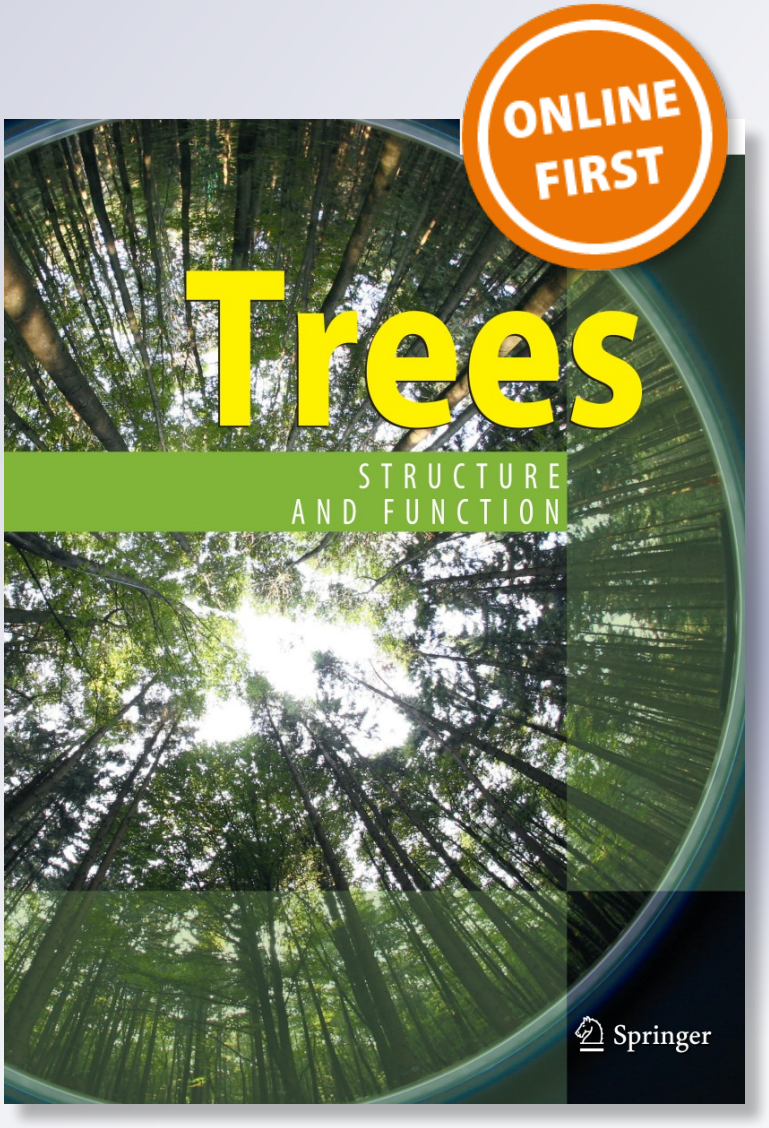
Restoration of high altitude forests in an area affected by a wildfire: Polylepis australis *Bitt. seedlings performance after soil inoculation*

Florencia Soteras, Daniel Renison & Alejandra G. Becerra

Trees
Structure and Function

ISSN 0931-1890

Trees
DOI 10.1007/s00468-013-0940-7



Springer

Trees
DOI 10.1007/s00468-013-0940-7

ORIGINAL PAPER

Restoration of high altitude forests in an area affected by a wildfire: *Polylepis australis* Bitt. seedlings performance after soil inoculation

Florencia Soteras · Daniel Renison · Alejandra G. Becerra

Received: 6 April 2013 / Revised: 8 September 2013 / Accepted: 17 September 2013

Abstract
Key message **Outplanted *Polylepis australis* seedling growth, survival and mycorrhizal response were not influenced by inoculation with soil from different vegetation types. Seedling inoculation would not be essential for reforestation practices.**
Abstract *Polylepis* forests are one of the most endangered high mountain ecosystems of South America and reforestation with native *Polylepis* species has been recommended. To determine whether native soil inoculation could help in reforestation success, a field trial was set up to evaluate the response of outplanted *P. australis* seedlings to the inoculation with soils from three vegetation types (a grassland, a mature forest and a degraded forest) and a sterile soil, used as control. We evaluated seedlings performance: growth and survival for 18 months, root/shoot ratio, phosphorous content and arbuscular mycorrhizal fungal (AMF) colonization. To interpret performance patterns we evaluated the colonization potential of the three inoculum soils and the changes of the AMF community composition of the seedlings rhizosphere in relation to inoculation treatment and season. Our main results showed no significant differences in seedlings survival and growth between treatments. The colonization potential of grassland and degraded forest soils was ~25 times greater than mature forest soil and specific spore density of some morphospecies varied with season. However, AMF spore community of seedlings rhizosphere became homogenized after outplanting and was similar between treatments after 12 months. Therefore, we conclude that soil inoculation is not essential for outplanted *P. australis* survival and increase in height, and thus all the tested soils could be used as inocula, including grassland soils which in practice are the easiest to collect.

Keywords Mountain forest · Reforestation · Performance · Natural soil inocula · Arbuscular mycorrhizal fungi

Communicated by P. E. Courty.

F. Soteras (✉) · A. G. Becerra
Laboratorio de Micología, Instituto Multidisciplinario de Biología Vegetal (IMBIV), CONICET, Universidad Nacional de Córdoba, CC 495, 5000, Córdoba, Argentina
e-mail: fsoteras@conicet.gov.ar

D. Renison
Centro de Ecología y Recursos Naturales Renovables "Dr Ricardo Luti", Instituto de Investigaciones Biológicas y Tecnológicas, CONICET, Universidad Nacional de Córdoba, Vélez Sarsfield 1611, X5016GCA Córdoba, Argentina

Introduction

Anthropogenic influence has greatly modified the structure of mountain forest ecosystems, reducing them through burning, cutting and livestock rearing (Ellenberg 1979; Zak and Cabido 2002). Forest loss hampers the ecosystem functioning, and therefore landscape changes usually are associated with the alteration of species composition, reduction of soil moisture, increase of soil erosion and compaction of soils (Kauffman et al. 2003).

Polylepis (Rosaceae) forests are one of the most endangered mountain forest ecosystems of South America; hence their conservation and reforestation are a priority (Fjeldså and Kessler 1996). The southernmost species of the genus, *Polylepis australis* Bitt., is distributed in the higher mountains of central and northwestern Argentina and is mostly restricted to the rocky outcrops and deep ravines (Cingolani et al. 2004; Renison et al. 2013). Forests

Published online: 04 October 2013

with different structural complexity have been created due to the effect of grazing lands managed by fires, browsing, trampling and logging (Renison et al. 2011). These changes have affected soil conditions (Renison et al. 2010) and may influence on arbuscular mycorrhizal fungi (AMF) (Picone 2000), that have been found colonizing *P. australis* roots (Menoyo et al. 2007).

