(1) Title: Distribution of *Frankia* and ectomycorrhizal fungi in a denuded volcanic soil exposed by a landslide during heavy rainfall caused by Typhoon No. 26 (Wipha) in 2013

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(4) Article type: original articles;

(5) Page count for the text: 13

(6) Number of Tables, 5; Number of figures 3

Abstract

In October 2013, shallow landslides occurred on the western slopes of Miharayama central cone on Izu-Oshima lsland, located about 120 km from Tokyo, Japan, caused by heavy rainfall during Typhoon Wipha (No. 26). In December 2013 and June 2014, we collected soils from different depths in a landslide-denuded area and an adjacent, intact forested area. We cultivated *Alnus sieboldiana* seedlings in these soils and examined the formation of root nodules of *Frankia*, a symbiotic nitrogen-fixing actinomycete, and ectomycorrhizal (EM) fungi on alder roots. Nodulation and EM formation were observed in samples from both areas, except in soil collected from 50 to 100 cm depths from the surface of the landslide-denuded soil. In June 2014, we found that *Aln. sieboldiana* seeds had dispersed from neighboring forests had germinated on the surface of the landslide-denuded soil. Root nodules and EM were found on the roots of 91.2 and 93.2 % of 148 seedlings, respectively. According to a bioassay test and field observations, the dominant types of EM fungi that formed on the seedlings were *Alpova* and *Tomentella*. These results show that *Frankia* and some EM species may be important for vegetation recovery after landslides on this island.

Key words: alder; ectomycorrhizal fungi; *Frankia*; Izu-Oshima; landslide

Introduction

Izu-Oshima is the largest island of a group of seven islands south of Tokyo. It is a basaltic stratovolcano with a caldera; the Miharayama central cone is situated at the center of the island (34°43′ N, 139°23′ E). On October 15 and 16, 2013, a heavy rainstorm caused by Typhoon No. 26 (Wipha) hit the island and shallow landslides occurred along the hillside of the Miharayama central cone. The mud flow originated from a layer of pyroclastic fall deposited during a volcanic eruption in 1338 (Yang et al. 2015). In this area, *Alnus sieboldiana* Matsum. and herbaceous plants would have typically colonized volcanic deposits after the 1338 eruption; subsequently, evergreen broadleaf trees became dominant (Ito et al. 2016). As these trees were harvested for fuel wood, they became shrub-like and grew densely; the roots of the trees became intertwined within a depth of 1–2 m (Figure 1). Thus, this shallow landslide occurred at the interface between the surface soil with the roots extended and the layer below (Murakami et al. 2016). The mud flow caused by the 2013 landslide seriously damaged the forests near to the residential area of Izu-Oshima; therefore, reforestation will be required for erosion control.

After an ecosystem is disturbed by a natural disaster, such as a volcanic eruption or landslide, residual soil microbes (especially symbiotic groups) survive in a resistant form or by colonizing dead plant materials saprotrophically in patches and thereby pulling the system back up to its original state via bootstrapping (Perry et al. 1989; Allen 1991). To achieve this, the association of symbiotic microbes with their specific host plants is important for reestablishing diverse plant communities (Wardle et al. 2004). After the volcanic eruption of Mijake-Jima island (70 km south of Izu-Oshima island), *Frankia* and ectomycorrhizal (EM) fungi contributed to the establishment and productivity of *Aln. sieboldiana*, an initial invader of the devastated area of the islands (Yamanaka and Okabe 2006).

In the present study, we investigated the distribution of *Frankia* and EM fungi in the underlying soil that appeared after the surface soil had been removed by the 2013 landslide. To do so, we cultivated *Aln. sieboldiana* seedlings in the soil collected (Yamanaka and Okabe 2006) and sampled one-year-old *Aln. sieboldiana* seedlings

grown after germinating the seeds dispersed on the ground following the 2013 landslide. This species of alder is an actinorhizal plant that fixes atmospheric nitrogen in the root nodules in association with an actinomycete, *Frankia* (Yamanaka et al. 2016), and also forms EM that improve the availability of mineral nutrients and water (Yamanaka et al. 2003). Therefore, these symbiotic associations between alder and the soil microbes are likely to be important for the recovery of vegetation in this area.

Material and methods

Study area

Izu-Oshima is the largest island of the Izu Islands of Japan; it has an area of 91.06 km^2. Climatic data from 1991 to 2020 were obtained from the meteorological station of the island (74 m above sea level). The mean annual precipitation was 2,859 mm, ranging from 406 mm in October to 118 mm in December. Annual temperatures averaged 16.4°C; the minimum monthly temperature was 7.5°C in January, whereas the maximum monthly temperature was 26.0°C in August.

