Colonization of native Andean grasses by arbuscular mycorrhizal fungi in Puna: a matter of altitude, host photosynthetic pathway and host life cycles

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altitude; Andean grasses; arbuscular mycorrhizas; arid environment; C3/C4 grasses; inter- and intraspecific colonization.

Abstract
The relationships of altitude, host life cycle (annual or perennial) and photosynthetic pathway (C3 or C4) with arbuscular mycorrhiza (AM) root colonization were analysed in 35 species of Andean grasses. The study area is located in north-western Argentina along altitudinal sites within the Puna biogeographical region. Twenty-one sites from 3320 to 4314 m were sampled. Thirty-five grasses were collected, and the AM root colonization was quantified. We used multivariate analyses to test emerging patterns in these species by considering the plant traits and variables of AM colonization. Pearson’s correlations were carried out to evaluate the specific relationships between some variables. Most grasses were associated with AM, but the colonization percentages were low in both C3 and C4 grasses. Nevertheless, the AM root colonization clearly decreased as the altitude increased. This distinctive pattern among different species was also observed between some of the populations of the same species sampled throughout the sites. An inverse relationship between altitude and AM colonization was found in this Southern Hemisphere Andean system. The effect of altitude on AM colonization seems to be more related to the grasses’ photosynthetic pathway than to life cycles. This study represents the first report for this biogeographical region.

Introduction
Arbuscular mycorrhizas (AM) are distributed from the Equator to the Antarctic and Arctic regions (Trappe, 1987; Smith & Read, 2008) and are even found at high altitudes up to 5250 m in the Andes (Schmidt et al., 2008). The majority of angiosperms are associated with symbiotic fungi forming AM. In the Poaceae, 99.6% of the species studied are AM symbionts (Wang & Qiu, 2006), constituting an AM group in Poales (Brundrett, 2009). Most of the studied species are mainly from the Northern Hemisphere and Eurasia, and only few of them are from South America.

In general, AM occur in environments with soils that have low organic material content, high nitrogen content and low phosphorous availability (Allen, 1991; Read, 1991). However, AM are also present in phosphorous-polluted soils where other factors are at limiting concentrations (Blanke et al., 2005) or AM could be dominant in the organic soil of montane cloud forests (Kottke et al., 2004). Ericoids and ectomycorrhizas are absolutely essential for their host plants (obligate mycotrophs); in contrast, arbuscular mycorrhizal fungi (AMF) can form associations with plant species that can live with or without mycorrhizal fungi (facultative mycotrophs) (Trappe, 1987). The degree of association with AMF depends on the photosynthetic pathway of the grass, and either C3 or C4. C3 grasses are considered to be facultative mycotrophs, while C4 species are obligate mycotrophs (Hetrick et al., 1990). In the Northern Hemisphere, cool-season (C3) grasses and warm-season (C4) grasses coexist. Considering the root systems, the C3 grasses have highly fibrous roots and are less dependent on mycorrhizal symbiosis despite the low phosphorus level available in the...
soil, and could develop an extensive root architecture as a strategy for nutrient acquisition (Hetrick et al., 1988). The C₄ grasses have coarse root systems and are obligate mycotrophs and use mycorrhizal association as their main nutritional strategy (Hetrick et al., 1988, 1990, 1991). In South American mountain grasslands, C₃ and C₄ native grasses display comparable mycorrhizal patterns (Lugo et al., 2003) to those described for the Northern Hemisphere (Hetrick et al., 1988, 1990, 1992).

The host’s life cycle (annual and perennial) also has an influence on the characteristics of the plant–mycorrhizal association. Annual grasses can avoid environmental stress, such as seasonal heating, cold, drought or flooding, by the production of large quantities of small seeds that have a dormancy period and are capable of long-distance dispersal. Species with a perennial life cycle usually experience competition and avoid stressed environments. Trappe (1987) reported that annuals are less associated with AM (about 15%) in contrast to perennials (85%) ‘possibly because they often occur in disturbed habitats’. Furthermore, when metabolic types and life cycles are considered, some trends emerge: while the C₄ perennial grasses are notably benefited by the AM, the annual C₄ and perennial C₃ grasses are less influenced (Wilson & Hartnett, 1998; Hartnett & Wilson, 2002).

