

Provided for non-commercial research and education use.
Not for reproduction, distribution or commercial use.



This article appeared in a journal published by Elsevier. The attached copy is furnished to the author for internal non-commercial research and education use, including for instruction at the authors institution and sharing with colleagues.

Other uses, including reproduction and distribution, or selling or licensing copies, or posting to personal, institutional or third party websites are prohibited.

In most cases authors are permitted to post their version of the article (e.g. in Word or Tex form) to their personal website or institutional repository. Authors requiring further information regarding Elsevier's archiving and manuscript policies are encouraged to visit:

<http://www.elsevier.com/copyright>



Contents lists available at ScienceDirect

Forest Ecology and Management

journal homepage: www.elsevier.com/locate/foreco

Arbuscular mycorrhizas and performance of *Polylepis australis* trees in relation to livestock density

Eugenia Menoyo^{a,*}, Daniel Renison^b, Alejandra G. Becerra^c

^a Cátedra de Diversidad Vegetal I, Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis, Ejército de los Andes 950, 5700 San Luis, Argentina

^b Cátedra de Ecología, F.C.E.F.yN., Universidad Nacional de Córdoba - CONICET, Av. Velez Sarsfield 299, X5000JJC Córdoba, Argentina

^c Instituto Multidisciplinario de Biología Vegetal (CONICET-Universidad Nacional de Córdoba), CC 495, X5000JJC Córdoba, Argentina¹

ARTICLE INFO

Article history:

Received 4 May 2009

Received in revised form 15 September 2009

Accepted 19 September 2009

Keywords:

Polylepis woodlands

Soil degradation

Mycorrhizal colonization

Arbuscular mycorrhizal fungi

ABSTRACT

Polylepis australis trees endemic to Argentina dominate the canopy of subtropical high altitude forests. Here, livestock rearing is the main economic activity and is suspect of the low performance of *P. australis* trees through direct and indirect effects which could include the reduction in arbuscular mycorrhizal fungi (AMF) and their benefit to trees. To elucidate the role of AMF, we compare plant performance indicators, arbuscular mycorrhizal (AM) colonization and AMF communities in 20 trees distributed in two areas of central Argentina which differed in livestock grazing intensity. The area with high livestock density presented more soil degradation and trees with a lower overall plant performance than the area with reduced livestock density. The AM colonization values of *P. australis* were considerably higher than reported for other tree species and the area with high livestock density had a lower proportion of arbuscules and higher proportion of hyphae, while vesicles and AM colonization – all structure considered together – did not differ between areas. Overall AMF spore number and of most species when considered separately was significantly higher in the area with high livestock density, suggesting a high tolerance and adaptation of AMF to livestock. We conclude that a reduction in livestock improves the performance of *P. australis*, that this improvement could be mediated by an increase in the proportion of arbuscules, but there does not appear to be any limitation in AM colonization or AMF spore number which could otherwise be limiting forest restoration.

© 2009 Elsevier B.V. All rights reserved.

1. Introduction

Forests dominated by trees of the genus *Polylepis* are endemic to the mountains of South America, where they occupy the upper forest belts. In many highland areas they are almost the only native tree species, and their forests are especially useful due to their capacity to retain water and reduce soil erosion (Fjeldså and Kessler, 1996). Additionally, *Polylepis* forests harbour an especially large richness of endemic species (Robledo et al., 2006; Cahill and Matthysen, 2007; Lloyd and Marsden, 2008). During centuries of human occupation *Polylepis* forests have been extensively destroyed due to browsing by livestock, human induced fires to promote plant re-growth and logging (Fjeldså and Kessler, 1996; Renison et al., 2006; Cingolani et al., 2008). In particular, livestock rearing is one of the main economic activities in mountain areas around the world and at least in South America there is evidence

these activities are producing irreversible changes due to soil erosion (Cingolani et al., 2008; Renison et al., in press).

During an attempt to restore high mountain forest areas, which are supposed to be dominated by *Polylepis australis* but which at present only have a few isolated individuals due to centuries of high livestock grazing, Renison et al. (2004) found seed viability of the remaining trees to be very low, with several trees in badly degraded areas having zero viability. Under these conditions, natural or assisted regeneration is slow or impossible. Further study revealed negative association between seed viability and indicators of long-term livestock pressure (Renison et al., 2004). This lack of viability could not be attributed to direct browsing effects as studied seeders trees were tall and out of reach by livestock browsing, nor could it be attributed to pollination problems as fragment size is not associated with seed viability (Seltmann et al., 2007). Furthermore, Renison et al. (2005) found *P. australis* seedlings grew extremely little in these areas. As arbuscular mycorrhizas (AM) have been found in *P. australis* from central Argentina (Menoyo et al., 2007) and other South American *Polylepis* species which also have low seed viability and high domestic livestock densities (Hensen, 1994), soil degradation and its negative impact on AM colonization and AMF diversity could be

* Corresponding author. Tel.: +54 2652 422644; fax: +54 2652 422644.

E-mail addresses: emenoyo@gmail.com (E. Menoyo), darenison@yahoo.com.ar (D. Renison), abecerra@efn.uncor.edu (A.G. Becerra).

¹ Institution where the study was carried out.

suspect of the low performance of *Polylepis* species. Studies in other plant species have determined a negative impact on AM colonization and AMF diversity due to soil degradation (Bethlenfalvy et al., 1985; Varma, 1999; Oehl et al., 2003).

The roots of most plants are symbiotically associated with AMF which facilitate nutrient and water uptake, reduce root infections, and improve the capacity of resisting biotic and abiotic stresses (Smith and Read, 1997; Entry et al., 2002). Large native and domestic herbivores, besides having a direct effect on plants through grazing and browsing, may also alter the symbiotic associations and fungal communities through soil compaction, reduction of water holding potential and nutrient movement. As a consequence plant performance may also be altered through these indirect mechanisms (Nadian et al., 1998; Eom et al., 2001; Entry et al., 2002; Jungk, 2002). When attempting to restore plant populations which are susceptible to large herbivores, it is thus important to understand both direct effects on plants and those more indirect effects on symbiotic associations.

