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FULL PAPER

Diversity and community structure of ectomycorrhizal fungi in *Pinus thunbergii* coastal forests in the eastern region of Korea

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Abstract We investigated the diversity and community structure of ectomycorrhizal (EcM) fungi in *Pinus thunbergii* stands on the eastern coast of Korea. We established two 10 × 10-m plots in six forest stands and sampled soil blocks containing rootlets of mature *P. thunbergii* trees. EcM roots were classified into morphological groups, and the fungal taxa associated with each morphotype were identified by sequencing the nuclear rDNA internal transcribed spacer region. *Cenococcum geophilum* and the Atheliales, Clavulinaceae, Russulaceae and Thelephoraceae species were the main members of the EcM fungal community, which included a total of 68 observed fungal taxa. As a whole, the community consisted of a few dominant fungal taxa, such as *C. geophilum* (28.6% relative abundance), and a large number of rare fungal taxa that showed low abundances and local distributions. Colonization patterns at the local site scale and at the scale of the study plots greatly differed among the EcM fungal taxa; *C. geophilum* was distributed extensively and was dominant in several study sites, whereas a certain *Lactarius* sp. was distributed locally but dominated in a given study site. We conclude with a discussion of the relationship between colonization patterns of EcM fungi and soil and environmental conditions.

Keywords Atheliales · *Cenococcum* · Clavulinaceae · Distribution · Thelephoraceae

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Introduction

Coastal forests perform functions important for humans, such as alleviating damage from salt and sand dispersion to inland areas and providing places for recreation (Konta 2001). *Pinus thunbergii* Parl., which grows naturally in China, Korea and Japan, is one of the major constituents of the coastal forests established on sand dunes and ocean-facing mountain slopes in Korea. This tree's prevalence is possibly due to its excellent tolerance of high salinity, drought stress and wind. In recent years, however, coastal pine forests have declined because of harmful pests such as the pine wood nematode *Bursaphelenchus xylophilus* (Steiner and Buhrer) Nickle (Yi et al. 1989) and also because of recent severe fire disasters in eastern Korean coastal areas (Son et al. 2006). Several studies and projects, including afforestation projects, have begun to recover and properly maintain coastal pine forests in Korea (Chun et al. 2008).

Ectomycorrhizal (EcM) associations are common in pine forests and play a significant role in plant establishment by promoting nutrient and water uptake in host plants and enhancing host tolerance of stressful situations (Smith and Read 1997). Consequently, EcM fungi are considered to be one of the most effective biological resources and have been put to practical use for the reforestation of degraded areas (Quoreshi 2008). The effect of EcM associations on host plant growth differs among different species of EcM fungi (van der Heijden and Kuyper 2003), possibly because of differences in growth and physiological characteristics, such as enzymatic activity (Courty et al. 2005). Therefore, diversity and community structure of EcM fungi are hypothesized to be important for the establishment of coastal pine forests.

A few studies have been conducted, mostly in Japan, describing the EcM fungal communities in *P. thunbergii*

coastal pine forests. Using molecular tools, several studies have revealed belowground EcM fungal communities in naturally regenerating *P. thunbergii* seedlings (Taniguchi et al. 2007; Matsuda et al. 2009b) and mature trees (Obase et al. 2009) of coastal pine forests. Matsuda et al. (2009a) have described the abundance and distribution of the dominant EcM fungus *Cenococcum geophilum* Fr. in soils of four coastal stands of *P. thunbergii* in Japan. All of these studies were conducted on forest stands established on maritime sand dunes. However, several coastal pine forests on ocean-facing mountain slopes are rooted in forest soil. It has been shown that EcM fungal communities can differ between host plants in different habitats (Iwański and Rudawska 2007). Therefore, it is possible that EcM fungal communities differ among forest stands established on different soil conditions, e.g., maritime sands and forest soils.

In this study, we investigated the diversity and community structure of EcM fungi in six stands of *P. thunbergii* coastal forests established on maritime sand dunes or on ocean-facing slopes on the eastern coast of Korea. We established two 10 × 10-m study plots in each forest stand and sampled soil blocks containing rootlets of mature *P. thunbergii* trees. EcM roots were classified by morphology, and the fungal taxa associated with each morphotype were identified by sequencing the nuclear ribosomal DNA internal transcribed spacer region. As previously reported, we expected *C. geophilum* to be the most prevalent and dominant species in the coastal pine forests and that the species composition of EcM fungi would differ among stands, especially between stands established on different soil conditions.