The dominant tree species of the mature forest on the middle and lower part of the hillside were *Eurya japonica* Thunb., *Ilex crenata* Thunb. var. *hachijoensis* Nakai, *Cerasus speciosa* (Koidz.) H. Ohba, *Camellia japonica* L., *Cinnamomum yabunikkei* H. Ohba, and *Castanopsis cuspidata* var. *sieboldii* (Makino) Nakai. The upper part of the hillsides contained pioneer forests consisting of *Aln. sieboldiana* with herbaceous plants, e.g., *Miscanthus condensatus* Hack., and *Polygonum cuspidatum* var. *terminalis* Honda. The mature forest was established on the pyroclastic fall deposited in 1338 (Yang et al. 2015) and the vegetation at this pioneer forest was recovered after recent eruptions.

Soil sampling

In December 2013, 2 months after the landslide, we visited the landslide-denuded area, which is approximately 2 km northwest of the Miharayama central cone. There, we collected soil samples from two sites (indicated by the A and B labels in Figure 1; 430–380 m above sea level), where the surface soil, which is ~1 m thick, had flowed

out. The A and B sites were approximately 0.3 and 0.17 ha in area, respectively. At each site, three soil samples were collected from points (Points 1, 2 and 3) that were at least 10 m from each other; sampling was performed only where no plant debris (e.g., branches, stems, or roots) was present. Before sampling, approximately 2 cm of the surface soil was removed with a trowel. Next, a soil sample was taken to a depth of 10 cm using a trowel. In addition, soil samples were collected from two points (shown as a and b in Figure 1) in an adjacent forested area unaffected by the landslide. Before sampling, litter accumulated on the soil surface was removed with a trowel. Soil was then collected at depths of 0–10, 10–20, 30–40, and 50–60 cm at one of the points and at 0–10 and 10–20 cm at another point. The trowel was cleaned with 70% ethanol between collection attempts. The amount of soil collected per sample ranged from 500 to 1,000 ml, which is equivalent to approximately 730–1,060 g by fresh weight. Soil samples were then placed in plastic bags, which were kept in a cold box during transportation to the laboratory. There they were stored in a refrigerator at 4°C before being used in bioassays, as described below. The chemical properties of the denuded soil have been reported by Sone et al. (2015).

In June 2014, soil samples were again collected from two points (Points 4 and 5) at site B (Figure 1) at depths of 0–10, 10–20, 30–40, 50–60, and 90–100 cm to examine the vertical distribution of *Frankia* and EM fungi.

Cultivation of Aln. sieboldiana in collected soils

Distribution of *Frankia* and EM fungi in the soil was examined by a bioassay test using *Aln. sieboldiana* seedlings (Yamanaka and Okabe 2006). Seeds of *Aln. sieboldiana* were immersed in running water for one day, surface-sterilized for 20 min in 30% H_2O_2, and then rinsed with sterilized water three times. The seeds were placed on 0.9% plain agar medium in 90-mm Petri dishes for germination. The Petri dishes were placed in a growth chamber at 28°C with continuous light at 124 μmol m^{-2} s^{-1} of photosynthetically active radiation (400–700 nm). Germinated seeds were transplanted into soil in a 66-ml RayLeach tube (RLC-4; Stuewe & Sons, OR, USA). First, 20 ml of a sterilized mixture (SM) of volcano ash (akadama soil)–diatomaceous earth [3:2 (v:v)]

was added to a tube and then 30 ml of the collected soil was added after removing the roots and plant debris from the soil. Subsequently, the soil was covered with 10 ml of SM. In this manner, soil samples were assayed without splashing out occurring when they were watered. Eight to 12 replicates were prepared for each of soil samples. The plants were grown in a naturally illuminated greenhouse for 7 months during the survey in 2013 or for 8 months during the survey in 2014. Temperature, light intensity, and photoperiod fluctuated throughout the study. At harvest, the seedlings were washed gently in running tap water and cleaned by removing soil particles with fine forceps.

Sampling of Alnus seedlings from the landslide area

In June 2014, we observed that *Aln. sieboldiana* seedlings had grown on denuded soil at both the A and B sites (Figure 1) used for soil sampling in December 2013. Our observations suggested that *Aln. sieboldiana* seeds had dispersed from neighboring forests after the landslide of October 2013 and had moved by the wind and/or by water flow to slightly concave points in the focal sites, where they then germinated (Figure 2). We collected alder seedlings from some of these concave points; sampled plants were at least 5–10 m from each other. We collected plants together with the soil using a shovel. We were careful not to damage the plant roots and placed sampled seedlings in plastic bags for transport. After returning to the laboratory, the soil was gently removed from the seedlings using running water. In total, 148 seedlings were used to observe the colonization states of both EM fungi and *Frankia*, as described below. In addition, we measured the shoot heights of the collected seedlings and counted their leaf numbers.

