Mycorrhiza (2011) 21:613–622
DOI 10.1007/s00572-011-0367-1

ORIGINAL PAPER

The best for the guest: high Andean nurse cushions of *Azorella madreporica* enhance arbuscular mycorrhizal status in associated plant species

M. Angélica Casanova-Katny ·
Gustavo Adolfo Torres-Mellado · **Goetz Palfner** ·
Lohengrin A. Cavieres

Received: 23 September 2010 / Accepted: 14 February 2011 / Published online: 8 March 2011

Abstract Positive interactions between cushion plant and associated plants species in the high Andes of central Chile should also include the effects of fungal root symbionts. We hypothesized that higher colonization by arbuscular mycorrhizal (AM) fungi exists in cushion-associated (nursling) plants compared with conspecific individuals growing on bare ground. We assessed the AM status of Andean plants at two sites at different altitudes (3,200 and 3,600 ma.s.l.) in 23 species, particularly in cushions of *Azorella madreporica* and five associated plants; additionally, AM fungal spores were retrieved from soil outside and beneath cushions. 18 of the 23 examined plant species presented diagnostic structures of arbuscular mycorrhiza; most of them were also colonized by dark-septate endophytes. Mycorrhization of *A. madreporica* cushions showed differences between both sites (68% and 32%, respectively). In the native species *Hordeum comosum, Nastanthus agglomeratus*, and *Phacelia secunda* associated to *A. madreporica*, mycorrhization was six times higher than in the same species growing dispersed on bare ground at 3,600 ma.s.l., but mycorrhiza development was less cushion dependent in the alien plants *Cerastium arvense* and *Taraxacum officinale* at both sites. The ratio of AM fungal spores beneath versus outside cushions was also 6:1. The common and abundant presence of AM in cushion communities at high altitudes emphasizes the importance of the fungal root symbionts in such situations where plant species benefit from the microclimatic conditions generated by the cushion and also from well-developed mycorrhizal networks.

Keywords Plant cushion communities · Mycorrhizal fungi · Positive interactions · Facilitation effects · Alien plants

Introduction

Cushion plants are well adapted to the extreme environmental conditions which prevail in high altitudes and latitudes. Due to their low stature, compact architecture, and thick canopy, they generate a microclimate which mitigates the adverse conditions of the surrounding environment (Arroyo et al. 2003; Cavieres et al. 2006; Körner 2003). Increased moisture and nutrient content of soil beneath cushions due to accumulation of plant bio- and necromass are additional beneficial factors which promote the establishment of seedlings of other plant species within cushions, especially herbs and grasses (e.g., Cavieres et al. 2006, 2007). For the high Chilean Andes, Cavieres and Badano (2009) reported that a mere 10% cover by cushion plants produces a substantial increase in species richness at the entire community level, and they attributed this

Electronic supplementary material The online version of this article (doi:10.1007/s00572-011-0367-1) contains supplementary material, which is available to authorized users.

M. A. Casanova-Katny (✉)
Centro de Biotecnología, Universidad de Concepción,
Concepción, Chile
e-mail: angecasanova@gmail.com

G. A. Torres-Mellado · G. Palfner · L. A. Cavieres
Departamento de Botánica, Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción,
Casilla 160-C,
Concepción, Chile

L. A. Cavieres
Instituto de Ecología y Biodiversidad (IEB),
Casilla 653,
Santiago, Chile

phenomenon to facilitation effects provided by the cushion matrix.

The presence and functional role of mycorrhizal fungi in this particular environment has been sparsely studied. As suggested by Callaway (2007), it can be assumed that mycorrhizal associations in cushion communities at high elevations profit from higher levels of moisture and organic matter inside the cushions, conditions which also enhance the establishment of mycorrhizal nursling plants through connections to a common mycorrhizal network, as has been shown for ectomycorrhizal trees where mycelia radiating from one plant function as a source of colonization for neighboring host plants (Newman 1988, Nara 2006). Transfer of mycorrhiza-mediated resources may also occur between plants (Heap and Newman 1980a, b). However, the term facilitation should be used with caution when referring to the positive effects of mycorrhizal networks on plant communities, considering that the original definition of facilitation excludes trophic benefits (Brooker et al. 2008).

Although pioneer research of interspecific resource fluxes mediated by mycorrhizal networks has been performed on AM associations (Grime et al. 1987; Heap and Newman 1980a, b), most related studies in alpine environments focus on ectomycorrhizal communities (Nara and Hogetsu 2004; Nara 2006). Van der Heijden and Horton (2009) reviewed the putative effect of mycorrhiza in facilitation scenarios for seedlings and showed that this effect is more pronounced in ectomycorrhizal communities than in AM associations but also stressed that more investigations of the latter are required.

Despite their omnipresence on a global scale, AM fungi have been reported to be less common in European alpine ecosystems (Körner 2003; Read and Haselwandter 1981) where their abundance decreases with altitude, and where partial or complete replacement by dark-septate endophytes (DSE) may occur (Dhillion 1994; Read and Haselwandter 1981; Ruotsalainen et al. 2004; Väre et al. 1997). Completely isolated plants above 3,000 m in the Alps or in alpine–arctic locations usually lack mycorrhizas (Read and Haselwandter 1981; Väre et al. 1992, 1997). Poor establishment of mycorrhizal associations at these sites has been attributed to the short growing season, low temperatures, and the abundance of nutrients in meltwater (Haselwandter et al. 1983). However, recent studies show that AM associations in alpine ecosystems are not necessarily rare (Cripps and Eddington 2005). For instance, Oehl et al. (2006, 2008) reported a remarkably high spore diversity of AM fungi at high altitudes in the Swiss Alps (1,000–3,000 ma.s.l.), where at least 60 species, some new to science, were found in mountain grasslands.

