ANEXO

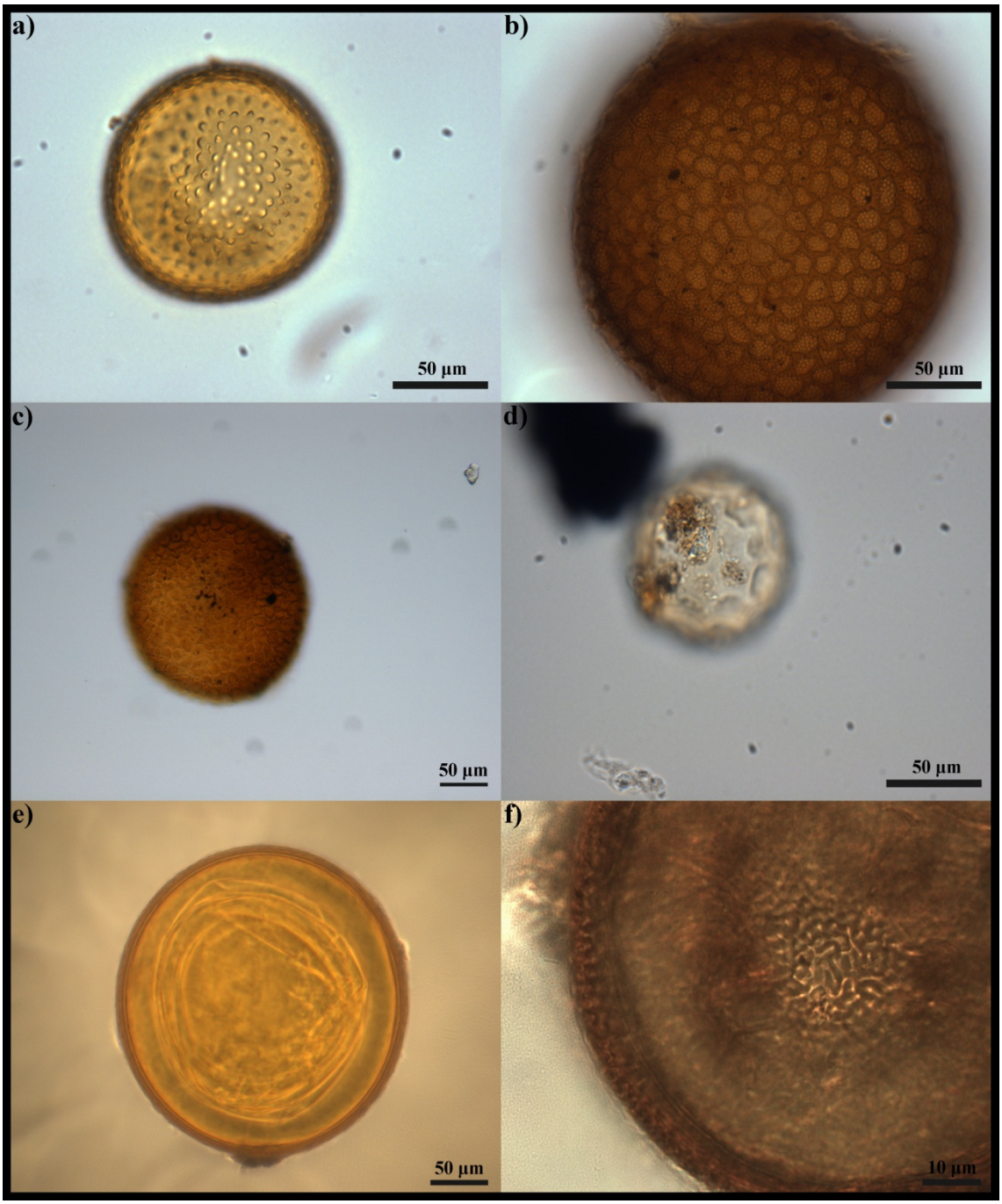

Figura 1 Morfoespecies de hongos micorrícico arbusculares encontradas en la rizosfera de *P. australis:* **(a)** *Acaulospora alpina,* **(b)** *Acaulospora bireticulata,* **(c)** *Acaulospora excavata*, **(d)** *Acaulospora foveata,* **(e)** *Acaulospora mellea* y **(f)** *Acaulospora rehmii*.

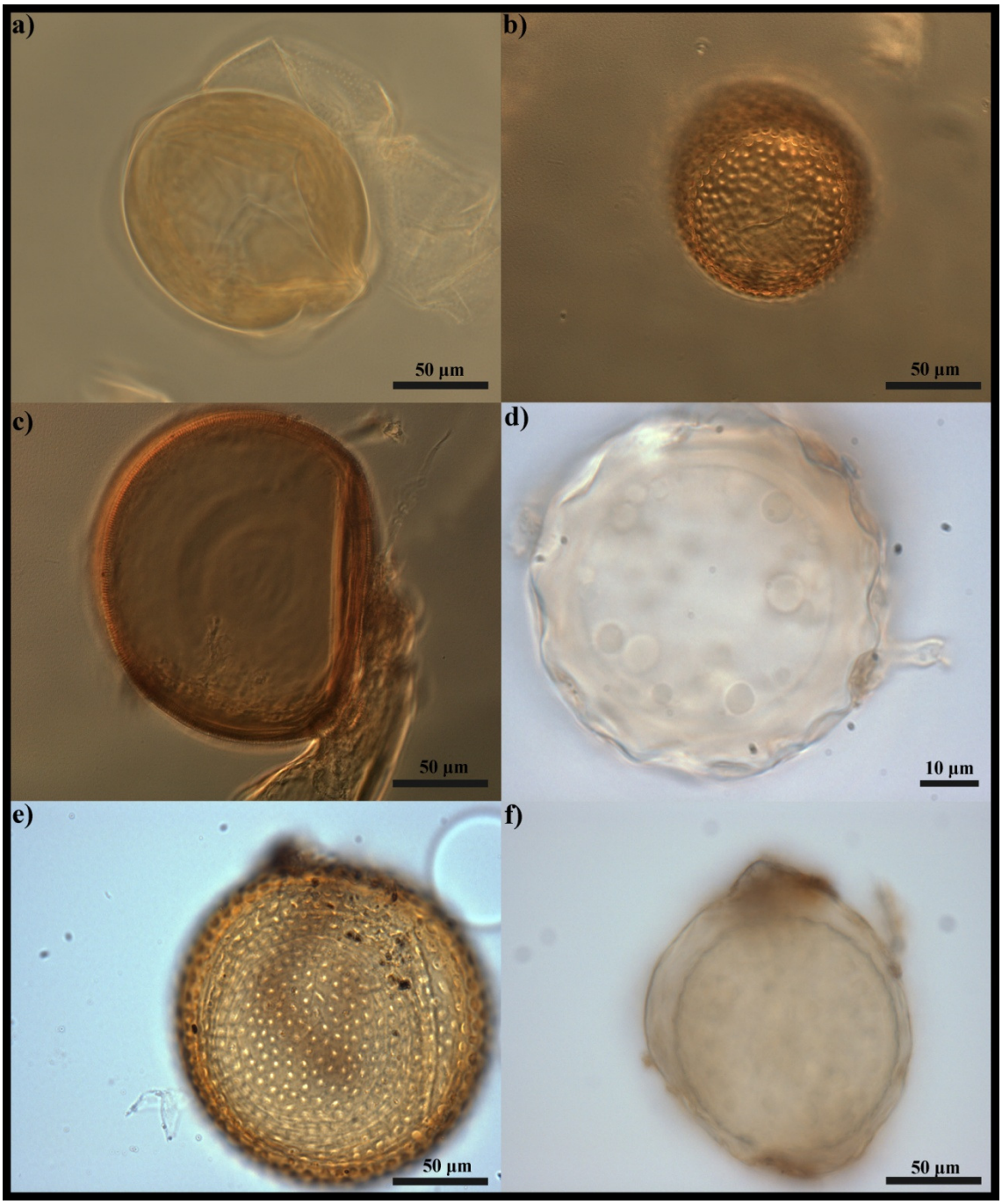

Figura 2 Morfoespecies de hongos micorrícico arbusculares encontradas en la rizosfera de *P. australis:* **(a)** *Acaulospora rugosa,* **(b)** *Acaulospora scrobiculata,* **(c)** *Acaulospora spinosa,* **(d)** *Acaulospora undulata,* **(e)** *Acaulospora lacunosa* y **(f)** *Ambispora leptoticha*.