Along with elevation gradients, changes in important factors such as precipitation, temperature and soil conditions can affect the structure of plant communities and, consequently, may determine the shifts in plant–mycorrhiza associations (Körner, 1999; Smith & Read, 2008). In alpine environments of the Northern Hemisphere, as altitude increases, (1) coverage of mycorrhizal plant species decreases (Väre et al., 1997), (2) mycorrhizal types change and AM plants are replaced by ectomycorrhizal, ericoid or nonmycorrhizal species and (3) mycorrhizal colonization decreases (Read & Haselwandter, 1981; Trappe, 1988; Kohn & Stasovski, 1990; Routalainen et al., 2004). In spite of the fact that mycorrhizal associations have been studied in relation to altitude in northern environments, data on AM “colonization patterns along altitudinal gradients at species-level generally is lacking” (Routalainen et al., 2004).

Puna is a harsh biogeographical province that contains varied floristic districts, and the vegetation is predominantly xerophytic, constituted of shrub steppes, low shrubs and mountain grasslands with only one tree species and few herbs (Cabrera & Willink, 1980; Morrone, 2001). The study area is located in north-western Argentina and experiences a dry season in winter and an annual precipitation of 100–400 mm in summer. Specifically, available data showed a monthly mean precipitation at Iturbe – nearly the lowest point of the gradient – of 88, 63 and 41 mm for January, February and March, respectively, and values of 95, 96 and 32 mm, respectively, for these months at Mina El Aguilar, the highest point (Ruthsatz, 1977). The solar radiation is intense, and the relative air humidity is low (10–15%), with large temperature differences between the maximum and the minimum during the day (16–20 °C). The resulting climate is of a desert type. The annual mean temperature oscillates between 8.5 and 9.5 °C (Cabrera & Willink, 1980). During the summer, the monthly mean temperatures at Mina El Aguilar were 6.3, 6.6 and 5.7 °C for January, February and March, respectively; no records exist for Iturbe.

**Materials and methods**

**Study area**

Puna is a harsh biogeographical province that contains varied floristic districts, and the vegetation is predominantly xerophytic, constituted of shrub steppes, low shrubs and mountain grasslands with only one tree species and few herbs (Cabrera & Willink, 1980; Morrone, 2001). The study area is located in north-western Argentina and experiences a dry season in winter and an annual precipitation of 100–400 mm in summer. Specifically, available data showed a monthly mean precipitation at Iturbe – nearly the lowest point of the gradient – of 88, 63 and 41 mm for January, February and March, respectively, and values of 95, 96 and 32 mm, respectively, for these months at Mina El Aguilar, the highest point (Ruthsatz, 1977). The solar radiation is intense, and the relative air humidity is low (10–15%), with large temperature differences between the maximum and the minimum during the day (16–20 °C). The resulting climate is of a desert type. The annual mean temperature oscillates between 8.5 and 9.5 °C (Cabrera & Willink, 1980). During the summer, the monthly mean temperatures at Mina El Aguilar were 6.3, 6.6 and 5.7 °C for January, February and March, respectively; no records exist for Iturbe.

**Sampling design**

Samples were collected during the summer growing season along the road that connects the town of Iturbe...
(Jujuy) with Iruya (Salta) heading up to Mina El Aguilar, Jujuy, the highest point considered. Thus, altitudinal sites were established between 3320 and 4314 m. Along the sites, 21 (30 × 40 m) sampling sites were established (at 3220, 3370, 3390, 3450, 3520, 3560, 3620, 3650, two at 3700, two at 3770, 3800, 3820, 3860, 3870, 3950, 4000, 4050, 4300 and 4314 m) with an interval of ≥ 50 m between them. However, in some instances, these elevation steps were ≤ 40 m because of perceived changes in the community composition of the grasses. No replicates were carried out within sites because of the changing conditions of the grasses, slope and exposition at each site.

**Hosts**

A total of 35 native species of annual and perennial Poaceae were sampled in the 21 sites (Supporting Information, Table S1). Twenty-two species of the 35 had a C3 photosynthetic pathway (three annual and 19 perennial), and 13 had a C4 pathway (seven annual and six perennial). Fifteen of these 35 species were sampled 2–6 times along the altitudinal sites. The remaining species were sampled only once.

**Root colonization**

For each of the studied species, four or five complete individuals were collected at each sampling site (i.e. if a species grew along the altitudinal sites, it was sampled at each altitudinal point) and stored in plastic bags that were refrigerated at 4 °C. At the laboratory, the roots were washed and set in formalin–acetic acid–alcohol solution for their subsequent clarification and staining (Grace & Stribley, 1991). The finest roots of each individual in each species were cut into approximately 1-cm segments, mixed and randomly selected for mounting on one or two slides in polyvinyl-lactic acid-glycerol. For each preparation, the roots were quantified in 100 intersections under the microscope (McGonigle et al., 1990) at 50× to determine the percentages of arbuscules (A%), hyphae (H%), vesicles (V%) and total root colonization (RC%).