Materials and methods

Study site

We established six study sites in the following *P. thunbergii* coastal forest sites on the eastern coast of Korea in the province of Kangwon-do: Sokcho, Yangyang, Gangneung, Samcheok (2 locations) and Uljin (Fig. 1). All of the coastal pine forests are located 60–250 m inland from the shoreline and measure from a few hundred to several thousand meters in width. The distances between adjacent study sites ranged from 15 to 40 km. The mature forests consisted primarily of *P. thunbergii* that were estimated to be approximately 40–50 years old. Several woody and herbaceous plants, such as *Artemisia* spp., *Commelina communis* L., *Festuca ovina* L., *Quercus dentata* Thunb., *Rosa rugosa* Thunb. and *Robinia pseudoacacia* L., were sparsely distributed in the understory. At the Sokcho, Yangyang, Gangneung and Samcheok 1 sites, the soils consisted entirely of maritime sand, which was classified as Regosols (IUSS Working Group WRB 2007), and organic horizons measuring 1–6 cm in thickness (Table 1). At the Samcheok 2 and Uljin sites, the soils consisted of forest soil, which was classified as Podzols (IUSS Working Group WRB 2007), and organic horizons measuring 5–10 cm in thickness. Gangneung, which was located roughly in the center of the study sites, had an annual precipitation of 1,401 mm and an annual average temperature of 12.9°C (the Korean Meteorological Administration).

In each study site, we established two 10 × 10-m square plots in areas where other EcM hosts besides *P. thunbergii* were absent and general environmental factors, such as vegetation and soil conditions, appeared to be similar. The two plots in each study site were separated by a distance of approximately 20–1,000 m. The density of mature trees ranged from 10.5 to 22.0 (/100 m^2) in the plots. Three soil cores (5 cm in diameter and depth) were collected at the soil surface in each plot, and soil pH (H_2O) and water content (w/w) were determined. Soil pH ranged from 5.0 to 6.0 in each plot (Table 1). Soil water content was low in Sokcho, Gangneung and Samcheok 1 (3.4–4.8%), intermediate in Yangyang (8.4%) and relatively high in Samcheok 2 and Uljin (12.8–14.0%).

Sampling methods and observation of EcM colonization

In September and October 2009, we sampled 16 soil blocks (5 × 5 × 20 cm length, width and depth, respectively) at regular intervals of 3 m in each study plot. We recognized that most of the EcM fungi in the soil blocks were associated with mature trees. All samples were stored in plastic bags at 4°C until further analysis.

Roots were separated from the adhering soil by careful washing in tap water over a 0.5-mm sieve. All root segments were viewed under a dissecting microscope after

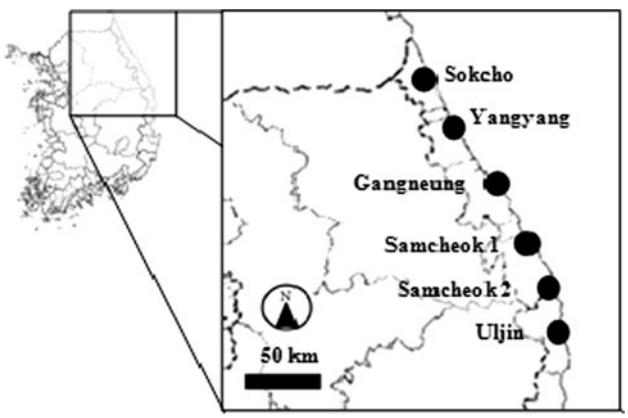

Fig. 1 Locations of the six study sites on the eastern coast of Korea

Table 1 Soil properties such as soil type, texture, pH, water content and depth of humus layer in each study site

Study sites	Sokcho	Yangyang	Gangneung	Samcheok 1	Samcheok 2	Uljin
Soil type[a]	Regosols	Regosols	Regosols	Regosols	Podzols	Podzols
Soil texture	Sandy	Sandy	Sandy	Sandy	Loamy	Sandy/loamy
pH (H_2O)[b]	5.4 ± 0.7	5.0 ± 0.5	5.0 ± 0.2	6.0 ± 0.4	5.0 ± 0.4	5.3 ± 0.4
Soil water content (%)[b]	4.8 ± 1.0	8.2 ± 2.2	4.2 ± 3.2	3.4 ± 2.4	14.0 ± 3.6	12.8 ± 4.1
Depth of humus layer (cm)	1–2	3–5	2–4	2–6	5–10	5–6

[a] Soils were classified according to IUSS Working Group WRB (2007)

[b] Averages and standard deviations were indicated

being cut into 5–10-cm sections. Vital EcM roots per soil block were classified into distinguishable groups by gross morphology. One to five root tips per group and soil block were then selected, and their microscopic characteristics, such as hyphal arrangement on the surface of mantle and emanating hypha, were observed (Ingleby et al. 1990) to further characterize the EcM root tips. EcM roots were classified into morphotypes that possessed distinguishable gross and/or microscopic morphologies. One EcM root tip of each morphotype was placed into 1.5-ml microtubes at −20°C for DNA extraction. The number of replicates ranged from 1 to 9 per morphotype, according to the abundance and/or frequency of each.

