

Ungulate browsing on introduced pines differs between plant communities: Implications for invasion process and management

LUCÍA B. ZAMORA-NASCA,^{1*}  M. ANDREA RELVA¹ AND MARTÍN A. NÚÑEZ²

¹*Instituto de Investigaciones en Biodiversidad y Medio Ambiente, CONICET-Universidad Nacional del Comahue, Quintral 1250, 8400 Río Negro, Argentina (Email: luciabzamora@gmail.com); and* ²*Grupo Ecología de Invasiones, Instituto de Investigaciones en Biodiversidad y Medio Ambiente, CONICET-Universidad Nacional del Comahue, Bariloche, Río Negro, Argentina*

Abstract The effects of herbivory on plant invasions are broadly discussed, and many studies have led to widely debated theories. In particular, the effects of herbivores on pine invasion found in different studies vary; in some cases, they controlled their expansion, and in others, they promoted it. On the other hand, vulnerability to invasion by pines differs between community types. Sites with dunes and bare ground are the most heavily invaded, followed by grasslands, while shrublands and forests are least invaded. Because current evidence is mostly observational, some of the varying responses of pine invasions to herbivory should be examined further through replicated experiments. Here, we address experimentally the extent to which preference for the non-native invasive *Pinus contorta* by domestic sheep (*Ovis aries*) depends on the vegetation type. We installed experimental enclosures within two adjacent communities, grassland and shrubland, and in each one, we planted seedlings of *P. contorta* Douglas and established a sheep density typically recommended for the study area. The number of browsed seedlings, the number and type of branches browsed per seedling, the reduction in height and probability of survival immediately after browsing period were recorded. The number of browsed seedlings and damage to the terminal bud were higher in grassland than in shrubland, while the number of browsed branches per seedling was higher in shrubland than grassland. The reductions in height and probability of survival immediately after browsing were similar in both communities. These results show that moderate levels of sheep herbivory could reduce 20% seedling survival in both communities; nevertheless, the damage patterns differ between them. The sheep browsed more substantial number of seedlings in grasslands than in shrublands. However, if sheep find the seedlings, they damage it more in shrublands. These results suggest that experimental studies comparing communities are important for pine invasion management.

Abstract in Spanish is available with online material.

Key words: biological invasion, grassland, *Pinus contorta*, sheep, shrubland, steppe.

INTRODUCTION

Causes and conditions needed for invasion by non-native species are the focus of numerous studies (Lockwood *et al.* 2013a). There are three recognised factors that could determine patterns of invasion success: (i) the number of individuals arriving (propagule pressure); (ii) intrinsic traits of the non-native species (invasiveness); and (iii) the susceptibility of the community to be invaded (invasibility; Lonsdale 1999). Regarding invasibility, since Elton (1958) coined the term ‘biotic resistance’, numerous theoretical and empirical studies have been carried out with variable results, leading to the so-called invasion paradox (Fridley *et al.* 2007). The invasion paradox

refers to the change in direction of the native diversity–invasion relationship as the scale of the study changes, being generally negative at small scales and positive at larger scales. Nunez-Mir *et al.* (2017) hypothesise that the variable results may be due to conditional interactions among the recognised factors that determine invasion success across study scales. For example, it is widely assumed that diverse and structurally complex ecosystems are less invaded (Levine 2000; Naeem *et al.* 2000; Iannone *et al.* 2016). However, some studies suggest, particularly for forest ecosystems, that low invasibility of complex ecosystems may be a result of the traits found in most of the species introduced deliberately (shade tolerance – fast-growing early successional species), major time-lag phenomena, isolation of the patches and distances to the propagule source (Martin *et al.* 2009; Essl *et al.* 2011, 2012). Thus, it must be taken

*Corresponding author.

Accepted for publication March 2019.

into account that biotic resistance is ecosystem-specific and will vary with propagule pressure, the traits of the invasive species and the fitness consequences of the interaction of the invasive species with the contemporary site characteristics and species assemblage.