Arbuscular mycorrhizal fungi are one of the most important soil microorganisms and can confer benefits to the host plant growth and development by increasing nutrient uptake and tolerance to stress conditions and soil-borne pathogens (Smith and Read 2008). Studies evaluating *P. australis* reforestation have highlighted the importance of considering soil characteristics, because at degraded microsites with exposed soil or rock, transplanted seedlings grow less, probably due to the lack of nutrients and water (Renison et al. 2005; Torres et al. 2008). Besides, under greenhouse conditions, the relevance of seedling inoculation with different native soils has been depicted (Martino et al. 2011; Soteras et al. 2013). However, the performance of seedlings previously inoculated with native soils in field trials has not yet been evaluated for reforestation practices with this species.

Inoculation with AMF generally increases survival and growth of outplanted tree seedlings (Allen et al. 2003; Cuenca et al. 2004; Amaranthus and Steinfeld 2005). However, the effects of different AMF inocula on host growth depends on plant physiology (Smith et al. 2011), AMF assemblage (Maherali and Klironomos 2007), inoculum potential (Asbjornsen and Montagnini 1994), plant successional stage (Asbjornsen and Montagnini 1994; Fischer et al. 1994; Kiers et al. 2000; Allen et al. 2003) and other biotic and abiotic factors (Hoeksema et al. 2010 and references therein).

The colonization potential of soil, the ability of soil-borne AMF to initiate root colonization (Plenchette et al. 1989) could determine the effectiveness of soil inocula (Cuenca et al. 2004). There is contrasting evidence of soil changes effect on the mycorrhizal colonization potential, higher soil colonization ability has been observed in pastures than in disturbed forest soils (Jasper et al. 1991), and in a disturbed than in an unmanaged forest (Closa and Goicoechea 2011). Nevertheless, Irrazabal et al. (2004) did not show variation of the colonization potential of soil with vegetation changes. Besides, to characterize accurately the AMF spore community it is important to consider different seasons, as it has been widely evidenced that the AMF community sporulation and colonization patterns vary with seasonal climatic changes (Lugo and Cabello 2002; Oehl et al. 2009; Sene et al. 2012).

In this study we contributed to reforestation practices by evaluating *P. australis* response to the inoculation with natural soil from three vegetation types differing in their structural complexity (a grassland, a mature forest and a degraded forest sensu Renison et al. (2011)) at a recently burnt grassland in Quebrada del Condorito National Park. According to Soteras et al. (2013) the three soils have different AMF communities with the grassland soils having the greatest number of species.

We focused in the following questions: (1) The soil from which vegetation type is better for inoculating *P. australis* seedlings for field trials? (2) Do soil inocula of different origin differ in their mycorrhizal colonization potential? (3) How does the AMF spore community of the seedlings rhizosphere change between treatments and among seasons?

Methods

Inoculum soil

We collected inoculum soil from Los Molles river basin (1,900 m a.s.l., 31°58′S, 64°56′W) as it is one of the most preserved river basins in the Córdoba mountains. The presence of several hectares of mature forest is exclusive to this site; extensive field explorations aided by satellite images have not yielded further findings (Cingolani et al. 2008; Renison et al. 2011). The landscape consists of a mosaic of rock outcrops, grasslands and *P. australis* forests in different stages of post-fire recovery (Cingolani et al. 2004; Renison et al. 2011).

Soil samples were collected from three sites differing in vegetation types due to different disturbance history: (1) a typical mesic tussock grassland with no *P. australis* trees, dominated by *Deyeuxia hieronymi* (Hack.) Türpe intermingled with *Alchemilla pinnata* Ruiz and Pav. and *Carex fuscula* Urv. (Cingolani et al. 2003; hereafter called "grassland"), (2) an area with sparse *P. australis* trees, little regeneration, abundant evidences of previous fire events and soil erosion (hereafter called "degraded forest") and (3) a *P. australis* mature forest with standing and fallen dead wood, fern cover and relatively greater abundance of shade-tolerant *Maytenus boaria* trees (hereafter called "mature forest") (Renison et al. 2011). The degraded forest was about 100 m from the mature forest and both were 600 m away from the grassland.

During the dry season (May 2009), soil samples were collected with a trowel from ten random points (0–20 cm depth) from each community type (inoculum type), placed in plastic bags and stored at 4 °C. In the laboratory, litter, stones and sticks were removed with a 1 cm mesh sieve. For more information about inoculum soils see Soteras et al. (2013).

Seedlings inoculation

Seeds of *P. australis* were collected from several forests fragments of the high mountains of central Argentina, were

surface sterilized for 10 min with 10 % sodium hypochlorite, washed with sterilized water and sown on trays with sterilized sand in March 2009. Three months later, seedlings were randomly transplanted to pots (19 cm height × 9 cm diameter) and were inoculated. Each soil inoculum was formed by a composite of the ten soil samples of each vegetation type and it consisted in spores, colonized root fragments, mycelia and other microbial organisms. The pots were filled with 500 g of the same composite used for inoculation previously autoclaved (1 h of heating at 120 °C for 3 days with intervals of 24 h). Then, 30 g of fresh soil inoculum (grassland, mature forest, degraded forest, sterile soil) was placed 3 cm beneath the autoclaved soil. We only recorded the presence of AMF, but as other symbiotic or pathogenic microbial organisms were probably together with AMF in the inocula, we called the treatment "inoculum soil". Extra pots were prepared for all treatments to ensure survival of 66 replicates per treatment.

Field transplant experiment

The seedlings grew at the greenhouse for 6 months, being 9-month old when outplanted, which is the usual practice in the region due to low survival in lowland production greenhouses during the warm summer (Renison et al. 2002). The field transplant experiment was carried out during the summer (December 2009) in a recently burnt grassland (July 2009) at Quebrada del Condorito National Park (1,900 m a.s.l., 31°40′S, 64°42′W). Seedlings were planted spaced 3 m apart, beside numbered metal pins with treatments randomly assigned, and watered abundantly.

AMF spore density was assessed at the burnt site and at an adjacent site not affected by the wildfire, collecting three randomly selected soil samples in each location. AMF spore density was 43 % reduced after the wildfire (at the burnt site: 68.75 ± 41.13 spores/100 g of dry soil, and at the adjacent site: 160.05 ± 69.75 spores/100 g of dry soil). Before the fire, the vegetation in the area consisted in a mosaic of tussock grasslands, grazing lawns, degraded grazing lawns, eroded areas with exposed rock surfaces, granite outcrops, and open *P. australis* shrublands covering <1 % of the site (Cingolani et al. 2004). Mean temperature is 5.0 and 14.4 °C for the coldest and warmest months, respectively, with no frost-free period (Cabido 1985). Mean annual precipitation is 920 mm, with 83 % of the rainfall being concentrated in the warmest months (October to April, Renison et al. 2002).

