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

3 2. Informative title

4 Mycorrhizal associations in woody plant species at volcano Usu.

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19

1 **Title:**

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3

4 **Authors:**

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6

7 **Abstract**

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

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

4

5 **Key words;**

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

7

8 **Introduction**

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

15 Ectomycorrhizal (ECM) hosts such as the Salicaceae often dominate areas
16 devastated by volcanic eruption (Goto 1937; Yoshii 1942; Tsuyuzaki 1987). The
17 dominant woody plant species at our Mt. Usu study site are *Salix sachalinensis* Fr.
18 Schm., *Salix hultenii* var. *angustifolia* Kimura and *Populus maximowiczii* A. Henry,
19 which belong to a family usually colonized by ECM and arbuscular mycorrhizal (AM)

1 fungi. These species are considered to be significant for future reforestation. ECM
2 fungi enhance the growth of host plant species: recent studies have revealed
3 coinoculation with various ECM fungi can alter host growth and nutrient acquisition
4 (Reddy and Natarajan 1997; Baxter and Dighton 2001). Thus, the composition of the
5 ECM fungal community influences establishment of host plant species and to
6 understand the effect of ECM associations on growth and survival of host plants, it is
7 important to know which species comprise a given community.

8 Although few studies have examined ECM associations in woody plant species
9 established in devastated areas, efforts have been made to describe the ECM fungi
10 involved in primary succession. Jumpponen et al. (2002) investigated the
11 chronosequence of ECM fungi occurring at the front of the Lyman Glacier. They noted
12 that the occurrence of ECM fungal sporocarps varies according to the time since
13 deglaciation, indicating an early and late stage model for succession. Allen et al.
14 (1992) noted that several years after the last eruption of Mt. St. Helens, several woody
15 plant species were associated with ECM fungi. Yang et al. (1998) investigated the
16 occurrence of ECM morphotypes in *Larix kaempferi* (Lamb.) Carr. at the Mt. Koma
17 volcano, Japan. They demonstrated that, as with litter accumulation and soil conditions,
18 the composition of ECM morphotypes varied with elevation, emphasizing the
19 importance of ECM diversity for survival and growth of seedlings. Recently, molecular

1 analyses have been applied to mycorrhizal research in order to differentiate and
2 identify ECM fungi. Using polymerase chain reaction (PCR) amplification of the
3 internal transcribed spacer (ITS) region of fungal nuclear ribosomal DNA (rDNA),
4 morphologically similar ectomycorrhizae can be distinguished and identified by their
5 restriction fragment length polymorphism (RFLP) patterns and sequencing,
6 respectively. Nara et al. (2003a, b) used both conventional morphotyping and
7 molecular analyses to reveal the presence of ECM flora in the roots of *Salix reinii*
8 Franch. et Savat. and demonstrate succession in underground ECM fungi from a
9 volcanic desert on Mt. Fuji. Ashkannejhad and Horton (2006) investigated the ECM
10 flora of *Pinus contorta* var. *contorta* seedlings on coastal sand dunes. However, little is
11 known about ECM flora in areas devastated by volcanoes, particularly in the period
12 immediately following cessation of volcanic activity.

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

17

18 **Materials and Methods**

19 Mt. Usu (42° 32' N, 140° 50' E; 773.1m asl) is an active volcano located in

1 southwest Hokkaido, Japan (Fig. 1) that has erupted repeatedly since 1663. It erupted
2 again on March 31, 2000, 22 years after the previous eruption. A number of small
3 craters formed at the foot of the Nishiyama and Konpira areas, and were accompanied
4 by the accumulation of a considerable amount of volcanic debris. Ejection of debris
5 subsided in autumn 2000, but the effects of thermal activity such as elevated soil
6 temperatures, as well as the emission of noxious gases, continued near the K-A, K-B
7 and N-B craters. Prior to the 2000 eruption, there was a natural secondary forest
8 comprising broadleaf species such as *Betula* spp., *Acer* spp. *Quercus* spp. and
9 *Magnolia* spp., and a partially planted forest of *L. kaempferi* and *Abies sachalinensis*
10 (Fr. Schm.) Masters. However, the deposition of 1-3m of volcanic debris (fine volcanic
11 ash and pumice) devastated ca. 71ha of forest around the craters. This study was
12 conducted in the devastated area around the N-A crater and at the foot of the
13 Nishiyama area, where it appeared that volcanic activity had ceased as we observed no
14 emissions of volcanic gases or elevation of soil temperature. In 2004, 15 woody plant
15 species had established near the Nishiyama area craters, reaching a total density of
16 1038ha⁻¹. The mean growth rate of the dominant species (*S. sachalinensis*) was ca.
17 10cm year⁻¹. Thus, conditions at present remain unfavorable for the establishment of
18 woody plant species. In 2004, climatic data from the Sapporo meteorological station at
19 Date (42° 30' N, 140° 54' E; 84.7m asl), indicated a mean annual precipitation of

1 835mm and annual temperature of 8.9°C, ranging between -12.0 and 30.8°C
2 (December to August, respectively).