Identification of EcM fungi

EcM fungal DNA was extracted from one EcM root tip of each morphotype using the DNeasy Plant Mini kit (QIAGEN, USA) according to the manufacturer's instructions. DNA amplification of ITS regions, including the 5.8S rDNA region, was performed using Takara Ex Taq (TAKARA, Japan) with a specific primer for higher fungi ITS1F (Gardes and Bruns 1993) and basidiomycete ITS4B (Gardes and Bruns 1993). In case the above primer pair failed to amplify the DNA, a primer set for ITS1F and the universal primer ITS4 (White et al. 1990) were used. We used the following polymerase chain reaction (PCR) amplification conditions: 94°C for 3 min, 30 cycles of 94°C for 30 s, 50°C for 30 s and 72°C for 2 min, with a final hold at 72°C for 10 min. Purification of PCR products and sequencing reactions were entrusted to Macrogen Inc. (Seoul, Korea). Sequencing reactions were conducted using the ABI 3730xl Analyzer (Applied Biosystems) with ITS1 and ITS4 primers. EcM root DNA sequences were submitted to the National Center for Biotechnology Information (NCBI: http://blast.ncbi.nlm.nih.gov) and compared with the GenBank database using the nucleotide-nucleotide basic local alignment search tool algorithm (BLAST program). Only library sequences that were derived from sporocarps and assigned names at the species or genus level were used for the comparison. The only exceptions to this criterion were identifications of *C. geophilum* and Ceratobasidiaceae, which were based on sequences from sclerotia or isolates from roots. Identifications of EcM fungal taxa followed the criteria described below. The EcM root sequences that matched library sequences with the greatest similarity (≥98%) to the full lengths of the ITS1 and ITS2 regions were designated by their species names. If the aligned sequences lacked a part or the full length of the ITS1 or ITS2 regions, genus or family names were assigned, respectively. If the highest sequence similarities were less than 98%, EcM root sequences and library sequences were submitted together to neighbor-joining analysis. EcM root sequences with affinities to only one genus were designated by the genus name. EcM root sequences with affinities to several genera within a family were designated by the family name. Each designation was followed by the numbers.

Estimation of mycorrhizal colonization

Soil blocks that contained <100 EcM root tips were excluded from all analyses. The EcM abundance, as indicated by the number of EcM root tips, of each morphotype was recorded separately for each soil block. The relative abundance was calculated in each study site as a ratio of abundance of a given morphotype to total EcM abundance and then averaged over all the study sites. The colonization frequencies of EcM fungal taxa, indicated by the ratio of the number of soil blocks that contained a given EcM morphotype to the total number of soil blocks, were also recorded in all the study sites.

Statistical analysis

To estimate whether sampling efforts were sufficient to describe EcM fungal flora, species accumulation curves were drawn by plotting the mean of the accumulated number of expected species in pooled samples after 1,000 randomizations without replacement using EstimateS program version 8.0 (Colwell 2005). The study site was selected as a sampling unit. In addition, the estimated

measurements of EcM fungal species richness were calculated using Chao 2 and Jackknife 2 estimators.

Principal component analysis (PCA) and redundancy analysis (RDA) were carried out to reveal trends within the EcM fungal community and the potential effects of measured environmental variables (soil type, pH, water content and depth of humus layer) on EcM fungal community structure, respectively. Relative abundances of EcM fungal taxa in each study site were standardized by arcsine square root transformation. Soil types were transformed to dummy variables (0 for Regosols and 1 for Podzols). Forty-two rare taxa that occurred at only one study site and showed <1.0% relative abundance and <10.0% colonization frequency were excluded from the analysis. Within the RDA, permutation tests ($n = 999$) were performed to test the significance of the relationship between community data and the environmental variables. The PCA and RDA were conducted within the Vegan package (Oksanen et al. 2008) in the statistical program R version 2.11.1 (R Development Core Team 2010).

To calculate the correlations between community similarities and spatial distances among study sites, a Mantel test was conducted within the Ecodist package (Goslee and Urban 2007) in the statistical program R version 2.11.1 (R Development Core Team 2010) with 10,000 permutation tests. Horn-Morisita distances based on the abundance of each EcM morphotype in each study site and Euclidian distances based on geographic coordinates of each study site were used in the analysis.

Results

Identification of EcM fungi

Because all sequences obtained from a given EcM morphotype were categorized into the same taxa, we judged that EcM root tips with identical morphologies might be formed by identical EcM fungal taxa. The ITS regions of all EcM roots were successfully amplified; however, we could not obtain complete sequences from 36% of the samples. Consequently, we identified a total of 68 EcM fungal taxa from 121 sequenced root tips (Table 2). Of these, we identified 11 and 30 taxa at the species and genus levels, respectively. The remaining 26 taxa were categorized at the family, order or phylum level. We were unable to obtain a clear sequence from the one remaining EcM morphotype, which was referred to as unidentified EcM fungi.

Diversity of EcM fungi

All soil samples contained EcM roots of *P. thunbergii*. The total number of EcM root tips was 120,762. The number of EcM root tips per soil block ranged from 12 to 3,767 and averaged 629. Six soil cores that contained <100 EcM root tips were excluded from further analysis. EcM fungal species richness per soil block ranged from 1 to 9 species with an average of 4.3. Between 15 and 22 species were observed at each study site. The species accumulation curve was ascending when all study sites were randomly sampled (not shown). The estimated species richness values using Chao 2 and Jackknife 2 estimators were 164.8 and 128.7, respectively.

