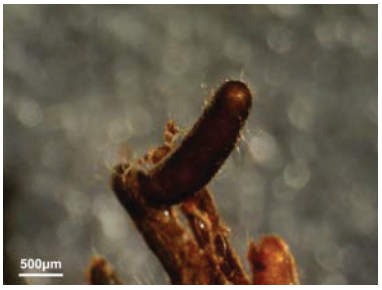
Suillus subaureus

Host : *Abies* 362

Suillus variegatus

Host : *Pinus* 363

Thelephora sp.1



Host : *Abies* 364

Thelephora sp.2

Host : *Fagus* 365

Thelephoraceae sp.1

Host : *Abies* 366

Thelephoraceae sp.2

Host : *Abies* 367

Tomentella badia

Host : *Betula* 368

Tomentella ramosissima

Host : *Abies* 369

Tomentella stuposa sp.1

Host : *Abies* 370

Tomentella stuposa sp.2

Host : unknown 371

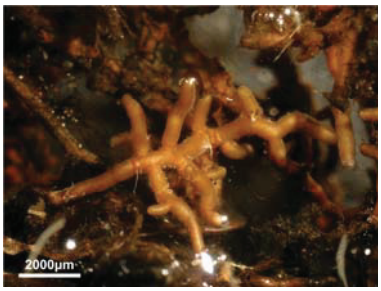
Tomentella sublilacina

Host : *Abies* 372

Tomentella terrestris

Host : *Abies* 373

Tomentella sp.1

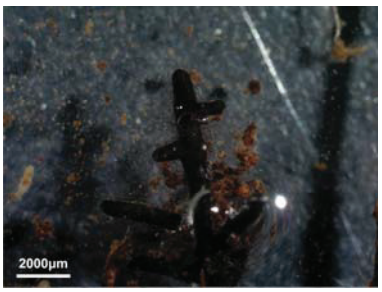


Host : *Abies* 374

Tomentella sp.2

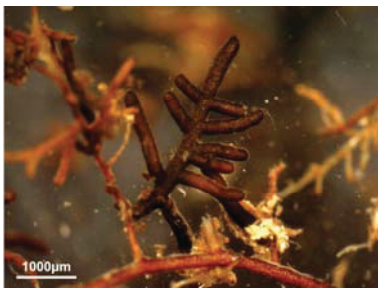
Host : *Abies* 375

Tomentella sp.3



Host : *Abies* 376

Tomentella sp.4



Host : *Fagus* 377

Tomentella sp.5

Host : *Abies* 378

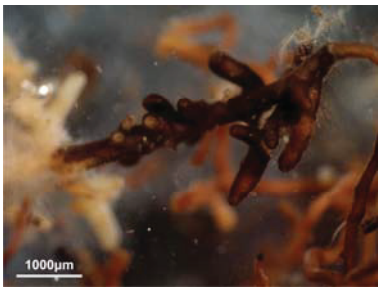
Tomentella sp.6

Host : *Fagus* 379

Tomentella sp.7

Host : *Tsuga* 380

Tomentella sp.8



Host : unknown 381

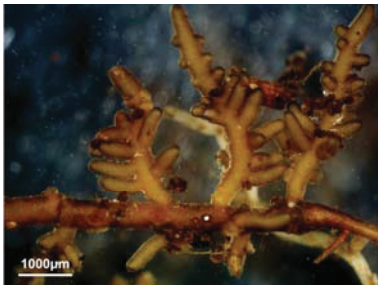
Tomentella sp.9

Host : *Fagus* 382

Tomentella sp.10

Host : *Abies* 383

Tomentella sp.11



Host : unknown 384

Tomentella sp.12

Host : *Abies* 385

Tomentella sp.13

Host : *Fagus* 386

Tomentella sp.14

Host : *Abies* 387

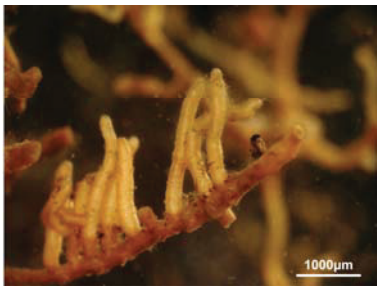
Tomentella sp.15

Host : *Abies* 388

Tomentella sp.16

Host : unknown 389