**Statistical analyses**

The variables considered to characterize AM colonization were the percentages of arbuscules (A%), hyphae (H%), vesicles (V%) and total root colonization (RC%). To determine the relationships between these variables and altitude, Pearson’s correlations were performed with the data for all species. Additionally, to analyse the effects of life cycles and photosynthetic pathways, linear regressions were conducted. To analyse the interspecific differences in each of the variables, we considered a whole data set (N = 69 samples corresponding to the 35 species studied, some of which were sampled in 2–6 sites) and a restricted data set (N = 35, the 35 species studied considered from only one site each; the selected site was the midpoint in the altitudinal sites for each of the species that were sampled at many sites). To analyse intraspecific patterns, regression lines were plotted for those species sampled four or more times within the altitudinal sites; rare species were not included.

We used multivariate analyses to generate hypotheses about the relationship between the AM colonization of 35 grass species and factors related to the environment (altitude) and those related to the plants (photosynthetic pathway and life cycle). Principal coordinate analysis (PCoA) and principal component analysis (PCA) were conducted according to Digby & Kempton (1996). Data were standardized prior to the analyses, with Gower distance in the case of PCoA. We carried out the statistical analyses and plotted the figures using SPSS Inc (2006) and InfoStat (2008).

**Results**

**Grass species composition along the studied gradient**

Grass species were considered rare (found at only one sampling site), mean rare (in two or three sites), less common (four or five sites) and common (six sites) depending on the number of occurrences in the 21 sites sampled along the altitudinal sites. The site at 3700 m had the highest number of rare grass species. Five C3 grasses were found only at this site; eight grasses were mean rare species; and six were less common species (Table S1). Along the studied sites, the proportion of annual and perennial grasses did not show a clear pattern. The proportion was equal at some points (3320, 3370 and 3450 m) but differed in most of the sampling sites (Table S1). For example, the perennial grasses were absent or less represented at some altitudinal points (sites 3390, 3520 and 3620 m), more frequent at other sites (sites 3700 and 3870 m) and 100% predominant over the annual grasses at most of the sites (3560, 3770, 3800, 3820, 3860, 3950, 4000, 4050, 4300 and 4314 m; Table S1).

In contrast, the distribution of species according to their photosynthetic pathways (C3 and C4) showed a clear pattern with the altitudinal sites (Table S1). At low altitudes (from 3320 to 3620 m), the C4 grasses were predominant over the C3 grasses in a proportion of 83–100%. At higher altitudes (above 3650 m), the
predominance changed, and C₃ grasses prevailed over C₄ from 75% to 100% when the altitude was higher than 3900 m (Table S1).

**Interspecific patterns of AM colonization along the altitudinal sites**

As the altitude increased, inverse relationships were found with the different variables that quantified AM colonization. There were statistically significant trends between altitude and the percentages of hyphae, vesicles and total root colonization values but no significant trend with arbuscules (Table 1). These trends were similar considering the whole data set and the restricted data set (N = 35, 35 species studied that were sampled at only one site or, for those species that were repeatedly sampled, the intermediate site within the altitudinal sites) (Table 1; Fig. 1). The general patterns are consistent in both the C₃ and C₄ grasses with a general decrease in AM colonization as the altitude increases (Fig. 1a–d). In contrast, when the relationship between altitude and AM colonization was considered for each life cycle, no clear patterns emerged (results not shown, but see Fig. 3).

Most grasses formed AM except for the perennial grasses Calamagrostis sp. (C₃), Chascolytrum subaristatum (C₃), Poa pratensis (C₃) and Eragrostis lugens (C₄). In general, the AM colonization values were low (Tables 2 and 3). The percentages of arbuscules among the C₃ annual grasses were higher than in the C₃ perennial or than in the annual or perennial C₄ grasses (Tables 2 and 3). Hyphal colonization and total root colonization were always observed in C₃ annual grasses but not always in perennial C₃/C₄ plants (Tables 2 and 3). The percentages of vesicles were lower in C₃ annual grasses than in C₃ perennial or C₄ species (Tables 2 and 3).