Taxa belonging to Thelephorales (e.g., *Tomentella*) were the most species rich (21 taxa), followed by Agaricales (11 taxa), Russulales (7 taxa) and Boletales (6 taxa) (Table 2).

Colonization pattern of each EcM taxon

Only one fungal taxon, *C. geophilum*, showed a high relative abundance (28.6%), while the others were found at relatively low abundances (Table 2). Fourteen taxa ranged from 1.0 to 8.8% in prevalence, and the remaining 53 taxa comprised <1.0% of the community. In addition, *C. geophilum* showed the highest colonization frequency (79.0%), while the others showed intermediate to low colonization frequencies; 8 taxa ranged from 10.8 to 33.9%, and the remaining 59 taxa made up <10.0%. Seven taxa were observed at multiple study sites. Two taxa (*C. geophilum* and Atheliales 1) were found in six sites, three taxa (Clavulinaceae 1, *Russula* sp. and Thelephoraceae 2) at five sites and two taxa (Atheliales 2 and *Lactarius hatsudake* Tanaka) in four sites. Forty-four taxa were observed only in one study site.

Among the 14 taxa aside from *C. geophilum* that had more than 1.0% relative abundance, Atheliales 1 showed the highest colonization frequency (33.9%), followed by *Russula* sp. (25.8%) and Clavulinaceae 1 (25.3%); these taxa were observed in 6, 5 and 5 study sites, respectively (Table 2). Clavulinaceae 2, Thelephoraceae 3 and *Lactarius* sp. were observed in two, two and one study sites, respectively, and they showed relatively low colonization frequencies of 8.6, 8.1 and 12.9%, respectively. However, these taxa showed high relative abundance and colonization frequencies in one study site (Fig. 2). Fifty-three rare taxa showing <1.0% relative abundance also had low colonization frequencies, ranging from 0.5 to 10.8%. Of the rare taxa, six [Sistotrema sp., *Pseudotomentella tristis* (P. Karst.) M. J. Larsen, *Suillus granulatus* (L.) Roussel, *Tomentella* sp. 1, *Tricholoma flavovirens* (Pers.) Lundell and *Tuber* sp.] were observed in three study sites, and the others were observed in two (5 taxa) or one study sites (42 taxa).

The Mantel test found no significant correlation (Mantel $r = -0.03$; $P = 0.51$) between community similarities and spatial distances among study sites.

Table 2 Possible identities of EcM fungal taxa based on comparisons of obtained sequences with references in Genebank database at NCBI, using BLAST program

