

Mycorrhizal status of alpine plant communities on Mt. Maedake Cirque in the Japan South Alps

Atsushi Kagawa¹, Masaaki Fujiyoshi^{2*}, Miki Tomita¹ and Takehiro Masuzawa¹

¹Department of Biology and Geosciences, Graduate School of Science and Technology, Shizuoka University, Ohya 836, Shizuoka 422-8017

²Department of Human Development, School of Humanities and Culture, Tokai University, Kitakaname 1117, Hiratsuka 259-1292

*Corresponding author. E-mail: masaakif@keyaki.cc.u-tokai.ac.jp

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Abstract: To clarify the mycorrhizal status of alpine plants, we sampled roots of 33 species from 15 families growing on Mt. Maedake Cirque in the Japan South Alps. Ericoid mycorrhiza, ectomycorrhiza and arbuscular mycorrhiza (AM) were observed in the study site. Colonization classified as ericoid mycorrhizal was observed with *Phyllodoce aleutica*, *Tripetaleia bracteata*, *Vaccinium uliginosum*, *Rhododendron aureum*, *Arctericia nana* and *Shortia soldanelloides* f. *alpina*, and ectomycorrhizal colonization with *Pinus pumila* and *Polygonum viviparum*. AM plants were found in species belonging to the following families: Compositae, Geraniaceae, Ranunculaceae, Rosaceae, Gramineae, Liliaceae, Violaceae, Crassulaceae, Gentianaceae and Campanulaceae. AM was the predominant mycorrhizal type in the study site and average colonization levels of AM fungi were within a wide range of 1.2 to 76.1%. In conclusion, mycorrhizal associations were observed in most (91%) of the plant species examined under the severe climate conditions of the study site.

Key words: mycorrhiza, arbuscular mycorrhizal fungi, alpine plant community, cirque, Japan South Alps

Introduction

In many alpine areas, the rate of mineralization of nutrients is slow due to the low mean annual temperature, and consequently, the availability of important major nutrients is restricted. Accordingly, poor soil nutrient concentration may be a major factor determining the productivity of alpine ecosystems (Bowman *et al.*, 1993).

Mycorrhizas are symbiotic relationships between plant roots and fungi, both of which potentially benefit from the association (Smith and Read, 1997). It is well known that most terrestrial vascular plants form mycorrhizas, which can be divided into seven distinct types: ecto-, ectoendo-, ericoid, arbuscular, arbutoid, orchid, and monotropoid (Harley, 1989; Smith and Read, 1997). There are a number of distinct qualitative differences in the nature of the substances that are moved in these different types of mycorrhizal association, and the ability of a host plant to access soil nutrients is largely dependent on its mycorrhizal type (Melin and Nilsson, 1953; Read and Stribley, 1973; Tinker, 1975; Smith and Read, 1997). For example, it is known that arbuscular mycorrhizal (AM) for-

mation influences uptake of nutrients, especially P (Tinker, 1975), while the major effect of ericoid mycorrhiza is the enhancement of host nitrogen content (Read and Stribley, 1973). Ectomycorrhizal formation appears to occupy an intermediate position. Mycorrhizal associations may therefore be especially important in severe environments such as alpine and sub-alpine regions where plant growth and nutrient availability is limited by environmental stress.

The mycorrhizal status of alpine plants has been studied to some extent in different alpine areas, and several authors have observed a variety of mycorrhizal types on roots of alpine herbs and shrubs (Haselwandter and Read, 1980; Read and Haselwandter, 1981; Blaschke, 1991; Treu *et al.*, 1996). It has also been reported that plants growing in the alpine zone are mycorrhizal to a high degree (Miller, 1982; Haselwandter, 1987; Barni and Siniscalco, 2000; Gai *et al.*, 2006). In Japan, however, little is known about the mycorrhizal types and colonization of plant species growing in alpine regions.

