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ORIGINAL ARTICLE

Responses of arbuscular mycorrhizal symbionts to contrasting environments: field evidence along a Tibetan elevation gradient

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Abstract Plant adaptation to alpine ecosystems is not fully explained by plant physiological and morphological traits. Arbuscular mycorrhizal (AM) associations may be involved in mediating plant performance in response to environmental differences. Little is known, however, as to whether or not a close relationship exists between plant performance and arbuscular mycorrhizal fungus status across environmental gradients. We conducted a field investigation of the performance of six plant species and their associated AM fungi along higher and lower elevation gradients on Mount Segrila in Tibet. In most of our species, we observed higher shoot and inflorescence biomass production and a lower root-to-shoot ratio in the populations at those sites where the species was dominant (intermediate elevation sites) than in populations sampled at the limits of the distribution. The elevation pattern of root colonization differed with plant species on both gradients, and the extraradical hypha development of most species showed a unimodal pattern as did plant growth. The relationship between plant and fungus traits shows that AM fungus development generally matched host plant performance on the lower elevation gradient but not on the higher elevation gradient. This study provides evidence that plant distribution and productivity were significantly related to root and soil colonization by AM fungi, especially under less physically stressful conditions.

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Introduction

Elevation gradients can provide useful information about how plant species and evolutionary dynamics are restricted by environmental conditions (Parmesan and Yohe 2003). Some recent work along elevation gradients has led to many insights regarding plant responses to climate change, directly via altered performance, distribution, and phenology and also indirectly through altered biotic interactions (Grabherr et al. 2009). However, most of these studies have ignored symbiont interactions under natural environmental conditions. Numerous environmental factors including climate, soil condition, and species interactions simultaneously determine symbiont function (Chagnon et al. 2013). In high alpine areas, plants and microbes are both constrained by low temperatures and short growing seasons, resulting in resource fluxes that are different from comparable temperate habitats and characteristic plant resource allocation strategies (Kytöviita 2005). Higher elevations rather than lower elevations are commonly dominated by plants of low stature and leaf area (Sundqvist et al. 2013), more reproductive investment (Fabbro and Körner 2004), large root systems as storage organs (Geng et al. 2014), and functional groups that are characterized by inherent adaptations to stressful environments. Although much work has focused on elevation patterns of alpine plant performance, less work has been done on the interactive effects of fungal symbioses along elevation ranges.

Arbuscular mycorrhizal (AM) fungi are obligate biotrophs with vascular plants, and they facilitate plant acquisition of limiting soil resources such as nutrients and water and increase

plant adaptation to environmental stress (Aroca et al. 2007). In high elevation sites, however, there are numerous unpredicted plant relationships with mycorrhizas in light of the important role of mycorrhizal symbiosis in comparable temperate habitats (Kytöviita 2005). For example, mycorrhizas should be especially advantageous for herbaceous plants, but there are many observations to suggest that the high alpine flora is non-mycorrhizal or has low colonization (Ruotsalainen et al. 2004). Explanations for this have focused mainly on plant performance and include decreasing species performance with increasing elevation due to lower temperatures (Körner 2003), reduced photosynthetic production, decreased supply of carbon to the fungal symbiont (Johnson 2010), and higher root-to-shoot ratios compared to low-altitude plants (Geng et al. 2014) which has been proposed to indicate a functional replacement of mycorrhizas. Another important idea is "asymmetric symbiont adaptation" which suggests that fungus performance limits the occurrence of mycorrhizal symbioses (Kytöviita 2005). Low temperatures may directly affect the germination of spores and the growth of hyphae and even the physiological activities of fungal hyphae (Kytöviita 2005) which limit nutrient transfer to the host and host carbon assimilation by mycorrhizal fungi. It must be noted that very few studies have been conducted on the simultaneous responses of plants and AM fungus symbionts to environmental change (Ruotsalainen et al. 2004). Studies to characterize the performance of symbionts along environmental gradients in alpine habitats are therefore necessary prior to accepting the ideas presented above.

The Tibetan plateau is a vast and high plateau with an average elevation of over 4000 m (Qiu 2008). Evidence indicates that the plateau is experiencing climatic warming (Wang and French 1994) leading to a dramatic decline in plant species diversity (Klein et al. 2004). Mount Segrila is situated on the southeastern part of the plateau and is representative of the typical montane frigid temperate forest of southeast Tibet. One approach to better understanding the generality of response to environmental change can be to compare several plant species or populations as "replicates" within an ecosystem (Parmesan et al. 2005). In the current study, we selected six plant species that are distributed over the lower (3280 to 3811 m (4140 m) a.s.l.) or higher (4140 to 4556 m a.s.l.) slopes of Mount Segrila to test whether a close relationship exists between plant performance and AM fungus status along elevation gradients.