Community invasibility, both in natural and productive ecosystems, could be determined by many factors like resource availability, habitat productivity, presence of facilitating species, competitive abilities of the resident species, or predation and herbivory (Maron & Vila 2001; Shea & Chesson 2002; Richardson & Pyšek 2006; Nunez-Mir *et al.* 2017). In particular, the effect of herbivory on plant invasions has been widely studied with different species and contexts and has been the subject of broadly debated theories like the Natural Enemies Hypothesis, Biotic Resistance and Enemy Release Hypothesis (Maron & Vila 2001; Keane & Crawley 2002; Lockwood *et al.* 2013a). A meta-analysis revealed that herbivory is one of the three factors that diminish the establishment success or performance of non-native species (Levine *et al.* 2004). Nevertheless, the results vary; in some cases, herbivores promote some invasive plants by avoiding them and eating their competitors, and in other cases, herbivores contribute to biotic resistance by consuming invading plants (Parker *et al.* 2006; Oduor *et al.* 2010; Averill *et al.* 2016). In particular, mammalian herbivory by native and exotic herbivores is a strong modulator of the plant community. Mammalian herbivory could influence, among other attributes, the structure and abundance of plants populations (Huntly 1991; Augustine & McNaughton 1998; Danell & Bergström 2003) and could sometimes promote plant invasions (Simberloff & Von Holle 1999; Knapp *et al.* 2008; Nuñez *et al.* 2008a; Moe *et al.* 2016). Several species of the Pinaceae family, native to the Northern Hemisphere, have been planted in the Southern Hemisphere mainly for forestry purposes, but also for soil erosion control or as ornamentals (Simberloff *et al.* 2010). Some of these species became invasive in many areas of Australia, New Zealand, South Africa and more recently of South America, a process well described in the literature (Richardson *et al.* 1994, 2007; Nuñez *et al.* 2017). Pine species vary in the degree of invasiveness, with differences explained by a set of characteristics, such as small seed mass, short juvenile period and short intervals between large seed crops (Rejmánek & Richardson 1996). However, many other factors influence pine invasion, such as propagule pressure (Pauchard *et al.* 2016), abiotic factors (Nuñez & Medley 2011) and biotic interactions like mycorrhizal fungi (Nuñez *et al.* 2013), herbivory (Relva *et al.* 2010), seed predation (Nuñez *et al.* 2008b) and vegetation type (Taylor *et al.* 2016).

Previous studies revealed that pine invasion pattern varies according to the invaded plant community; in

increasing order of invasibility are forest and shrubland, then grassland, and finally dunes and bare ground (Richardson *et al.* 1994). From a spatially explicit individual-based model, Higgins and Richardson (1998) observed the same pattern of differential invasibility between communities, but they note the importance of the mechanisms and interactions between plant type, environment type and disturbance regime, since the conclusions are context-specific. However, invasions in open native forest, canopy gaps and short shrubland are frequently observed (Sarasola *et al.* 2006; Simberloff *et al.* 2010; Taylor *et al.* 2016). This pattern could be influenced by multiple factors interacting in a complex manner, such as disturbance regimes (Hobbs & Huenneke 1992; Lonsdale 1999), ground cover, structure and composition of the receiving community (Richardson *et al.* 1994; Taylor *et al.* 2016), and herbivory (Relva *et al.* 2010). Specifically, the effects of herbivores on pines found in different studies vary. In some cases, they promote invasion (Bartolomé *et al.* 2008; Nuñez *et al.* 2008a; Relva *et al.* 2010; Osem *et al.* 2011; de Villalobos *et al.* 2011), and in others, they hinder the invasion (Chauchard *et al.* 2006; Sarasola *et al.* 2006; Boulant *et al.* 2008; Becerra & Bustamante 2009). With respect to cases where herbivores promoted pine invasion, Bartolomé *et al.* (2008) observed that grazing by sheep and cows could be favouring *Pinus sylvestris* L. and *Pinus mugo* Turra invasion in areas of Spain, possibly by lowering competition from nearby vegetation and opening gaps that favour these shade-intolerant pine species. In the same study, *Abies alba* P. Mill. was regulated by the presence of ungulates, as it is much more sensitive to herbivory than the other two species. Nuñez *et al.* (2008a) and Relva *et al.* (2010) observed in Argentina that exotic invasive deer preferred native species and consumption of the exotic Pinaceae *Pseudotsuga menziesii* (Mirb.) Franco and *Pinus ponderosa* Douglas ex Laws. was low. Finally, de Villalobos *et al.* (2011) observed in Pampean grasslands that the combination of direct and indirect effects generated by long-term grazing by wild horses favoured the establishment of *Pinus halepensis* Mill. seedlings. In contrast, Chauchard *et al.* (2006) observed that semi-wild cattle (*Bos taurus* L.) controlled the regeneration of the non-native species *Pinus nigra* subsp. *nigra* Arn. Likewise, Boulant *et al.* (2008) observed a negative effect of grazing on recruitment of *P. nigra* subsp. *nigra* and *P. sylvestris* L. in France, where recruitment was two to four times lower in grazed than non-grazed areas. Moreover, the proportion of pine seedlings near shrubs was higher in grazed than in ungrazed areas, indicating a protective effect of shrubs. Lastly, Becerra and Bustamante (2009) observed that, independently of plant cover, herbivory by exotic vertebrates negatively affected survival of *Pinus radiata*

D. Don in Mediterranean areas of Chile. Probably, these contradictory results may be explained by the variation in herbivore species, grazing pressure, pine species, plant community and the experimental design of these studies.

