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Mycorrhizal and Dark-Septate Fungi in Plant Roots above 4270 Meters Elevation in the Andes and Rocky Mountains

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Abstract

Arbuscular mycorrhizal (AM) and dark-septate endophytic (DSE) fungi were quantified in plant roots from high-elevation sites in the Cordillera Vilcanota of the Andes (Perú) and the Front Range of the Colorado Rocky Mountains (U.S.A.). At the highest sites in the Andes (5391 m) AM fungi were absent in the two species of plants sampled (both Compositae) but roots of both were heavily colonized by DSE fungi. At slightly lower elevations (5240–5250 m) AM fungi were present in roots while DSE fungi were rare in plants outside of the composite family. At the highest sites sampled in Colorado (4300 m) AM fungi were present, but at very low levels and all plants sampled contained DSE fungi. Hyphae of coarse AM fungi decreased significantly in plant roots at higher altitude in Colorado, but no other structures showed significant decreases with altitude. These new findings indicate that the altitudinal distribution of mycorrhizal fungi observed for European mountains do not necessarily apply to higher and drier mountains that cover much of the Earth (e.g. the Himalaya, Hindu Kush, Andes, and Rockies) where plant growth is more limited by nutrients and water than in European mountains. This paper describes the highest altitudinal records for both AM and DSE fungi, surpassing previous reported altitudinal maxima by about 1500 meters.

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Introduction

Microorganisms and plants in alpine ecosystems are adapted to low temperatures, nutrient-limited soils, and short growing seasons (Fisk et al., 1998; Meyer et al., 2004). For example, many temperate alpine plants invest in extensive root systems and have high root-to-shoot ratios (Billings 1988) whereas others, especially in tropical alpine regions, do not (Cuatrecasas, 1968; Ramsay and Oxley, 1997; Körner, 1999). Alpine plants can also utilize symbioses with bacteria and fungi to access limiting nutrients such as nitrogen (Lipson et al., 1999a; Bowman et al., 1996) and phosphorus (Mullen and Schmidt, 1993). Especially well studied have been mycorrhizal relationships of plants of alpine regions of Europe (Haselwandter and Read, 1980; Read and Haselwandter, 1981; Haselwandter, 1987). In general, it has been reported that mycorrhizal colonization overall decreases with increasing altitude while non-mycorrhizal plants increase in abundance with altitude. For example, Read and Haselwandter (1981) found AM fungi colonized 20% of plants sampled at 1600 m, and only 7% at 3200 m.

Our understanding of mycorrhizal fungi in high-alpine soils outside of Europe is very limited. Most of the high mountains of the world (above 4000 m) are in climatic zones that are quite different from prevailing conditions in the Alps. For example, much of the Himalayas, Hindu Kush, Andes, and Rocky Mountains are drier than the Alps and have vegetation at much higher elevations (Halloy, 1989). Our previous work indicates that even plants in wet snow bed sites (elevation 3500 m) in the Rocky Mountains may depend on mycorrhizal fungi for nutrient uptake. For example, development of arbuscules in the snow buttercup (*Ranunculus adoneus*) corresponded with late-season phosphorus accumulation (Mullen and Schmidt, 1993), and over-wintering dark septate endophytic (DSE) fungi corresponded with early season nitrogen uptake in *R. adoneus* (Mullen et al., 1998).

Despite the growing awareness of the importance of mycorrhizal fungi in alpine environments, we still do not have an understanding of the distribution of mycorrhizal fungi in the highest alpine regions of the world. Surprisingly, there has been no exploration to ascertain the presence of mycorrhizal fungi at elevations above 3800 m. Therefore, the two main objectives of this study were to: (1) examine the occurrence of AM and DSE fungi in roots of plants growing near the high altitude limits for plant growth in the Rocky Mountains and Andes, and (2) carry out a preliminary assessment to determine if these fungi decrease with altitude in the roots of alpine plants. To our knowledge, this paper presents the highest elevation investigations of AM and DSE fungi yet undertaken.