Analysis

Under a stereomicroscope, the number of *Frankia* root nodules was counted, and the EM were classified into morphotypes based on the external color and conditions of emanating hyphae (Agerer 1991). Several EM tips from each morphotype were used for DNA extraction for identification of EM fungal species (Taniguchi et al. 2021). Briefly, DNA was extracted from 1 or 2 EM tips using the cetyltrimethylammonium bromide (CTAB) method (Gardes and Bruns 1993). The extracted DNA was amplified with

AmpliTaq Gold 360 Master Mix (Applied Biosystems) and fungi-specific primer pairs, ITS1F (Gardes and Bruns 1993) and ITS4 (White et al. 1990). Amplicons were visualized on 0.7% agarose gels stained with SYBR Green I (Molecular Probes). The successfully amplified PCR products were cleaned with exonuclease I and shrimp alkaline phosphatase and then sequenced by Eurofins Genomics K.K. (Tokyo, Japan). After grouping the sequences at the species level as described in Taniguchi et al. (2018), the representative DNA sequences were blasted against the UNITE databases (Nilsson et al. 2019) (https://unite.ut.ee/analysis.php#) to assign a taxonomic name to each sequence. Individual sequences were deposited in GenBank under the accession numbers LC646347–LC646352. The dry weight of shoots and roots was determined after oven-drying to a constant weight at 50℃.

Two-way analysis of variance (ANOVA) and the Tukey–Kramer multiple comparison test (BellCurve for Excel, Social Survey Research Information Co., Japan) were used to statistically analyze data on plant growth and the numbers of root nodules and EM fungi formed in the bioassay of the soil samples collected from the forested area in December 2013 and from the landslide area in June 2014. Data from the bioassay test of soil samples collected from the forested area in December 2013 were analyzed using one-way ANOVA and the Tukey–Kramer multiple comparison test. Values of plant sizes collected by the June 2014 survey were presented as the mean ± standard error.

Results

Plant bioassay

In the forested (nonlandslide) area surveyed in December 2013, a statistically significant difference existed in the number of nodule lobes between the two collection points according to a two-way ANOVA including data from two soil layers collected at 0–10 and 10–20 cm depths (Tables 1 and 2). At each of these points, the number of root nodules did not differ in the soils collected from depths of 0–10 and 10–20 cm. Shoot growth following cultivation in soil taken from a depth of 0–10 cm was higher than that observed in the lower soil layer at Point a. Although total numbers of EM in

soils collected from 0–10 and 10–20 cm depths were not statistically different, EM numbers were lower in soil taken from depths of 30–40 and 50–60 cm at Point b. The types of EM observed in the forested area could be roughly divided into two groups based on morphology and color (Figure 3a, b). EM1 was monopodial to irregularly compound with many lateral root tips, 0.4–0.6 mm in diameter, cylindrical or tapering toward the tip, with a surface that was silky, golden in young then dark yellow or brown in old, with yellow extramatrical hyphae, and with well-developed yellow to light brown hyphal strands. EM1 was identified as *Alpova diplopholoeus* (Figure 3a) based on DNA analysis (Table 3); this was the dominant EM type, which accounted for 84.3% of all EM formed in this bioassay test. EM1 was detected in all of the soil layers in the forested area. EM2 was monopodial, straight or slightly flexuous, cylindrical or tapering to the apex, occasionally had the nonmycorrhizal root apex exposed, ca. 0.3 mm in diameter, with a wooly to downy surface, and with scattered extramatrical hyphae. EM2 was identified as *Tomentella* spp. because the morphotype included three *Tomentella* species according to DNA analysis (*Tomentella testaceogilva* (Figure 3b), *Tomentella ellisii*, and an unidentified *Tomentella* species), but they could not be clearly distinguished based on their morphology. EM2 comprised 15.7% of total EM formed. The number of EM2 was higher in the upper soil layer (0–10 cm) compared with the numbers in the other soil layers examined (Table 1).