In this work, we carried out an experiment controlling some of the variables that determine the outcome of plant–herbivore interactions in the invasion success mentioned in the previous paragraph: herbivore type, stocking rate, pine species and resident plant community. In particular, we sought to determine whether the herbivores are promoting or preventing the observed gradient of pine invasion in two systems of Argentinian Patagonia. To this end, we worked with seedlings of *Pinus contorta* of 2 years old. This species is one of the most invasive pine species of the region. We worked with sheep, an exotic herbivore widely distributed in the area (Coronato 2010), and in two characteristically invaded systems (grass steppe and shrub-steppe; León *et al.* 1998; Bran *et al.* 2002). The preference of sheep can vary depending on the vegetation context (Villagra *et al.* 2013). If they prefer to consume more pine seedlings in shrub-steppe communities (henceforth shrubland) than in grass steppe communities (henceforth grassland), they could be favouring the invasion pattern observed of relatively more pronounced invasion in grasslands than in shrublands. By contrast, if sheep select more pine seedlings in grasslands than in shrublands, they could be acting to mitigate the observed gradient of invasion. In previous experiments (Zamora Nasca *et al.* 2018), we observed that sheep could effectively control pine seedlings in grasslands. The knowledge of the differential sheep preference for pine in relation to the vegetation context is key information for pine invasion management.

METHODS

Study area

This research was conducted in the Andean Precordillera, in NW Patagonia on the Fortín Chacabuco ranch, located in Neuquén Province in Argentina (41°0′16.67″S and 71°10′46.62″W). The landscape is composed of mountains, mountain ranges and hills and is crossed by many rivers and streams. The climate is Mediterranean, with 60% of precipitation occurring in autumn and winter. Annual rainfall ranges between 300 and 700 mm per year, and the average annual temperature does not exceed 10°C (Bran *et al.* 2002). The vegetation corresponds to a semiarid Patagonian steppe in the Andean piedmont and forms a mosaic dependent on exposure and soil.

The experiment was conducted in two characteristic Patagonian plant communities, grassland and shrubland (Fig. 1). In grassland, the dominant vegetation is tussock grasses, predominantly *Pappostipa speciosa* and *Festuca pallescens*, with scattered shrubs of *Acaena splendens*, *Senecio bracteolatus* and *Mulinum spinosum*. There are many flood

meadows with *Juncus balticus*, *Poa pratensis* and *F. pallescens*. The vegetation of the shrubland is dominated by *Berberis microphylla*, *Ochetophila trinervis*, *Maytenus boaria* and the introduced *Rosa eglanteria*. Other species are found to a lesser extent: *Discaria chacaye*, *Escallonia virgata*, *Acaena pinnatifida*, *Achillea millefolium* and patches of tussock grasses, predominantly *P. speciosa* and *F. pallescens* (Bran *et al.* 2002; Paramidani *et al.* 2014). For a detailed list of the plant species recorded in each community, their general characteristics and their use as forage by sheep, see Appendix S1.

The ranch also contains plantations of *P. contorta* and *P. ponderosa*, and pine has invaded some areas. Many pines that have escaped from plantations show signs of herbivore damage. Beside domestic herbivores (cattle, sheep and horses), invasive non-native herbivores are present, such as red deer (*Cervus elaphus*) and European hare (*Lepus europaeus*), as well as native herbivores such as guanaco (*Lama guanicoe*).

Even though sheep production in Patagonia decreased in recent decades owing to a combination of factors like the worldwide decline of the meat and wool markets, government and social decisions and climatic factors (Teixeira & Paruelo 2006; Coronato 2010), sheep production remains the main agricultural activity in Patagonia, and the main production is the Merino breed. For this reason, sheep are among the most abundant and influential herbivores in several ecosystem processes, such as plant invasions.

Experimental design

To evaluate the effect of browsing on non-native pines by ungulates in different communities, we conducted an experiment exposing seedlings of *P. contorta* to the recommended stocking rate of sheep (*Ovis aries*) on each site in two communities: grassland and shrubland. For these experiments, we selected Merino wethers (castrated male sheep) of the same age, all of whom had a similar initial diet with similar nutritional components and all of whom had been exposed to pines before the experiment. The latter feature is important because individuals may develop a preference for one species or another over time (Squibb *et al.* 1990; Walker *et al.* 1992).

Within each community, we installed five experimental enclosures of 25 × 25 m with a 1.50-m tall fence (each enclosure constituted a replicate). Also, each enclosure had hardware cloth 0.6 m tall buried in the soil to prevent access by other herbivores, such as European hare (*L. europaeus*). Each enclosure received a sheep density typically recommended for the study area (0.2–0.3 wethers ha⁻¹ year⁻¹ for grassland and 0.1–0.2 wethers ha⁻¹ year⁻¹ for shrubland). This density was based on assessment at forage condition of the sites and on the pasture guidelines developed by specialists from INTA (Instituto Nacional de Tecnología Agropecuaria, Argentina; Bonvissuto *et al.* 2008; Siffredi *et al.* 2013). These guidelines are the standard for the region and are widely used by ranchers. Since these guidelines provided stocking rate recommendations per hectare per year and dry forage production per year, we calculated the area and grazing time corresponding to our experimental design. We took into account that wethers consume 0.920 kg of