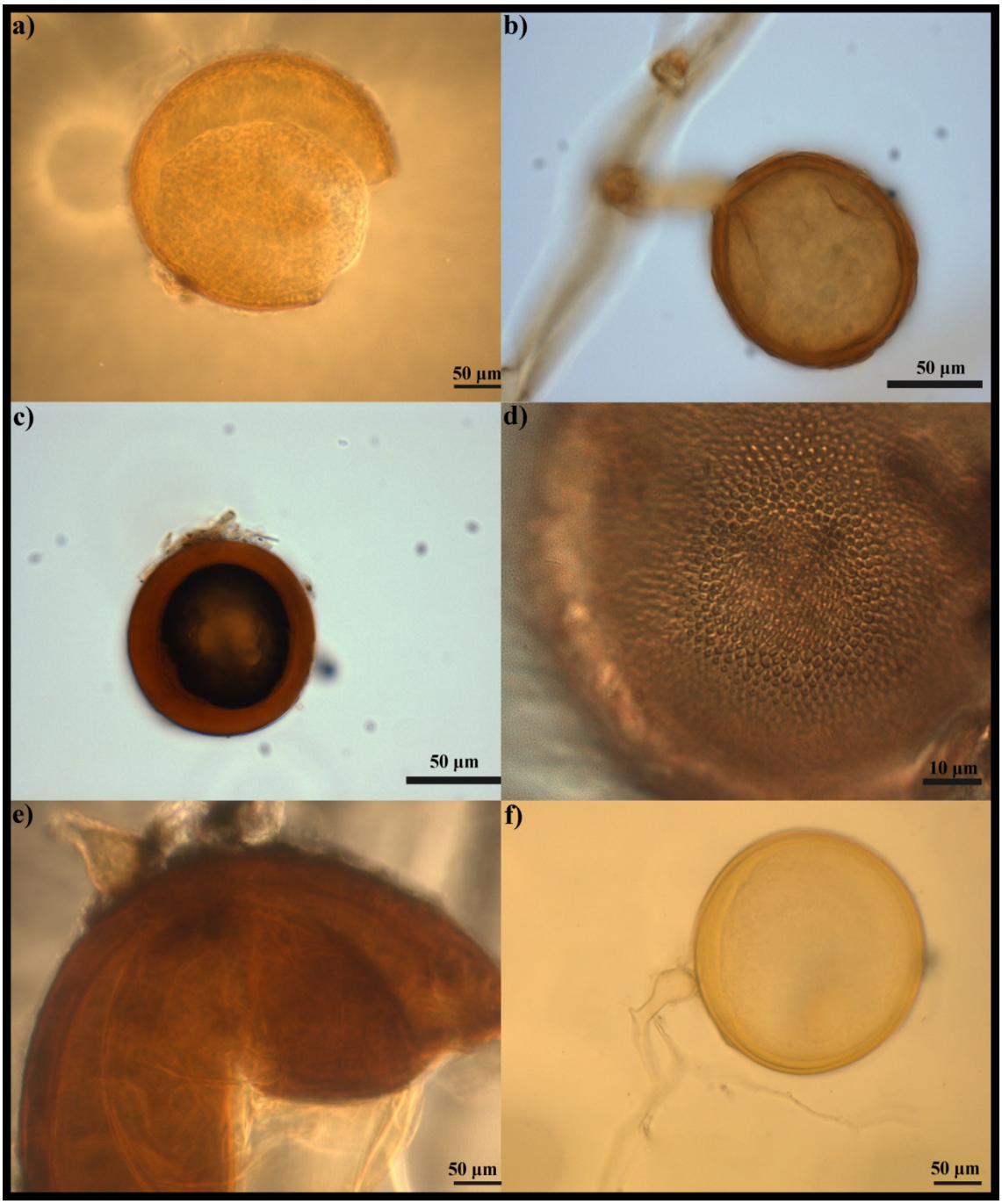

Figura 3 Morfoespecies de hongos micorrícico arbusculares encontradas en la rizosfera de *P. australis:* **(a)** *Ambispora appendicula,* **(b)** *Claroideoglomus claroideum,* **(c)** *Claroideoglomus luteum,* **(d)** *Entrophospora infrequens* **(e)** *Gigaspora margarita* y **(f)** *Gigaspora rosea*.

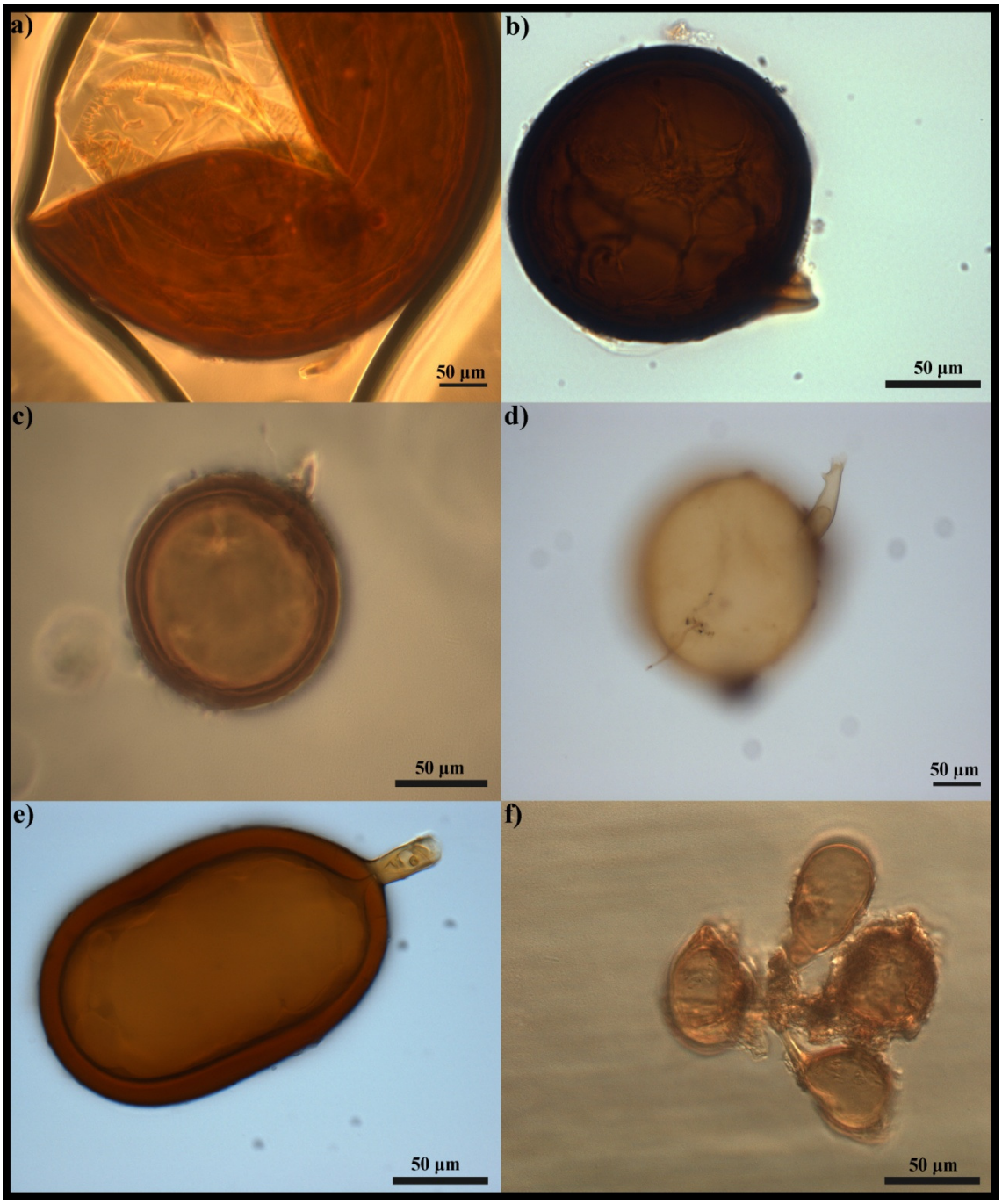

Figura 4 Morfoespecies de hongos micorrícico arbusculares encontradas en la rizosfera de *P. australis:* **(a)** *Scutellospora biornata,* **(b)** *Funneliformis badium,* **(c)** *Funneliformis geosporum,* **(d)** *Funneliformis mosseae* **(e)** *Glomus brohultii* y **(f)** *Glomus fuegianum.*