Few studies have been conducted on mycorrhiza in Andean plants (Barnola and Montilla 1997; Lugo et al. 2003; Schmidt et al. 2008). Menoyo et al. (2007) found that in a *Polylepis* forest above 2,800 m in central Argentina, AM and colonization by DSE are widespread. High mycorrhizal and fungal diversity has been found in mountain grasslands in Argentina (Lugo and Cabello 2002; Lugo et al. 2003). A study realized in the Peruvian Andes by Schmidt et al. (2008) indicates that the upper limit for AM can be as high as 5,250 m, opening new fields of research in this zone of scarce soil nutrients where mycorrhizal fungi should be particularly beneficial.

Although most plant families present in the high Andean cushion communities are known to form arbuscular mycorrhiza, no specific information about status and abundance of mycotrophy in this particular environment exists. In this study, we evaluate the presence of AM fungi and DSE in plant communities dominated by cushion-forming *Azorella madreporica* in the Andes of central Chile at two sites at different altitudes. The working hypothesis is that inside cushions, AM associations develop better than outside cushions and that this difference is expressed in the relative frequency of mycorrhizal structures associated with plants growing under both conditions. Relative root colonization and number of soil-borne AM fungal spores were compared between *A. madreporica* and associated plants growing inside and outside cushions to assess the potential role of the symbiotic association in the facilitation effects on cushion plants.

Materials and methods

Study site

Research was carried out at two sampling sites of approximately 5,000 m^2 at Cerro Franciscano (3,600 ma. s.l) and at Valle Nevado (3,200 ma.s.l) in the Andes of central Chile (33°19′ S, 70°15′ W) where the vegetation is dominated by cushion-forming plants. Climatic conditions are alpine with a Mediterranean-type influence, characterized by cold and rainy winters followed by dry summers which last around 4–6 months. The annual average precipitation above 3,000 ma.s.l. is 943 mm, with main snowfall between May and October. The monthly average air temperature at 3,150 ma.s.l., during the growth season, fluctuates between 3°C in April and 7.6°C in February (Cavieres and Arroyo 1999). Soil at both sampling sites corresponds to a clay type and is characterized by values for total nitrogen between 13–14 ppm, total phosphorus between 4–7 ppm, and pH between 6.4–6.5 (Cavieres et al. 2000).

Vegetation is dominated by dwarf shrubs and cushion plants, with the occurrence of perennial and rosette herbs at higher elevations. Above 2,800 ma.s.l., the dominant cushion- forming species is *Laretia acaulis* (Cav.) Gillies and Hook. whereas above 3,200 m.a.s.l. it is replaced by *A. madreporica* Clos (Cavieres et al. 2000). *A. madreporica* (Apiaceae), or "leña de piedra", is distributed in Chile between 33° to 55° S along the Andes. The cushion diameter in the study area fluctuates between 10 and more than 100 cm (Hoffmann et al. 1998).

Analysis of AM colonization

During the summer of 2005–2007 (between February and March), roots of the most frequent plant species, belonging to several families (Table 1), were collected. Roots were dug out, cut at the hypocotyl, moistened and put with the adhering soil into plastic bags. All plants were identified and root samples were stored in a cooler while being transferred to the laboratory. The chosen plants growing both inside and outside *Azorella* cushions were the native species *Nastanthus agglomeratus* Miers, *Hordeum comosum* J. Presl, and *Phacelia secunda* J.F. Gmel., as well as the alien species *Cerastium arvense* L., and *Taraxacum officinale* F.H. Wigg. The specimens on bare ground were collected at a minimum distance of 1 m from the cushions to avoid root contact effects. Once in the laboratory, roots were carefully washed in tap water to remove soil particles and organic matter, fixed in 70% ethanol and subsequently cleared and stained with Trypan blue or aniline blue according to Brundrett et al. (1996). Selected stained root segments were mounted on glass slides in lactoglycerol and observed with an Olympus CX31 microscope (Olympus, Tokyo, Japan) at ×100 to ×1,000 magnification. Details of root and mycorrhizal structures were documented by digital photomicrographs using a Moticam 2000 digital microscope camera, and edited using Motic Images Plus 2.0 software (Motic, Hong Kong, China).

Percentage mycorrhization (presence of AM fungal structures per cm root length) was calculated using the Trouvelot et al. (1986) method (cited in Covacevich et al. 2001). Twenty 1-cm segments of stained fine roots were mounted on a slide and observed at ×40 magnification. Every root segment was assigned to a relative category of mycorrhization from 0 (0% of mycorrhization) to five (>95% mycorrhization).

The mycorrhization percentage was then calculated as follows:

$$M_{\text{Trouvelot}}(\%) = (n_1 + 5n_2 + 30n_3 + 70n_4 + 95n_5)/N$$

$M_{\text{Trouvelot}}$ (%) is symmetrical in the 5–95% range, N is the number of observed segments, n_1 to n_5, represents the number of segments categorized as 1 to 5, respectively. The mycorrhization percentage was obtained for each plant (Covacevich et al. 2001). The presence of DSE was only qualitatively recorded.

AM fungal spore extraction

Soil samples were taken for spore extraction inside and outside the *Azorella* cushions at 3,600 m.a.s.l. and deposited in plastic bags. Soil samples from around three individuals of each plant species were pooled into one sample of approximately 1 kg and transferred in a cooler to the laboratory. Spore extraction and separation was carried out according to Brundrett et al. (1996) by passing water-suspended 20 g subsamples through a series of sieves (500-, 250-, 53-μm mesh size), followed by resuspension and centrifugation of each fraction in a 50% sucrose solution. The supernatant containing spores was filtered (Advantec GA55 glass fiber filter) using a vacuum pump and filters were stored in Petri dishes. The extraction procedure was repeated twice for each soil sample. For quantification, filters with spores were observed under a dissecting microscope (Nikon SMZ645, Tokyo, Japan) with a cold light source (ECO Light 150, 180 W), and homogeneously distributed on the filter with dissection needles for counting. The most frequent spore morphotypes were mounted on slides and documented by photomicrographs.

