

Ectomycorrhizal Fungal Communities of Red Pine (*Pinus densiflora*) Seedlings in Disturbed Sites and Undisturbed Old Forest Sites

Eun-Hwa Lee and Ahn-Heum Eom*

Department of Biology Education, Korea National University of Education, Cheongwon 363-791, Korea

Abstract This study aimed to investigate differences in ectomycorrhizal (ECM) fungal communities between disturbed sites and undisturbed old forest sites. ECM root tips of *Pinus densiflora* were collected from 4 sites disturbed by human activities and 3 undisturbed old forest sites adjacent to the disturbed sites. Results in this study showed that the number of ECM root tips, species diversity, and number of species were significantly higher in the disturbed sites than in the undisturbed sites, suggesting that the ECM fungal community structure was affected by the degree of disturbance.

Keywords Community structure, Disturbance, Ectomycorrhizas, *Pinus densiflora*, Species diversity

Ectomycorrhizal (ECM) fungi are ubiquitous organisms that form symbiotic associations with most woody plants in temperate and boreal forests, thereby improving the absorption of nutrients, especially phosphorus, and protecting the host plants from root pathogens and environmental stress [1]. In addition, the mycelia of ECM fungi allow host plants to access carbon and other nutrients [2, 3]. Therefore, the characteristics of the belowground ECM fungal community directly and indirectly affect aboveground plants.

Many researchers have shown that ECM fungi affect plant communities; however, the dynamics of ECM fungal communities have not been clearly established. Several factors are known to influence or alter the ECM community structures. For instance, ECM fungal community structure is related to forest age [4], composition of the host plant [5], and above- or belowground disturbances [6]; however, the factors and processes that influence and maintain fungal

ECM community structures remain poorly understood. Disturbance to forests might affect the structure and maintenance of ECM fungal communities, because of changes to the soil condition and composition of host plants. In turn, such changes could cause the dominant ECM species or species diversity of the forest to change. In disturbed sites, where the environmental conditions have been subject to major alteration, ECM fungi contribute to reforestation or recovery of the forest. These fungi supply nutrients and water to plant seedlings, and help them overcome environmental stresses.

This study aimed to compare the ECM fungal community structure between disturbed sites and undisturbed forests. Further, the colonization rate and number of ECM fungal species were investigated in all sites. An understanding of the ECM community structure of disturbed sites could provide information on the ECM fungal community structure during early succession and on the tolerant species for according clearing of plant or removal of top soil.

MATERIALS AND METHODS

Study site. Four disturbed sites (D1~D4) and 3 undisturbed old forest sites (OF1~OF3) located in the middle and northeastern parts of Korea were selected for this study (Fig. 1). The sites D1~D4 were disturbed only by human activities and not by any natural disaster. Sites D1 and D4 were disturbed by similar activities; site D1 was a mountain trail and D4, a burial ground. Sites D2 and D3 were construction sites that had been abandoned for about 5 years, with only a few grass species and pine seedlings, because most of the woody plants and top soil were removed. Sites OF1, OF2, and OF3 were located near the

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*Corresponding author
E-mail: eomah@knue.ac.kr

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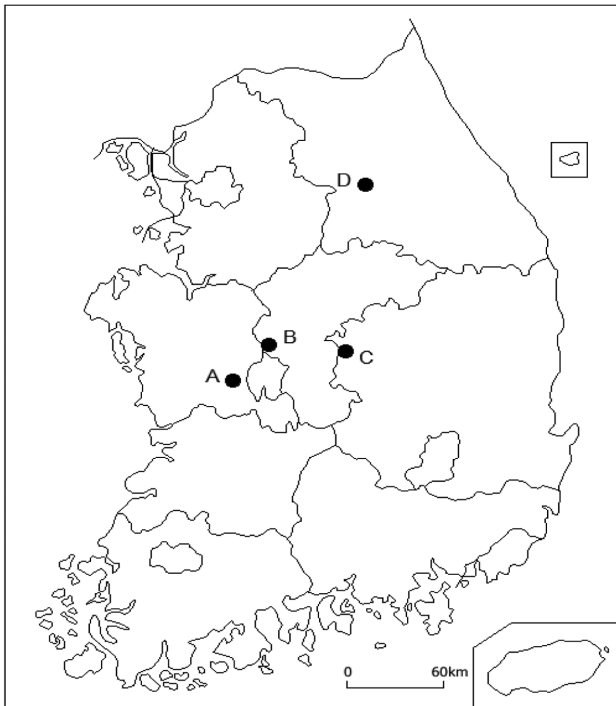


Fig. 1. Sampling sites of ectomycorrhizal fungi in this study. A, 36°21'59.60" N, 127°11'11.37" E, sites D1 and OF1; B, 36°33'39.13" N, 127°25'39.59" E, sites D2 and OF2; C, 36°35'39.61" N, 127°49'31.99" E, sites D3 and OF4; D, 37°30'42.27" N, 128°13'10.78" E, site D4.

sites D1~D3 and represented undisturbed old forests.