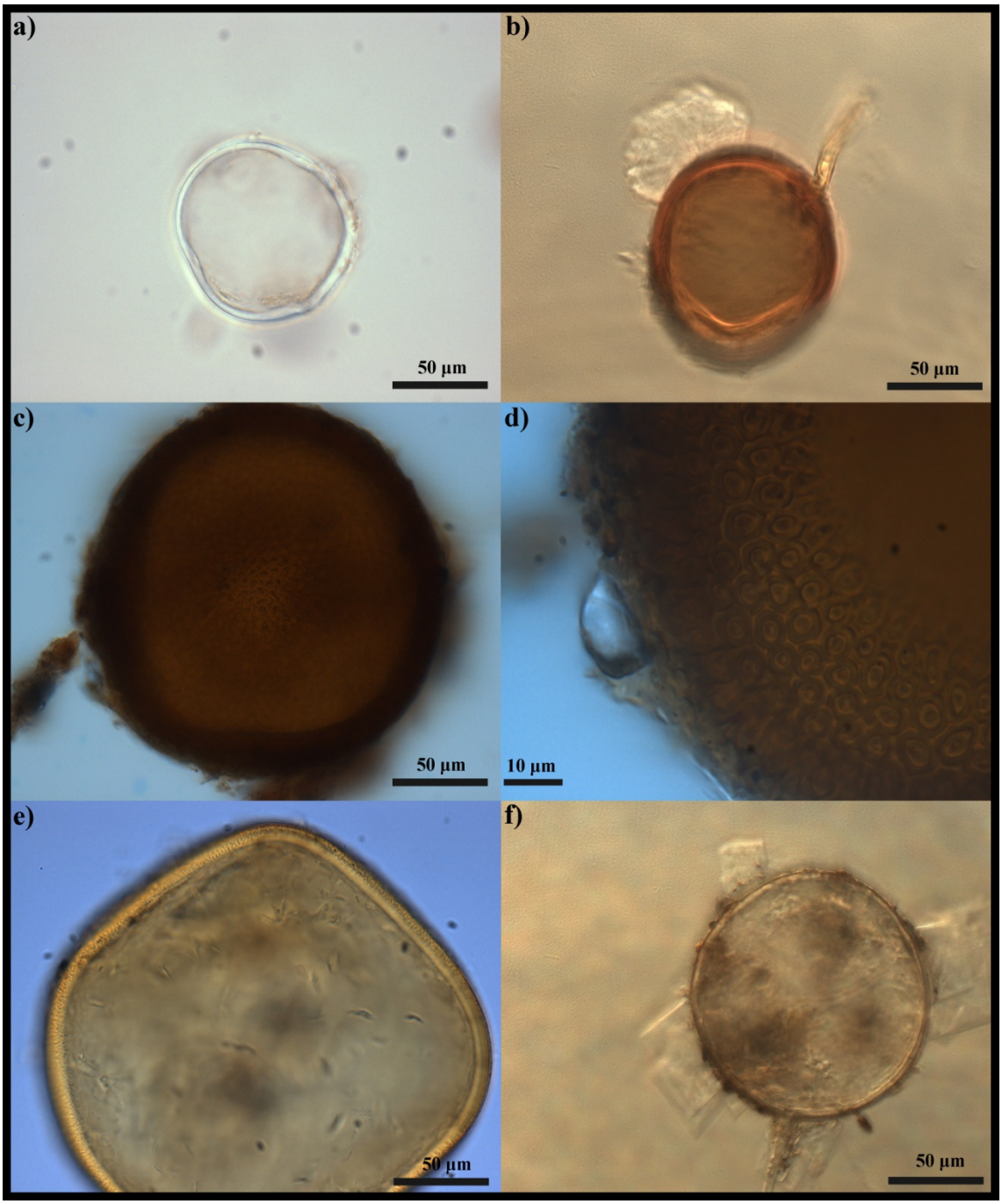

Figura 5 Morfoespecies de hongos micorrícico arbusculares encontradas en la rizosfera de *P. australis:* **(a)** *Rhizophagus clarus,* **(b)** *Rhizophagus intraradices,* **(c)** *Pacispora patagonica* **(d)** detalle de la pared de *Pacispora patagonica* **(e)** *Pacispora dominikii* y **(f)** *Pacispora robigina*.

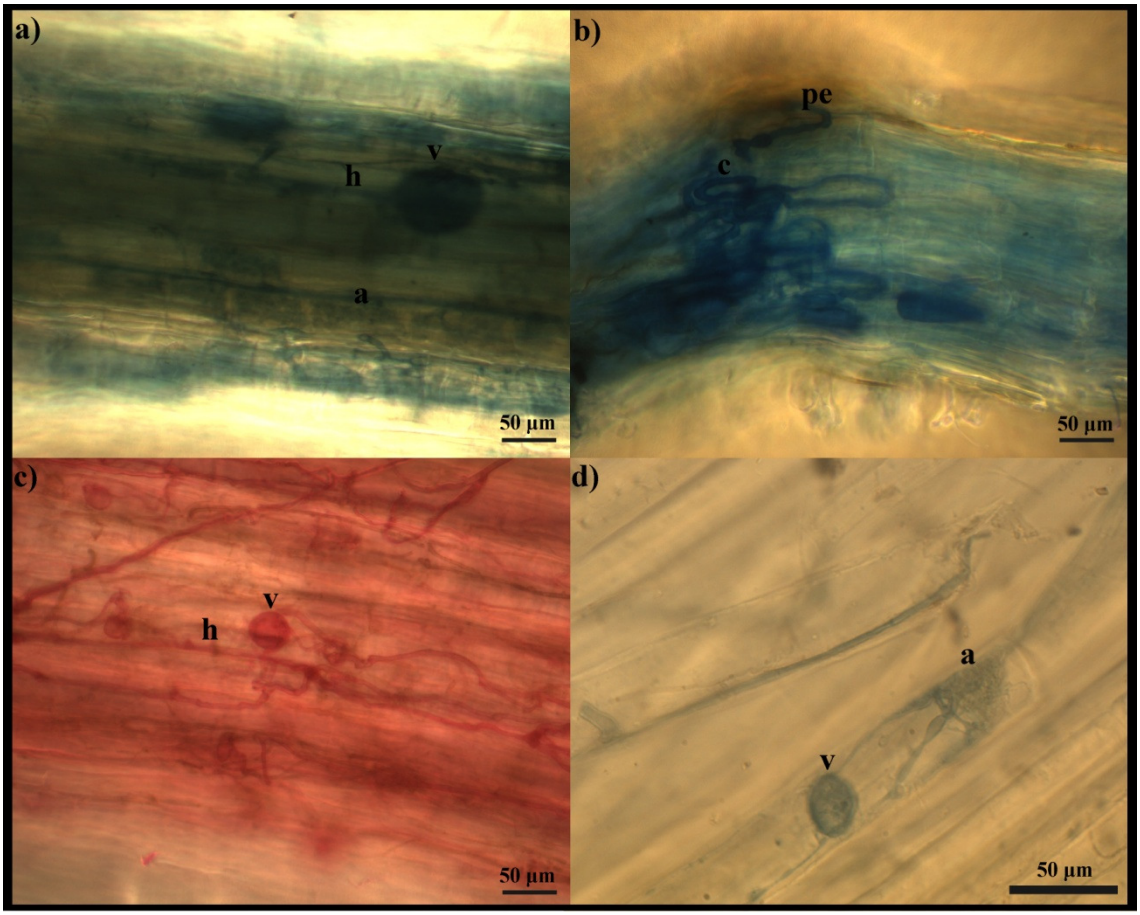

Figura 6 Colonización micorrícica arbuscular de raíces de *P. australis:* **(a)**, **(b)** y **(d)** tinción con azul de anilina, **(c)** tinción con fucsina ácida. Estructuras intra-radicales: hifa - h -, vesícula -v-, circunvolución -c-, punto de entrada -pe-.

Growth response, phosphorus content and root colonization of Polylepis australis *Bitt. seedlings inoculated with different soil types*

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Growth response, phosphorus content and root colonization of *Polylepis australis* Bitt. seedlings inoculated with different soil types

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Abstract *Polylepis* forests are one of the most endangered high mountain ecosystems of South America and reforestation with native *Polylepis* species has been highly recommended. Greenhouse bioassays were set up to determine the influence of three different soils on growth and phosphorous nutrition of *Polylepis australis* seedlings. Soils were collected from a grassland, a rare mature forest and a forest degraded due to repeated fires. We identified the arbuscular mycorrhizal fungi (AMF) present in the three soils and after 12 months we harvested the seedlings to evaluate root and shoot biomass, plant P content and root colonization by native AMF and dark septate endophytes (DSE). The soil inocula contained 26 AMF morphospecies. Grassland inoculum showed the highest AMF richness, and mature forest showed a different AMF community assembly from grassland and degraded forest inocula. Root biomass and root colonization were highest in seedlings inoculated with mature forest soil, meanwhile shoot biomass and plant P content were similar between all treatments. AMF colonization correlated negatively with DSE and root biomass was negatively correlated with DSE colonization, thus these fungal symbionts could be competing for resources. Our results indicate that AMF inoculum from the mature forest stand has the potential to improve *P. australis* performance, probably due to the dominance of Glomeraceae and Acaulosporaceae families. However, other soil microorganisms could be together with AMF in the natural inocula, affecting the growth response of *P. australis* seedlings. Future studies evaluating the effect of these inocula under field conditions should be carried out.

