Mycorrhiza (2007) 17:495–506
DOI 10.1007/s00572-007-0114-9

ORIGINAL PAPER

Community structure of arbuscular mycorrhizal fungi in a primary successional volcanic desert on the southeast slope of Mount Fuji

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Received: 20 July 2006 / Accepted: 23 January 2007 / Published online: 6 March 2007
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Abstract Community structure of arbuscular mycorrhizal fungi (AMF), evaluated as spore samples and mycorrhizal roots of four herbaceous plant species, was investigated at different altitudes in a primary successional volcanic desert on Mount Fuji using molecular methods (fragment and sequence analysis of the large ribosomal subunit RNA gene). In total, 17 different AMF clades were identified, and most were members of the Glomaceae, Acaulosporaceae, and Gigasporaceae. The AMF community structures detected by spore sampling were inconsistent with those from plant roots. Of all AMF clades, six (35.3%) were detected only on the basis of spores, six (35.3%) only in roots, and five corresponded to both spores and roots (29.4%). Although an *Acaulospora* species was the most dominant among spores (67.1%), it accounted for only 6.8% in root samples. A species analysis of AMF communities at different altitudes demonstrated that AMF species diversity increased as altitude decreased and that the species enrichment at lower altitudes resulted from the addition of new species rather than species replacement. The inconsistencies in the species composition of spore communities with those in roots and the change in species diversity with altitude are discussed.

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Keywords Altitude · AMF · Diversity · Large ribosomal subunit RNA gene (LSU rDNA) · Primary succession · Terminal restriction fragment-length polymorphism (t-RFLP)

Introduction

In nature, communities of arbuscular mycorrhizal fungi (AMF) occurring in different ecosystems have different species composition (Öpik et al. 2006) and exert different symbiotic functions, depending on their community structure. Thus, many researchers have investigated the structure and function of AMF communities, and relationships between AMF and host communities. Recently, it has been recognized that the species composition of AMF communities can in fact affect plant community structure and productivity, as well as plant invasion success (Grime et al. 1987; Gange et al. 1993; van der Heijden et al. 1998; Hartnett and Wilson 1999; Bever 2003; Stampe and Daehler 2003), and several experimental investigations have demonstrated that a single AMF species induces different growth responses in different plant species (Johnson et al. 1992; Sanders and Fitter 1992; Eom et al. 2000). Conversely, the diversity of the plant community can influence AMF community structure (Burrows and Pfleger 2002; Johnson et al. 2003). The actual function of AMF symbiosis in nature should be considered at the community level of both the AMF and host plants.

AMF symbiosis is thought to play a particularly important role in the successional process on bare or disturbed lands such as volcanic deserts, where the availability of nutrients such as nitrogen and phosphorus is quite limited. According to an AMF-related basic model of primary vegetational succession on volcanic substrates, nonmycotrophic plants are the dominant colonizing species during early successional stages, followed by facultative

mycotrophic species at the intermediate stages, whereas obligate mycotrophic species dominate in later seral communities (Janos 1980; Allen 1991). A study of AMF colonization on volcanic substrates indicated an increase in mycorrhizal colonization with successional progress (Gemma and Koske 1990). More case studies are required to determine a general scheme of AMF community development in the successional volcanic desert by identifying AMF species composition.

During the most recent eruption of Mount Fuji in 1707, a large amount of volcanic ash and scoria was deposited, destroying all vegetation on the southeast slope of the volcano. Primary succession has been underway at this site since that time. Whereas the timberline is located at approximately 2,500 m above sea level (asl) on the undisturbed sides of the mountain, it is located at approximately 1,300 m asl on the southeast slope and continues to move upward even after 300 years of vegetation recovery. Several perennial herbs of the Polygonaceae, Asteraceae, and Brassicaceae were the first colonizers because of their ability to adapt to the unstable scoria surface (Masuzawa 1997). *Polygonum cuspidatum* Sieb. et Zucc. plays an important role in subsequent vegetation succession. This species forms large patches by vegetative and sexual reproduction, thus providing stable patchy habitats on the unstable scoria desert for subsequent plant species (Adachi et al. 1996; Zhou et al. 2003). Many pioneer herbaceous and woody species grow in these patches at relatively low altitudes on the slope.

Previously, we reported the extent to which AMF colonize substantially some pioneer herbaceous species on Mount Fuji (*Cirsium purpuratum*, *Clematis stans*, and *Campanula punctata* subsp. *hondoensis*), only slightly *Polygonum cuspidatum*, and never *P. weyrichii* var. *alpinum* (Wu et al. 2004). Furthermore, we found more frequent AMF colonization and larger spore densities at lower altitudes where vegetational succession was more advanced. Because these quantitative features of AMF colonization and spore density on Mount Fuji may reflect both the quantitative and qualitative development of AMF community structure, an analysis of the species composition of AMF communities was undertaken by describing the AMF community structures based on spores and colonized roots at different altitudes on Mount Fuji. We hypothesize that AMF are more abundant and have greater species richness at lower altitudes where plant succession is more developed. To determine AMF community structures, we collected AMF as spores from the soil, as well as roots of four herbaceous plant species, i.e., *Polygonum cuspidatum*, *Cirsium purpuratum*, *Clematis stans*, and *Campanula punctata* ssp. *hondoensis*, and identified the species composition of the AMF communities using molecular analysis of the large ribosomal subunit RNA gene (LSU rDNA).

Materials and methods

Research sites

Our research sites were those described previously (Wu et al. 2004) and were located from 1,500 to 1,930 m asl on the southeast slope of Mount Fuji (35°20′18″N, 138°47′23″E). We established sampling sites at four different altitudes: 1,500, 1,600, 1,700, and 1,930 m asl. Except for *Polygonum weyrichii* var. *alpinum*, *Cirsium purpuratum*, *Arabis serrata*, and *Carex doenitzii*, all other herbaceous and woody species, including *Clematis stans* and *Campanula punctata* ssp. *hondoensis*, preferentially invade *P. cuspidatum* patches. *Polygonum weyrichii* var. *alpinum*, *Arabis serrata*, and *Carex doenitzii* grow on open ground and never invade vegetation patches, whereas *Cirsium purpuratum* can invade both vegetation patches and open ground. Vegetation coverage and plant species abundance were higher at lower altitudes than at higher altitudes (Table 1). Respectively, 28 and 20 species of plant were found at the 1,500- and 1,600-m sites, whereas only eight and four herbaceous species were found at the 1,700- and 1,930-m sites (Table 1). Woody species were found only at lower altitudes (1,500 m and 1,600 m), where ectomycorrhizal *Salix reinii* is the dominant woody species (Nara et al. 2003; Wu et al. 2005).