Materials and methods

Study areas

This study was conducted at six elevation sites from 3280 to 4556 m a.s.l. along the west slope of Mount Segrila (29° 21′–29° 50′ N, 94° 28′–94° 51′ E) in Nyingchi region, southeast Tibet. The mountain is located at the convergence of the Nyainqentanglha range and Himalaya range, and the altitude of the peak is 5200 m. The vegetation is typical alpine meadow, and the climatic conditions are typically alpine. The mean annual air temperature of the mountain is −0.73 °C (minimum −13.98 °C in January and maximum 9.23 °C in July), the mean annual total precipitation is 1134.1 mm, and the annual evaporation is 544.0 mm. There are four distinct vegetation types, namely, warm temperate montane with mixtures of conifer and broadleaved trees (<4000 m), temperate montane with *Abies* and *Picea* trees (4000–4300 m), cold alpine temperate composed of shrub meadow (4300–4500 m), and cold alpine composed of meadow (>4500 m) (Chai et al. 2004). The corresponding soil properties and climatic conditions are presented in Table 1.

Host plant species

Six plant species were selected for study. *Fragaria nubicola* (*Rosaceae*), *Trollius ranunculoides* (*Ranunculaceae*), and *Tibetia himalaica* (*Leguminosae*) inhabit the lower elevation gradient (3280 to 3811 (4140) m a.s.l.) with a typical temperate montane climate, and *Geranium sibiricum* (*Geraniaceae*), *Poa crymophila* (*Gramineae*), and *Phlomis younghusbandii* (*Labiatae*) are generally distributed on the higher elevation gradient (4140 to 4556 m a.s.l.) with a subalpine to alpine cold climate. All six species are perennial herbs. *P. crymophila* is monocotyledonous and the other five species are dicotyledonous.

Sample collection

Plant and soil samples were collected at the flowering stage from three sites of both the lower (3280, 3485, 3811 (4140) m a.s.l.) and higher (4140, 4361, 4556 m a.s.l.) elevation gradients from June to July 2014. Within each study site, we chose a belt ca. 50 m long and 10 m wide within which we randomly selected six or seven plots (1 × 1 m, at least 10 m apart) for each plant species. The number of plots was 21 at 3280, 3485, 3811 m a.s.l., 26 at 4140 m a.s.l., and 20 at 4361and 4556 m a.s.l., respectively. We counted the number of plant species in each plot and estimated the total cover of the study species. Five flowering individuals per plot of every study species were excavated with a shovel to a depth of 20 cm and separated from other plants by hand. Inflorescences and leaves were cut and stored in paper bags. Other plant parts were transported to the laboratory intact. Rhizosphere soil of five individuals was shaken from the roots and collected. A total of 129 plots and 645 individual plant and soil samples were collected from the six elevation sites.

Table 1 Location, soil properties, and climatic conditions at the sampling sites

Elevation (m)	Location	pH	Olson-P (g kg^{-1})	Total C(g kg^{-1})	Total N (g kg^{-1})	GST(°C)	GSP (mm)
3280	29° 33′ N 94° 32′ E	5.84 ± 0.06ab	8.67 ± 1.42b	21.65 ± 1.78b	2.29 ± 0.21a	13.24	795
3485	29° 33′ N 94° 33′ E	6.04 ± 0.06a	9.28 ± 0.94b	32.74 ± 5.33ab	2.78 ± 0.49a	11.78	770
3811	29° 33′ N 94° 34′ E	5.51 ± 0.09bc	16.32 ± 2.31a	53.67 ± 17.26ab	4.83 ± 1.61a	9.45	750
4140	29° 35′ N 94° 36′ E	5.43 ± 0.09bcd	13.33 ± 1.48ab	53.13 ± 1.32ab	4.98 ± 0.08a	7.24	930
4361	29° 35′ N 94° 36′ E	5.25 ± 0.10cd	9.99 ± 0.82ab	58.20 ± 0.92a	5.03 ± 0.17a	5.52	855
4556	29° 37′ N 94° 37′ E	5.01 ± 0.14d	7.33 ± 0.41b	48.93 ± 4.63ab	4.05 ± 0.25a	4.12	740

Data are averages (±SE) of five replicates at each elevation. Values in each column followed by the same letter do not differ significantly at $P \leq 0.05$ by Tukey's HSD test among the six elevations

GST growing season temperature (mean temperature from May to September), *GSP* growing season precipitation (total precipitation from May to September)

Measurement and analysis

The frequency and cover coefficients of target plant species were calculated with a 1-m^2 point frame in each plot (shown in Table S1). The frequency was calculated as the number of plots with the target species divided by the total number of plots; the cover coefficient was calculated as the total coverage of all individuals of a target species per plot averaged across the total number of plots. Richness (species number per plot) also was recorded. Roots were washed carefully with tap water, weighed, and divided into two subsamples, one of which was used to determine root colonization by AM fungi. The roots were cleared in 10 % KOH and stained with 0.05 % trypan blue. We then used the magnified intersection method to assess fungal colonization percentage including the presence of fungal structures (vesicles, arbuscules, and hyphae) in the roots at 300 random intersections (McGonigle et al. 1990). The remaining root samples together with leaves, inflorescences, and other plant parts were dried at 65 °C for a minimum of 48 h and weighed to determine the dry biomass.