Statistical analyseis

Prior to analyses, data were tested for normality with the Shapiro Wilkes test. As the percentage mycorrhization (%*M*) was not normal, a non parametric Kruskal–Wallis test was carried out to compare %*M* between plants growing within and outside cushions, among plant species and sampling sites (Cerro Franciscano and Valle Nevado). For spore number, data were normal and one-way ANOVA were used, considering growing position (inside vs. outside cushions) as the independent variable.

Results

Arbuscular mycorrhiza and dark-septate endophytes

Twenty-three common plant species found at both sites on Cerro Franciscano and Valle Nevado, principally perennial herbs and cushion plants belonging to 13 families, were analyzed for presence or absence of mycorrhizal structures and DSE (Table 1). Fifteen species belonging to 11 families (65% of the sampled species) presented arbuscular mycorrhiza (Table 1; Fig. 1), whereas 18 plants showed DSE structures (Table 1; Fig. 2). Diagnostic AM structures included intra- and extracellular hyphae, arbuscules, vesicles, and hyphal coils (Table 2; Fig. 2).

Mycorrhization in *A. madreporica* cushions was different at both sampling sites (p=0.03), reaching 68% at Cerro

Table 1 Fungal associations (arbuscular mycorrhiza (AM) and dark-septate endophytes (DSE)) in plant families collected from Cerro Franciscano (3,600 m a.s.l.) and at Valle Nevado (3,200 ma.s.l.) in the Andes of central Chile

Plant species	Life-form	Altitude (m a.s.l.)	AM	DSE
APIACEAE				
Azorella madreporica Clos	C	3,200, 3,600	+	+
Laretia acaulis (Cav.) Gillies and Hook.	C	3,200	+	+
Pozoa coriacea Lag.	PH	3,600	+	+
ASTERACEAE				
Chaetanthera lycopodioides Cabrera	ANN	3,200	−	−
Erigeron andicola DC.	PH	3,600	+	+
Nassauvia lagascae (DON) F. Meigen	PH	3,200	−	+
Perezia carthamoides Hook. and Arn.	PH	3,200	−	−
Senecio bustillosianus Remy	PH	3,200	+	+
Senecio francisci Phil.	PH	3,200	+	+
Taraxacum officinale F.H. Wigg.[a]	PH	3,200, 3,600	+	+
CALYCERACEAE				
Nastanthus agglomeratus Miers	PH	3,200, 3,600	+	+
CARYOPHYLLACEAE				
Cerastium arvense L.[a]	PH	3,200, 3,600	+	+
FABACEAE				
Adesmia sp.	PH	3,600	−	−
HYDROPHYLLACEAE				
Phacelia secunda J.F. Gmel.	PH	3,200, 3,600	+	+
OXALIDACEAE				
Oxalis compacta Gillies ex Hook. and Arn.	PH	3,600	+	+
POACEAE				
Hordeum comosum J. Presl	PH-G	3,200, 3,600	+	+
PORTULACACEAE				
Calandrinia caespitosa Gill. ex Arn.	PH	3,600	−	−
Montiopsis sericea (Hook. and Arn.) D.I. Ford	PH	3,200	−	+
RANUNCULACEAE				
Barneoudia major Phil.	PH-R	3,600	+	+
ROSACEAE				
Acaena pinnatifida Ruiz and Pav.	PH-R	3,200	+	+
SCROPHULARIACEAE				
Melosperma andicola Benth.	SUFF	3,200	−	+
VIOLACEAE				
Viola atropurpurea Leyb.	ANN	3,600	+	+
Viola philippii Leybold	ANN	3,200	−	−

Life-forms are: *C* cushion species, *ANN* annual species, *PH* perennial herb, *PH-R* rhizomatous perennial herb, *PH-G* perennial grass, *SUFF* suffruticose

[a] Alien species

Franciscano and 32% at Valle Nevado (at 3,600 ma.s.l. and 3,200 ma.s.l., respectively). Similarly, the plant species *P. secunda* and *C. arvense* associated with the cushions showed colonization levels of 81% versus 54% (Fig. 3b, f) and 74% versus 38% (Fig. 3a, e), respectively. The other plant species either did not show significant differences or were not found at both sites. Consistent and highly significant differences were found for percentage mycorrhization in most associated plant species when growing on bare ground (0.3% to 32%) compared with growing within *A. madreporica* cushions (29% to 80%, $p=0.0001$; Fig. 3, Table 3). Across all the examined associated species, mycorrhization inside cushions was about six times higher than on bare ground. When considering the combination of sampling sites and relative position, a highly significant interaction between both parameters was observed ($p \leq 0.0001$), with the lowest mycorrhization found in plants growing on bare ground at Cerro Franciscano (Fig. 3e–h).

Interestingly, the two alien plants species, *C. arvense* and *T. officinale* (Fig. 3a, d), showed the highest mycorrhization values when growing on bare ground at Valle Nevado, in contrast to the always higher values inside cushions of the native associated species (Fig. 3).

