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## *Pinus halepensis* invasion in mountain pampean grassland: Effects of feral horses grazing on seedling establishment

A.E. de Villalobos<sup>a,b,c,\*</sup>, S.M. Zalba<sup>a,b,c</sup>, D.V. Peláez<sup>a,b,c,d</sup><sup>a</sup> GEKKO (Grupo de Estudio en Conservación y Manejo), Departamento de Biología Bioquímica y Farmacia, Universidad Nacional de Sur., Argentina<sup>b</sup> Departamento de Agronomía, Universidad Nacional del Sur, San Juan 670, Bahía Blanca (8000), Argentina<sup>c</sup> CONICET, Consejo Nacional de Investigaciones Científicas y Técnicas, Argentina<sup>d</sup> CIC Comisión Científica y Técnica, Buenos Aires, Argentina

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## ABSTRACT

We evaluated the mechanisms that determine the increasing abundance of *Pinus halepensis* in mountain pampean grasslands in Argentina that is associated with the continuous presence of feral horses. We hypothesized that direct and indirect effects of feral horse grazing on grassland may affect the establishment of *P. halepensis*. We conducted a field experiment to evaluate this hypothesis, studying the response of seedling emergence, survival and growth to herbaceous vegetation defoliation and soil disturbance in sites with contrasting grazing histories. We also evaluated the composition and structure of plant communities of each experimental site. Direct effects such as heavily defoliated perennial grasses and indirect effects such as reduced specific and functional richness, diversity and evenness enhanced the emergence, survival and early growth of the seedlings. High bare ground percentage and low grass biomass also positively affected seedling establishment. The experimental evidence demonstrates that the combination of invasional meltdown hypothesis and weakened biotic resistance are the mechanisms involved in *P. halepensis* invasion.

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### 1. Introduction

Invasive species threaten natural communities and ecosystems worldwide (Vitousek et al., 1996) and many studies have focused on understanding the factors that control alien plant invasions. Many hypotheses dealing with the success of invasive plant species concern plant–herbivore interactions. The “invasional meltdown hypothesis” suggests that exotic herbivores may directly or indirectly facilitate the invasion of an exotic plant species by either favourably changing environmental conditions or reducing competition from native plant species (Simberloff and Von Holle, 1999). On the other hand, the “biotic resistance hypothesis” suggests that native herbivores and competitors can reduce the ability of non-native plant species to invade a new territory (Levine et al., 2004).

Meltdown is a community-level process in which the net effect of facilitations would lead to an increasing rate of establishment of one or more of the introduced species and accelerate its impact. Introduced grazers could affect natural grassland, reducing its biotic resistance to the invasion of exotic plants, having influenced the ecosystem processes and plant community dynamics both directly

and indirectly. Direct effects are those related to alterations in plant physiology and morphology resulting from defoliation and trampling. Indirect effects result from changes in the microenvironment, soil physical and chemical properties and plant competitive interactions (Archer and Smeins, 1991; Mitchell et al., 2006). In turn, alterations caused by both the direct and indirect effects of grazing may reduce the resistance of the system, creating conditions suitable for the establishment of woody plant seedlings (Jacoby, 1986; DeGasperis and Motzkin, 2007).

The severity of the effects of introduced herbivores on natural grassland depends on the intensity and frequency of grazing and the evolutionary relationship between grasslands and grazers (Milchunas et al., 1988). Where the historical record of grazing was light or moderate, areas that are frequently and intensely grazed are more exposed to invasion by exotic species due to alteration of the competitive relationship (Hobbs, 1991; Mitchell et al., 2006). Most grasslands in South America have evolved under low grazing pressure and are more vulnerable to plant invasion, compared with those that included large hooved congregating ungulates in their evolutionary past, e.g. the North American grasslands and African savannas (Milchunas et al., 1988; Mack, 1989; Cingolani et al., 2005; de Villalobos and Zalba, 2010).

Frequently disturbed areas are more susceptible to the establishment and dispersal of invasive species (Levine et al., 2004). Harper (1977) demonstrated that the invasion of exotic species is

\* Corresponding author at: Departamento de Biología, Bioquímica y Farmacia, Universidad Nacional del Sur, San Juan 670, Bahía Blanca (8000), Argentina.  
 E-mail address: [avillalo@criba.edu.ar](mailto:avillalo@criba.edu.ar) (A.E. de Villalobos).