Japan, having been linked in the past to the Eurasian landmass, has a number of alpine plant species in its alpine zone, including those endemic to the area. The alpine zone of Japan is characterized by mosaic vegetation, with mixed plant communities consisting of, for example, herbaceous plants, snow-bed and fell-field communities and *Pinus pumila* scrub. The *Pinus pumila* scrub is unique in that it is not an extension of the forest zone regarded as the timberline ecotone (Okitsu, 1985). In addition, Japan has an exceptional number of ground-hugging heath shrubs able to tolerate acid soils and with strong, deep roots that secure them to steep scree slopes (Olver, 1991). For example, 20 genera from the heath family Ericaceae are known to exist such as the woody plants *Rhododendron*, *Phyllodoce*, *Cassiope*, *Arctous* and *Vaccinium*. Since the alpine zone of Japan has a variety of flora and vegetation types, it is possible that various types of mycorrhiza associations also exist.

This study aimed to survey mycorrhizal formation on plants growing in an alpine region in Japan. We also examined the colonization and spore density of AM fungi in the observed alpine plant communities.

Methods

Investigations were carried out in an alpine region in the Japan South Alps. The Japan South Alps are the largest mountain range in Japan, spreading over three prefectures, Shizuoka, Yamanashi and Nagano, in central Honshu and including 12 peaks higher than 3000 m above sea level. A typical glacial landform remains in the Arakawa Mountains (Mt. Maedake, Mt. Nakadake and Mt. Warusawa) in the central South Alps. This glacial landform is located at the southern limit of the Japanese archipelago. The Maedake Cirque (35°29'35"N, 138°10'00"E), one of three glacier geographical features formed on the southwest of the Arakawa Mountains, was selected as the study site (Fig. 1).

A number of different types of established plant communities are found in the study site (Masuzawa *et al.*, 2005, Masuzawa *et al.*, 2006a, b) (Fig. 2A), with (I) a fell-field community at the cirque bottom, (II) an alpine plant community dominated by perennial herbs, and (III) *Pinus pumila* moraine scrub being the predominant vegetation types. Community (I) contains several pioneer plants and is dominated by *Deschampsia flexuosa*; the coverage in community (I) ranges from 20 to 30%. Tall perennial herbs such as

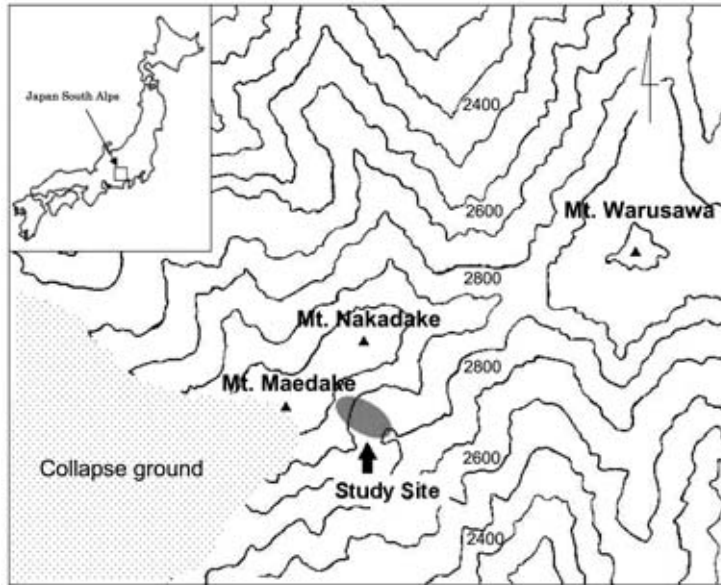


Fig. 1. Location of the study site in the Japan South Alps.

Polygonum weyrichii var. *alpinum*, *Lilium medeoloides* and *Ranunculus acris* var. *nipponicus* are found in community (II), with coverage ranging from 90 to 100%. In community (III), *P. pumila* dominates the central area, while herbs and grasses such as *Viola biflora*, *Artemisia sinanensis*, *Geranium yesoense* var. *nipponicum* and *Gramineae* sp. are found in peripheral areas. The coverage in community (III) ranges from 90 to 100%.