Root sampling and spore collection

Root samples were collected from August to September 2003. At each of the four altitudes, three *Polygonum cuspidatum* roots were randomly sampled from each of ten patches. One root was also harvested from each of ten *Cirsium purpuratum* individuals at each of the four altitudes. Because *Clematis stans* and *Campanula punctata* ssp. *hondoensis* were not found above 1,700 and 1,600 m, respectively, root samples of *C. stans* were collected at 1,500, 1,600, and 1,700 m, and those of *C. punctata* ssp. *hondoensis* were collected at 1,500 and 1,600 m. At each altitude, ten roots were sampled from each of the two species. The root samples were carefully washed with tap water. A total length of about 3 cm of short fine-root fragments was excised from different parts of each root sample and washed five times with sterilized distilled water in a 2.0-ml tube. These fragments were then dried at room temperature by packing with silica gel in plastic bags until use for DNA extraction.

Soil sampling to collect AMF spores was carried out in June 2004. Soil samples were taken from sites at the four altitudes. At each altitude, three soil samples (10×10×10 cm) were haphazardly taken from each of ten *P. cuspidatum* patches. The soil samples were stored at 4°C until spore collection. Within 1 week of storage, glomeromycotan

Table 1 Vegetation coverage and plant composition at four sites at different altitudes on Mount Fuji

Site altitude (m)	Vegetation coverage (%)	Plant composition		
		Plant species abundance	Plant species	Composition (%)
1,500	15	28	*Polygonum cuspidatum*	25
			Miscanthus oligostachyus	30
			Salix reinii	15
			Cirsium purpuratum	5
			Clematis stans	10
			Others: *Polygonum weyrichii* var. *alpinum*, *Campanula punctata* ssp. *hondoensis*, *Arabis serrata*, *Carex doenitzii*, *Artemisia pedunculosa*, *Senecio nemorensis*, *Stellaria nipponica*, *Artemisia princeps*, *Aster ageratoides* var. *ovatus*, *Anaphalis margaritacea*, *Hedysarum vicioides*, *Astragalis adsurgens*, *Cirsium effusum*, *Picris hieracioides* ssp. *Japonica*, *Angelica hakonensis*, *Fragaria nipponica*, *Larix kaempferi*, *Spiraea japonica*, *Rosa fujisanensis*, *Betula ermanii*, *Ligustrum obtusifolium*, *Salix bakko*, *Weigela decora*	15
1,600	3	20	*Polygonum cuspidatum*	65
			Miscanthus oligostachyus	10
			Cirsium purpuratum	10
			Clematis stans	5
			Others: *Polygonum weyrichii* var. *alpinum*, *Campanula punctata* ssp. *hondoensis*, *Arabis serrata*, *Carex doenitzii*, *Artemisia pedunculosa*, *Senecio nemorensis*, *Stellaria nipponica*, *Artemisia princeps*, *Aster ageratoides* var. *ovatus*, *Anaphalis margaritacea*, *Hedysarum vicioides*, *Astragalis adsurgens*, *Cirsium effusum*, *Picris hieracioides* ssp. *Japonica*, *Salix reinii*, *Larix kaempferi*	10
1,700	1	8	*Polygonum cuspidatum*	65
			Miscanthus oligostachyus	10
			Polygonum weyrichii var. *alpinum*	7
			Cirsium purpuratum	8
			Clematis stans	5
			Others: *Arabis serrata*, *Carex doenitzii*, *Artemisia pedunculosa*	5
1,930	5	4	*Polygonum cuspidatum*	85
			Polygonum weyrichii var. *alpinum*	10
			Cirsium purpuratum	5
			Arabis serrata	<1

fungal spores were separated from about 200 g of each soil sample using a wet sieving and sucrose centrifugation method according to Brundrett et al. (1996). Spore density was calculated as the number of spores per weight of air-dried soil. The spores were classified into morphotypes under a dissecting microscope according to their morphology such as color, size, and some structural characters (Brundrett et al. 1996) and were counted.

DNA extraction from roots and spores

The dried fine-root fragments were pulverized in a 2.0-ml tube containing five 2.0-mm zirconium balls using an MS-100 microhomogenizing system (Tomy Digital Biology, Tokyo, Japan) at 2,800 rpm for 2 min. After the addition of 500 μl of 2× cyltrimethylammonium bromide (CTAB) solution [2% CTAB, 0.1 M Tris–HCl (pH 8.0), 20 mM ethylenediaminetetraacetic acid (EDTA; pH 8.0), 1.4 M NaCl, and 0.5% β-mercaptoethanol], the sample was homogenized again at 2,800 rpm for 2 min with the system and incubated in a block heater at 65°C for 1 h. After mixing with 500 μl of chloroform:isoamyl alcohol mixture (24:1, v:v), it was centrifuged at 20,400×*g* for 7 min. The supernatant was transferred to a 1.5-ml tube, mixed with an equal volume of isopropyl alcohol and kept at −30°C for 10 min to precipitate the DNA. After centrifugation at 5,800×*g* at 4°C for 10 min, the DNA pellet was washed once with 80% ethanol, dried, resuspended in 120 μl of Tris–EDTA (TE) buffer [10 mM Tris–HCl (pH 8.0) and 1 mM EDTA (pH 8.0)], and stored at −30°C until use.

One to ten spores of each morphotype were washed five times with sterilized distilled water and transferred to a 0.5-ml tube containing 20 μl of sterilized distilled water and five 2.0-mm zirconium balls, and were then homogenized at 4,000 rpm for 1 min with the system. After adding a small amount of sterilized distilled water, the

pellet in the tube was homogenized again at 4,000 rpm for 1 min and then centrifuged at 5,800×*g* for 2 min. The supernatant was transferred to a 1.5-ml tube and stored at −30°C until use for polymerase chain reaction (PCR).

Molecular analysis

The DNA samples extracted from the plant roots and spores were used as PCR templates after 20-fold dilution and without dilution, respectively. The amplification of a region in the fungal LSU rDNA was conducted using a nested PCR method (Gollotte et al. 2004). The fungus-specific primers LR1 and FLR2 (Trouvelot et al. 1999) were used in the first round of PCR to amplify the 5′ end of the LSU rDNA region. The first-round PCR products were diluted 100-fold and used as templates for the second round of PCR using the nested primers FLR3 and FLR4 (Gollotte et al. 2004) under the same PCR conditions. The FLR3 or FLR4 primer was labeled with Texas Red at 5′-end to generate forward- or reverse-labeled products ($FLR_{3\text{-}4}$), respectively, for electrophoresis in the sequencer.

PCR was performed in a 10-µl reaction mixture containing 1 µl of template DNA, 1 µl of 10× PCR buffer, 0.2 mM of each deoxyribonucleotide triphosphate, 0.3 µM of each primer, and 0.25 U of TaKaRa *Taq* DNA polymerase (Takara Shuzo, Tokyo, Japan), using a thermal cycler (GeneAmp 9700, Applied Biosystems). The PCR protocol was composed of an initial treatment at 94°C for 1 min; 30 cycles of treatments at 94°C for 30 s, 54°C for 30 s, and 72°C for 1 min; and a final treatment at 72°C for 10 min.