Tomentella sp.17



Host : *Carpinus* 390

Tomentella sp.18

Host : *Abies* 391

Tomentella sp.19

Host : *Abies* 392

Tomentella sp.20

Host : unknown 393

Tomentella sp.21

Host : *Abies* 394

Tomentella sp.22

Host : *Fagus* 395

Tomentella sp.23

Host : *Fagus* 396

Tomentella sp.24

Host : *Fagus* 397

Tomentella sp.25

Host : unknown 398

Tomentella sp.26

Host : *Fagus* 399

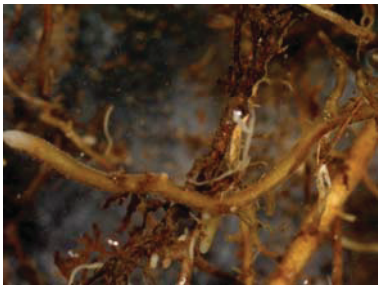
Tomentella sp.27

Host : *Tsuga* 400

Tomentella sp.28

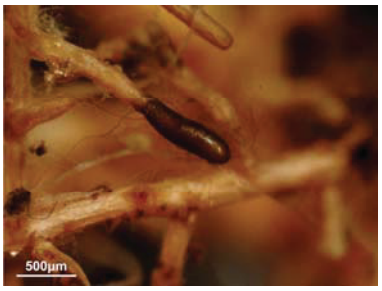
Host : *Fagus* 401

Tomentella sp.29



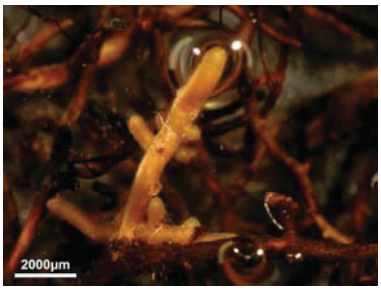
Host : *Fagus* 402

Tomentella sp.30



Host : unknown 403

Tomentella sp.31



Host : *Abies* 404

Tomentella sp.32

Host : unknown 405

Tomentella sp.33

Host : *Tsuga* 406

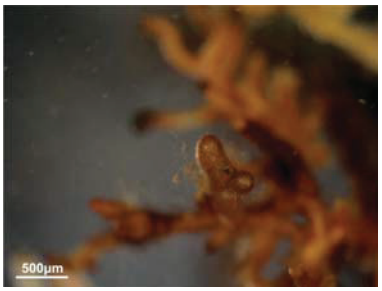
Tomentella sp.34

Host : unknown 407

Tomentella sp.35

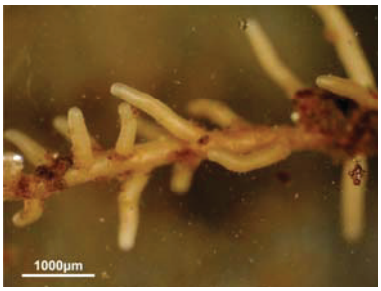
Host : *Abies* 408

Tomentella sp.36



Host : *Fagus* 409

Tomentella sp.37



Host : unknown 410

Tomentella sp.38

Host : *Fagus* 411

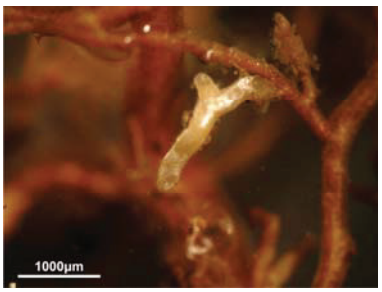
Tomentella sp.39

Host : *Carpinus* 412

Tomentella sp.40

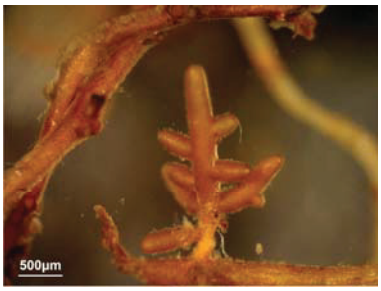
Host : *Fagus* 413

Tomentella sp.41



Host : unknown 414

Tomentella sp.42

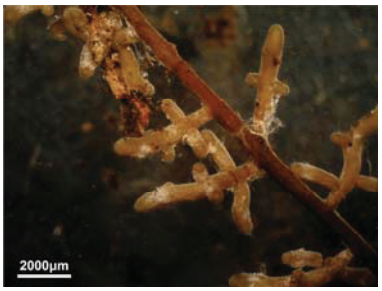


Host : unknown 415

Tomentella sp.43

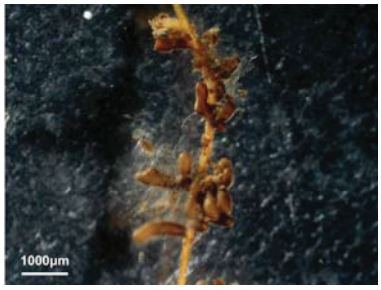