Here, we contribute to *Polylepis* forest restoration by comparing plant performance indicators, mycorrhizal colonization and AMF diversity in relation to grazing impact, in two otherwise similar areas but which differed in livestock grazing history. If livestock grazing negatively affects symbiotic associations and plant performance, in areas with reduced livestock density, we expected to find: (1) higher AM root colonization and AMF diversity; and (2) higher *P. australis* performance.

2. Materials and methods

2.1. Study area and field sampling

The mountains of Central Argentina (31°34'S, 64°50'W), rise up to 2884 m a.s.l. Mean temperatures of the coldest and warmest months are 5.0 and 11.4 °C, respectively, with no frost-free period. Mean annual precipitation is 854 mm, with 83% of the rainfall concentrated in the warmer months, between October and April (Renison et al., 2002). We selected two study areas with different grazing intensities. The area with reduced livestock density was selected in "Quebrada del Condorito" National Park (31°37'S; 64°49'W; 2190 m a.s.l.). This area was managed under traditional high stocking rates until 1997 when livestock was completely excluded (6 years before our sampling). In 2001, livestock were re-introduced for conservation purposes at very low densities of 0.08 cattle equivalents/usable hectare (Teich et al., 2005). The area with high livestock density in "Los Gigantes" (31°25'S; 64°47'W; 2140 m a.s.l., Córdoba-Argentina) was chosen due to its physiognomic and topographical similarity to the low grazing intensity area. This area has been managed under traditional livestock densities for centuries, and since at least 1986 it had similar densities as at present (Renison pers. obs.) which has been estimated at 1.68 cattle equivalents/usable hectare (Teich et al., 2005). Both areas had a similar mixture of granite rock outcrops, areas with rock exposed by soil erosion and vegetation patches as observed in situ and in a vegetation map elaborated with Landsat TM images (Cingolani et al., 2004). *P. australis* individuals are isolated or in groups of 2–5 individuals and with a total estimated cover of less than 5% of the area (Cingolani et al., 2004). At each study area, during autumn of 2003 we selected 10 similar *P. australis* trees which were large enough to escape browsing (diameter at breast height—DAP of 26–30 cm). To better attain sampling independence, distance between trees was always larger than 50 m.

2.2. Site characterization and livestock indicators

To characterize the area around the study trees we determined solar exposure, slope, orientation and depth. Additionally, from

each study tree we collected a composite soil sample of 10 subsamples up to a depth 10 cm. After oven-drying for 48 h at 105 °C and removal of coarse soil particles (>2 mm), the samples were subjected to the following chemical analyses: soil pH and electric conductivity were measured in water (20 g soil, 50 ml H₂O) with a standard probe (SenTix 21, WTW); total carbon (corrected for carbonate-born C) and total nitrogen content were analyzed by the Dumas method (CN Analyzer Vario EL, ELEMENTAR); cations were extracted with NH₄Cl solution (0.1 mol/l); Ca²⁺ and Mg²⁺ were analyzed by atomic absorption spectrometry and K⁺ and Na⁺ with flame spectrometry using a Flame-AAS (Vario EL, Analytik Jena); available phosphate was extracted with Ca-lactate at pH 3.6 and measured with a photometer (EPPSTEIN); and soil water content was measured as percent water by weight.

As site indicators of livestock grazing intensity we measured in a 30 m × 30 m area around each tree: (1) dung frequency by randomly placing 50 times a 30 cm × 30 cm square and registering the presence of livestock dung (mainly cattle); (2) soil impedance (kg/cm²) by randomly inserting 10 times a pocket penetrometer (Forestry Suppliers Inc.) up to a depth of 0.7 cm. We considered soil impedance a good short-term grazing indicator as Cingolani et al. (2003) found a strong correlation between soil impedance and dung frequency; (3) proportion of the area covered by grazing lawns, mosses, thin tussocks (*Festuca hieronymi*, *Festuca tucumanica* and *Deyeuxia hieronymi*) and ferns (%). Grazing lawns consist of grasses and forbs maintained short by livestock, mosses cover soils with sparse vegetation due to excessive livestock, while thin tussocks and ferns are typical of areas with low grazing (Cingolani et al., 2003); (4) surface of vertical erosion edges, measured by their length and average height. We considered them a short-term grazing indicator when the erosion surface was active (plant cover < 50%). We considered erosion edges as indicators of grazing because Cingolani et al. (2003) found a strong correlation between dung deposition and the proportion of active soil erosion edges.

2.3. Plant performance

As plant performance indicators we estimated seed germination capacity, crown cover and two indicators of health condition: insect damage and the presence of wood decay and fungal fructifications. *P. australis* seed germination capacity was found to be directly linked to site degradation by livestock (Renison et al., 2004) and is an important barrier to regeneration in many plant species (e.g., Du et al., 2007). Crown cover (often mentioned by its converse: crown transparency) is directly related to photosynthetic capacity and plant performance and has been used as an indicator of tree performance in other studies (e.g., Dobbertin, 2005). Additionally, lack of nutrients or adverse site conditions may predispose trees to predators like insect defoliators or wood decaying fungi which in turn further reduce plant fitness (e.g., McGraw et al., 1990; Flückinger and Braun, 1999; Dobbertin and Brang, 2001; Horsley et al., 2002). In particular, *P. australis* leaves may be eaten by at least 15 species of insects including *Bucculatrix* sp., *Libytheana carinenta*, *Dichroplus* sp. and *Cephalocoema lancea* (Lett, 2006), and their live stems are attacked by at least 8 wood decaying fungi species including *Fomitiporia tabaquilio*, *Bjerkandera adusta*, *Ganoderma adspersum*, *Phellinus uncisetus*, *Inonotus venzuelicus* and *Datronia orcomanta* (Robledo et al., 2006).