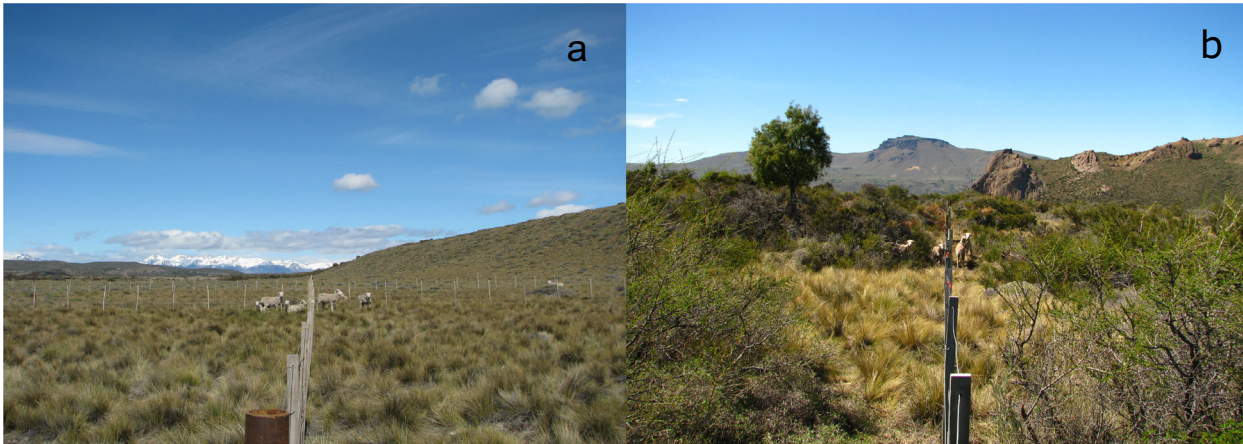


Fig. 1. Photographs of the two plant communities where the enclosures were established. The study area was located in NW Patagonia on the Fortín Chacabuco ranch, Neuquén Province in Argentina (41°0′16.67″S and 71°10′46.62″W). (a) Enclosures in grassland community. (b) Enclosures in shrubland community.

forage daily and that dry forage production per year and per hectare estimated for each community is 200–300 kg for grassland communities and 100–150 kg of grasses and 100–200 kg of new foliage, branches, fruits and flowers of shrubs for shrubland communities. We considered the vegetation use factor (UF), a value corresponding to a proportion of effectively consumable forage that can be grazed by sheep without compromising a sustainable forage production over time (Golluscio *et al.* 2009). The UF assumed for grassland sites was 50%, and the UF assumed for shrubland sites was 30%. Thus, the calculated stocking rate was two wethers in each grassland enclosure for 4 days and two wethers in each shrubland enclosure for 3 days. Keeping the same herbivore pressure in each community would have been problematic, because we know that forage availability differs, so we regulated for no overgrazing, resulting in sheep present for different numbers of days. We realise that our experiments are limited by the fact that sheep were kept in small plots, which is not ideal because of the variability in plant forage production per year and because it could change sheep feeding behaviour. However, we believe this work nevertheless provides a tool to carry out replicated experiments with detailed analyses that are needed in this type of study. The experiment was conducted in spring 2016, the season with the maximum availability and quality of forage in each community, minimising the risk of overgrazing. Also, we established the enclosures adjacent to each other, and no sheep were alone, so as not to alter their gregarious behaviour. A similar design was used successfully in a previous study in the region (Zamora Nasca *et al.* 2018).

Similar environmental conditions and forage availability occurred in the enclosures, thus each enclosure can be considered a replicate. In each enclosure, we planted 40 seedlings of *P. contorta* (35.8 SD \pm 7.9 cm height) in a systematic way; these came from a greenhouse where they had been grown under identical conditions. The seedlings were less than 2 years old (20 months old), because it has been suggested that before that age, they are more vulnerable to herbivores (Crozier & Ledgard 1990). The average initial branches per seedling (main branch plus side branches) were

4.52 \pm 0.18 (mean \pm SE) in grassland and 3.99 \pm 0.15 (mean \pm SE) in shrubland. The number of browsed seedlings, damage to the terminal bud, number of browsed branches, maximum height before and after treatment and probability of survival immediately after the treatment were recorded for all seedlings. The probability of survival was estimated based on the assumption that only seedlings with all branches browsed would die. This was a conservative approach given we could not control the seedlings for long term owing to external factors, such as an unusually dry summer and difficulty watering the seedlings properly, and difficult to maintain the seedlings protected from posterior herbivory damages. All seedlings were removed at the end of the experiment.