Materials and Methods

OVERALL SAMPLING STRATEGY

In both the high Andes of Perú and the Rockies of Colorado, U.S.A., our strategy was to sample all of the plants present at our highest sampling sites (5391 and 4298 m in Perú and Colorado, respectively). We then sampled several lower elevation sites for comparative purposes and with the goal of sampling the same species of plants (when they were present) that were present at the highest elevation sites and then to randomly sample some of the many other plants present at the lower elevation sites. We realize that this is an unbalanced experimental design, but this was unavoidable given the distribution of high alpine plants in these understudied systems.

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STUDY SITES

Andes

The study site is located in the Laguna Sibinacocha basin (Cordillera Vilcanota range, (14°S, 71°W) in the Peruvian Andes. A large glacier covered much of this basin until the end of the Little Ice Age, approximately 120 years ago. The moraine-derived soils are composed of rock with significant quartz and calcite content (Nemergut et al., 2007). Precipitation (~1 m per year) is highly seasonal, and approximately 60% falls between December and March. During the dry season, when our sampling took place, surface soil temperatures oscillated between −5 and 25 °C on a diurnal basis (Nemergut et al., 2007). Roots were sampled from plants along an altitudinal gradient from 4700 to 5400 m which includes a site of the Global Observation Research Initiative in Alpine Environments (GLORIA) Network. The aim of the GLORIA Network is "to establish an effective long-term observation network for detecting the effects of climate change on mountain biota on a global scale" (http://www.gloria.ac.at/gloria_home/). The high Andean vegetation of our study area is relatively diverse and is moving upwards in elevation as climate warms, with some 50 species of vascular plants recorded outside the Little Ice Age boundary and 12 species within that boundary above 5250 m (Halloy et al., 2005). Detailed descriptions of this site can be found in Nemergut et al. (2007), Seimon et al. (2007), and Krajick and Peter (2006).

For each root sample from Perú, a voucher plant was collected for identification (or compared to a previous voucher plant), designated with a collection number, and deposited at the Herbario Vargas of the Universidad Nacional San Antonio Abad del Cusco, Perú. Root samples for the present study were collected during August 2003 from all of the plants present at the highest altitude site and from the same plants at lower elevation sites (if they were present) in addition to randomly selected plants at the lower elevation sites. Roots were washed and stored in FAA (90% formalin, 5% acetic acid, 5% ethanol) and transported back to Boulder, Colorado.

Rockies

Plants with roots attached were collected from high elevations at two sites in Colorado. The highest site was 4298 m on Mt. Evans, Colorado (39°35′N, 105°38′W). Plants of 10 species (all of the vascular species present at the site) were collected from near the summit of Mt. Evans in early July. The site sampled consisted of patches of vegetation separated by gravelly soil. Because this site was about to be excavated for the construction of a road, we were able to excavate the plants to depths of up to 0.5 m in order to obtain adequate root samples.

Numerous plants were also collected from near the D1 weather station (see map in Brooks et al., 1996, and photo in West et al., 1999) at elevations of up to 3743 m at the Niwot Ridge Long Term Ecological Research site (40°03′N, 105°35′W) in Colorado in late July. This site encompasses attributes of both dry and moist alpine meadows as described elsewhere (Fisk et al., 1998; Trappe and Luoma, 1992) and is a more typical closed alpine tundra community compared to the Mt. Evans site. Care was taken not to remove any rare species. All plants were identified to species using Weber (1976).

In order to collect intact root systems, 10 blocks of soil containing plants were excavated (to a depth of approximately 15 cm) and transported back to the laboratory where they were soaked in water for 2 h and then washed under running tap water to remove loose soil. Only roots that could be traced back to the identified plant were collected. Roots were then fixed in vials containing FAA for at least 24 h before staining.

STAINING AND QUANTIFICATION

Roots were stained with 0.05% lactophenol-trypan blue following the procedures outlined in Mullen and Schmidt (1993). AM structures stained blue using this technique, and DSE structures maintained their dark brown pigment. Roots were first rinsed in distilled water to remove FAA and then boiled in 10% KOH solution for 1 to 2 h. After clearing, heavily pigmented roots were bleached in 3% hydrogen peroxide (3 mL of NH_4OH added to 30 mL of 10% H_2O_2 in 567 mL H_2O) for 10–20 min until the root color was removed. The roots were then soaked in 0.5% HCl for 20 min and finally in 0.05% lactophenol-trypan blue overnight at room temperature. The roots were then rinsed in deionized water and mounted lengthwise in lactophenol-glycerol on microscope slides. For each plant species sampled at least 10 randomly chosen 2 cm sections of roots were examined in order to quantify mycorrhizal colonization.

