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Intra- and Interspecific Variation in Mycorrhizal Associations across a Heterogeneous Habitat Gradient in Alpine Plant Communities

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Abstract

Spatial and temporal variation in mycorrhizal associations may significantly impact plant community dynamics. In this study we evaluated the distribution and abundance of mycorrhizal associations in alpine plant communities to gain a better understanding of the potential effects of microhabitat and host identity on plant-fungus mutualisms. We surveyed the abundance of ectomycorrhizae (ECM) associated with *Salix* sp. and the abundance of arbuscular mycorrhizae (AMF) associated with *Taraxacum ceratophorum*, *T. officinale*, *Polemonium viscosum*, and *P. delicatum* in plots under willow canopies and in adjacent open meadows. AMF colonization of *T. ceratophorum*, *T. officinale*, and *P. viscosum* was greater in open meadow than in understory habitats. Conversely, ECM abundance was greater in the willow understory than in the surrounding open meadow. AMF abundance in three of the four host species was negatively correlated with ECM abundance in the soil microsite. *Taraxacum ceratophorum* showed consistently high colonization by AMF regardless of habitat or ECM abundance. Our results suggest that willow-mediated heterogeneity in light and nutrient availability influence the distribution of AMF associations across the willow-meadow ecotone. Furthermore, species-specific plant life history traits related to growth strategies, carbon allocation patterns, and stress tolerance likely affect mycorrhizal dependence and interspecific variation in mycorrhizal associations.

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Introduction

Mycorrhizal associations, mutualisms between plants and root-colonizing fungi, play an important role in plant communities (Bever, 2002; Koide and Dickie, 2002). Several studies have shown that fungal communities can influence interactions among plants and other organisms; individual responses to environmental stress, and overall community composition and structure (Callaway et al., 2001; O'Connor et al., 2002; Auge, 2004; Booth, 2004). Mycorrhizal associations are ubiquitous in nature and found in an estimated 80% of plant species (Wang and Qiu, 2006). Despite the prevalence and hypothesized importance of mycorrhizal associations to plant communities, many host plants show great variation in mycorrhizal status over space and time (Mullen and Schmidt, 1993; Smith and Read, 1997; Cripps and Eddington, 2005). Thus, even though mycorrhizal fungi are obligate biotrophs (Smith and Read, 1997), their host plants can be viewed as facultative mutualists. This variation raises interesting questions about the underlying factors determining where mycorrhizal associations occur and the importance of spatial and temporal variation in mycorrhizal status for plant community dynamics.

In this study we examined variation in the distribution and abundance of mycorrhizal associations in alpine plant communities of krummholz habitats. Krummholz vegetation represents a mosaic of open meadow and shrub or stunted tree islands at the upper limits of timberline. Some alpine plants are thought to be nonmycorrhizal; however, many plant species in these communities associate with a variety of arbuscular mycorrhizae (AMF), ectomycorrhizae (ECM), and dark septate fungi (Read and Haselwandter, 1981; Kernaghan, 2001; Cripps and Eddington, 2005; Wang and Qiu, 2006). Previous surveys have found that mycorrhizal status varies significantly for many of the plant families and species found in krummholz communities (Read and Haselwandter, 1981; Cripps and Eddington, 2005). To gain a better understanding of the mechanisms underlying this variation we evaluated patterns in mycorrhizal status associated with the willow-meadow ecotone and with host identity across this ecotone.

Our first objective was to evaluate mycorrhizal status in the willow understory and open meadow to determine whether microhabitat affects mycorrhizal associations, generating fungal mosaics that parallel aboveground patchiness in vegetation structure. In many temperate regions, alpine willows function as ecosystem engineers by modifying resource availability above- and belowground. Willows affect nutrient resources indirectly via impacts on snow depth and litter accumulation (Totland and Esaete, 2002; Dona and Galen, 2007) and directly via nutrient uptake in partnership with ECM (Nara and Hogetsu, 2004; Cripps and Eddington, 2005; Dudley, 2006). Since mycorrhizal associations are predicted to be important when and where nutrients are limiting, the effects of willows on nutrient availability could impact mycorrhizal relationships in the surrounding community.

In addition to their impact on soil nutrients, willows create a heterogeneous aboveground environment for understory plant species. Herbaceous plants under willow canopies frequently experience significant positive and negative fitness effects due to variation in light intensity, quality, and exposure (Totland and Esaete, 2002; Dona and Galen, 2006). Insofar as these effects

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restrict plant carbon budgets, they may also indirectly affect the AMF associated with understory species, providing an additional source of variation in mycorrhizal status over small spatial scales.