The second-round PCR products ($FLR_{3\text{-}4}$) were subjected to terminal restriction fragment-length polymorphism (t-RFLP) analysis by digestion with *Alu*I. Both the $FLR_{3\text{-}4}$ PCR products and their restriction fragments ($FLR_{3\text{-AluI}}$ and $FLR_{4\text{-AluI}}$) were diluted appropriately with TE buffer, denatured at 95°C for 5 min, and loaded on 6% sequencing gels (Long Ranger; Cambrex Bio Science Rockland, Rockland, ME, USA) buffered by 1.2× TBE (0.1 M Tris, 0.1 M boric acid, and 3.0 mM EDTA) containing 6.1 M urea. The samples were electrophoresed in 0.6× TBE on a DNA sequencer (SQ-5500E; Hitachi Electronics Engineering, Tokyo, Japan). DNA size standards were loaded into every tenth lane on the gel. The length of each fragment was estimated from the size standards using FRAGLYS 3.0 software (Hitachi Electronics). AMF with the same rDNA fragment lengths ($FLR_{3\text{-}4}$, $FLR_{3\text{-AluI}}$, and $FLR_{4\text{-AluI}}$) were classified into the same t-RFLP group. The numbers of terminal restriction fragments (TRFs) of each t-RFLP group were determined by counting the number of bands on the electrophoresis images.

More than one quarter of the DNA samples from each t-RFLP group were randomly selected for sequence analysis. The second-round $FLR_{3\text{-}4}$ PCR products were reamplified from the diluted first-round PCR products (LR1 to FLR2) of these samples and subcloned into pT7 Blue using the Perfectly Blunt cloning kit (Novagen, Madison, WI, USA) following the manufacturer's instructions. The plasmid DNA was extracted from transformed *Escherichia coli* cells suspended in 50 µl of sterile water in a 1.5-ml tube by boiling for 5 min. The plasmid-containing supernatants were amplified by PCR using M13 forward and reverse primers (RPN 2337 and RPN 2338; Amersham International, Buckinghamshire, England), and the products were electrophoresed on agarose gels to confirm the insertion of the $FLR_{3\text{-}4}$ PCR fragments into the plasmids. The plasmids containing the $FLR_{3\text{-}4}$ fragments were sequenced in both directions using Texas Red M13 forward or T7 primers by cycle sequencing using Thermo Sequenase premixed cycle sequencing kits (RPN 2444, Amersham International) following the manufacturer's instructions and then analyzed on the DNA sequencer. The consistency of t-RFLP patterns inferred from the obtained sequences with the observed t-RFLP patterns was confirmed for all sequences.

Data analysis

The species of AMF were inferred from sequence homologies with sequences registered in the DNA Data Bank of Japan (DDBJ, http://www.ddbj.nig.ac.jp/). Multiple alignments and neighbor-joining phylogenic trees were constructed using ClustalX; *Mortierella verticillata* (accession no. AF157199) was used as an outgroup.

The diversity of AMF spore morphotypes (SMTs) was estimated using Shannon's index, H, calculated as:

$$H = -\sum p_i \ln p_i$$

where p_i is the frequency of the ith SMT. H was calculated for each altitude at two levels: the total diversity of AMF spores (H_{total}) and the diversity of AMF spores within each patch (H_{patch}). A coefficient ($G_{\text{p–p}}$) representing the relative contribution of diversity between patches to the total diversity was also calculated as:

$$G_{\text{p–p}} = (H_{\text{total}} - \bar{H}_{\text{patch}})/H_{\text{total}},$$

where $\bar{H}_{\text{patch}}$ is the average of all H_{patch} at that altitude.

The range of $G_{\text{p–p}}$ is 0 to 1. For example, when the total diversity is derived from only within-patch diversity with no between-patch diversity, $G_{\text{p–p}}=0$; when the total diversity is derived from only between-patch diversity with no within-patch diversity, $G_{\text{p–p}}=1$. Thus, a larger value indicates the larger contribution of between-patch diversity to total diversity.

The significance of the differences in the number of spores, number of SMTs, and AMF species diversity

