Root traits and arbuscular mycorrhiza on perennial grasses exposed to defoliation after a controlled burning

Atributos radicales y micorrizas arbusculares en gramíneas perennes expuestas a defoliación luego de una quema controlada

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Abstract. Plant competition for soil resources is common in semiarid rangelands. Plants have various, alternative mechanisms to deal with soil resource acquisition. They include (1) length, weight and proliferation of roots, (2) root length density, and (3) root colonization by arbuscular mycorrhiza. In rangelands of Argentina, plants are exposed to herbivory after natural fires. As a result, knowledge on how these disturbances impact root traits is important for outlining guidelines focused on rangeland management and improvement. Our aim was to analyze the effects of defoliation after a controlled burning on root traits (1) to (3) on two preferred (*Poa ligularis* and *Nassella tenuis*) and one unpreferred (*Amelichloa ambigua*) perennial grasses. Applied treatments did not affect neither root length nor percentage colonization by arbucular mycorrhiza. The smaller diameter and greater root length found in *P. ligularis* might contribute to explain its lower root mycorrhizal colonization in comparison to *A. ambigua* and *N. tenuis*, respectively. The greatest root length and weight, on *P. ligularis* will contribute to explain the already known greater competitive ability in this than in the other two study species. Our results suggest that defoliating *P. ligularis* after fire would not compromise its competitive ability, thus contributing to rangeland management.

Keywords: Fire; Defoliation; Root; Mycorrhizae; Rangelands.

Resumen. En pastizales semiáridos, la competencia por los recursos subterráneos es una de las principales formas de competencia entre plantas. Las plantas poseen varios mecanismos alternativos para la adquisición de los recursos del suelo. Éstos incluyen (1) la longitud, peso y proliferación radical, (2) la densidad de longitud de raíces y (3) la colonización del sistema radical por hongos micorrízicos arbusculares. En los pastizales de Argentina las plantas están expuestas a herbivoría luego de fuegos naturales. En consecuencia, resulta crucial conocer cómo impactan dichos disturbios sobre los atributos radicales, con el objeto de emplearlos como herramientas de manejo para hacer un uso sustentable de estos ecosistemas. El objetivo del estudio fue analizar el efecto de la defoliación luego de una quema controlada sobre los parámetros subterráneos mencionados (1 a 3), en dos especies deseables (*Poa ligularis* y *Nassella tenuis*) y una indeseable (*Amelichloa ambigua*). Los tratamientos aplicados no afectaron la longitud radical ni la colonización por hongos micorrízicos arbusculares. El menor diámetro y la mayor longitud radical de *P. ligularis*, contribuyeron a explicar la menor colonización radical de esta especie respecto de *A. ambigua* y *N. tenuis*, respectivamente. Los resultados encontrados sugieren que la defoliación de *P. ligularis* luego de una quema controlada no comprometería la habilidad competitiva de esta especie, contribuyendo al mejoramiento de los pastizales.

Palabras clave: Fuego; Defoliación; Raíces; Micorrizas; Pastizales naturales.

INTRODUCTION

Many of plant species survival strategies in arid and semi-arid rangelands, where water and nutrients are limiting, depend on their radical systems (Distel & Fernández, 1988). Root proliferation, root length density (RLD) and association with mychorrhizal-forming fungi are often alternative strategies for plant nutrient acquisition (Kothari et al., 1990; Eissenstat & Volder, 2005). Koide and Li (1991) reported that species with less RLD might be more dependent on mycorrhizal colonization for nutrient acquisition than species with greater root length densities. In addition, higher RLD and vesicular-arbuscular mycorrhizae (VAM) have been associated with greater nutrient acquisition in perennial grasses, thus contributing to competitive ability (Mommer et al., 2011).

Rangelands in northeastern Patagonia, Argentina, are often exposed to various, interacting biotic (e.g., grazing) and abiotic (e.g., fire) factors, causing vegetation defoliation (Fernández & Busso, 1999). Factors that reduce photosynthetic tissue availability will also reduce soil resource acquisition mechanisms because these are dependent upon plant C fixation (Briske & Richards, 1995). The role of grazing by domestic livestock after fire must be considered when evaluating strategies that contribute to competitive ability of rangeland vegetation. However, the effects of post-fire grazing on mechanisms of soil resource acquisition have scarcely been studied.

The net effect of grazing after fire on root length may not be equivalent to the summation of both disturbances on that plant trait when they occur separately. Johnson and Matchett (2001) found that fire stimulates root growth, while defoliation has the opposite effect. These authors reported that the combination of both disturbances resulted in an exponential decrease of root length with increasing soil depth.