Mycorrhizal colonization was quantified following McGonigle et al. (1990) at 160× using a compound microscope fitted with an ocular crosshair. Passes at random intervals were made vertically over the mounted roots. Only roots with the cortex intact were examined and only fungal structures inside the roots were counted. At each intersection of a root and the vertical axis of the crosshair, the presence of coarse and fine AM hyphae, vesicles, arbuscules, dark septate endophytic fungi, or no colonization was noted. A percentage was then calculated for each category scored by dividing the number per category by the total number of intersections. If no fungal structures were observed after examining 200 intersections, then the entire length of the root sample was examined to determine if the roots were completely free of fungi.

STATISTICAL ANALYSES

In order to determine if fungal root colonization varied with altitude, we used the model utility test (MVPStats, Vancouver, Washington, U.S.A.) to determine if the slope of the linear regression of root colonization versus altitude was significantly different from zero.

Results

ANDES

Of the 18 plant species from high elevation sites in the Andes, 14 contained AM and/or DSE fungal structures (9 species were colonized by both DSE and AM fungi, 4 species only by DSE, 1 only by AM, and 4 by no fungi; Table 1). All of the plants sampled in the Andes came from families that have been reported to contain AM fungi in previous studies (except the Ephedraceae; see references in Table 1). However, the roots of several composite species (*Perezia, Werneria orbignyana*, and *Xenophyllum*) sampled at the highest site (5391 m) contained no AM features, but were colonized by DSE fungi (Table 1). All other composites sampled at lower elevations contained some AM structures (Table 1), although levels were still quite low compared to composites in Colorado (see below).

The Andean Compositae also contained all of the plants most heavily colonized by DSE fungi. DSE fungi were rare or completely absent in the roots of most other plants sampled in the Andes, except *Bartsia pumila* (Scrophulariaceae), which

TABLE 1

Percent colonization of roots collected in the Cordillera Vilcanota of the Andes (Perú). The plants sampled are arranged by family in order to facilitate comparison of our data to previous studies (see footnotes) that have determined that most plants in a given family are either mycorrhizal (M) or nonmycorrhizal (NM). Voucher specimen numbers for each plant species are in parentheses after the species name. DSE = dark-septate endophytic fungi.

Family	Species (Myc. Status)	Elevation (a.s.l.)	Coarse hyphae	Fine hyphae	Arbuscules	Vesicles	DSE
Aspleniaceae (M[a])							
	Asplenium castaneum (SH4695)	4960	0	0	0	0	0
Compositae (M[b])							
	Perezia coerulescens (SH4681)	5247–5391	0	0	0	0	8–29
	Senecio "rititica" sp. (SH4660)	4850	9	29	6	37	39
	Werneria sp. (SH4687)	5247	5	0	1	1	66
	Mnioides sp. (SH4661)	4720	29	6	0	35	39
	W. orbignyana Wedell (SH4618)	5170	0	0	0	0	34
	Unknown (SH4691)	5242	0	6	0	2	5
	Xenophyllum rosenii (SH4622)	5389–5391	0	0	0	0	74–79
Ephedraceae (Unknown)							
	Ephedra rupestris Bentham (SH4617)	4940	0	0	0	0	0
Geraniaceae (M[c])							
	Erodium cicutarium (SH4679)	5245	0	0	0	0	0
Gramineae (M[b])							
	Calamagrostis antoniana (SH4642)	5250	11	26	27	10	0
	Calamagrostis ovata (SH4657)	~5000	0	0	0	0	0
Leguminosae (M[d])							
	Astragalus cf. *arequipensis* (SH4694)	4960	95	1	57	56	7
	Lupinus cf. *aridulus* (SH4693)	5116	0	1	0	1	0
Lycopodiaceae (M[d,e])							
	Lycopodium (Huperzia) sp. (SH4696)	4960	0	41	8	41	5
Malvaceae (M[d])							
	Nototriche sulfurea (SH4653)	5250	7	0	0	10	0
Scrophulariaceae (M[b])							
	Bartsia pumila Bentham (SH4616)	4940	1	6	6	1	28
Valerianaceae (M[f])							
	Valeriana cf. *pycnantha* (SH4685)	5242	8	39	26	63	6

