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Mycorrhizal associations in woody plant species at the Mt. Usu volcano, Japan.

2. Informative title

Mycorrhizal associations in woody plant species at volcano Usu.

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Title:

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Authors:

Keisuke Obase, Yutaka Tamai, Takashi Yajima, Toshizumi Miyamoto.

Abstract

We investigated the association between ectomycorrhizal (ECM) and arbuscular mycorrhizal (AM) fungi and pioneer woody plant species in areas devastated by the eruption of Mt. Usu, Japan, in 2000. We observed 8 woody plant species at the research site, most of which were associated with ECM and/or AM fungi. In particular, dominant woody plant species *Populus maximowiczii*, *Salix hultenii* var. *angustifolia* and *Salix sachalinensis* were consistently associated with ECM fungi and erratically associated with AM fungi. We found 1 to 6 morphotypes in the roots of each ECM host and on average 2 in the roots of each seedling, indicating low ECM fungal diversity. ECM colonization ranged from 17 to 42% of root tips. Using morphotyping and molecular analyses, 15 ECM fungi were identified. ECM fungi differed greatly between hosts. However, *Laccaria amethystea*, *Hebeloma mesophaeum*, *Thelephora terrestris* and other Thelephoraceae had high relative colonization, constituting the

majority of the ECM colonization in the roots of each plant species. These ECM fungi may be important for the establishment of pioneer woody plant species and further revegetation at Mt. Usu volcano.

Key words;

Mycorrhizal association, Ectomycorrhizal fungi, Woody plant, Disturbed area, Volcano.

Introduction

Woody plant species invade and become established in devastated areas immediately following volcanic eruption, despite the presence of environmental stresses such as low soil nutrients, instability of the soil surface and drought (Goto 1937; Yoshii 1942; Tsuyuzaki 1987). These woody plant species, called pioneer species, contribute to vegetation recovery by facilitating the establishment of later seral vegetation (Walker and del Moral 2003).

Ectomycorrhizal (ECM) hosts such as the Salicaceae often dominate areas devastated by volcanic eruption (Goto 1937; Yoshii 1942; Tsuyuzaki 1987). The dominant woody plant species at our Mt. Usu study site are *Salix sachalinensis* Fr. Schm., *Salix hultenii* var. *angustifolia* Kimura and *Populus maximowiczii* A. Henry, which belong to a family usually colonized by ECM and arbuscular mycorrhizal (AM) fungi. These species are considered to be significant for future reforestation. ECM fungi enhance the growth of host plant species: recent studies have revealed coinoculation with various ECM fungi can alter host growth and nutrient acquisition (Reddy and Natarajan 1997; Baxter and Dighton 2001). Thus, the composition of the ECM fungal community influences establishment of host plant species and to understand the effect of ECM associations on growth and survival of host plants, it is important to know which species comprise a given community.

Although few studies have examined ECM associations in woody plant species established in devastated areas, efforts have been made to describe the ECM fungi involved in primary succession. Jumpponen et al. (2002) investigated the chronosequence of ECM fungi occurring at the front of the Lyman Glacier. They noted that the occurrence of ECM fungal sporocarps varies according to the time since deglaciation, indicating an early and late stage model for succession. Allen et al. (1992) noted that several years after the last eruption of Mt. St. Helens, several woody plant species were associated with ECM fungi. Yang et al. (1998) investigated the occurrence of ECM morphotypes in *Larix kaempferi* (Lamb.) Carr. at the Mt. Koma volcano, Japan. They demonstrated that, as with litter accumulation and soil conditions, the composition of ECM morphotypes varied with elevation, emphasizing the importance of ECM diversity for survival and growth of seedlings. Recently, molecular analyses have been applied to mycorrhizal research in order to differentiate and identify ECM fungi. Using polymerase chain reaction (PCR) amplification of the internal transcribed spacer (ITS) region of fungal nuclear ribosomal DNA (rDNA), morphologically similar ectomycorrhizae can be distinguished and identified by their restriction fragment length polymorphism (RFLP) patterns and sequencing, respectively. Nara et al. (2003a, b) used both conventional morphotyping and molecular analyses to reveal the presence of ECM flora in the roots of *Salix reinii* Franch. et Savat. and demonstrate succession in underground ECM fungi from a volcanic desert on Mt. Fuji. Ashkannejhad and Horton (2006) investigated the ECM flora of *Pinus contorta* var. contorta seedlings on coastal sand dunes. However, little is known about ECM flora in areas devastated by volcanoes, particularly in the period immediately following cessation of volcanic activity.