Approximately 100 g soil was collected from the rhizosphere of the study species roots by the shaking method. Extraradical hyphae were isolated from 5-g soil by an aqueous extraction and membrane filter technique (Jakobsen et al. 1992). Intersections between trypan blue-stained hyphae and an eyepiece grid were counted in 25 random fields of view at ×200 magnification under a light microscope, and hyphal density was calculated according to the method described by Miller et al. (1995).

Shoot tissue P concentrations were determined after wet digestion with sulfuric acid and hydrogen peroxide of dried plant material ground and passed through a 20-μm mesh. Phosphorus concentrations in the digests were determined using the molybdenum blue method (Murphy and Riley 1962).

Calculations and statistics

Plant traits consisted of the shoot biomass (leaves and stem), inflorescence biomass, reproductive allocation (calculated as inflorescence biomass divided by the shoot biomass), root-to-shoot ratio (calculated as the root biomass divided by the shoot biomass), and the leaf mass ratio (dry weight of leaves divided by plant total biomass). Five individual samples from each plot were combined to give one composite value per plot for subsequent statistical analyses. Shoot biomass, inflorescence biomass, reproductive allocation, leaf mass ratio, root-to-shoot ratio, shoot P concentration, colonization rate, and hyphal length density values all were z-score transformed prior to correlation analyses in order to minimize interspecific differences resulting from plant size. A series of bivariate correlation analyses were conducted to examine the relationship between plant traits and AM fungus root and soil colonization. Moreover, two separate stepwise regressions of AM fungal growth vs. the biotic variables and abiotic variables were used to find the most important influencing factors which could be determined on the two elevation gradients. To obtain the same sample sizes, we averaged soil pH and soil phosphorus concentration per elevation, and we similarly averaged the richness of those plots pertaining to particular plant study species per elevation for use in the stepwise regressions. Significant differences among elevations in shoot biomass, inflorescence biomass, total biomass, root-to-shoot ratio, reproductive allocation, shoot P concentration, proportion of organ investment, colonization rate, and hyphal length density values were detected by Tukey's honestly significant difference (HSD) at $P < 0.05$. ANCOVA was used to analyze the effect of elevation site on reproductive allocation and root-to-shoot ratio, and the aboveground biomass and total biomass were used as co-variables,

respectively. Statistical analyses were performed with the SPSS 21.0 software package (SPSS Inc., Chicago, IL).

Results

Host response to different environments associated with elevation

Elevation greatly influenced plant biomass. Total biomass and shoot biomass of five species—*F. nubicola*, *T. ranunculoides*, and *T. himalaica* on the lower elevation gradient and *G. sibiricum* and *P. younghusbandii* on the higher elevation gradient (Fig. 1a, b and Table S2)—were highest at the intermediate sites (3485 or 4361 m a.s.l.) where the highest frequency value and cover coefficient of those target species were detected (shown in Table S1). On the higher elevation gradient, the biomass of *P. crymophila* did not differ significantly at the three sites (Fig. 1b).

A significant elevation trend in inflorescence biomass also was detected. Irrespective of elevation gradient conditions, inflorescence biomass of all species was highest at the intermediate sites at 3485 or 4361 m a.s.l along the two elevation gradients (Fig. 1c, d).

Reproductive allocation and root-to-shoot ratio were highly variable among elevations and also among plant species (Table S2). Elevation was the main factor affecting the reproductive allocation of *F. nubicola* ($F_{3,\ 19}=6.61$, $P<0.01$). Both elevation and aboveground biomass influenced the reproductive allocation of *T. ranunculoides* ($F_{2,\ 17}=7.20$, $P<0.01$; $F_{1,\ 17}=20.45$, $P<0.01$, respectively) and *P. crymophila* ($F_{2,\ 17}=9.90$, $P<0.01$; $F_{1,\ 17}=5.16$, $P<0.05$, respectively). For the other three plant species, no significant elevation effect on reproductive allocation was observed. Elevation was the main factor affecting the root-to-shoot ratio of *F. nubicola* ($F_{3,\ 19}=4.47$, $P<0.05$), *P. crymophila* ($F_{2,\ 17}=17.40$, $P<0.01$), and *P. younghusbandii* ($F_{2,\ 14}=38.46$, $P<0.01$). An effect of elevation ($F_{2,\ 17}=16.86$, $P<0.01$) as well as of total biomass ($F_{1,\ 17}=5.54$, $P<0.05$) on root-to-shoot ratio was observed for *T. himalaica* only. No significant effect of elevation on the root-to-shoot ratio was observed for *T. ranunculoides* or *G. sibiricum*.