3

4 **Sampling procedure**

5 In May 2004, we established a 4-ha research site encompassing several craters in
6 which no trees had survived the 2000 eruption and where all understory vegetation had
7 disappeared due to the deposition of volcanic debris (> 1m). From June to September
8 2004, we randomly selected 1-12 seedlings from each woody plant species and
9 sampled their lateral roots, which extended from the soil surface to a depth of 15cm.

10

11 **ECM and AM associations**

12 We investigated the ECM and AM associations in each woody plant species with > 6
13 seedlings. Adhering soil was separated from the roots by soaking and careful washing
14 of samples in tap water. Appearance and the presence of a mantle and Hartig net was
15 used to identify ECMs under differential interference microscopy (400-1000x
16 magnification). AMs were identified using the staining procedure described by Phillips
17 and Hayman (1970), with some modifications. Roots were rinsed with distilled water,
18 cleared with 10% KOH for 80 min at 80°C, bleached in 0.5% H₂O₂ for 10-20 min at
19 60°C, acidified in 1% HCl at room temperature (ca. 15-20°C), and stained using 0.05%

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

3

4 **Determination of mycorrhizal colonization**

5 We focused on ECM hosts and investigated their underground ECM fungal flora.

6 The overall morphologies of ECMs were observed under stereoscopic microscopy.

7 ECMs from each woody plant species were classified into morphological groups and

8 divided into two subsamples: one was placed in FAA solution (formaldehyde: acetic

9 acid: ethyl alcohol: distilled water = 1:1:9:9) for microscopic investigation and the

10 other stored at -80°C for DNA extraction.

11 ECM abundance was estimated as the proportion of each morphotype relative to the

12 total ECM. Frequency was estimated as the proportion of seedlings colonized by one

13 morphotype relative to all seedlings.

14

15 **DNA extraction, PCR amplification and RFLP**

16 The samples contained one ECM root tip of each morphotype from each seedling; 3

17 to 5 samples from each morphotype identified in a given woody plant species were

18 categorized individually by PCR-RFLP. ECM fungal DNA was extracted from 5-10mg

19 ground, lyophilized tissue using the DNeasy Plant Mini kit (QIAGEN, USA) according

1 to the manufacturer's instructions. The ITS region, including the 5.8S rDNA, was
2 amplified using a specific primer for higher fungi (ITS1-f; Gardes and Bruns 1993) and
3 a universal primer (ITS4; White et al. 1990). The following PCR amplification
4 conditions were used: 94°C for 3 min, followed by 30 cycles of 94°C for 1 min, 50°C
5 for 1 min and 72°C for 3 min, then a final extension at 72°C for 10 min (Landeweert et
6 al. 2005).

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

12

13 **Sequencing**

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

1 program, and species names were assigned to BLAST matches exhibiting > 95%
2 homology.

3

4 **Sporocarps of ECM fungi**

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

17

18 **Results**

19 **Mycorrhizal association**

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

15

16 **ECM morphotype and colonization**

17 We found between 1 and 6 morphotypes in the roots of each ECM host (Table 2) and
18 17 to 42% of all root tips were colonized by ECM fungi. On average, 2 morphotypes
19 were observed in the roots of each seedling, except for those of *Q. crispula* and *B.*

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

2

3 **PCR-RFLP patterns and genetic identification**

4 Although DNA amplification using the primers ITS1f and ITS4 resulted in nearly
5 100% amplification of PCR products, some types produced multiple PCR products,
6 possibly because of the presence of other fungi within or around the root tissues. As
7 some samples could not be determined by RFLP analysis alone, we digested the most
8 well-separated and abundant PCR products from each sample with *Hinf*I and *Alu*I, and
9 thus categorized each ECM morphotype (Table 3). With the exception of *Lk*-2, *Ss*-2
10 and *Bp*-2, the banding patterns were identical from different samples within each ECM
11 morphotype.