successful when the biotic resistance of the system is weakened. Frequent and intense grazing might reduce the resistance of the system to be invaded. Many *Pinus* species are highly invasive in natural systems worldwide and they threaten grassland communities in the southern hemisphere (Richardson et al., 1994; Rouget et al., 2001), for instance in South America (Richardson et al., 2008; Simberloff et al., 2010). In particular, *Pinus halepensis* is considered an important invasive species in the southern hemisphere in general and in South American in particular (Richardson and Higgins, 1998). In the Argentine pampas, pine plantations were established between 1930 and 1960 and 20 years later they are spreading over relictual areas of native grassland (Simberloff et al., 2010). An increased abundance of *P. halepensis* dispersed from cultivated stands has been recorded in the natural grassland in Ernesto Tornquist Provincial Park (ETPP), a natural protected area in the mountain pampean grassland of Argentina, and its abundance has increased 25 times since 1987 (Zalba, 1994; Zalba et al., 2008). The ETPP has been extensively grazed by feral horses for more than 30 years. Exotic ungulates would strongly enhance the relative abundance of exotic woody plants (Parker et al., 2006; Relva et al., 2010). In general, selective grazing by domestic herbivores affects grassland by preventing its natural regeneration, reducing the abundance of grass species, delaying its growth and competitive ability and altering the composition of the plant community, which generally leads to the dominance of less preferred and more grazing-tolerant herbaceous species (Augustine and McNaughton, 1998; de Villalobos and Zalba, 2010). Usually these effects are more severe in the case of feral herbivores since they are free-ranging and non-managed, which differs from the typical situation of cattle ranching.

An understanding of the causes of biological invasions would offer fundamental knowledge for predicting both the impact of exotic species on the community and the chance of management or eradication. The aim of our work was to identify key factors that influence the establishment of *P. halepensis* in relation to grazing by feral horses. We carried out experiments to evaluate the emergence, survival and early growth of *P. halepensis* seedlings under different defoliation regimes of the herbaceous vegetation and soil compaction treatments at sites with contrasting grazing histories.

## 2. Materials and methods

### 2.1. Study species

*P. halepensis*, commonly known as the Aleppo Pine, is a conifer tree native to the Mediterranean region and it is also common in plantations within and outside its natural range, spreading rapidly from plantations to nearby natural habitats. It is considered to be one of the most invasive pine species, threatening native biodiversity in different habitats and causing severe financial losses, especially across the southern hemisphere (Richardson and van Wilgen, 2004; Richardson, 2006). It is an obligate seeder and pollination takes place in spring, fertilization a year later and wind dispersal of the seed begins in the third year after pollination (Thanos and Daskalou, 2000). Mature seeds are retained in persistent cones, and seed release is often delayed for many years, building up a canopy-stored seed bank (serotiny). Seed release is induced by hot, dry weather during late spring and summer or by hot temperatures reached during forest fires (Thanos and Daskalou, 2000). An endogenous rhythm regulates germination to the late autumn–early winter period (Nathan et al., 2000).

### 2.2. Study area

This study was conducted during the 2003/2004 growing season in the Ernesto Tornquist Provincial Park (ETPP), established in 1942, one of the last relicts of pampean grassland that is in a relatively good state of conservation (Cabrera, 1976; Bilenca and Miñarro, 2004). ETPP is located in the Ventania mountain range in the central Pampas region, between 38°00' and 38°10'S, and 61°45' and 62°08'W. It has an area of ca. 6700 ha and includes some of the highest peaks in the region, reaching up to 1200 m. Climate is temperate, with an average

annual temperature of 14 °C (Burgos, 1968). Average precipitation is 800 mm, falling mostly in spring and autumn, with occasional snow-fall in the winter. The region belongs to the Southern District of the Pampas Phytogeographic Region, where grass steppe is the dominant vegetation (Cabrera, 1976). The most abundant perennial tussock grasses are *Stipa* and *Piptochaetium* spp. The shrubs *Discaria americana* and *Eupatorium buniifolium* are the dominant woody species. Native trees are absent in the system (Long and Grassini, 1997). Native herbivores present in the pampean grasslands since the pre-hispanic period include the Pampas Deer (*Ozotoceros bezoarticus*) and Guanaco (*Lama guanicoe*), but both species have become extinct or they only occur in extremely low numbers in the area. Domestic livestock (cattle, horses, sheep and goats) became abundant in the 18th century and other exotic herbivores (*Cervus elaphus* and *Dama dama*) were introduced for hunting in this region. Nevertheless, feral horses have become the main herbivore in ETPP, reaching a population of 700 horses (Scorolli, 2007). In 1942, five horses were introduced with the aim of maintaining a “native” breed (caballos criollos) and the population has increased by 6% p.a. reaching a density of 0.35 horses ha<sup>-1</sup> in 2003 (Scorolli, 2007).

### 2.3. Experimental sites and field experiments

In summer 2003 two experimental sites with contrasting grazing histories were selected within the study area to evaluate the effect of different grass defoliation intensity and soil compaction on the establishment of *P. halepensis* seedlings. Both sites were located on adjacent piedmont grasslands, with 5–11% steepness, north-facing slopes and characterized by shallow soils with occasional rocky outcrops. Feral horses prefer these environments for grazing on account of the high availability of grass and facility of displacement (Scorolli, 2007). Each experimental site comprised an area of 15 ha. One site had been subject to continuous intensive grazing by free-ranging feral horses (grazed site) and the other had been protected from horse grazing for seven years (ungrazed site). The latter was the only available site in the study area that had been closed to feral horses.