Roots of 33 native species belonging to 15 families were collected from different plant communities including I–III from 25 July to 1 August 2004. Root samples were carefully excavated with shoots and adhering soil. Any soil particles and organic debris adhering to the roots were carefully removed in a water bath using a paintbrush. Special care was taken to eliminate contaminants originating from the roots of other species.

To determine the density of AM fungal spores in the soil, three quadrats measuring 2m×2m (I) or 1m×1m (II–III) were placed in each of the three plant communities. Three core soil samples (0–10 cm in depth) were randomly collected from each quadrat, and to eliminate litter and gravel, were air-dried and passed through a 2-mm mesh sieve prior to analysis.

Part of each root sample (0.01–1 g in dry weight) was used for mycorrhizal type identification. The roots were cut into 1–2 cm segments, washed with 2.5% (w/v) KOH solution, and stained with 0.05% (w/v) trypan blue in acidic glycerol according to the method of Koske and Gemma (1989). Root segments were then viewed under light microscopy at either ×100 or ×400 and screened for mycorrhizal infection. If a fungal sheath-like structure was observed, a cross section of the root sample was viewed under light microscopy at ×100 or ×400 to determine whether or not it was ectomycorrhizal.

Mycorrhizal types were determined according to the criteria of Harley (1989). To avoid confusion with non-mycorrhizal fungi, which also have non-septate internal hy-

phae, AM fungi were recorded only when arbuscules or vesicles were present. Ectomycorrhizas were recorded when both a fungal sheath and Hartig net were observed. Determinations of other mycorrhizal types were made by observations of the fungal sheath, Hartig net, fungal coil, or a combination of these three structures. Each root system was examined for the presence or absence of each mycorrhizal type.

To estimate the extent of AM colonization, 10 randomly selected root fragments were mounted on slides. The presence or absence of AM colonization (internal hyphae, vesicles or arbuscules) was determined using light microscopy ($\times 100$) on intersecting vertical gridlines (McGonigle *et al.*, 1990; Klironomos *et al.*, 1996). AM colonization (AC) was quantified as follows:

$$AC = Ni/Nt \times 100 (\%),$$

where Ni is the number of vertical gridlines intersected by infected roots (AM) and Nt is the total number of vertical gridlines intersected by roots (both infected and noninfected).

The number of spores in the soil samples was determined by modification of the sucrose centrifugation method (Daniels and Skipper, 1982; Nishio, 1987). Three grams of air-dried soil was suspended in water and centrifuged at 1500 rpm for 5 min. The precipitate was then suspended in 50% sucrose and centrifuged at 1500 rpm for 1 min. The supernatant was poured into a 45- μm sieve and rinsed with distilled water. After washing the inner surface, the contents of the sieve were transferred to a Petri dish containing distilled water and the number of spores was counted under a dissecting microscope ($\times 10\text{--}65$).

Results and discussion

In this study, 33 species of plants from 15 plant families were examined, representing most of the study site. Most of these species had mycorrhizal associations. Colonization classified as ericoid mycorrhizal was observed with *Phyllodoce aleutica*, *Tripetaleia bracteata*, *Vaccinium uliginosum*, *Rhododendron aureum*, *Arctericia nana* and *Shortia soldanelloides* f. *alpina* (Fig. 2B, Table 1); most of these species are in the family Ericaceae. It has been reported that most ericaceous plants have ericoid mycorrhizal associations (Haselwandter, 1979; Haselwandter and Read, 1980; Miller, 1982; Treu *et al.*, 1996; Cazares *et al.*, 2005), although AM mycorrhizas were also observed by Chaurasia *et al.* (2005) in several species of Ericaceae. Ericoid mycorrhizal fungi are known to have the ability to utilize complex organic nitrogen, which is unavailable to uninfected plants (Read, 1983; Smith and Read, 1997). Therefore, ericoid mycorrhizal associations are especially important in alpine areas where low temperatures cause reduced mineralization of nitrogen, and hence, accumulation of organic nitrogen.