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

16

17 **Colonization by each ECM morphotype**

18 The ECM fungal flora differed between hosts (Table 4) and 11 of the 15 fungi were
19 observed in the roots of only one host. However, *Laccaria amethystea*, *H. mesopaeum*,

1 *Thelephora terrestris* Fr. and Thelephoraceae 1 were observed in the roots of 2, 3, 4
2 and 4 ECM hosts, respectively. These fungi were abundant and represented most of the
3 ECM colonization in the roots of each woody plant species.

4

5 **Discussion**

6 In 2000, the study site was strongly disturbed by the eruption, which resulted in the
7 loss of nearly all the plant species that had colonized the site before 2000. Thus, almost
8 all seedlings were new recruits that had become established independently on the new
9 substrate. Although the deposition of a thick layer of new volcanic debris around
10 craters must presumably make it problematic for woody plant species to associate with
11 mycorrhizal fungi, such associations were nonetheless observed in the roots of
12 newly-recruited seedlings. Following volcanic eruptions, AM and ECM associations
13 reestablish immediately (Allen et al. 1992), and are of major importance to the primary
14 succession of plant species in volcanic areas (Titus and Tsuyuzaki 2002; Fujiyoshi et al.
15 2005; Tsuyuzaki et al. 2005).

16 At the time of eruption, the scale of disturbance in our study area was relatively
17 small (ca. 71ha) and the surrounding forest edge recovered quickly. It would appear
18 that there was a rapid recovery of, or minimal damage to the fungal flora at the forest
19 edge, as Obase et al. (2005) identified a variety of fungal species by investigating

1 sporocarp occurrence. The speed of recovery of both vegetation and fungal flora in
2 these edge areas, as well as their proximity to the study site, both play a role in the
3 recruitment of mycorrhizal inocula to the devastated area.

4 Almost all seedlings of the dominant woody plant species *P. maximowiczii*, *S.*
5 *hultenii* var. *angustifolia* and *S. sachalinensis* exhibited ECM fungal associations. In
6 2002, only two years after the volcanic eruption, a preliminary study revealed the
7 presence of ECM colonization in the roots of *Salix*. ECM and AM fungi both colonize
8 the Salicaceae (Harley and Harley 1987). In the present study, we observed a very low
9 percentage of AM colonization, with less than half of the seedlings exhibiting an AM
10 association. Thus, it appears that AM fungi represent a relatively insignificant factor in
11 the establishment of Salicaceae seedlings, compared to ECM fungi. In a study on Mt.
12 Fuji, Nara (2006) reported a strong relationship between established *S. reinii*
13 individuals and ECM fungal association, but found only rare associations with AM
14 fungi. In contrast, in about 50% the seedlings of *A. mono* var. *marmoratum* f. *dissectum*
15 associated with AM fungi and no ECM fungi were observed in present study. *Acer* spp.
16 have been observed with AM, ECM or non-mycorrhizal associations (Harley and
17 Harley 1987). Under different environmental conditions, some seedlings of *A. mono*
18 var. *marmoratum* f. *dissectum* associated with AM fungi but others did not form
19 mycorrhizal associations (unpublished data). Thus, it seems *A. mono* var. *marmoratum*

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

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

1 that are undergoing primary succession are only able to associate with a limited range
2 of early-stage ECM fungi.

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

13

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18

1 **References**

- 2 Allen MF, Crisafulli C, Friese CF and Jeakins SL (1992) Re-formation of mycorrhizal
3 symbioses on Mount St Helens, 1980-1990 : interactions of rodents and mycorrhizal
4 fungi. *Mycological Research* 96: 447-453
- 5 Ashkannejhad S and Horton TR (2006) Ectomycorrhizal ecology under primary
6 succession on coastal sand dunes: interactions involving *Pinus contorta*, suilloid
7 fungi and deer. *New Phytologist* 169: 345-354.
- 8 Baxter JW and Dighton J (2001) Ectomycorrhizal diversity alters growth and nutrient
9 acquisition of grey birch (*Betula populifolia*) seedlings in host-symbiont culture
10 conditions. *New Phytologist* 152: 139-149
- 11 Fujiyoshi M, Kagawa A, Nakatubo T and Masuzawa T (2005) Successional changes in
12 mycorrhizal type in the pioneer plant communities of a subalpine volcanic desert
13 on Mt. Fuji, Japan. *Polar Bioscience* 18: 60-72
- 14 Gardes M and Bruns TD (1993) ITS primers with enhanced specificity for
15 basidiomycetes - application to the identification of mycorrhizae and rusts.
16 *Molecular Ecology* 2: 113-118
- 17 Goto H (1937) Revegetation of Volcano Tokachidake after the peculiar eruption in
18 1926. *Journal of the Japanese Forest Society* 19: 537-550
- 19 Harley JL and Harley EL (1987) A check-list of mycorrhiza in the British flora. *New*