Using morphotyping and molecular analyses we investigated: 1) the status of mycorrhizal associations in seedlings of woody plant species; and 2) the underground ECM fungal community associated with pioneer woody plant species in areas devastated by the 2000 eruption of Mt. Usu.

Materials and Methods

Mt. Usu (42° 32’ N, 140° 50’ E; 773.1m asl) is an active volcano located in southwest Hokkaido, Japan (Fig. 1) that has erupted repeatedly since 1663. It erupted again on March 31, 2000, 22 years after the previous eruption. A number of small craters formed at the foot of the Nishiyama and Konpira areas, and were accompanied by the accumulation of a considerable amount of volcanic debris. Ejection of debris subsided in autumn 2000, but the effects of thermal activity such as elevated soil temperatures, as well as the emission of noxious gases, continued near the K-A, K-B and N-B craters. Prior to the 2000 eruption, there was a natural secondary forest comprising broadleaf species such as *Betula* spp., *Acer* spp. *Quercus* spp. and *Magnolia* spp., and a partially planted forest of *L. kaempferi* and *Abies sachalinensis* (Fr. Schm.) Masters. However, the deposition of 1-3m of volcanic debris (fine volcanic ash and pumice) devastated ca. 71ha of forest around the craters. This study was conducted in the devastated area around the N-A crater and at the foot of the Nishiyama area, where it appeared that volcanic activity had ceased as we observed no emissions of volcanic gases or elevation of soil temperature. In 2004, 15 woody plant species had established near the Nishiyama area craters, reaching a total density of 1038ha^{-1}. The mean growth rate of the dominant species (*S. sachalinensis*) was ca. 10cm year^{-1}. Thus, conditions at present remain unfavorable for the establishment of woody plant species. In 2004, climatic data from the Sapporo meteorological station at Date (42° 30’ N, 140° 54’ E; 84.7m asl), indicated a mean annual precipitation of

835mm and annual temperature of 8.9°C, ranging between -12.0 and 30.8°C (December to August, respectively).

Sampling procedure

In May 2004, we established a 4-ha research site encompassing several craters in which no trees had survived the 2000 eruption and where all understory vegetation had disappeared due to the deposition of volcanic debris (> 1m). From June to September 2004, we randomly selected 1-12 seedlings from each woody plant species and sampled their lateral roots, which extended from the soil surface to a depth of 15cm.

ECM and AM associations

We investigated the ECM and AM associations in each woody plant species with > 6 seedlings. Adhering soil was separated from the roots by soaking and careful washing of samples in tap water. Appearance and the presence of a mantle and Hartig net was used to identify ECMs under differential interference microscopy (400-1000x magnification). AMs were identified using the staining procedure described by Phillips and Hayman (1970), with some modifications. Roots were rinsed with distilled water, cleared with 10% KOH for 80 min at 80°C, bleached in 0.5% H_2O_2 for 10-20 min at 60°C, acidified in 1% HCl at room temperature (ca. 15-20°C), and stained using 0.05%

trypan blue in lactophenol for 15 min at 80°C. AM colonization was identified by the presence of vesicles or arbuscules, as well as internal hyphae.

Determination of mycorrhizal colonization

We focused on ECM hosts and investigated their underground ECM fungal flora. The overall morphologies of ECMs were observed under stereoscopic microscopy. ECMs from each woody plant species were classified into morphological groups and divided into two subsamples: one was placed in FAA solution (formaldehyde: acetic acid: ethyl alcohol: distilled water = 1:1:9:9) for microscopic investigation and the other stored at -80°C for DNA extraction.

ECM abundance was estimated as the proportion of each morphotype relative to the total ECM. Frequency was estimated as the proportion of seedlings colonized by one morphotype relative to all seedlings.