To determine seed germination capacity, over 200 seeds per tree were collected in January 2004 when fruit set was at its optimum and stored in paper bags at ambient temperature. In June 2004 we sowed 200 seeds per tree in a greenhouse using sterile soil as in Renison and Cingolani (1998). Germination trays were watered regularly and rotated at random every 4–6 days to reduce biases due to greenhouse position. Seeds were checked daily until

germination was almost zero and a seed was considered as germinated when the root emerged.

Crown cover was visually evaluated as the proportion of the sky which was covered by the studied individual (% cover). Damaged leaves included leaves with signs of being chewed, mined or perforated, and presence of wood decay and polypore fungi fructification was evaluated on both main stems and smaller branches. Each *P. australis* individual was revised for signs of damaged leaves and wood decaying fungi, and assigned a value of presence on a 0–5 or 0–4 scale, respectively. For leaf damage, numbers corresponded to (0) no detected leaf damage; (1) 1–5% of the leaves with signs; (2) 6–10%; (3) 11–15%; (4) 16–20% and (5) >20%. For wood decaying fungi, numbers corresponded to (0) no signs of wood decay or polypore fructifications; (1) only polypore fructifications in thin branches; (2) wood decay detected in an area of less than 200 cm² of the stem surface; (3) wood decay in an area of 200–500 cm² of the stem surface; (4) wood decay over 500 cm² of the stem area.

2.4. Arbuscular mycorrhizas

We sampled soil and roots of each tree at a distance of 15–50 cm from the main stem by extracting a 15 cm × 15 cm soil and root cube to a depth of 25 cm. The samples were placed in plastic bags and stored at 4 °C. The mycorrhizal analysis was determined from fine-root samples. Root samples were washed to remove soil and adhering organic particles and the root system of each plant was preserved with FAA (formalin–acetic acid–ethanol) and later cleared and stained for observation (Grace and Stribley, 1991). Roots were cleared with 10% KOH (15 min at 90 °C). Dark roots were further bleached with 30% H₂O₂ (5 min, room temperature). Roots were then acidified with 1% HCl (1 min, room temperature) and stained in 0.05% aniline blue. Around 30, 1 cm long, root samples from each plant were mounted on slides and viewed under a compound microscope at 400× magnification (Mc Gonigle et al., 1990). The presence of AM fungal structures was scored for 100 intersections of root and reticle line per plant. An intersection was considered mycorrhizal if the reticle intersected an arbuscule, coil, vesicle or internal hypha attached to one of these structures. The root colonization percentages are expressed as colonized intersects/total number of intersects × 100.

We used soil spores to quantify AMF number. The rhizospheric soil from *P. australis* was pooled and mixed for each area. Spores and sporocarps were extracted from 100 g air-dried soil samples in triplicate by wet sieving and decanting (Gerdemann and Nicolson, 1963), followed by centrifugation in water and in 80% sucrose solution (Walker et al., 1982). Sieves (125 and 38 μm) were used to collect the spores. The spores were collected on a grid patterned (1 cm × 1 cm) and only apparently healthy spores were directly counted using a dissecting microscope at 50× magnification. A sporocarp was counted as one unit. For observation and identification of spore characters (color, size and wall structure), spores were mounted on glass slides in polyvinyl alcohol–lactoglycerol (PVLG) and PVLG + Melzer's reagent and then identified to species level using current taxonomic criteria (Schenck and Pérez, 1990; Morton and Redecker, 2001) and information published by INVAM (<http://www.invam.caf.wvu.edu>). Furthermore, to provide fresh spores for positive identification of AMF species present in field soil samples (Stutz and Morton, 1996), trap cultures were used. A fraction of the original soil samples was diluted with sterilized sand (1:1, v:v), transferred to 250 ml plastic pots where *Sorghum vulgare* (Hack.) Haines seeds were sown and kept in a greenhouse (22/18 °C day/night, photoperiod 16/8 h day/night). No fertilizer was used on the trap plants. At the end of the trapping phase, 6 months, the green parts of the plants were cut off and the pots left to dry in the greenhouse. The dry soil was stored at 4 °C

before examination of the fungi. The trap culture substrate was first decanted and then sieved as described above. The number of spores is expressed as the mean of three replicates. Spore number in soil was expressed as number of AMF spores in 100 g dry soil. Voucher mycorrhizas and AMF spores were deposited in the “Museo Botánico de Córdoba”, Herbarium (CORD).

2.5. Data analysis

We compared AM colonization (arbuscules, vesicles or internal hyphae), plant performance (seed germination capacity, crown cover and health condition), site physical–chemical characteristics and livestock grazing indicators, between areas (10 trees per area) using parametric *T*-tests or non-parametric Mann–Whitney *U*-tests in the case of non-normal residuals. Slope orientation was divided into an East–West component with the sine transformation, and a North–South component using the cosine transformation (Renison and Cingolani, 1998). Wilcoxon sign test was used to determine whether overall differences in spore number – paired by species – were significantly different between livestock treatments. All statistics were performed using the Infostat program.

3. Results

3.1. Site characterization and livestock indicators

Our study areas were topographically similar in most measured variables except that trees in the area with reduced livestock density were slightly less exposed to the sun and had more westerly slope orientations than the area with high livestock density (Table 1). Soil characteristics were typical of the study area and did not differ between areas and were relatively acid, with low electric conductivity, high carbon and nitrogen, and moderate phosphorus contents (Table 1). As expected, the area with reduced livestock density had lower dung frequency, soil impedance, proportion of grazing lawns, mosses and active surface of erosion, while proportion of the area covered by thin tussocks and ferns were higher than the area with high livestock grazing intensity (Table 2).

3.2. Plant performance

Average germination rates of *P. australis* seed were low, varying from 0 to 14% (Mean = 3.15%; SE = 0.79; *N* = 20 trees). As expected, germination rates were almost three times higher in the area with reduced livestock density (reduced livestock: Mean = 4.65%, SE = 1.43; high livestock, Mean = 1.65%, SE = 0.33; Wilcoxon test: *W* = 78.5, *N* = 10; *P* = 0.042; Fig. 1). Trees in the area with reduced livestock density had lower insect predation and fungal fructifications than trees in the area with high livestock density (*W* = 137.5, *P* = 0.013 and *W* = 142.5, *P* = 0.002, respectively), while no significant differences were found for crown cover (*T* = –0.97, *P* = 0.34; Fig. 1).