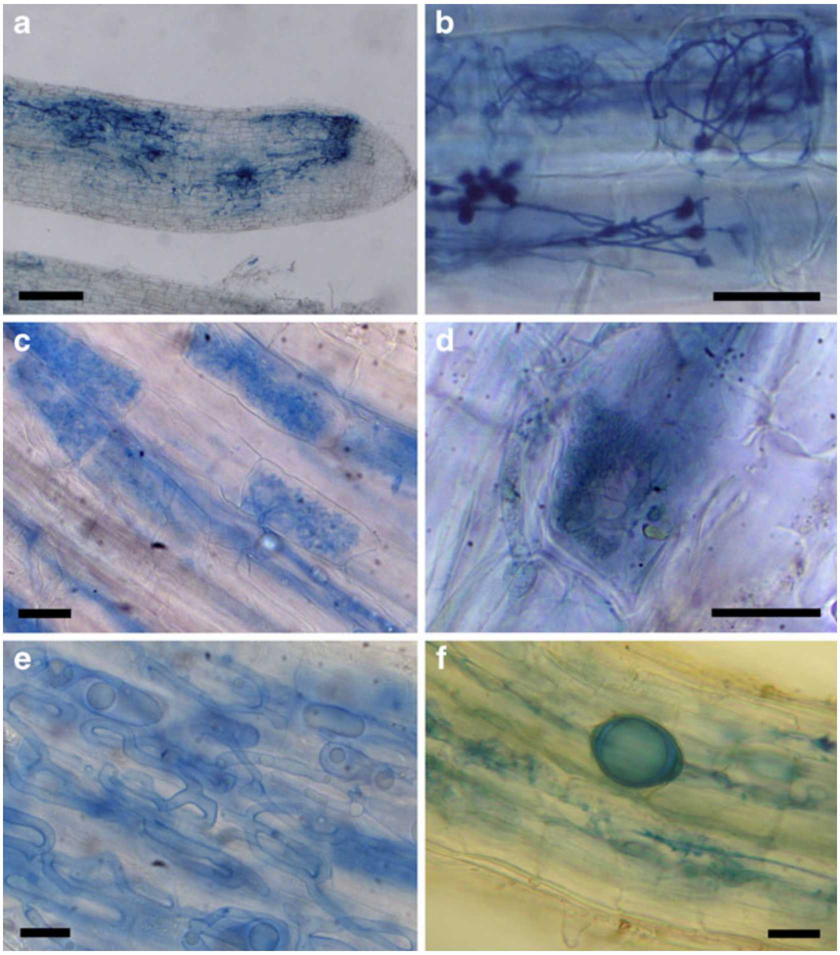

Fig. 1 Examples of AM fungal structures in roots of plants from inside cushions (IC) of *Azorella madreporica* or growing on bare ground outside cushions (OC), stained with Trypan blue; **a** root tip of *Hordeum comosum* with strong AM colonization IC, *bar*=200 μm; **b** fine AM endophytes in root of *Phacelia secunda* IC, *bar*=20 μm; **c** arbuscules in root of *Erigeron andicola* OC, *bar*=20 μm; **d** arbuscule in root of *Cerastium arvense* IC, *bar*=20 μm; **e** hyphal coils and vesicles in root of *Erigeron andicola* OC, *bar*=20 μm; **f** vesicle in root of *Acaena pinnatifida* OC, *bar*=20 μm

Soil-borne spores

About six times more spores per volume of soil were found beneath *Azorella* cushions and associated species compared with soil outside cushions (43.8±13.8 and 14±2.4 spores g^{-1}, respectively, Table 3). Two spore morphotypes, a *Glomus* type (Fig. 4a in the Electronic supplementary material) and an *Acaulospora* type (Fig. 4b–d in the Electronic supplementary material) could be distinguished, based on spore wall characteristics.

Discussion

This study, based on the first mycorrhizal survey of Andean plants from central Chile, contributes to the little existing information about the mycorrhizal status of alpine cushion plants. So far, there have been only few reports on AM in plants growing at high altitudes in the Andes, in Venezuela (Barnola and Montilla 1997) and in Peru (Schmidt et al. 2008). Our findings that about 65% of plant species growing above 3,000 ma.s.l. present AM is in accordance with the global presence of this association in the tundra/alpine biome (Dhillion 1994; Cripps and Eddington 2005; Johnston and Ryan 2000; Oehl et al. 2006, 2008; Treseder and Cross 2006). In contrast to the observation of decreases in AM with increasing elevation in the northern hemisphere (Read and Haselwandter 1981; Ruotsalainen et al. 2004; Väre et al. 1992, 1997), our results confirm that AM fungi can follow their plant hosts without an altitudinal limit, confirming the observation by Schmidt et al. (2008) who found AM associations above 5,000 ma.s.l. in the Peruvian Andes.

DSE were also present in 78% of roots of the studied plant species growing on bare ground and inside Andean nurse cushions. These fungi, whose identity and possible mutualistic role remain to be fully understood (Haselwandter 1987; Jumpponen and Trappe 1998; Mandyam and Jumpponen 2005), are well described in alpine ecosystems (Read and Haselwandter 1981) and have also been reported in the Peruvian Andes (Schmidt et al. 2008).

Nevertheless, not all analyzed plant species were found to be mycorrhizal although belonging to families where AM associations are common (Table 1). This was the case for two Asteraceae (*Chaetanthera lycopodioides* and *Perezia carthamoides*) and a Violaceae (*Viola philippii*).

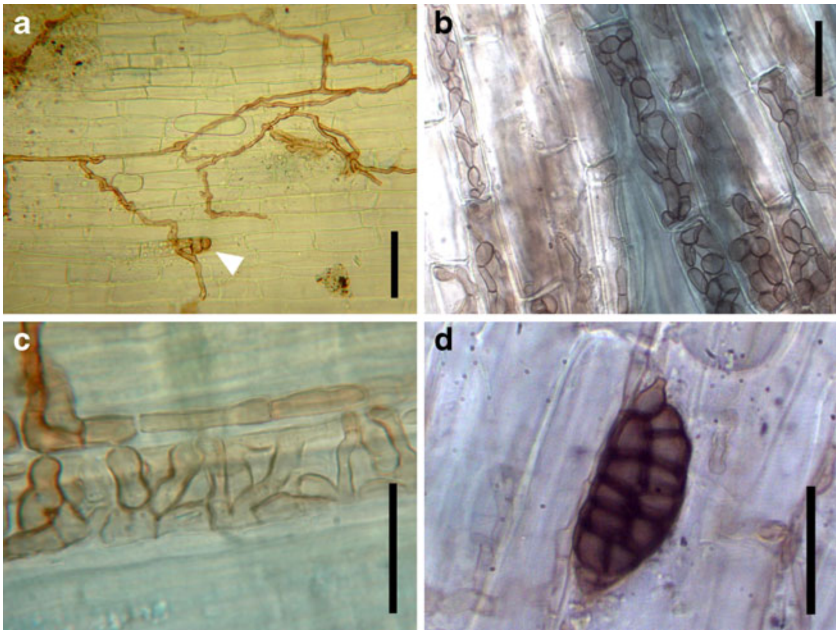

Fig. 2 Examples of dark-septate endophytic fungal structures in fine roots of plants growing in cushions of *Azorella madreporica*; **a** overview of root of *Melosperma andicola* with pigmented runner hyphae and early-stage sclerotium-like structure (*arrowhead*), *bar*=50 µm; **b** hyphae and sclerotium-like intracellular structures in root of *Hordeum comosum*, *bar*=20 µm; **c** close-up of hyphae and sclerotium-like intracellular structure in root of *Hordeum comosum*, *bar*=20 µm; **d** hyphae and dictyospore-like intracellular structure in root of *A. madreporica*, *bar*=20 µm