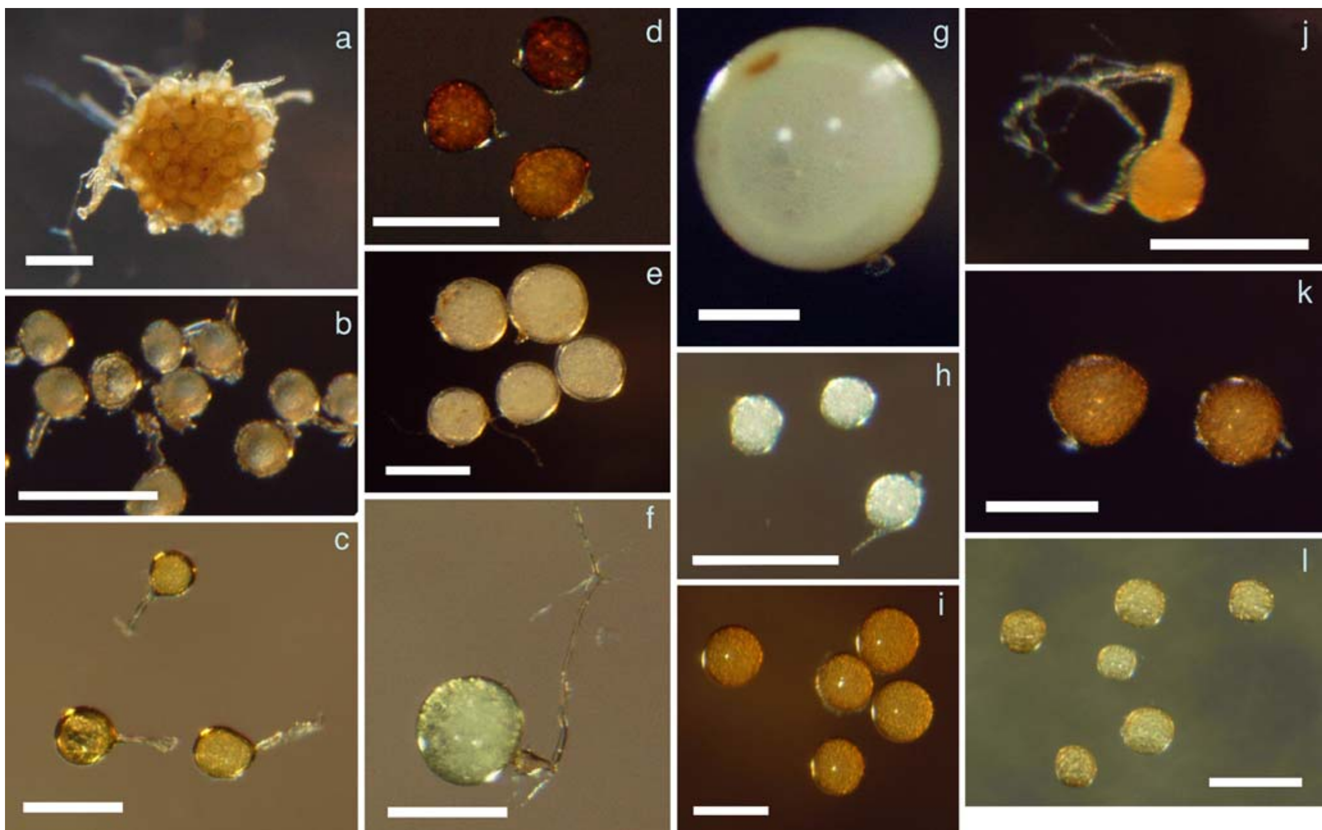

Fig. 1 Spores of AMF collected from the primary successional volcanic desert on the southeast slope of Mount Fuji showing 11 spore morphotypes. **a** and **b**, SMT 1; **c**, SMT 2; **d**, SMT 3; **e**, SMT 4; **f**, SMT 5; **g**, SMT 6; **h**, SMT 7; **i**, SMT 8; **j**, SMT 9; **k**, SMT 10; **l**, SMT 11; scale bars, 200 μm

(Shannon's index) among sites at different altitudes was tested using one-way analysis of variance followed by a Tukey honestly significant difference test at $P < 0.05$.

Results

AMF SMTs

AMF spores collected from the four altitudes were classified into 11 morphotypes (Fig. 1). SMT 1 was light yellow in color, 70–90 μm in diameter, and occurred in clusters. SMT 2 was golden yellow and 80–120 μm in diameter. SMTs 3 and 4 were *Glomus*-like, but differed in color and size; SMT 3 was brown to dark brown and 100–120 μm in diameter, whereas SMT 4 was pale yellow to yellow and 150–200 μm in diameter. SMT 5 was light yellow, about 200 μm in diameter, and had bulbous subtending hyphae. SMT 6 was creamy white, about 500 μm in diameter, and had swollen subtending hyphae. SMT 7 was also *Glomus*-like, white, and 70–90 μm in diameter. SMTs 8 to 11 were light yellow to brown and differed in spore size, with a range of 70–180 μm in diameter. SMTs 1 to 4 were identified as the genus *Glomus*, SMT 5 belonged to *Scutellospora*, SMT 6 belonged to *Gigaspora*, and SMTs 8 to 11 were identified as members of the Acaulosporaceae.

Abundance and community structure of AMF spores

AMF spore abundance was highest at the lowest site (1,500 m) and decreased with altitude (Fig. 2). The number of morphotypes (abundance) also decreased with altitude (Fig. 3). Of all 11 spore morphotypes, only SMT 11, which was predominant at altitudes of 1,500, 1,600, and 1,700 m, was present at all four sites. The AMF morphotype abundance was correlated with plant species abundance (Fig. 4).

The average diversity of AMF spores within a patch (H'_{patch}) at 1,500 and 1,600 m was significantly higher than at 1,700 and 1,930 m, but the $G_{\mathrm{p-p}}$ coefficients at the lower sites (0.65 and 0.45 at 1,500 and 1,600 m, respectively) were lower than those at the higher sites (0.92 and 0.94 at 1,700 and 1,930 m, respectively; Fig. 5). This indicates that the AMF diversity at lower altitudes was due more to within-patch diversity than at the higher altitudes.

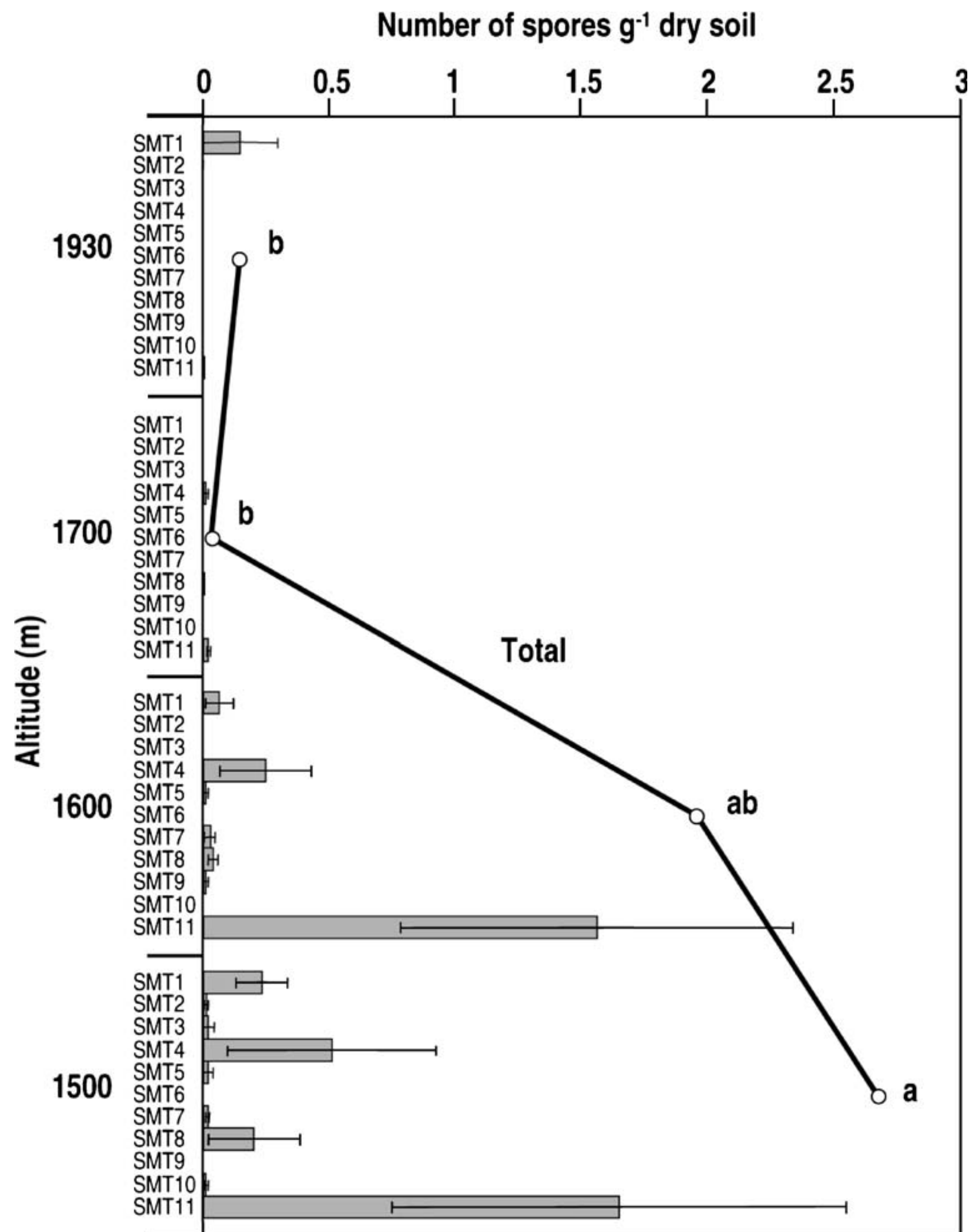

Fig. 2 Total spore abundance and spore abundance of each morphotype (mean ± SE, n=10) at four altitudes on the southeast slope of Mount Fuji. Total spore abundances with different letters differ statistically at P<0.05 according to Tukey HSD tests after one-way ANOVA