Our second objective was to compare mycorrhizal colonization in host species that vary in niche breadth as a first step in exploring whether habitat specialization is reflected in plant-fungal mutualisms. Habitat specialization influences species interactions and mutualist dependence aboveground (Richardson et al., 2000; Fine et al., 2004). Niche breadth may also impact interactions belowground, resulting in different responses to mycorrhizal colonization depending on the degree of mutualist dependence. These responses may, in turn, affect habitat specialization by the host species. For example, if mycorrhizal associations enhance host survival in stressful environments, then fungal partners may enable plants to expand their ecological niche. Here we compare the mycorrhizal status of two pairs of congeneric host species that vary in niche breadth.

Due to the ecological connection between above- and belowground communities, understanding the distribution of mycorrhizae in heterogeneous habitats will provide insight into factors regulating plant community structure (Wardle et al., 2004). In this paper we address this topic across multiple spatial scales in alpine ecosystems. Specifically, we ask whether patterns in mycorrhizal associations correlate with variation in (1) habitat, (2) life history traits, and (3) host plant species.

Materials and Methods

STUDY SYSTEM

This study was conducted in the alpine krummholz habitat, largely composed of a willow-meadow ecotone on Pennsylvania Mountain over an altitudinal range of 3598–3698 m a.s.l. (Pike National Forest, Park County, Colorado, U.S.A.). At this location, two willow species, *Salix glauca* and *S. brachycarpa*, and their potential hybrids form a large shrub population. As reported for other willow populations (Cripps and Eddington, 2005), individuals in this habitat are frequently colonized by multiple species of ECM (K. Becklin, unpublished data). Willows elsewhere are also known to host AMF (van der Heijden and Vosatka, 1999; Hashimoto and Higuchi, 2003); however, we have not observed AMF infection of willows at our field site over 3 years of study (K. Becklin, personal observation).

To evaluate the distribution of AMF associations in herbaceous host plants within this habitat we used two pairs of congeneric species. Species in each pair vary in niche breadth, but overlap in distribution. *Polemonium delicatum* and *P. viscosum* (Polemoniaceae) often co-occur in alpine communities in the Rocky Mountains (Scott, 1995). At our study site *P. delicatum* is found only beneath willow or spruce canopies, whereas *P. viscosum* is found in open meadows and under willow shrubs. Both species are native alpine plants, yet *P. delicatum* has a more specialized niche. *Taraxacum ceratophorum* and *T. officinale* (Asteraceae) co-occur in most habitats at the study site, including the willow understory (Brock et al., 2005). *Taraxacum ceratophorum*, the alpine dandelion, is native to North America and restricted to higher elevations (Chaney and Mason, 1936; Scott, 1995). In contrast, *T. officinale*, the common dandelion, was introduced to North America during European settlement and is common throughout the continent (Solbrig, 1971; Mack, 2003). Thus, compared to the common dandelion, *T. ceratophorum* has a more specialized niche. All four species form facultative AMF associations.

INTRA- AND INTERSPECIFIC VARIATION IN MYCORRHIZAL ASSOCIATIONS

In late July and early August 2007 we surveyed the distribution of AMF associations in the willow-meadow ecotone by determining the abundance of AMF associated with *P. delicatum*, *P. viscosum*, *T. ceratophorum*, and *T. officinale* in plots beneath and just outside the willow canopy. Surveys were conducted at three sites (3598, 3626, 3698 m) spanning the main altitudinal distribution of alpine krummholz habitat at this location. This sampling design allowed us to compare spatial patterns in mycorrhizal status at multiple scales. At each site, we located 10 plots (0.25 m^2) in the open meadow and 10 plots under the willow canopy that contained one of the four focal species. Where possible we selected understory and open meadow plots separated by no more than 3 m to minimize variation in other environmental factors (i.e. slope, aspect, and soil type). Due to the understory habit of *P. delicatum* we were only able to survey this species under willows. *Taraxacum officinale* also has a limited distribution at our study site; consequently, we were only able to survey this species at the lower two elevations.