Ectomycorrhizal associations were found in woody (*Pinus pumila*: Pinaceae) and herbaceous plant species (*Polygonum viviparum*: Polygonaceae) (Fig. 2C–F, Table 1). Excavated root systems of these species had many ectomycorrhizal lateral roots attached to long roots (Fig. 2C–F). *Pinus* species are known to be strongly ectomycorrhizal (Maeda, 1954; Harley and Harley, 1987a, b). On the other hand, several plant species belonging to the genus *Polygonum* have a variety of mycorrhizal types on their roots (Harley and Harley, 1987a, b), although many *Polygonum* species have been shown to be non-

Table 1. The mycorrhizal types of plants growing on Maedake Cirque in the Japan South Alps.

Family	Species	n ^a	Type of mycorrhizal association ^b				AM colonization level (%) ^c
			Non	Ericoid	Ecto	AM	
COMPOSITAE	<i>Artemisia sinanensis</i>	6	2			4	8.7±7.3
COMPOSITAE	<i>Anaphalis alpicola</i>	1				1	5.7
COMPOSITAE	<i>Solidago virga-aurea</i>	1				1	12.0
COMPOSITAE	<i>Senecio takedanus</i>	1				1	19.9
COMPOSITAE	<i>Leontopodium japonicum</i> f. <i>shiroumense</i>	1				1	19.7
COMPOSITAE	<i>Arnica unalascensis</i> var. <i>tschonoskyi</i>	1				1	2.8
ERICACEAE	<i>Phyllodoce aleutica</i>	6		6			0
ERICACEAE	<i>Tripetaleia bracteata</i>	3	1	2			0
ERICACEAE	<i>Vaccinium uliginosum</i>	1		1			0
ERICACEAE	<i>Rhododendron aureum</i>	3		3			0
ERICACEAE	<i>Arctericia nana</i>	1		1			0
GERANIACEAE	<i>Geranium yesoense</i> var. <i>nipponicum</i>	6	2			4	9.8±11.5
RANUNCULACEAE	<i>Anemone narcissiflora</i>	3				3	22.2±6.2
RANUNCULACEAE	<i>Ranunculus acris</i> var. <i>nipponicus</i>	3	1			2	34.6±8.8
RANUNCULACEAE	<i>Aconitum senanense</i>	3	1			2	44.5±3.4
ROSACEAE	<i>Sibbaldia procumbens</i>	3				3	30.4±22.4
ROSACEAE	<i>Sorbus sambucifolia</i>	3	1			2	1.6±1.8
ROSACEAE	<i>Geum calthaeifolium</i> var. <i>nipponicum</i>	1				1	63.4
ROSACEAE	<i>Geum pentapetalum</i>	1				1	76.1
POLYGONACEAE	<i>Polygonum weyrichii</i> var. <i>alpinum</i>	3	3				0
POLYGONACEAE	<i>Polygonum viviparum</i>	3				3	0
GRAMINEAE	<i>Deschampsia flexuosa</i>	9	5			4	6.6±6.9
GRAMINEAE	<i>Gramineae</i> sp.	3	2			1	1.2
LILIACEAE	<i>Veratrum stamineum</i>	3	1			2	14.8±19.2
LILIACEAE	<i>Lilium medeoloides</i>	2				2	40.0±23.6
CYPERACEAE	<i>Carex</i> sp.1	3	3				0
CYPERACEAE	<i>Carex</i> sp.2	3	3				0
PINACEAE	<i>Pinus pumila</i>	3				3	0
VIOLACEAE	<i>Viola biflora</i>	6	3			3	3.2±2.0
CRASSULACEAE	<i>Sedum roseum</i>	1				1	21.4
GENTIANACEAE	<i>Gentiana algida</i>	1				1	2.5
CAMPANULACEAE	<i>Campanula lasiocarpa</i>	1				1	15.4
DIAPENSIACEAE	<i>Shortia soldanelloides</i> f. <i>alpina</i>	1		1			0

a: number of plants examined.

b: number of plants associated with mycorrhiza, Non: non-mycorrhiza, Ericoid: ericoid mycorrhiza, Ecto: ectomycorrhiza, AM: arbuscular mycorrhiza.

c: average ± SD.