Schmidt et al. (2008) also reported that *Perezia coerulescens* lacked AM in a Peruvian Andes site above 5,200 ma.s.l., although it was colonized by DSE. It is worthy to note for *V. philippii* that the congeneric species *V. atropurpurea*, which was found only at Cerro Franciscano (3,600 ma.s.l.), showed the presence of both AM fungi and DSE in roots. The lack of mycorrhiza in the above species which all came from bare areas outside *Azorella* cushions may relate to a local low level or lack of AM fungal inoculum in soil, an aspect which is discussed below.

It has been demonstrated that cushion-forming plants are highly efficient engineers of new habitats in high altitude Andean ecosystems. Due to their particular growth form, the underlying soil maintains higher moisture and a more even temperature than in open areas, facilitating the establishment and growth of different plant species with increasing species richness at the entire community level (Cavieres et al. 2006, 2007, 2008). How important are mycorrhizal fungi as mediators or direct contributors of positive interactions in these particular communities?

Our results clearly indicate that *A. madreporica* cushions promote the development of AM associations compared with scattered plants outside cushions. This is in agreement with the results obtained by Genney et al. (2001) who showed higher AM colonization in swards of *Nardus stricta* than in isolated plants of the same species. The involvement of mycorrhiza in the facilitation phenomena has also been observed in an alpine ectomycorrhizal community at Mount

Table 2 AM fungal structures found in fine roots of *Azorella madreporica* and associated plant species from Cerro Franciscano (3,600 ma.s.l.) and Valle Nevado (3,200 ma.s.l.) in the Andes of Central Chile

Plant species	Hyphal coils	Intraradical hyphae	Arbuscules	Vesicles
Acaena pinnatifida	X	X	X	X
A. madreporica		X	X	X
Barneoudia major		X	X	X
Cerastium arvense	X	X	X	X
Erigeron andicola	X	X	X	X
Hordeum comosum	X	X	X	X
Laretia acaulis		X		X
Nastanthus agglomeratus		X		X
Oxalis compacta	X	X		X
Phacelia secunda	X	X	X	X
Pozoa coriacea		X	X	X
Senecio bustillosianus		X		X
Senecio francisci		X		X
Taraxacum officinale	X	X	X	X
Viola atropurpurea		X	X	X