Keywords Arbuscular mycorrhizal fungi · Dark septate endophytes · Mountain forest · Natural soil inocula

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Abbreviations

AMF	Arbuscular mycorrhizal fungi
DSE	Dark septate endophytes
P	Phosphorus
N	Nitrogen

Introduction

The higher forest belt of many South American mountains has a canopy dominated by species of the genus *Polylepis* (Kessler and Schmidt-Lebuhn 2006). Human activities, as livestock rearing and forest burning and logging, have greatly modified the appearance of these forest ecosystems (Ellenberg 1979). Such activities have produced loss of forest soils and litter cover, changes in the structural complexity of remnant forests, increased soil compaction and reduced soil moisture (Cingolani et al. 2008; Renison et al. 2010, 2011). Thus, *Polylepis* forests are one of the most endangered high mountain ecosystems in the world and their conservation and reforestation are considered a priority (Fjeldså and Kessler 1996).

Polylepis australis Bitt. (Rosaceae), the southernmost *Polylepis* species, provides important ecosystem services in the higher central mountains of Argentina, such as clean water, soil generation, habitat for endemic species and maintenance of biodiversity (Renison et al. 2010, 2011). Since the seventeenth century, livestock browsing and intentional fires to promote the growth of forage have greatly reduced the forested area of the region (Cingolani et al. 2008). Therefore, at present forest restoration is being implemented and several studies have developed methods to facilitate the restoration with *P. australis* (Renison et al. 2005, 2011; Seltmann et al. 2006).

Arbuscular mycorrhizal fungi (AMF) may 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). These fungi usually coexist with dark septate endophytes (DSE) in almost all plant species (Jumpponen and Trappe 1998; Jumpponen 2001) including *P. australis* (Menoyo et al. 2007). However, the DSE ecological functions are poorly understood and they might range, as AMF, from mutualism to parasitism (Johnson et al. 1997).

AMF show differences in their colonizing strategies, taxonomically based at the family level (Hart and Reader 2002). AMF communities formed by phylogenetically over dispersed species promote higher biomass increase than AMF communities formed by closely related species (Maherali and Klironomos 2007). However, the relationship between AMF abundance and richness in soils and plant growth response is uncertain. It has been shown that soils with the highest inoculum potential (Asbjornsen and Montagnini 1994) or with highest AMF richness (Cuenca et al. 2004) did not promote the best plant response. This could be due to AMF specific sporulation patterns and differential effect of increasing colonization on plant cost-benefit balance. Furthermore, there is contrasting evidence regarding forest successional stage and the effectiveness of their soils as inoculum. A positive response of late- or early-successional trees has been observed with an early-successional AMF inoculum (Asbjornsen and Montagnini 1994; Allen et al. 2003). Meanwhile, Kiers et al. (2000) showed that a late-successional inoculum was the best for a pioneer tree and early-successional inoculum for a mature forest tree. Hence, it is important to study the influence of a particular AMF inoculum on each plant species, because mycorrhizal effectiveness depends on the specific interaction between each fungus and plant species (Smith et al. 2011).

Studies evaluating livestock density impacts on *P. autralis* growth and AMF root colonization have been performed (Menoyo et al. 2009; Martino et al. 2011). *P. australis* seedlings inoculated with soil collected from an area with null livestock density showed the highest shoot biomass but the lowest AMF root colonization (Martino et al. 2011).

In this work we used natural soil inocula from three vegetation types differing in their structural complexity: a grassland, a mature forest and a degraded forest sensu Renison et al. (2011), to evaluate the growth, root colonization and P nutrition of *P. australis* seedlings under greenhouse conditions. We hypothesized that AMF and DSE from three vegetation types will promote differentially the growth and nutrition on *P. australis* seedlings.

Materials and methods

Study area

The study area is located in the Córdoba Mountains of Central Argentina (up to 2,790 m asl). Mean temperatures at 2,100 m asl are 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) and a long dry season during the coldest months (May to September). Since the beginning of the seventeenth century livestock rearing has been the main economic activity (Cingolani et al. 2003).

We collected inocula soils from Los Molles river basin (1,900 m asl, 31°58′S, 64°56′W) because it is one of the best preserved river basins in the Córdoba mountains. At Los Molles basin the landscape is a mosaic of rock outcrops, grasslands and *P. australis* forests in different stages of post-fire recovery. There are sites where successive fires have impacted on forests structure, causing extensive soil erosion and hampering forest regeneration. On the other hand, there is a unique mature forest stand with great volumes of standing dead wood, a typical fern understory and the presence of the rare, shade-tolerant tree *Maytenus boaria* Molina (Renison et al. 2011). This situation with presence of several hectares of mature forest is exclusive to the Los Molles river basin; extensive field explorations aided by satellite images have not yielded further findings (Renison et al. 2011; Cingolani et al. 2008).

Soil inocula

In Los Molles river basin, soil samples were collected from three vegetation types: (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) a *P. australis* mature forest with standing and fallen dead wood, fern cover and abundance of shade-tolerant *M. boaria* trees (hereafter called "mature forest"), and (3) an area with sparse *P. australis* trees, little regeneration, evidences of previous fire events and soil erosion (hereafter called "degraded forest") (Renison et al. 2011). The degraded forest was about 100 m from the mature forest and both degraded and mature forests were 600 m away from the grassland community.

During the dry season (May 2009), soil samples were collected with a trowel from 10 random points (0–20 cm depth) from each community type (inoculum type), placed in plastic bags and stored at 4 °C. Ten soil samples per inoculum type were analyzed to determine: soil texture, total N (%), nitrate (%), organic matter (%), pH (25 % water solution) and extractable P (%) determined by Bray and Kurtz I method.

Soil inoculum of each vegetation type consisted in a composite of the 10 soil samples. In the laboratory, litter, stones and sticks were removed with a 1-cm mesh sieve. In order to determine the AMF diversity of each inoculum type, AMF spores were extracted from each soil by wet sieving and decanting, 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 with stereomicroscope and recorded as mean spores per 100 g of dry soil. 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. AMF morphospecies identification was based on current species descriptions and the identification manuals of Schenk and Perez (1990) and INVAM http://invam.caf.wvu.edu/Myc_Info/Taxonomy/species.htm. Total fungal number was calculated as total spore number per 100 g of dry soil. Shannon biodiversity index, species richness and evenness were also calculated (Magurran 1988). Vouchers were deposited in the herbarium at the Museo Botánico de Córdoba (CORD).

Inoculation

Seeds of *P. australis* were surface sterilized with 10 % sodium hypochlorite for 10 min, washed several times with sterilized distilled 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) containing approximately 500 g of fresh autoclaved soil of the same composite used for inoculation (1 h of heating at 120 °C, repeated 3 times with intervals of 24 h). Thirty grams of the three inocula, composed of fresh homogeneous mixture of rhizospheric soil, were placed 3 cm beneath the surface in a hole at the time of transplanting. Extra pots were prepared for all treatments to ensure survival of 18 replicates, 6 per treatment. Uninoculated controls (30 g of sterile soil) were also established to make sure that the mycorrhizal colonization would be only explained by soil inoculation and not due to contamination during the greenhouse trial.

Growing conditions were 25 °C and 12 h photoperiod and plants were watered daily as necessary from the start of the experiment.

Assessment of variables

At the end of the experiment, after 12 months, the following plant growth parameters were analyzed: shoot and root dry weight and plant P content (percentages of P per g of dry weight) by the acid dry digestion method (Jones et al. 1991). Although many essential nutrients can be acquired through mycorrhizal symbiosis (Smith et al. 2011; van der Heijden et al. 2008), only seedling P content was considered here. This is because P is limiting in *P. australis* degraded forest soils (Renison et al. 2010) and trees are highly dependent on mycorrhizas for P acquisition under these conditions (Plassard and Dell 2010).

The percentage of AMF was assessed according to the techniques described by Phillips and Hayman (1970) and by Grace and Stribley (1991). Roots were cleared with 10 % KOH (15 min at 90 °C), then acidified with 1 % HCL (1 min, root temperature) and stained in 0.05 % aniline blue. Colonization was estimated counting 100 line intersections per root sample (McGonigle et al. 1990) under Leica 4-100X light microscope. AMF structures were distinguished by bright hyphal color and the presence of vesicles, coils and arbuscules; and the DSE structures by dark hyphae color and microsclerotia (Jumpponen and Trappe 1998; Smith and Read 2008).

Data analysis

Polylepis australis plant performance (shoot and root biomass, plant P content, AMF colonization, DSE colonization), and the AMF spore measurements (Shannon index, richness and evenness) were analyzed using a one-way analysis of variance (ANOVA) with inoculum types (three levels: grassland, mature and degraded forest) as fixed factor. Fisher's least significant difference (LSD) post hoc test was applied with a significance level of 0.05 to determine differences between treatments. All residuals were tested for normality and homocedasticity (with Shapiro–Wilks and Levene's tests, respectively). The following transformations were applied before analysis: AMF spore number and evenness were rank-transformed. Soil nutrients were compared among the three inoculum types using the non parametric Kruskal–Wallis test. The following Spearman rank correlations with a significance level of 0.05 were performed: root biomass × AMF, root biomass × DSE colonization, DSE colonization × AMF colonization, shoot biomass × shoot P content and root biomass × shoot P content. All statistics were performed using Infostat program.