Possible identity	Obtained sequence		BLAST match with high similarity			EcM colonization		
	Accession no.	Length (bp)	Definition	Accession No.	Similarity (%)	RA (%)	CF (%)	Stand (/6)
Amanita spissa	AB587730	661	*Amanita spissa*	AJ889924	658/661 (99)	0.7	2.2	1
Atheliales 1	AB587731	560	*Amphinema byssoides*	AY219839	494/585 (85)	4.6	33.9	6
Atheliales 2	AB587732	569	*Amphinema byssoides*	AY219839	378/439 (87)	3.4	17.2	4
Atheliales 3	AB587733	576	*Amphinema byssoides*	AY838271	501/597 (84)	3.7	22.6	3
Basidiomycetes 1	AB587734	632	*Tylospora asterophora*	AF052558	239/256 (94)	0.2	5.9	2
Basidiomycetes 2	AB587735	599	*Alloclavaria purpurea*	AY228345	470/630 (75)	<0.1	0.5	1
Boletus sp.	AB587736	581	*Boletus hiratsukae*	EU231960	581/581 (100)	0.7	4.3	1
Cenococcum geophilum	AB587740	451	*Cenococcum geophilum*	AF495462	446/451 (99)	28.6	79.0	6
Ceratobasidiaceae 1	AB587741	643	*Ceratobasidium* sp.	GQ175300	590/650 (91)	0.7	5.4	1
Ceratobasidiaceae 2	AB587742	687	Vouchered mycorrhizae	AB303056	601/605 (99)	0.3	1.6	1
Clavulinaceae 1	AB587737	612	*Clavulina* sp.	FN669173	576/638 (91)	7.8	25.3	5
Clavulinaceae 2	AB587738	597	*Clavulina* sp.	FN669173	486/555 (88)	5.3	8.6	2
Cortinarius sp. 1	AB587743	588	*Cortinarius californicus*	FJ039588	585/588 (99)	0.4	3.2	1
Cortinarius sp. 2	AB587744	623	*Cortinarius* aff. *pauperculus*	GQ159858	594/623 (96)	0.3	2.2	1
Cortinarius sp. 3	AB587745	651	*Cortinarius* aff. *pauperculus*	GQ159858	627/648 (97)	0.1	2.2	1
Entolomataceae	AB587746	923	*Entoloma sinuatum*	GQ397994	775/894 (87)	0.4	3.2	1
Hymenochaetaceae	AB587747	936	*Coltricia* cf. *oblectans*	AM412246	413/416 (99)	<0.1	1.6	1
Inocybe sp. 1	AB587748	660	*Inocybe* sp.	GQ892990	587/618 (95)	0.9	9.1	2
Inocybe sp. 2	AB587749	638	*Inocybe flocculosa* var. *flocculosa*	HQ604084	625/653 (96)	0.2	1.6	1
Inocybe sp. 3	AB587750	689	*Inocybe arthrocystis*	AM882856	632/664 (96)	<0.1	0.5	1
Laccaria sp.	AB587751	542	*Laccaria amethystina*	AB211270	534/543 (98)	<0.1	1.1	1
Lactarius akahatsu	AB587752	704	*Lactarius akahatsu*	AB301609	704/704 (100)	1.1	3.8	2
Lactarius hatsudake	AB587753	677	*Lactarius hatsudake*	AB301611	677/677 (100)	2.7	9.1	4
Lactarius sp.	AB587754	807	*Lactarius alnicola*	DQ099898	750/809 (93)	8.8	12.9	1
Otidea sp.	AB587756	580	*Otidea bufonia*	EU784387	563/583 (97)	0.5	1.6	1
Peziza sp.	AB587757	578	*Peziza* sp.	FN669234	565/579 (98)	0.1	2.7	1
Pezizales	AB587758	910	*Helvella elastica*	AF335455	435/505 (87)	0.1	1.6	1
Pseudotomentella tristis	AB587759	708	*Pseudotomentella tristis*	GQ267480	703/711 (99)	0.3	4.8	3
Psudotomentella sp.	AB587761	706	*Pseudotomentella tristis*	AJ889968	523/556 (95)	1.3	5.4	1
Pyronemataceae	AB587763	560	*Trichophaea* cf. *hybrida*	DQ200834	509/560 (91)	0.1	2.2	1
Rhizopogon roseolus	AB587764	683	*Rhizopogon roseolus*	HM036649	678/682 (99)	0.4	1.1	1
Rhizopogonaceae	AB587765	819	*Rhizopogon succosus*	AF062933	505/538 (94)	0.3	3.8	1
Russula nauseosa	AB587768	637	*Russula* cf. *nauseosa*	GU371293	629/637 (99)	0.1	0.5	1
Russula sp.	AB587766	640	*Russula* cf. *fuscorubroides*	HQ604842	613/645 (96)	6.2	25.8	5
Russulaceae 1	AB587755	640	*Lactarius* cf. *piperatus*	AB459515	569/667 (86)	0.3	1.6	1
Russulaceae 2	AB587767	646	*Russula mariae*	EU819426	593/653 (91)	<0.1	1.1	1
Sebacina sp. 1	AB587769	582	*Sebacina incrustans*	EU819442	550/584 (95)	2.7	9.7	2
Sebacina sp. 2	AB587770	579	*Sebacina* sp.	DQ974768	557/579 (97)	0.1	1.1	2
Sebacinaceae 1	AB587771	638	*Sebacina* sp.	DQ974768	516/620 (84)	0.1	1.1	2
Sebacinaceae 2	AB587772	511	*Sebacina* sp.	FN669252	453/511 (89)	<0.1	0.5	1
Sistotrema sp.	AB587739	581	*Sistotrema* sp.	FN669255	548/588 (94)	0.6	7.0	3
Suillus granulatus	AB587774	653	*Suillus granulatus*	AY898617	643/656 (99)	0.3	7.0	3
Suillus sp. 1	AB587773	510	*Suillus bovinus*	AB284446	509/519 (99)	0.9	7.0	1
Suillus sp. 2	AB587775	409	*Suillus luteus*	AB284448	409/409 (100)	1.1	3.2	2
Thelephora terrestris	AB587776	625	*Thelephora terrestris*	EU427330	619/625 (99)	<0.1	1.6	1
Thelephoraceae 1	AB587762	631	*Pseudotomentella* sp.	GQ267479	566/636 (89)	<0.1	1.6	1