DNA extraction, PCR amplification and RFLP

The samples contained one ECM root tip of each morphotype from each seedling; 3 to 5 samples from each morphotype identified in a given woody plant species were categorized individually by PCR-RFLP. ECM fungal DNA was extracted from 5-10mg ground, lyophilized tissue using the DNeasy Plant Mini kit (QIAGEN, USA) according to the manufacturer's instructions. The ITS region, including the 5.8S rDNA, was amplified using a specific primer for higher fungi (ITS1-f; Gardes and Bruns 1993) and a universal primer (ITS4; White et al. 1990). The following PCR amplification conditions were used: 94°C for 3 min, followed by 30 cycles of 94°C for 1 min, 50°C for 1 min and 72°C for 3 min, then a final extension at 72°C for 10 min (Landeweert et al. 2005).

Single enzyme digests using *Hin*fI and *Alu*I were performed on PCR products from 3 to 5 ECM root tips of each ECM morphotype. Using 2.5% agarose gel electrophoresis, we determined the quality and quantity of the PCR products, as well as the size of restriction fragments. Band lengths were calculated using KiloACE (http://www.nih.go.jp/%7Ejun/cgi-bin/kiloace.pl).

Sequencing

We used the primer ITS1f to sequence samples of each PCR product arising from different ECM morphotypes and exhibiting differences in RFLP analysis. Sequencing reactions were performed using the BigDye Terminator v3.1/1.1 Cycle Sequencing Kit (Applied Biosystems, USA), followed by ethanol precipitation and analysis with an ABI Auto Sequencer 310 (Applied Biosystems, USA). ECM sequences were compared with the GenBank database at the DNA Data Bank of Japan (DDBJ) using the BLAST program, and species names were assigned to BLAST matches exhibiting > 95% homology.

Sporocarps of ECM fungi

Although no ECM fungal sporocarps were found during preliminary work at the study site, we identified 9 taxa in the area that contained surviving mature trees and herbaceous plants (Obase et al. 2005). These were identified microscopically as *Laccaria* sp., *Inocybe nitidiuscula* (Britzelm.) Sacc., *Inocybe dulcamara* (Pers. Albertini and Schweinitz) P. Kumm., *Hebeloma crustuliniforme* (Bull. Fr.) Quel., *Hebeloma mesophaeum* complex, *Hebeloma* sp., *Suillus laricinus* (Berk. in Hook.) O. Kuntze, *Suillus grevillei* (Klotzsch Fr.) Singer and *Scleroderma bovista* Fr. In order to perform alignments between above- and below-ground fungal sequences, we extracted and sequenced DNA from these ECM sporocarps. The procedures for DNA extraction, PCR amplification and sequencing were as described above, except that the ratio of DNA template to sterilized distilled water was altered from 9:16 to 1:24 in the PCR procedure.

Results

Mycorrhizal association

We observed 8 woody plant species at the research site (Table 1) and observed ECM colonization in almost all seedlings of *Betula platyphylla* Sukatchev var. *japonica* (Miq.) Hara, *Quercus crispula* Blume, *P. maximowiczii*, *S. hultenii* var. *angustifolia*, *Salix integra* Thunb. and *S. sachalinensis*. AM colonization was detected in the roots of *B. platyphylla* var. *japonica*, *P. maximowiczii*, *Q. crispula*, *S. hultenii* var. *angustifolia*, *S. sachalinensis*, *Acer mono* Maxim. var. *marmoratum* (Nichols.) Hara f. *dissectum* (Wesmael) Rehder and *Rosa multiflora* Thunb. We also observed AM and ECM co-colonization in some seedlings of *B. platyphylla* var. *japonica*, *P. maximowiczii*, *Q. crispula* and *S. hultenii* var. *angustifolia*, but the association frequency of the former was lower than that of the latter. In general, we observed association with ECM and/or AM fungi in most woody plant species and with the exception of *A. mono* var. *marmoratum* f. *dissectum*, we found that the dominant woody plant species were associated consistently with ECM fungi and erratically with AM fungi.

ECM morphotype and colonization

We found between1 and 6 morphotypes in the roots of each ECM host (Table 2) and 17 to 42% of all root tips were colonized by ECM fungi. On average, 2 morphotypes were observed in the roots of each seedling, except for those of *Q. crispula* and *B.*

platyphylla var. *japonica*, which had 1 and 4, respectively.