[a] Cooper (1976).
[b] Smith and Read (1997).
[c] O'Connor et al. (2001).
[d] Schmidt and Scow (1986).
[e] Laursen et al. (1997).
[f] Gorsi (2002).

showed moderate levels of DSE colonization (28%). Typical mycorrhizal and DSE structures observed in our Andean samples are shown in Figure 1. The only plant sampled above 4900 m that had significant levels of coarse AM hyphae was *Astragalus arequipensis*, which also had root nodules indicative of N-fixing bacteria. Roots of four plant species (*Asplenium castaneum*, *Ephedra rupestris, Calamagrostis ovata,* and *Erodium cicutarium*) were completely devoid of all internal fungal structures (AM or DSE), a situation that is rare in studies of natural plant communities. There was no significant relationship between mycorrhizal or DSE colonization with altitude in the limited data set from the Andes (Table 2).

ROCKY MOUNTAINS

Levels of coarse AM-type colonization were low in plants (zero in 5 species) collected from near the top of Mt. Evans (all samples at elevation 4298 m in Table 3), whereas all plants from Mt. Evans, except those in the Cruciferae, were infected with fine AM hyphae. All plants examined from Mt. Evans, including the two *Draba* spp. (Cruciferae), were colonized by DSE fungi with colonization levels ranging from 1% in *Claytonia megarhiza* (Portulacaceae) to 68% in *Festuca brachyphyla* (Graminae) (Table 3). One plant (*Trifolium nanum* Torr.) from 4298 m also had large root nodules, up to 2 cm in diameter, that were presumably perennial and housed N-fixing bacteria. This plant was also colonized by DSE and fine hyphae, but not coarse AM fungi (Table 3).

At the lower elevation tundra sites (all plants at 3719 m and lower in Table 3), levels of AM colonization were similar to levels observed in low elevation plants in the same plant families that we sampled (see references in Table 3). Exceptions were unusually high levels of colonization in members of the Polemoniaceae and moderate levels in members of two families that are most often