In the landslide area, the numbers of root nodules and EM in the soil differed depending on the collection point within the site (Table 4). Accordingly the growth of alder seedlings cultivated in these soils was different. In addition to the two types of EM observed in the forested area, another type of EM (EM3; Figure 3c) was observed in the landslide area. EM3 was monopodial, 0.3–0.4 mm in diameter, straight, cylindrical or tapering slightly toward the tip, with a silky surface, white occasionally with the nonmycorrhizal root apex exposed, and with hyaline extramatrical hyphae. EM3 was identified as *Hebeloma submelinoides* by molecular identification (Table 3). EM3 was observed only in the roots of a plant from the landslide area.

The survey of the landslide area in June 2014 showed that nodulation and EM formation occurred in the soil taken from depths of 0–10, 10–20, and 30–40 cm at

Point 4 (Table 5). The growth of the seedlings cultivated in soil collected from a depth of 0–10 cm was higher than that of seedlings cultivated in the deeper soil layer. At Point 5, nodulation was observed in the soil taken from a depth of 0–10 cm; however, no EM fungi were formed in any soil layer collected and there was no significant difference in the plant growth among the soil layers.

Alnus seedlings growing in the landslide area

We examined 148 *Aln. sieboldiana* seedlings. The mean seedling shoot height was 1.5 ± 0.0 cm and the mean number of leaves per a plant was 3.4 ± 0.0. Among them, 135 seedlings were nodulated and 138 seedlings formed EM. The average numbers of nodule lobes and EM tips were 3.5 and 12.5 per a seedling, respectively. Of 13 seedlings without nodulation, four had no EM. The EM1 and EM2 from the bioassay test were also observed in this survey. Additionally, another type of EM (EM4) was observed (Figure 3d). EM4 was monopodial, 0.3–0.4 mm in diameter, straight, cylindrical, with a net-like surface, and with black emanating hyphae. EM4 was identified as a species from the *Ceratobasidiaceae* family. Among 138 EM seedlings, 92 were associated with one type of the three EM types, 43 were associated with EM1 and EM2 or EM4, and 3 seedlings were associated with all 3 EM types.

Discussion

In the forested area, *Frankia* nodulation was observed in the soil taken from depths at 0–60 cm (Table 2) where tree roots had developed in pyroclastic materials deposited presumably during the volcanic eruption in 1338. At the early stage of vegetation recovery in this area, alder had most probably been dominant with *Frankia* nodules on their roots as is the case in similar forests today (Figure 2 in Ito et al. (2016)). Afterwards, as well as vegetation changes from primary forests to mature forests during plant succession, *Aln. sieboldiana* should have been inhibited by late-successional trees. However, the alders have persisted in the forests because eruptions occurred repeatedly in Izu-Oshima (Yang et al. 2015) and *Frankia* most probably existed as a symbiont in root nodules of alder. *Frankia* can survive as a dormant form

such as spores, or as a saprotroph in soil (Samant et al. 2016).

The nodulation capacity identified during a bioassay was found to differ between samples taken at two points in the forested area (Tables 1 and 2). Tree species in the forested area were the non-actinorhizal *Eurya*, *Ilex*, *Cerasus*, *Camellia*, *Cinnamomum*, and *Castanopsis*. However, *Frankia* strains capable of infecting alders were also present without a host plant (Benson and Dawson 2007). The nodulation capacity varies depending on soil properties, plant species composition, and cultivation conditions (Chaia et al. 2010).

Ectomycorrhizal species that are symbiotic with alder were dominantly *Alp. diplopholoeus* and *Tomentella* spp. in the bioassay tests (Table 1 and 4) and in the field study. The former species is well known as an alder-specific species (Miller et al. 1992) and has also been detected in the soil of an *Aln. sieboldiana* forest on Miyake Island (Yamanaka and Okabe 2003). In contrast, *Tomentella* species are reported to be widespread EM fungi that grow in the organic soil horizon (Põlme et al. 2013; Nouhra et al. 2015). These findings from previous reports is consistent with detection of *Tomentella* species in the surface soil layer in the present study. Conversely, *Alp. diplopholoeus* species were detected in deeper soil layers (Table 1). The bioassay test using *Aln. sieboldiana* seedlings showed that *Alpova* was detected in the soil at depths of 160–170 cm, which was collected 34 months after the alder forest was destroyed in the volcanic eruption of July 2000 (Yamanaka and Okabe 2006). Miller et al. (1992) reported that EM of *Alp. diplopholoeus* type was detected in the soil of a 450-year-old conifer forest as well as in that of a 22-year-old alder forest in the bioassay test. Thus, *Alpova* can survive for many years in soil; it becomes activated again when the appropriate host is established after natural disasters such as volcanic eruptions and landslides.