**Table 1. Pearson’s correlation analyses of AM root colonization vs. altitude of sites**

| Data set | Statistics | Arbuscules | Vesicles | Hyphae | Root colonization |
|----------|------------|------------|----------|--------|-------------------|
| Extended | r          | –0.054     | –0.275   | –0.271 | –0.267            |
|          | P          | 0.661      | 0.022    | 0.024  | 0.027             |
| Reduced  | r          | –0.127     | –0.402   | –0.343 | –0.351            |
|          | P          | 0.466      | 0.017    | 0.044  | 0.039             |

Pearson’s correlation analyses of AM root colonization vs. altitude considering all data (i.e. 69 data corresponding to all the collected samples along the altitudinal sites) or a reduced data set (i.e. 35 data corresponding to each species, independently if some of them were sampled at many sites; the selected site was the midpoint among the altitudinal sites for each of the species sampled at many sites). References: r = Pearson’s correlation coefficient, P = probability.

**Intraspecific patterns of AM colonization along the altitudinal sites**

The total root colonization percentages were plotted to analyse the general tendencies for the C₄ species sampled four or more times within the altitudinal sites (Aristida asplundii, Bouteloua barbata, Bouteloua simplex and Microchloa indica; Fig. 2). A pattern emerged, with an inverse relationship between the AM colonization and altitude (Fig. 2). The same patterns were observed for the other two variables of AM colonization, the percentages of hyphae and vesicles (results not shown). In contrast, the trends are not clear for the C₃ species sampled at more than four sites (Bromus catharticus, Poa calachiquiensis and Poa lilloi; results not shown). However, the reduced sample size used for the observation of intraspecific tendencies does not allow for generalizations.

**Multivariate analyses**

PCoA showed that the principal two axes provide a clear ordination of the species into two separate groups. The two principal axes together account for 62.9% of the variability in AM colonization, with 39.4% and 23.5% accounted for by the first and second axes, respectively (Fig. 3a). There is a clear distinction between the photosynthetic pathway of the species, with C₃ on the upper-left part of the figure and C₄ species on the lower-right side (Fig. 3a and Table S1 for the identification of the species’ photosynthetic pathway). PCA showed that the main two factors related to the separation of the two groups of species are altitude and the photosynthetic pathway of the species, especially on the second axis (Fig. 3b). In the PCoA figure, the C₄ species are on the upper part and the C₃ species on the lower one (Fig. 3b). The plant life cycle seems to be of minor importance in the distinction between the two groups of species (Fig. 3b). PCA explained a comparable proportion of the total variation in the AM colonization, with the first two axes accounting for 74.8% of the variability (47.1% and 27.7% for the first and second axes, respectively; Fig. 3b).

In brief, the PCoA and PCA showed a clear distribution of AM colonization of C₃/C₄ grasses in this altitudinal sites in the Puna.

**Discussion**

**Photosynthetic pathway distribution of grasses**

C₄ plants are more competitive under limited water stress, while C₃ plants are better adapted to cool and shady conditions (e.g. Ehleringer & Monson, 1993 and...
However, some exceptions were found considering altitude, temperature and C₄ and C₃ grass distribution (Pittermann & Sage, 2000, 2001; Sage, 2001a). The proportion of C₃/C₄ grasses changed along the studied altitudinal sites in the Puna. The C₄ grasses were common at the lowest altitudes and decreased in abundance up to 3870 m, and C₃ was the unique photosynthetic pathway found above this altitude up to 4314 m. The C₄ grass distribution could be predicted by temperatures warmer than 8 °C corresponding to the minimum temperature of the warmest month and the mean minimum temperature of the rainy season (Teeri & Stowes, 1976; Ehleringer & Monson, 1993). In the Puna sites studied, the historic reported mean minimum temperatures for rainy season were 0.7–4.8 °C above 4000 m (Ruthsatz, 1977). These temperatures can explain the absence of C₄ grasses above this altitude. It has been proposed that a species transition between C₃/C₄ grasses

Fig. 1. Dispersion diagrams of AM colonization vs. altitude along altitudinal sites of the Puna (Argentina) using the whole data set (69 samples of 35 species). (a) hyphae, (b) total root colonization, (c) vesicles, (d) arbuscules. Filled squares = C₄ species; open circles = C₃ species; m.a.s.l. = metres above sea level.
Host photosynthetic pathways (C$_3$, C$_4$) and life cycle (annual, perennial) for each species are indicated. Data correspond to mean ± standard error of the different populations sampled at many sites (previously, data for individual plants were used to characterize each population).