Another factor that can affect root growth are climatic conditions (Distel & Fernández, 1988). During wet years, it most likely that a great root growth is not produced, since water and nutrient needs by the plant can be satisfied by a modest radical system. However, a greater root system is needed during dry years, but the amount of carbohydrate reserves and/or the water deficit can by themselves limit growth of new roots (Fernández et al., 1988; Benning & Seastedt, 1997). After the nutrient release ends because of burning, root length is only affected by the climatic conditions on ungrazed sites (Benning & Seastedt, 1997; Hubbard, 2003).

The direct effects of fire on vesicular-arbuscular mycorrhizae fungi propagules depend on the intensity, duration and frequency of such disturbance (Hartnett et al., 2004). Aguilar-Fernandez et al. (Aguilar Fernández et al., 2009) reported that controlled, moderate burnings would not have a significant impact on the root colonization degree by VAM. This would be the result of the low thermic conductivity of the soil and the rapid combustion of herbaceous vegetation (Aguilar Fernández et al., 2009). Another disturbance, which can influence VAM colonization, is defoliation. It has been reported that VAM colonization decreases as defoliation severity increases (Gehring & Whitham, 2002). This has been attributed to a shortage of photoassimilates available to the fungi (Klironomos et al., 2004). If defoliation severity is moderate, however, VAM colonization is either not affected (Pietikaäinen et al., 2005) or can even increase (Wearn & Gange, 2007). Finally, species with high root length are less dependent of the association with VAM (Brundrett, 2002).

Poa ligularis Ness. (highly competitive, palatable, late-seral grass; Distel & Bóo, 1996; Cano, 1998), *Nassella tenuis* (Phil.) Barkworth (intermediate-competitive, palatable, mid-seral grass) (Cano, 1998; Saint Pierre et al., 2002) and *Amelichloa ambigua* (Speg.) Arriaga & Barkworth, (less-competitive, unpalatable, early-seral grass; Cano, 1998; Saint Pierre et al., 2004 a, b) are three species of native perennial grasses, abundant in the grasslands of Argentina (Mommer et al., 2011). Although this abundance depends at least partially on the relative effects of fire and of domestic livestock grazing (Distel & Bóo, 1996).

Hypotheses were that (1) fire and severe defoliation diminish root traits associated with nutrient uptake and inhibit percentage VAM colonization by compromising carbohydrate supply to both the root system and fungi as an energy source, and (2) values for root length, but not percentage colonization by VAM, are greater on the late- than on the earlier-seral perennial grass species. Our objectives were to examine the effects of defoliation after a controlled burning on various root traits (i.e., root growth and length; RLD) and percentage VAM colonization on *P. ligularis*, *N. tenuis* and *A. ambigua*. This paper will add to the literature concerning the ecophysiology of grass growth following disturbance and provide comparative data for grasslands.

MATERIALS AND METHODS

Study site. This study was conducted within a 15-year-exclosure to domestic livestock in the Chacra Experimental Patagones, southwest of the Province of Buenos Aires (40° 39' 49.7" S, 62° 53' 6.4" W; 40 m a.s.l.), Argentina, during 2011 and 2012. Climate is temperate semi-arid, with precipitations concentrated in summer and autumn. Mean annual precipitation was 421 mm during 1981-2012. A meteorological station located at the study site registered that total annual precipitation was 444 mm during 2011, and 513 mm during 2012. For the same period (1981-2012), mean annual, and absolute minimum and maximum temperatures were 14.1, -10.5 (July) and 42.1 °C (January), respectively.

Landscape on the region is mostly a plain although there are undulations and isolated microdepressions. Soil was classified as a typical Haplocalcid. Mean pH is 7 and there are no limitations of depth in the soil profile. The plant community is an open shrubby stratum that includes herbaceous species of different quality for livestock production (Giorgetti et al., 1997).

Experimental design. We followed a completely randomized experimental design with balanced replicates (n=6). Evaluated factors were (1) species, (2) treatments and (3) sampling dates.

Forty-eight vegetation patches (1 m² each) were selected for each of the species at the study site [(*P. ligularis*, *N. tenuis* and *A. ambigua*); 48 × 3 species= 144 patches]. Each vegetation patch, which contained at least 6 plants of any of the study species, constituted an experimental unit.

Treatments. Each treatment consisted of a combination of burning either without or with defoliation at the vegetative or internode elongation or both developmental morphology stages during the second (2012) or both study years (Table 1).

The controlled, experimental burning was conducted on 23 March 2011 in the area that included 126 patches. The 18 remaining patches, unburnt, were not defoliated and used as a control. The climatic conditions during burning (from 12:30 to 1:00 PM) were: 21.8 – 22.4 ˚C air temperature, 28% air relative humidity, 19.3 - 20 km/h wind speed (wind direction: NW – WNW).