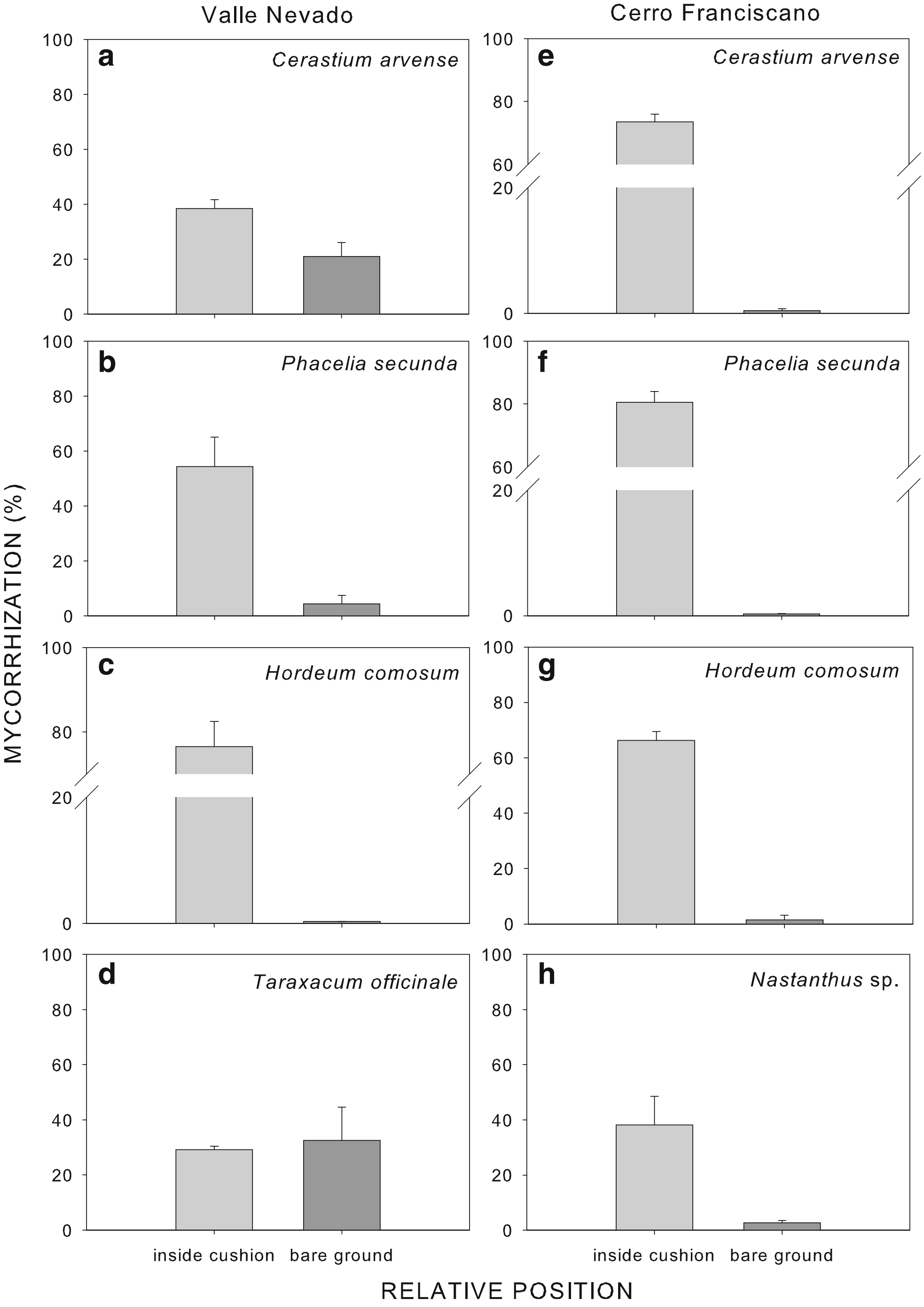

Fig. 3 Relative mycorrhization (%) of plants growing in *Azorella madreporica* cushions or on bare ground at Valle Nevado (3,200 ma.s.l.) and Cerro Franciscano (3,600 ma.s.l.). Values are means+SE (n=3) for each plant species

Fuji, Japan, where seedlings of *Salix reinii* and two other dwarf tree species do not form ectomycorrhiza unless they are located near established *S. reinii* shrubs (Nara and Hogetsu 2004).

Cushions of *A. madreporica* grow very slowly, and it can be assumed that larger cushions, due to their old age and stable rhizosphere conditions (Halloy 2002; Körner 2003), possess a well-developed mycorrhizal community which enhances colonization of nursling species roots. Mycorrhization of conspecific plants outside cushions, contrastingly, should be restricted due to the limited dispersal of AM fungal propagules (Genney et al. 2001) and less dense mycorrhizal mycelia in the unprotected upper soil layer where hyphal proliferation may be hampered by the combined disturbance of oscillating climatic extremes and soil movement, typical for alpine soils (Körner 2003). Lower density of AM soil mycelia and less root colonization has also been observed in Sonoran desert cacti growing outside nurse tree islands as compared with individuals inside these islands (Carrillo-Garcia et al.

Table 3 Statistical analyses for mycorrhizal status in plant species growing in *Azorella madreporica* cushions or growing on bare ground, in the Andes of central Chile

Source of variation	H	*P*	SS	*df*	MS	*F*
Mycorrhizal percentage						
Species	0.4	0.9947				
Sampling sites	10.86	0.2099				
Position	12.91	<0.0001*				
Species × sites	0.42	0.8594				
Species × position	14.82	0.0006*				
Position × sampling sites	13.08	<0.0001*				
Species × sampling sites × position	15.65	0.0035*				
Spore number						
Position (outside/inside)		0.0001*	3,328,660.17	1	3,328,660.17	209.88
Error			63,440.67	4	15,860.17	

Kruskal–Wallis for percentage of mycorrhization and ANOVA for spore number

*$p<0.05$, significant differences

1999). The same study reported a 1:7 ratio of soil-borne AM fungal propagules from bare area versus resource island habitat, which is strikingly similar to our data of spore counts. If we assume that spore number and density of mycelium in soil is positively related to the frequency of AM fungal hypha entry points and colonization units in host plant roots, both parameters could help to explain the six- to sevenfold higher relative mycorrhization under cushions of *A. madreporica* compared with the situation on bare ground, although a high number of AM fungal spores in soil is not always correlated with high mycorrhization levels of roots (Smith and Read 2008).

Consequently, facilitation effects should also be regarded from the viewpoint of mycorrhizal networks where fungus-root associations formed or maintained by one plant are beneficial to other plants (van der Heijden and Horton 2009). As has been shown for alpine grasslands, closed and diverse plant communities are characterized by higher mycorrhizal colonization than in more spatially separated plants of the same environment, and this phenomenon has been explained by the "close contact between roots" which enhances the spread of mycorrhizal fungi through infective hyphae (Read and Haselwandter 1981). Root exudates, which should be more abundant in dense cushion communities, have been shown to enhance the germination of spores as well as the primarily branching of extraradical hyphae (Buée et al. 2000; Elfstrand et al. 2005). It has also been proposed that closer root contact decreases the competitive effect between plant individuals in a dense community, and may thus promote denser mycorrhizal networks.

A. madreporica, which is the main facilitator species in the cushion belt of the Andes Cordillera in central Chile (Fajardo et al. 2008), presented a high level of AM colonization. At both sites, four of the associated plant species growing within *A. madreporica* cushions (*C. arvense, P. secunda, H. comosum*, and *N. agglomeratus*) also showed higher levels of mycorrhization compared with conspecific individuals growing on bare ground, whereas *P. secunda* grows well inside and outside cushions at both altitudes (Quiroz et al. 2009). For *C. arvense* and *T. officinale*, which are introduced species from the northern hemisphere (Matthei 1995), no strong difference in mycorrhization was observed between plants growing inside and outside cushions at 3,200 ma.s.l., although mycorrhiza levels of both species were highest on bare ground at both the sites. This may explain their high invasive success (Matthei 1995; Cavieres et al. 2005). *C. arvense* has been previously classified as non-mycorrhizal (Harley and Harley 1987; Fontenla et al. 1998; Tester et al. 1987) and at the same time it is hardly found above 1,000 ma.s.l. in central Europe (Seybold 1993). It has been previously shown that cushions of native plants like *L. acaulis* and *A. madreporica* offer favorable microclimatic conditions for seed germination and seedling establishment of other native and introduced plants (Cavieres et al. 2005, 2007; Quiroz et al. 2009). Our finding that *C. arvense* is regularly mycorrhizal in the high Andes indicates that it may also be the well-developed mycorrhizal community within these cushions which makes them stepping stones for the expansion of plants in this environment which are normally restricted to lower altitudes. This hypothesis is supported by Read and Haselwandter (1981) who observed fine AM endophytes in *Cerastium uniflorum* in alpine ecosystems at 2,500 ma.s.l.