3.3. Arbuscular mycorrhizas

P. australis root colonization percentages varied from 70 to 90%, with a mean of 81% (SE = 1.54; *N* = 20 trees). Root colonization did not differ between areas (*W* = 108, *P* = 0.82); however, there were differences when considering the types of studied mycorrhizal structures separately: the area with reduced livestock density had a higher proportion of arbuscules (*W* = 74, *P* = 0.02) and lower proportion of hyphae (*W* = 139, *P* = 0.01) than the area with high livestock density, while proportion of vesicles did not differ between areas (*W* = 83, *P* = 0.1; Fig. 2).

Table 1

Characterization of our two study areas with reduced and high livestock densities. Values represent mean ± standard error of 10 sample trees. Mann–Whitney *U*- or *T*-test and *P*-test values are indicated.

Characteristic	Reduced livestock density	High livestock density	<i>T</i> -test (<i>T</i>) Mann–Whitney (<i>W</i>)
Solar exposure (°)	83.5 ± 6.19	116.5 ± 8.23	<i>T</i> = 3.20, <i>P</i> = 0.005 [*]
Slope (°)	35 ± 5	44.2 ± 4.84	<i>T</i> = 1.32, <i>P</i> = 0.203
Orientation NS (°)	-0.38 ± 0.13	-0.17 ± 0.19	<i>W</i> = 114.5, <i>P</i> = 0.44
Orientation EW (°)	-0.24 ± 0.27	0.61 ± 0.17	<i>W</i> = 132, <i>P</i> = 0.03 [*]
Soil depth (cm)	42.51 ± 6.86	42.98 ± 6.86	<i>W</i> = 102, <i>P</i> = 0.821
pH 1:2.5 (in H ₂ O)	4.93 ± 0.1	4.93 ± 0.1	<i>T</i> = 0, <i>P</i> = 1
Electric conductivity (dS/m)	0.2 ± 0.03	0.2 ± 0.03	<i>W</i> = 104.5, <i>P</i> = 0.97
Total C (%)	1.27 ± 0.15	1.32 ± 0.2	<i>T</i> = 0.22, <i>P</i> = 0.83
Total N (%)	0.11 ± 0.01	0.12 ± 0.02	<i>T</i> = 0.511, <i>P</i> = 0.61
Ca ²⁺ (cmolc/kg of soil)	10.91 ± 0.96	11.12 ± 1.36	<i>T</i> = 0.126, <i>P</i> = 0.9
Mg ²⁺ (cmolc/kg of soil)	1.48 ± 0.22	1.25 ± 0.18	<i>T</i> = -0.83, <i>P</i> = 0.42
K ⁺ (cmolc/kg of soil)	1.13 ± 0.24	0.9 ± 0.35	<i>T</i> = -0.88, <i>P</i> = 0.39
Na ⁺ (cmolc/kg of soil)	0.83 ± 0.23	0.97 ± 0.15	<i>W</i> = 115, <i>P</i> = 0.45
Available P (ppm)	16.3 ± 4.27	10.17 ± 3.29	<i>W</i> = 89, <i>P</i> = 0.225
Soil water content (%)	39.18 ± 5.67	38.07 ± 5.51	<i>W</i> = 108, <i>P</i> = 0.82

Note: cmolc, centimols of charge. The analyses were as follows: Ca-lactate extract (pH 3.6) for available phosphorus; Dumas method for total C and N; electric conductivity and pH in 1:2.5 suspension of soil in water; cations after equilibrium of soil in 0.1N NH₄Cl; percent water by weight for soil water content.

^{*} *p* < 0.05.

Table 2

Livestock densities indicators in the study areas. Mean ± standard error of 10 samples, Mann–Whitney *U*- or *T*-test values are indicated.

Variables	Reduced livestock density	High livestock density	<i>T</i> -test (<i>T</i>) Mann–Whitney (<i>W</i>)
Dung counts	0.7 ± 0.3	8.4 ± 1.36	<i>W</i> = 155, <i>P</i> = 0.0001 [*]
Soil impedance (kg/cm ²)	0.33 ± 0.03	1.05 ± 0.12	<i>W</i> = 155, <i>P</i> = 0.0002 [*]
Grazing lawns (%)	1.7 ± 1.05	21.2 ± 3.7	<i>W</i> = 152, <i>P</i> = 0.0003 [*]
Mosses (%)	2.6 ± 1.33	10.9 ± 2.43	<i>W</i> = 138, <i>P</i> = 0.01 [*]
Thin tussocks (%)	45.3 ± 7.88	37 ± 5.83	<i>T</i> = -0.85, <i>P</i> = 0.41
Ferns (%)	27.5 ± 3.89	11.2 ± 2.83	<i>T</i> = -3.39, <i>P</i> = 0.003 [*]
Active surface of erosion (m ²)	0.59 ± 0.36	21.1 ± 4.85	<i>W</i> = 152, <i>P</i> = 0.0003 [*]

^{*} *p* < 0.05.

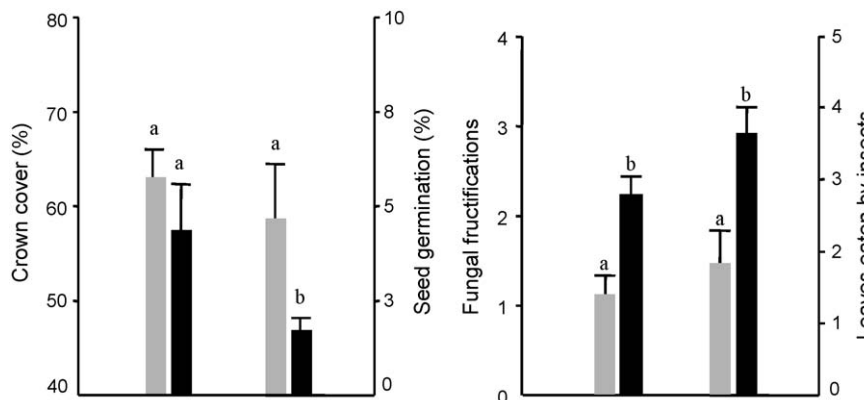


Fig. 1. Indicators of plant performance for an area with reduced livestock density (grey columns) and an area with high livestock grazing (black columns). Mean ± standard error of 10 samples per area is indicated. Different letters indicate significant differences, *p* < 0.05.