Amplification of the rRNA gene of AMF from plant roots

Partial sequences of LSU rDNA that were homologous to AMF sequences in the DDBJ database were amplified from almost all root samples of *C. purpuratum*, *C. stans*, and *C. punctata* ssp. *hondoensis*. The AMF LSU rDNA was amplified from 100% of the samples of all three species at each site, except for *C. purpuratum* samples collected at 1,930 m, 83% of which were positive for AMF DNA. The rate of amplification of AMF genes from the roots of *P. cuspidatum* was comparatively low, particularly at the higher altitudes. AMF DNA was amplified from 33, 30, 13, and 10% of *P. cuspidatum* samples collected at 1,500, 1,600, 1,700, and 1,930 m, respectively (Fig. 6). The partial LSU rDNA fragments ranged from 309 to 378 bp in length had sequences homologous to AMF sequences. A non-AMF PCR product, 229 or 234 bp long, was amplified from 35% of *P. cuspidatum* root samples using the FLR3 and FLR4 primer pair, which is considered to amplify the LSU rDNA of members of the Glomeromycota only (Gollotte et al. 2004). These products showed sequence homology to the LSU gene of an unknown species of the basidiomycete genus *Tremella* (AY18879). These sequences were not detected in any *P. cuspidatum* root samples in which AMF sequences were detected.

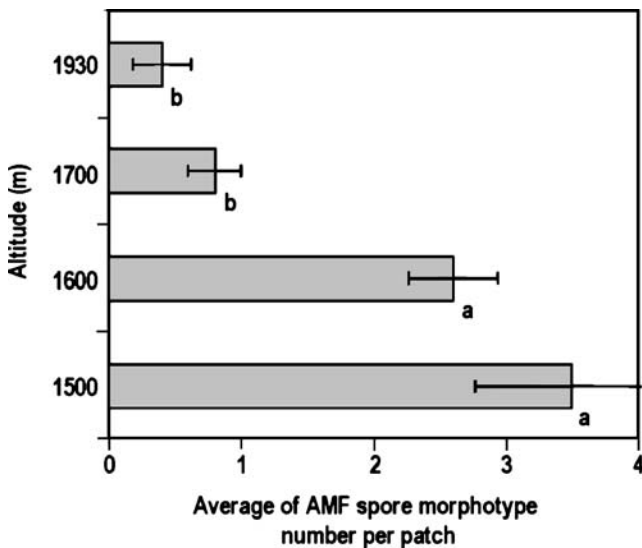

Fig. 3 Spore morphotype abundance (mean ± SE, n=10) at four altitudes on the southeast slope of Mount Fuji. *Bars with different letters* differ statistically at P<0.05 according to Tukey HSD tests after one-way ANOVA

Phylogenetic analysis of AMF based on LSU rDNA sequences

In total, 19 different t-RFLP patterns were detected in the root and spore samples (Table 2). The partial LSU rDNA sequences of 80 cloned fragments representing these patterns were determined, and 55 different sequences from root samples and 18 different sequences from AMF spores were found. These sequences have been deposited in the DDBJ database (AB206183–AB206255). Phylogenetic analysis of these representative sequences revealed 17 phylogenetic clades, of which nine and four, respectively, were grouped in the genera *Glomus* and *Acaulospora* (Glo and Aca clades, respectively; Fig. 7). Most sequences sharing the same t-RFLP pattern

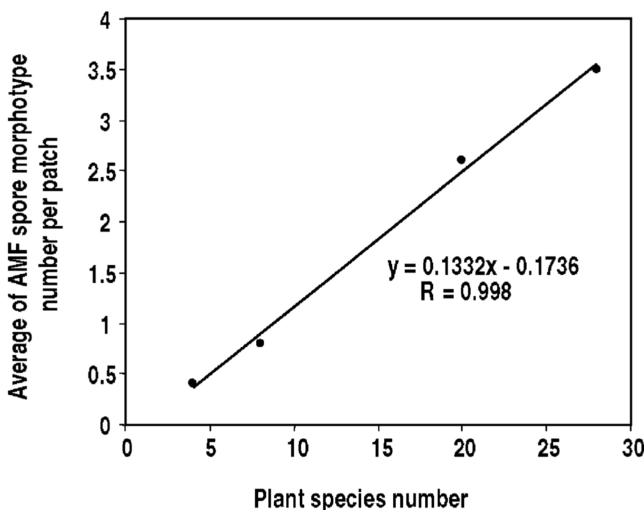

Fig. 4 Correlation between plant species abundance and AMF spore morphotype abundance at four altitudes on the southeast slope of Mount Fuji

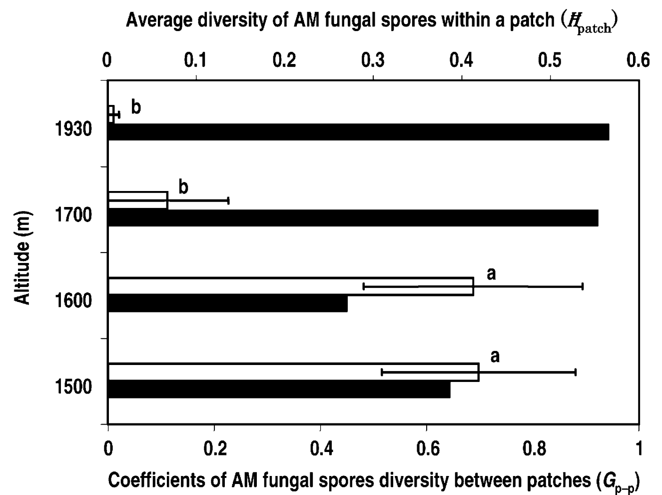

Fig. 5 Average diversity of AMF spores within a patch (H_{patch}, *open bars*; mean ± SE, n=10) and coefficients of AMF spore diversity between patches (G_{p-p}, *black bars*) at each site. *Bars with different letters* differ statistically at P<0.05 according to Tukey HSD tests after one-way ANOVA

formed monophyletic clades, although samples in some clades (Glo 1, Glo 3, Glo 5, and clade B) contained more than one t-RFLP pattern. Clade Glo 1 contained two known *Glomus intraradices* sequences (AF396787 and AY541855) from the database. Clades Glo 3 and Glo 4 took the nearest position to a sequence of *G. microaggregatum* (AF389021). Clades Glo 7, Glo 8, and Glo 9 contained sequences of *G. etunicatum* (AJ623309), *G. claroideum* (AY639342), and *G.* cf. *eburneum*