Twelve experimental plots were established in the ungrazed site and eight experimental plots in the grazed site. The location of each experimental plot was randomly determined in both study sites in order to obtain the interspersed treatments recommended by Hurlbert (1984) and Oksanen (2001) which ensures the independence of the measured variables so that the responses of each treatment can be analysed. Each plot comprised an area of 0.1 m<sup>2</sup>. The plots in the ungrazed site were randomly assigned to each of the following treatments: herbaceous vegetation not defoliated ( $n=4$ ), herbaceous vegetation moderately defoliated (herbaceous vegetation clipped at height of 15 cm) ( $n=4$ ) and herbaceous vegetation heavily defoliated (herbaceous vegetation clipped at height of 5 cm) ( $n=4$ ). Defoliation treatments were carried out every 15–20 days and the herbaceous biomass removed was exported from the experimental plots. The treatments assigned in the grazed site were undisturbed bare ground ( $n=4$ ) and disturbed bare ground (soil disturbed mechanically down to 15 cm depth) ( $n=4$ ). Four plots were used for each treatment and fifty seeds of *P. halepensis* were planted at a depth of 1 cm in each plot in March 2003. The seeds were collected from mature cones that opened in summer 2003. The emergence of *P. halepensis* (seedlings/number of seeds planted) was recorded weekly after each planting date and seedling survival (percentage of surviving seedlings) was noted every 15 days for one year. In April 2004, the surviving seedlings in each treatment and study site were collected, oven-dried and weighed to determine their above- and below-ground biomass. The height and main root length of each surviving seedling was also determined.

In order to evaluate the state of the vegetation of each experimental site in spring 2003 (late November/early December), 20 plots of 1 m<sup>2</sup> were randomly distributed in the grazed and ungrazed sites and the cover was recorded for each plant species present in each plot using the canopy-cover method of Braun Blanquet (Mueller-Dombois and Ellenberg, 2003). Species richness, Shannon's diversity (Krebs, 2001), evenness (Pielou, 1975), percentage of exotic species and bare ground were calculated for each experimental site. All species recorded were grouped together according to their similar biological traits that are notable for their response to grazing, e.g. growth form (erect or prostrate) and life cycle (annual or perennial) (Vesk and Westoby, 2001). The abundance of the resulting functional groups was calculated from the cover of each species, recorded in the 20 randomly distributed plots (1 m<sup>2</sup>) in each experimental site. Finally, in order to evaluate how the two main components of the plant community (grasses and herbs) respond to grazing, we clipped the herbaceous above-ground biomass of grasses and herbs at ground level in 5 plots ( $n=5$ ; 1 m<sup>2</sup>) in each experimental site at the end of the growing season (autumn 2004). The samples were oven-dried at 60 °C for 48 h and weighed.

### 2.4. Data analysis

The combined effects of grazing history, defoliation and soil treatment on the emergence and early growth (height, main root length, above- and below-ground biomass) of *P. halepensis* seedlings between experimental sites were analysed using one-way analysis of variance for a completely randomized design. Prior to

the analysis all data were square-root transformed and the means were separated using Tukey's test (Zar, 1999).

Survival differences between grazing history, defoliation and soil treatments were investigated by a multiple comparisons for log-rank test and Bonferroni's correction for multiple comparisons was applied (Krebs, 2001). The log-rank test is a chi-squared test that is calculated using the observed and the expected number of surviving individuals that occur in each time interval of the study, assuming as null hypothesis that all the treatments have the same survival distribution.

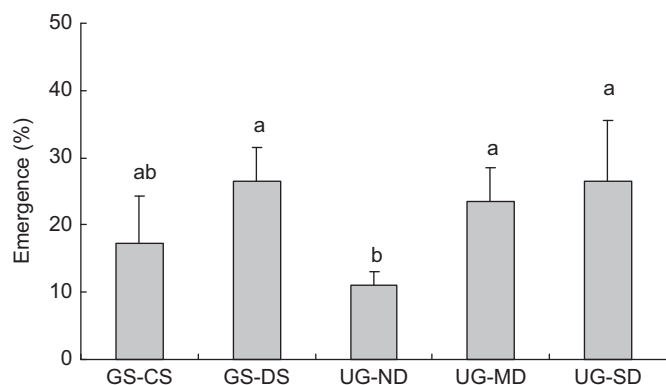
We used multivariate analysis of variance (MANOVA) to evaluate the effects of contrasting feral horses grazing histories on species richness, diversity, evenness, percentage of exotic species and bare ground. Prior to analysis all the data were log-transformed to achieve normally distributed residuals and homogeneous variances. In the case of significance, the analysis was followed by a Tukey's test for equal sample sizes (Scheiner and Gurevitch, 1993). The relative abundance of each functional group and the above-ground biomass of grasses and herbs recorded in each study site was evaluated using Student's *t*-test for equal sample sizes and unequal variance (Zar, 1999). All data were square-root transformed (Zar, 1999).