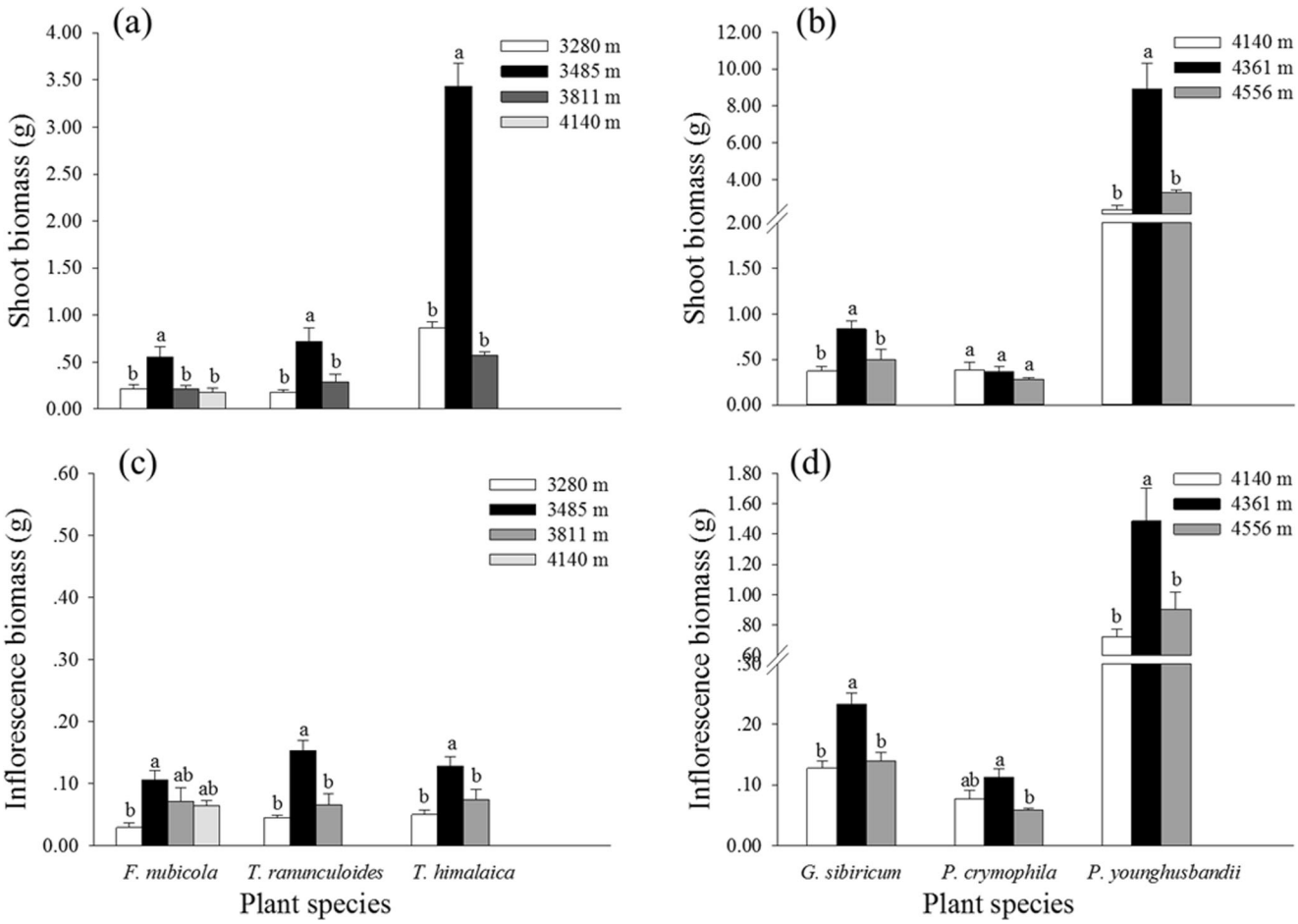

Fig. 1 Shoot biomass and inflorescence biomass from two groups of three different plant species across a range of seven elevations. *Error bars* are standard errors of the mean (SEM). *Bars* with the same *letter* do not differ significantly at $P \leq 0.05$ by Tukey' s HSD test

AM fungus performance along the elevation gradients

No consistent elevation trend in root colonization was detected regardless of whether the host plants were on the higher or lower elevation gradient (Fig. 2a, b). A higher percentage of root colonization at the intermediate site (3485 m a.s.l.) than at other elevation sites was found in *F. nubicola* and *T. himalaica* only (40.5 and 43.3 %, respectively). Root colonization of *G. sibiricum* tended to increase and of *P. younghusbandii* tended to decrease with increasing elevation. Elevation did not significantly affect the level of root colonization in *T. ranunculoides* or *P. crymophila*.

For four of the plant species, the elevation patterns of soil colonization (hyphal length density) were similar to that of shoot and inflorescence biomass. AM fungi produced much more extraradical hyphae at intermediate sites (3485 or 4361 m a.s.l.) where the shoot and inflorescence biomass of the host plants were highest than at any other sites (Fig. 2c, d). Soil colonization associated with *G. sibiricum* decreased with increasing elevation, with the highest (4.21 m g^{-1}soil) and lowest (2.79 m g^{-1}soil) values occurring at 4140 and 4556 m a.s.l., respectively. No significant difference was found in the soil colonization associated with *T. ranunculoides* among its three sites.

