論 文 の 内 容 の 要 旨

論文題目　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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1. Background

Distribution patterns of organisms provide fundamental knowledge on their ecology, which are 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 via the mycelia of EM fungi extending into soil. EM associations occur on many ecologically and economically important tree species covering a large proportion of global forests, including Pinaceae, Fagaceae, and Betulaceae. These trees cannot survive without EM fungi under natural conditions. Thus, EM fungi are essential in forest establishment and functions. More than >20,000 fungal species are estimated to exist globally, resulting in high functional and taxonomic diversity. Clarifying distribution patterns and community structures of EM fungi is prerequisite to evaluate fungus-environment relationships and to predict how fungal communities respond to the global environmental change.

The main objectives of this study are 1) to examine distribution patterns of individual EM fungal species and community structures of EM fungi, and 2) to examine the relative importance of geographic distance, environmental factors, and host trees on EM fungal composition. These questions are addressed at various scales, forest types, and fungal developmental stages (i.e., spore and existing root), which are discussed in each chapter.

Chapter 1 provides the background of this study. Chapter 2 clarifies EM fungal distributions and community structures at a stand scale (~1ha) and examines 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 the 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 roots. Chapter 5 provides a summary of this thesis and overall discussion. A supplementary material includes a comprehensive database of EM fungi, including images of EM root tips, DNA sequences (ITS, LSU), and site and host information for all the EM fungal species found in this study.

2. Materials and Methods

Seven study sites were established in closed-canopy natural forests along elevation gradients on two mountains in Japan. The study sites were characterized by typical vegetation in the Pacific Ocean side of Japan. Field sampling was conducted on the northwest slope of Mt. Fuji, Yamanashi (N35º E138º) in 2011, and on the south slope of Mt. Ishizuchi, Ehime (N33º E133º), in 2012. The highest sampling sites on Mt. Fuji (2250 m) and on Mt. Ishizuchi (1850 m) were located just below the tree lines. Fifty soil cores (5 × 5 cm to 10 cm deep) were collected from a 1-ha area at each site. Litter depth and geographic coordinates were recorded at each sampling point. Tree species and diameter at breast height (1.3 m) were recorded for all trees within a 5-m radius at every other sampling point. Soil pH, total carbon (C) and total nitrogen (N) of each soil sample were analyzed 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 referred to as 'species') at ≥97% similarities. Hosts 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 by bioassay experiments. Conifer (*Pinus densifolia*) and deciduous (*Salix reinii* or *Betula maximowicziana*) host seedlings were grown in 15ml tubes containing soils collected in the field. Fifty bioassay seedlings per host per site were prepared, resulting in 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 in 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 phylogeny.

3. Results and discussion

In total, 454 EM fungal species were identified on existing root tips in 330 soil samples, representing the highest richness reported from a single study using similar identification approaches. The richness ranged from 55 to 113 with an average of 89 species per site. Estimated richness (using Chao 2 non-parametric estimator) was 475 ± 38.3 on Mt. Fuji and 355 ± 40.6 on Mt. Ishizuchi. Most fungal

species belonged to lineages that typically dominate in temperate forests (/russula-lactarius, /thelephora-tomentella, and /cortinarius).

Overlaps of individual EM fungal species between sites mostly occurred at adjacent sites along an elevation gradient (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 550 km distance. Therefore, EM fungal distributions may not be restricted by geographical distance at this spatial scale but constrained by contemporary environmental factors.

EM fungal composition within a stand was differentiated by host identity in conifer-broadleaf mixed forests, and the strength of host effects increased with host phylogenetic diversity of a site (fitted in a Quadratic model: $R^2 = 0.97$, $P < 0.001$). 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 was insignificant in explaining 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 effect is pronounced at the stand scale where climate and soil properties remain constant, but it becomes less prominent at larger scales encompassing wider climate variance, which are more influential in structuring EM fungal communities. 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 independently but synchronously.

Twenty-nine EM fungal species were detected in spore communities from a total of 668 bioassay seedlings. The communities were composed of many pioneer fungal species including *Rhizopogon*, *Laccaria* and *Scleroderma*, which were rarely found in the existing EM roots. Host identity significantly separated spore communities across sites, while site conditions (including both distance and environmental factors) were insignificant. These results suggest that the dormant spores of EM fungi in soil are less affected by contemporary environments, while the germination of these spores is triggered by compatible host roots. Thus, host specificity of EM fungi may be more notable in germination stages than in root colonizing stages.