In the present study, only two types of EM were dominant (Tables 1 and 3). Such low diversity of *Alnus*-associated EM communities was also observed in previous studies (Miller et al. 1992; Bogar and Kennedy 2013; Nouhra et al. 2015). In addition to these two types, *H. submelinoides* (syn. *Alnicola submellinoides*) was also detected in the soil of the landslide area (Figure 3c; Table 3); this species has previously been

reported from alder forests (Moreau 2005). In addition, a species belonging to *Ceratobasidiaceae* was obtained from the seedlings of *Aln. sieboldiana* growing in the landslide area (Figure 3d; Table 3). *Ceratobasidiaceae* is a family that contains species with all major nutritional modes (i.e., saprotrophic, pathogenic, orchid mycorrhizal, and EM); these modes are phylogenetically conserved (Veldre et al. 2013). The *Ceratobasidiaceae* species identified in the present study was included in a clade of the orchid mycorrhizal group. However, orchid mycorrhizal species also formed EM in a previous inoculation study (Yagame et al. 2008). Further research on mycorrhization via *in vitro* inoculation studies will be necessary to confirm the association between alder and *Ceratobasidiaceae* species.

We did not analyze genes from *Frankia* strains in *Aln. sieboldiana* root nodules in our bioassay experiment or in our sampling of seedlings growing in the landslide-denuded site. Three major groups or clusters of *Frankia* strains have been identified by analyzing nucleotide sequences from different *Frankia* strains (Pawlowslki and Demchenko 2012). *Frankia* strains from cluster 1 form nodules on plants of *Betulaceae* (*Alnus*), *Myricaceae* (*Myrica*, *Mozella*, and *Comptonia*), and *Casuarinaceae* (*Casuarina* and *Allocasuarina*). Pokharel et al. (2011) reported different subgroups within *Alnus* infection groups (cluster 1) by sequence analyses of *nif*H gene fragments in nodules. Nagashima et al. (2008) reported that a *Frankia* culture obtained from *Aln*. *sieboldiana* growing in Okayama, western Japan, belonged to cluster 1a (Jeong et al. 1999). Schwob et al. (2018) showed that *in planta* sporulation of *Frankia* in alders differed depending on the host plant species and on soil conditions. They hypothesized that the *in planta* sporulation type may affect nutrient conditions in its host plant and determine the symbiotic association.

After the October 2013 landslide (Figure 1), *Frankia* and EM fungi were present at the surface layer of the denuded soil (Tables 3 and 4) prior to the establishment of host plants (i.e., alder). These symbiotic microbes may be repeatedly dispersed by wind and water from neighboring forests (Dawson et al. 2005) and may be present in a dormant form (i.e., spores) (Schwob et al. 2018; Glassman et al. 2016) because environmental conditions, such as temperature and humidity, may be severe on the denuded soil

surface. Vegetation recovery began with the germination of *Aln. sieboldiana* seeds originating from neighboring forests (Figure 2). Compounds from the roots of host plants can stimulate the germination of *Frankia* spores and EM fungi (Krumholz et al. 2003; Kikuchi et al. 2007), which can, in turn, improve the growth of the host plant via the formation of symbiotic associations. *Frankia* and EM fungi develop in the soil by obtaining carbon from host trees. Conversely, *Frankia* fix nitrogen in the root nodules, and EM fungi solubilize mineral nutrients that have poor mobility in soil, such as phosphate and iron. Vegetation recovery after landslides is thereby faster and the fertility of the soil is improved by the supply of fixed nitrogen and minerals from these microbes.

In the present study, we focused on *Frankia* and EM fungi improving soil fertility in denuded soil after landslides. Accordingly, a variety of soil microbes grow in this soil and, in turn, may affect multiple symbioses among actinorhizal plants, *Frankia*, and mycorrhizal fungi (Chaia et al. 2010). The coexistence of interacting microbes in the rhizosphere implies a complex network of nutrient dynamics during vegetation recovery. Further studies on the effects of these rhizosphere microbes on multiple symbioses involving actinorhizal plants, *Frankia*, and mycorrhizal fungi, as well as on ecological succession, are necessary.

Disclosure statement

No potential conflict of interest was reported by the authors.

Acknowledgments

This study was financially supported by a research grant (#201405) from the Forestry and Forest Products Research Institute. We would like to thank the members of Oshima Island branch office of Tokyo metropolitan government and of the office of Oshima-town, Tokyo, for arranging our survey at Izu-Oshima Island.