AM fungi produced significantly more root colonization and extraradical hyphae on the lower elevation gradient than on the higher elevation gradient (colonization, $F_{2,\ 108} = 12.49$, $P = 0.000$; HLD, $F_{2,\ 70} = 3.53$, $P = 0.035$).

Symbiont interactions and environmental effects

A series of bivariate correlation analyses were conducted to reveal relationships between host plant traits and AM fungus growth (Table 2). Hyphal densities were significantly and positively correlated with inflorescence biomass and shoot P concentration on both the lower and higher elevation gradients, but were negatively correlated with root-to-shoot ratio and positively correlated with shoot biomass and leaf mass ratio on the lower elevation gradient only. Moreover, AM fungal colonization rate was further influenced by plant performance on the lower elevation gradient but not on the higher elevation gradient. The colonization rates on the lower gradient were

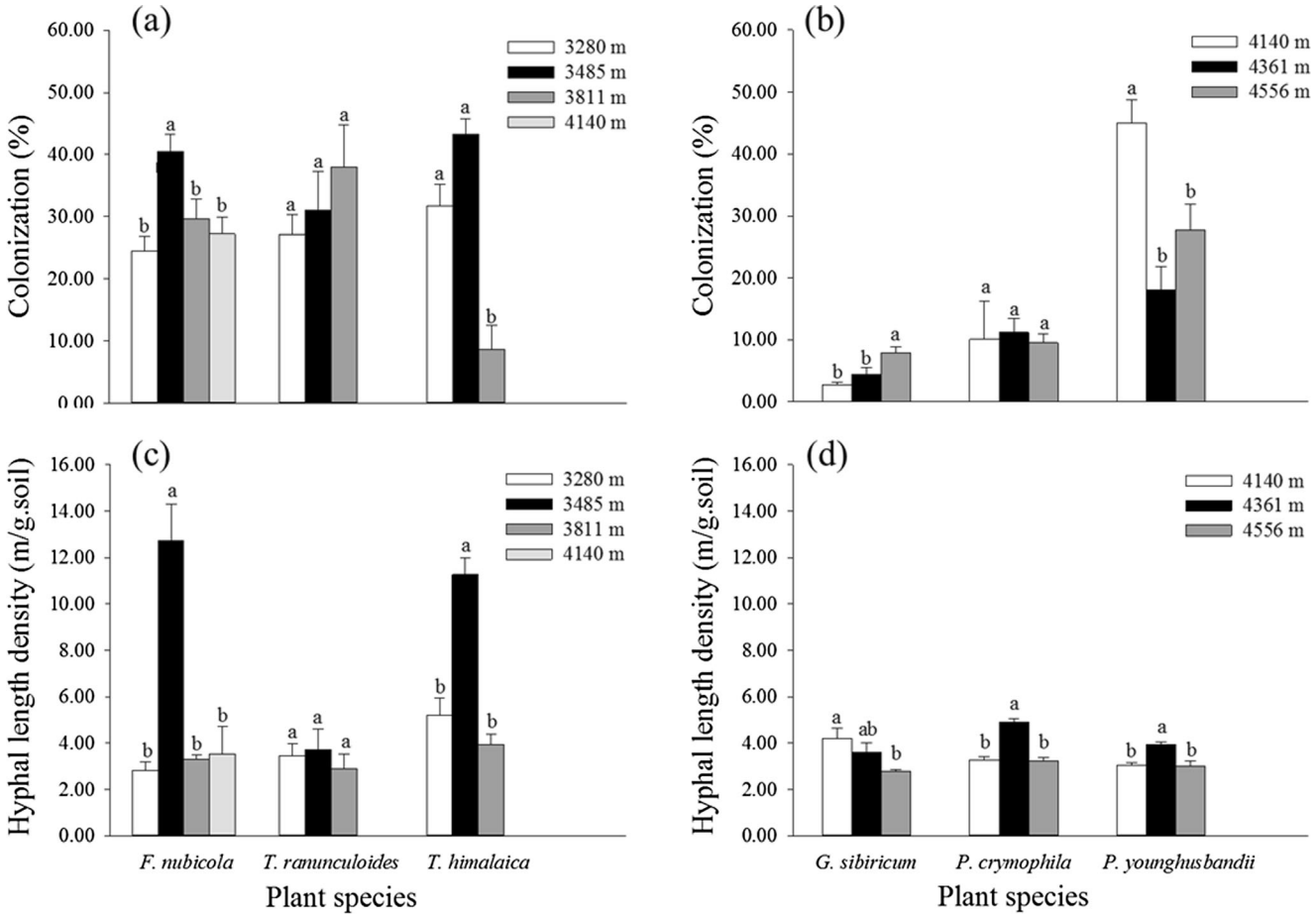

Fig. 2 Root colonization and extraradical hyphal length density from two groups of three different plant species across a range of seven elevations. *Error bars* are standard errors of the mean (SEM). *Bars* with the same *letter* do not differ significantly at $P \leq 0.05$ by Tukey's HSD test