We surveyed a flowering individual closest to the center of each plot. To determine the abundance of AMF associated with that individual we excavated the plant, carefully removed the soil from around its root system, and collected a root sample for analysis. Roots were kept damp until processed later that same day. We cleared the roots for 48 h at room temperature in 10% KOH. We then rinsed the roots with 0.1N HCl before placing them in a staining solution consisting of 0.05% trypan blue in lactic acid and glycerol for 24 h (Phillips and Hayman, 1970). The effectiveness of a given staining procedure can vary with plant species (Gange et al., 1999), however, all four species consistently showed identifiable AMF using this protocol. We examined the roots for mycorrhizae under a light microscope (Carl Zeiss, D-7082) at 250× magnification. We scanned a total of 100 points along ten 1–2 cm root fragments for arbuscules, vesicles, or coils. Due to inconsistent staining of hyphae and the difficulty of distinguishing mycorrhizal from nonmycorrhizal hyphae we did not count root lengths containing hyphae as mycorrhizal unless the hyphae were clearly connected to an arbuscule, vesicle, or coil. We then calculated AMF abundance as the proportion of colonized root length.

To evaluate the distribution of ECM in these habitats we collected a soil core (6 × 10 cm) from a random point in each plot, isolated the roots, and examined them for ECM under a dissecting microscope (Carl Zeiss, Stemi SV 6) at 10× magnification. Mycorrhizal root tips were identified based on the presence of a mantle and external hyphae (Agerer, 1993). Since we were interested in the distribution of ECM as a functional group, we focused on the abundance of these fungi in our analyses. We calculated ECM abundance as the proportion of root tips colonized by ECM. Most morphotypes displayed a simple branching pattern allowing us to count individual root tips as mycorrhizal or nonmycorrhizal. Since willow shrubs are the dominant woody plant in these habitats, the woody roots colonized by ECM were likely from *Salix* hosts; however, we did not attempt to identify the isolated roots to species.

A subsample of the herbaceous roots isolated from each soil core was stained as above to evaluate the average abundance of AMF in each plot. Again, we did not attempt to identify the plant species represented by these root samples. We followed the same methodology as previously described to calculate the proportion of root length colonized by AMF per plot.

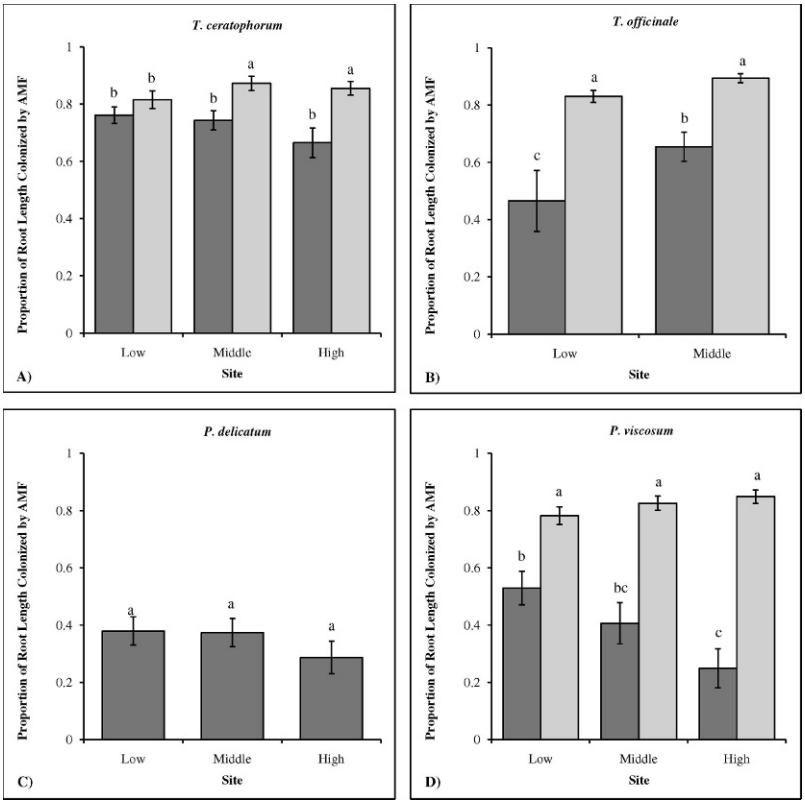

FIGURE 1. Proportion of root length colonized by AMF in the target host species (mean ± SE). Dark bars represent plants in the willow understory and light bars represent plants in the open meadow ($n = 10$ individuals per habitat per site). Sites were located at 3598 m (low), 3626 m (middle), and 3698 m (high) elevation. Different letters indicate means that are significantly different at $p < 0.05$ by a Tukey-Kramer HSD.