Root sampling and morphotyping. Sampling was conducted at both the disturbed sites and undisturbed old forest sites from March 2006 to August 2007. Five root samples of pine seedlings (*Pinus densiflora*) were randomly collected from the study sites, and the ECM root tips were observed under a microscope for morphotyping using color, branching systems, texture, and hyphae and luster characteristics [7]. The representative specimen of each morphotype was selected for DNA sequence analysis, to identify the ECM species at a molecular level.

PCR and sequence analysis. DNA was extracted from the ECM root tip. Then, the partial internal transcribed spacer (ITS) of the rDNA was amplified using ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3'), which represent a fungal specific primer pair [8]. Thermocycling for PCR was conducted as follows: 94°C for 3 min during 1 cycle, 94°C for 30 sec, 50°C for 30 sec, and 72°C for 1 min for 30 cycles, then at 72°C for 5 min for 1 cycle. The amplified sequence was submitted to further PCR sequencing, after which a sequence similarity search was conducted using the BLAST algorithm in National Center for Biotechnology Information (NCBI). The sequences were aligned using MEGA 5 [9] and a neighbor-joining phylogeny tree was

constructed [10] by using *Rhizopus stolonifer* as the outgroup.

Statistical analysis. The species diversity (Shannon-Wiener index [H]), evenness, and number of ECM species were calculated. Statistical analysis was conducted using one-way analysis of variance (ANOVA) with the statistical program SPSS ver. 18 (SPSS Inc., Chicago, IL, USA). The similarity of the ECM community structure was analyzed using principle component analysis.

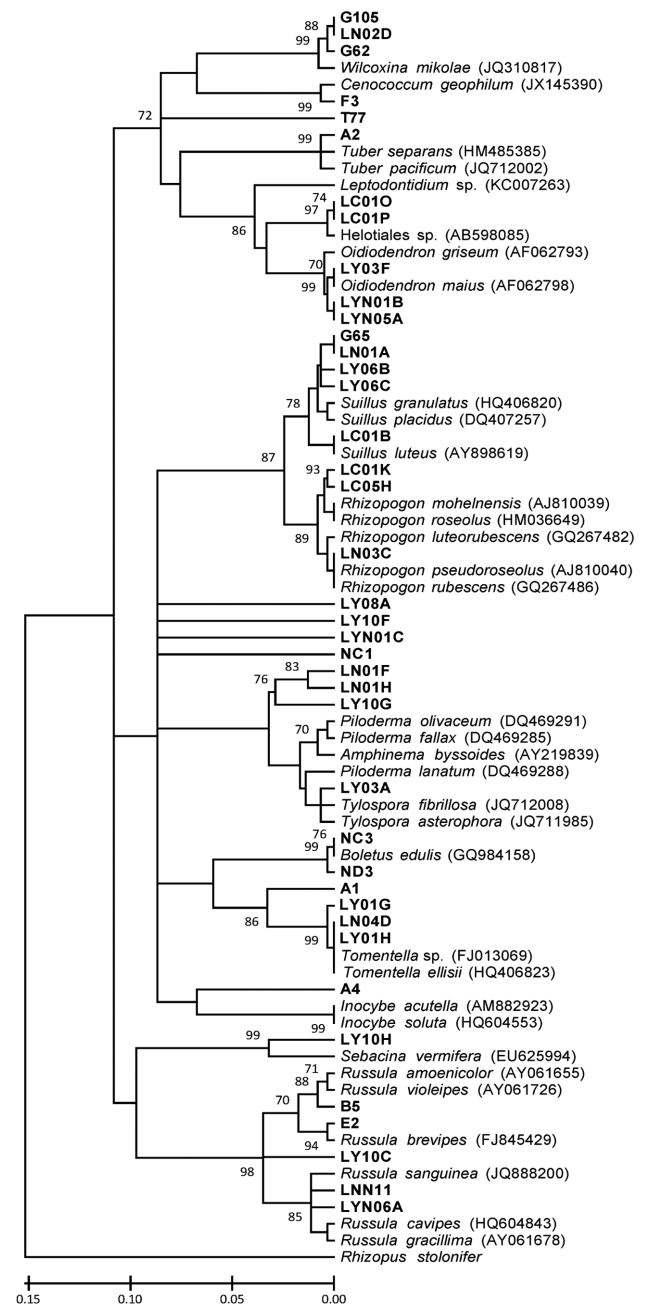


Fig. 2. Neighbor-joining tree to illustrate the taxonomical topology of the ectomycorrhizal fungi from this study (written in bold). *Rhizopus stolonifer* was used as an outgroup.