### 3. Results

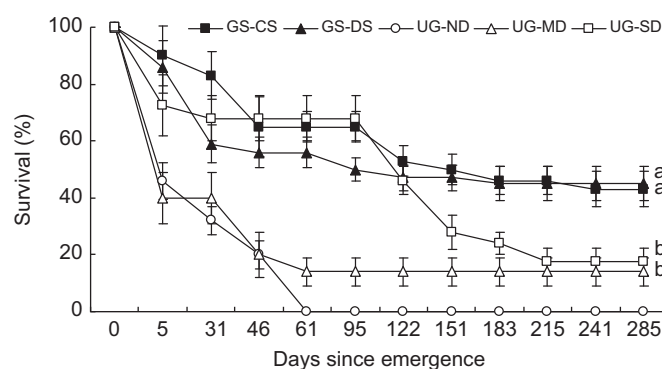
#### 3.1. Seedling establishment

We found that all the parameter descriptors of the successful establishment of *P. halepensis* were influenced by grazing history and the intensity of herbaceous defoliation. Total *P. halepensis* seedling emergence was recorded during the first month after planting date, and was always lower than 30% in both study sites. We found that the grazing history of the study site and the intensity of herbaceous defoliation had different affects on the emergence of seedlings ( $F=4.44$ ;  $p=0.014$ ;  $df=4, 12$ ). The lowest emergence was recorded in the ungrazed site where the herbaceous vegetation was untouched, while the highest emergence was recorded where the herbaceous vegetation was heavily defoliated (Fig. 1). Different results were obtained in the grazed site, where the soil disturbance treatment did not affect seedling emergence (Fig. 1).

The highest rate of seedling mortality was observed during the first 2 months after seedling emergence when mean survival of *P. halepensis* decreased to 60%. In most of the treatments seedling mortality was very low after this period (Fig. 2). Seedling survival was dependent on the grazing history and defoliation treatments (chi-squared=83.1;  $p=3.8 \times 10^{-17}$ ;  $df=4$ ). During the next 7 months, the highest survival rate from all the experimental plots was recorded for the *P. halepensis* seedlings that emerged in the grazed site in both the undisturbed and disturbed soil treatments (chi-squared=71.5;  $p=1.1 \times 10^{-14}$ ;  $df=4$ ). However, seedling survival declined by more than 40% in the defoliation



**Fig. 1.** Emergence (%) ± SE of *P. halepensis* seedlings in plots with the following treatments: grazed site and undisturbed soil (GS-CS), grazed site and disturbed soil (GS-DS), ungrazed site and herbaceous vegetation not defoliated (UG-ND), ungrazed site and herbaceous vegetation moderately defoliated (UG-MD) and ungrazed site and herbaceous vegetation heavily defoliated (UG-SD). Columns with the same letter are not significantly different (Tukey test at  $p < 0.05$ ).



**Fig. 2.** Survival (%) ± SE of *P. halepensis* seedlings in plots with the following treatments: grazed site and undisturbed soil (GS-CS), grazed site and disturbed soil (GS-DS), ungrazed site and herbaceous vegetation not defoliated (UG-ND), ungrazed site and herbaceous vegetation moderately defoliated (UG-MD) and ungrazed site and herbaceous vegetation heavily defoliated (UG-SD). Lines followed by the same letter are not significantly different (log-rank test of survival curves,  $p < 0.05$  after Bonferroni's correction for multiple comparisons).

treatments in the ungrazed site over the same period and there were no surviving seedlings in the herbaceous vegetation not defoliated treatment (Fig. 2).