The source of the seedlings could be a limitation of our experiments because nursery seedlings could have grown in nutritional conditions that rendered them particularly palatable for herbivores. However, the use of nursery seedlings allowed us to work with numerous seedlings with identical characteristics as was necessary for a replicated experiment.

Data analysis

We used generalised linear mixed models (GLMM; Zuur *et al.* 2009; Crawley 2013) to test whether sheep herbivory at the recommended stocking rate affected *P. contorta* seedlings differentially in grassland or shrubland. The models were fitted with enclosure as a random effect to control for possible variation between replicates in adjacent but different enclosures. The predictor variable was community type, a factor with two levels, grassland and shrubland, and we modelled the following response variables: (i) the probability of a seedling being browsed (browsing incidence); (ii) the probability of a terminal bud being browsed; (iii) the probability of survival; (iv) the proportion of browsed branches per seedling (browsing intensity), all with a *logit* link function and binomial error distribution; and (v) the relative reduction in height (initial height – final height/initial height) with a *logit* link function and beta error

distribution. For the analysis of (iv) and (v), unbrowsed individuals were excluded from the data.

The function `glmer` from the R package 'lme4' (Bates *et al.* 2015) was used to fit the probability of a seedling being browsed, the probability of a terminal bud being browsed, the probability of survival and the proportion of browsed branches per seedling. The function `glmmadmb` from the 'glmmADMB' package (Skaug *et al.* 2012) was used to fit the relative reduction in height (initial height – final height/initial height). The R package 'ggplot2' (Wickham 2009) was used for visualisation of the models. R version 3.5.0 was used (R Development Core Team 2018).

RESULTS

On average, pines were consumed more in grassland than in shrubland. The average initial height of the seedlings was 35.97 ± 0.62 cm (mean \pm SE) in grassland and 35.8 ± 0.52 cm (mean \pm SE) in shrubland, while the average final height of the seedlings was 25.55 ± 0.64 cm (mean \pm SE) in grassland and 29.05 ± 0.58 cm (mean \pm SE) in shrubland. The observed proportion of browsed *P. contorta* seedlings (i.e. browsing incidence, number of browsed seedlings/number of seedlings) was 89% in grassland and 56% in shrubland. The probability that a *P. contorta* was browsed, as estimated by the model, was significantly higher in grassland than in shrubland, 93% and 62%, respectively ($z = -6.1$, $P < 0.05$; Fig. 2a; Table 1). Similarly, the observed proportion of terminal buds browsed was 89% in grassland and 55% in shrubland. The probability that a terminal bud was browsed, as estimated by the model, was significantly higher in grassland than in shrubland, 93% and 60%, respectively ($z = -6.26$, $P < 0.05$; Fig. 2b; Table 1).

Of the subset of browsed seedlings, the observed proportion of browsed branches per *P. contorta* seedling (i.e. browsing intensity, number of browsed branches/number of branches) was 61% in grassland and 70% in shrubland. The proportion of browsed branches per *P. contorta* seedling estimated by the model was 53% in grassland and 64% in shrubland; the differences were statistically significant ($z = 3.45$, $P < 0.05$; Fig. 2c; Table 1).

Of the subset of browsed seedlings, the observed average of relative reduction in seedling height (i.e. initial height – final height/initial height) as a result of sheep herbivory was 32% for grassland and shrubland. The relative reduction in seedling height estimated by the model was similar in both vegetation types: 31% and 29% for grassland and shrubland, respectively ($z = -0.92$, $P = 0.36$; Fig. 2d; Table 1).

The probability of survival of *P. contorta* seedlings immediately after the treatment was similar in both vegetation types: 75% and 78% for grassland and shrubland, respectively. The probability estimated by

the survival model of *P. contorta* in both types of vegetation was 80% ($z = 0.2$, $P = 0.84$; Table 1).

DISCUSSION

Our results show that sheep herbivory can affect the invasion process of *P. contorta* by browsing seedlings both in grassland and in shrubland. Although at the recommended stocking rate the mortality level was not enough to prevent invasion, our previous study shows that medium to high densities (commonly used in some livestock management approaches like silvopastoral practices or holistic management) could prevent pine invasion (Zamora Nasca *et al.* 2018). Treeless communities such as temperate grassland are recognised as being relatively more susceptible to invasion by pines than are diverse and structurally more complex communities (e.g. shrublands; Richardson *et al.* 1994; Rundel *et al.* 2014). Then, the large number of browsed pine seedlings in our grasslands would mitigate their greater invasibility.

Even though the proportion of browsed seedlings in grasslands exceeded that in shrublands, when a seedling was browsed in shrubland, the severity of damage was greater because the sheep damaged more branches per seedling than in grassland. This measure (proportion of branches browsed per seedling) is an important factor in invasion management because low levels of damage retard growth (Zamora *et al.* 2001) but do not guarantee seedling mortality (Crozier & Ledgard 1990). If sheep cannot access an invaded site every growing season, the efficiency of the pine control will be low.