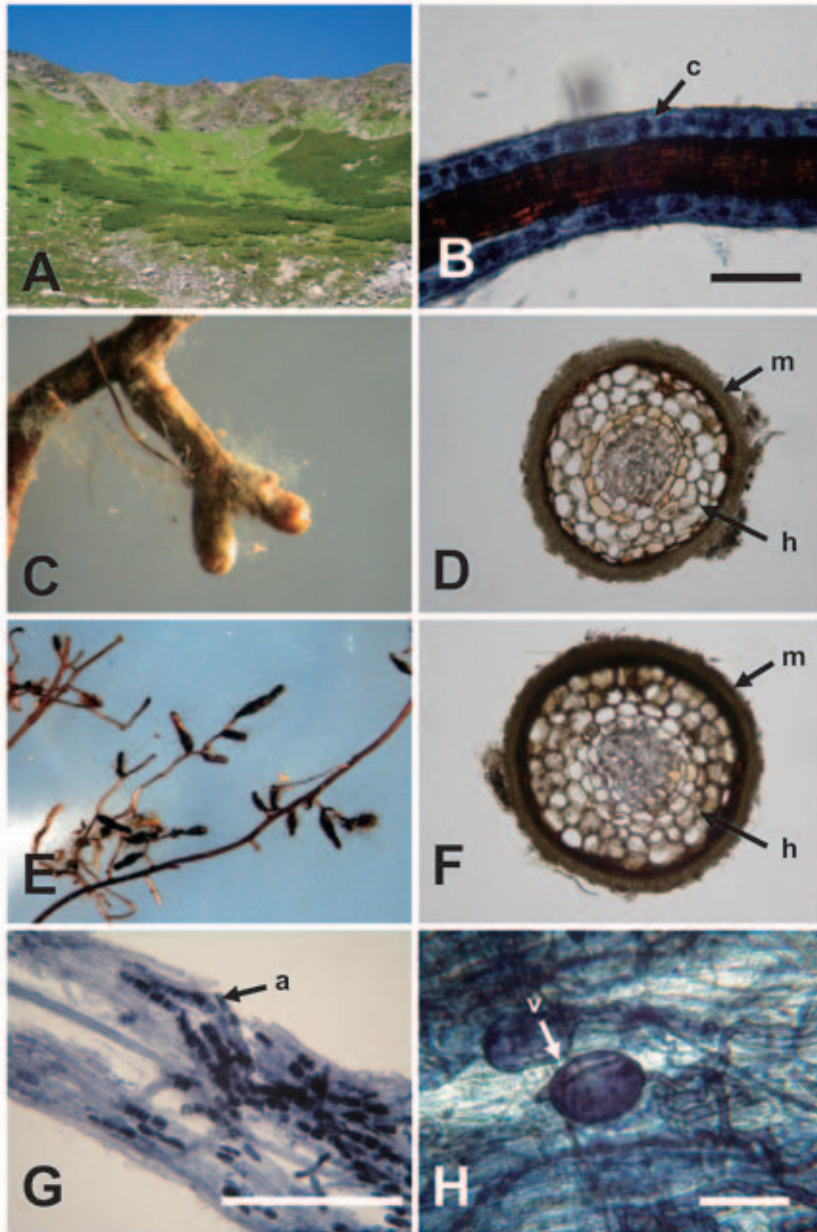


Fig. 2. A) The alpine plant communities on Mt. Maedake Cirque, B) ericoid mycorrhiza of *Phyllodoce aleutica*, C) ectomycorrhiza of *Pinus pumila*, D) transverse section of ectomycorrhiza on *Pinus pumila*, E) ectomycorrhiza of *Polygonum viviparum*, F) transverse section of ectomycorrhiza on *Polygonum viviparum*, G) arbuscular mycorrhiza of *Lilium medeoloides*, H) arbuscular mycorrhiza of *Geum calthaeifolium* var. *nipponicum*. Shown are arbuscules (a), vesicles (v), mantle hyphae (m), Hartig net hyphae (h) and fungal coils (c). The scale bars represent 50 μm (B and H) or 1 mm (G).

Table 2. Colonization level and spore density of arbuscular mycorrhiza in subalpine and alpine ecosystems.

Vegetation type	Location	Elevation (m)	n ^a	Genus name of plant species examined	AM colonization level (%)	Spore density (spores/g soil)	Reference
Base poor grass-heath and dwarf shrub vegetation	Tyrolean Central Alps (47°13'N, 11°20'E)	2000-2200	15	<i>Poa</i> , <i>Agrostis</i> , <i>Deschampsia</i> , <i>Festuca</i> , <i>Anthoxanthum</i> , <i>Nardus</i> , <i>Carex</i> , <i>Senecio</i> , <i>Leucanthemopsis</i> , <i>Homogyne</i> , <i>Geum</i> , <i>Viola</i> , <i>Antennaria</i> , <i>Primula</i>	1-80	—	Read and Haselwander, 1981
Base rich grass-heath	Northern Calcareous Alps (47°20'N, 11°23'E)	1900	10	<i>Briza</i> , <i>Festuca</i> , <i>Anthoxanthum</i> , <i>Potentilla</i> , <i>Scabiosa</i> , <i>Linum</i> , <i>Homogyne</i> , <i>Erigeron</i> , <i>Saxifraga</i> , <i>Campanula</i>	10-90	—	Read and Haselwander, 1981
Alpine meadow	Hohe Tauern, Central Alps (47°4'N, 12°50'E)	2000-2400	14	<i>Poa</i> , <i>Anthoxanthum</i> , <i>Festuca</i> , <i>Luzula</i> , <i>Ranunculus</i> , <i>Trollius</i> , <i>Pulsatilla</i> , <i>Aster</i> , <i>Doronicum</i> , <i>Geranium</i> , <i>Potentilla</i> , <i>Achillea</i> , <i>Primula</i> , <i>Botrychium</i>	10-100	—	Read and Haselwander, 1981
Alpine meadow	Beartooth Mountains, Montana (45°4'N, 109°57'W)	2975-3050	8	<i>Deschampsia</i> , <i>Agrophyon</i> , <i>Poa</i> , <i>Epilobium</i> , <i>Sibbaldia</i> , <i>Potentilla</i> , <i>Geum</i>	3-60	1-76	Allen <i>et al.</i> , 1987