STATISTICAL ANALYSIS

We used JMP 5.1 statistical software (SAS Institute, Inc.) to conduct all analyses. Due to the incomplete sampling design we conducted the following analyses for each plant species separately. To meet the assumptions of ANOVA we arcsine–square root transformed the proportion data. To evaluate the distribution of AMF associations in the willow-meadow ecotone we conducted an analysis of variance (ANOVA) on the abundance of AMF in the target plants. This analysis was repeated using the abundance of arbuscules, vesicles, or coils to evaluate the distribution of these fungal structures across the ecotone. We conducted a second ANOVA on the abundance of ECM in the plots to evaluate the distribution of these associations. We included site (elevation), habitat (open vs. understory), and interactions between these variables as fixed factors in both models. To compare the distributions of ECM and AMF associations we conducted an analysis of covariance with habitat and site as independent variables, AMF abundance in the surveyed plants as the dependent variable, and ECM abundance in the plots as the covariate. To determine whether the target plants were representative of the surrounding herbaceous community in terms of their associations with AMF, we compared AMF abundance in roots from the target plants to AMF abundance in roots from the soil cores. Since the samples were paired within plots, we used a repeated measures ANOVA with habitat and site as fixed factors and plot as the subject. To compare trends between congeneric host species we repeated these analyses using data from the sites (*Taraxacum*) and habitats (*Polemonium*) where we sampled both species. To evaluate whether habitat specialization is reflected in mycorrhizal dependence we conducted an ANOVA on the abundance of AMF in the target plant species. We restricted this analysis to plants growing in the willow understory since mycorrhizal colonization was most variable in this habitat. Life history (specialist vs. generalist), host plant genus, and interactions between these variables were included as fixed factors.

Results

The abundance of AMF associated with *T. ceratophorum*, *T. officinale*, and *P. viscosum* was significantly higher in plants growing in the open meadow than in those under the willow canopy (Fig. 1 and Table 1; $p < 0.0001$). Host identity impacted the strength of this effect in the *Taraxacum* species, while site was important to trends in *P. viscosum*. The difference in AMF abundance between willow and open meadow habitats was reduced in *T. ceratophorum* compared to *T. officinale* (Figs. 1a and 1b; habitat by host interaction, $p = 0.004$), suggesting that *T. ceratophorum* may be more dependent on mycorrhizae than *T. officinale*. In *P. viscosum* the abundance of AMF in the open meadow habitat did not vary among sites; however, AMF abundance in the willow understory decreased significantly with elevation (Fig. 1d and Table 1; habitat by site interaction, $p = 0.008$). Consequently, the difference in AMF abundance between habitats increased with elevation for this species.

Arbuscules were the most abundant AMF structure observed in all four host species (Table 2); however, the effects of habitat on the abundance of each structure depended on the host. Arbuscules ($df = 1$, $F = 5.7$, $p = 0.02$) and coils ($df = 1$, $F = 16.7$, $p = 0.0002$) were more abundant in the open meadow for *T. ceratophorum*, while arbuscules ($df = 1$, $F = 4.9$, $p = 0.03$) and vesicles ($df = 1$, $F = 16.8$, $p = 0.0002$) were more abundant in the open meadow for *T. officinale*. Habitat did not affect the abundance of arbuscules in *P. viscosum* ($df = 1$, $F = 0.1$, $p = 0.08$); however, coils were more abundant in understory plants of this species ($df = 1$, $F = 4.7$, $p = 0.04$). Site did not affect the direction of these trends ($p > 0.05$ in all cases).

TABLE 1

Analysis of variance in the abundance of AMF. Habitat refers to willow vs. open meadow vegetation and site refers to elevation. AMF were surveyed in target individuals.

	T. ceratophorum			*T. officinale*			*P. delicatum*			*P. viscosum*		
Factor	*F*	*df*	*P*	*F*	*df*	*P*	*F*	*df*	*P*	*F*	*df*	*P*
Site	1.07	2	0.35	5.25	1	0.03	1.15	2	0.33	1.64	2	0.20
Habitat	18.89	1	0.0001	27.01	1	0.0001	—	—	—	82.21	1	0.0001
Site*Habitat	1.60	2	0.21	0.92	1	0.34	—	—	—	5.34	2	0.008

T. officinale was surveyed at the low and middle sites only. *P. delicatum* was surveyed in willow habitats only.
AMF = arbuscular mycorrhizae.

TABLE 2

Mean proportion of root length colonized by arbuscules, vesicles, and coils. AMF structures were surveyed in target individuals growing in the willow understory and open meadow.