Table 2 continued

Possible identity	Obtained sequence		BLAST match with high similarity			EcM colonization		
	Accession no.	Length (bp)	Definition	Accession No.	Similarity (%)	RA (%)	CF (%)	Stand (/6)
Thelephoraceae 2	AB587777	632	*Thelephora penicillata*	U83484	592/632 (94)	3.8	14.0	5
Thelephoraceae 3	AB587778	620	*Tomentella ferruginea*	EU819497	564/634 (89)	3.2	8.1	2
Thelephoraceae 4	AB587781	628	*Tomentella* cf. *sublilacina*	AJ889982	574/630 (92)	0.3	1.6	1
Thelephoraceae 5	AB587789	625	*Tomentella* sp.	EF644116	568/635 (90)	0.3	1.1	1
Thelephoraceae 6	AB587790	627	*Tomentella cinerascens*	U83483	547/633 (87)	<0.1	0.5	1
Thelephoraceae 7	AB587791	633	*Tomentella* sp.	DQ974780	571/634 (91)	<0.1	0.5	1
Thelephorales	AB587760	374	*Pseudotomentella tristis*	AJ889979	306/375 (81)	0.2	0.5	1
Tomentella badia	AB587779	636	*Tomentella badia*	AF272917	574/588 (98)	0.8	6.5	2
Tomentella sp. 1	AB587780	634	*Tomentella* sp.	DQ822830	599/636 (95)	0.6	9.7	3
Tomentella sp. 2	AB587782	419	*Tomentella* cf. *coerulea*	AY010274	404/416 (98)	0.2	3.2	1
Tomentella sp. 3	AB587783	628	*Tomentella ellisii*	DQ974775	583/627 (93)	0.1	0.5	1
Tomentella sp. 4	AB587784	628	*Tomentella fuscocinerea*	GU214810	581/627 (93)	<0.1	0.5	1
Tomentella sp. 5	AB587785	626	*Tomentella* sp.	FM955848	575/618 (94)	<0.1	0.5	1
Tomentella sp. 6	AB587786	629	*Tomentella* sp.	EF655702	581/625 (93)	0.8	5.4	1
Tomentella sp. 7	AB587787	628	*Tomentella* sp.	DQ822830	594/628 (95)	0.6	5.9	1
Tomentella sp. 8	AB587788	629	*Tomentella ramosissima*	U83480	607/629 (97)	0.5	4.3	1
Tomentellopsis sp.	AB587792	652	*Tomentellopsis submollis*	AJ410774	574/606 (95)	<0.1	1.1	1
Tremellodendron sp.	AB587793	591	*Tremellodendron pallidum*	GQ166897	570/590 (97)	0.4	0.5	1
Tricholoma flavovirens	AB587794	666	*Tricholoma flavovirens*	AF377181	652/667 (98)	0.3	4.3	3
Tricholoma sp.	AB587795	666	*Tricholoma flavovirens*	AF377181	634/668 (95)	0.2	1.1	1
Tuber sp.	AB587796	650	*Tuber separans*	HM485385	575/615 (94)	0.7	10.8	3
Unidentified EcM fungus	–	–	–	–	–	<0.1	1.1	1

Relative abundance (RA), colonization frequency (CF) of each fungal taxa and frequency of forests stands in which each fungal taxon was observed were also indicated

PCA revealed no clear impact of soil type on the EcM fungal community, probably because of soil type variations among the study sites (Fig. 3a); one of the study sites containing Podzol soil (Samcheok 2) was largely segregated from study sites with Regosol soil, whereas the other study sites (Uljin) appeared close together on a plot of the first two axis scores with Sokcho, where the soil type was Regosols. The RDA showed similar study site distributional patterns to those of the PCA on the ordination (Fig. 3b). No measured environmental variables significantly influenced the EcM fungal community composition.

Discussion

Ectomycorrhizal fungal diversity

We found species-rich and diverse EcM fungal communities in mature *P. thunbergii* coastal forests; 68 total taxa of ectomycorrhizal (EcM) fungi were observed in 6 stands of *P. thunbergii* coastal forests (Table 2). However, it appeared that our results only showed a small fraction of the total EcM fungal diversity in coastal pine forests. An ascending species accumulation curve that estimated a species richness approximately 2–3 times higher (128.7 or 164.8 taxa) than the actual richness (68 taxa) indicated that much of the new additional fungal taxa would be detected if sampling efforts were increased. Additionally, the low ratio of EcM roots subjected to DNA analysis (121 root tips) to the total number of EcM roots observed (120,762 root tips) may have increased errors in accurately discriminating EcM roots (i.e., two or more closely related species could be classified together into one group by morphotyping because of their similarity in morphology), perhaps resulting in an underestimation of the actual fungal diversity. Increasing the number of EcM root tips for DNA analysis is preferable in order to more accurately understand the fungal community and its diversity.

Several dozen species of EcM fungi have been recorded in pine forests dominated by a single woody plant, such as natural stands of bishop pine (*Pinus muricata* D. Don) (20 taxa; Gardes and Bruns 1996), *P. thunbergii* coastal forests (27 taxa; Obase et al. 2009), red pine (*Pinus resinosa* Ait.) plantations (39 taxa; Koide et al. 2005), boreal Scots