Host : *Carpinus* 416

Tomentella sp.44



Host : *Abies* 417

Tomentella sp.45

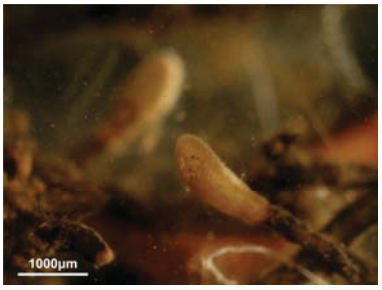


Host : unknown 418

Tomentella sp.46

Host : *Pinus* 419

Tomentella sp.47



Host : *Tsuga* 420

Tomentella sp.48

Host : *Quercus* 421

Tomentella sp.49

Host : unknown 422

Tomentella sp.50

Host : *Fagus* 423

Tomentella sp.51

Host : unknown 424

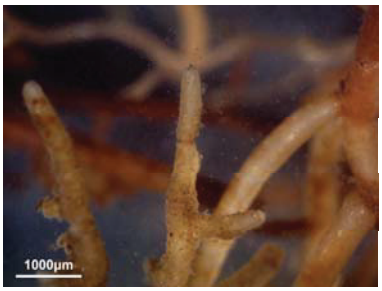
Tomentella sp.52

Host : unknown 425

Tomentella sp.53

Host : *Tsuga* 426

Tomentella sp.54



Host : *Fagus* 427

Tomentella sp.55

Host : *Quercus* 428

Tomentella sp.56

Host : Fagaceae 429

Tomentella sp.57

Host : unknown 430

Tomentella sp.58

Host : *Quercus* 431

Tomentella sp.59

Host : *Abies* 432

Tomentella sp.60

Host : *Abies* 433

Tomentella sp.61

Host : *Abies* 434

Tomentella sp.62

Host : *Abies* 435

Tomentella sp.63

Host : *Abies* 436

Tomentella sp.64

Host : *Fagus* 437

Tomentella sp.66

Host : *Fagus* 438

Tomentella sp.67

Host : *Quercus* 439

Tomentella sp.68

Host : *Fagus* 440

Tomentella sp.69

Host : *Abies* 441

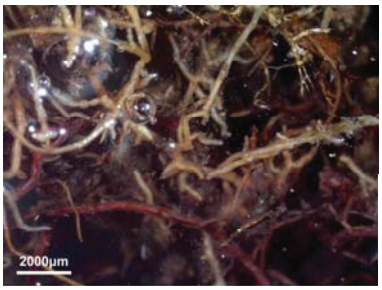
Tomentella sp.70

Host : *Quercus* 442

Tomentella sp.71

Host : *Fagus* 443

Tomentella sp.72



Host : *Quercus* 444

Tomentella sp.73

Host : *Fagus* 445

Tomentella sp.74

Host : *Betula* 446

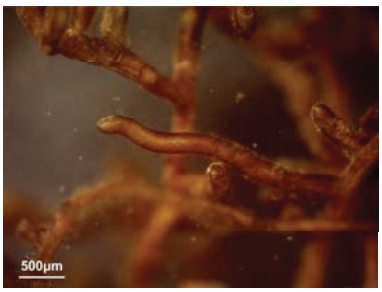
Tomentella sp.75

Host : *Betula* 447