	T. ceratophorum		*T. officinale*		*P. delicatum*		*P. viscosum*	
AMF Structure	Willow	Open	Willow	Open	Willow	Open	Willow	Open
Arbuscules	0.41	0.52*	0.45	0.60*	0.26	—	0.38	0.39
Vesicles	0.28	0.22	0.15	0.39*	0.07	—	0.12	0.06
Coils	0.14	0.26*	0.08	0.12	0.06	—	0.06	0.03*

Asterisks indicate means that were significantly different between open and willow habitats ($P < 0.05$). Differences among sites followed the trends represented here. *P. delicatum* was surveyed in willow habitats only.

Mycorrhizal dependence as indicated by AMF abundance in understory plants differed between habitat specialist and generalist plants for the *Taraxacum* species, but not for the *Polemonium* species (Table 3; life history by genus interaction, $p = 0.01$). Compared to *T. officinale*, AMF abundance was significantly higher in *T. ceratophorum* plants growing in the willow understory ($p = 0.006$), again suggesting that *T. ceratophorum* is more dependent on mycorrhizae across the willow-meadow ecotone than *T. officinale*. AMF abundance did not differ between *P. delicatum* and *P. viscosum* understory plants ($p = 0.44$).

ECM abundance was positively correlated with the presence of willows (Fig. 2; $p < 0.0001$) and negatively correlated with the abundance of AMF (Fig. 3). Specifically, the abundance of AMF associated with *T. ceratophorum*, *T. officinale*, and *P. viscosum* decreased as the abundance of ECM in the corresponding plots increased (Fig. 3 and Table 4; $p = 0.0001$). This correlation was not significant for *P. delicatum* ($p = 0.6$), except at the high site ($p < 0.0001$). The strength of the inverse relationship between the abundance of ECM and AMF tended to increase with elevation for all four host species, though this trend was not significant for *T. ceratophorum* or *P. viscosum* (Table 4; ECM by site interaction). ECM were significantly more abundant at the high site than at the lower two sites (Fig. 2; $p = 0.02$), which could contribute to that trend. The absence of a significant ECM by habitat interaction indicates that the inverse relationship between ECM and AMF abundance was independent of habitat (Table 4; $p > 0.1$).

The correlation between AMF abundance in the surveyed plants and in roots collected from the plots at large depended on the focal species and habitat (Table 5; plot effect and plot by habitat interaction). In the open meadow AMF abundance in the target plant was not significantly different than AMF abundance in the corresponding plot for *T. officinale* (Fig. 4b; $p = 0.9$) and *P. viscosum* (Fig. 4d; $p = 0.7$). In the willow understory AMF colonization tended to be lower than the plot average for *T. officinale* (Fig. 4b; $p = 0.1$), *P. delicatum* (Fig. 4c; $p = 0.002$), and *P. viscosum* (Fig. 4d; $p < 0.0001$). The strength of these trends was greater at the middle and high sites for *P. viscosum* (Table 5; plot by habitat by site interaction, $p = 0.04$). For *T. ceratophorum* AMF were significantly more abundant in the target individual

TABLE 3

Analysis of variance in the abundance of AMF in understory plants. Genus refers to *Polemonium* and *Taraxacum* congeners and life history refers to habitat specialists and generalists. AMF were surveyed in target individuals growing under willows.

Factor	*F*	*df*	*P*
Genus	40.63	1	0.0001
Life History	2.59	1	0.11
Genus*Life History	6.85	1	0.01

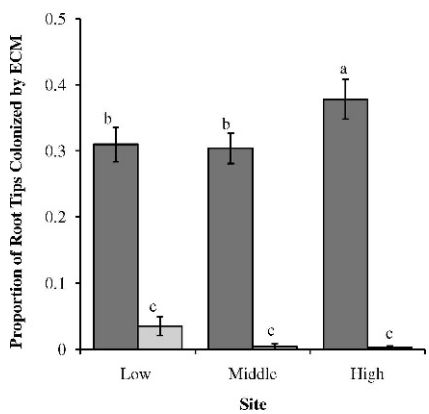

FIGURE 2. Proportion of root tips colonized by ECM in the survey plots (mean ± SE). Dark bars represent samples from understory plots ($n = 40$ plots per site) and light bars represent samples from open meadow plots ($n = 30$ plots per site). Sites were located at 3598 m (low), 3626 m (middle), and 3698 m (high) elevation. Different letters indicate means that are significantly different at $p < 0.05$ by a Tukey-Kramer HSD.