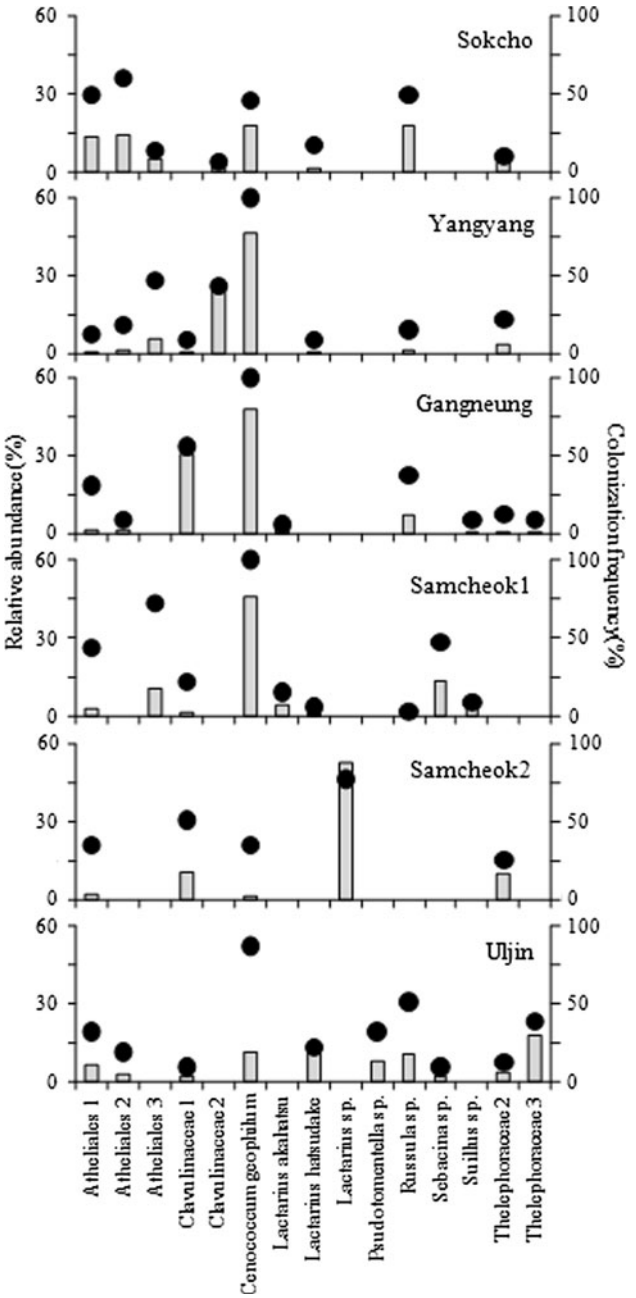

Fig. 2 Relative abundance (*bars*) and colonization frequency (*black circles*) of ectomycorrhizal fungal taxa that showed an average relative abundance >1.0% over all of the study sites

pine (*Pinus sylvestris* L.) forests (43 taxa; Jonsson et al. 1999a, 135 taxa; Jonsson et al. 1999b), *Pinus muricata* D. Don coastal forests (48 taxa; Peay et al. 2007) and old stands of lodgepole pine (*Pinus contorta* Douglas ex Louden) (81 taxa; Douglas et al. 2005). It is difficult to compare the species richness of EcM fungi among various studies because of differences in sampling strategies; however, EcM fungal species richness in *P. thunbergii* coastal forests was as high, as has been reported in other pine forests.

Cenococcum geophilum and species of Atheliales, Clavulinaceae, Russulaceae and Thelephoraceae were the main members of the EcM fungal communities (Table 2). *Cenococcum geophilum* is one of the most common EcM fungi in the world (Trappe 1964; LoBuglio 1999) and often abundantly colonizes in several forest ecosystems (e.g., Valentine et al. 2004; Blom et al. 2009). *Cenococcum geophilum* has also been reported as a dominant fungus in the roots of *P. thunbergii* seedlings (Taniguchi et al. 2007; Kataoka et al. 2008; Matsuda et al. 2009b) and mature trees (Matsuda et al. 2009a; Obase et al. 2009) in coastal pine forests. Some fungal taxa that were presumed to belong to Atheliaceae (e.g., *Tylospora* and *Amphinema*) (Erland 1995), Clavulinaceae (e.g., *Clavulina*) (Koide et al. 2005), Russulaceae (e.g., *Russula* and *Lactarius*) and Thelephoraceae (e.g., *Thelephora* and *Tomentella*) (Horton and Bruns 2001) have often been detected in inland forest ecosystems and have also been observed as the second or third most dominant species or a rare species in coastal pine forests of Japan (Taniguchi et al. 2007; Kataoka et al. 2008; Matsuda et al. 2009b). It appears that the EcM fungi that have colonized in *P. thunbergii* coastal forests are similar between Korea and Japan, and that the structures of the EcM fungal communities comprising those fungal taxa are likely similar among *P. thunbergii* coastal forests.

Community structure of ectomycorrhizal fungi

Overall, *C. geophilum* was the most common and dominant taxon at the study sites (Table 2). A few fungal taxa, such as *C. geophilum*, *Lactarius* sp., Clavulinaceae 1 and *Russula* sp., showed high relative abundances, but the others showed intermediate to low relative abundances. A similar pattern was also observed for colonization frequency. The presence of a few dominant fungi and a large number of rare fungi is a common pattern in EcM fungal communities associated with *P. thunbergii* coastal forests (Matsuda et al. 2009b; Obase et al. 2009) or other tree species (e.g., Valentine et al. 2004). Most of the rare fungal taxa were distributed locally at both the stand scale and plot scale, and tended to show low relative abundances. These results indicate that the local distributions of rare EcM fungal taxa contributed to the species-rich EcM fungal communities in *P. thunbergii* coastal forests.