Seedlings response to inoculation

Seedlings height and survival were measured at planting (time 0), during the wet season, 6, 12 and 18 months later. Growth of each treatment was calculated as the height differences between dates.

To evaluate seedlings response to AMF inoculation, after 12 months growing at the field site, six randomly chosen seedlings per inoculum type were harvested, leaving 60 seedlings per treatment in the field. Shoot and root biomass, plant phosphorous content and root colonization were measured. Root system was preserved in FAA (formalin–acetic acid–ethanol), cleared and stained for observation of AMF structures (Phillips and Hayman 1970; Grace and Stribley 1991). AMF colonization was measured under Nikon- E200 light microscope at 400× magnification, following the technique of McGonigle et al. (1990).

Mycorrhizal colonization potential of soil and AMF of seedlings rhizosphere

The same composite used as inoculum soil was utilized for the colonization potential assay. Three soil dilutions (1:0, 1:4 and 1:40) were done mixing the original soil with sterilized (100 °C, 1 h) perlite:vermiculite in 1:1 (v:v) proportions (Díaz and Honrubia 1993). Soil was then transferred to 250 ml plastic pots and two *Medicago sativa* L. seedlings per pot were planted with 12 replicates per dilution per inoculum type. *M. sativa* was chosen due to its good response to mycorrhizal inoculation (Cabello 1997). Plants were placed in a greenhouse (22/19 °C day/night, photoperiod 16/8 h day/night) watered daily and were not fertilized. After 15, 30 and 60 days four pots per dilution and soil inoculum were harvested at each time. The whole root system was collected, carefully washed under tap water, cleared and stained (Phillips and Hayman 1970; Grace and Stribley 1991). AMF colonization was quantified according to the grid-line intercept method under a Nikon Ni-150 stereomicroscope (Giovannetti and Mosse 1980).

To assess AMF community composition and changes among seasons, rhizospheric soil samples from six randomly chosen seedlings per treatment were collected with a corer (3 cm diameter), after 6 and 12 months (hereafter "dry and wet seasons", respectively). AMF spores were extracted by wet sieving and decanting (Gerdemann and Nicolson 1963), followed by centrifugation in sucrose (Walker et al. 1982). A fine sieve (38 µm) was used to collect small spores, and the material remaining on the top sieve (125 µm) was checked for sporocarps and larger spores. Only apparently healthy spores were counted by direct observations under a stereomicroscope (Nikon Ni-150) and recorded as mean spores per 100 g of dry soil (spores density). For taxonomic identification, fungal spores and sporocarps were mounted onto slides using PVA with and without Melzer reagent (Omar et al. 1979) and examined with a compound microscope (Nikon-

E200). AMF morphospecies identification was based on current species descriptions and the identification manuals of Schenk and Perez (1990), INVAM (http://invam.caf.wvu.edu/fungi/taxonomy/speciesID.htm) and Oehl et al. (2011). Shannon biodiversity index, species richness and evenness were also calculated (Magurran and McGill 2011).

Data analyses

Differences in seedlings height and growth along dates and between treatments were detected with a repeated measures analysis of variance (ANOVA), with dates as within factor (four levels: 0, 6, 12 and 18 months) and treatment as between factor (four levels: three inoculum types and the sterile soil). Tukey post hoc test was applied with a significance level of 0.05 to determine differences between treatments.

Seedlings survival probability was analyzed using Kaplan–Meier survival estimation curves of SPSS 15.0 package. Log-rank non-parametric test was used with a significance level of 0.05 to detect differences between treatments.

For harvested seedlings root dry weight/shoot dry weight ratio, AMF colonization and plant P content among treatments were compared using a one-way analysis of variance (ANOVA) with treatments (four levels: three inoculum types and the sterile soil) as fixed factors. Tukey post hoc test was applied with a significance level of 0.05 to determine differences between treatments. All residuals were tested for normality and homoscedasticity (with Shapiro–Wilks and Levene's tests, respectively). Shoot biomass was log10 transformed before analysis. These analyses were performed using R (R Development Core Team 2011).

To test for differences in the AMF composition (density, richness, Shannon diversity and specific spore density) a repeated measures ANOVA, with dates as within factor (two levels: 6 and 12 months) and treatment as between factor (four levels: three inoculum types and the sterile soil) was performed. Tukey post hoc test was used for differences among treatments ($\alpha = 0.05$).

Results

Seedlings response to inoculation

Seedlings survival and growth over the four dates averaged 90 % and 0.29 ± 0.19 cm, respectively, and were not significantly influenced by soil inoculation treatment (for survival: Log Rank test, $L = 1.20$, $P = 0.75$; for growth: $F = 0.54$, $P = 0.65$). Between the second and the third dates, all transplanted seedlings underwent a decrease in height, because terminal buds dried during the cold and dry winter (Fig. 1).

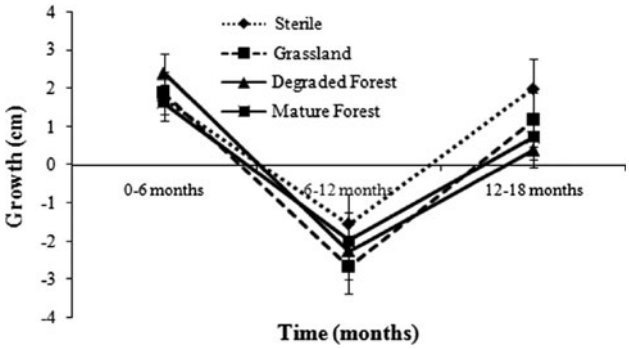


Fig. 1 Difference in height of *Polylepis australis* seedlings between dates (from outplanted date to 6 months, from 6 to 12 months and from 12 to 18 months), grown in sterile soil (initially non-mycorrhizal) or inoculated with grassland, degraded forest or mature forest soils. *Vertical bars* show ± standard error ($n = 51$ replicates)

Seedlings that were harvested after 12 months growing in the field did not show significant differences among treatments of root/shoot ratio, phosphorus content and root colonization ($F = 0.79$, $P = 0.51$; $F = 0.94$, $P = 0.44$; $F = 0.73$, $P = 0.55$; respectively; Tukey post hoc test was not needed). Seedlings from all treatments were found colonized by AMF and mycorrhizal colonization varied from 13 to 80 % (average: 47.99 ± 4.33 %). The percentage of vesicles and arbuscules was similar between treatments ($F = 1.41$, $P = 0.27$; $F = 0.83$, $P = 0.49$; respectively; Tukey post hoc test was not needed). Considering all treatments, root colonization by vesicles ranged between 2 and 44 % (average: 18.86 ± 2.44 %) and by arbuscules among 0–5 % (average: 0.50 ± 0.23 %).