The diversity of AM fungi inside and outside cushions remains to be assessed, as well as an eventual phenomenon of species specificity between the mycobionts and Phytobionts. Previous studies have shown that increased richness of AM fungal species and the presence of particular fungal taxa in defined plant communities also enhance plant diversity and productivity (Vogelsang et al. 2006). A preliminary analysis of intraradical structures, spores, and ITS sequences indicate that the same AM fungal species may be present not only in the cushion plants and their associates *H. comosum, Erigeron andicola* and *Nasthantus* spp. but also outside cushions (Torres-Mellado et al., unpublished data).

In conclusion, the impact which facilitation by cushion plants has on plant diversity and community structure in the high Andes (Cavieres et al. 2005, 2006, 2007) may be generated not only through the improvement of abiotic conditions but also through the promotion of denser mycorrhizal networks which are supposed to enhance resource mobilization and transfer in plants. In addition for native Andean plants, mycorrhization could be an essential factor of cushion-mediated facilitation, whereas alien plant species may be less dependent on the mycorrhizal community inside the cushion. Results from the present study raise more questions about the interactions between mycorrhizal fungi, *A. madreporica* cushions and associated plants, and whether these enhance seedling survival as has been shown for *H. comosum* in cushions of *L. acaulis* (Cavieres et al. 2005).

Acknowledgments We would like to thank Dr. Ewald Sieverding for his valuable help with the identification of Glomeromycotan spores. Many thanks also to Drs. Guillermo Pereira and Angela Machuca from Los Angeles for providing the sieves for spore extraction and Drs. León Bravo of Plant Physiology and Eugenio Sanfuentes of Biotechnology Center, University of Concepción, for laboratory facilities used to carry out our study. Important improvements of the original manuscript were possible due to the valuable comments of two anonymous reviewers which are highly appreciated. This research was supported by grants from FONDECYT 1090389, P05-02F ICM, and PBF-23 CONICYT.

References

Arroyo MTK, Cavieres LA, Peñaloza A, Arroyo-Kalin MA (2003) Positive interactions between the cushion plant *Azorella monantha* (Apiaceae) and alpine plant species in the chilean patagonian andes. Plant Ecol 169:121–129

Barnola LG, Montilla MG (1997) Vertical distribution of mycorrhizal colonization, root hairs, and belowground biomass in three contrasting sites from the tropical high mountains, Merida, Venezuela. Arct Alp Res 29:206–212

Brooker RW, Maestre FT, Callaway RM, Lortie CL, Cavieres LA, Kunstler G et al (2008) Facilitation in plant communities: the past, the presence and the future. J Ecol 96:18–34

Brundrett M, Bougher N, Dell B, Grove T, Malajczuk N (1996) Working with mycorrhizas in forestry and agriculture. ACIAR Monograph 32. Australian Centre for International Agricultural Research, Canberra

Buée M, Rossignol M, Jauneau A, Ranjeva R, Becard G (2000) The pre-symbiotic growth of arbuscular mycorrhizal fungi is induced by a branching factor partially purified from plant root exudates. Mol Plant Microbe 13:693–698.

Callaway RM (2007) Positive interactions and interdependence in plant communities. Springer, Berlin, p 415

Carrillo-García A, León de la Luz JL, Bashan Y, Bethlwnfalvay GJ (1999) Nurse plants, mycorrhizae, and plant establishment in a disturbed area of the Sonoran desert. Restor Ecol 7: 321–335.

Cavieres LA, Arroyo MTK (1999) Tasa de enfriamiento adiabático del aire en el Valle del Río Molina, Provincia de Santiago, Chile central (33º S). Rev geogr Chile Terra Australis 44:79–86

Cavieres LA, Badano EI (2009) Do facilitative interactions increase species richness at the entire community-level? J Ecol 97:1181–1191

Cavieres LA, Peñaloza A, Arroyo MTK (2000) Altitudinal vegetation belts in the high Andes of Central Chile (33°C). Rev Chil Hist Nat 73:331–344

Cavieres LA, Quiroz CL, Molina-Montenegro MA, Muñoz AA, Pauchard A (2005) Nurse effect of the native cushion plant *Azorella monantha* on the invasive non-native *Taraxacum officinale* in the high-Andes of Central Chile. Persp Plant Eco Evol Syst 7:217–226

Cavieres LA, Badano EI, Sierra-Almeida A, Gómez-González S, Molina-Montenegro MA (2006) Positive interactions between alpine plant species and the nurse cushion plant *Laretia acaulis* do not increase with elevation in the Andes of Central Chile. New Phytol 169:59–69

Cavieres LA, Badano EI, Sierra-Almeida A, Molina-Montenegro MA (2007) Microclimatic modifications of cushion plants and their consequences for seedling survival of native and non-native herbaceous species in the high Andes of Central Chile. Arct Ant Alp Res 39:229–236

Cavieres LA, Quiroz CL, Molina-Montenegro MA (2008) Facilitation of the non-native *Taraxacum officinale* by native nurse cushion species in the high Andes of Central Chile: are there differences between nurses? Funct Ecol 22:148–156

Covacevich F, Echeverría HE, Aguirrezabal LAN (2001) Comparación de dos técnicas de cuantificación de infección micorrítica. Cienc Suelo 19:155–158

Cripps CL, Eddington LH (2005) Distribution of mycorrhizal types among alpine vascular plants families on the Beartooth Plateau, Rocky Mountains, U.S.A., in reference to large-scale patterns in Arctic–Alpine habitats. Arct Antar Alp Res 37:177–188

Dhillion S (1994) Ectomycorrhizae, arbuscular mycorrhizae, and *Rhizoctonia* sp. of alpine and boreal *Salix* spp. in Norway. Arct Antar Alp Res 26:304–307

Elfstrand M, Feddermann N, Ineichen K, Nagaraj VJ, Wiemken A, Boller T, Salzer P (2005) Ectopic expression of the mycorrhiza-specific chitinase gene *Mtchit 3–3* in *Medicago truncatula* root-organ cultures stimulates spore germination of glomalean fungi. New Phytol 167:557–570

Fajardo A, Quiroz CL, Cavieres LA (2008) Distinguishing colonization modes from spatial structures in populations of the cushion plant *Azorella madreporica* in the high-Andes of central Chile. Austral Ecol 33:703–712