| Host                | Poaceae                            | AM Root colonization (%) |
|---------------------|------------------------------------|--------------------------|
|                     | Arbuscules | Vesicles | Hyphae | Root colonization |
| Annual C$_3$        | Bromus catharticus Vahl | 1.43 ± 0.51 | 2.91 ± 0.93 | 17.09 ± 3.58 | 21.70 ± 4.70 |
|                     | Vulpia myuros (L.) Gmel. f. megalura (Nutt.) Stace et Cotton | 7.25 ± 3.05 | 0.75 ± 0.62 | 19.88 ± 7.83 | 27.88 ± 10.73 |
| Perennial C$_3$     | Danthonia annableae P. M. Peterson et Rügolo | 0 | 0.77 ± 0.30 | 1.69 ± 0.52 | 2.40 ± 0.65 |
|                     | Festuca rigezensis (J. Presl) Kunth | 0.03 ± 0.03 | 0.83 ± 0.37 | 3.67 ± 1.10 | 4.73 ± 1.48 |
|                     | Jarava plumosula (Nees ex Steud) F. Rojas | 0.13 ± 0.13 | 1.88 ± 0.95 | 8.13 ± 3.54 | 10.00 ± 3.96 |
|                     | Koeleria praeditanda A. M. Molina | 5.00 ± 2.03 | 4.67 ± 1.86 | 11.11 ± 4.50 | 20.80 ± 8.31 |
|                     | Poa calchaquensis Hack. | 0 | 0.16 ± 0.07 | 0.56 ± 0.14 | 0.71 ± 0.21 |
|                     | Poa lilloi Hack. | 0 | 1.20 ± 0.94 | 2.27 ± 0.85 | 3.50 ± 1.72 |
| Annual C$_4$        | Aristida adscensionis L. | 1.67 ± 0.93 | 2.75 ± 0.91 | 15.29 ± 4.30 | 19.70 ± 5.04 |
|                     | Bouteloua barbarata Lag. | 0.53 ± 0.15 | 1.53 ± 0.41 | 13.35 ± 1.90 | 16.12 ± 2.41 |
|                     | Bouteloua simplex Lag. | 1.82 ± 0.96 | 2.53 ± 0.71 | 16.00 ± 3.10 | 20.32 ± 3.92 |
|                     | Microchloa indica (L. f.) P. Beauv. | 0 | 0.94 ± 0.34 | 3.71 ± 1.23 | 4.59 ± 1.51 |
| Perennial C$_4$     | Aristida asplundii Henriard | 0.09 ± 0.07 | 3.87 ± 0.87 | 8.91 ± 1.94 | 12.85 ± 2.71 |
|                     | Cynodon dactylon (L.) Pers. var. biflorus Merino | 0 | 0.75 ± 0.22 | 1.75 ± 0.48 | 2.50 ± 0.63 |
|                     | Eragrostis sp$_1$ | 0.25 ± 0.25 | 5.43 ± 0.89 | 22.94 ± 4.22 | 28.81 ± 3.90 |

Host photosynthetic pathways (C$_3$, C$_4$) and life cycle (annual, perennial) for each species are indicated. Data correspond to mean ± standard error of a sample of plant individuals of each species at the sampled site.