Mycorrhizal colonization potential of soil

The colonization potential assay showed an increasing root colonization of *M. sativa* seedlings with time in grassland and degraded forest inocula. In contrast, mature forest soil showed similar colonization values among all harvesting dates (Fig. 2). The three inoculum types showed the highest colonization in the undiluted soil (1:0) (78, 77 and 49 %, for grassland, degraded forest and mature forest soil, respectively), intermediate colonization values in the 1:4, and the lowest colonization in the 1:40 diluted soil (0 % in grassland, 4 % degraded forest and 2 % in mature forest soil, respectively). The highest colonization value in grassland and in degraded forest soils was achieved after 60 days of experiment initiation. In mature forest soil the highest colonization value was achieved after 30 days of experiment initiation (Fig. 2).

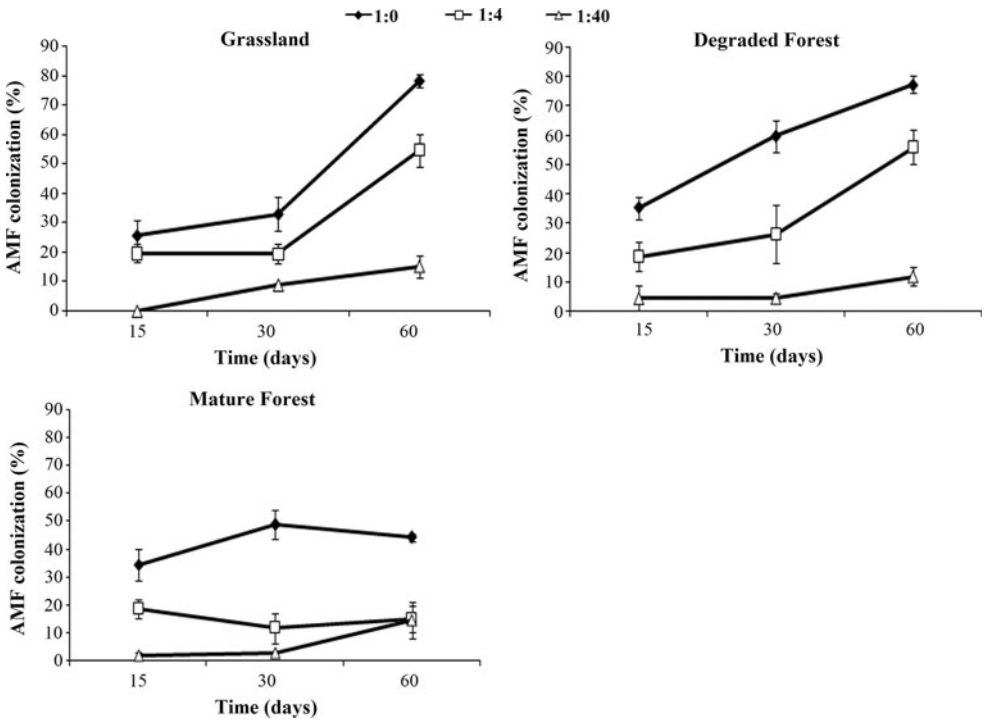


Fig. 2 Arbuscular mycorrhizal fungi (*AMF*) colonization of *Medicago sativa* seedlings grown in the three soil types (grassland, degraded forest and mature forest), in three soil dilutions (1:0, 1:4 and 1:40) during three harvesting dates (15, 30 and 60 days). Values are the mean ± standard error ($n = 4$)

An equivalent root colonization of 40 % was reached in a degraded forest soil dilution of 1:4, a grassland soil dilution of 1:4 or an undiluted (1:0) mature forest soil (Fig. 2). Therefore, grassland and degraded forest soils showed ~25 times more colonization potential than the mature forest soil.

AMF spore community of seedlings rhizosphere

AMF spore community of seedlings rhizosphere showed similar AMF density, richness and Shannon diversity index among treatments ($F = 0.07$, $P = 0.97$; $F = 0.31$, $P = 0.82$; $F = 0.82$, $P = 0.49$; respectively; Tukey post hoc test was not needed). Spore density varied from 7 to 153/100 g of soil after 6 months (dry season) and from 43 to 325/100 g of soil after 12 months (wet season). We identified 30 AMF morphospecies that belonged to nine genera (*Acaulospora*, *Claroideoglomus*, *Entrophospora*, *Funneliformis*, *Glomus*, *Gigaspora*, *Pacispora*, *Rhizophagus* and *Scutellospora*), 27 could be identified to species level.

Season significantly influenced the AMF density of seedlings with sterile and grassland inocula ($F = 33.88$, $P < 0.001$; $F = 6.26$, $P < 0.05$; respectively) (Fig. 3a). AMF richness and Shannon diversity index were not significantly different between seasons ($F = 0.83$, $P = 0.48$; $F = 0.82$, $P = 0.49$; respectively) (Fig. 3b, c).

Specific spore density (number of each morphospecies) significantly varied with season, except for seedlings inoculated with mature forest soil ($F = 0.07$, $P = 0.79$). In the rhizosphere of seedlings grown with sterile soil, *Rhizophagus intraradices* showed the highest spore density during the wet season ($F = 5.61$, $P < 0.05$) (Fig. 4). In grassland seedlings rhizosphere, *Acaulospora* sp. 1 showed the highest spore density during the dry season ($F = 5.88$, $P < 0.05$) (Fig. 4). In the rhizosphere of seedlings grown with degraded forest soil: *Acaulospora undulata*, *Glomus* sp. 1 and *Rhizophagus intraradices* showed the highest spore density during the wet season ($F = 10.28$, $P < 0.05$; $F = 18.57$, $P < 0.001$; $F = 6.69$, $P < 0.05$; respectively) (Fig. 4). The remaining AMF morphospecies were not significantly influenced by season.

Discussion

The soil from which vegetation type is better for inoculating *P. australis* seedlings for field trials?

The lack of differences between inoculation treatments in the survival and growth of the seedlings suggests that *P. australis* inoculation is not essential and that all the tested soil inocula could be used for the growing stage at the greenhouse. We are confident that the sample size used was adequate as studies using the same species have shown that 60 planted seedlings (as used in the present study) are enough to determine significant differences between treatments, i.e. season of planting, type of microsite and differences between provenances (Renison and Cingolani 2002; Renison et al. 2002, 2005).