Table 1. Treatments applied to the patches (n=6) of *P. ligularis, N. tenuis* or *A. ambigua* during the study years (2011 and 2012). UB = Unburnt; B = Burnt; UD = Undefoliated; DV = Defoliated at the vegetative stage; DE = Defoliated during internode elongation; D (V + E) = Defoliated at the vegetative + internode elongation stages.
Tabla 1. Tratamientos aplicados a los parches (n = 6) de *P. ligularis, N. tenuis* o *A. ambigua* durante los años de estudio (2011 y 2012). UB = Sin quemar; B = Quemado; UD = No defoliado; DV = Defoliado en estado vegetativo; DE = Defoliado durante la elongación de los entrenudos; D (V + E) = Defoliado en el estado vegetativo y durante la elongación de los entrenudos.

Treatments	2011	2011	2012
1 (control)	UB	UD	UD
2	B	UD	UD
3	B	UD	DV
4	B	UD	DE
5	B	UD	D (V + E)
6	B	DV	DV
7	B	DE	DE
8	B	D (V + E)	D (V + E)

Temperatures during burning were measured with 8 type-K (chromel-alumel) thermocouples at 1 second-intervals. They were located at the soil surface level in areas with different fine-fuel accumulation (high, intermediate, low) [25]. Temperatures were registered connecting the thermocouples to a datalogger (Campbell 21 XL) which was buried to 1 m soil depth. Maximum temperature reached was 560 ˚C.

Soil moisture content was determined by gravimetry previous to burning (mean ± 1 S.E., n= 5 ± 0.4%, 14). The amount of fine fuel [i.e., plant material over the soil surface (including litter) of a diameter less than or equal to 3 mm] was also determined. This plant material was collected using 10 quadrats of 1 m² each. After drying it in an oven (72 h at 70 ˚C), a total 3,887.6 kg dry matter/ha were obtained; with 9.1 ± 1.5% plant tissue moisture (mean ± 1 S.E., n=10).

Defoliations were conducted to 5 cm stubble height from the soil surface at various developmental morphology stages. These stages were either (1) vegetative (15 August 2011 and 06 May 2012) or (2) during internode elongation (14 October 2011 and 14 September 2012) or (3) vegetative + internode elongation. Neighbouring plants were also burnt and/or defoliated similarly to those measured to provide of a uniform competitive environment.

Sampling procedures

Root proliferation. One hundred and forty-four cylindric (8 cm diameter, 40 cm length), iron structures (8 treatments/species × 3 species × 6 replicates/treatment/species) were prepared. These structures ("cylinders") were wrapped with a plastic screen of 1 × 1 cm apertures. Each cylinder was buried diagonally to each plant using a powered auger, from its periphery downward to the center of each plant, with an angle of approximately 30˚ with respect to the soil surface. Each cylinder was subsequently filled with soil of the study site, which had been previously cleaned out of any residue using a 0.355 mm screen. Since the marked plants were separated at least 30 cm from neighbouring plants, it was considered that most roots that grew into the cylinders came from the study plants (Saint Pierre et al., 2002). Cylinders were placed on March 2011 and were first extracted, and then replaced by a new set of cylinders, in March 2012. The replacement set remained under the studied plants until the end of the study period in December 2012. Data reported represent the cumulative growth during both growing seasons (2011-2012).

Cylinders were destructively harvested using a shovel, and roots extending out of the plastic screen surrounding the cylinders were cut using a scissor. Roots were manually washed free of soil using a 35-mesh screen (Williams & Baker, 1957). Root proliferation was estimated through measurements of root length (cm) and ash-free, dry weight of the organic matter grown into the cylinders. Root length (a measure of root growth) was determined on images obtained after the scanning of roots placed between two glasses of 20 × 35 cm, which were thereafter read by the software ROOTEDGE 2.3b (Kaspar & Ewing, 1997). Roots were dried to 105 ˚C during 72 h to obtain the sample dry weight. Thereafter, samples were placed in a furnace to 550 ˚C during 6 h (McNaughton et al., 1998). The dry weight of the ash-free organic matter was obtained after subtracting the dry weight to 550 ˚C from that to105 ˚C.

Root length density. Samplings were conducted after burning and before the first defoliation (11 May 2011), and after 40-50 days from each defoliation treatment during 2011 and 2012 (22 September and 3 December 2011, and 3 July and 18 October 2012). At each sampling time, an auger (62.83 cm³ volume) was used to get a soil + root sample at 10 cm soil

depth in the plant periphery. Roots were obtained and measured as indicated under the Root Proliferation subheading. Since the cylinder volume was known, RLD (cm roots cm³ soil) was determined.