Results

Soil analysis

Grassland soils had a sandy loam texture and slightly acidic pH. Mature forest inoculum also had a sandy loam texture, with a moderately acidic pH and a higher P concentration (%). Degraded forest inoculum showed a loam texture, with a moderately acidic pH, higher total N content and percentage of organic matter. Nitrate (%) was not different between soil types (Table 1).

Soil AMF community

Shannon biodiversity index, spore evenness and spore abundance were not different between inocula. Spore richness was significantly higher in grassland inoculum (Table 1). Twenty-six AMF morphospecies that belong to eight genera: *Funneliformis*, *Glomus*, *Acaulospora*, *Pacispora*, *Rhizophagus*, *Scutellospora*, *Gigaspora* and *Entrophospora* were identified in the three inocula and 21 were identified to species level. Most frequent morphospecies were *Rhizophagus intraradices* in degraded and mature forest inocula and *Glomus fuegianum* in grassland inoculum. The mature forest soil showed the dominance of morphospecies of Acaulosporaceae and Glomeraceae families. Whereas degraded forest and grassland inocula, also showed morphospecies belonging to Claroideoglomeraceae and Gigasporaceae (Table 2).

Growth parameters and plant P content

After 12 months growing under greenhouse conditions, *P. australis* seedlings achieved a mean height of 22.56 ± 2.94 cm. All seedlings were healthy and no root browning, changes of leaf color, lesions nor wilting were observed. Root biomass was enhanced in plants inoculated with mature forest soil ($F = 10.64$, $P < 0.001$) (Fig. 1a). Shoot biomass did not show differences among inocula ($F = 1.52$, $P = 0.25$), although a tendency of higher shoot biomass could be observed in plants inoculated with mature forest soil in comparison with the other inocula (Fig. 1b).

Table 1 Soil characterization and arbuscular mycorrhizal fungi ecological indexes (Shannon biodiversity index, evenness, richness and abundance) of the three studied inocula (grassland, mature forest and degraded forest)

	Grassland	Mature forest	Degraded forest	Test and P value
Total nitrogen (%)	0.51 (0.03) b	0.56 (0.08)b	0.81 (0.07)a	$H = 8.80$, $P = 0.01$
Nitrate (%)	0.022 (0.0017)a	0.026 (0.00066)a	0.021 (0.0066)a	$H = 4.77$, $P = 0.09$
Phosphorus (%)	0.001 (0.00004)b	0.002 (0.0001)a	0.0004 (0.0002)b	$H = 19.95$, $P < 0.0001$
Organic matter (%)	9.53 (0.61)b	12.62 (1.71)ab	16.68 (1.39)a	$H = 10.95$, $P < 0.01$
pH 1:2.5 (in H_2O)	6.23 (0.04)a	5.9 (0.07)b	5.85 (0.09)b	$H = 11.73$, $P < 0.01$
Shannon biodiversity index	2.28 (0.14)a	2.14 (0.12)a	2.00 (0.20)a	$F = 0.83$, $P = 0.45$
Evenness	0.65 (0.04)a	0.64 (0.04)a	0.66 (0.07)a	$F = 0.34$, $P = 0.72$
Richness	12 (0.73)a	10 (0.55)ab	9 (1.00)b	$F = 3.93$, $P = 0.03$
AMF spore abundance (number of AMF spores/ 100 g of dry soil)	234(67)a	229 (33)a	281 (114)a	$F = 0.27$, $P = 0.77$

Means (standard error) of 10 samples followed by different letters indicate significant differences ($P < 0.05$) among inocula as determined by Kruskal–Wallis test and Tukey HSD test

AMF and DSE colonized all the seedlings analyzed. AMF root colonization varied from 7 to 75 %. AMF vesicles colonization ranged between 2 and 66 % and arbuscules from 0 to 14 %. Plants inoculated with mature forest soil showed marginally significant higher AMF colonization than plants inoculated with grassland soil ($F = 4.17$, $P = 0.06$) (Fig. 1c). DSE colonization ranged from 0.5 to 32 % and was significantly lower in plants inoculated with mature forest soil (Fig. 1d).

Inoculum types did not increase seedlings P content (Fig. 1e). A positive Spearman correlation was found between root biomass × AMF colonization ($r = 0.50$, $P < 0.05$) (Fig. 2a). Negative Spearman correlations were observed between DSE colonization × root biomass ($r = -0.73$, $P < 0.05$) (Fig. 2b), DSE colonization × AMF colonization ($r = -0.59$, $P < 0.01$) (Fig. 2c), plant P content × shoot biomass ($r = -0.49$, $P < 0.05$) (Fig. 2d) and plant P content × root biomass ($r = -0.71$, $P < 0.01$) (Fig. 2e).

Discussion

Different forest management systems have revealed similar AMF spore diversity of rhizospheric soils (e.g., Opik et al. 2008; Menoyo et al. 2009) and in plant roots (Uibopuu et al. 2009). In concordance with these findings, in our study Shannon's diversity index was similar between soils from the three vegetation types. As Opik et al. (2006) evidenced, a higher AMF spore richness was found in the grassland soil since this community shows high plant species-richness (Cingolani et al. 2003) that are highly mycotrophic (Lugo et al.

Table 2 Relative frequencies of arbuscular mycorrhizal fungi morphospecies found in soil inocula from a grassland, a mature and a degraded forest

	Grassland	Mature forest	Degraded forest
Family Acaulosporaceae			
Acaulospora bireticulata Rothwell & Trappe	0.044	0	0
A. foveata Rothwell & Trappe	0	0.015	0.002
A. mellea Spain & Schenck	**0.070**	0.049	0.014
A. rehmii Sieverd. & Toro	0	0.006	0.035
A. scrobiculata Trappe	0.014	**0.063**	**0.186**
A. spinosa Walker & Trappe	0.015	0.010	0.007
Acaulospora sp. 1	0.004	0	0
Acaulospora sp. 2	0.0003	0	0
Acaulospora sp. 3	0	0	0.012
Family Claroideoglomeraceae			
Claroideoglomus claroideum (Schenck & Sm.) Walker & Schüßler	**0.020**	0	0
C. luteum (Kenn. Stutz & Morton) Walker & Schüßler	0.001	0	**0.019**
Family Entrophosporaceae			
Entrophospora infrequens (Hall) Ames & Schneid	0.005	0.003	0.012
Family Glomeraceae			
Funneliformis badium (Oehl, Redecker & Sieverd.) Walker & Schüßler	0.035	0.006	0.014
F. geosporum (Nicolson & Gerd.) Walker & Schüßler	0.069	0	0
F. mosseae (Nicolson & Gerd.) Walker & Schüßler	0.016	0.028	0.017
Glomus brohultii Sieverd. & Herrera	0.049	0.044	0.161
G. fuegianum (Speg.) Trappe & Gerd	**0.413**	0.254	0.063
G. hoi Berch & Trappe	0.028	0.018	0.015
Glomus sp. 1	0.006	0.077	0.020
Glomus sp. 2	0.012	0	0
Rhizophagus clarus (Nicolson & Schenck) Walker & Schüßler	0.014	0.023	0.033
R. intraradices (Schenck & Sm.) Walker & Schüßler	0.129	**0.398**	**0.360**
Family Gigasporaceae			
Gigaspora margarita Becker & Hall	0.006	0	0.002
Gigaspora rosea Nicolson & Schenck	0.010	0	0.002
Scutellospora biornata Sieverd. & Toro	0.014	0	0.002
Family Pacisporaceae			
Pacispora dominikii (Blaszk.) Sieverd. & Oehl	0.024	0	0.012

The highest values are highlighted in bold

2003; Menoyo et al. 2007). Further studies using trap culture experiments will be carried out, to explore if there are AMF morphospecies that did not sporulate at the sampling time but are present in all inoculum soils (Lopes Leal et al. 2009). We also found different AMF family assemblies between inoculum types. Maherali and Klironomos (2007) suggested that competitive exclusion of AMF species phylogenetically over dispersed (from different AMF families), prevents functionally redundant species to co-occur. In addition these communities with less related species appeared to be the best functional assembly for improving plant productivity. Taking into account the evidence of AMF communities'