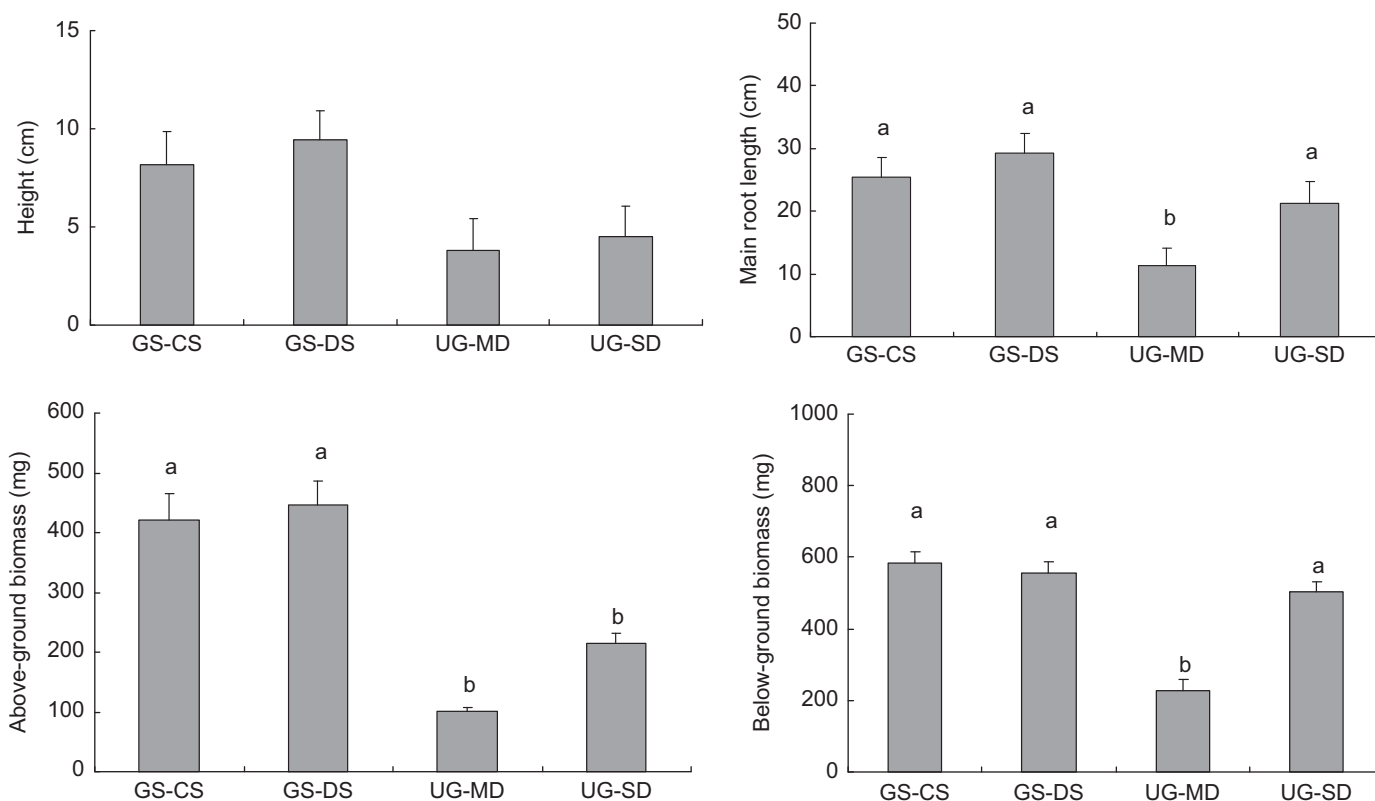
Early seedling growth was influenced by the grazing history of the experimental site and the intensity of the herbaceous defoliation treatment. At the end of the study period, seedlings that grew in the grazed site were significantly higher ( $F=22.30$ ;  $p=3.4 \times 10^{-5}$ ;  $df=3, 12$ ) than seedlings in the ungrazed site, regardless of the soil or defoliation treatments (Fig. 3). The above-ground biomass of *P. halepensis* seedlings showed a similar response ( $F=36.5$ ;  $p=2.6 \times 10^{-6}$ ;  $df=3, 12$ ) (Fig. 3). Below-ground growth variables were favoured by the presence of feral horses and the intensity of defoliation of herbaceous vegetation, but they were not affected by soil compaction. Lower below-ground biomass ( $F=16.53$ ;  $p=0.0001$ ;  $df=3, 12$ ) and main root length ( $F=36.6$ ;  $p=2.5 \times 10^{-6}$ ;  $df=3, 12$ ) were recorded on plots with moderate defoliation than in the case of seedlings from the intensively defoliated plots in the ungrazed site and with or without soil disturbance in the grazed site (Fig. 3).

#### 3.2. Evaluation of the vegetation at experimental sites with contrasting grazing histories

The multivariate variance analysis (MANOVA) showed that the performance of the plant community variables recorded in both study sites was significantly affected (Wilks'  $\lambda=0.21$ ;  $F=104.8$ ;  $P=2.06 \times 10^{-19}$ ;  $df=12, 27$ ) by the contrasting grazing histories.

There was a negative effect of continuous feral horse grazing on the species richness in the mountain grassland of ETPP. Out of a total of 120 species recorded in our study, 53 were present in the grazed site and 67 in the ungrazed site, which showed a significant difference (Table 1). Species diversity, evenness and percentage of exotic species also were associated with contrasting histories of grazing by feral horses in these mountain grasslands. Significantly higher species diversity and evenness were recorded for the ungrazed site in comparison with the grazed one (Table 1), whereas the percentage of exotic species was significantly higher in the site under long-term continuous grazing by feral horses (Table 1).