Vesicular arbuscular mycorrhizae. Vesicular-arbuscular mycorrhizae were evaluated on those roots utilized to determine RLD. Immediately after scanning roots to determine their length, they were placed on closed flasks containing a FAA solution (formaldehyde, glacial acetic acid, ethanol; Phillips & Hayman, 1970).

Percentage root colonization by VAM was determined following Giovannetti and Mosse (1980). Ten 1 cm-segments of stained roots were placed parallel on each of 3 glass slides per sample. Three trajectories were made on each glass slide, and 10 scores (i.e., observation fields) were made per trajectory [i.e., one score per root segment/trajectory/ glass slide × 10 root segments/glass slide × 3 trajectories/ glass slide × 3 glass slides/per sample=90 observation scores/ sample] looking for the presence (i.e., colonized fields) or absence of hyphae, vesicles and/or arbuscules. These determinations were made under a microscope (Leica ICC50 40-400X). Finally, the percentage colonization by VAM on each of 6 replicate samples/treatment/species/sampling date was obtained from the relationship between the number of colonized fields (CF) with respect to the total number of observed fields (OF) as follows:

$$\text{Percentage colonization} = \frac{CF}{OF} * 100$$

Statistical analysis. Data were analyzed with the statistical software INFOSTAT (Di Rienzo et al., 2013). Before analysis, they were transformed to √x for root proliferation and RLD, arcsine √x for VAM, and ln (x+1) for ash-free dry weight to comply with assumptions of normality and homocedasticity. Values without transformation are presented in the figures. Root proliferation data were analyzed with two-way ANOVA (species × treatments); if the interaction was significant, one-way ANOVAs were made for each factor separately. Mean comparisons were made using Tukey test. Data of RLD and VAM, were analyzed with three-way ANOVA (species × treatments × sampling dates). Since data of RLD and VAM corresponded to repeated measures, mixed linear models were used for RLD with a structure of autoregressive residual correlation of order 1, heteroscedastic residual variances and random effects for plants. When appropriate, contrasts were made to compare treatment effects. Mixed linear models were also used for VAM with a residual correlation without structure and heterogeneous residual variances through time. Mean comparisons were made using the protected LSD test of Fisher at a significant level of 5%.

RESULTS

Root proliferation

Root length. There was neither significant interaction between species and treatments ($F_{14, 120}$= 1.61; n= 6; p= 0.0866) nor treatment effects ($F_{7, 120}$=1.38; n= 18; p=
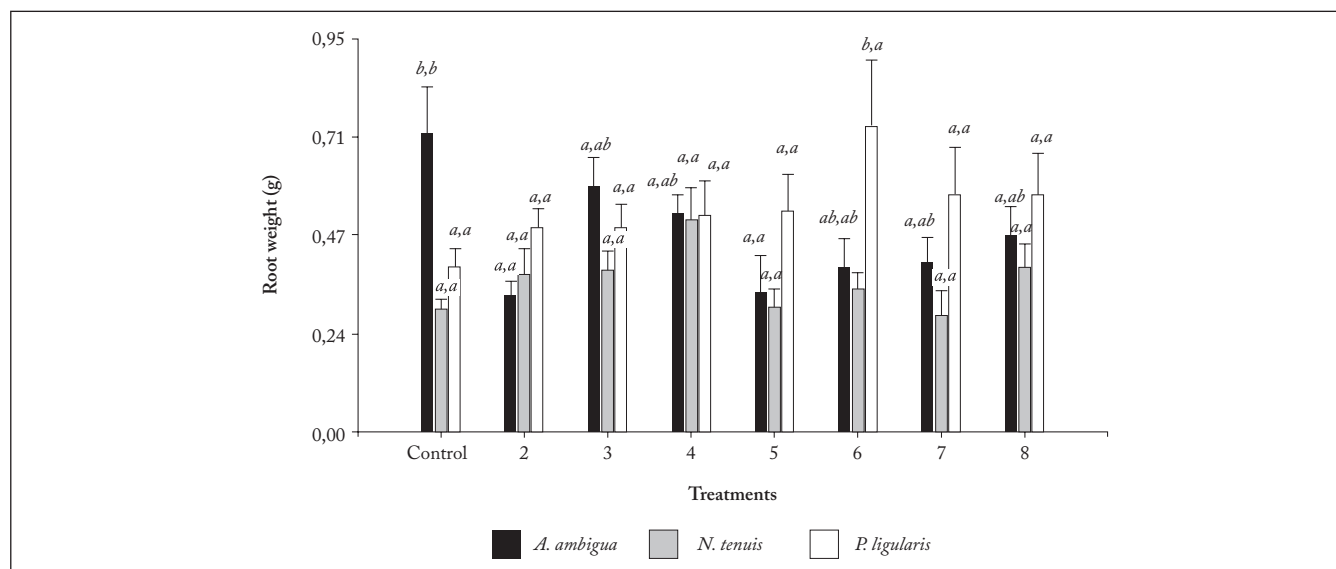