Ruderal and early-successional community	Western Italian Alps (45°7'N, 6°58'E)	1090	12	<i>Veronica</i> , <i>Consolida</i> , <i>Viola</i> , <i>Convolvulus</i> , <i>Galium</i> , <i>Bromus</i> , <i>Poa</i> , <i>Trifolium</i> , <i>Arrhenatherum</i> , <i>Vicia</i> , <i>Agrostis</i>	10-75	—	Barni and Siniscalco, 2000
Pioneer plant community of grass, herbs and shrubs	Mt. Fuji, Japan (35°20'N, 138°47'E)	1550-1650	39	<i>Calamagrostis</i> , <i>Polygonum</i> , <i>Campanula</i> , <i>Senecio</i> , <i>Cirsium</i> , <i>Picris</i> , <i>Miscanthus</i> , <i>Clematis</i> , <i>Spiraea</i> , <i>Rosa</i> , <i>Hedysarum</i> , <i>Aster</i> , <i>Pterygocalyx</i> , <i>Abies</i> , <i>Larix</i> , <i>Pinus</i> , <i>Aletris</i> , <i>Polygonatum</i> , <i>Salix</i> , <i>Betula</i> , <i>Fragaria</i> , <i>Prunus</i> , <i>Sorbus</i> , <i>Maackia</i> , <i>Astragalus</i> , <i>Acer</i> , <i>Elaeagnus</i> , <i>Cornus</i> , <i>Pyrula</i> , <i>Ligustrum</i> , <i>Fraxinus</i> , <i>Euphrasia</i> , <i>Anaphalis</i> , <i>Solidago</i> , <i>Artemisia</i>	0.1-72	0.7-26	Fujiyoshi <i>et al.</i> , 2005
Dry meadow and high alpine communities	Lyman glacier, North Cascades Mountains (48°10'N, 120°54'W)	1800-2600	41	<i>Carex</i> , <i>Juncus</i> , <i>Luzula</i> , <i>Erytronium</i> , <i>Veratrum</i> , <i>Deschampsia</i> , <i>Poa</i> , <i>Anaphalis</i> , <i>Aster</i> , <i>Crepis</i> , <i>Erigeron</i> , <i>Senecio</i> , <i>Minuartia</i> , <i>Phyllodoce</i> , <i>Epilobium</i> , <i>Phlox</i> , <i>Oxyria</i> , <i>Anemone</i> , <i>Luetkea</i> , <i>Potentilla</i> , <i>Sorbus</i> , <i>Salix</i> , <i>Saxifraga</i> , <i>Casilleja</i> , <i>Mimulus</i> , <i>Pedicularis</i> , <i>Penstemon</i> , <i>Veronica</i> , <i>Equisetum</i> , <i>Cystopteris</i> , <i>Cryptogramma</i>	1-100	—	Cazares <i>et al.</i> , 2005
Mountain scrub grasslands and alpine meadow	South Tibet (28°-31°N, 87°-93°E)	3500-4800	18	<i>Ajanta</i> , <i>Salsola</i> , <i>Carex</i> , <i>Cyperus</i> , <i>Kobresia</i> , <i>Agrostis</i> , <i>Festuca</i> , <i>Hordeum</i> , <i>Imperata</i> , <i>Poa</i> , <i>Stipa</i> , <i>Caragana</i> , <i>Medicago</i> , <i>Oxytropis</i> , <i>Potentilla</i>	10-50	0.1-3.3	Gai <i>et al.</i> , 2006