- 1 Phytologist (Supplement) 105: 1-102
- 2 Horton TR and Bruns TD (2001) The molecular revolution in ectomycorrhizal ecology:
3 peeking into the black-box. *Molecular Ecology* 10: 1855-1871
- 4 Jumpponen A, Trappe JM and Cazares E (2002) Occurrence of ectomycorrhizal fungi
5 on the forefront of retreating Lyman Glacier (Washington, USA) in relation to time
6 since deglaciation. *Mycorrhiza* 12: 43-49
- 7 Landeweert R, Leeflang P, Smit E and Kuyper T (2005) Diversity of an
8 ectomycorrhizal fungal community studied by a root tip and total soil DNA approach.
9 *Mycorrhiza* 15: 1-6
- 10 Nara K, Nakaya H and Hogetsu T (2003a) Ectomycorrhizal sporocarp succession and
11 production during early primary succession on Mount Fuji. *New Phytologist* 158:
12 193-206
- 13 Nara K, Nakaya H, Wu B, Zhou Z and Hogetsu T (2003b) Underground primary
14 succession of ectomycorrhizal fungi in a volcanic desert on Mount Fuji. *New*
15 *Phytologist* 159: 743-756
- 16 Nara K (2006) Ectomycorrhizal networks and seedling establishment during early
17 primary succession. *New Phytologist* 169: 169-178
- 18 Obase K, Tamai Y, Miyamoto T and Yajima T (2005) Macrofungal flora on the volcano
19 Usu, deforested by 2000 eruptions. *Eurasian journal of Forest Research* 8: 65-70

- 1 Phillips JM and Hayman DS (1970) Improved procedures for clearing roots and
2 staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of
3 infection. *Trans Br Mycol Soc* 55: 158-161
- 4 Püttsepp Ü, Rosling A and Taylor AFS (2004) Ectomycorrhizal fungal communities
5 associated with *Salix viminalis* L. and *S. dasyclados* Wimm. clones in a
6 short-rotation forestry plantation. *Forest Ecology and Management* 196: 413-424
- 7 Reddy MS and Natarajan K (1997) Coinoculation efficacy of ectomycorrhizal fungi on
8 *Pinus patula* seedlings in a nursery. *Mycorrhiza* 7: 133-138
- 9 Titus JH and Tsuyuzaki S (2002) Arbuscular mycorrhizal distribution in relation to
10 microsites on recent volcanic substrates of Mt. Koma, Hokkaido, Japan. *Mycorrhiza*
11 12: 271-275
- 12 Trowbridge J and Jumpponen (2004) Fungal colonization of shrub willow roots at the
13 forefront of a receding glacier. *Mycorrhiza* 14: 283-293
- 14 Tsuyuzaki S (1987) Origin of plants recovering on the volcano Usu, northern Japan,
15 since the eruptions of 1977 and 1978. *Vegetatio* 73: 53-58
- 16 Tsuyuzaki S, Hase A and Niinuma H (2005) Distribution of different mycorrhizal
17 classes on Mount Koma, northern Japan. *Mycorrhiza* 15: 93-100
- 18 van der Heijden EW and Vosatka M (1999) Mycorrhizal associations of *Salix repens* L.
19 communities in succession of dune ecosystems. . *Mycorrhizal dynamics and*

1 interactions of ectomycorrhizal and arbuscular mycorrhizal fungi. Canadian journal
2 of Botany 77: 1833-1841

3 Walker LR and del Moral R (2003) Primary succession and ecosystem rehabilitation.
4 Cambridge University press, Cambridge, UK

5 White TJ, Bruns T, Lee S and Taylor J (1990) Amplification and direct sequencing of
6 fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky
7 JJ and White TJ (ed) PCR protocols: A Guide to Methods and Applications.
8 Academic Press, San Diego, pp 315-322

9 Yang G, Cha JY, Shibuya M, Yajima T and Takahashi K (1998) The occurrence and
10 diversity of ectomycorrhizas of *Larix kaempferi* seedlings on a volcanic mountain in
11 Japan. Mycological Research 102: 1503-1508

12 Yoshii Y (1942) Plant communities on Mount Koma after eruption. Ecological Review,
13 Sendai 8: 170-226

14

15 **Table and Figure Legends**

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

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

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

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

3 *Standard deviations are indicated.