Fig. 1. Ash-free root weight (g) on plants of *P. ligularis*, *A. ambigua* and *N. tenuis* either exposed or not to different combinations of burning and defoliation (see Table 1). Each data is the mean ± 1 standard error of n=6. Different letter before the comma indicate significant differences (P≤0.05) among species within each treatment. Different letters after the comma indicate significant differences (P≤0.05) among treatments within each species.

Fig. 1. Peso radical libre de cenizas (g) de plantas de *P. ligularis*, *A. ambigua* y *N. tenuis*, expuestas o no a distintas combinaciones de quema y defoliación (ver Tabla 1). Cada dato es el promedio ± 1 error estándar de n = 6. Las letras distintas delante de la coma indican diferencias significativas (P≤0,05) entre especies, dentro de cada tratamiento. Las letras distintas detrás de la coma indican diferencias significativas (P≤0,05) entre tratamientos, dentro de cada especie.

0.2191). *Poa ligularis* showed a greater ($F_{2, 120}$= 15.19; n= 48; p= 0.0001) root growth (total root length/cylinder: 39.54 m ± 1.76; n= 48) than *A. ambigua* (33.75 m ± 1.82; n= 48). At the same time, *A. ambigua* showed a greater ($F_{2, 120}$= 15.19; n= 48; p= 0.0001) root growth than *N. tenuis* (27.46 ± 1.18; n= 48).

Root weight. There was a significant interaction ($F_{14, 120}$= 2.28; n= 6; p= 0.0084) between species and treatments. Roots of *P. ligularis* ($F_{7, 40}$= 1.02; n= 6; p= 0.4326) and *N. tenuis* ($F_{7,40}$= 1.99; n= 6; P= 0.0808) did not show differences among treatments, while those of *A. ambigua* exhibited greater root weight ($F_{7, 40}$= 3.33; n= 6; P= 0.0069) in the control (UB UD UD) than in treatments 2 (B UD UD) and 5 (B UD DV+E) (Fig. 1). *Amelichloa ambigua* showed a greater ($F_{2, 15}$= 10.45; n= 6; P= 0.0014) root weight than *P. ligularis* and *N. tenuis* in the control (UB UD UD), while *P. ligularis* had a greater ($F_{2, 15}$= 4.60; n= 6; P= 0.0277) root weight than *N. tenuis* in treatment 6 (B DV DV). There were no differences (P>0.05) among the species in the remaining treatments (Fig. 1).

Root length per unit root weight. The interaction between species and treatments was not significant ($F_{14, 120}$= 1.28; n= 144; P= 0.2313). Also, no significant differences were evident neither among species ($F_{2, 120}$= 1.40; n= 48; P= 0.2500) nor treatments ($F_{7, 120}$= 0.86; n= 18; P= 0.5411). The mean root length per unit root weight was 76.98 ± 1.37 m/g (mean ± 1 S.E.; n= 144).

Root length density. There was a significant interaction ($F_{28, 480}$= 1.77; n= 18; P= 0.0098) among treatments and sampling dates. Forty-nine days after the controlled burning, root length density was greater ($F_{7, 136}$= 3.16; n= 18; P= 0.0040) on burnt and/or defoliated than on control, unburnt plants (Fig. 2). Thereafter, however, there were no differences (P>0.05) among treatments. All treatments followed a similar (P>0.05) pattern through time, increasing (T1= $F_{4, 85}$= 17.24; n= 18; P<0.0001; T2= $F_{4,85}$= 14.90; n= 18; P<0.0001; T3= $F_{4,85}$= 4.74; n= 18; P= 0.0017; T4= $F_{4,85}$= 20.07; n= 18; P<0.0001; T5= $F_{4,85}$= 6.23; n= 18; P= 0.0002; T6= $F_{4,85}$= 7.46; n= 18; P<0.0001; T7= $F_{4,85}$= 9.59; n= 18; P<0.0001; T8= $F_{4,85}$= 16.78; n= 18; P<0.0001) the root length density from the first to the last sampling date (Fig. 2).