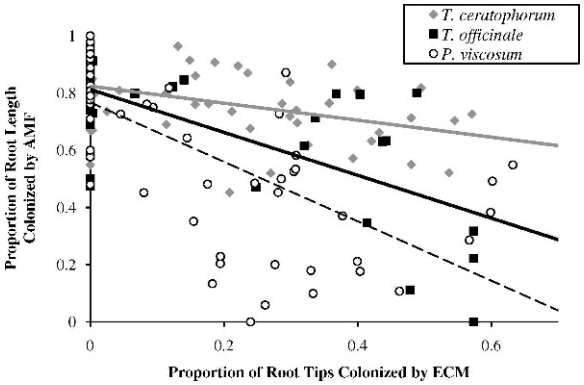

FIGURE 3. Negative correlation between ECM abundance in the survey plots and AMF abundance in *T. ceratophorum* (gray line; $r = -0.26$), *T. officinale* (black line; $r = -0.63$), and *P. viscosum* (dashed line; $r = -0.72$). This relationship was not significant in *P. delicatum* (not shown).

than in the corresponding plot regardless of habitat (Fig. 4a; $p < 0.0001$). This difference was greater in the open meadow (Fig. 4a).

Discussion

Previous research on mycorrhizal associations in alpine habitats emphasized broad patterns in plant-fungus associations. These studies indicate that many alpine plants form dynamic or facultative associations with mycorrhizal fungi, including multiple ECM and AMF species. Beyond this fundamental information there is relatively little resolution regarding variation in mycorrhizal status and its impact on plant communities. Our study indicates that interspecific interactions, life history or ecological characteristics, and habitat heterogeneity account for some of the variation in mycorrhizal colonization rates within local alpine communities. Results support the strong association between alpine willows and ECM observed in previous studies (Read and Haselwandter, 1981; Kernaghan, 2001; Cripps and Eddington, 2005). At the same time, our surveys indicate that willows and/or ECM may negatively affect the distribution of AMF associations in herbaceous alpine plants. Plant species identity had a significant impact on these trends, indicating that the four host species may vary in mycorrhizal dependence. Site variability also affected these patterns, emphasizing the role of habitat heterogeneity in plant-fungal interactions.

HABITAT HETEROGENEITY AND MYCORRHIZAL ASSOCIATIONS

Patchy resources and environmental gradients in alpine habitats are often associated with mosaics of plant and fungal communities (Read and Haselwandter, 1981; Gardes and Dahlberg, 1996). For example, krummholz vegetation is defined by a patchwork of willow shrubs and alpine meadow openings at treeline. Our results suggest that variation in mycorrhizal status within plant populations creates a belowground fungal mosaic that mirrors aboveground plant distributions. Several mechanisms may contribute to this pattern. First, willows significantly reduce the amount of photosynthetically active radiation that reaches understory plants (Totland and Esaete, 2002; Dona and Galen, 2006). Given the costs of AMF associations, reduced light availability could negatively influence carbon allocation to mycorrhizal partners and the overall abundance of these relationships in understory habitats. Alternatively, resource depletion and/or competition between ECM and AMF for soil nutrients may negatively affect the efficiency of AMF associations

TABLE 4

Analysis of covariance between ECM and AMF abundance. Habitat refers to willow vs. open meadow vegetation and site refers to elevation. ECM were surveyed in soil cores and AMF were surveyed in target individuals.

	T. ceratophorum			*T. officinale*			*P. delicatum*			*P. viscosum*		
Factor	*F*	*df*	*P*	*F*	*df*	*P*	*F*	*df*	*P*	*F*	*df*	*P*
ECM	19.67	1	0.0001	30.74	1	0.0001	0.25	1	0.62	69.93	1	0.0001
ECM*Site	0.96	2	0.39	7.83	1	0.008	16.65	2	0.0001	3.05	2	0.06
ECM*Habitat	0.88	1	0.35	0.73	1	0.40	—	—	—	2.55	1	0.12

T. officinale was surveyed at the low and middle sites only. *P. delicatum* was surveyed in willow habitats only.
ECM = ectomycorrhizae.

TABLE 5

Repeated measures analysis of variance in the abundance of AMF. Habitat refers to willow vs. open meadow vegetation and site refers to elevation. AMF were surveyed in target individuals and soil cores.