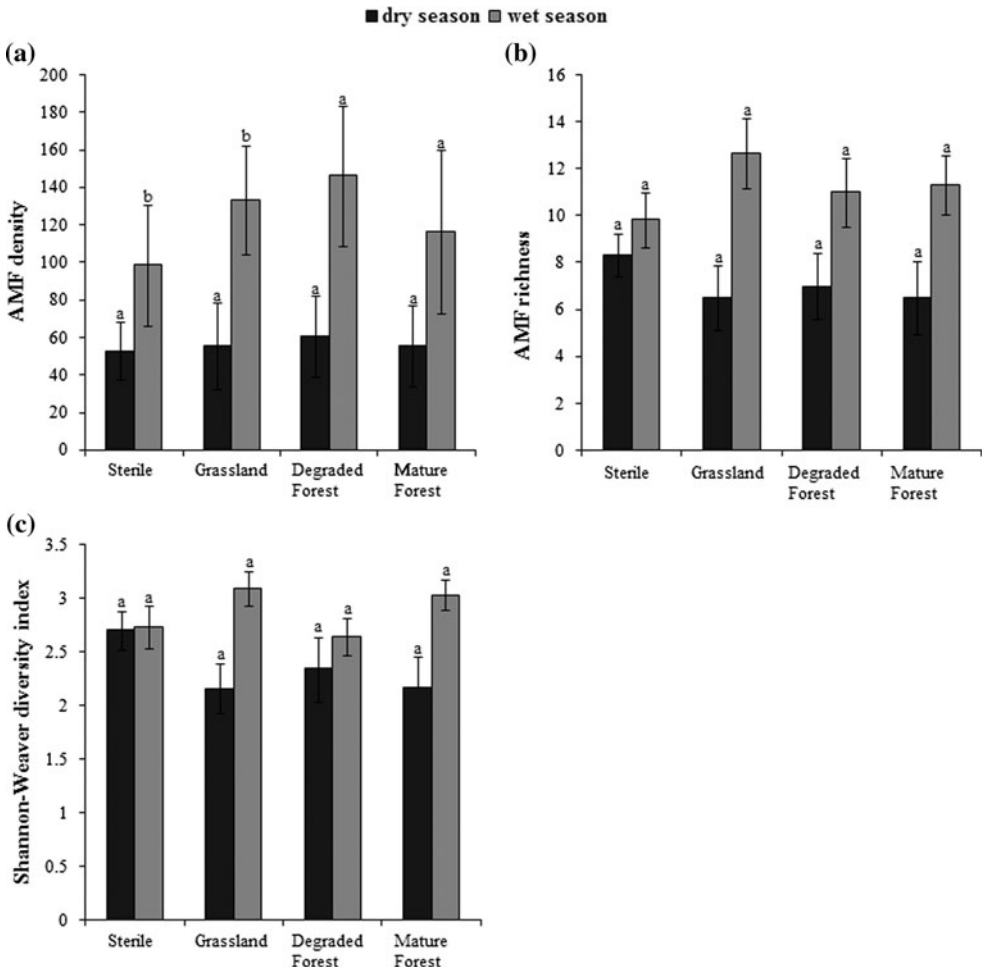


Fig. 3 Mean ± standard error of **a** arbuscular mycorrhizal fungi (*AMF*) density (number of spores/100 g of dry soil), **b** AMF richness, and **c** Shannon diversity index in the rhizosphere of *Polylepis australis* seedlings 6 and 12 months (dry and wet seasons, respectively) after transplanted at the restoration site, grown in sterile soil (initially non-mycorrhizal) or inoculated with grassland, degraded forest or mature forest soils. *Different letters* indicate significant differences between dates according to Tukey post hoc test at $P = 0.05$ ($n = 6$ replicates)

In greenhouse trials we found that mature forest inocula promoted root biomass (Soteras et al. 2013), but this effect did not translate into a better performance in the field under the conditions of the present study. Therefore, as was shown by other authors, caution must be taken when extrapolating greenhouse results to the ecosystem scale (Rillig 2004; Amaranthus and Steinfeld 2005; Lekberg and Koide 2005).

It should be borne in mind that other AMF propagules (external mycelia and colonized root fragments) (Klironomos and Hart 2002) and symbiotic or pathogenic microbial organisms that could be influencing seedlings response (Maron et al. 2011) were in the inoculum soil together with the AMF spores recorded here. Hence, the inoculation of *P. australis* with AMF isolates from natural communities and with other AMF propagule forms (López-García et al. 2013) should be considered to promote seedlings growth and survival.

In accordance with other AMF-inoculation field experiments (Allen et al. 2003; Cuenca et al. 2003; Amaranthus and Steinfeld 2005), after 12 months at the restoration site seedlings grown in sterile soil, initially non-mycorrhizal, became colonized by AMF. Moreover, as was shown by Cuenca et al. (2003), here seedlings root colonization was similar among treatments after 1 year at the field site.

Do soil inocula of different origin differ in their mycorrhizal colonization potential?

The soil inocula of different vegetation types differed in the patterns of mycorrhizal colonization potential. The mycorrhizal colonization of *M. sativa* seedlings increased with time, except for the mature forest soil treatment that remained almost constant, which is consistent with other studies of mycorrhizal colonization potential of soil (Plenchette et al. 1989; Díaz and Honrubia 1993; Irrazabal et al. 2004). AMF communities of grassland and degraded forests soils were similar in their ability to colonize *M. sativa* roots. Other studies have also found grassland as a soil with high mycorrhizal colonization potential (Jasper et al. 1991; Sene et al. 2012). The improved soil colonization potential, as well as the highest AMF richness observed in this inoculum (Soteras et al. 2013), are probably due to the increasing richness and density of highly mycotrophic annual plants present in this vegetation