Vesicular arbuscular mycorrhizae. We did not find significant interactions neither among factors (T= treatment, S= species, D= sampling date; T × S= $F_{14, 120}$= 1.27; n= 30; P=0.2215; T × D= $F_{28, 480}$= 1.48; n= 18; P=0.0600; T × S × D= $F_{56, 480}$= 1.08; n= 6; P=0.3111) nor treatment effects ($F_{7, 120}$= 1.37; n= 90; P=0.2148). Percentage colonization of VAM was greater ($F_{2, 120}$= 7.89; n= 240; P=0.0004) on *A. ambigua* and *N. tenuis* than on *P. ligularis* (Fig. 3). On a time scale, greatest ($F_{4, 480}$= 72.93; n= 144; P<0.0001) values were found at the first sampling date, then at the reproductive stages of the second and first sampling years, respectively, and lastly at the vegetative stages of the first and second study years, respectively (Fig. 3).
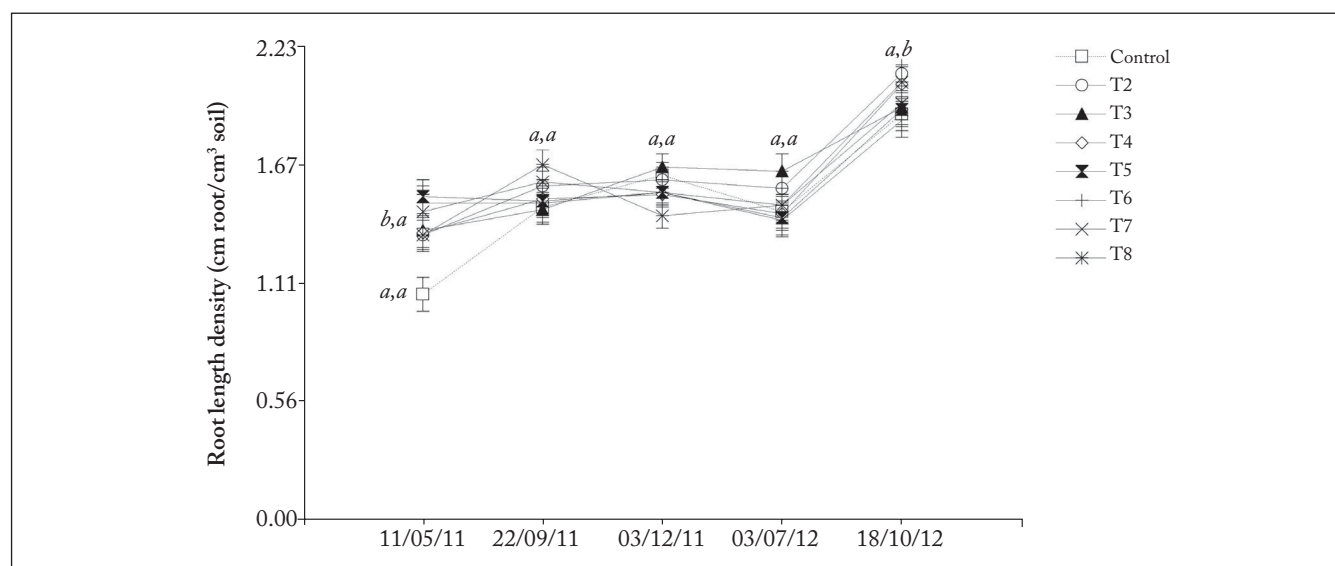

Fig. 2. Root length density (cm root/cm³ soil) for each sampling date on plants of *P. ligularis*, *A. ambigua* and *N. tenuis* either exposed or not to the various combinations of burning and defoliation (see Table 1). Each symbol is the mean ± 1 standard error of n=18. Different letters before the comma indicate significant differences (P≤0.05) among treatments, within each sampling date. Different letters after the comma indicate significant differences (P≤0.05) among sampling dates within each treatment.