	T. ceratophorum			*T. officinale*			*P. delicatum*			*P. viscosum*		
Factor	*F*	*df*	*P*	*F*	*df*	*P*	*F*	*df*	*P*	*F*	*df*	*P*
Between Subjects												
Site	0.68	2	0.51	0.2	1	0.66	0.16	2	0.85	1.31	2	0.28
Habitat	51.65	1	0.0001	27.25	1	0.0001	—	—	—	65.69	1	0.0001
Site*Habitat	0.24	2	0.79	0.29	1	0.59	—	—	—	3.50	2	0.04
Within Subjects												
Plot	47.44	1	0.0001	0.02	1	0.89	12.21	1	0.002	0.17	1	0.68
Plot*Site	0.84	2	0.44	8.94	1	0.005	0.89	2	0.42	0.77	2	0.47
Plot*Habitat	3.07	1	0.09	3.36	1	0.08	—	—	—	37.89	1	0.0001
Plot*Site*Habitat	1.55	2	0.22	0.70	1	0.41	—	—	—	3.52	2	0.04

T. officinale was surveyed at the low and middle sites only. *P. delicatum* was surveyed in willow habitats only.

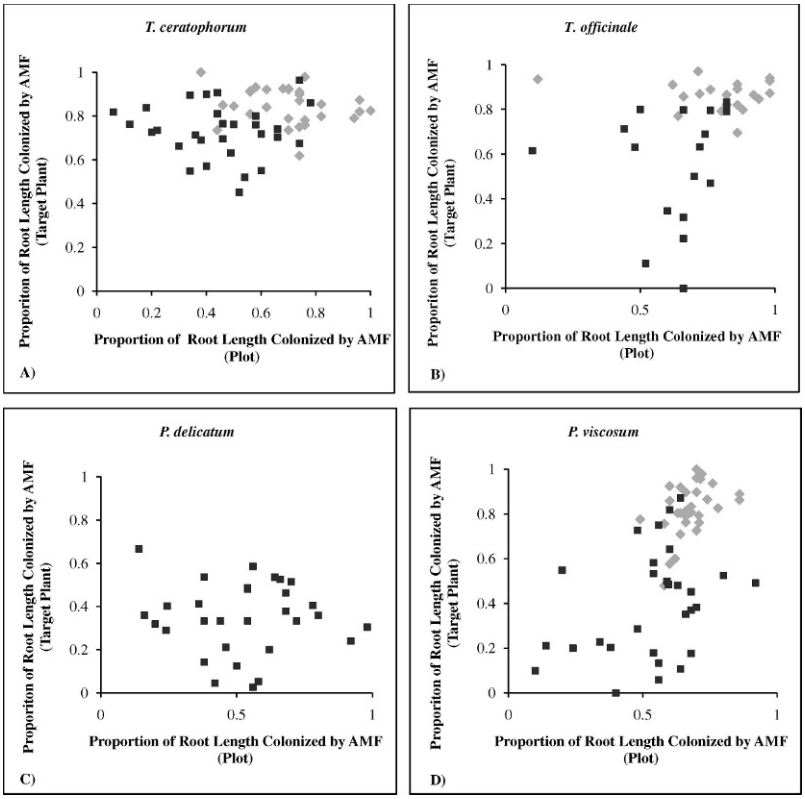

FIGURE 4. Correlation between AMF abundance in the target host species and corresponding survey plots. Dark symbols represent understory plots and light symbols represent open meadow plots.

(Koide and Dickie, 2002; Booth, 2004). By providing their willow hosts with soil nutrients, ECM may usurp resources that could otherwise be allocated to AMF hosts. As the potential benefits of AMF associations decrease, herbaceous hosts may be less likely to support symbiotic fungi. Thus, carbon limitation and/or resource depletion and competition may contribute to the decline in AMF abundance under the willow canopy. Additionally, indirect effects of willows mediated through other environmental factors could influence AMF associations. For example, litter input by willows may increase nutrient availability in the understory, reducing the need for AMF. Plant secondary compounds released by *Salix* may also affect the soil biota and nutrient cycling (Schmidt et al., 2000) leading to shifts in AMF colonization across the willow-meadow ecotone. Experiments are needed to dissect these and other hypotheses.

Broader environmental heterogeneity related to resource distribution and abiotic stress may also affect variation in mycorrhizal status within plant communities. Studies indicate that plant-fungus interactions vary along environmental gradients in resource availability, disturbance, and abiotic stress (Jumpponen et al., 2002; Hasselquist et al., 2005; Nilsson et al., 2005). Our results suggest that alpine mycorrhizal distributions follow similar trends. Specifically, we found that the impact of willow canopies on AMF abundance increased with elevation. This trend suggests that the combination of biotic (willow canopy) and abiotic (altitude) conditions had a greater impact on mycorrhizal colonization than either factor alone.