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Figure legends

Figure 1. Shallow landslides occurred on the western side of Izu-Oshima Island in October 2013. Landslide-denuded soils were collected from two sites (A and B), and soil from the forested site was collected at two points (a and b). Photo taken on December 11, 2013.

Figure 2. *Alnus sieboldiana* seedlings growing on the ground in a landslide area. Seeds were dispersed from the neighboring forests after the landslides on October 2013 and they germinated in 2014. Photo taken on June 24, 2014

Figure 3. Ectomycorrhizae observed in the present study. a) EM1: *Alpova diplopholoeus*; b) EM2: *Tomentella testaceogilva*; c) EM3: *Hebeloma*

submelinoides; d) EM4: *Ceratobasidiaceae* sp. Scale bars = 1 mm.

Figure

Figure 1

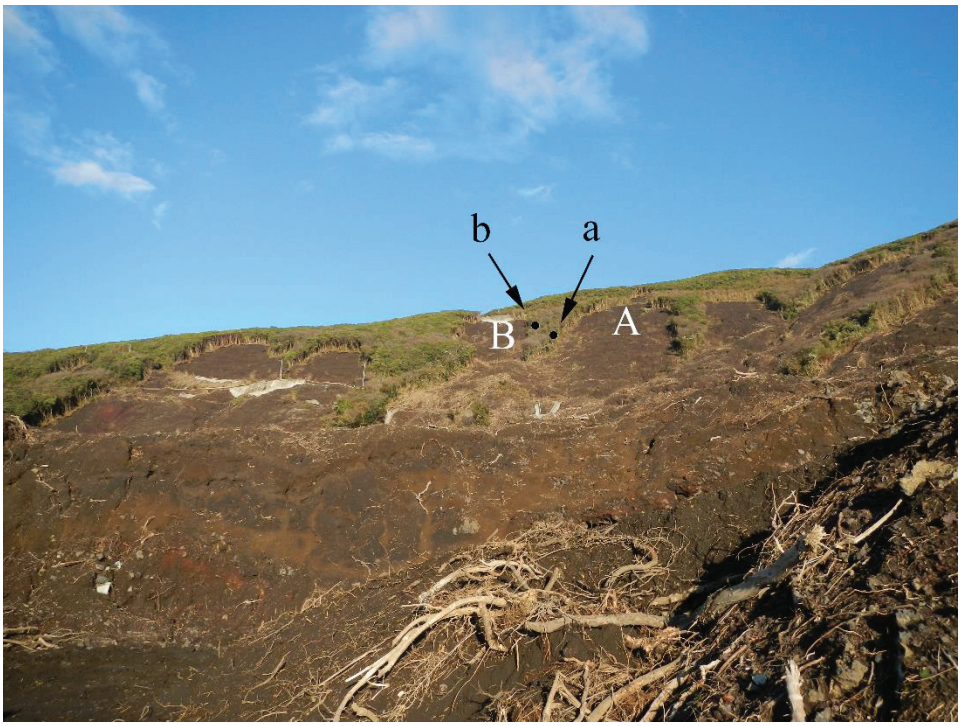

Figure 2

Figure 3

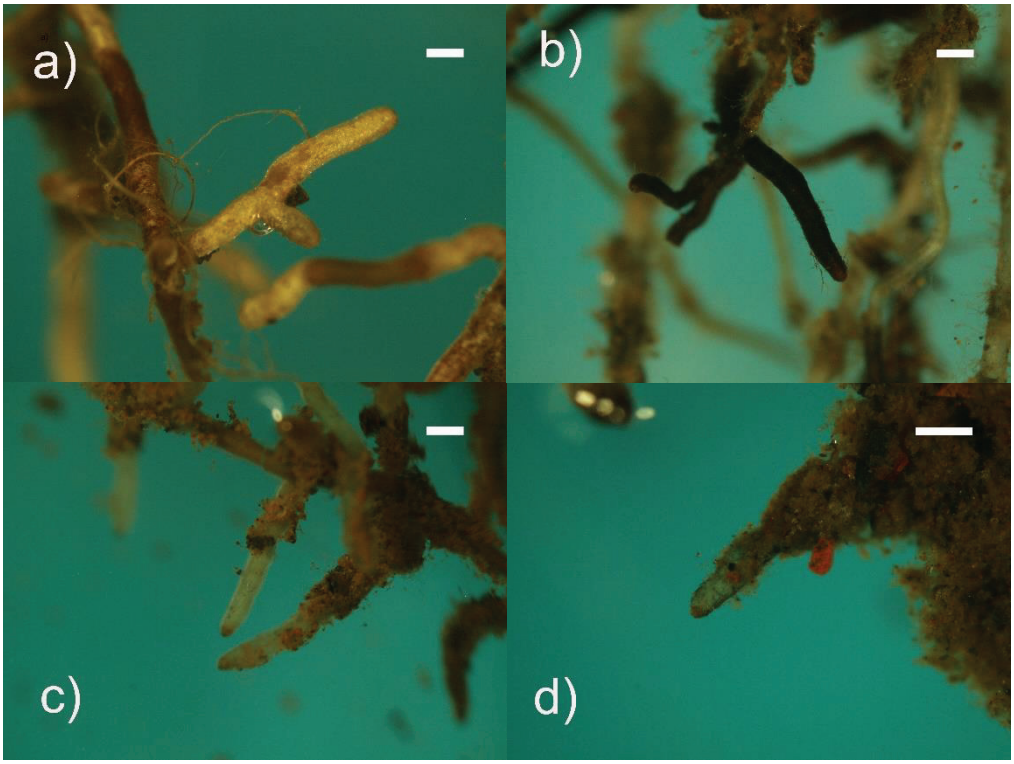

Table 1

Table 1. Growth of *Alnus sieboldiana* seedlings cultivated in soil collected from a forested area in a survey conducted in December 2013.

Sampling point	Depth (cm)	Replicate	Dry weight of plant (g)			Number of nodule lobes per plant	Number of ectomycorrhizal (EM) tips per plant		
			Total	Shoots	Roots		Total	EM1	EM2
a	0-10	11	0.23 (0.05)	0.14 (0.03)	0.10 (0.03)	20.1 (6.1)	162 (41)	148 (44)	14 (19)
	10-20	11	0.21 (0.05)	0.11 (0.02)	0.10 (0.02)	16.4 (3.5)	147 (38)	147 (37)	0 (0)
b	0-10	9	0.21 (0.04)	0.09b (0.02)	0.12 (0.02)	3.9 (1.8)	126b (16)b	48 (22)	78b (14)
	10-20	8	0.07 (0.01)	0.02a (0.00)	0.04 (0.01)	0.0 (0.0)	58b (13)b	58 (13)	0.0a (0.0)
	30-40	11	0.09 (0.05)	0.01a (0.00)	0.09 (0.05)	1.5 (0.7)	9a (5)a	9 (5)	0.0a (0.0)
	50-60	11	0.09 (0.04)	0.02a (0.01)	0.06 (0.03)	5.1 (1.8)	32a (22)a	31 (22)	0.2a (0.2)
Sterilized soil (control)		6	0.02 (0.00)	0.00 (0.00)	0.01(0.01)	0 (0)	0 (0)	0 (0)	0 (0)