PCR-RFLP patterns and genetic identification

Although DNA amplification using the primers ITS1f and ITS4 resulted in nearly 100% amplification of PCR products, some types produced multiple PCR products, possibly because of the presence of other fungi within or around the root tissues. As some samples could not be determined by RFLP analysis alone, we digested the most well-separated and abundant PCR products from each sample with *Hin*fI and *Alu*I, and thus categorized each ECM morphotype (Table 3). With the exception of *Lk*-2, *Ss*-2 and *Bp*-2, the banding patterns were identical from different samples within each ECM morphotype.

Alignment of these sequences with those from GenBank resulted in potential matches for 15 ECM fungi (Table 4). Sequences of 2 ECM morphotypes matched the *H. mesopaeum* complex and *S. bovista* sporocarps that were observed in the preliminary study (Obase et al. 2005).

Colonization by each ECM morphotype

The ECM fungal flora differed between hosts (Table 4) and 11 of the 15 fungi were observed in the roots of only one host. However, *Laccaria amethystea*, *H. mesophaeum*, *Thelephora terrestris* Fr. and Thelephoraceae 1 were observed in the roots of 2, 3, 4 and 4 ECM hosts, respectively. These fungi were abundant and represented most of the ECM colonization in the roots of each woody plant species.

Discussion

In 2000, the study site was strongly disturbed by the eruption, which resulted in the loss of nearly all the plant species that had colonized the site before 2000. Thus, almost all seedlings were new recruits that had become established independently on the new substrate. Although the deposition of a thick layer of new volcanic debris around craters must presumably make it problematic for woody plant species to associate with mycorrhizal fungi, such associations were nonetheless observed in the roots of newly-recruited seedlings. Following volcanic eruptions, AM and ECM associations reestablish immediately (Allen et al. 1992), and are of major importance to the primary succession of plant species in volcanic areas (Titus and Tsuyuzaki 2002; Fujiyoshi et al. 2005; Tsuyuzaki et al. 2005).

At the time of eruption, the scale of disturbance in our study area was relatively small (ca. 71ha) and the surrounding forest edge recovered quickly. It would appear that there was a rapid recovery of, or minimal damage to the fungal flora at the forest edge, as Obase et al. (2005) identified a variety of fungal species by investigating sporocarp occurrence. The speed of recovery of both vegetation and fungal flora in these edge areas, as well as their proximity to the study site, both play a role in the recruitment of mycorrhizal inocula to the devastated area.

Almost all seedlings of the dominant woody plant species *P. maximowiczii*, *S. hultenii* var. *angustifolia* and *S. sachalinensis* exhibited ECM fungal associations. In 2002, only two years after the volcanic eruption, a preliminary study revealed the presence of ECM colonization in the roots of *Salix*. ECM and AM fungi both colonize the Salicaceae (Harley and Harley 1987). In the present study, we observed a very low percentage of AM colonization, with less than half of the seedlings exhibiting an AM association. Thus, it appears that AM fungi represent a relatively insignificant factor in the establishment of Salicaceae seedlings, compared to ECM fungi. In a study on Mt. Fuji, Nara (2006) reported a strong relationship between established *S. reinii* individuals and ECM fungal association, but found only rare associations with AM fungi. In contrast, in about 50% the seedlings of *A. mono* var. *marmoratum* f. *dissectum* associated with AM fungi and no ECM fungi were observed in present study. *Acer* spp. have been observed with AM, ECM or non-mycorrhizal associations (Harley and Harley 1987). Under different environmental conditions, some seedlings of *A. mono* var. *marmoratum* f. *dissectum* associated with AM fungi but others did not form mycorrhizal associations (unpublished data). Thus, it seems *A. mono* var. *marmoratum*

f. *dissectum* intrinsically forms erratic relationships with AM fungi during seedling stage, that also appeared in primary succession.