The classification obtained using growth form and life cycle attributes of all species recorded resulted in eight functional groups: grasses and graminoids (including annual and rhizomatous perennial grasses and other monocotyledonous species), perennial tussock (caespitose) grasses, perennial herbs, annual herbs, shrubs, rosette and prostrate herbs. The relative importance of the different plant functional groups varied between experimental sites. The abundance of shrubs, rosettes and prostrate herbs was significantly higher in the



**Fig. 3.** Height (cm), main root length (cm), above-ground biomass (mg) and below-ground biomass (mg) ( $\pm$  SE) of *P. halepensis* seedlings in plots with the following treatments: grazed site and undisturbed soil (GS-CS), grazed site and disturbed soil (GS-DS), ungrazed site and herbaceous vegetation moderately defoliated (UG-MD) and ungrazed site and herbaceous vegetation heavily defoliated (UG-SD). Columns followed by the same letter are not significantly different (Tukey test at  $p < 0.05$ ).

**Table 1**

Mean species diversity (Shannon index), evenness (Pielou index) and percentage of bare ground and exotic species, and grass and herb above-ground biomass ( $\text{g m}^{-2}$ ) ( $\pm$  SE) ( $n=20$ ) in grazed and ungrazed experimental sites. Values followed by the same letter are not significantly different ( $p > 0.05$ , Tukey test) for each variable.

	Grazed site	Ungrazed site
<b>Species richness</b>	23.0 $\pm$ 0.60b	37.50 $\pm$ 0.50a
<b>Diversity</b>	1.90 $\pm$ 0.05b	3.00 $\pm$ 0.04a
<b>Evenness</b>	0.68 $\pm$ 0.02b	0.97 $\pm$ 0.01a
<b>Exotic species (%)</b>	17.0 $\pm$ 1.50a	5.80 $\pm$ 0.30b
<b>Bare ground (%)</b>	44.0 $\pm$ 0.03a	5.00 $\pm$ 0.01b

grazed than the ungrazed site, whereas for grasses and graminoids and tall perennial grasses it was lower (Table 2). The abundance of other perennial and annual herbs was similar between sites.

The above-ground biomass of grasses measured in the ungrazed site was 10 times higher ( $t=6.74$ ;  $p=0.002$ ;  $df=4$ ) than that measured in the grazed site ( $133.0 \pm 17.10$  vs  $12.5 \pm 2.2 \text{ g m}^{-2}$ ). Also significant differences ( $t=3.64$ ;  $p=0.022$ ;  $df=4$ ) were detected in herb biomass between experimental sites ( $12.0 \pm 5.05$  vs  $44.5 \pm 19.30 \text{ g m}^{-2}$  in grazed and ungrazed sites, respectively).

#### 4. Discussion

Our results provide strong evidence to support the hypothesis that a combination of direct and indirect effects generated by long-term feral horse grazing enhanced the establishment of *P. halepensis* seedlings. Direct effects of feral horses on ETPP grassland, such as the reduction of above-ground herbaceous cover and lower grass biomass with a consequent increase in

the percentage of bare ground, diminished the competitive interaction between the resident vegetation and the pine seedlings (Gurevitch et al., 2000), generating favourable microsites for seedling emergence and survival, as has been suggested for other invasive woody species (Augustine and McNaughton, 1998; Lonsdale, 1999; Camarero et al., 2005).

The nature of the microenvironment immediately surrounding the *P. halepensis* seedlings and its effect on the seedlings are of critical importance in determining the permanence and dissemination of this species in the plant community. Conditions of high soil nutrient concentration and full sunlight can stimulate germination in early successional species such as *P. halepensis* and would be expected to enhance their establishment (Broncano et al., 1998). Richardson and Bond (1991) affirmed that pine recruitment is principally determined by interactions between pine seedlings and resident vegetation. According to these authors, pine invasion is most prevalent where competition is limited in the regeneration niche and it occurs more often in habitats where pines differ the most from the dominant vegetation, e.g., in grasslands.

In an exhaustive review about the factors that can precipitate the pine invasion in South America, Simberloff et al. (2010) suggest that propagule pressure is a determinant key, however, we found that at the same level of sowed seeds, the establishment of *P. halepensis* seedlings was encouraged by feral horse activities. Disturbance generated by horses may lead to a reduction of biotic pressures including competition, thus indirectly influencing the susceptibility of communities to pine invasion. The results presented in our study add to the findings of Richardson and Bond (1991), where the introduction of highly intensive grazing coincides with the initiation of pine invasions. Grazing reduces the cover of vigorous grasses and thus competition with pine seedlings. Continuous and frequent feral horse grazing would decrease

**Table 2**

Cover (%) ( $\pm$  SE) ( $n=20$ ) of grasses and graminoids, perennial tussock grasses, shrubs, rosettes, prostrate herbs, annual herbs and perennial herbs in grazed and ungrazed experimental sites.