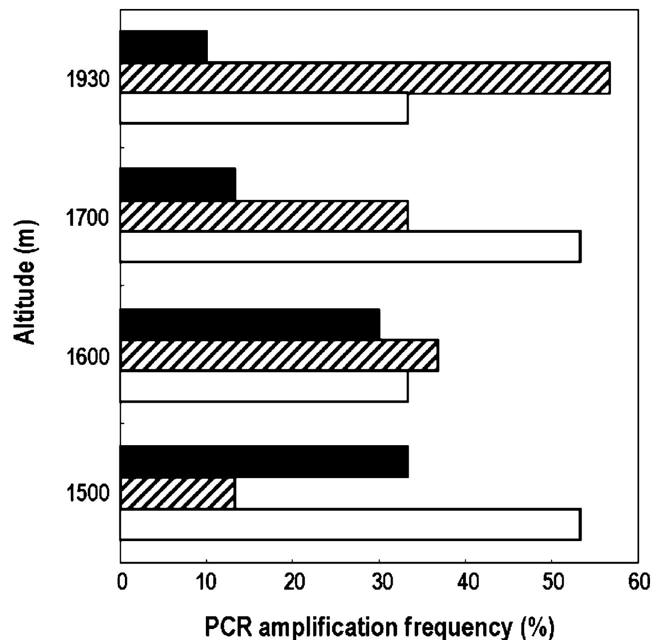

Fig. 6 PCR-amplified percentage frequency of AMF DNA (*black bars*), PCR-amplified percentage frequency of a non-AMF DNA (*cross-hatched bars*), and unamplified percentage frequency of roots samples (*open bars*), using the FLR3 and FLR4 primer pair, from DNA samples extracted from the roots of *Polygonum cuspidatum* collected at four sites at different altitudes in a primary successional volcanic desert on the southeast slope of Mount Fuji

Table 2 Patterns of t-RFLP detected among LSU rDNA PCR products amplified from plant root and AMF spore specimens

t-RFLP pattern	Length of FLR_{3-4} PCR product (bp)	Length of $FLR_{3\text{-}AluI}$ terminal restriction fragment (bp)	Length of $FLR_{4\text{-}AluI}$ terminal restriction fragment (bp)	Clade
A	370–373, 378	179–181	71–72, 76	Glo 1
B	372, 373	301, 302	71	Glo 1
C	372	77	71	Glo 1
D	371–373	180, 181	191, 192	Glo 2, 3
E	361	45	181	Glo 3
F	366, 374	45	72	Glo 4
G	368	100	70	Glo 5
H	368	176	192	Glo 5
I	326	139	72	Glo 6
J	367, 368	295	72, 73	Glo 7, 8
K	333, 335	148, 149	185, 186	Scu
L	312	127	185	Gig
M	309	45	70	Clade A
N	312	45	267	Clade B
O	312	45	184	Clade B
P	318	246	72	Aca 1
Q	368	296	72	Aca 2
R	334, 335	261, 263	72, 73	Aca 3, 4
S	369	181	188	Glo 9

(AY639228), respectively. Although the sequences of *Entrophospora colombiana* (AF389016) and *Acaulospora spinosa* (AF378434) were most homologous to species in clades Aca 1 and Aca 2, basic local alignment search tool homology values were relatively low, at approximately 85%, making it difficult to define these clades as either the *Acaulospora* or *Entrophospora* genus of the Acaulosporaceae. The sequences in clades Aca 2, Aca 3, and Aca 4 were closely related to *Acaulospora longula* (AF389007). The predominant AMF spore (SMT 11) belonged to clade Aca 4. Two clades, A and B, did not cluster with any species in the three traditional families of Glomeromycota, i.e., Glomaceae, Gigasporaceae, and Acaulosporaceae (Fig. 7). These clades may thus represent species in undescribed families. Of the 17 clades, six (Glo 2, Glo 3, Glo 4, Glo 6, clade A, and Glo 9) were found only on plant root samples, six (Glo7, Glo 8, Scu, Gig, Aca 2, and Aca 3) were found only in spore samples, and five (Glo 1, Glo 5, clade B, Aca 1, and Aca 4) were found in both plant root and spore samples.

Community structure of AMF in roots

The numbers of TRFs detected for each AMF clade colonizing the four examined host plant species at the collection sites at different altitudes are shown (Table 3). Clades Glo 1, Glo 3, A, and B were the most common in all four plant species. Of the 205 TRFs detected, 159 (77.6%) belonged to these four clades. The AMF from the roots of *P. cuspidatum*, *C. purpuratum*, *C. stans*, and *C. punctata* ssp. *hondoensis* were grouped into eight, ten, six, and six clades, respectively. Clade Glo 1 appeared to be the dominant clade in all four plant species. Of the 205 TRFs, 75 (36.6%) belonged to this clade. The TRFs of clade Aca 4 from root samples only had a low frequency, 14 (6.8%), although the spores in this clade were predominant. Members of the Acaulosporaceae (clades Aca 1 and Aca 4) occurred in the roots of all host species except *C. stans*. Clades Glo 6 and Glo 9 were only detected in the roots of *C. purpuratum*, clade Glo 2 was found only in *P. cuspidatum* and *C. purpuratum*, clade Glo 4 was found only in *C. purpuratum* and *C. stans*, and clade Glo 5 was found only in *P. cuspidatum* and *C. stans*. The number of clades of AMF occurring in the roots of *P. cuspidatum*, *C. purpuratum*, and *C. stans* was lower in the sites at higher altitudes (1,700 or 1,930 m).

