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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'

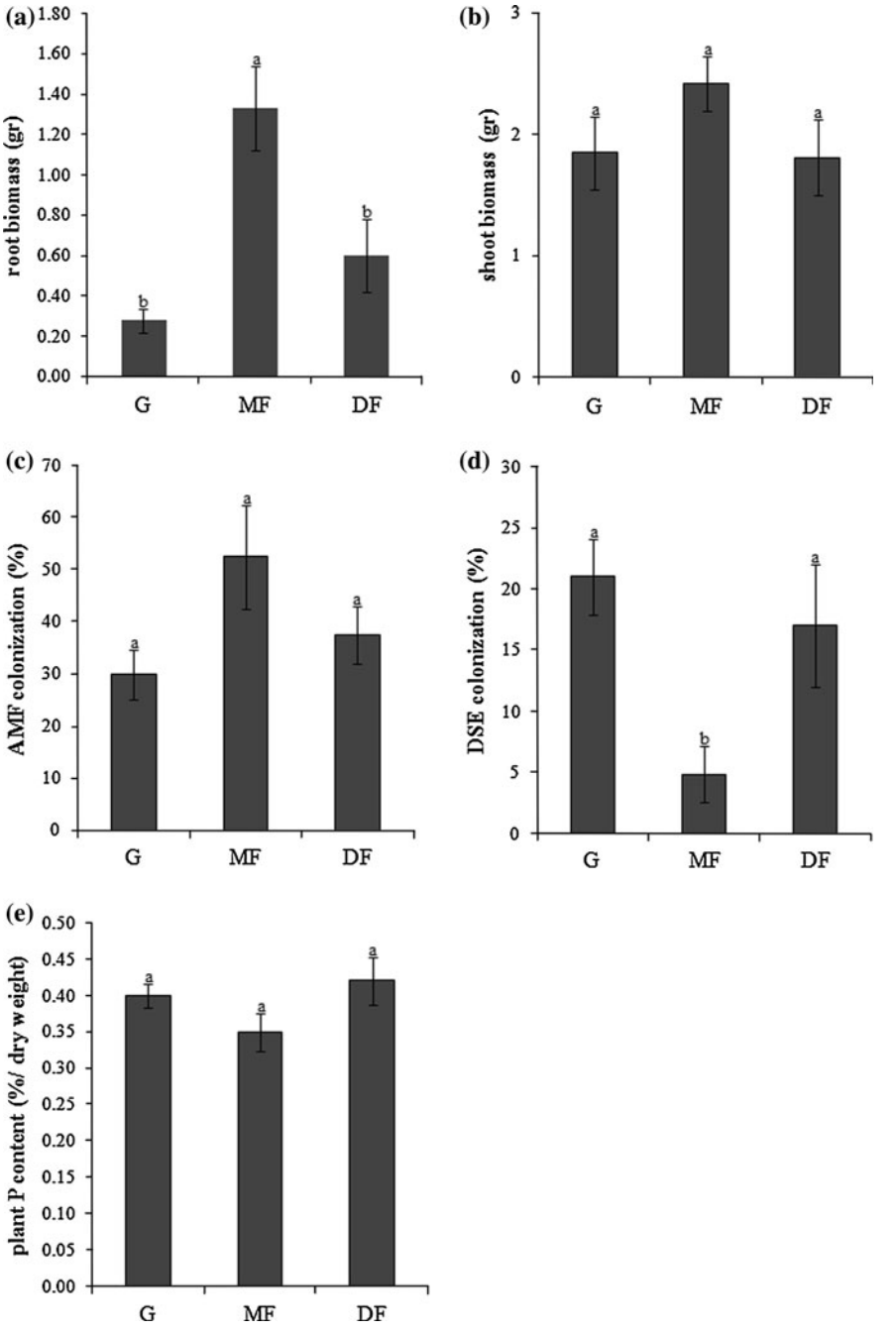

Fig. 1 Mean ± standard error of **a** root biomass, **b** shoot biomass, **c** arbuscular mycorrhizal fungi—AMF—colonization, **d** dark septate endophytes—DSE—colonization and plant P content (percentage/g dry weight) of *P. australis* seedlings inoculated with soil inocula from a grassland (*G*), a 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)

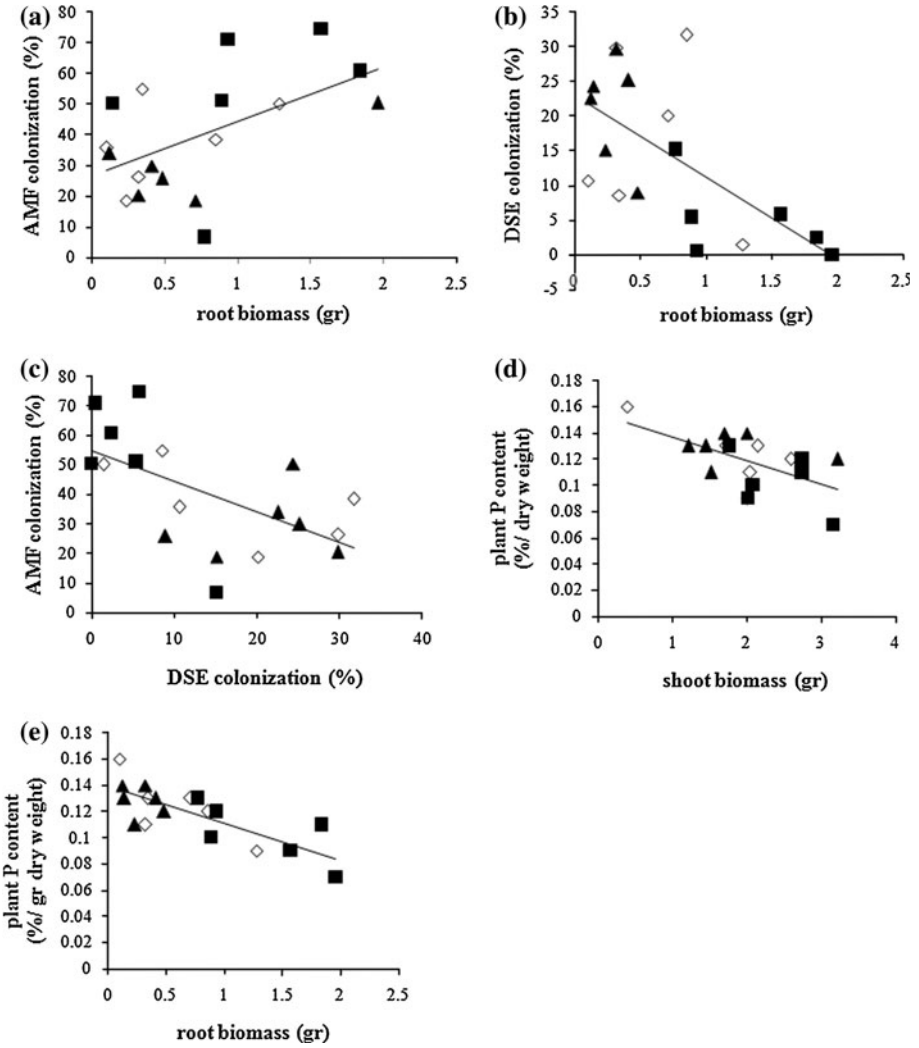

Fig. 2 Relationship between **a** root biomass and arbuscular mycorrhizal fungi—AMF—colonization, **b** root biomass and dark septate endophytes-DSE—colonization, **c** DSE colonization and AMF colonization **d** shoot biomass and plant P content, **e** root biomass and plant P content of *P. australis* 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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