Fig. 2. Densidad de longitud de raíces (cm raíz/cm³ suelo) para cada fecha de muestreo en plantas de *P. ligularis*, *A. ambigua* y *N. tenuis* expuestas o no a distintas combinaciones de quema y defoliación (ver Tabla 1). Cada símbolo representa la media ± 1 error estándar de n = 18. Las letras distintas antes de la coma indican diferencias significativas (P≤0,05) entre tratamientos, dentro de cada fecha de muestreo. Las letras distintas después de la coma indican diferencias significativas (P≤0,05) entre fechas de muestreo, dentro de cada tratamiento.
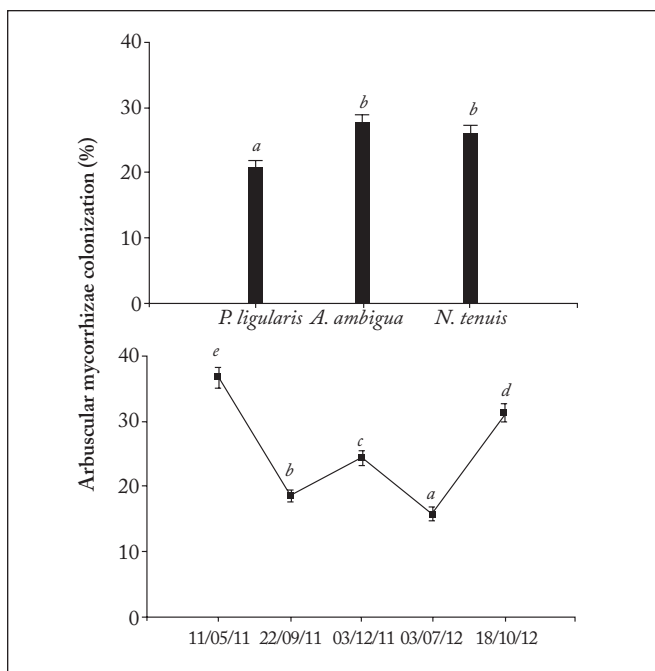
Fig. 3. Percentage colonization by vesicular-arbuscular mycorrhizae on plants of *P. ligularis*, *A. ambigua* and *N. tenuis* for each sampling date. Histograms are the mean ± 1 standard error of n=240 (species; 6 samples/treatment/date x 8 treatments/date x 5 dates) (A), and Symbols are the mean of n=144 (sampling dates; 6 samples/species/treatment/date x 3 species x 8 treatments) (B). Different letters above either histograms or symbols indicate significant differences (P≤0.05) among species or sampling dates, respectively.

Fig. 3. Porcentaje de colonización por micorrizas vesiculo-arbusculares en plantas de *P. ligularis*, *A. ambigua* y *N. tenuis* para cada fecha de muestreo. Los histogramas representan la media ± 1 error estándar de n = 240 (especie, 6 muestras / tratamiento / fecha x 8 tratamientos / fecha x 5 fechas) (A), y los símbolos representan la media de n = 144 (fechas de muestreo; 6 muestras / especie / tratamiento / fecha x 3 especies x 8 tratamientos) (B). Letras distintas sobre los histogramas o símbolos indican diferencias significativas (P≤0,05) entre especies o fechas de muestreo, respectivamente.

DISCUSSION

Root proliferation. In disagreement with the first hypothesis, the study parameters of root proliferation (i.e., root length and weight; root length/unit root weight) were similar in all study treatments and in the control. The only exception was on plants of *A. ambigua*, where the control showed a greater root weight than plants which were (1) burnt and undefoliated in 2011 and 2012, or (2) burnt and undefoliated in 2011 but defoliated at both study developmental stages (i.e., V+E) in 2012. Information on the effects of disturbances (such as defoliation management after fire) is scarce and contradictory in some cases. In agreement with our findings, Melgoza and Nowak (1991) also did not find differences in root proliferation between burnt and unburnt areas. These authors suggested that fire would not have a short-term effect on root production. Plant survival after fire would be related more to the capacity of rapidly utilizing soil available water and nutrients during regrowth than to direct effects from fire.

Saint Pierre et al. (2002) found a similar root proliferation on defoliated than on undefoliated plants of *Nassella longiglumis*, *N. tenuis* and *A. ambigua*. Out of nine studies conducted by Johnson and Matchett (2001) three showed a positive, four showed a neutral, and two showed a negative effect of rangeland grazing on root productivity. These results indicate that the effects of grazing on root productivity would be highly tied to the characteristics of the (1) study site (soil, climate, etc.), (2) vegetation (species composition, photosynthetic pathway, etc.), and (3) disturbance (e.g., grazing pressure, animal species, etc) (Johnson & Matchett, 2001).

Although in this study no differences were found among the species in root length per unit root weight, *P. ligularis* showed a greater root length than the other two species in all treatments. Also, that species obtained a greater ash-free root biomass than *N. tenuis* on burnt and defoliated plants at the vegetative developmental morphology stage during the two study years. *Amelichloa ambigua*, however, showed a greater ash-free root weight than the other two species when it was neither burnt nor defoliated. These results might be due to the competitive ability and the response mechanisms to a given disturbance of each species. Crick and Grime (1987) reported a higher plasticity for rapidly modifying root growth in the more than less competitive species. This would allow plants to explore a greater soil volume, coming up to nutrient-rich microsites (Caldwell, 1979; Fernández et al., 1988). However, this greater growth does not necessarily secure a greater soil resource acquisition to the plant. Determination of nutrient uptake rates by the plants would be necessary to assure it (Saint Pierre et al., 2002). However, our results suggest that the greatest root length (> 14%) and greater weight (overall mean among disturbed soils > 21%) on disturbed soils on plants of *P. ligularis* might be some of the mechanisms which allow a greater competitive ability in this species.