The main factors that diminish the establishment success of non-native species are competition with native plants, species richness of the local community and herbivory by insects and mammals (Levine *et al.* 2004). It is also important to take into account the evolutionary history of grazing of the community and changes in disturbance frequencies (Vila *et al.* 2007; Chytrý *et al.* 2008). However, none of these factors act alone, but rather they all act in combination, and the context-specific characteristics are important (Lockwood *et al.* 2013b). In this study, we observed that herbivory could be an important factor in deterring invasive conifer establishment, but it may not be one of the causes of the observed pattern of invasion (i.e. grasslands more invaded than shrublands and shrublands more invaded than forest), and other factors influence this observed pattern. Recently, Taylor *et al.* (2016) quantified *P. contorta* abundance and size in six invaded ecoregions and observed that the gradient of invasion grassland > shrubland > forests is not so clear because pine establishment differed if the shrubland structure varied. Thus, specific habitat characteristics and biotic factors (e.g. vegetation

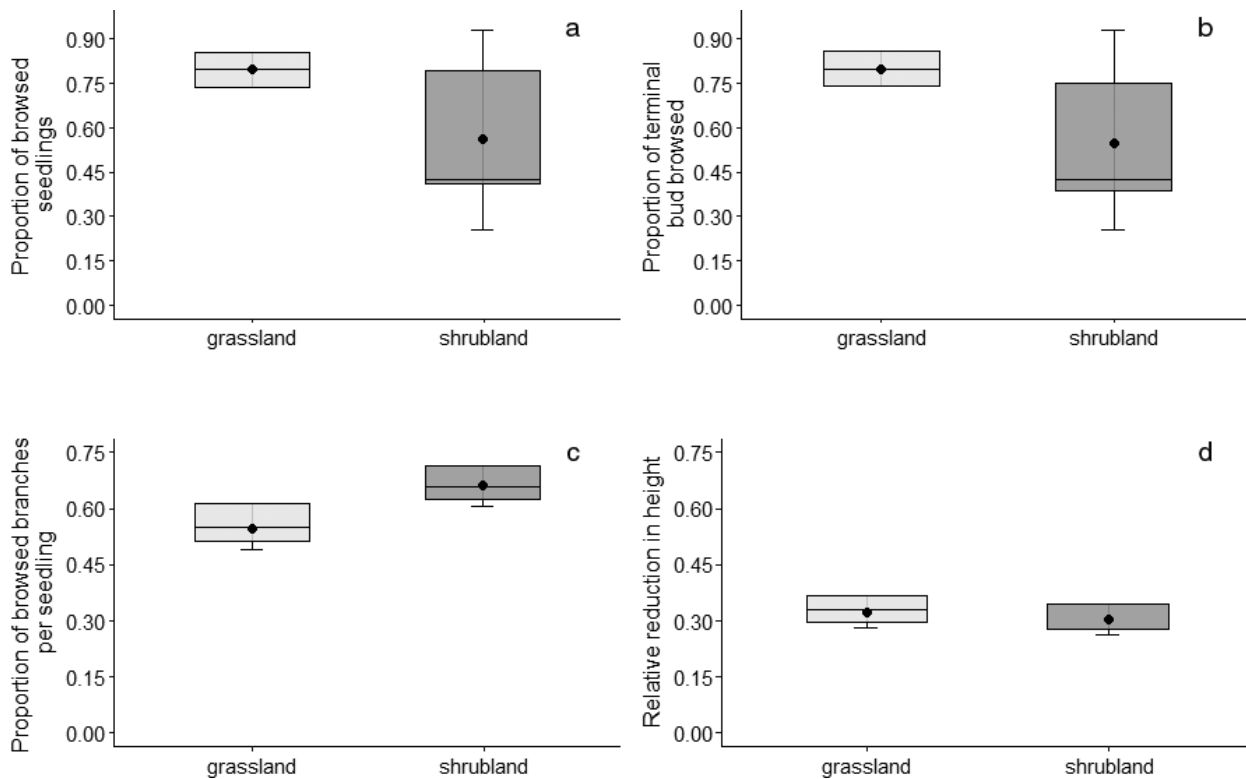


Fig. 2. Herbivory on *Pinus contorta* seedling by sheep in two vegetation types, grassland and shrubland. (a) Estimated proportion of browsed seedlings ($z_{(d.f.: 1)} = -6.1$, $P < 0.05$). (b) Estimated proportion of terminal buds browsed ($z_{(d.f.: 1)} = -6.26$, $P < 0.05$). (c) Estimated proportion of browsed branches per seedling (browsed branches/total branches; $z_{(d.f.: 1)} = 3.45$, $P < 0.05$). (d) Estimated relative reduction in seedling height after treatment. Relative reduction in height = (initial height – final height/initial height) ($z_{(d.f.: 1)} = -0.92$, $P = 0.36$). Black point refers to the mean and black line to the median.