| Host                | Poaceae                            | AM Root colonization (%) |
|---------------------|------------------------------------|--------------------------|
|                     | Arbuscules | Vesicles | Hyphae | Root colonization |
| Annual C$_3$        | Polygono interruptus Kunth | 1.44 ± 0.67 | 1.00 ± 0.33 | 9.78 ± 3.40 | 12.22 ± 4.20 |
| Perennial C$_3$     | Calamagrostis sp. | 0 | 0 | 0 | 0 |
|                     | Calamagrostis breviaristata (Wedd.) Pilg. | 0.40 ± 0.22 | 1.10 ± 0.66 | 1.50 ± 0.86 |
|                     | Calamagrostis trichodonta (Wedd.) Soreng | 0.40 ± 0.24 | 2.20 ± 0.37 | 28.00 ± 0.55 | 34.00 ± 0.55 |
|                     | Chascolytrum subariatum (Lam.) Desv. | 0 | 0 | 0 | 0 |
|                     | Danthonia boliviensis Renvoize | 0.22 ± 0.15 | 2.56 ± 1.20 | 2.80 ± 1.20 |
|                     | Festuca humilior Nees et Meyen | 0 | 3.50 ± 0.29 | 18.25 ± 2.59 | 21.75 ± 2.75 |
|                     | Hordeum muticum J. Presl | 0.89 ± 0.51 | 0.89 ± 0.42 | 5.78 ± 1.78 | 7.56 ± 2.37 |
|                     | Nassella meyeriana (Trin. & Rupr.) Parodi | 0.40 ± 0.22 | 1.90 ± 0.91 | 2.30 ± 1.11 |
|                     | Piptochaetium indentum Parodi | 0 | 0.80 ± 0.37 | 1.20 ± 0.58 | 2.00 ± 0.89 |
|                     | Poa laetevirens R. E. Fries | 0.25 ± 0.25 | 2.75 ± 0.85 | 6.75 ± 1.89 | 9.75 ± 2.75 |
|                     | Poa pratensis L. | 0 | 0 | 0 | 0 |
|                     | Poa superata Hack. | 0 | 6.00 ± 3.35 | 4.80 ± 2.40 | 11.60 ± 5.54 |
|                     | Trisetum spicatum (L.) K. Rich | 0.20 ± 0.20 | 0.80 ± 0.58 | 3.80 ± 1.36 | 4.80 ± 1.66 |
| Annual C$_4$        | Eragrostis nigricans (Kunth) Steud. var. nigricans | 0 | 2.00 ± 0.91 | 3.25 ± 1.49 | 5.25 ± 2.29 |
|                     | Eragrostis nigricans var. punensis Nicora | 0 | 0.75 ± 0.48 | 0.75 ± 0.48 |
|                     | Eragrostis mexicana (Hornem.) Link ssp. virescens J. Presl. | 0 | 6.63 ± 3.12 | 6.63 ± 3.12 |
| Perennial C$_4$     | Cynodon dactylon (L.) Pers. | 0 | 0.60 ± 0.24 | 0.60 ± 0.24 |
|                     | Eragrostis lugens Nees | 0 | 0 | 0 | 0 |
|                     | Muhlenbergia rigida (Kunth) Kunth | 0 | 1.00 ± 0.71 | 1.00 ± 0.71 |

occurs at similar altitudes around the world regardless of the phytogeographic region (Rundel, 1980). Nevertheless, in the South American semi-arid and arid Monte and PrePuna biogeographical provinces, Cavagnaro (1988) found a transition between C$_3$ and C$_4$ grasses at 1500 m and that C$_4$ grasses were absent above 2200 m. Also, in the arid Chaco province, Cabido et al. (1997) met the same transitional trend, and C$_4$ grasses were rare above 1000 m but were found up to 2100 m. In the studied Puna altitudinal sites, the transition between C$_3$ and C$_4$ grasses was higher (at 3650 m) than in other arid and semi-arid South American biogeographical provinces, and
C4 grasses were found up to 3870 m (e.g. Bouteloua barbata), although C4 species became rare above 3520 m. Moreover, some of the Puna environmental characteristics such as the occurrence of open sites, arid zones with low CO2 partial pressure, large amounts of animal disturbance and high soil salinity create excellent conditions for C4 grass establishment (Pittermann & Sage, 2000, 2001; Sage, 2001a, b).

**AM colonization, altitude and host’s photosynthetic pathway**

Altitude has been recognized as an important factor in European alpine ecosystems affecting the distribution of many organisms (e.g. Körner, 2007). Although autotrophy is the norm for alpine plants at the highest elevations (Trappe, 1987; Brundrett, 2009), mycotrophy also can be found at high altitudes as in the Alps (Nespiak, 1953; Read & Haselwandter, 1981), Bolivian Andean highlands (Urcelay et al., 2011), Peruvian Andes (Schmidt et al., 2008), western North America mountains (Trappe, 1988) and the Puna (this work). Gardes & Dahlberg (1996) found similar associations in arctic and alpine tundra. In high mountain grasslands, AM are common and well documented (Read & Haselwandter, 1981; Mullen & Schmidt, 1993; Onipchenko & Zobel, 2000). In general, our results showed a clear pattern of a decrease in the AM colonization in the roots of Puna grasses with an increase in altitude. This trend could be related to a mean temperature decrease as the altitude is increased and consequently to a reduction in soil fungal diversity as well as in fungal growth rate (Koske, 1987; Entry et al., 2002), which negatively influence the AM colonization capacity (Smith & Read, 2008). In fact, along Puna sites, the diversity of AM fungi (richness and density of spores) decreased with the altitudinal increase (Lugo et al., 2008). Although spores constitute only a part of the propagules of AMF, their decline along the sites would imply a lower availability of AMF in soil and therefore a decrease in root colonization with increasing altitude. Thus, the decrease in spore diversity could be related to the diminishing pattern of the root colonization along the Puna sites studied.