Table 2 Pearson's correlation coefficients (r) and P value between plant traits and root colonization and hyphal length density

Plant trait parameters	Lower-elevation gradient				Higher-elevation gradient			
	Root colonization		Hyphal length density		Root colonization		Hyphal length density	
	r	P value	r	P value	r	P value	r	P value
Shoot biomass	0.391	**0.003**	0.659	**0.000**	−0.219	0.092	0.312	0.064
Inflorescence biomass	0.193	0.161	0.467	**0.002**	−0.121	0.357	0.340	**0.043**
Reproductive allocation	−0.345	**0.011**	−0.199	0.219	0.235	0.071	0.047	0.786
Leaf mass ratio	0.371	**0.006**	0.465	**0.003**	−0.200	0.126	−0.003	0.987
Root-to-shoot ratio	−0.415	**0.002**	−0.438	**0.005**	0.334	**0.009**	−0.092	0.595
Shoot P concentration	0.278	**0.048**	0.513	**0.001**	−0.230	0.077	0.369	**0.027**

Lower elevation gradient: species range of *F. nubicola, T. ranunculoides*, and *T. himalaica* from 3280 to 4140 m. Higher elevation gradient: species range of *G. sibiricum*, *P. crymophila*, and *P. younghusbandii* from 4140 to 4556 m. Significant P values are indicated in bold letters

positively related to shoot biomass, leaf mass ratio, and shoot P concentration and negatively related to reproduction allocation and root-to-shoot ratio.

A stepwise regression analysis (AM fungal colonization rates and hyphal length density values vs. pH, Olson-P, temperature, precipitation, plant cover, plant frequency, and plant richness) was conducted, and variables were selected into the model (criteria: probability of F to enter ≤0.05, probability of F to remove ≥0.100). The results show that only plant frequency entered the models of AM colonization rate, suggesting that this was the most significant influencing factor on both the lower and higher elevation gradients (Table 3; Fig. 3). This regression also shows that pH value displayed a greater effect on hyphal length density value ($R^2 = 0.351$) than temperature ($R^2 = 0.165$) on the lower elevation gradient. Moreover, plant cover had a significant effect on hyphal length density values on the higher elevation gradient (Table 3; Fig. 4). Consequently, these models were able to represent the influence of abiotic and biotic factors on AM fungal growth on both elevation gradients on the mountain.

Discussion

This field investigation has highlighted the variability in plant-AM symbioses along an elevation gradient in terms of plant traits and AM fungal colonization. Studying the performance of plant species along an elevation gradient is associated with the "null" hypothesis of a decrease in performance with elevation as a result of lower temperatures and a shorter growing season (Körner 2003). Our results do not support this hypothesis because higher shoot and inflorescence biomass was observed in all six plant species studied at the intermediate elevation sites rather than at their limits of their distributions (Fig. 1). Grassein et al. (2014) also detected two co-dominant plant species from two sites (at 650 and 2000 m a.s.l.) and observed higher biomass production in the population in the site where the species was dominant (intermediate population) compared to the marginal population. The different responses of intermediate and edge populations across the geographical range have become an important component of species response to different environments (Davis and Shaw 2001). In this context, the current results may be explained by

Table 3 Stepwise regression coefficients of AM fungal colonization and hyphal density vs. abiotic factors (pH, Olson-P, air temperature, precipitation) and biotic factors (plant cover, plant frequency, and plant richness).

Plant range	Dependent variables	Abiotic variables				Biotic variables		
		pH	Olson-P	Temperature	Precipitation	Plant cover coverage	Frequency	Richness
Lower elevation	Colonization	0.219	−0.085	0.041	−0.122	0.243	0.302*	−0.074
	Hyphal density	0.592**	−0.373*	0.212**	−0.153	0.122	0.306	−0.339*
Higher elevation	Colonization	0.086	0.112	0.111	0.079	−0.497	−0.576**	−0.043
	Hyphal density	0.246	0.181	0.184	0.262	0.516**	0.410	−0.335

Significance of the regression equations is indicated as *$P < 0.05$ and **$P < 0.01$