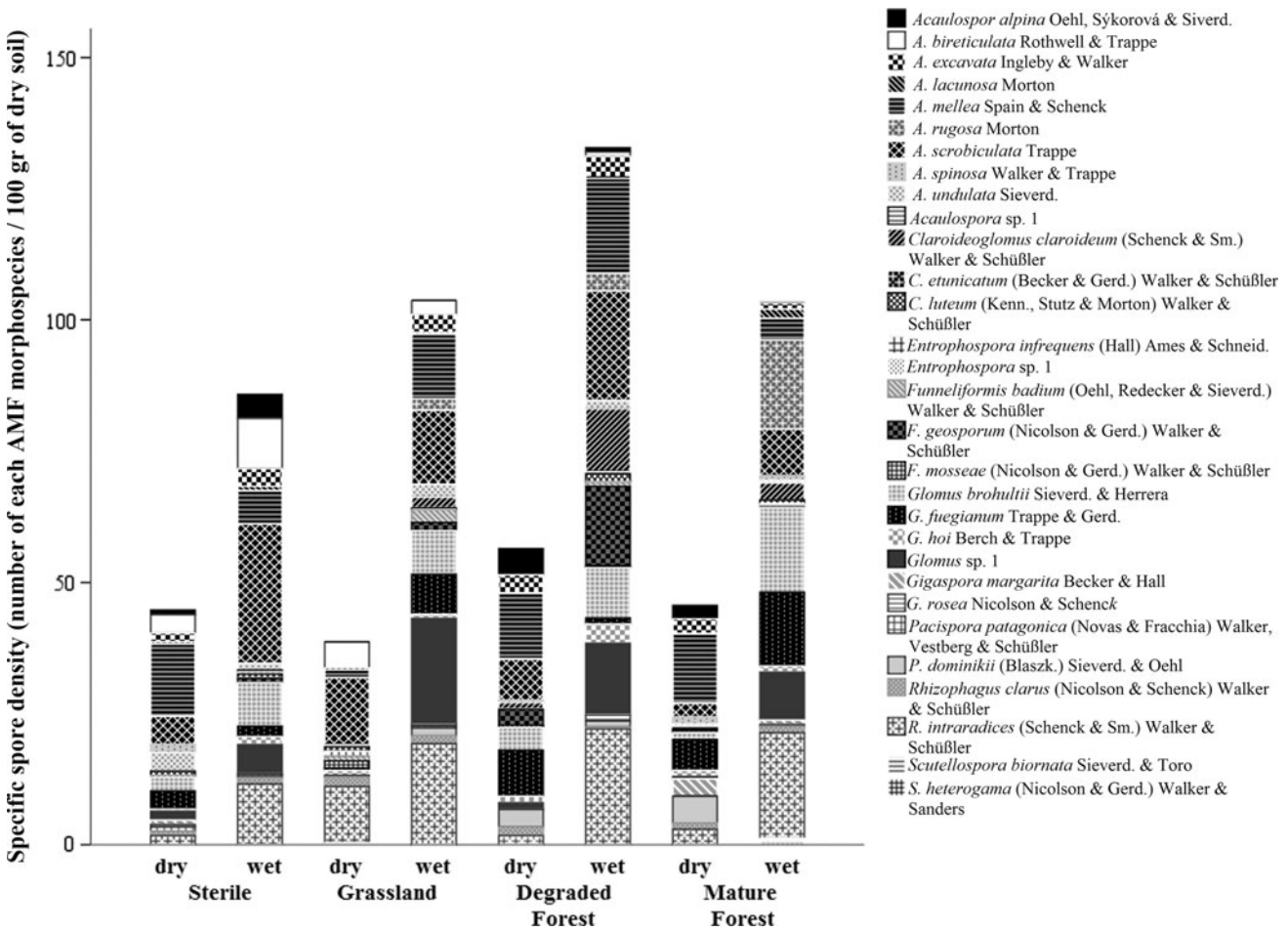


Fig. 4 Specific spore density (number of each morphospecies of arbuscular mycorrhizal fungi (*AMF*)/100 g of dry soil) of the rhizosphere of *Polylepis australis* seedlings 6 and 12 months (dry and wet seasons, respectively) after transplanted at the restoration site, grown in sterile soil (initially non-mycorrhizal) or inoculated with grassland, degraded forest or mature forest soils

type (Cingolani et al. 2003; Menoyo et al. 2007) that enhance the amount of AMF propagules. Since livestock rearing and fire events have determined the vegetation structure of grassland and degraded forests (Cingolani et al. 2008; Renison et al. 2011), spores may be readily available to colonize plant roots, having short dormancy periods as an adaptation to stressful conditions (Bever et al. 2001). However, soil colonization potential is not only related with AMF spores because other AMF propagules (colonized root fragments and external mycelia) and soil characteristics influence the soil ability to initiate root colonization (Jasper et al. 1991; Irrazabal et al. 2004; Smith and Read 2008), but could not be determined from the experiment carried out.

How does the AMF spore community of the seedlings rhizosphere change between treatments and among seasons?

Despite the fact that initially the reforestation site was strongly reduced in AMF spores, probably dispersion from nearby sites (Koske and Gemma 1990; Friese and Koske 1991; Salgado Salomón et al. 2010) or the original AMF spore community recovering after the fire event (Heneghan et al. 2008), homogenized all treatments regarding AMF spore composition and root colonization, including the rhizosphere of control seedlings. Nevertheless, AMF community is not only represented by sporulating morphospecies, thus the phylotypes present as other propagule forms, like external mycelia or colonized roots, could be differing between inoculation treatments (Hempel et al. 2007; Torrecillas et al. 2012). Further molecular analyses should be carried out to accurately describe the whole AMF community present in the rhizosphere of transplanted seedlings.

The most common AMF families (Glomeraceae and Acaulosporaceae) reported in worldwide forests soils (Irrazabal et al. 2004; Stürmer et al. 2006; Velázquez and Cabello 2011) were present in all treatments. Overall, AMF diversity, richness and density were highest during the wet season, which is also the warmest period of the year;

similar results were reported by Lugo and Cabello (2002) in the high mountains of Central Argentina. AMF composition changes with abiotic factors, such as soil phosphorous, pH or temperature (Tommerup 1983; Dumbrell et al. 2010), thus probably respond to environmental seasonal variations (Dumbrell et al. 2011; Sánchez-Castro et al. 2012).

AMF specific sporulation dynamics among seasons was evidenced as well as in other studies (Lugo and Cabello 2002; Becerra et al. 2009; Oehl et al. 2009; Soteras et al. 2012). It has been pointed out that AMF species have niche changes according to their physiological activity, being more abundant in the soil or inside the roots depending on the season (Merryweather and Fitter 1998; Pringle and Bever 2002; Dumbrell et al. 2010).

Conclusions

This is the first field trial that tests the response of *P. australis* seedlings to the inoculation with different inoculum types. We did not find significant differences between seedlings grown in sterile soil and in the other soil inocula. Moreover, the differences of the AMF spore community and root colonization of the different treatments quickly became homogeneous in the outplanted seedlings rhizosphere. Therefore, we conclude that soil inoculation is not essential for outplanted *P. australis* survival and increase in height, all the tested soils could be used as inocula including grassland soils which in practice are the easiest to collect.

Acknowledgments This work was financially supported by Agencia de Promoción Científica y Tecnológica—PICT 438-2008, Consejo Nacional de Investigaciones Científicas y Técnicas—PIP 0269 and Secretaría de Ciencia y Técnica de la Universidad Nacional de Córdoba (SECyT-UNC). F.S. is grateful to CONICET for providing her fellowship. A.B. and D.R. are researchers of CONICET. We thank to the Quebrada del Condorito National Park authorities that generously allowed the use of the lands for the reforestation assay. This research would not have been possible without numerous volunteers (especially Barberá I., Berizzo A., Bernaschini M.L., Domínguez J., Farias G., Flores S., Galli E., Gatica L., Peralta G., Street E., and researchers and PhD students of the Laboratorio de Micología-IMBIV-) who provided exceptional field assistance.