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

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

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

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

11 **Table 4.** Percentage of colonization (Ec)** and frequencies (F) of each ECM fungus

12 observed in ECM hosts*** established at the study site on Mt. Usu, Hokkaido, Japan.*

13 Percentage of colonization and frequencies of these fungi are obscured because two

14 fungal species were included in one ECM type.

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

16 ***See Table 3.

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

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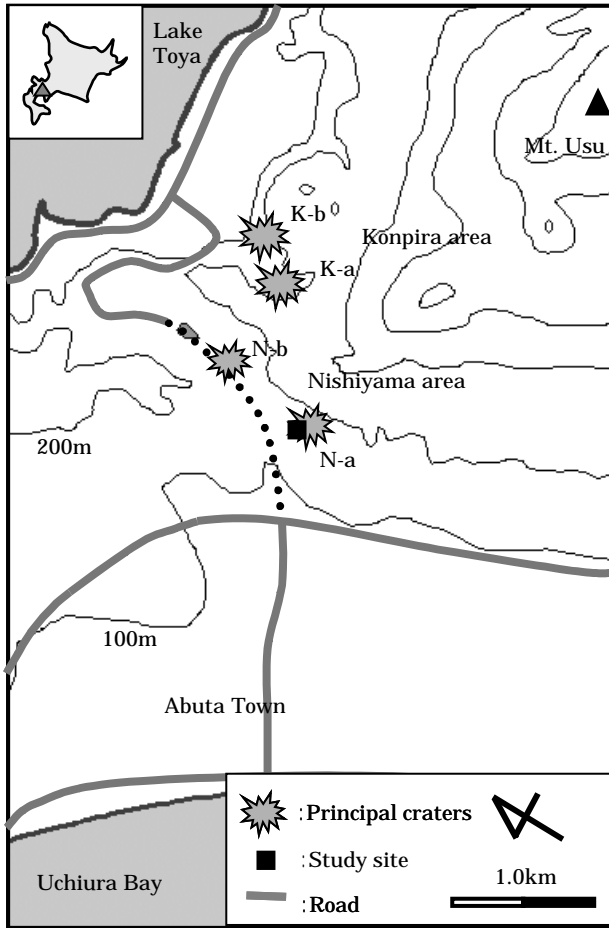


Fig. 1 Location of study site on Mt. Usu, Hokkaido, Japan

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

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

*ECM ectomycorrhizal, AM arbuscular mycorrhizal

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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 ECM (%) [*]
	total	per seedling [*]	
<i>Betula platyphylla</i> var. <i>japonica</i>	6	4.0 ± 1.5	41.8 ± 22.4
<i>Populus maximowiczii</i>	4	1.7 ± 0.5	39.5 ± 24.8
<i>Quercus crispula</i>	1	0.8 ± 0.4	17.4 ± 16.7
<i>Salix hultenii</i> var. <i>angustifolia</i>	4	1.9 ± 0.8	29.3 ± 16.0
<i>Salix integra</i>	4	2.3 ± 0.5	17.3 ± 9.2
<i>Salix sachalinensis</i>	3	2.0 ± 0.9	24.7 ± 12.7

^{*}Standard deviations are indicated

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

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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	<i>Bp</i>		<i>Qc</i>		<i>Pm</i>		<i>Sh</i>		<i>Si</i>		<i>Ss</i>	
	Ec (%)	F (/6)	Ec (%)	F (/6)	Ec (%)	F (/9)	Ec (%)	F (/9)	Ec (%)	F (/6)	Ec (%)	F (/12)
<i>Laccaria amethystea</i>					12.1 (14.2)	6			9.6 (6.9)	6		
<i>Inocybe lacera</i>					18.9 (9.1)*	7*						
<i>Hebeloma mesophaeum</i>	2.2 (1.8)*	4*					18.3 (12.9)	8	3.4 (3.0)	5		
<i>Hebeloma</i> sp.											12.8 (12.9)	7
<i>Scleroderma bovista</i>					74.1	1						
<i>Leccinum scabrum</i>	48.8	1										
<i>Thelephora terrestris</i>			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

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