Table 1. Model adjustment measures in relation to the null model of the generalised linear mixed models realised to test whether sheep herbivory at the recommended stocking rate affected *Pinus contorta* seedlings differentially in grassland or shrubland

Model	d.f.	ChiSq	Pr (>ChiSq)
Proportion of browsed seedlings	1	44.913	$2.06e^{-11}$
Proportion of terminal bud browsed	1	47.671	$5.041e^{-12}$
Proportion of browsed branches per seedling	1	12.052	0.0005174
Relative reduction in height	1	0.838	0.36
Probability of survival	1	0.0411	0.8394

ChiSq, chi-square test statistic; d.f., degrees of freedom; Pr (>ChiSq), associated P -value.

type and structure, herbivory, or mutualisms) are important to determine invasion success because the rate of invasion and invasion extent are context-dependent (Chytrý *et al.* 2008; Taylor *et al.* 2016).

Several studies show that herbivory can control plant invasion (Cadenasso *et al.* 2002; Chauchard

et al. 2006; Boulant *et al.* 2008; Becerra & Bustamante 2009; Capó *et al.* 2016). However, herbivore preference will vary depending on the structure and composition of the plant community and the relative presence of palatable plants (Facelli & Temby 2002). There were observed cases where the height of surrounding vegetation affected the discovery rate, severity and extent of browse damage of *P. radiata* seedlings by generalist mammalian herbivores (Pietrzykowski *et al.* 2003). Boulant *et al.* (2008) observed that grazing decreased sapling recruitment rates of both *P. nigra* (non-native) and *P. sylvestris* (native) in grasslands; however, in grazed areas, shrubs favoured their establishment. Taylor *et al.* (2016) found *P. contorta* density varied with vegetation structure. Forested areas and areas dominated by tall shrubs had lower *P. contorta* densities than grasslands, while areas dominated by short shrubs had higher densities than grasslands. If the vegetation surrounding the pines is less palatable than the pines, this could promote herbivory if the herbivore prefers pine, or it could protect the pines by ‘associative resistance’ (Hjältén *et al.* 1993; Gómez *et al.* 2001; Baraza *et al.* 2006; Kuijper *et al.* 2010).

Bertiller and Ares (2008) observed that sheep feeding selection depends first on structural/visual characteristics and later depends on anti-herbivore defences and the presence of preferred plants. An associative resistance process could be acting in shrublands in our study system. Shrubs could be protecting *P. contorta* seedlings by hindering sheep access to seedlings; thus, we observed fewer damaged seedlings in shrublands than in grasslands. This seedling protection could allow pines to surpass the height that renders them immune to herbivory. The different results seen in our study for the variable number of browsed seedlings and the variable browsing intensity highlight the importance of conducting experiments comparing different ecosystems because the invasion pattern between communities is not a clear line; instead, it results from many interactions between variables.

Sheep as controller of plant invasion

Sheep are predominantly grazers, but, based on dental and forestomach characteristics, should have less problems in eating shrubs than browsing grasses (Clauss *et al.* 2008). Thus, because sheep process plants with high fibre content better than other herbivores do, they have been used successfully for weed control (Frost & Launchbaugh 2003). However, several factors must be taken into account regarding this interaction, such as herbivore intensity and the age of pine seedlings. First, regarding herbivory intensity, at low densities sheep select more productive habitats, while at high densities they use different habitats equally (Mobæk *et al.* 2009). Although sheep (at the recommended stocking rate) consumed *P. contorta* in our experiment, the probability of seedling survival immediately after browsing period in both plant communities still exceeded 75%. In contrast, in previous experiments we found moderate to high stocking rates produced high rates of seedling mortality in grasslands (Zamora Nasca *et al.* 2018). Therefore, for effective invasion control, one should apply a grazing schedule that includes high stocking rates for short periods (Butterfield *et al.* 2006; Kurtz *et al.* 2016; Lalampaa *et al.* 2016; Chara *et al.* 2018). Moreover, with respect to the seedling age, seedlings younger than 2 years old are more vulnerable to herbivore damage (Crozier & Ledgard 1990; Smit *et al.* 2006). If the pine exceeds this age, herbivory could still damage the plant and delay its growth (Zamora *et al.* 2001) but would not necessarily kill it. This fact highlights another limitation of the use of sheep as a way to control pine invasions and the need to use them to this end only when pines are very young and not very lignified.