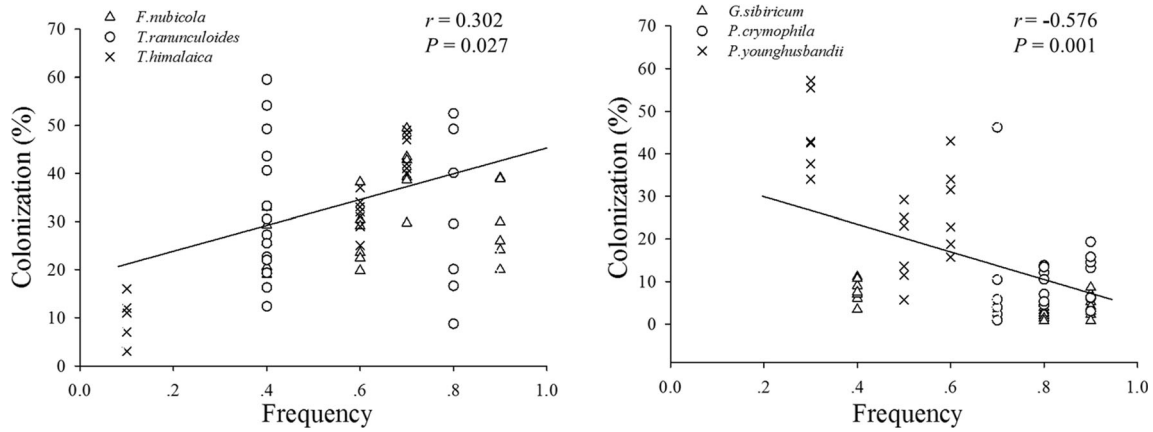

Fig. 3 Relationships between root colonization and plant frequency across the lower and higher elevation gradients

similar responses of co-occurring species to different environments occurring at a local scale both for biotic (such as interspecific competition, e.g., Callaway et al. 2002; Hülber et al. 2011) and abiotic environmental conditions (such as temperature or moisture, e.g., Vittoz et al. 2009) related to their environmental requirements.

We detected some differences in AM fungal root colonization within the host elevation range, but no consistent pattern was observed, especially for the high-elevation plants (Fig. 2). A previous investigation of *Pennisetum centrasiaticum* and *Kobresia* sp. from eight elevations (3446 to 4556 m a.s.l.) on Mount Segrila and an additional two sedge species, *Kobresia pygmaea* and *Carex pseudofoetida*, from six elevations (4149 to 5033 m a.s.l.) on the mountain showed that root colonization had no significant elevational pattern (Li et al. 2014; 2015). Differences in fungal phenology might, as suggested by Schmidt et al. (2008), account at least in part for the discrepancy between the high- and low-elevation sites for populations in the present study in which plant frequency and cover were related to the colonization pattern of AM fungi (Table 3).

We found that the distribution pattern of extraradical hyphal was similar to that of plant biomass, showing highest values at the intermediate sites in most cases (Fig. 2). This result is in accord with previous studies that have found close relationships between a high density of AM hyphae and high shoot biomass (Aggarwal et al. 2011). Plant communities and soil properties are greatly affected by elevation gradients (Sundqvist et al. 2013), and this may affect the development of extraradical hyphae (Dodd 2000). We observed a marked correlation between hyphal length value and soil pH, Olson-P, temperature, and plant richness on the lower elevation gradient and with plant cover on the higher elevation gradient (Table 3). However, the current hyphal density measurements did not include hyphae from decomposing leaves and live roots, which are well positioned to obtain and efficiently recycle mineral nutrients released by decomposer microorganisms (Aristizábal et al. 2004) and then might affect host nutrition indirectly.

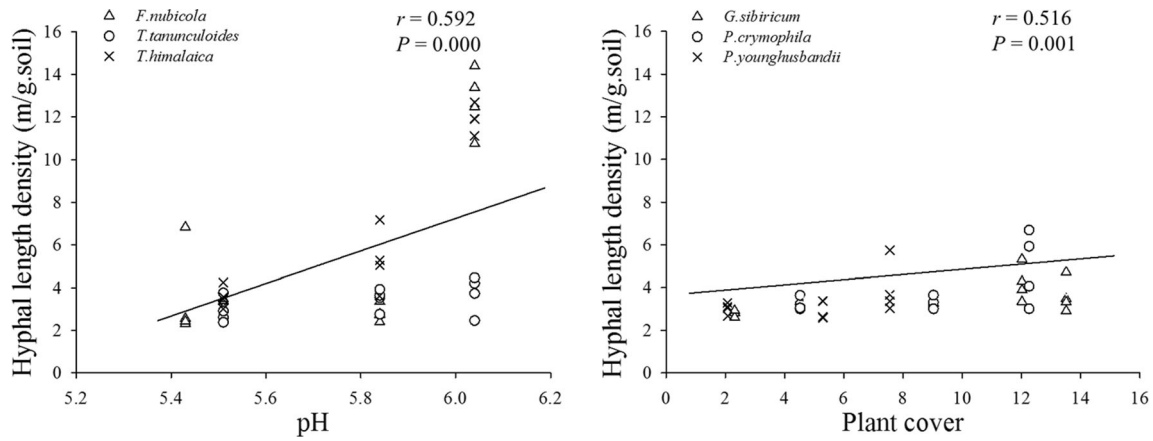

Fig. 4 Relationships between extraradical hyphal length density and soil pH on the lower elevation gradient and between extraradical hyphal length density and plant cover on the higher elevation gradient