RESULTS AND DISCUSSION

The distribution of ECM fungal species in the 3 old forest sites and 4 disturbed sites was investigated. In total, 251 ECM root tips were collected from all the study sites and classified into 40 morphotypes based on their characteristics [7]. DNA sequence analysis was conducted using the fungal specific primers ITS1F and ITS4 for each morphotype. As a result, 7 species of ascomycetes and 24 species of basidiomycetes were identified. Most ascomycetes identified in this study belonged to the genera *Oidiodendron* and *Wilcoxina*, while most basidiomycetes belonged to the genera *Russula*, *Tomentella*, *Suillus*, and *Rhizopogon* (Table 1, Fig. 2).

In the old forest sites, 16 ECM fungi of basidiomycetes and 2 of ascomycetes were identified, with the most frequent species being *Suillus* (Basidiomycetes). Moreover, the 3 old forest sites had an average of 7.6 identified ECM fungal species, whereas the 4 disturbed sites had an average of 12.5 identified ECM fungal species. Overall, the disturbed sites contained more ECM species than those in the old

forest sites, with 22 species of basidiomycetes and 7 species of ascomycetes. The most frequent species were species belonging *Oidiodendron*, *Suillus*, *Tomentella*, *Rhizopogon*, and *Russula*.

From the results, disturbance appeared to influence the species composition of the ECM fungal community. Most ECM fungi were found in the disturbed sites, with about 74% of the species belonging to basidiomycetes. The fungi belonging to the genera *Suillus*, *Tomentella*, *Rhizopogon*, and *Russula* were abundant in D1~D4 and they are known as early- or multi-stage species in ECM fungal succession [4, 6, 11]. Although the sites D1~D4 were disturbed, the ECM fungal community was at the primary stage of succession, mostly because of the time for which these sites were left undisturbed after the initial disturbance. Therefore, the responses to disturbance seemed to have been initiated early in the belowground fungal community and were then followed on by the aboveground plant community. The undisturbed old forests exhibited a fungal community structure different from that in the disturbed sites. The forests contained a clearly dominant fungal group, belonging

Table 1. Relative abundance of ectomycorrhizal fungal species colonizing roots of pine seedlings in disturbed sites and old forests

ECM fungi species	ECM morphotypes	Relative abundance of ECM fungi (%)						
		D1	D2	D3	D4	OF1	OF2	OF3
<i>Ascomycetes</i> sp.	T77	-	2.4	-	-	-	-	-
<i>Cenococcum geophilum</i>	F3	2.4	-	5.4	-	-	-	-
<i>Helotiales</i> sp.	LC01O, LC01P	2.4	-	3.6	-	-	-	-
<i>Oideodendron</i> sp.1	LY03F	17.1	-	5.4	-	-	-	-
<i>Oideodendron</i> sp.2	LYN01B, LYN05A	-	2.4	25.0	2.5	-	-	2.6
<i>Tuber</i> sp.	A2	12.2	-	-	-	-	-	-
<i>Wilcoxina mikolae</i>	LYN02D, G62, G105	-	2.4	-	15.0	6.7	-	7.9
<i>Agaricomycetes</i> sp.	A1	-	-	-	-	13.3	-	-
<i>Atheliales</i> sp.1	NC1	-	-	-	-	-	15.0	-
<i>Atheliales</i> sp.2	LN01H, LN01F	-	-	-	17.5	-	-	-
<i>Atheliales</i> sp.3	LY10G	2.4	-	5.4	-	-	-	15.8
<i>Boletus edulis</i>	NC3	-	-	1.8	-	13.3	5.0	-
<i>Boletus</i> sp.	ND3	19.5	2.4	-	-	-	-	-
<i>Inocybe</i> sp.	A4	-	7.3	-	-	6.7	-	-
<i>Rhizopogon</i> sp.1	LC05H, LC01K	-	-	1.8	15.0	-	-	7.9
<i>Rhizopogon</i> sp.2	LN03C	-	-	-	2.5	-	-	2.6
<i>Rhizopogon</i> sp.3	LY10F	9.8	-	-	-	6.7	-	-
<i>Rhizopogon</i> sp.4	LYN01C	-	2.4	12.5	-	-	-	-
<i>Russula bravipes</i>	E2	-	26.8	-	-	-	-	-
<i>Russula</i> sp.1	B5	-	9.8	5.4	-	-	-	5.3
<i>Russula</i> sp.2	LY10C	-	-	1.8	-	-	-	-
<i>Russula</i> sp.3	LNN11	-	12.2	-	-	-	-	-
<i>Russula</i> sp.4	LYN06A	-	7.3	-	2.5	-	15.0	-
<i>Sebacina vermifera</i>	LY01H	-	7.3	-	-	-	-	-
<i>Suillus luteus</i>	LC01B	-	-	1.8	7.5	-	-	23.7
<i>Suillus</i> sp.1	LN01A, G65	-	-	7.1	2.5	-	-	5.3
<i>Suillus</i> sp.2	LY06B, LY06C	2.4	2.4	7.1	10.0	-	10.0	26.3
<i>Suillus</i> sp.3	LY08A	-	-	1.8	-	33.3	40.0	-
<i>Tomentella</i> sp.1	LY01G	-	-	-	5.0	-	-	-
<i>Tomentella</i> sp.2	LN04D, LY01H	29.3	14.6	-	17.5	20.0	15.0	-
<i>Tylospora</i> sp.	LY03A	2.4	-	14.3	2.5	-	-	2.6