Arbuscular mycorrhizal fungi spore number varied from 360 to 825 spores/100 g of soil, with a richness of 20 AMF species and five genera. Three AMF species were not present in the area with high livestock density and another three species were not present in the area with reduced livestock density, but all were rare species so the difference is probably be due to low sample sizes (Table 3). Contrary to expectations, overall AMF spore numbers were significantly higher in the area with high livestock density (Mean = 802.33; SE = 13.59) than in the area with reduced livestock density (Mean = 546.33; SE = 133.87). When we considered spore number by species, 13 AMF species had higher spore numbers in the area with high livestock density while only seven AMF species had similar or lower spore numbers in the area with high livestock density (Sign test: *Z* = -2.65; *P* = 0.008; Table 3).

4. Discussion

As occurs in other environments, in our study area livestock activity initiates soil degradation. Soils are compacted; therefore, water holding capacity and plant growth are reduced (Martínez and Zinck, 2004; Powers et al., 2005). Soil erosion and export of nutrients accelerates with decreased plant cover, with further repercussions on soil productivity (Belsky and Blumenthal, 1997; Cingolani et al., 2003, 2008; Castellano and Valone, 2007). In addition, bare soils, at least under conditions of high solar radiation, dry out more rapidly than soils covered by vegetation. Thus, not surprisingly our area with high livestock density had more signs of soil erosion and higher compaction values than the area with reduced livestock density. In accordance, *P. australis* trees

Table 3
Specific arbuscular mycorrhizal (AM) spores numbers in the study areas. Mean \pm standard error of three samples. For the ease of comparison the highest value for each species is highlighted in bold.

AM species	Reduced livestock density	High livestock density
<i>Acaulospora bireticulata</i> Rothwell & Trappe	3.29 \pm 1.17	11.89 \pm 5.26
<i>A. denticulata</i> Sieverding & Toro	3.84 \pm 1.92	0
<i>A. excavata</i> Ingleby & Walker	38.38 \pm 15.71	125.76 \pm 14.39
<i>A. laevis</i> Gerdemann & Trappe	61.86 \pm 8.5	91.37 \pm 20.70
<i>A. mellea</i> Spain & Schenck	23.48 \pm 5.54	23.32 \pm 9.51
<i>A. scrobiculata</i> Trappe	35.45 \pm 6.21	64.78 \pm 19.89
<i>A. spinosa</i> Walker & Trappe	6.39 \pm 1.21	3.81 \pm 3.81
<i>Acaulospora</i> sp.	2.83 \pm 1.66	19.51 \pm 8.27
<i>A. tuberculata</i> Janos & Trappe	0	3.81 \pm 3.81
<i>Archaeospora leptoticha</i> (Schenck & Smith) Morton & Redecker	7.68 \pm 1.92	0.91 \pm 0.91
<i>Entrophospora infrequens</i> (Hall) Ames & Schneider	2.38 \pm 2.38	3.81 \pm 3.81
<i>Glomus claroidium</i> Schenck & Smith	1.92 \pm 1.92	15.24 \pm 8.31
<i>G. fasciculatum</i> (Thaxter) Gerd. & Trappe emend. Walker & Koske	0.46 \pm 0.46	0.45 \pm 0.45
<i>G. fuegianum</i> (Spegazzini) Trappe & Gerdemann	270.18 \pm 80.05	275.74 \pm 47.21
<i>G. geosporum</i> (Nicol. & Gerd.) Walker	0	13.79 \pm 13.12
<i>G. intraradices</i> Schenck & Smith	81.04 \pm 44.55	125.76 \pm 5.72
<i>G. rubiforme</i> (Gerd. & Trappe) Almeida & Schenck	6.21 \pm 5.54	15.70 \pm 15.02
<i>Glomus</i> sp. 1	0	7.62 \pm 3.81
<i>Glomus</i> sp. 2	0.46 \pm 0.46	0
<i>Scutellospora biornata</i> Spain, Sieverding & Toro	0.46 \pm 0.46	0

did not perform as well, as has been found in other study areas (Renison et al., 2004, 2005). This negative effect on performance due to soil degradation could be explained by a restriction in the function and development of roots under soil compaction (Bengough and Mullins, 1990; Nadian et al., 1998). Roots in degraded soils could have difficulties in absorbing nutrients with low mobility like phosphorus, which together with a diminished availability of water could be reducing plant performance (Nadian et al., 1996, 1998).

The AM colonization values of *P. australis* were considerably higher than reported for other trees (Onguene and Kuyper, 2001; Tawaraya et al., 2003), suggesting that they could have an important role in *P. australis* nutrition. Livestock reduction and consequent soil improvement did not appear to affect total AM colonization values, however, there were differences when considering the types of studied mycorrhizal structures separately.