A decrease in arbuscular mycorrhizal colonization along altitudinal gradients had been found when the altitudes were stretched out over 1245–1300 m (Haselwandter, 1979; Haselwandter & Read, 1980; Routsalainen et al., 2004) but the colonization showed intermediate trends when the altitude gradients were smaller (Read & Haselwandter, 1981). However, Väre et al. (1997) reported that AM colonization decreased along a gradient of 300 m. Although the Puna altitudinal difference was shorter (994 m) than most gradients studied in the Northern Hemisphere, the decrease in root colonization in the grasses agrees with the patterns found for larger gradients in alpine environments (Haselwandter & Read, 1980; Routsalainen et al., 2004).

The general trends among different species of the Puna grasses were also observed at the intraspecific level. Intraspecific colonization in four Puna C4 species showed a decreasing trend with altitude. In contrast, the C3 species did not show a consistent pattern. Although these are preliminary results because of the low sample size and the sampling design (without replications), this is the first field report from the Puna Region for native grasses. Experimental mycorrhizal influence within a single species and among different species was previously reported from grasses of North American tallgrass prairie. The competitive advantage of the dominant prairie C4 host was strongly reduced during the absence of mycorrhiza, and the mycorrhizal effect on competition for the C3 host was smaller (Hartnett et al., 1993).

**AM colonization and life cycle**

In the Puna grassland, both annual and perennial grasses had mycorrhizal colonization; however, the life cycle was a subordinate factor compared to the photosynthetic pathways, according to the results of the multivariate analyses. The presence of AM in grasses with both life
cycles is striking because in the Northern Hemisphere, annual species are frequently nonmycorrhizal, while the perennial forms are predominantly mycorrhizal (Trappe, 1987). This difference could be related to environmental conditions because the reported nonmycorrhizal annual plants in the Northern Hemisphere come from disturbed areas and behave as ruderal weeds, while annual grasses in the Puna differ functionally because they are native and well established in this biogeographical region.

The mycorrhizal root colonization of the Puna grasses had opposite characteristics to Northern tallgrass prairie grasses. In North American tallgrass prairie, annual grasses of both C3 and C4 photosynthetic pathways showed a low responsiveness to mycorrhizal colonization (Wilson & Hartnett, 1998). Thus, although the roots of annual and perennial C4 grasses in the Puna were less colonized than those from Northern tallgrass prairie, the annual and perennial C3 colonization was higher in the Puna. The higher root colonization of C4 grasses in tallgrass prairies could be explained by the lower altitudes at which these species grow, and the higher mycorrhizal colonization in the perennial C3 Puna grasses could indicate that these grass species were able to acclimate to the low temperatures of the highest sites in the Puna.

**AM colonization: linking altitude, photosynthetic pathway and life cycle**

Our results are the first field report showing complex shifts in arbuscular mycorrhizal colonization in a sample of 35 species of Andean grasses along 21 altitudinal sites at high elevation. Results suggest that altitude is the most important factor, the host photosynthetic pathway is the second most relevant and the plant life cycle seems to be subordinated. Routsalainen et al. (2002) proposed a model for optimal mycorrhizal colonization along altitudinal gradients that considered photosynthetic nutrient use efficiency (PNUE) as the driver of altitudinal mycorrhizal trends and suggested that associations cannot always be favoured at low PNUE. Furthermore, PNUE is related to photosynthetic pathways; C3 plants have a lower PNUE than C4 plants, and consequently, a lower root colonization in C3 than in C4 grasses would be expected. Besides, different PNUE values among C3 plant species occur, and interspecific differences are also related to root activity (Hikosaka, 2010); that is, species with higher PNUE have higher root activity to maintain higher leaf nitrogen concentration.