Analysis of RFLP and sequence data derived from root materials demonstrated that 3 Salicaceae woody plant species that are dominant in the study area harbored 9 ECM fungal taxa, with one woody plant species alone containing 3 to 5 ECM fungal taxa. These numbers are very low compared to the high ECM fungal diversity in temperate and boreal forests (Horton and Bruns 2001). In the roots of *Salix repens* L. established in sand dunes, 78 ECM fungal species were recorded as sporocarps (van der Heijden 1999). Nara et al. (2003a, b) reported 23 ECM species as sporocarps and 21 ECM species in the roots of *S. reinii* established in the volcanic desert on Mt. Fuji. However, in a 6-year-old plantation, a study on the ECM community associated with *Salix viminalis* L. and *Salix dasyclados* Wimm. identified only 4 and 7 ECM taxa, respectively (Püttsepp et al. 2004). In addition, Nara et al. (2003a, b) observed only 5 ECM taxa in young *S. reinii* seedlings. As the seedlings investigated in the present study were young, their age and isolation from mature trees will have had an influence on the diversity of their ECM communities. Ashkannejhad and Horton (2006) revealed that both the ECM diversity per seedling and the total number of ECM fungi was lower in isolated dunes than in forests. They also showed that some ECM fungi found in the forest also colonized seedlings in sand dunes. Thus, it appears that isolated seedlings that are undergoing primary succession are only able to associate with a limited range of early-stage ECM fungi.

We observed *Hebeloma*, *Laccaria*, Thelephoraceae species consistently in the roots of the dominant woody plant species *P. maximowiczii*, *S. hultenii* var. *angustifolia* and *S. sachalinensis* in the study area. These ECM fungi are well known colonizers of plants in disturbed or primary habitats (e.g. Nara et al. 2003a, b; Trowbridge and Jumpponen 2004) and may be important for the establishment of pioneer woody plant species, as well as the further revegetation of Mt. Usu. In general, however, the role played by mycorrhizal fungi in the growth and survival of seedlings of woody plant species remains unclear, since their interactions may vary according to the combination of species and environmental conditions. In the future, it would be useful to examine the effects of inoculation of these mycorrhizal fungi in the field.

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Table and Figure Legends

Table 1. Frequencies (F) of ECM and AM associations with woody plant species, observed at a research site on the Mt. Usu volcano, Hokkaido, Japan in 2004.

*Non, Non-mycorrhizal; ECM, ectomycorrhizal; and AM, arbuscular mycorrhizal.

Table 2. Number of ECM morphotypes, mean number of ECM morphotypes per

seedling and percentage of all types of ECM colonization (Ec) in the roots of woody plant species on the Mt. Usu volcano, Hokkaido, Japan.

*Standard deviations are indicated.

Table 3. ECM fungi detected according to type and best BLAST match.

****Ss***, *S. sachalinensis*; ***Pm***, *P. maximowiczii*; ***Sh***, *S. hultenii* var. *angustifolia*; ***Si***, *S. integra*; ***Qc***, *Q. crispula*; ***Bp***, *B. platyphylla* var. *japonica*; ***Bm***, *B. maximowicziana*; and ***Lk***, *L. kaempferi*.

**The assignment of two names for one ECM type indicates that some ECM types were identified initially as identical but were differentiated later by PCR-RFLP.

*** n.d., not detected; + not cleaved.

Table 4. Percentage of colonization (Ec)** and frequencies (F) of each ECM fungus observed in ECM hosts*** established at the study site on Mt. Usu, Hokkaido, Japan.* Percentage of colonization and frequencies of these fungi are obscured because two fungal species were included in one ECM type.

** Mean and standard deviations (in parenthesis) are presented.

***See Table 3.

Fig. 1. Location of study site on Mt. Usu, Hokkaido, Japan.