Higher elevation sites are commonly dominated by functional groups that are characterized by inherent adaptations to stressful environments (Zhao et al. 2006). In this study, we found a significantly higher reproductive allocation rate (a higher reproductive investment) at the three higher elevation sites than the three lower ones in accordance with numerous former studies (Fabbro and Körner 2004). These results suggest that higher-elevation plants need to allocate a much higher proportion of their carbon to maintain a certain amount of inflorescence for reproduction (Fan and Yang 2009). Moreover, we observed a higher root colonization percentage and hyphal length density at the three lower elevation sites than at the higher elevation sites (Fig. 2). This finding is in accord with reports from the Andes and the Rocky Mountains (Schmidt et al. 2008) and the South American Puna grasslands (Lugo et al. 2012). A possible explanation is that the host can vary in mutualistic quality, for example, by providing less carbon to fungal partners during the early spring or under carbon limitation (Grman and Robinson 2013). Hosts also can reduce the allocation to non-beneficial AM fungi, and this may affect the distribution and abundance of AM symbionts (Grman 2012). Another possible explanation might be that AM symbionts on higher elevation gradients are frequently challenged by extreme cold temperatures in winter that may suppress their development (Ruotsalainen et al. 2002).

Plants are faced with variable environments aboveground and belowground, and this may affect the plants themselves and also their symbiotic partners (Goh et al. 2013). In the present study, we explored small-scale co-variation of AM fungi and their host plants on the lower elevation gradient; i.e., root and soil colonization was closely related to plant performance on the lower elevation gradient (Table 2). AM fungi often performed better (higher root and soil colonization) when the plants had higher shoot biomass and reproductive investment and lower root-to-shoot ratio and reproductive allocation (Figs. 1 and 2; Table S2). The evidence indicates relationships between plant distribution and productivity and root and soil colonization by AM fungi as previously indicated by some former studies (Hodge and Fitter 2010; Grman and Robinson 2013).

A classic assumption in life history theory is that there is a trade-off between resource allocation to reproduction and that to other functions related to fitness (Hempel et al. 2013; Zheng et al. 2015). Individuals of *F. nubicola* and *T. himalaica* appeared to increase reproductive and root allocation (Table S2) and form less AM fungal colonization in roots and soil to maintain a certain amount of reproductive investment at the trailing and leading edges, while the plants had sufficient carbon to supply to the host and AM fungal biomass at the intermediate sites. Work by Grman (2012) also suggests that mycorrhizal plants with the ability to effectively regulate their mycorrhizal association will benefit most from the association. *T. ranunculoides* individuals showed similar elevation pattern of shoot and inflorescence biomass to the other two species but did not show the differences in reproduction allocation and AM fungal development in different environments. This phenomenon might imply that the resource allocation of AM associations is related to plant identity in particular conditions.

Compared to the lower elevation gradient, we observed a quite different symbiotic interaction on the higher elevation gradient (Table 2), especially interactions between plant traits and root colonization. Plant performance along the higher elevation gradient was similar to the low elevation gradient, but fungal performance was quite different (Figs. 1 and 2). AM fungi associated with different plant species showed different responses to contrasting environments, indicating that there was likely to be no close relationship between symbionts at the high-elevation sites. Some other studies have found that host plants and AM fungi show contrasting responses to temperature change (Shi et al. 2015) or that the responses of fungal intraradical structures and hyphal networks to soil temperatures are largely independent of plant size and photosynthetic rate (Hawkes et al. 2008). This phenomenon seems to be quite common, especially in high Arctic ecosystems (Kytöviita 2005). There was generally lower root colonization at the high elevation sites than at the low sites and, consequently, a lower signal-to-noise ratio at high elevations compared with the lower elevations which may have precluded finding statistically significant patterns.

Even though the interactions among plant traits and mycorrhizal colonization were complex on the higher elevation gradient, the extraradical hyphal pattern matched plant performance in most cases. It seems that the responses of the external hyphae were more stable with environmental change than those of the internal hyphae. Some studies have shown that extraradical hyphae can form water-stable soil aggregates and can be protected by soil structure (Aggarwal et al. 2011). Addy et al. (1997) also suggest that extraradical hyphae are well adapted to tolerate low temperatures and natural soil disturbance associated with freezing may not eliminate AM fungal hyphae. We also found that hyphal length values were significantly and positively correlated with shoot P concentration on both elevation gradients (Table 2), a finding consistent with previous studies (Wang et al. 2011), with the possibility that high hyphal density can directly increase phosphorus uptake.

In summary, we found that plant distribution and productivity were significantly related to root and soil colonization by AM fungi, especially on the lower elevation gradient. Moreover, plant biomass and AM fungus biomass can vary independently among species and populations depending on their sensitivity to different environments (Chevin et al. 2013; Shi et al. 2014). Even though no consistent pattern of AM fungal distribution was detected for both the lower and higher elevation gradients, our results indicate effects of

environmental change on populations of plant species sampled at the intermediate elevations or at the edges of species distributions (Davis and Shaw 2001). These results confirm the need to incorporate plant species variation and AM fungal symbiont development into predictions of response to global climate change. Although this presentation has provided some information on the relationships of symbionts across different elevation gradients, there remain other important factors that could not be accounted (such as genotypic differences among plants and AM fungal communities), as the present results were derived from field observations without experimental manipulation. Detailed future experimental work is required on the performance of AM symbionts prior to generalization of these current results based on natural ecosystems.

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