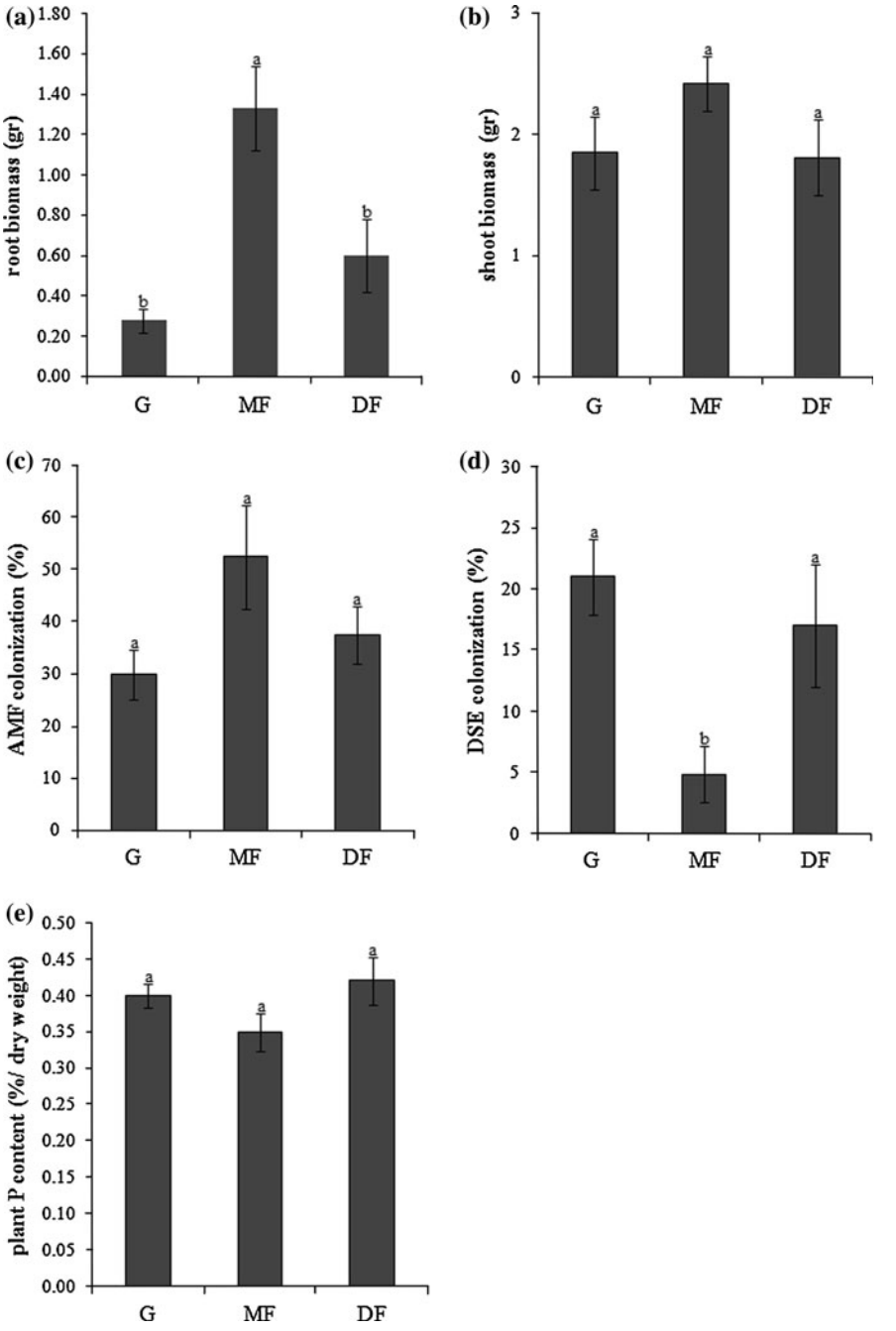

Fig. 1 Mean ± standard error of **a** root biomass, **b** shoot biomass, **c** arbuscular mycorrhizal fungi—AMF—colonization, **d** dark septate endophytes—DSE—colonization and plant P content (percentage/g dry weight) of *P. australis* seedlings inoculated with soil inocula from a grassland (*G*), a mature forest (*MF*) and a degraded forest (*DF*). *Different letters* indicate significant differences ($P < 0.05$) as determined by Fisher LSD test ($n = 6$ replicates)

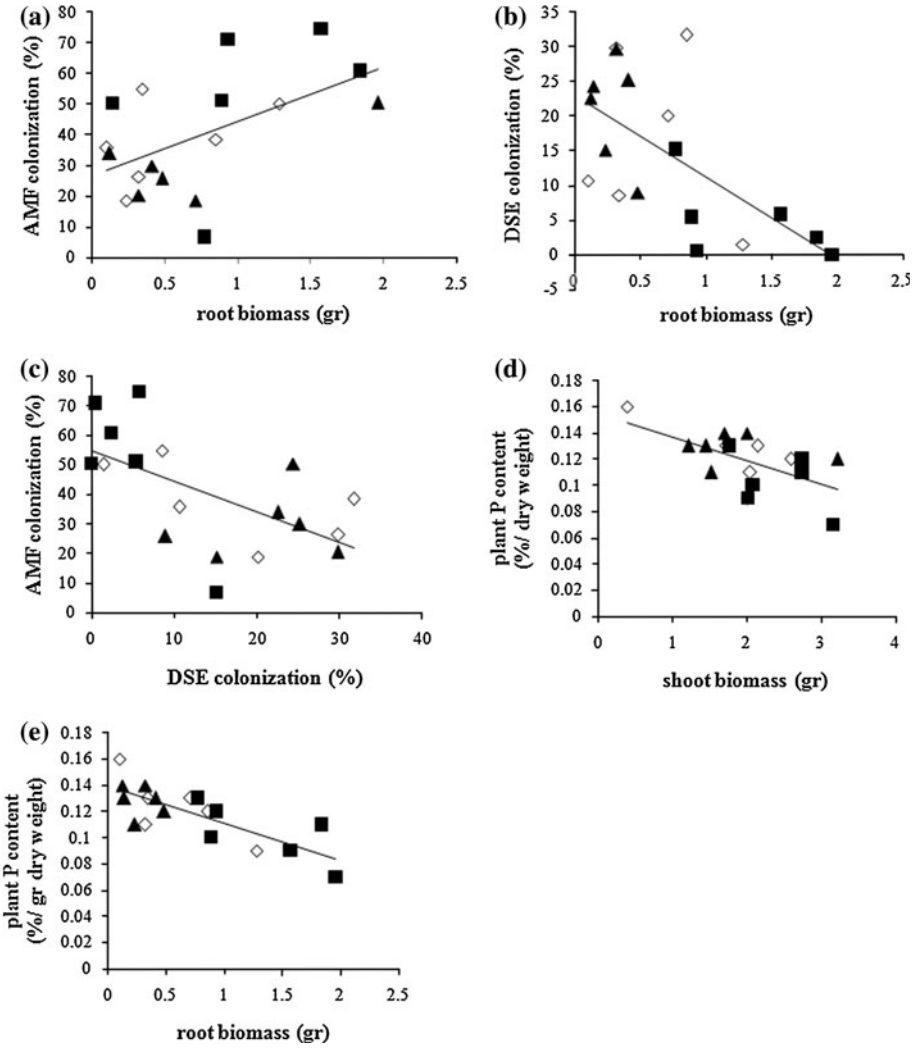

Fig. 2 Relationship between **a** root biomass and arbuscular mycorrhizal fungi—AMF—colonization, **b** root biomass and dark septate endophytes-DSE—colonization, **c** DSE colonization and AMF colonization **d** shoot biomass and plant P content, **e** root biomass and plant P content of *P. australis* seedlings inoculated with grassland (*filled triangle*), mature forest (*filled square*) and degraded forest (*open diamond*) inocula

variation with time (Oehl et al. 2009), and considering that the AMF community was only assessed here at the beginning, it probably could have changed at the end of the experiment.

After 12 months *P. australis* seedlings were healthy and achieved similar height to previous greenhouse and field trials (Renison et al. 2005), showing that no negative effect was exerted by growing in the pots under greenhouse conditions for such a long period. The effect of different natural soil inocula on plant growth have been widely depicted (Allen et al. 2003; Uibopuu et al. 2009; Urgiles et al. 2009; Martino et al. 2011). In this study, the AMF inoculum from the mature forest was the most beneficial for *P. australis* seedlings. It is in concordance with AMF successional models that point out that when late-

successional plants colonize a site, late successional AMF species density increases and colonize these plants as well (Hart et al. 2001). Thus, late-successional trees may find more compatible species of late-successional AMF communities. However, other soil microbes could be together with AMF in the natural inocula, affecting the growth response of *P. australis* seedlings. Thus we cannot be completely sure that the effect of soil inocula is due to AMF communities (van der Heijden et al. 2008).