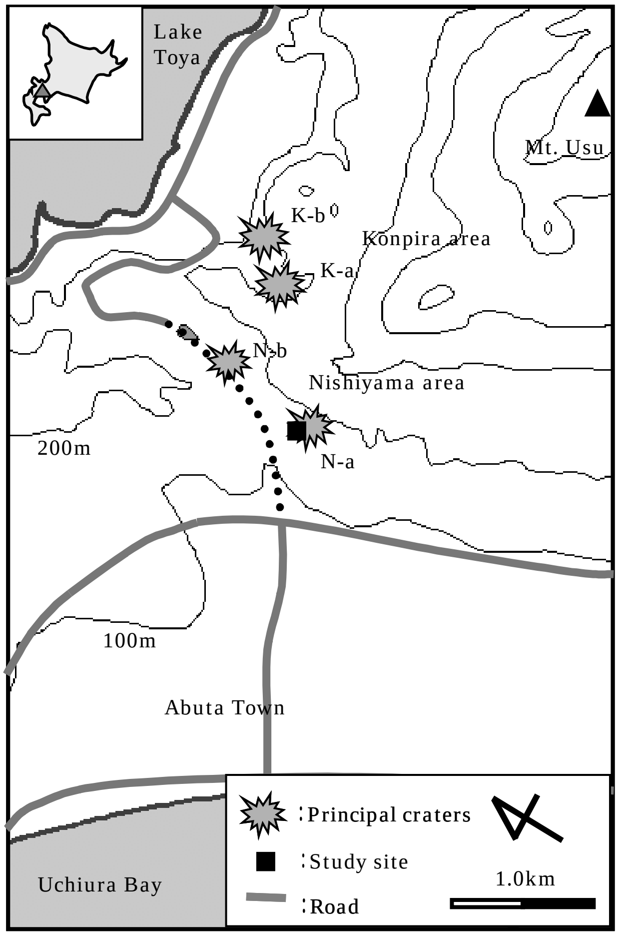

Fig. 1 Location of study site on Mt. Usu, Hokkaido, Japan

Table 1 The frequency (F) of ECM and AM association with woody plant species, observed at a research site on Mt. Usu, Hokkaido, Japan in 2004.

Woody plant species	Mycorrhiza*	F	
		ECM	AM
Betula platyphylla var. *japonica*	ECM, AM	6/6	3/6
Populus maximowiczii	ECM, AM	9/9	4/9
Quercus crispula	ECM, AM	5/6	1/6
Salix hultenii var. *angustifolia*	ECM, AM	9/9	3/9
Salix integra	ECM	6/6	0/6
Salix sachalinensis	ECM, AM	12/12	1/12
Acer mono	AM	0/6	3/6
Rosa multiflora	AM	0/6	4/6

*ECM ectomycorrhizal, AM arbuscular mycorrhizal

Table 2 The number of ECM morphotypes, the average number of ECM morphotypes per seedling and the percentage of all types of ECM colonization in the roots of woody plant species on Mt. Usu, Hokkaido, Japan

Woody plant species	ECM morphotype		Total EC (%)*
	total	per seedling*	
Betula platyphylla var. *japonica*	6	4.0 ± 1.5	41.8 ± 22.1
Populus maximowiczii	4	1.7 ± 0.5	39.5 ± 24.8
Quercus crispula	1	0.8 ± 0.4	17.4 ± 16.7
Salix hultenii var. *angustifolia*	4	1.9 ± 0.8	29.3 ± 16.0
Salix integra	4	2.3 ± 0.5	17.3 ± 9.2
Salix sachalinensis	3	2.0 ± 0.9	24.7 ± 12.7