	Grazed site	Ungrazed site	<i>t</i>	<i>p</i>	df
<b>Grasses and graminoids</b>	58.0 $\pm$ 10.60b	87.50 $\pm$ 20.50a	7.55	< 0.001	36
<b>Perennial tussock grasses</b>	17.50 $\pm$ 5.0b	37.00 $\pm$ 9.50a	6.85	< 0.001	33
<b>Shrubs</b>	16.0 $\pm$ 7.20a	5.70 $\pm$ 2.10b	7.37	< 0.001	22
<b>Rosettes</b>	37.0 $\pm$ 5.50a	12.50 $\pm$ 2.30b	4.90	< 0.001	30
<b>Prostrate herbs</b>	62.0 $\pm$ 6.50a	30.50 $\pm$ 12.50b	5.04	< 0.001	31
<b>Annual herbs</b>	30.0 $\pm$ 12.50a	26.0 $\pm$ 8.50a	0.32	0.755	38
<b>Perennial herbs</b>	41.0 $\pm$ 15.50a	35.00 $\pm$ 8.0a	2.30	0.115	37

*t*: Statistical index value; *p*: probability value; df: freedom degree.

resource uptake by the resident vegetation due to mortality or debilitation of the resident species by heavy defoliation, leaving more resources available for the *P. halepensis* seedlings. In the same way, increased light availability as a result of grass canopy defoliation and reduction of competitive aerial interferences may stimulate the germination and growth of pine seedlings in the grazed site. Similar results were reported by Relva et al. (2010) who found that introduced deer have strong detrimental effects on several abundant native plants, and that deer promote the invasion of non-native conifers. It was also noteworthy that the height and above-ground dry mass of *P. halepensis* seedlings were significantly reduced when they grew in the ungrazed site, diminishing the chance of definitive establishment.

Seedling establishment was also strongly influenced by the intensity of herbaceous defoliation. In the ungrazed site, heavy defoliation of herbaceous cover favoured the survival and early growth of *P. halepensis* seedlings. Moreover, seedling survival was totally suppressed in the undefoliated treatment. According to Coutts (1987), the ability of many woody plants to become established may be related to the rapid development of a root system which enables the plant to access soil moisture at depths not effectively utilized by grasses, even more when drought appear as responsible of temporal failure in pine seedling establishment (Simberloff et al., 2010). Concomitant reductions in leaf area, root biomass and root activity associated with the grazing of grasses may result in greater superficial soil moisture and nutrients which would enhance the establishment of woody seedlings and the growth of shallow rooted species (Gurevitch et al., 2000).

Soil compaction may negatively affect root growth and development, which might contribute to the failure of woody plant establishment (Taylor, 1990; Clarke and Davison, 2001). Moreover, compaction could act as a physical barrier to seedling emergence (Bassett et al., 2005). However, soil compaction did not affect the establishment of *P. halepensis* seedlings that grew in the grazed site, which had a greater root length and underground biomass than those that emerged in the ungrazed site, quite independently of the soil treatment. This differential growth would have improved the efficiency of soil resource uptake by the pines compared with that of the herbaceous vegetation, ensuring their establishment. Hobbs and Huenneke (1992) and Wiser and Allen (2006) reported that the trampling of large herbivores can favour the growth of exotic woody plants and increase the rate of incorporation of nutrients into the soil and also alter their distribution in the plant community. These changes in the availability of resources constitute a trigger factor for the processes of invasion (Davis et al., 2000).

The effect of disturbance on grassland invasibility by pine species acts at different levels, modifying the properties of the system (Higgins and Richardson, 1998). We found that the composition and structure of the grassland of ETPP might be affected by indirect effects related to the presence of feral horses,

favouring the *P. halepensis* establishment. The continuous presence of feral horses was associated with alterations in the functional groups and grassland conditions, with a reduction in plant richness, diversity and evenness, and an increase in the abundance of exotic plants. However, in contrast with our findings, the results reported by Sala et al. (1986), Hongo et al. (1995), Fahnestock and Detling (1999), Sternberg et al. (2000), Rodríguez et al. (2003) and Altesor et al. (2005) showed higher values for plant species diversity and evenness in grazed ecosystems than in those excluded from grazers. These differences might correspond to variations in the intensity and persistence of grazing and in the type of herbivores. There are important differences in the impacts of different herbivore species on grazed communities. Cattle are more selective of specific plant parts or the species that they consume, while horses are well adapted to grazing a wider range of species and pasture types (Gudmundsson and Dyrmundsson, 1994). Moreover, feral horses are not managed in a way that would reduce the grazing impact, whereas the grazing of domestic livestock is usually controlled in order to avoid deterioration of the grassland.