References

Allen EB, Allen MF, Egerton-Warburton LM, Corkidi L, Gómez-Pompa A (2003) Impacts of early-and late-seral mycorrhizae during restoration in seasonal tropical forest. Ecol Appl 13:1701–1717

Amaranthus M, Steinfeld D (2005) Arbuscular mycorrhizal inoculation following biocide treatment improves *Calocedrus decurrens* survival and growth in nursery and outplanting sites. In: Dumroese R, Riley LE, Landis T (eds) National Proceedings: forest and conservation nursery associations 2004; 2004 July 12–15; Charleston, NC; and 2004 July 26–29; Medford, OR. Proc. RMRS-P-35. Fort Collins, pp 103–108

Asbjornsen H, Montagnini F (1994) Vesicular-arbuscular mycorrhizal inoculum potential affects the growth of *Stryphnodendron microstachyum* seedlings in a costa rican human tropical lowland. Mycorrhiza 5:45–51

Becerra AG, Cabello M, Zak MR, Bartoloni N (2009) Arbuscular mycorrhizae of dominant plant species in Yungas forests, Argentina. Mycologia 101:612–621

Bever JD, Schultz PA, Pringle A, Morton JB (2001) Arbuscular mycorrhizal fungi: more diverse than meets the eye, and the ecological tale of why. Bioscience 51:923–932

Cabello MN (1997) Hydrocarbon pollution: its effect on native arbuscular mycorrhizal fungi (AMF). FEMS Microbiol Ecol 22:233–236

Cabido M (1985) Las comunidades vegetales de Pampa de Achala, Sierras de Córdoba. Argen Doc Phytosociol 9:431–443

Cingolani AM, Cabido M, Renison D, Solís Neffa V (2003) Combined effects of environment and grazing on vegetation structure in argentine granite grasslands. J Veg Sci 14:223–232

Cingolani AM, Renison D, Zak MR, Cabido M (2004) Mapping vegetation in a heterogeneous mountain rangeland using landsat data: an alternative method to define and classify land-cover units. Remot Sens Environ 92:84–97

Cingolani AM, Renison D, Tecco PA, Gurvich DE, Cabido M (2008) Predicting cover types in a mountain range with long evolutionary grazing history: a GIS approach. J Biogeogr 35:538–551

Closa I, Goicoechea N (2011) Infectivity of arbuscular mycorrhizal fungi in naturally regenerating, unmanaged and clear-cut beech forests. Pedosphere 21:65–74

Cuenca G, De Andrade Z, Lovera M, Fajardo L, Meneses E (2003) Mycorrhizal response of *Clusia pusilla* growing in two different soils in the field. Trees 17:200–206

Cuenca G, De Andrade Z, Lovera M, Fajardo L, Meneses E (2004) The effect of two arbuscular mycorrhizal inocula of contrasting richness and the same mycorrhizal potential on the growth and survival of wild plant species from La Gran Sabana, Venezuela. Can J Bot 82:582–589

Díaz G, Honrubia M (1993) Infectivity of mine soils from southeast Spain. II. Mycorrhizal population levels in spoilt sites. Mycorrhiza 4:85–88

Dumbrell AJ, Nelson M, Helgason T, Dytham C, Fitter AH (2010) Relative roles of niche and neutral processes in structuring a soil microbial community. ISME J 4:337–345

Dumbrell AJ, Ashton PD, Aziz N, Feng G, Nelson M, Dytham C, Fitter AH, Helgason T (2011) Distinct seasonal assemblages of arbuscular mycorrhizal fungi revealed by massively parallel pyro-sequencing. New Phytol 190:794–804

Ellenberg H (1979) Man's influence on tropical mountain ecosystems in South America. J Ecol 67:401–416

Fischer CR, Janos DP, Perry DA, Linderman RG, Sollins P (1994) Mycorrhiza inoculum potentials in tropical secondary succession. Biotropica 26:369

Fjeldså J, Kessler M (1996) Conserving the biological diversity of *Polylepis* woodlands of the highland of Peru and Bolivia. A contribution to sustainable natural resource management in the Andes, Nordeco

Friese CF, Koske RE (1991) The spatial dispersion of spores of vesicular-arbuscular mycorrhizal fungi in a sand dune: microscale patterns associated with the root architecture of American beachgrass. Mycol Res 95:952–957

Gerdemann JW, Nicolson TH (1963) Spores of mycorrhizal endogone species extracted from soil by wet sieving and decanting. Trans Br Mycol Soc 46:235–244

Giovannetti M, Mosse B (1980) An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots. New Phytol 84:489–500

Grace C, Stribley DP (1991) A safer procedure for routine staining of vesicular-arbuscular mycorrhizal fungi. Mycol Res 95: 1160–1162

Hempel S, Renker C, Buscot F (2007) Differences in the species composition of arbuscular mycorrhizal fungi in spore, root and soil communities in a grassland ecosystem. Environ Microbiol 9:1930–1938

Heneghan L, Miller SP, Baer S, Callaham MA, Montgomery J, Pavao-Zuckerman M, Rhoades CC, Richardson S (2008) Integrating soil ecological knowledge into restoration management. Rest Ecol 16:608–617

Hoeksema JD, Chaudhary VB, Gehring CA, Collins Johnson N, Karst J, Koide RT, Pringle A, Zabinski C, Bever JD, Moore JC, Wilson GWT, Klironomos JN, Ubanhowar J (2010) A meta-analysis of context-dependency in plant response to inoculation with mycorrhizal fungi. Ecol Lett 13:394–407

Irrazabal G, Velázquez S, Cabello MN (2004) Infectividad y diversidad de HMA de la rizosfera de los talares de Magdalena, provincia de Buenos Aires, Argentina. Bol Micol 19:49–57

Jasper DA, Abbot LK, Robson AD (1991) The effect of soil disturbance on vesicular arbuscular mycorrhizal fungi in soils from different vegetation types. New Phytol 118:471–476

Kauffman JB, Steele MD, Cummings DL, Jaramillo VJ (2003) Biomass dynamics associated with deforestation, fire, and conversion to cattle pasture in a mexican tropical dry forest. For Ecol Man 176:1–12

Kiers ET, Lovelock CE, Krueger EL, Herre EA (2000) Differential effects of tropical arbuscular mycorrhizal fungal inocula on root colonization and tree seedling growth: implications for tropical forest diversity. Ecol Lett 3:106–113

Klironomos JN, Hart MM (2002) Colonization of roots by arbuscular mycorrhizal fungi using different sources of inoculum. Mycorrhiza 12:181–184

Koske RE, Gemma JN (1990) VA mycorrhizae in strand vegetation of Hawaii: evidence for long-distance codispersal of plants and fungi. Am J Bot 77:466–474

Lekberg Y, Koide RT (2005) Is plant performance limited by abundance of arbuscular mycorrhizal fungi? A meta-analysis of studies published between 1988 and 2003. New Phytol 168:189–204

López-García A, Hempel S, Miranda JdeD, Rillig MC, Barea JM, Azcón-Aguilar C (2013) The influence of environmental degradation processes on the arbuscular mycorrhizal fungal community associated with yew (*Taxus baccata* L.), an endangered tree species from mediterranean ecosystems of southeast Spain. Plant Soil 370:355–366

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

Magurran AE, McGill BJ (2011) Biological diversity: frontiers in measurement and assessment. Oxford University Press, Oxford, UK

Maherali H, Klironomos JN (2007) Influence of phylogeny on fungal community assembly and ecosystem functioning. Science 316:1746–1748