*Standard deviations are indicated

Table 3 ECM fungi detected on each ECM type with their best Blast match

Tree*	ECM type**	Possible identity	Blast match	overlap (bp)	Similarity (%)	RFLP pattern (bp)***						
						*Hin*fⅠa	*Hin*fⅠb	*Hin*fⅠc	*Alu*Ⅰa	*Alu*Ⅰb	*Alu*Ⅰc	*Alu*Ⅰd
Bp	*Bp*-1	Thelephoraceae 1	DQ195592.1	413	94	310	220	150	300	260	200	100
	Bp-2	*Hebeloma mesophaeum*	AY311521.1	313	99	n.d.			n.d.			
		Unidentified 5	—	410	—	n.d.			n.d.			
	Bp-3	*Leccinum scabrum*	AF454585.1	436	99	860	440		570	400	120	
	Bp-4	Thelephoraceae 5	AB211278.1	404	96	350	160	120	500	130		
	Bp-5	Unidentified 3	—	358	—	240	190	120	+			
	Bp-6	Thelephoraceae 4	AF184742.1	421	95	360	200	150	470	130		
Pm	*Pm*-1	*Scleroderma bovista*	AB099901.1	216	95	n.d.			n.d.			
	Pm-2	Thelephoraceae 1	DQ195592.1	438	95	290	200	140	310	280	210	100
	Pm-3	*Laccaria amethystea*	AB211270.1	431	99	400	350		420	380	100	
	Pm-4	*Inocybe lacera*	AY750157.1	405	100	380	250		330	210	180	
		Thelephora terrestris	AF272921.1	470	98	350	190	100	440	140		
Qc	*Qc*-1	*Thelephora terrestris*	AJ549972.1	417	98	380	200	100	450	150		
Sh	*Sh*-1	*Hebeloma mesophaeum*	AY311521.1	431	99	410	340		320	270	210	
	Sh-2	Thelephoraceae 1	DQ195592.1	460	95	320	200	140	290	260	190	100
	Sh-3	*Thelephora terrestris*	AY230241.1	468	98	370	210	100	440	130		
	Sh-4	Thelephoraceae 2	U83475.1	391	97	350	180		520	210		
Si	*Si*-1	*Laccaria amethystea*	AB211270.1	405	100	390	340		420	370	120	
	Si-2	*Hebeloma mesophaeum*	AY311521.1	408	99	400	340		290	220	190	
	Si-3	Thelephoraceae 3	AF184742.1	400	95	210	190	160	470			
	Si-4	*Thelephora terrestris*	AY230241.1	431	99	370	210	110	450	150		
Ss	*Ss*-1	*Hebeloma* sp.	AY320395	500	98	420	350		320	280	250	210
	Ss-2	Thelephoraceae 1	DQ195592.1	488	95	290	190	140	300	260	190	100
		Unidentified 1	AB096869	350	97	380	190	100	+			
	Ss-3	Unidentified 1	AB096870	505	96	n.d.			n.d.			

Ss* *S. sachalinensis*, ***Pm* *P. maximowiczii*, ***Sh*** *S. hultenii* var. *angustifolia*, ***Si*** *S. integra*, ***Qc*** *Q. crispula*, ***Bp*** *B. platyphylla* var. *japonica*, ***Bm*** *B. maximowicziana*, ***Lk*** *L. kaempferi*.

**The assigned two names for one ECM type indicates that some ECM types were misunderstood as identical but were differentiated by PCR-RFLP.

*** n.d. not detected, + not craved.

Table 4 Percentage of colonization (Ec)** and frequencies (F) of each ECM fungi observed in ECM hosts*** established at the study site on Mt. Usu, Hokkaido, Japan.

ECM fungi	*Bp*		*Qc*		*Pm*		*Sh*		*Si*		*Ss*	
	Ec (%)	F (/6)	Ec (%)	F (/6)	Ec (%)	F (/9)	Ec (%)	F (/9)	Ec (%)	F (/6)	Ec (%)	F (/12)
Laccaria amethystea					12.1 (14.2)	6			9.6 (6.9)	6		
Inocybe lacera					18.9 (9.1)*	7*						
Hebeloma mesophaeum	2.2 (1.8)*	4*					18.3 (12.9)	8	3.4 (3.0)	5		
Hebeloma sp.											12.8 (12.9)	7
Scleroderma bovista					74.1	1						
Leccinum scabrum	48.8	1										
Thelephora terrestris			17.4 (16.7)	5	18.9 (9.1)*	7*	30.0 (21.3)	3	1.3 (1.6)	2		
thelephoraceae 1	19.8 (23.0)	5			76.7	1	3.2 (3.7)	5			17.3 (9.0)*	12*
thelephoraceae 2							11.4	1				
thelephoraceae 3									26.5	1		
thelephoraceae 4	1.2 (0.9)	4										
thelephoraceae 5	8.2 (9.9)	5										
Unidentified 1											17.3 (9.0)*	12*
Unidentified 2	4.8 (3.6)	6										
Unidentified 3	2.2 (1.8)*	4*										

* Percentage of colonization and frequencies of these fungi is obscured because two fungal species were included in one ECM type.

** Average and standard deviation (in parenthesis) were presented.

***See Table 3