The association with AMF is one of the strategies by which plants extend their belowground surface for nutrient acquisition (Neumann and Eckhard 2010). In this work, AMF root colonization was positive correlated with root biomass. Besides, the highest root biomass of plants inoculated with mature forest soil could be explained by the dominance of Acaulosporaceae and Glomeraceae families in this inoculum. These families tend to have very delicate and diffuse hyphae which may be less "costly" to its host (Hart and Reader 2002). No differences ($F = 1.52$, $P = 0.25$) were found between the other two inocula (grassland and degraded forest), that showed similar AMF family assemblies. Moreover the Gigasporaceae morphospecies, only present in these soils, may be acting as a carbon drain (Hart and Reader 2002), reducing plant biomass in comparison with seedlings inoculated with mature forest inoculum. Moreover, other parasitic/pathogenic fungi present in the soil inocula could be negatively influencing plant biomass (Maron et al. 2011).

For the response variables considered here, seedlings grown with soil inocula collected from the most degraded natural communities, both grassland and degraded forest, showed similar tendencies and different from seedlings inoculated with mature forest soil. Probably land use history may have produced changes in the structure of the vegetation and thus in soil food webs (Wardle 2006) driving these soil ecosystems to an AMF community which is less favorable for the growth of the late-successional *P. australis* seedlings.

A negative relationship between DSE and seedling root biomass was shown. As reported by other authors, DSE could reduce plants growth (Jumpponen 2001). In addition, the negative correlation between AMF and DSE colonization suggest that these fungi could be competing for resources (Scervino et al. 2009). Many studies have reported this negative relationship but mostly for altitudinal increases and stressful conditions, where DSE are suggested to replace the function of AMF (Haselwandter and Read 1980; Medina-Roldán et al. 2008). This is in accordance with our results, where seedlings grown with the most degraded soils (grassland and degraded forest) showed the lowest AMF colonization and had higher DSE colonization. Nevertheless, more studies are necessary to elucidate the role of these coexisting fungi on *P. australis* performance.

A higher AMF colonization and root biomass of seedlings inoculated with mature forest soil was observed, though inocula did not increase plant P content. This could be explained by a dilution effect of plant biomass on P content, as observed by Allen et al. (2003), and supported by the negative correlation observed between these variables. However, it is widely known that AMF can contribute to P uptake without increasing total plant P content (Smith et al. 2004).

Our study tested the response of *P. australis* seedlings to the inoculation with three natural soils from different vegetation types and yielded different seedling responses, leading to an improvement of reforestation techniques with this species. One of the drawbacks was the methodological limitation of lacking a control treatment to infer the AMF effect, thus future studies should be carried out to specifically test the mycorrhizal response.

Conclusion

Since 1997 *P. australis* reforestation practices have been carrying out to contribute with forests restoration in central Argentina (http://www.reforestacion.com.ar/). Given that livestock browsing impairs seedling growth and survival at restoration fields, the major challenge so far has been to promote seedling growth in order to avoid livestock exclusion for prolonged periods. As a part of this main project, we were interested in evaluating *P. australis* response to three natural soil inocula and thus help to provide larger and healthier seedlings. As was expected, our results showed that soil inocula from communities with different structural complexity could differentially influence seedling performance of *P. australis* which can be considered a late successional species in the area. Particularly, plants inoculated with mature forest soil showed the highest root biomass and AMF colonization, and the lowest DSE colonization. Additional studies inoculating *P. australis* seedlings with DSE strains to test the possible negative associations reported here are necessary. The AMF community found in mature forest inoculum, with the dominance of Acaulosporaceae and Glomeraceae families, seems to be the best functional assembly to promote *P. australis* seedlings performance under greenhouse conditions. Seedling responses to natural soil inoculation should be further studied under natural conditions.

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

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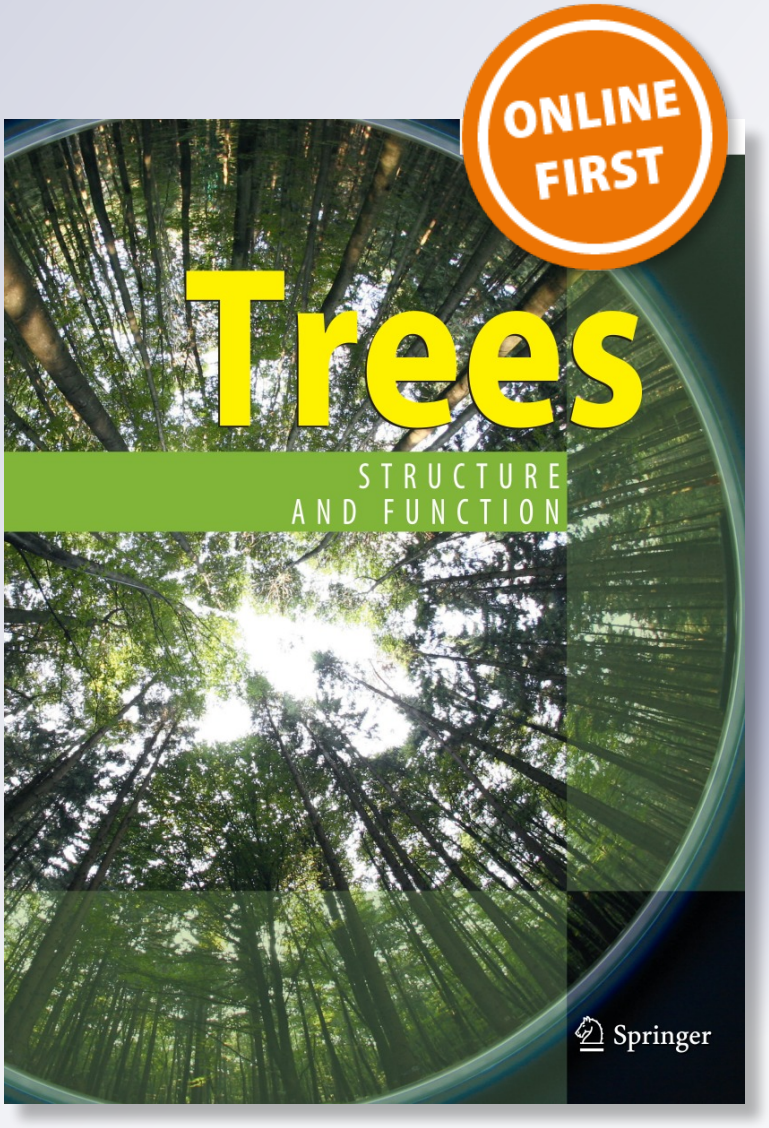

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ORIGINAL PAPER

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

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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

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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

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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).

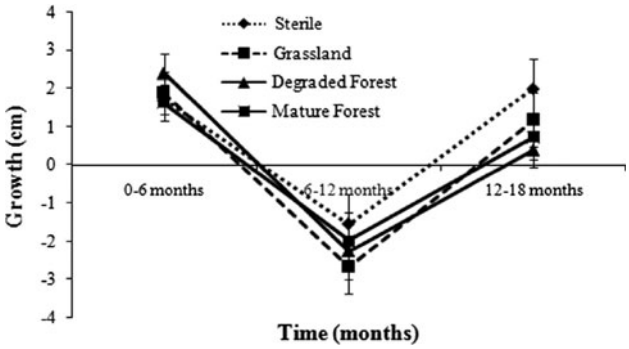

Fig. 1 Difference in height of *Polylepis australis* seedlings between dates (from outplanted date to 6 months, from 6 to 12 months and from 12 to 18 months), grown in sterile soil (initially non-mycorrhizal) or inoculated with grassland, degraded forest or mature forest soils. *Vertical bars* show ±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

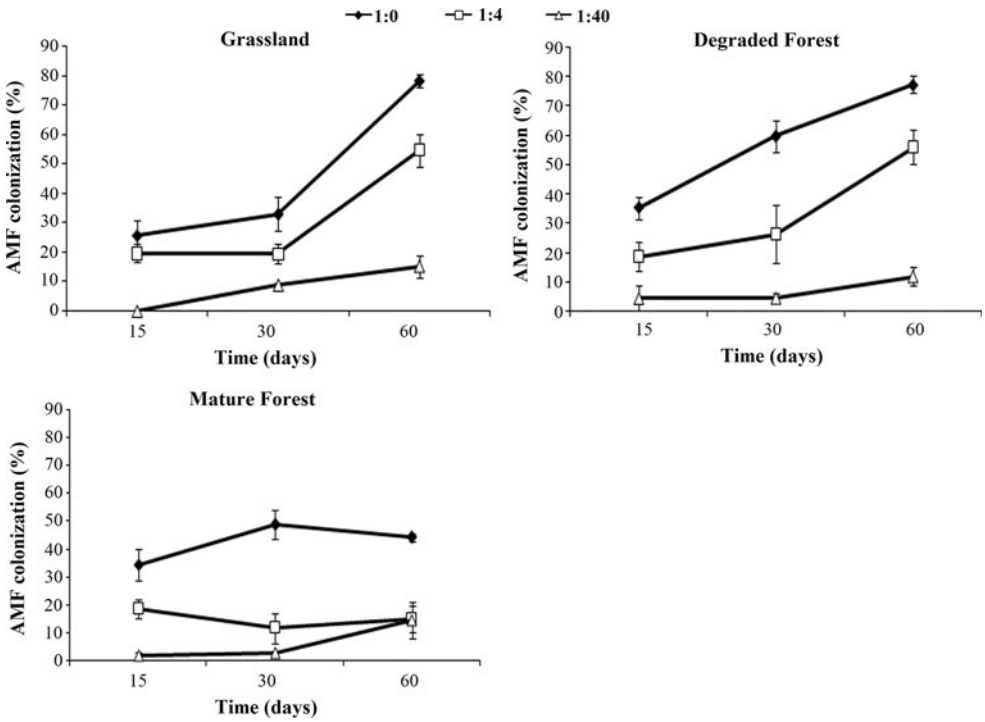

Fig. 2 Arbuscular mycorrhizal fungi (*AMF*) colonization of *Medicago sativa* seedlings grown in the three soil types (grassland, degraded forest and mature forest), in three soil dilutions (1:0, 1:4 and 1:40) during three harvesting dates (15, 30 and 60 days). Values are the mean ± standard error ($n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

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).