Data are given as means (with SE in parentheses) calculated from 6–11 replicates. Yields followed by different letters within each point are significantly different at $P < 0.05$ according to Tukey–Kramer tests after one-way analysis of variance.

Table 2

Table 2. Analysis of variance for the effects of site and soil depth on the dry weights (DWs) of shoots and roots, numbers of nodule lobes, and numbers of ectomycorrhizal (EM) tips of *Alnus sieboldiana* in forested sites in a survey conducted in December 2013.

Sources of variation	Degrees of freedom	DW of shoots		DW of roots		Numbers of nodule lobes		Numbers of EM tips	
		F	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Site	1	7.991	0.008	0.516	0.477	15.904	0.000	3.548	0.068
Depth	1	3.214	0.082	2.754	0.106	0.870	0.357	1.565	0.219
Site × depth	1	0.698	0.409	3.745	0.061	0.000	0.984	0.649	0.426

Table 3

Table 3 Results of BLAST search against the UNITE database and putative identification of ectomycorrhizal (EM) morphotypes.

EM morphotypes	Accession number	Putative identification	Division	Family	Length (bp)	Closest BLAST match (accession number)	Score	E-value	Percent identity (%)
EM1	LC646347	*Alpova diplophloeus*	Basidiomycota	Paxillaceae	788	*Alpova diplophloeus* (UDB037694)	1287	0.0	97
EM2	LC646350	*Tomentella ellisii*[1]	Basidiomycota	Thelephoraceae	644	Thelephoraceae (MK285935)	1137	0.0	99
	LC646351	*Tomentella testaceogilva*[2]	Basidiomycota	Thelephoraceae	642	Thelephoraceae (MK285920)	1154	0.0	99
	LC646352	*Tomentella* sp.[3]	Basidiomycota	Thelephoraceae	645	Thelephoraceae (KU924719)	1148	0.0	99
EM3	LC646348	*Hebeloma submelinoides*	Basidiomycota	Hymenogastraceae	677	*Hebeloma submelinoides* (AY900095)	1073	0.0	99
EM4	LC646349	Ceratobasidiaceae sp.	Basidiomycota	Ceratobasidiaceae	544	Ceratobasidiaceae (KJ716222)	817	0.0	94

[1] DNA sequence (LC646350) had 99% similarity with *T. ellisii* (UDB039489) according to a BLAST search in UNITE (https://unite.ut.ee/), although the highest score was obtained with the sequence of Thelephoraceae (MK285935).