Maron JL, Marler M, Klironomos JN, Cleveland CC (2011) Soil fungal pathogens and the relationship between plant diversity and productivity. Ecol Lett 14:36–41

Martino J, Urcelay C, Renison D (2011) Crecimiento y colonización micorrícica de *Polylepis australis* Bitter (Rosaceae) en suelos con distinta historia de pastoreo. Kurtziana 36:69–77

McGonigle TP, Miller M, Evans DG, Fairchild GL, Swan JA (1990) A method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. New Phytol 115:495–501

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

Merryweather J, Fitter AH (1998) The arbuscular mycorrhizal fungi of hyacinthoides non-scripta II. Seasonal and spatial patterns of fungal populations. New Phytol 138:131–142

Oehl F, Sieverding E, Ineichen K, Mäder P, Wiemken A, Boller T (2009) Distinct sporulation dynamics of arbuscular mycorrhizal fungal communities from different agroecosystems in long-term microcosms. Agric Ecosys Environ 134:257–268

Oehl F, Sieverding E, Palenzuela J, Ineichen K, Alves da Silva G (2011) Advances in *Glomeromycota* taxonomy and classification. IMA Fungus 2:191–199

Omar MB, Bolland L, Heather WA (1979) P.V.A. (polyvinyl alcohol). A permanent mounting medium for fungi. Bull Br Mycol Soc 13:31–32

Phillips JM, Hayman DS (1970) Improved procedures for clearing roots and staining parasitic and vesicular arbuscular mycorrhizal fungi for rapid assessment of infection. Tran Br Mycol Soc 55:158–161

Picone C (2000) Diversity and abundance of arbuscular-mycorrhizal fungus spores in tropical forest and pasture. Biotropica 32:734–750

Plenchette C, Perrin R, Duvert P (1989) The concept of soil infectivity and a method for its determination as applied to endomycorrhizas. Can J Bot 67:112–115

Pringle A, Bever JD (2002) Divergent phenologies may facilitate the coexistence of arbuscular mycorrhizal fungi in a North Carolina grassland. Am J Bot 89:1439–1446

R Development Core Team (2011) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org

Renison D, Cingolani AM (2002) Evaluación de la supervivencia y crecimiento de plantines de *Polylepis australis* (Rosaceae) para la elección de plantas semilleras. Agriscientia 19:63–66

Renison D, Cingolani AM, Schinner D (2002) Optimizing restoration of *Polylepis australis* woodlands: when, where and how to transplant seedlings to the mountains? Ecotropica 8:219–224

Renison D, Cingolani AM, Suarez R, Menoyo E, Coutsiers C, Sobral A, Hensen I (2005) The restoration of degraded mountain woodlands: effects of seed provenance and microsite characteristics on *Polylepis australis* seedling survival and growth in central Argentina. Res Ecol 13:129–137

Renison D, Hensen I, Suarez R, Cingolani AM, Marcora P, Giorgis MA (2010) Soil conservation in *Polylepis* mountain forests of central Argentina: Is livestock reducing our natural capital? Austral Ecol 35:435–443

Renison D, Hensen I, Suarez R (2011) Landscape structural complexity of high-mountain *Polylepis australis* forests: a new aspect of restoration goals. Res Ecol 19:390–398

Renison D, Cuyckens GAE, Pacheco S, Guzmán GF, Grau HR, Marcora P, Robledo GL, Cingolani AM, Dominguez J, Landi M, Bellis L, Hensen I (2013) Distribución y estado de conservación de las poblaciones de árboles y arbustos del género *Polylepis* (Rosaceae) en las montañas de Argentina. Ecol Austral 23:27–36

Rillig MC (2004) Arbuscular mycorrhizae and terrestrial ecosystem processes. Ecol Lett 7:740–754

Salgado Salomón ME, Barroetaveña C, Rajchenberg M (2010) Do pine plantations provide mycorrhizal inocula for seedlings establishment in grasslands from Patagonia, Argentina? New For 41:191–205

Sánchez-Castro I, Ferrol N, Cornejo P, Barea JM (2012) Temporal dynamics of arbuscular mycorrhizal fungi colonizing roots of

representative shrub species in a semi-arid mediterranean ecosystem. Mycorrhiza 22:449–460

Schenk NC, Perez Y (1990) Manual of identification of vesicular-arbuscular mycorrhizal fungi. Gainesville, USA

Sene G, Thiao M, Manga A, Kane A, Samba-Mbaye R, Samba-Mbaye M, Khasa D, Sylla SN (2012) Arbuscular mycorrhizal soil infectivity and spores distribution across plantations of tropical, subtropical and exotic tree species: a case study from the forest reserve of Bandia. Senegal Afr J Ecol 50:218–232

Smith SE, Read D (2008) Mycorrhizal symbiosis, 3rd edn. Academic Press, Great Britain

Smith SE, Jakobsen I, Grønlund M, Smith FA (2011) Roles of arbuscular mycorrhizas in plant phosphorus nutrition: interactions between pathways of phosphorus uptake in arbuscular mycorrhizal roots have important implications for understanding and manipulating plant phosphorus acquisition. Plant Physiol 156:1050–1057

Soteras F, Becerra A, Cofré N, Bartoloni J, Cabello M (2012) Arbuscular mycorrhizal fungal species in saline environments of Central Argentina: seasonal variation and distribution of spores at different soil depths. Sydowia 64:301–311

Soteras F, Renison D, Becerra AG (2013) Growth response, phosphorus content and root colonization of *Polylepis australis* Bitt. seedlings inoculated with different soil types. New For 44:577–589

Stürmer SL, Filho OK, De Queiroz H, De Mendonça MM (2006) Occurrence of arbuscular mycorrhizal fungi in soils of early stages of a secondary succession of Atlantic forest in south Brazil. Acta Bot Bras 20:513–521

Tommerup IC (1983) Temperature relations of spore germination and hyphal growth of vesicular-arbuscular mycorrhizal fungi in soil. Trans Brit Mycol Soc 81:381–387

Torrecillas E, Alguacil del Mar M, Roldán A (2012) Differences in the AMF diversity in soil and roots between two annual and perennial gramineous plants co-occurring in a mediterranean, semiarid degraded area. Plant Soil 354:97–106

Torres R, Renison D, Hensen I, Suarez R, Enrico L (2008) *Polylepis australis*' regeneration niche in relation to seed dispersal, site characteristics and livestock density. For Ecol Man 254:255–260

Velázquez S, Cabello M (2011) Occurrence and diversity of arbuscular mycorrhizal fungi in trap cultures from El Palmar national park soils. Eur J Soil Biol 47:230–235

Walker C, Mize W, McNabb HS (1982) Populations of endogonaceous fungi at two populations in central Iowa. Can J Bot 60:2518–2529

Zak MR, Cabido M (2002) Spatial patterns of the Chaco vegetation of central argentina: integration of remote sensing and phytosociology. Appl Veg Sci 5:213–226