The differences in the composition of species and functional groups between the two study sites with contrasting grazing histories might indicate the possible loss of grazing-vulnerable species and the consequent invasion of opportunistic grazing-tolerant species due to the selective grazing of feral horses. In plant communities with a short evolutionary grazing history, such as the Argentine pampas grassland, the response of competitors in the herbaceous canopy to the removal of above-ground biomass dominated by grasses might create an increase in species that are usually subordinated, such as alien or opportunistic plants that displace the dominant vegetation (Westoby et al., 1989; de Villalobos and Zalba, 2010). In the grassland of ETPP long-term continuous and intense grazing by feral horses of their preferred species, e.g. perennial tussock grasses could have reduced the capacity of growth and production of reproductive structures, diminishing their abundance in the grazed grassland. The reduction in perennial grass abundance was at least partially compensated by an increase in shrubs, rosettes and prostrate herbs.

The term 'biotic resistance' is the degree of resistance offered by the resident biota to external factors such as external disturbance and invasion of exotic species (Levine et al., 2004). When the resistance threshold of grassland is exceeded, the diversity declines rapidly as mortality due to grazing becomes a greater force in shaping the composition of the plant community than competitive interactions (Milchunas et al., 1988). Plant richness may play an important role in biotic resistance, as greater native species richness might be expected to decrease nutrient availability and increase competition (Shea and Chesson, 2002). A dense herbaceous cover also helps to prevent the invasion of woody species in grassland (Mazía et al., 2010). Moreover, additional resources (e.g. nutrients from urine and

dung) would be introduced into the plant community by feral horses (Davis et al., 2000). According to Milchunas et al. (1988), the response of inferior canopy competitors to the removal of above-ground grass biomass at relatively high grazing intensities is a decline in diversity and so grazing mortality becomes a relatively greater force in shaping the composition of the community than the relaxation of competitive interactions. In consequence, only species capable of tolerating or avoiding feral horse grazing become dominant in the plant community, resulting in a decline in plant species richness and diversity in the grazed grassland. The reduction of evenness could also be determined by greater dominance of the few species that are tolerant of heavy grazing. Thus, the ETPP grassland could be described as a system constituted by two alternative states, one of them characterized by a grazing-resistant pool of species that increases in periods of high grazing intensity, with low herbaceous cover and perennial grass biomass, which is more vulnerable to *P. halepensis* invasion; and another that shows greater plant diversity in periods of lower grazing intensity, with a set of species that are less resistant to grazing, greater herbaceous cover and above-ground grass biomass, leading to greater resistance to *P. halepensis* establishment.

The determination of ecological traits and interactions that affect invasion success is therefore critical for predicting, preventing, and mitigating the negative effects of biological invasions. Simberloff and Von Holle (1999) established that one of the key types of facilitative interactions related to the invasional meltdown hypothesis appears to be the way animals modify the habitat, such as by grazing, that favours introduced plant species over natives. In our particular case, the relationship described between feral horses and *P. halepensis* in the mountain grassland of ETPP would be a weaker version of meltdown, in which one invader facilitates population survival and/or growth of another invader without evidently receiving any benefit itself (Simberloff, 2006). The experimental evidence summarized in our study could help to demonstrate that invasional meltdown is a mechanism more than a metaphor in invasion processes. Moreover, although some authors still consider invasion meltdown and biotic resistance as mutually exclusive hypotheses (Von Holle, 2005; Ricciardi and Kipp, 2008; La Pierre et al., 2010), in the case of our study, we found that they are complementary processes, which would explain the increasing abundance of *P. halepensis* in natural mountain grassland of Argentina.

Meltdowns might be exacerbated by the introduction of exotic species into native ecosystems by humans, potentially both lowering the capacity for biotic resistance and increasing the potential for positive feedbacks among exotic species. Our analyses suggest that the alteration of grazing patterns by feral horses introduced into natural mountain grassland in the Argentine pampas positively influenced the establishment of *P. halepensis*. The described effects about the interaction between pines and introduced herbivores must be considered as relevant information in reference to current knowledge about the pine invasion process discussed in Simberloff et al. (2010), because these findings have considerable implications for ecosystem conservation. The effect of introduced herbivores often retards ecosystem recovery after disturbance even when control practices on adult and sapling pines are realized successfully, because it might unleash shifts in the composition, function and structure of grassland, modifying entire plant communities towards undesirable conditions that might permanently affect the resilience of the native community. In order to minimize or avoid the risk of woody plant invasion we recommend a full understanding of the mechanisms implicated in the synergetic relationship between exotic ungulates and exotic plants and a strategy of adaptive management designed to include active monitoring actions for the control of feral ungulate activities.

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