Root length density. All treatments showed a greater RLD than the control approximately a month and a half after burning. However, these differences disappear afterwards. The increased soil nutrient availability immediately after fire could favour root growth under adequate soil water contents (Melgoza et al., 1990). In agreement with this, Ithurrart et al. (2017) reported that the results of this study might be explained, at least in part, due to the increased soil available nitrogen contents (ammonium + nitrate) during the first sampling dates immediately after burning (compared with the unburnt control) at the study site. Hubbard (2003) also found a high increase in root length density immediately after a controlled burning; this increase, however, was of short duration. In agreement with our findings, Melgoza and Nowak (1991)

reported similar root length density values between burnt and unburnt sites a year after burning. These authors concluded that plants might rapidly increase carbon allocation to roots under nutrient deficiency conditions. This mechanism might contribute to explain the lack of significant differences in root length density between burnt and unburnt sites since the second sampling date onwards.

Temperature and soil water availability are factors that directly affect root growth (Distel & Fernández, 1988; Fernández et al., 1988). In some C3 perennial grasses, maximum root production was associated with soil temperatures in the range of 15 to 25 °C (Troughton, 1957). Drew (1979) showed that even small deviations from optimal soil temperatures for root development could have a marked effect on the process of root growth. This is because temperature and soil water content have an important influence on the rates of root production and senescence thereby affecting the final length of both processes (Nyhan, 1976). In this way, the maximum root length density values found in October 2012 might be the result of soil temperatures of at least 15 °C during that month of the year at the study site, under adequate soil moisture content conditions. Distel and Fernández (1988) showed that the maximum root growth of the C3 *N. tenuis* and *P. napostaense* during the period from October to April could be attributed to an adequate soil water availability. Becker et al. (1997) also found the greater root length density values in these perennial grass species during October at a similar site than ours.

Vesicular-arbuscular mycorrhizae. We did not observe effects of the combination of both disturbances on the percentage root colonization by VAM on any of the three study species. The lack of response of VAM to the controlled burning could be related to the rapid combustion of grasslands dominated by perennial grasses and the low thermic conductivity of the soil (Aguilar Fernández et al., 2009).

Amelichloa ambigua and *N. tenuis* showed a greater percentage colonization than *P. ligularis*. Corkidi and Rincón (1997) showed that the vesicular-arbuscular mycorrhizal fungi are essential for growth of early-seral plant species. This association pioneer plant – vesicular-arbuscular mycorrhizal fungi is likely to increase the success probability of establishment and survival of such plants (Carmona Escalante et al., 2013). In addition, a greater diameter of fibrous roots can be tied to a greater colonization percentage of VAM (Reinhardt & Miller, 1990; Koltai & Kapulnik, 2010). *Amelichloa ambigua* is a species of early-seral stages (Busso et al., 2008) and it has a greater root diameter (mean ± 1 SE, n=48: 2.18 ± 0.1mm) than *P. ligularis* (mean ± 1 SE, n=48: 1.84 ± 0.1mm). This would contribute to explain the greater colonization percentage determined in *A. ambigua*.

The degree of benefit that plants can experience from their association with vesicular-arbuscular mycorrhiza fungi can vary with their root architecture. Since root length density

and growth are tied to water and nutrient uptake (Caldwell & Richards, 1986), plants with a large root length are less dependent on vesicular-arbuscular mycorrhizal fungi (Miller et al., 1997; Brundrett, 2002). *Poa ligularis* and *N. tenuis* were the species with the greatest and lowest root length, respectively. This might contribute to explain the lower and higher root colonization observed in *P. ligularis* and *N. tenuis*, respectively.

The information about the temporal variation of VAM percentage is scarce. Bellgard (1993) suggested that the pattern of root colonization by VAM is related to the seasonal temperatures throughout the year. As a result, high levels of colonization would be associated with the spring and summer because of these two seasons characterize by having high diurnal temperatures. In our study, the greatest values of root colonization by VAM were obtained before the first defoliation, and in the spring and summer of the first and second study years. Heinemeyer et al. (2003) also detected a greater degree of colonization during the spring. During this season, they reported a root colonization increase from 12 to 25% during a six-week-period. This increase pattern could be due to a greater proportion of young roots during the spring, and an increased proportion of either old or senescent roots later on. Similar results were found by Bentivenga and Hetrick (1991), who suggested that VAM dependency increases with temperatures that foster growth of the host.

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