Colonization patterns at the local scale of study sites and the small scale of study plots differed among EcM fungal taxa. *Cenococcum geophilum* was distributed extensively at the local scale and showed various levels of colonization, often being the dominant taxon, among study sites, while *Lactarius* sp. was distributed locally but dominated at only one study site (Fig. 2). Different distribution patterns among EcM fungal taxa have been well documented in several studies at small scales (e.g., within 20 × 20 m

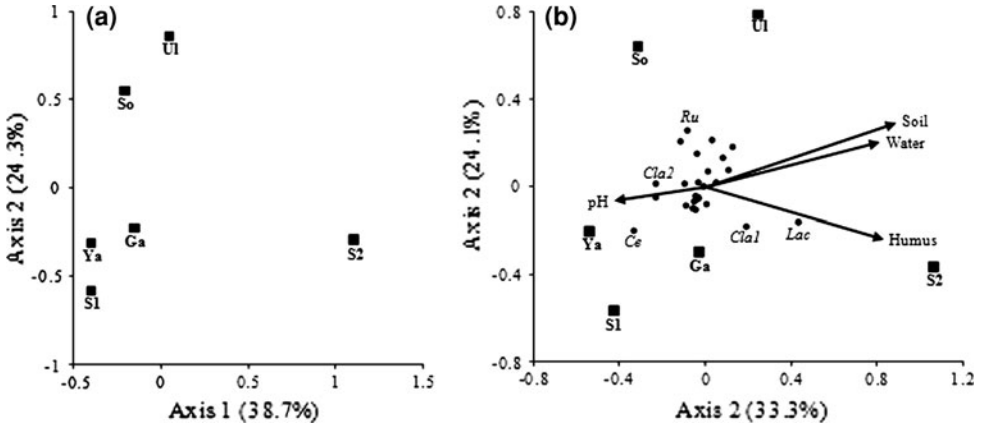

Fig. 3 **a** Principal component analysis displaying the positions of study sites. Axis 1 and axis 2 explained 38.7 and 24.3% of the variability, respectively. **b** Redundancy analysis displaying the positions of study sites (*squares*) and EcM fungal taxa (*circles*), and the effects of soil type, soil pH, soil water content and depth of humus layer (*arrows*) on ectomycorrhizal fungal community structure. Axis 1 and axis 2 explained 33.3 and 24.1% of the variability, respectively. *Cla1* Clavulinaceae 1, *Cla2* Clavulinaceae 2, *Ce Cenococcum geophilum*, *Lac Lactarius* sp., *Ru Russula* sp., *So* Sokcho, *Ya* Yangyang, *Ga* Gangneung, *S1* Samcheok 1, *S2* Samcheok 2, *Ul* Uljin

plots) (Lilleskov et al. 2004; Pickles et al. 2010), but only a few studies have described the distribution of certain EcM fungi at a local scale. In *P. thunbergii* coastal forests, Matsuda et al. (2009a) investigated the distribution of *C. geophilum* at the local scale (several tens of kilometers in extent) in coastal pine forests of Japan and found that this fungus was distributed ubiquitously and dominant, but its colonization ratio varied among study sites (20.0 to 62.6% of EcM roots).

The presence of colonization pattern differences among EcM taxa and in certain EcM fungal taxa among study sites could be related to several factors, such as spatial heterogeneity of soil conditions (e.g., Bruns 1995) and interspecific interactions among EcM fungi (i.e., competence and coexistence) (Pickles et al. 2010). Although we could not find distinct effects of the measured environmental variables on EcM fungal colonization, possibly because the most important soil parameters went unmeasured or because the number of replicated study sites was insufficient to understand the trends within the whole EcM fungal community (Fig. 3), field observations provided several insights into the distribution patterns of given EcM fungal taxa. In this study, low abundance and local colonization of *C. geophilum* were observed in one forest stand (Samcheok 2) where *Lactarius* sp. was dominant (Fig. 2). In study sites where *C. geophilum* was dominant, the soils consisted entirely of maritime sand with relatively shallow litter and low water content. Previous studies have suggested that *C. geophilum* is an excellent colonizer in areas where drought stress is high and where the soil is very weakly developed (Obase et al. 2009). In contrast, in a study site where *Lactarius* sp. was dominant, soils consisted of forest soil that appeared to be compacted, had poor drainage and contained a relatively thick and humid humus layer. Most EcM roots extended into the humus layer, and *Lactarius* sp. exhibited distinct clumps in this layer. Dense root colonization by *Lactarius* species in organic soil layers has also been reported in a previous study (Genney et al. 2006).

Conclusions

We investigated the colonization status of EcM fungi in six stands of *P. thunbergii* coastal forests and found species-rich and diverse communities in which each EcM fungal taxon showed various distribution patterns at local scales of the study sites and at small scales of the study plots. Estimation of species richness by an extrapolation method revealed that high sampling efforts are required to elucidate the entire EcM fungal community in coastal pine forests within an area that extends for several hundred kilometers. The dominance of *C. geophilum* is likely to be a common occurrence in *P. thunbergii* coastal forests; however, these results also show that EcM fungal communities could differ among study sites and that other taxa could be superior to *C. geophilum* in colonization at the study plot scale. It is important to understand the ecological characteristics of EcM fungi and their colonization patterns in various environmental conditions when attempts are undertaken to apply EcM fungi for revegetation techniques in disturbed coastal pine forests.

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