a: number of plant species examined.

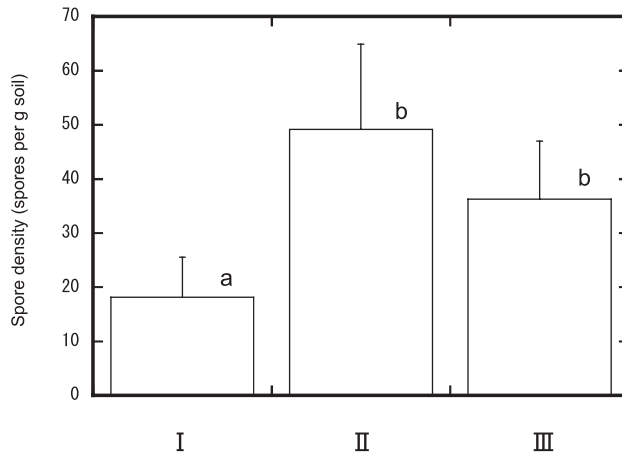


Fig. 3. AM fungal spore density in the soil of (I) fell-field community at the cirque bottom, (II) alpine plant community dominated by perennial herbs, (III) *Pinus pumila* moraine scrub. Vertical bars represent the SD ($n=9$). Columns with identical letters are not significantly different ($P>0.05$) according to Scheffé's test after one-way ANOVA.

mycorrhizal (Harley and Harley, 1987a, b). Several authors have reported *P. viviparum* and *Polygonum weyrichii* to be ectomycorrhizal (Haselwandter and Read, 1980; Read and Haselwandter, 1981; Blaschke, 1991; Treu *et al.*, 1996; Massicotte *et al.*, 1998; Titus and Tsuyuzaki, 2002). Moreover, AM associations have been found in *Polygonum cuspidatum* growing in a sub-alpine zone on Mt. Fuji (Wu *et al.*, 2004; Fujiyoshi *et al.*, 2005).