Tomentella sp.76

Host : *Betula* 448

Tomentella sp.77



Host : *Betula* 449

Tomentella sp.78

Host : *Betula* 450

Tomentella sp.79

Host : *Betula* 451

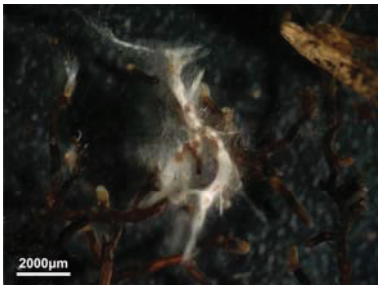
Tricholoma bufonium

Host : *Quercus* 452

Tricholoma flavovirens

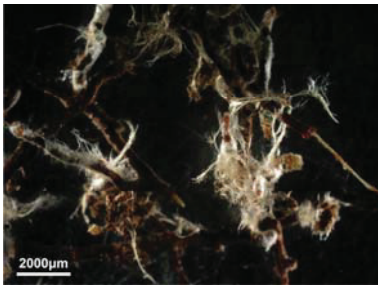
Host : *Abies* 453

Tricholoma sejunctum sp.1



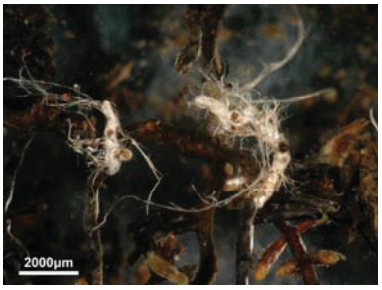
Host : *Tsuga* 454

Tricholoma sejunctum sp.2



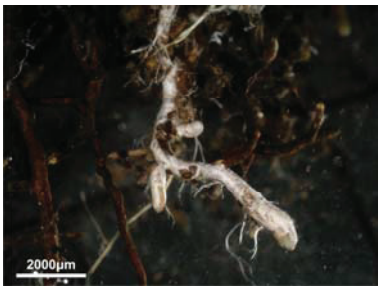
Host : *Tsuga* 455

Tricholoma sp.1



Host : *Tsuga* 456

Tricholoma sp.2



Host : *Abies* 457

Tricholoma sp.3

Host : *Abies* 458

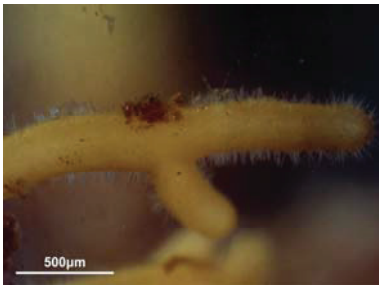
Tricholomataceae sp.1

Host : *Carpinus* 459

Tuber sp.1

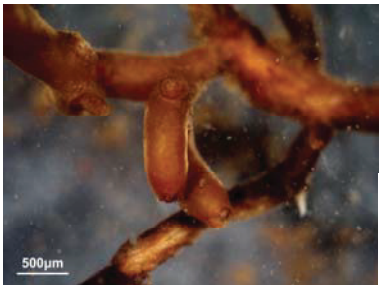
Host : *Carpinus* 460

Tuber sp.2



Host : Fagaceae 461

Tuber sp.3

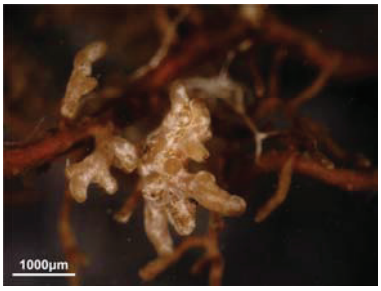


Host : *Abies* 462

Tuber sp.4

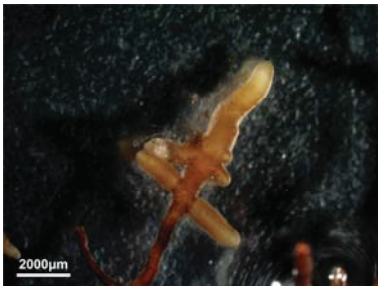
Host : *Fagus* 463

Tylopilus felleus



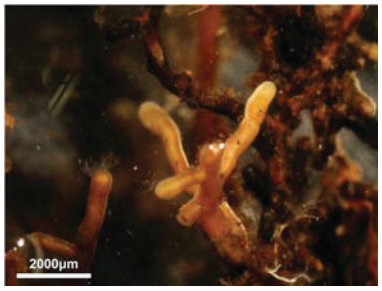
Host : *Fagus* 464

Tylospora fibrillosa sp.1



Host : *Larix* 465

Tylospora fibrillosa sp.2



Host : *Abies* 466

Tylospora sp.1

Host : *Abies* 467

Tylospora sp.2

Host : *Abies* 468

Xerocomus sp.1

Host : *Quercus* 469

Xerocomus sp.2

Host : unknown 470

Xerocomus sp.3

Host : *Fagus* 471

Xerocomus sp.4

Host : *Quercus* 472

Xerocomus sp.5

Host : *Betula* 473