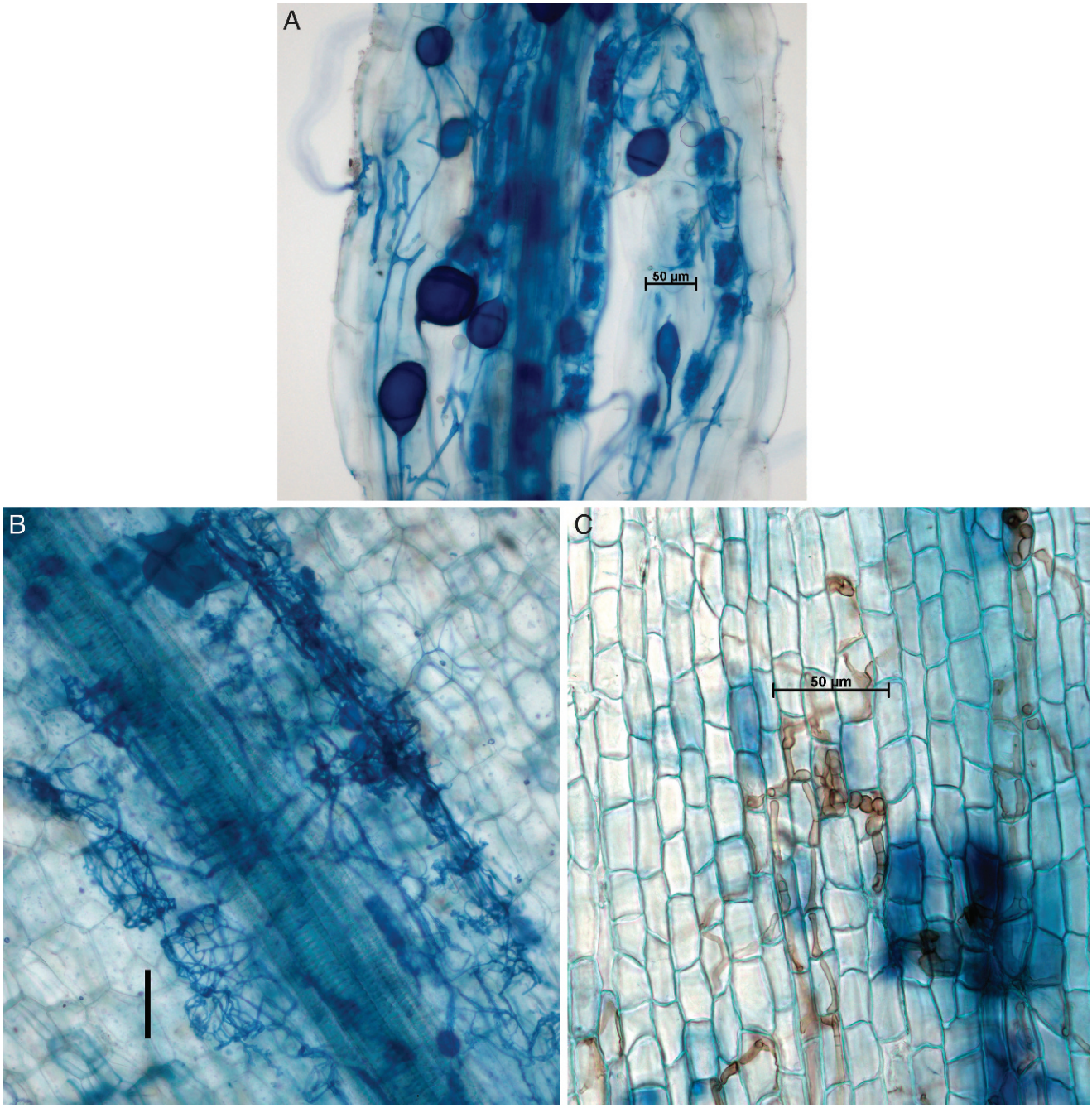

FIGURE 1. (A) Coarse AM fungi in *Astragalus* sp. growing at 4960 m in elevation (Perú). These roots also had root nodules. (B) Fine AM fungi in *Valeriana* roots at 5242 m. (C) Dark-septate endophytic fungi in roots of *Xenophyllum rosenii* at 5389 m. Scale bars are 50 µm in all pictures.

TABLE 2

Significance table for comparison of % root colonization by different fungal structures with altitude. Slopes of regression lines were all negative except DSE vs. altitude for Perú. AM = arbuscular mycorrhizal fungi.

	Coarse AM	Fine AM	Arbuscules	Vesicles	DSE
Perú					
r^2	0.109	0.038	0.017	0.155	0.072
P	0.155	0.408	0.58	0.086	0.254
Colorado					
r^2	0.395	0.109	0.002	0.093	0.007
P	0.0001	0.065	0.815	0.09	0.65

TABLE 3

Percent colonization of roots collected in the Front Range of the Colorado Rocky Mountains (U.S.A.). Plants were collected from the top of Mt. Evans (all plants from 4298 m), the D1 weather station (all plants from 3719 m.), and near the Tundra Lab on Niwot Ridge (all other elevations).