Discussion

Until recently, ecological studies of the community structure of AMF have been mainly conducted based on spore morphological taxonomy (Landis et al. 2004; Chaurasia et al. 2005; Oehl et al. 2005). However, as Sanders (2004) pointed out, AMF community based only on the morphological taxonomy of spores may be seriously flawed because an AMF species could be present in low frequency but produce many spores and another could be very abundant but hardly produce any spores. Furthermore, detailed classification based on morphology requires tremendous expertise. Recently, molecular identification methods have been used for the taxonomic identification of AMF species as reliable and somewhat standardized techniques (Clapp et al. 1995; Helgason et al. 1998; Husband et al. 2002; Jansa et al. 2002;

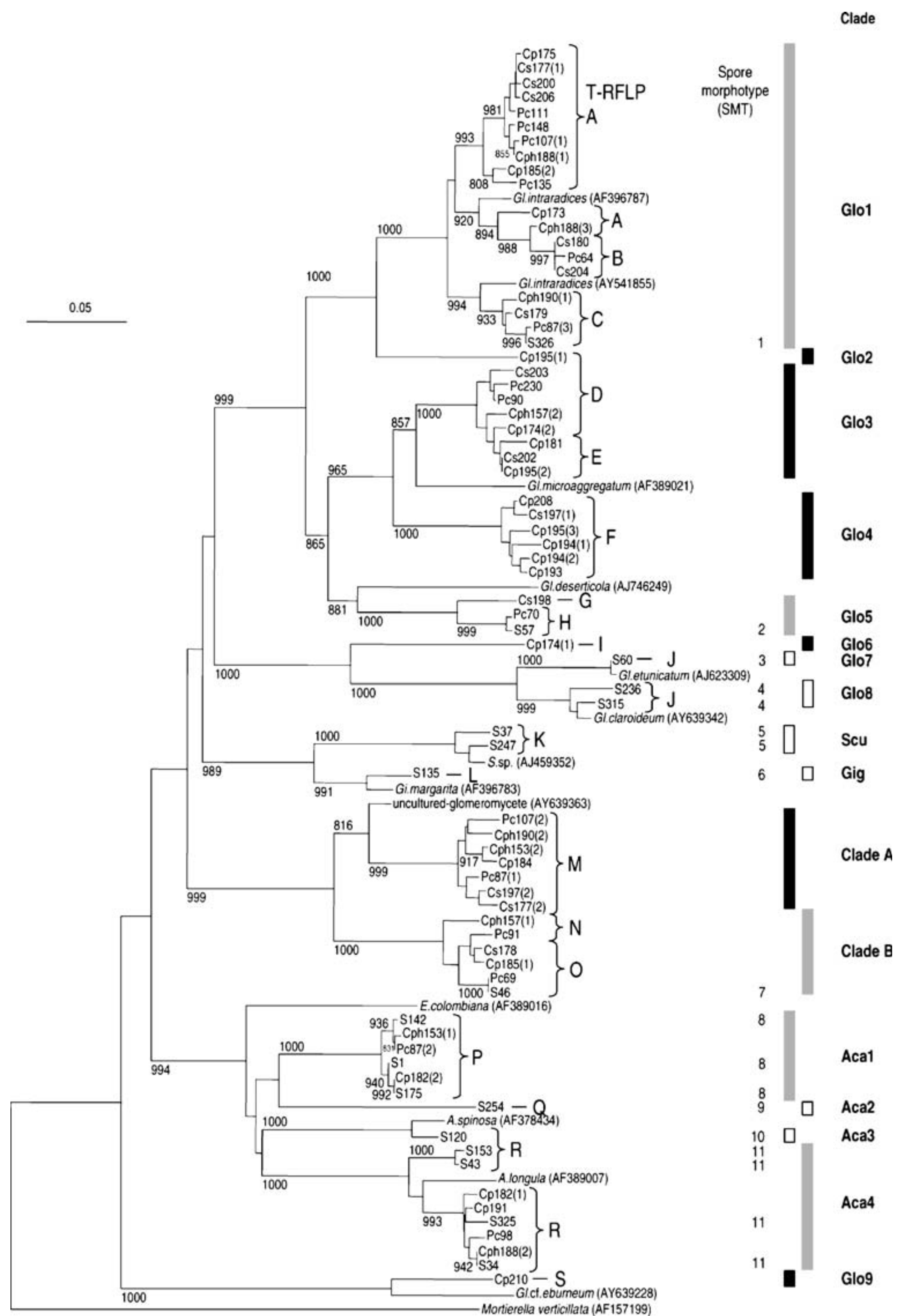

Fig. 7 Neighbor-joining tree of partial LSU rDNA sequences from AMF, isolated from AMF spore and plant root samples collected from the primary successional volcanic desert on the southeast slope of Mount Fuji, including known AMF sequences from the DDBJ database for comparison. *Bootstrap values* (only values >800 are shown) were estimated from 1,000 replicates. Each individual clone from root samples is labeled with a prefix (*Pc*, *Cp*, *Cs*, or *Cph* representing *Polygonum cuspidatum*, *Cirsium purpuratum*, *Clematis stans*, and *Campanula punctata* ssp. *hondoensis*, respectively) followed by a clone number. Each individual clone from AMF spores is lableled with the letter *S* followed by a clone number. *Letters A–S* indicate the t-RFLP pattern (see Table 2). *Numbers 1–11* indicate the SMT. *Grey bar* indicates that the phylogenetic clade contained AMF sequences from both plant roots and spores; *black bar* indicates that the clade contained AMF sequences only from plant roots; *open bar* indicates that the clade contained AMF sequences only from spores

Vandenkoornhuyse et al. 2002; Johnson et al. 2003; Öpik et al. 2003, 2006; Renker et al. 2003; Wubet et al. 2003; Gollotte et al. 2004; Oba et al. 2004; Rosendahl and Stukenbrock 2004; Scheublin et al. 2004). In the present study on AMF community structures in a volcanic desert, we used molecular methods to identify several representative spores for each morphotype after roughly grouping AMF spores into obvious morphotypes.

The phylogenetic analysis of the LSU rDNA of AMF from both spores and colonized roots of four plant species in the volcanic desert of Mount Fuji clustered all AMF species into 17 clades. The abundance of AMF species found is comparable to the eight phylotypes identified in the lahar area of Mount Pinatubo (Oba et al. 2004) and the ten phylotypes detected in an unimproved pasture in the Scottish Borders (Gollotte et al. 2004), but is greater than

Table 3 Numbers of TRFs detected for each AMF clade colonizing the roots of four plant species in four sites at different altitudes

Plant species	Site	AMF clade												No. of clades occurring
		Glo 1	Glo 2	Glo 3	Glo 4	Glo 5	Glo 6	A	B	Aca 1	Aca 4	Glo 9	Subtotal	
Polygonum cuspidatum	1,500 m	9	–	2	–	2	–	3	1	–	2	–	19	6
	1,600 m	5	1	1	–	2	–	1	2	1	1	–	14	8
	1,700 m	7	–	–	–	–	–	–	–	–	–	–	7	1
	1,930 m	2	–	1	–	–	–	–	–	–	–	–	3	2
	Subtotal	23	1	4	–	4	–	4	3	1	3	–	43	–
Cirsium purpuratum	1,500 m	10	–	4	–	–	2	–	2	2	–	–	20	5
	1,600 m	4	–	4	–	–	–	2	4	2	2	–	18	6
	1,700 m	6	1	4	8	–	–	–	–	–	1	–	20	5
	1,930 m	–	–	–	3	–	–	–	–	–	6	1	10	3
	Subtotal	20	1	12	11	–	2	2	6	4	9	1	68	–
Clematis stans	1,500 m	7	–	10	–	–	–	4	1	–	–	–	22	4
	1,600 m	2	–	2	2	4	–	4	–	–	–	–	14	5
	1,700 m	8	–	6	–	–	–	–	–	–	–	–	14	2
	1,930 m	–	–	–	–	–	–	–	–	–	–	–	–	–
	Subtotal	17	–	18	2	4	–	8	1	–	–	–	50	–
Campanula punctata ssp. *hondoensis*	1,500 m	3	–	7	–	–	–	4	1	1	–	–	16	5
	1,600 m	12	–	–	–	–	–	8	6	–	2	–	28	4
	1,700 m	–	–	–	–	–	–	–	–	–	–	–	–	–
	1,930 m	–	–	–	–	–	–	–	–	–	–	–	–	–
	Subtotal	15	–	7	–	–	–	12	7	1	2	–	44	–
Total		75	2	41	13	8	2	26	17	6	14	1	205	–