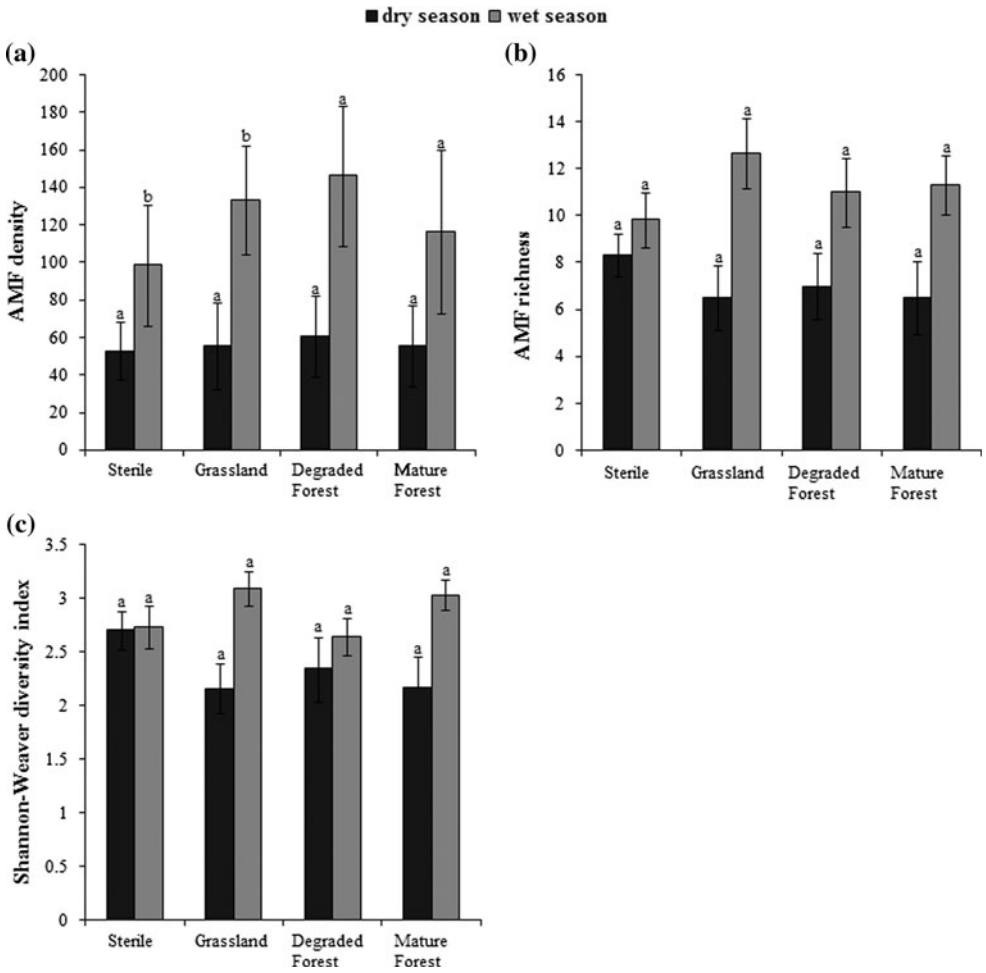

Fig. 3 Mean ± standard error of **a** arbuscular mycorrhizal fungi (*AMF*) density (number of spores/100 g of dry soil), **b** AMF richness, and **c** Shannon diversity index in the rhizosphere of *Polylepis australis* seedlings 6 and 12 months (dry and wet seasons, respectively) after transplanted at 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

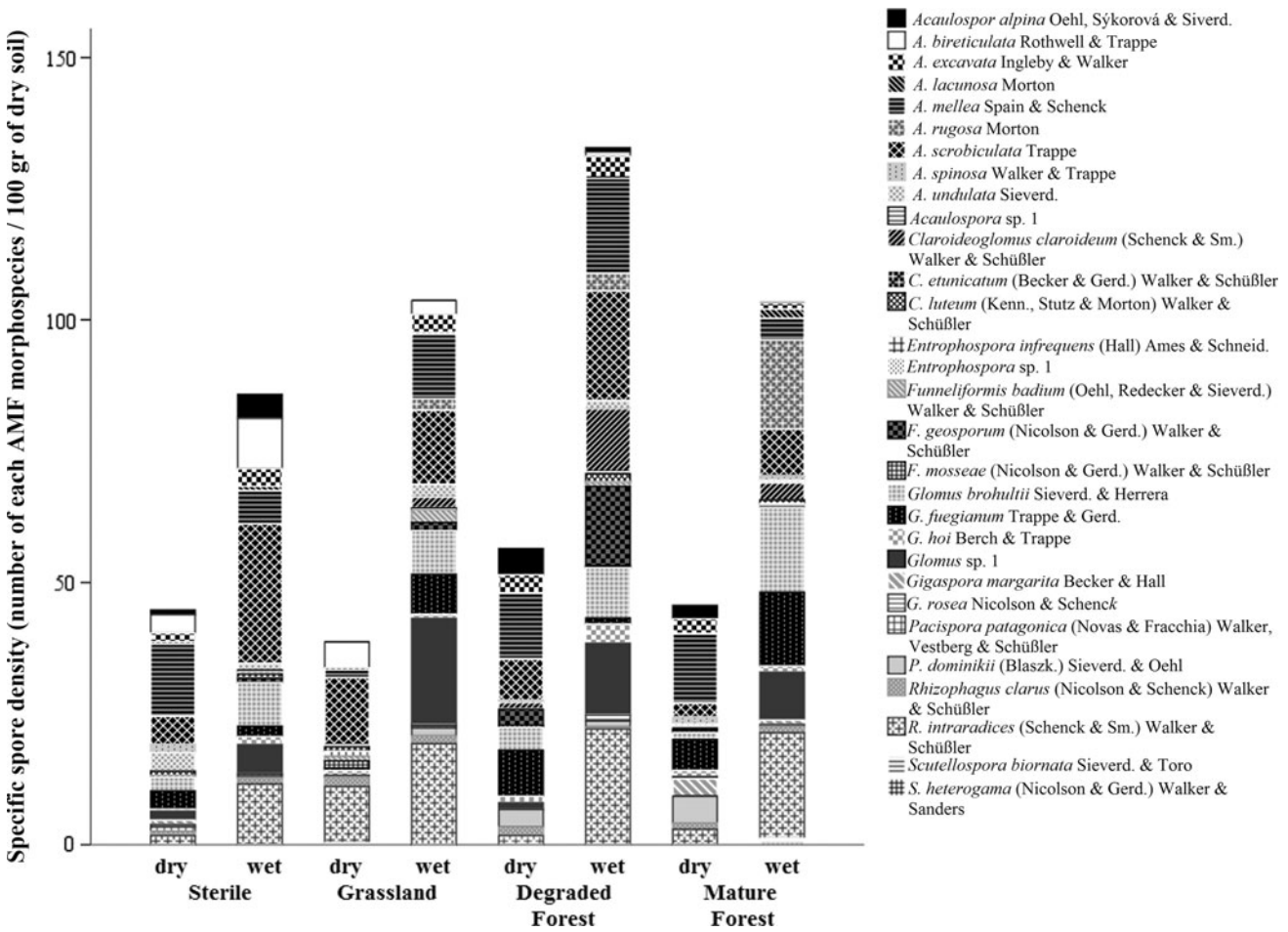

Fig. 4 Specific spore density (number of each morphospecies of arbuscular mycorrhizal fungi (*AMF*)/100 g of dry soil) of the rhizosphere of *Polylepis australis* seedlings 6 and 12 months (dry and wet seasons, respectively) after transplanted at the restoration site, grown in sterile soil (initially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

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