4. Conclusions

Demonstrating geographical distributions of microorganisms is challenging, and few previous studies have shown species ranges of EM fungi. The relatively intensive sampling (i.e. collecting many samples per site), which has rarely been applied in microbial studies, enabled me to obtain large EM

fungal community data at each site and demonstrate the existence of species ranges of EM fungi for the first time. The importance of climate factors in structuring EM fungal composition implies that global climate change possibly affects the distribution and composition of EM fungi, which play critical roles in forest functions.

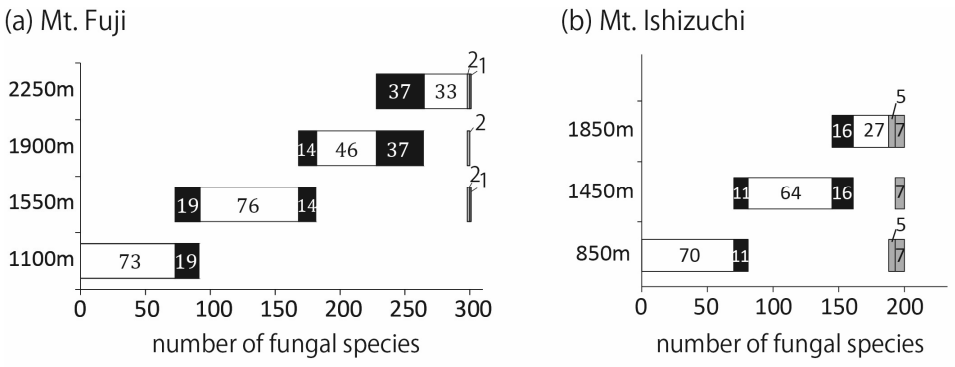

Figure 1 Overlap of EM fungal occurrence along the elevation gradients on (a) Mt. Fuji and (b) Mt. Ishizuchi. Cumulative number of fungal species, from low to high elevation sites is shown. Values in the columns are the numbers of species. Open columns indicate site-specific species and closed columns 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.

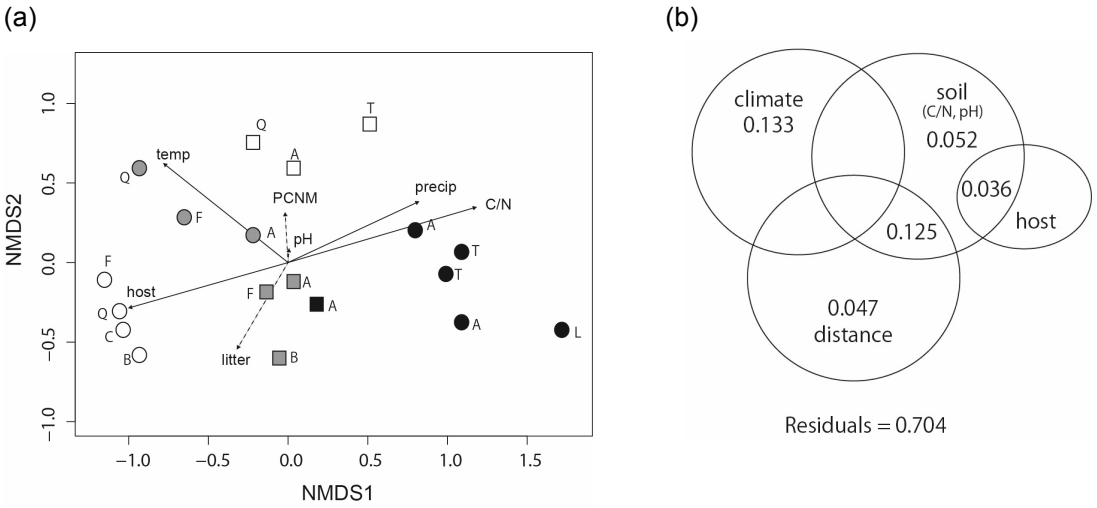

Figure 2 (a) Nonmetric multidimensional scaling (NMDS) graph of 19 ectomycorrhizal fungal communities. Circles and squares indicate sites on Mt. Fuji and Mt. Ishizuchi, respectively. Predictor variables are fitted to the NMDS ordination. The abbreviations are temp (temperature), precip (precipitation), host (host phylogenetic eigenvector), 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.