Family (Myc. Status)	Species	Elevation (a.s.l.)	Coarse hyphae	Fine hyphae	Arbuscules	Vesicles	DSE
Alliaceae (M[a])	*Allium geyeri* Wats.	3511	48	8	3	1	60
Boraginaceae (M[b])	*Eritrichum aretioides* (Cham.) DC	4298	6	26	3	2	4
		3511	17	30	0	1	10
Campanulaceae (M[a])	*Campanula rotundifolia* L.	3474	22	59	0	4	20
Caryophyllaceae (NM[c])	*Silene acaulis* L. ssp. *subacaulescens* (F. N. Williams) C. L. Hitchc. & Maguire	4298	0	3	0	0	41
Compositae (M[a])	*Artemisia scopulorum* Gray	4298	1	22	4	0	20
	Artemisia arctica Less. ssp. *saxicola* (Rydb.) Hultén	3719	22	7	3	0	10
	Erigeron simplex Greene	3719	72	27	3	8	42
	Achillea lanulosa Nutt.	3400	32	22	10	2	5
Cruciferae (NM[c])	*Draba aureae* Vahl	4298	0	0	0	0	5
	Draba nivalis Lilj.	4298	0	0	0	0	4
	Erysimum nivale (Greene) Rydb.	3719	77	3	0	5	11
Gentianaceae (M[a])	*Gentianodes algida* (Pallas) Löve & Löve	3505	48	9	0	0	36
Gramineae (M[a])	*Festuca brachyphyla* Schult.	4298	7	1	4	2	68
	Poa lettermanii Vasey	4298	1	9	2	0	28
Leguminosae (M[b])	*Trifolium nanum* Torr.	4298	0	16	4	0	36
	Trifolium spp.	3719	70	33	0	7	0
Liliaceae (M[a])	*Lloydia serotina* (L.) Sw.	3719	62	38	7	3	35
Polemoniaceae (M[a])	*Phlox sibirica* L.	3719	14	0	2	8	15
	ssp. *pulvinata* (Wherry) W. A. Weber	3719	86	12	50	7	8
	Polemonium viscosum Nutt.	3511	64	32	0	15	30
	Polemonium delicatum Rydb.	3488	64	0	0	0	46
Portulacaeae (NM[c])	*Claytonia megarhiza* (Gray) Parry	4298	4	2	0	1	1
	Lewisia pygmaea (Gray) Robinson	3488	8	38	0	0	38
Primulaceae (M[d])	*Primula angustifolium* Torr.	3719	40	6	5	1	48
Ranunculaceae (M[e])	*Caltha leptosepala* DC.	3719	62	25	7	8	74
Rosaceae (M[a])	*Acomastylis rossii* (R. Br.) Greene ssp. *turbinata* (Rydb.) W. A. Weber	3511	42	33	0	2	21
Saxifragaceae (M[f])	*Saxifraga serpyllifolia* Pursh ssp. *chrysantha* (Gray) W. A. Weber	4298	0	2	0	1	20
Scrophulariaceae (M[a])	*Castilleja occidentalis* Torr.	3511	9	0	0	0	18
	Pedicularis parryi Gray	3511	31	5	0	0	27
	Penstemon whippleanus Gray	3505	48	10	0	0	18
Violaceae (M[g])	*Viola adunca* Smith	3474	85	5	2	11	18

[a] Smith and Read (1997).
[b] Schmidt and Scow (1986).
[c] Tester et al. (1987).
[d] Haselwandter and Read (1980).
[e] Mullen and Schmidt (1993).
[f] Dalpé and Aiken (1999).
[g] Väre et al. (1997).

considered to be non-mycorrhizal: the Cruciferae and Portulacaceae (Table 3).

Levels of coarse AM hyphae in roots showed a significant ($P = 0.0001$) decrease with increasing elevation in our Colorado samples (Fig. 2a), whereas all other fungal structures did not change significantly with elevation (Table 2 and Fig. 2b).

Discussion

This paper reports the first survey of mycorrhizal and DSE fungi at elevations above 4000 m. The observation of AM fungi at 5250 m and DSE at the highest elevations we sampled (5391 m) exceeds previous altitudinal records for both fungal groups by 1500 m. Examples of previous reports of high elevation mycorrhizae include Read and Haselwandter (1981) who found AM fungi up to 3200 m in Austria and Lesica and Antibus (1986) who reported AM colonization at 3300 m in Montana and Wyoming, U.S.A. The absolute highest previous reports of mycorrhizal fungi were in South America where Barnola and Montilla (1997) found AM fungi at 3800 m in the Sierra de la Culata, Venezuela, and Fehse et al. (2002) found mycorrhizal *Polylepis* trees in Ecuador at elevations of up to 3600 m.