[2] DNA sequence (LC646351) had 99% similarity with *T. testaceogilva* (GQ398248) according to a BLAST search in UNITE (https://unite.ut.ee/), although the highest score was obtained with the sequence of Thelephoraceae (MK28592).

[3] Closest sequence (KU924719) was identified as Thelephoraceae in UNITE, but the original annotation of the DNA sequence was *Tomentella* sp.

Table4

Table 4. Growth of *Alnus sieboldiana* seedlings cultivated in soil collected from a landslide-denuded area in a survey conducted in December 2013.

Sampling		Replicate	Dry weight of plant (g)			Number of nodule lobes per plant	Number of ectomycorrhizal (EM) tips per plant			
Site	Point		Total	Shoot	Root		Total	EM1	EM2	EM3
A	1	11	0.23a (0.05)	0.10b (0.02)	0.13a (0.03)	16.4b (6.1)	166b (46)	161b (45)	2 (2)	2 (2)
	2	11	0.04a (0.01)	0.01a (0.00)	0.03a (0.01)	1.8a (0.8)	8a (6)	8a (6)	0 (0)	0 (0)
	3	11	0.10ab (0.03)	0.03a (0.01)	0.08ab (0.02)	4.7ab (1.3)	41a (14)	0a (0)	41 (14)	0 (0)
B	1	11	0.03x (0.01)	0.01x (0.00)	0.02x (0.01)	0.5x (0.4)	0x (0)	0x (0)	0x (0)	0 (0)
	2	11	0.09x (0.02)	0.04x (0.01)	0.05x (0.01)	3.0x (1.0)	49x (19)	47xy (18)	2x (2)	0 (0)
	3	11	0.28y (0.07)	0.11y (0.03)	0.18y (0.04)	16.5y (4.2)	181y (40)	119y (38)	61y (26)	0 (0)
Sterilized soil (control)		6	0.02 (0.00)	0.00 (0.00)	0.01(0.01)	0.0 (0.0)	0 (0)	0 (0)	0 (0)	0 (0)

Data are given as means (with SE in parentheses) calculated from 6–11 replicates. Yields followed by different letters within each site are significantly different at $P < 0.05$ according to Tukey–Kramer tests after one-way analysis of variance.

Table 5

Table 5. Growth of *Alnus sieboldiana* seedlings cultivated in soil collected from Site B in a survey conducted in June 2014.

Sampling point	Depth (cm)	Replicate	Dry weight of plant (mg)			Number of nodule lobes per plant	Number of ectomycorrhizal tips per plant
			Total	Shoots	Roots		
4	0-10	12	128b (32)	30b (10)	98b (22)	2.8b (1.0)	51b (18)
	10-20	11	37a (11)	7a (3)	30a (8)	0.4ab (0.4)	8a (8)
	30-40	11	28a (4)	6a (1)	22a (4)	1.5ab (1.5)	0.5a (0.5)
	50-60	12	39a (7)	4a (1)	35a (7)	0.0a (0.0)	0.0a (0.0)
	90-100	11	21a (3)	3a (1)	18a (2)	0.0a (0.0)	0.0a (0.0)
5	0-10	12	23 (2)	5 (1)	19 (2)	0.0 (0.0)	0.0 (0.0)
	10-20	12	20 (2)	4 (0)	16 (3)	0.0 (0.0)	0.0 (0.0)
	30-40	12	21 (1)	4 (0)	16 (1)	0.0 (0.0)	0.0 (0.0)
	50-60	12	27 (4)	4 (0)	23 (4)	0.0 (0.0)	0.0 (0.0)
	90-100	11	25 (2)	4 (0)	21 (2)	0.0 (0.0)	0.0 (0.0)
Sterilized soil (control)		11	26 (2)	6 (1)	19 (1)	0.0 (0.0)	0.0 (0.0)

Data are given as means (with SE in parentheses) calculated from 11 or 12 replicates. Yields followed by different letters within each point are significantly different at $P < 0.05$ according to Tukey–Kramer tests following one-way analysis of variance.