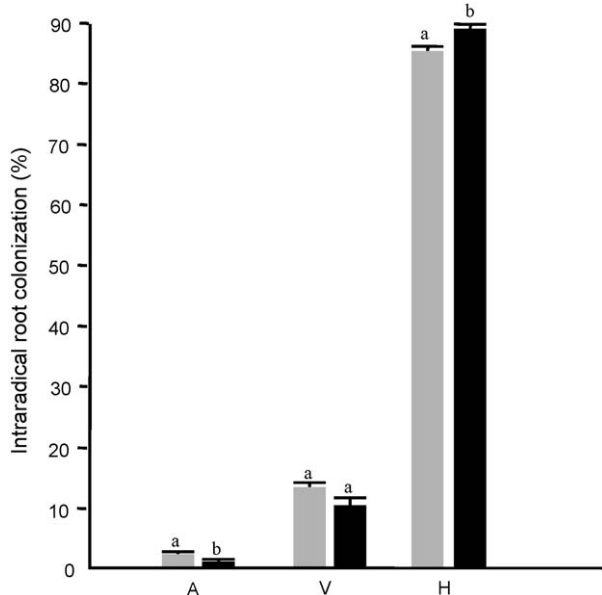


Fig. 2. The proportion of arbuscules (%A), vesicles (%V) and internal hyphal (%H) root colonization in an area with reduced (grey columns) and high livestock density (black columns). Mean \pm standard error of 10 samples are indicated. Different letters indicate significant differences, $p < 0.05$.

The proportion of arbuscules was notably reduced in the site with high livestock density; the different responses between total AM colonization and a type of mycorrhizal structure has been reported in other studies (Nadian et al., 1997; Grigera and Oesterheld, 2004; Klironomos et al., 2004). In particular, arbuscules are sensitive to oxygen contents of soils (Saif, 1981, 1983), and soil compaction reduces oxygen availability (Nadian et al., 1998; Arvidsson, 1999). Arbuscular mycorrhiza may diminish the stressful effects of soil compaction on plant growth through enhancing nutrient uptake (Nadian et al., 1998; Miransari et al., 2009). As arbuscules are the principal unit of nutrient exchange between the plant and AMF, the reduced number of arbuscules could be contributing to a reduced nutrient uptake by the plant.

Contrary to our expectations under a scenario of negative grazing effects, arbuscular mycorrhizal fungal spore number was increased in the site with high livestock density. In our study area livestock exclusion reduces plant species richness at a local scale due to the proliferation of a few tussock species which in the past were controlled by native camelids now replaced in their function by domestic livestock (Cingolani et al., 2003). This variation in host plants may have influenced AMF spore numbers, as occurs in other ecosystems (Bethlenfalvay and Dakessian, 1984; Jasper et al., 1991; Bever et al., 1996; Korb et al., 2003; Zhang et al., 2004). Also, many studies found AMF species could be tolerant to livestock (Gehring and Whitman, 1994; Cuenca et al., 1998; Carpenter et al., 2001; Oehl et al., 2003; Zhang et al., 2004). Therefore, the result we obtained could be due to a tolerance of AMF to conditions present under high livestock density combined with an adaptation of the AMF community to the enhanced plant richness found in areas with livestock.

Our main conclusion is that a reduction in livestock grazing intensity appears to improve the performance of *P. australis* trees and that this improvement could be mediated by an increase in arbuscule colonization. Furthermore, there does not appear to be any limitation in AM colonization or AMF spore abundance or richness in the area with high livestock density as compared to the area with reduced livestock, implying that lack of AM spore in degraded areas is not the main limiting factor in *P. australis* poor performance.

We chose our study areas for them to be representative of the Córdoba mountains, notwithstanding this is a subjective statement, thus our study has all the limitations associated to the comparison of only two study areas, and the inference is restricted

to these two areas, and to a lesser extent to similar situations within the Córdoba Mountains or *Polylepis* forests in other regions. We thus suggest further research including more replicates, and more extreme situations with pristine forests and complete forest disappearance.

Acknowledgements

This work was supported by a Rufford Small Grant, Secretaría de Ciencia y Técnica – Universidad Nacional de Córdoba and Volkswagen Foundation Germany. The National Parks authorities provided permits. We thank D. Devin for assisting in the field and greenhouse surveys and R. Suarez for his assistance in the soil analysis. E.M. is grateful to CONICET for the provided fellowship, while A.B. and D.R. are researchers of CONICET.