AM associations were widespread among herbaceous plants in the families Campanulaceae, Compositae, Crassulaceae, Gentianaceae, Geraniaceae, Gramineae, Liliaceae, Ranunculaceae, Rosaceae, and Violaceae (Fig. 2G–H, Table 1), and AM fungal colonization was observed in 22 (67%) out of the 33 species. In subalpine and alpine areas, the colonization of AM fungi associated with host plants has been studied by several authors (Read and Haselwandter, 1981; Allen *et al.*, 1987; Barni and Siniscalco, 2000; Cazares *et al.*, 2005; Fujiyoshi *et al.*, 2005; Gai *et al.*, 2006), revealing significantly differing colonization levels between plant species (Table 2); this was also the case in the present study. The average colonization levels of AM fungi were within a wide range of 1.2 to 76.1% (Table 1). *Arnica unalascensis* var. *tshonoskyi*, *Sorbus sambucifolia* and *Gentiana algida* showed very low levels of colonization (<3%), while on the other hand, *Geum calthaefolium* var. *nipponicum*, *Geum pentapetalum* and *Aconitum senanense* showed high levels of colonization (>44%).

A positive relationship between the AM colonization level and growth response of the host plant to AM colonization has been observed (Hirata *et al.*, 1988; Kelly *et al.*, 2001). However, studies on the response of grasses and herbaceous plants in early successional stages to AM fungal colonization have often failed to find any positive relationship (Titus and Del Moral, 1998; Sasaki *et al.*, 2001; Funatsu *et al.*, 2005; Fujiyoshi *et al.*, 2006). Fujiyoshi *et al.* (2006) showed that the effect of AM colonization on plant

growth and nutrient absorption varies widely among plant species. It is therefore necessary to carefully interpret the effect of colonization of AM fungi.

AM fungi were abundant in the soil of all examined plant communities (I–III), ranging from 18.2 to 49.2 spores per g soil (Fig. 3). However, the spore density differed significantly, with high spore density being restricted to communities II and III, where vegetation cover was heavy. Similar ranges were observed in grass and herbaceous plant communities in Beartooth Mountains and Mount Fuji (Allen *et al.*, 1987; Fujiyoshi *et al.*, 2005), although the low spore density of AM fungi in an alpine meadow in southern Tibet has also been reported (Gai *et al.*, 2006) (Table 2). A pot culture experiment was conducted to examine recolonization of AM fungi in soil collected from the study site, with AM fungi showing high inoculum potential (unpublished data). Therefore, the high spore density observed in the study site is thought to facilitate the recolonization of AM fungi in host plants.

In conclusion, mycorrhizal associations were observed in most (91%) of the plant species examined under the severe climate conditions of the study site. In particular, AM was the predominant mycorrhizal type. AM mycorrhizal association not only increases the uptake of nutrients (especially P), but also protects plant roots from pathogens (Newsham *et al.*, 1995; Graham, 2001) and increases drought tolerance (Klironomos *et al.*, 2001; Bever *et al.*, 2001). Moreover, the importance of AM fungi on plant diversity and productivity in the field and pot culture experiments has also been recognized (Van der Heijden *et al.*, 1998; Bever, 2002; Burrows and Pflieger, 2002). However, the role of the AM associations observed in the present study were not elucidated, and therefore, to clarify the importance of each mycorrhizal type in the study site, further investigation and pot culture experiments are required.

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