Fontenla S, Godoy R, Rosso P, Havrylenko M (1998) Root associations in *Austrocedrus chilensis* forests and seasonal dynamics of arbuscular mycorrhizas. Mycorrhiza 8:29–33

Genney DR, Hartley SH, Alexander IJ (2001) AM colonization increases with host density in a heathland community. New Phytol 152:355–363

Grime JP, Mackey JML, Hillier SH, Read DJ (1987) Floristic diversity in a model system using experimental microcosms. Nature 328:420–422

Halloy SRP (2002) Variations in community structure and growth rates of high-Andean plants with climatic fluctuations. In: Körner C, Spehn EM (eds) Mountain Biodiversity: A Global Assessment. Parthenon Publishing, Londres, pp 225–237

Harley JL, Harley EL (1987) A check-list of mycorrhiza in the British flora. New Phytol 105:1–102

Haselwandter K (1987) Mycorrhizal infection and its possible ecological significance in climatically and nutritionally stressed alpine plant communities. Angew Bot 61:107–114

Haselwandter K, Hoffmann A, Holzmann HP, Read DJ (1983) Availability of nitrogen and phosphorus in the nival zone of the Alps. Oecol 57:266–269

Heap AJ, Newman EI (1980a) The influence of vesicular-arbuscular mycorrhizas on phosphorus transfer between plants. New Phytol 85:173–179

Heap AJ, Newman EI (1980b) Links between roots by hyphae of vesicular–arbuscular mycorrhizas. New Phytol 85:169–171

Hoffmann A, Kalin-Arroyo MTK, Liberona F, Muñoz M, Watson J (1998) Plantas altoandinas. Imprenta Salesianos, Santiago

Johnston S, Ryan M (2000) Occurrence of AM fungi across a range of alpine humus soil conditions in Kosciuszko National Park, Australia. Arct Ant Alp Res 32:255–261

Jumpponen A, Trappe JM (1998) Dark septate endophytes: a review of facultative biotrophic root colonizing fungi. New Phytol 140:295–310

Körner C (2003) Alpine plant life: functional plant ecology of high mountain ecosystems, 2nd edn. Springer, Heidelberg

Lugo MA, Cabello MN (2002) Native AM fungi (AMF) from mountain grassland (Córdoba, Argentina) I. Seasonal variation of fungal spore diversity. Mycologia 94:579–586

Lugo MA, González-Maza ME, Cabello MN (2003) AM fungi in a mountain grassland II: seasonal variation of colonization studied, along with its relation to grazing and metabolic host type. Mycologia 95:407–415

Mandyam K, Jumpponen A (2005) Seeking the elusive function of the root-colonising dark septate endoPhytolic fungi. Stud Mycol 53:173–189

Matthei O (1995) Manual de las malezas que crecen en Chile. Alfabeta Impresores, Santiago

Menoyo E, Becerra AG, Renison D (2007) Mycorrhizal associations in *Polylepis* woodlands of Central Argentina. Can J Bot 85:526–531

Nara K (2006) Ectomycorrhizal networks and seedling establishment during early primary succession. New Phytol 169: 169–178

Nara K, Hogetsu T (2004) Ectomycorrhizal fungi on established shrubs facilitate subsequent seedling establishment of successional plant species. Ecol 85:1700–1707

Newman EI (1988) Mycorrhizal links between plants: their functioning and ecological significance. Adv Ecol Res 18:243–270

Oehl F, Sýkorová Z, Redecker D, Wiemken A, Sieverding E (2006) *Acaulospora alpina*, a new AM fungal species characteristic for high mountainous and alpine regions of the Swiss Alps. Mycologia 98:286–294

Oehl F, de Souza FA, Sieverding E (2008) Revision of *Scutellospora* and description of five new genera and three new families in the arbuscular mycorrhiza–forming Glomeromycetes. Mycotaxon 106:311–360

Quiroz CL, Badano EI, Cavieres LA (2009) Floristic changes in alpine plant communities induced by the cushion plant *Azorella madreporica* (Apiaceae) in the Andes of Central Chile. Rev Chil Hist Nat 82:171–184

Read DJ, Haselwandter K (1981) Observations on the mycorrhizal status of some alpine plant communities. New Phytol 88:341–352

Ruotsalainen AL, Väre H, Oksanen J, Tuomi J (2004) Root fungus colonization along an altitudinal gradient in North Norway. Arct Ant Alp Res 36:239–243

Schmidt SK, Sobieniak-Wiseman LC, Kageyama SA, Halloy SRP, Schadt CW (2008) Mycorrhizal and dark-septate fungi in plant roots above 4270 meters elevation in the Andes and Rocky Mountains. Arct Ant Alp Res 40:576–583

Seybold S (1993) Caryophyllaceae. In: Sebald O, Seybold S, Philippi G (eds) Die Blütenpflanzen Baden Württembergs, vol 1. Germany, Ulmer

Smith SE, Read DJ (2008) Mycorrhizal symbiosis. Academic, Cambridge, p 800

Tester M, Smith SE, Smith FA (1987) The phenomenon of non-mycorrhizal plants. Can J Bot 65:419–431

Treseder KK, Cross A (2006) Global distributions of AM fungi. Ecosystems 9:305–316

Van der Heijden MGA, Horton TR (2009) Socialism in soil? The importance of mycorrhizal fungal networks for facilitation in natural ecosystems. J Ecol 97:1139–1150

Väre H, Vestberg M, Eurola S (1992) Mycorrhiza and root-associated fungi in Spitsbergen. Mycorrhiza 1:93–104

Väre H, Vestberg M, Ohtonen R (1997) Shifts in mycorrhiza and microbial activity along an oroarctic altitudinal gradient in Northern Fennoscandia. Arct Alp Res 29:93–104

Vogelsang KM, Reynolds HL, Bever JD (2006) Mycorrhizal fungal identity and richness determine the diversity and productivity of a tallgrass prairie system. New Phytol 172:554–562