References

- Arvidsson, J., 1999. Nutrient uptake and growth of barley as affected by soil compaction. *Plant and Soil* 208, 9–19.
- Bengough, A.G., Mullins, C.E., 1990. Mechanical impedance to root growth: a review of experimental techniques and root growth responses. *European Journal of Soil Science* 41, 341–358.
- Belsky, J.A., Blumenthal, D.M., 1997. Effects of livestock grazing on stand dynamics and soils in upland forests of the interior west. *Conservation Biology* 11, 315–327.
- Bethlenfalvay, G.J., Dakessian, S., 1984. Grazing effects on mycorrhizal colonization and floristic composition of the vegetation on a semiarid range in Northern Nevada. *Journal of Range Management* 37, 312–316.
- Bethlenfalvay, G.J., Evans, R.A., Lesperance, A.L., 1985. Mycorrhizal colonization of created wheatgrass as influenced by grazing. *Agronomy Journal* 77, 233–236.
- Bever, J.D., Morton, J.B., Antonovics, J., Schultz, P.A., 1996. Host-dependent sporulation and species diversity of arbuscular mycorrhizal fungi in a mown grassland. *Journal of Ecology* 84, 71–82.
- Carpenter, F., Palacios Mayorga, L., Gonzalez Quinteros, S., Schroeder, E.M., 2001. Land-use and erosion of a Costa Rica Ultisol affect soil chemistry, mycorrhizal fungi an early regeneration. *Forest Ecology and Management* 144, 1–17.
- Cahill, J.R.A., Matthysen, E., 2007. Habitat use by two specialist birds in high-Andean *Polylepis* forests. *Biological Conservation* 40, 62–69.
- Castellano, M.J., Valone, T.J., 2007. Livestock, soil compaction and water infiltration rate: evaluating a potential desertification recovery mechanism. *Journal of Arid Environments* 71, 97–108.
- Cingolani, A.M., Cabido, M.R., Renison, D., Solis Neffa, V., 2003. Combined effects of environment and grazing on vegetation structure in Argentina granite grasslands. *Journal of Vegetation Science* 14, 223–232.
- Cingolani, A., Renison, D., Tecco, P., Gurvich, D., Cabido, M., 2008. Predicting cover types in a mountain range with long evolutionary grazing history: a GIS approach. *Journal of Biogeography* 35, 538–551.
- Cingolani, A.M., Renison, D., Zak, M.R., Cabido, M.R., 2004. Mapping vegetation in a heterogeneous mountain rangeland using Landsat data: an alternative method to define and classify land-cover units. *Remote Sensing of Environment* 92, 84–97.
- Cuenca, G., De Andrade, Z., Escalante, G., 1998. Diversity of glomalean spores from natural, disturbed and revegetated communities growing on nutrient-poor tropical soils. *Soil Biology and Biochemistry* 30, 711–719.
- Dobbertin, M., Brang, P., 2001. Crown defoliation improves tree mortality models. *Forest Ecology and Management* 141, 271–284.
- Dobbertin, M., 2005. Tree growth as indicator of tree vitality and of tree reaction to environmental stress: a review. *European Journal of Forest Research* 124, 319–333.
- Du, X., Guo, Q., Ma, K., 2007. Seed rain, soil seed bank, seed loss and regeneration of *Castanopsis fargesii* (Fagaceae) in a subtropical evergreen broad-leaved forest. *Forest Ecology and Management* 238, 212–219.
- Entry, J.A., Rygielwicz, P.T., Watrud, L.S., Donnelly, P.K., 2002. Influence of adverse soil conditions on formation and function of Arbuscular mycorrhizas. *Advances in Environmental Research* 7, 123–138.
- Eom, A., Wilson, G.W.T., Hartnett, D.C., 2001. Effects of ungulate grazers on arbuscular mycorrhizal symbiosis and fungal community structure in tallgrass prairie. *Mycologia* 93, 233–242.
- Fjeldsá, 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. Nordco, Copenhagen, Denmark, p. 250.
- Flückinger, W., Braun, S., 1999. Stress factors of urban trees and their relevance for vigour and predisposition for parasite attacks. *Acta Horticulturae* 496, 325–334.
- Gehring, C.A., Whitman, T.G., 1994. Interactions between above ground herbivores and the mycorrhizal mutualists of plants. *Tree* 9, 251–255.
- Gerdemann, J.W., Nicolson, T.H., 1963. Spores of a mycorrhizal *Endogone* species extracted from the soil by wet sieving and decanting. *Transactions British Mycological Society* 46, 235–244.
- Grace, C., Stribley, D.P., 1991. A safer procedure for root staining of vesicular arbuscular mycorrhizal fungi. *Mycological Research* 95, 1160–1162.
- Grigera, G., Oesterheld, M., 2004. Mycorrhizal colonization patterns under contrasting grazing and topographic conditions in the flooding pampa (Argentina). *Journal of Range Management* 57, 601–605.
- Hensen, I., 1994. Estudios ecológicos y fenológicos sobre *Polylepis bessi* Hieron. en la Cordillera Oriental Boliviana. *Ecología en Bolivia* 23, 21–32.
- Horsley, S.B., Long, R.P., Bailey, S.W., Hallett, R.A., Wargo, P.M., 2002. Health of Eastern North American sugar maple forests and factors affecting decline. *Northern Journal of Applied Forestry* 19, 34–44.
- Jasper, D.A., Abbott, L.K., Robson, A.D., 1991. The effect of soil disturbance on vesicular-arbuscular mycorrhizal fungi in soils from different vegetation types. *New Phytologist* 118, 471–476.
- Jungk, A.O., 2002. Dynamics of nutrient movement at the soil–root interface. In: Waisel, Y., Eshel, A., Kafkafi, U. (Eds.), *Plant Roots. The Hidden Half*. 3rd ed. Marcel Dekker, Inc., New York, Basel, p. 1120.
- Klironomos, J.N., Mc Cune, J., Moutoglou, P., 2004. Species of arbuscular mycorrhizal fungi affect mycorrhizal responses to simulated herbivory. *Applied Soil Ecology* 26, 133–141.
- Korb, J.E., Johnson, N.C., Covington, W.W., 2003. Arbuscular mycorrhizal propagule densities respond rapidly to ponderosa pine restoration treatments. *Journal of Applied Ecology* 40, 101–110.
- Lett, I., 2006. Efectos de la fragmentación del bosque de *Polylepis australis* Bitt. sobre la herbivoría causada por insectos y su incidencia en la viabilidad de las semillas. Graduation thesis. Universidad Nacional de Córdoba, Argentina, p. 41.
- Lloyd, H., Marsden, S.J., 2008. Bird community variation across *Polylepis* woodland fragments and matrix habitats: implications for biodiversity conservation within a high Andean landscape. *Biodiversity and Conservation* 17, 2645–2660.
- Martínez, L.J., Zinck, J.A., 2004. Temporal variation of soil compaction and deterioration of soil quality in pasture areas of Colombian Amazonia. *Soil and Tillage Research* 75, 3–17.
- Miransari, M., Bahrami, H.A., Rejali, F., Malakouti, M.J., 2009. Effects of soil compaction and arbuscular mycorrhiza on corn (*Zea mays* L.) nutrient uptake. *Soil and Tillage Research* 103, 282–290.
- Mc Gonigle, T.P., Miller, M.H., Evans, D.G., Fairchild, G.L., Swan, J.A., 1990. A method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. *New Phytologist* 115, 495–501.
- Menoyo, E., Becerra, A.G., Renison, D., 2007. Mycorrhizal associations in *Polylepis* woodlands of Central Argentina. *Canadian Journal of Botany* 85, 526–531.
- Morton, J.B., Redecker, D., 2001. Two new families of Glomales, Archaeosporaceae and Paraglomaceae, with two new genera *Archaeospora* and *Paraglomus*, based on concordant molecular and morphological characters. *Mycologia* 93, 181–195.
- McGraw, J.B., Gottschalk, K.W., Vavrek, M.C., Chester, A.L., 1990. Interactive effects of resource availabilities and defoliation on photosynthesis, growth, and mortality of red oak seedlings. *Tree Physiology* 7, 247–254.
- Nadian, H., Smith, S.E., Alston, A.M., Murray, R.S., 1996. The effects of soil compaction on growth and P uptake by *Trifolium subterraneum*: interactions with mycorrhizal colonisation. *Plant and Soil* 182, 39–49.
- Nadian, H., Smith, S.E., Alston, A.M., Murray, R.S., Siebert, B., 1998. Effects of soil compaction on phosphorus uptake and growth of *Trifolium subterraneum* colonized by four species of vesicular-arbuscular mycorrhizal fungi. *New Phytologist* 139, 155–165.
- Nadian, H., Smith, S.E., Alston, A.M., Murray, R.S., 1997. Effects of soil compaction on plant growth, phosphorus uptake and morphological characteristics of vesicular-arbuscular mycorrhizal colonization of *Trifolium subterraneum*. *New Phytologist* 135, 303–311.
- Oehl, F., Sieverding, E., Ineichen, K., Mäder, P., Boller, T., Wiemken, A., 2003. Impact of land use intensity on the species diversity of arbuscular mycorrhizal fungi in agroecosystems of central Europe. *Applied and Environmental Microbiology* 69, 2816–2824.
- Onguene, N.A., Kuyper, T.W., 2001. Mycorrhizal associations in the rain forest of South Cameroon. *Forest Ecology and Management* 140, 277–287.
- Powers, R.F., Scott, D.A., Sanchez, F.G., Voldseth, R.A., Page-Dumroese, D., Elio, J.D., Stone, D.M., 2005. The North American long-term soil productivity experiment: findings from the first decade of research. *Forest Ecology and Management* 220, 31–50.
- Renison, D., Cingolani, A.M., 1998. Experiencias en germinación y reproducción vegetativa aplicada a la reforestación con *Polylepis australis* (Rosaceae) en las Sierras Grandes de Córdoba, Argentina. *Agriscientia* 15, 47–53.
- Renison, D., Cingolani, A.M., 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, A.M., Suarez, R., Menoyo, E., Coutsiere, C., Sobral, A., Hensen, I., 2005. The restoration of degraded mountain forests: effects of seed provenance and microsite characteristics on *Polylepis australis* seedling survival and growth in Central Argentina. *Restoration Ecology* 13, 129–137.
- Renison, D., Hensen, I., Cingolani, A.M., 2004. Anthropogenic soil degradation affects seed viability in *Polylepis australis* mountain forests of central Argentina. *Forest Ecology and Management* 196, 327–333.
- Renison, D., Hensen, I., Suarez, R., Cingolani, A.M., 2006. Cover and growth habit of *Polylepis* woodlands and shrublands in the mountains of central Argentina: human or environmental influence? *Journal of Biogeography* 33, 876–887.
- Renison, D., Hensen, I., Suarez, R., Cingolani, A.M., Marcora, P., Giorgis, M.A., in press. Soil conservation in *Polylepis* mountain forests of Central Argentina: is livestock reducing our natural capital? *Australian Ecology*.