Table 2. Characteristics of ectomycorrhizal (ECM) fungal communities of disturbed sites and undisturbed old forests in this study

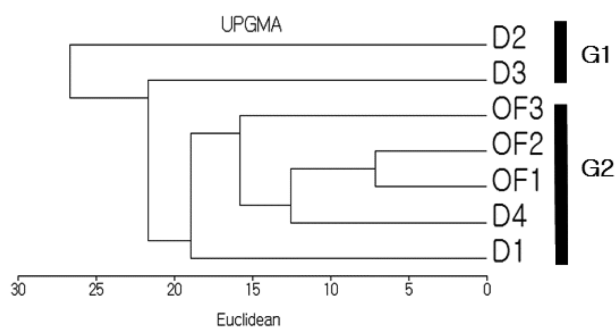
	Disturbed sites	Old forests	<i>p</i> -value
Shannon index (H)	2.18 ± 0.05	1.78 ± 0.06	< 0.05
Species evenness	0.87 ± 0.01	0.89 ± 0.01	0.328
No. of species	12.50 ± 0.52	7.67 ± 0.69	< 0.05
No. of ECM root tips	44.50 ± 1.92	24.33 ± 4.03	< 0.05

to the genus *Suillus* (49%), with only 2 species (7%) of ascomycetes being identified.

The number of ECM root tips, species diversity, evenness, and species number were estimated to determine the effects of disturbance on the ECM fungal community. The ECM root tip numbers, species diversity, and species number were significantly higher in the disturbed sites than in the old forest sites; however, no significant difference was observed in the evenness (Table 2).

Disturbed sites of this study showed higher species diversity, more abundant species, and more ECM root tips than those in the 3 undisturbed old forest sites. Previous studies have shown that the species diversity and community structure of ECM fungi are altered by disturbance [12-14]; however, the effect of disturbance may vary with the extent and type of disturbance. For instance, severe disturbances might reduce the number of ECM fungal species and biodiversity, whereas mild disturbances generate the opposite effect on the community, promoting the appearance of new or different species that are better adapted to surviving under altered environment conditions [15]. Therefore, ECM fungal species diversity might increase in mildly disturbed sites such as D1~D4.

Cluster analysis of the fungal communities showed 2 different groups, G1 and G2 (Fig. 3). ECM fungal communities of the old undisturbed forest sites in G2 were different from those of the abandoned construction sites in G1, although disturbed sites D1 and D4 were included in G2 with undisturbed sites. From this, the ECM fungal community structure was considered to be influenced by

**Fig. 3.** The cluster analysis of ectomycorrhizal (ECM) fungal communities of study sites. G1 and G2 are the group including similar composition of ECM fungal species by cluster analysis. D1~D4, disturbed sites; OF1~OF3, undisturbed old forests.

the degree of disturbance and proximity to the other forests. The degree of disturbance in G1 was the same, because both the sites were abandoned construction sites. On the other hand, D1, D4, and the undisturbed old forest sites were clustered as G2. D1 was a mountain trail and D4 was a burial area on a mountain. The species composition of ECM fungi at D1, D4, and the undisturbed old forest sites was similar because D1 and D4 were areas that were located near the forest sites and were only mildly disturbed when compared to D2 and D3.

The results of this study showed that the ECM fungal communities of disturbed sites differ from those of the undisturbed old forest sites, with some species being more abundant in the disturbed sites. The cause and extent of disturbance might have affected the biodiversity and composition of the ECM fungal community. The ECM fungal species identified at the disturbed sites were regarded as the “early-successional species” and were considered tolerant to sterile conditions. Therefore, these fungi in disturbed sites could potentially be used to revegetate or reforest sites subjected to harsh conditions or disturbed sites and it may provide useful information to guide future revegetation efforts in these sites.

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