Our data showed some support for a general decrease in coarse fungal hyphae in Colorado with altitude (Fig. 2, Table 2). These results may indicate that coarse AM fungi are not well adapted to high elevations, but they may also be due to differences in plant and fungal phenology with elevation. All samples were taken in July in Colorado. It is likely that plants at the highest elevations sampled were at an earlier phenological state than plants from lower elevations. Previous work in Colorado showed a

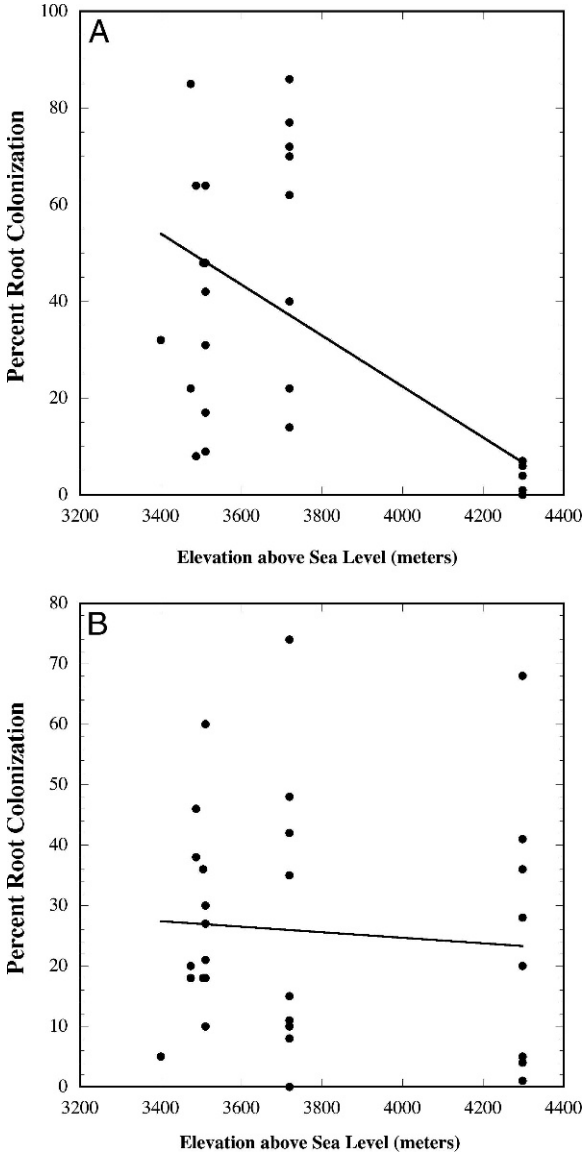

FIGURE 2. (A) Linear regression of percent colonization by coarse AM fungi and altitude above sea level in the Front Range of the Rocky Mountains ($r^2 = 0.395$; $P = 0.0001$). This relationship appears to be driven completely by the low levels of AM fungi in plant roots at the highest elevation sampled. (B) In contrast, DSE fungi showed no decrease with elevation ($r^2 = 0.007$; $P = 0.65$).

strong correlation between plant phenology and AM colonization of *Ranunculus adoneus* roots, with AM levels increasing throughout the growing season (Mullen and Schmidt, 1993). However, Mullen and Schmidt (1993) showed this same pattern for both coarse and fine AM hyphae, which would not explain the lack of decrease in fine AM hyphae and DSE with elevation in both Colorado and Perú.

No AM structures of any kind were found in the highest (elevation 5389 and 5391 m; Table 1) plants we sampled in this study despite these plants being in the Compositae, a family that is generally considered to be mycorrhizal. However, these plants were heavily colonized by DSE (Table 1), perhaps supporting a more important role for DSE fungi at high elevations. An even more surprising finding was the complete lack of any fungal structures in four species sampled in Perú (*Asplenium castaneum*, *Ephedra rupestris, Calamagrostis ovata,* and *Erodium cicutarium*), even though they were not sampled at the highest sites there. However, two of these species (*Ephedra rupestris* and *Calamagrostis ovata*) were sampled from early successional sites where they are the first plants to colonize soils that had recently been deglaciated (Nemergut et al., 2007). Based on field observations, these sites had only been deglaciated for 10–20 years, which may not be enough time for mycorrhizal inoculum to have become established at this very remote site. Other workers have noted that early successional plants often lack AM fungi, either because of a lack of inoculum and/or because early colonists are non-mycorrhizal ruderal species (Miller, 1979; Janos, 1980; Schmidt and Scow, 1986).