Arbuscular mycorrhizas are considered to be ubiquitous symbiotic associations because of their broad host spectrum (approximately 80% of land plants) in spite of the scant number of fungi forming this type of association (only c. 230 species of Glomeromycota) (Smith & Read, 2008; Krüger et al., 2011). However, glomalean fungi have been shown to select their host based on ecological factors (Sanders & Fitter, 1992a, b; Eon et al., 2000; Helgason et al., 2002; Husband et al., 2002), and this selection may partly explain the interspecific differences found experimentally in root colonization in grasses from the Northern Hemisphere (Hartnett et al., 1993; Wilson & Hartnett, 1998). Moreover, when the AMF community was analysed in the grasses’ rhizosphere at five sites in Puna (Lugo et al., 2008), the AMF diversity was not related to the grasses’ photosynthetic pathway, although AM root colonization along the 21 sites was associated with the pathway. In microcosm experiments, AMF from a mown grassland showed host dependence of sporulation rates and diversity (Bever et al., 1996); also AMF identity rather than AMF diversity affected the plant community diversity and productivity in North American tallgrass prairie (Vogelsang et al., 2006). Besides, AMF community can present spatial patterns at a local scale, and this spatial heterogeneity of AMF communities might have an impact on plant communities in structure and processes (Pringle & Bever, 2002). Therefore, AMF communities in the 21 sites sampled in this work, instead of five sites (Lugo et al., 2008), possibly include new AMF communities with spatial heterogeneity that may involve different AMF species with their specific host dependence, affecting the plant community composition. Furthermore, AMF sporulation host dependence (Bever et al., 1996) could suggest a host preference effect and therefore interspecific differences in root colonization as were found in grasses of Puna and that represent a first field report from the Southern Hemisphere of this variation in AM colonization. All of the factors studied here, including altitude, host photosynthetic pathway and host life cycle, confirm an interesting pattern in the variability of hyphae, vesicles and root colonization percentages that is not evident in arbuscules. The lack of a clear pattern for arbuscules may occur because their presence is usually related to other factors such as the soil phosphorus content (Smith & Read, 2008). Additionally, the proportion of annual mycorrhizal species seems to be related to the type of habitat; that is, in dry, mesophytic and calcareous grasslands, a large proportion of the species are annual.

**Fig. 3.** (a) PCoA and (b) PCA for 35 grasses species growing along altitudinal sites of the Puna (Argentina). The variables of AM colonization were percentages of hyphae, vesicles, arbuscules and total root colonization. Altitude, photosynthetic pathways (C3 and C4), and life cycle (annual and perennials) for each species can be seen in Table S1.
mycorrhized grasses (Peat & Fitter, 1993). The different amount of colonization observed by Newsham et al. (1995) could be due to the differential effect of AM on the host: in perennial grasses, phosphorus recovery is more efficient, while in the annuals, phosphorous recovery is not affected, although their resistance to pathogens is increased. Newsham & Watkinson (1998) found that some grass species that are normally nonmycorrhizal or slightly colonized when the soil is fertile are strongly infected when the soil is poor. Furthermore, according to Fitter & Merryweather (1992), the level of mycorrhizal colonization of roots is a characteristic that plants may regulate depending on the benefit they receive. In the Puna grasses, the slight or subordinate influence of the host life cycle on root colonization could be a result of the low nutrient availability in the soil, which would favour an association with annual as well as with perennial hosts (Entry et al., 2002) and could also be compelled by a cost–benefit trade-off that varies with the characteristics of the hosts, such as their photosynthetic pathways or life cycle, at different altitudes. Although AM–plant interactions in grasses were mostly driven by the altitude, more questions result if we consider that ‘altitude’ is conceptually more than a single factor and includes a reduction in the land area along with many climatic trends such as decreasing total atmospheric pressure, decreasing partial pressures of O₂ and CO₂ (among other atmospheric gases), reduction in atmospheric temperature and following implications for ambient humidity, increasing radiation under a cloudless sky, incoming increasing solar radiation and outgoing night-time thermal radiation and higher UV-B radiation (Körner, 2007). Moreover, it has been shown that in grasslands, root production, turnover and respiration also decrease along altitudinal gradients, while solar radiation increases and the resource availability remains low (Fitter et al., 1998). This trend of root traits would result in a diminishing of the AM colonization along the Puna sites because of a decreasing availability of roots to colonize. Therefore, this trend of decreasing AM colonization with altitude could be considered to be a general pattern regardless of the geographical location of the biome considered. Moreover, different relationships between AM and plants can be understood along the altitudinal gradient as complex strategies depending on the characteristics of the hosts at different altitudes, including their photosynthetic pathways or life cycles.

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**Supporting Information**

Additional Supporting Information may be found in the online version of this article:

**Table S1.** Grasses species distribution along altitudinal sites of the Puna (Argentina).

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