This study built upon previous surveys of mycorrhizal associations by looking at the distribution and abundance of these interactions within and among host species over multiple spatial scales. Most of the variation we saw was at the local scale between microhabitats. If we had limited our study to large-scale patterns we would have underestimated the amount of variation in mycorrhizal associations and missed some of the differences due to host identity and life history traits. Thus, our results demonstrate the importance of incorporating multiple spatial scales in ecological studies.

HOST IDENTITY, LIFE HISTORY, AND MYCORRHIZAL ASSOCIATIONS

Although mycorrhizal associations are common in most plant species (Wang and Qiu, 2006), host plants frequently vary in the degree to which they depend on fungal partners for survival and growth (Callaway et al., 2001; Bever, 2002). Some species or groups, such as orchids and club mosses, are completely dependent upon mycorrhizae for carbon and/or nutrients during different life stages (Cameron et al., 2006; Winther and Friedman, 2008). However, as our results show, many herbaceous plants engage in facultative mutualisms ranging from strong to relatively weak associations (Smith and Read, 1997). Factors that influence the efficiency of mycorrhizal associations, such as habitat and resource heterogeneity, may impact hosts differently based on their degree of mycorrhizal dependence. Our data suggest that *T. ceratophorum* is more dependent on mycorrhizae than its congener, *T. officinale*. In particular, *T. ceratophorum* consistently supported a higher abundance of AMF compared to *T. officinale*. This difference was most apparent when comparing colonization rates in understory habitats where environmental conditions seemed to negatively impact mycorrhizal associations. *Polemonium viscosum* also showed greater colonization by AMF than *P. delicatum*. However, since *P. delicatum* is only found in the willow understory where colonization was reduced for both species it is difficult to determine whether these species differ in mycorrhizal dependence. The lack of an apparent difference in mycorrhizal dependence between *P. delicatum* and *P. viscosum* may reflect their similar evolutionary histories in alpine environments. Overall, our

results partially support the view that plant-fungus mutualisms may promote local adaptation to a particular habitat or ecological niche (Read, 1993; Richardson et al., 2000).

The observed interspecific variation in mycorrhizal status may correlate with host-specific ecological strategies in the *Taraxacum* species. For example, *T. officinale* exhibits traits characteristic of weedy plants, including rapid growth and greater vegetative plasticity (Brock et al., 2005). Increased carbon allocation to leaf growth may reduce a host's ability to support AMF, particularly if carbon is limiting as may be expected for understory habitats. In contrast, *T. ceratophorum* is adapted to tolerate stressful, dry conditions (Brock and Galen, 2005; Brock et al., 2005). As part of this strategy, *T. ceratophorum* tends to be smaller with a slower growth rate. Plants exhibiting these stress-tolerant characteristics may not experience the same carbon trade-offs as their weedy relatives (Tinker et al., 1994). Furthermore, mycorrhizae are known to positively affect plant-water relations and drought tolerance (Auge, 2004). Consequently, these associations may be an important component of *T. ceratophorum*'s ability to tolerate dry alpine conditions. As this comparison shows, multiple factors likely influence interspecific variation in mycorrhizal associations and ecological or life history traits.

LIMITATIONS AND FUTURE DIRECTIONS

An important consideration when interpreting these results is the limitations of our approach. We surveyed these plants at a single time during the 2007 growing season and we focused our survey on flowering adults. However, mycorrhizal colonization is known to vary temporally and with plant developmental stage (Mullen and Schmidt, 1993; Ruotsalainen et al., 2002; Correa et al., 2006). Thus, it is likely that the observed patterns would change over time. Preliminary research from 2008 indicates that AMF abundance differed significantly between willow understory and open meadow habitats in mid- to late July, but not in early June (M. Pallo and K. Becklin, unpublished data). Other studies of alpine plants have shown that AMF colonization peaks in August when nutrient availability is reduced, indicating that these relationships may be particularly important at that time (Mullen and Schmidt, 1993). In our survey we concentrated our efforts at a time and developmental stage when we thought AMF might play a bigger role in plant communities. However, future research should consider other sources of variation.

Another limitation of this study comes from our focus on AMF and ECM abundance. Mycorrhizal fungi differ in their associated costs and benefits just as plants differ in their resource needs and photosynthetic abilities. Consequently, habitat heterogeneity and host identity likely influence the composition of fungal communities (Gehring et al., 1998). Future research should explore variation in fungal assemblages across a broad scale of environmental gradients to better describe the dynamic nature of mycorrhizal associations.

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