Our findings of a decrease of AM fungi at the highest elevations sampled parallels observations made in studies of root-associated fungi in high latitude soils of the Arctic and Antarctica. For example, AM fungi were absent from the roots of 55 plant species sampled in the high Arctic (Devon Island; Bledsoe et al., 1990). Likewise Kohn and Stasovski (1990) reported that AM fungi were conspicuously absent from all but one species sampled at another high Arctic site (Ellesmere Island). In contrast to the high Arctic, low Arctic plants have moderate to high levels of AM fungi in their roots (reviewed in Gardes and Dahlberg, 1996). In the southern hemisphere, AM fungi are found on subantarctic islands (Christie and Nicolson, 1983; Frenot et al., 2005) but are reported as missing or in very low abundance in roots of plants in Antarctica (Christie and Nicolson, 1983; DeMars and Boerner, 1995). The reasons for the paucity of AM fungi in high latitude and high altitude environments remain obscure (Gardes and Dahlberg, 1996), and it will be interesting to see if AM fungi expand their range into high latitude and high altitude environments as global warming moderates conditions of plant and fungal growth in these extreme environments (Frenot et al., 2005).

Our findings of high levels of DSE fungi in alpine plants of both Perú and Colorado add to an extensive literature of the ubiquitous distribution of DSE fungi in alpine regions (Currah and Van Dyk, 1986; Laursen et al., 1997; Read and Haselwandter, 1981; Urcelay, 2002), but their ecological significance and taxonomic identity remain mysterious. DSE fungi are defined in the literature as any melanized, septate fungi that colonize plant roots either intercellularly or intracellularly (Jumpponen and Trappe, 1998). It is likely that there are a number of different fungal taxa that fall under the general heading of DSE fungi (Jumpponen and Trappe, 1998; Schadt et al., 2001), and much more work is needed to identify the taxonomic position of DSE fungi present in alpine plants. Molecular-phylogenetic analyses using small subunit (SSU) and internal transcribed spacer (ITS) rDNA sequences indicated that a common DSE fungus (DS16B) in Colorado (Mullen et al., 1998; Schadt et al., 2001) is similar to an endophyte of winter wheat in Japan (sp. K89, GenBank Acc# AB016175) and to *Leptodontidium orchidicola* strain ZT98 022 (GenBank Acc# AF486133; Grünig et al., 2002). DS16B has also been detected in clone libraries of unvegetated glacial forelands in the Cascade Range of Washington, U.S.A. (Jumpponen, 2003). Jumpponen (2003) found that sequences 99% similar to DS16B from Colorado (Schadt et al., 2001) made up 6% of fungi in clone libraries from young unvegetated soils, and sequences 95% similar to DS16B made up 11% of fungal clones in older deglaciated soils. The very broad distribution of DS16B in cold soils (and especially in unvegetated soils) indicates that this fungus must be quite versatile metabolically and/or is very widely dispersed to high elevations. In either case it seems to be better poised to colonize high elevation plants than coarse AM fungi that are not well adapted for long-distance dispersal (Schmidt and Scow, 1986). In addition, AM fungi are obligate biotrophs and thus cannot survive vegetatively in the absence of plants, making them likely secondary

colonists of plants as they move into new habitats opened up by glacial retreat and general warming at high elevations.

Overall, our results indicate that we have only just scratched the surface of understanding the distribution and identity of DSE and AM fungi in alpine environments, especially in very high mountains such as the Andes. The fine AM hyphae and DSE structures (Fig. 1) observed in our samples from the Andes are very similar to those observed in past studies of alpine regions of the world (e.g. Crush, 1973; Schadt et al., 2001), but much more work is needed to determine the taxonomic position and functioning of DSE and AM fungi near the elevational limits for plant growth. More work is also needed to assess the functional role of DSE fungi in alpine plants. There is some indication that DSE fungi aid alpine plants in uptake of phosphorus (Haselwandter, 1987) and nitrogen (Mullen et al., 1998) but more research is needed to corroborate these studies. Nonetheless, our discovery of high levels of DSE fungi in the highest (5391 m) roots ever sampled opens the door to further studies of how these fungi affect plant growth under some of the most extreme environmental conditions for plants on Earth.

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