- Robledo, G., Urcelay, C., Domínguez, L., Rajchenberg, M., 2006. Taxonomy, ecology and biogeography of polypores (Basidiomycetes) from Argentinian *Polylepis* woodlands. *Canadian Journal of Botany* 84, 1561–1572.
- Saif, S.R., 1981. The influence of soil aeration on the efficiency of vesicular-arbuscular mycorrhizae. I. Effect of soil oxygen on the growth and mineral uptake of *Eupatorium odoratum* L. inoculated with *Glomus macrocarpus*. *New Phytologist* 88, 649–659.
- Saif, S.R., 1983. The influence of soil aeration on the efficiency of vesicular-arbuscular mycorrhizas. II. Effect of soil oxygen on growth and mineral uptake in *Eupatorium odoratum* L., *Sorghum bicolor* (L.) Moench and *Guizotia abyssinica* (L. f.) Cass. inoculated with vesicular-arbuscular mycorrhizal fungi. *New Phytologist* 95, 405–417.
- Schenck, N.C., Pérez, Y., 1990. Manual for the Identification of Mycorrhizal Fungi. Synergistic Publications, Gainesville, p. 286.
- Seltmann, P., Renison, D., Cocucci, A., Hensen, I., Jung, K., 2007. Fragment size, pollination efficiency and reproductive success in natural populations of wind-pollinated *Polylepis australis* (Rosaceae) trees. *Flora* 202, 547–554.
- Smith, S.E., Read, D.J., 1997. Mycorrhizal Symbiosis, 2nd ed. Academic Press, London, p. 605.
- Stutz, J., Morton, J.B., 1996. Successive pot cultures reveal high species richness of arbuscular endomycorrhizal fungi in arid ecosystems. *Canadian Journal of Botany* 74, 1883–1889.
- Tawaray, K., Takaya, Y., Turjaman, M., Tuah, S.J., Limin, S.H., Tamai, Y., Cha, J.Y., Wagatsuma, T., Osaki, M., 2003. Arbuscular mycorrhizal colonization of tree species grown in peat swamp forests of Central Kalimantan, Indonesia. *Forest Ecology and Management* 182, 381–386.
- Teich, I., Cingolani, A.M., Renison, D., Hensen, I., Giorgis, M.A., 2005. Do domestic herbivores retard *Polylepis australis* Bitt. woodland recovery in the mountains of Córdoba, Argentina? *Forest Ecology and Management* 219, 229–241.
- Varma, A., 1999. Functions and application of arbuscular mycorrhizal fungi in arid and semi-arid soils. In: Varma, A., Hock, B. (Eds.), *Mycorrhiza*. 2nd ed. Springer-Verlag, Berlin, Heidelberg, p. 704.
- Walker, C., Mize, W., McNabb, H.S., 1982. Populations of endogonaceous fungi at two populations in central Iowa. *Canadian Journal of Botany* 60, 2518–2529.
- Zhang, Y., Guo, L.D., Liu, R.J., 2004. Survey of arbuscular mycorrhizal fungi in deforested and natural forest land in the subtropical region of Dujiangyan, southwest China. *Plant and Soil* 261, 257–263.