the two to four AMF phylotypes found to colonize crops on arable land in North Yorkshire (Daniell et al. 2001).

Whereas nine, four, and two clades among spores were inferred to be species of *Glomus*, *Acaulospora*, and Gigasporaceae, respectively, no Gigasporaceae species was identified in plant roots. Two remaining clades (A and B) were not clustered with any sequences of the three traditional families of Glomeromycota, i.e., Glomaceae, Gigasporaceae, and Acaulosporaceae. Thus, these might be placed in genera within a new family of AMF.

The phylogenetic analysis also demonstrated diversity within the same cluster. For example, in clade Glo 1, there were two subclusters containing two known sequences of *G. intraradices* from the database. Moreover, some clades contained more than one t-RFLP pattern. Such a diversity of AMF phylotypes within a clade corresponds to the high rates of sequence variation in some DNA regions that have been found even within single AMF spores (Sanders 2002; Pawlowska and Taylor 2004; Hijri and Sanders 2005).

The AMF community structures detected from spores were different from those in roots. This is consistent with a report by Clapp et al. (1995) in which the roots of the common understory plant, bluebell (*Hyacinthoides non-scripta*), in a woodland in North Yorkshire were colonized ubiquitously by *Glomus* species, whereas a spore survey indicated the ubiquity of the AMF types *Acaulospora* and *Scutellospora*, but no *Glomus* spores. Sanders (2004) commented that this inconsistency may be common in nature.

Species in only five of 17 AMF clades were detected from both spores and roots. SMT 11 in clade Aca 4, the most dominant species among spores, occupied 67.1% of spores, but 6.8% of AMF-colonized plant roots. Some AMF species such as Glo 7 and Glo 8 were found only in spores and never in roots. This indicates that they could not colonize roots effectively, but they produce abundant spores in soil. In contrast, AMF species such as Glo 2, Glo 3, Glo 4, Glo 6, Glo 9, and clade A, which were detected in roots, were not found in spores. This indicates that these species produced few spores, but efficiently colonized host roots. Thus, the efficiency of both spore formation and root colonization from spores or extraradical mycelia are determinants of the maintenance and expansion of AMF occupation in ecosystems.

AMF spores are resistant to environmental stresses and are viewed as long-term propagules in places where viable host plants are rarely present (Kabir 2005). They can be dispersed by wind, water flow, and mammals such as rodents and ungulates (Allen 1987; Warner et al. 1987; Allen and MacMahon 1988). Therefore, AMF spores are considered to be effective propagules for the persistence, colonization, and propagation of the AMF community,

especially in primary successional volcanic areas. If the viability of spores is maintained for a long period, they would be effective symbiotic propagules. Thus, the longevity of spore viability is also a determinant of the maintenance and expansion of AMF occupation. For a deeper understanding of AMF symbiosis, it may be necessary to separately estimate spore formation, germination, the infectivity of spores or extraradical mycelia, and the longevity of spore infectivity for each AMF species.

AMF species in roots showed host preferences among the four plant species. Although four clades of AMF (including the dominant clade Glo 1) commonly colonized all four plant species, some clades colonized only one or two plant species. This difference in the AMF communities in host plant species indicates that a symbiotic preference may occur between the host plant and AMF species. This result is consistent with several previous reports (Johnson et al. 1992; Bever et al. 1996; Gollotte et al. 2004).

Although the detection of AMF in the roots of *P. cuspidatum* contradicts the conclusion that Polygonaceae are often regarded as nonmycorrhizal, despite several reports of ectomycorrhizal species (Massicotte et al. 1998; Titus and Tsuyuzaki 2002; Yamato 2004), a low level of mycorrhizal colonization in *P. cuspidatum* was also observed in a pot culture experiment (Fujiyoshi et al. 2006).

We demonstrated for the first time the successional change in AMF species richness at different altitudes in a primary successional volcanic desert. Both the abundance and species diversity of AMF spores increased significantly with decreasing altitude. The dominant species among spores at the higher altitudes, Aca 4 and Glo 1, remained dominant at lower altitudes with the addition of several nondominant species. Thus, the increase in AMF species among spores at lower altitudes resulted from the addition, rather than the replacement, of new AMF species. The species richness of AMF in plant roots also tended to decrease at higher altitudes. In this case, the dominant species colonizing roots at higher altitudes, Glo 1, remained dominant at lower altitudes. This also suggests the addition, but not replacement, of AMF symbionts in roots with vegetational succession. Although the rates of successful AMF PCR amplification from plant root samples differed depending on the host plant species and the sampling site altitude, they corresponded well with the rates of mycorrhization that we reported previously (Wu et al. 2004). This also indicates an increase in AMF abundance with decreasing altitude.

The change in species diversity with altitude may be attributed to several causes. First, because vegetational succession in this area is progressing from lower to higher altitudes, the time after host plant establishment is greater at lower altitudes, and thus, there have been more colonization opportunities for new AMF species. Second, the higher vegetation abundance and plant species diversity at lower altitudes may result in an increased probability that an AMF spore finds a suitable host. Because the AMF species at these sites showed some host preference, as described above, an increase in the variety of host plant species may provide partnerless AMF with partner hosts and accelerate the additional colonization of new AMF species. Third, edaphic factors may also affect the AMF community during the successional process. Soil becomes more fertile with the development of plant community structure and productivity during primary succession (Fujiyoshi et al. 2006), providing AMF species with additional habitats. In fact, the diversity of the plant community can influence AMF community structure (Burrows and Pfleger 2002; Johnson et al. 2003). Additional experimental analyses of the interactions between the AMF and host plant communities will provide clues to the dynamism of AMF communities in volcanic deserts.

Acknowledgement We thank Takuro Urashima for assistance in the field. This work was supported in part by grants from the 21st Century Center of Excellence Programs and a Grant-in-Aid for Scientific Research (No. 16101008) from the Ministry of Education, Science, Sports, and Culture of Japan.

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