Finally, although we did not investigate this factor, seasonality could affect the intensity of herbivore damage to seedlings. Season is a key factor in herbivore effects on plant establishment for two reasons. One, the nutritional quality of surrounding forage available will vary seasonally (Cavagnaro & Golluscio 2017). Sheep have feeding plasticity according to seasonal variation, complementing their diet with shrubs in autumn or winter according to their physiological needs (Posse *et al.* 1996; Villagra *et al.* 2013). Our experiments were in the spring, with high surrounding forage availability, but the proportions of damage observed could differ in other seasons (and likely be even higher, since pines are evergreen). Two, the degree of damage and the response of the seedlings after browsing also could differ depending on the stage of the pine and the functions of the damaged part of the plant at the time they were removed (i.e. whether was physiologically a sink or a source of carbon; Honkanen *et al.* 1994; Cingolani *et al.* 2005).

Management implications

Invasion by introduced species for commercial purposes often entails a trade-off between the economic return and the negative environmental impact that they could cause; this trade-off should be taken into account at the political, social and economic levels (Richardson 1998). It could be better to plant forestry trees with species vulnerable to ungulate herbivory in early growth stages, since it is possible to control them in the first years of the plantation and also to control subsequent surrounding pine dispersion (Moe *et al.* 2016). McAlpine *et al.* (2016) studied how tree control methods affected subsequent invasive *P. contorta* seedlings establishment and observed that the most efficient method included low intensity of intervention, poisoning the tree but leaving dead trees standing to allow native plants to establish. Also, they observed that excluding introduced mammal herbivores favoured the establishment of pines. These studies suggest that combining other practices (Nuñez *et al.* 2017) and domestic herbivores could possibly control the establishment of pine seedlings. This approach will be more efficient in grassland than shrubland and in areas with a recent invasion, with young seedlings, rather than in areas with many reproductive individuals already established. Our results highlight the complexities of management of biological invasions and emphasise the need for deep ecological knowledge for appropriate management.

ACKNOWLEDGEMENTS

We are grateful to The Nature Conservancy and the Fortin Chacabuco ranch for allowing us to work in

the area, especially to F. Montenegro and N. Rodríguez for the invaluable help. We thank the CONICET technicians P. Alvear and A. Mayoral for their field assistance. We also thank J. Bailey and two anonymous reviewers for valuable comments on earlier drafts that greatly improved the manuscript and D. Simberloff for the detailed English review. This work was supported by a fellowship (LZN) and a grant from Agencia Nacional de Promoción Científica y Tecnológica of Argentina (PICT 2012-2283).

REFERENCES

- Augustine D. J. & McNaughton S. J. (1998) Ungulate effects on the functional species composition of plant communities: herbivore selectivity and plant tolerance. *J. Wildl. Manage.* **62**, 1165–83.
- Averill K. M., Mortensen D. A., Smithwick E. A. H. & Post E. (2016) Deer feeding selectivity for invasive plants. *Biol. Invasions* **18**, 1247–63.
- Baraza E., Zamora R. & Hódar J. A. (2006) Conditional outcomes in plant-herbivore interactions: neighbours matter. *Oikos* **113**, 148–56.
- Bartolomé J., Boada M., Saurí D., Sánchez S. & Plaixats J. (2008) Conifer dispersion on subalpine pastures in northeastern Spain: characteristics and implications for rangeland management. *Rangel. Ecol. Manag.* **61**, 218–25.
- Bates D. M., Machler M., Bolker B. & Walker S. (2015) Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* **67**, 1–48.
- Becerra P. I. & Bustamante R. O. (2009) The effect of herbivory on seedling survival of the invasive exotic species *Pinus radiata* and *Eucalyptus globulus* in a Mediterranean ecosystem of Central Chile. *For. Ecol. Manage.* **256**, 1573–8.
- Bertiller M. B. & Ares J. O. (2008) Sheep spatial grazing strategies at the arid Patagonian Monte, Argentina. *Rangel. Ecol. Manag.* **61**, 38–47.
- Bonvissuto G. L., Somlo R. C., Lanciotti M. L., Carteau A. G. & Busso C. A. (2008) *Guías de Condición para Pastizales Naturales de “Precordillera”, “Sierras y Mesetas” y “Monte Austral” de Patagonia*. Instituto Nacional de Tecnología Agropecuaria – INTA, Buenos Aires.
- Boulant N., Kunstler G., Rambal S. & Lepart J. (2008) Seed supply, drought, and grazing determine spatio-temporal patterns of recruitment for native and introduced invasive pines in grasslands. *Divers. Distrib.* **14**, 862–74.
- Bran D., Ayesa J. & Lopez C. (2002) *Áreas ecológicas de Neuquen*. Instituto Nacional de Tecnología Agropecuaria – INTA, Bariloche.
- Butterfield J., Bingham S. & Savory A. (2006) *Holistic Management Handbook. Healthy Land, Healthy Profits*. Island Press, Washington.
- Cadenasso M. L., Pickett S. T. A. & Morin P. J. (2002) Experimental test of the role of mammalian herbivores on old field succession: community structure and seedling survival. *J. Torrey Bot. Soc.* **129**, 228–37.
- Capó E. A., Aguilar R. & Renison D. (2016) Livestock reduces juvenile tree growth of alien invasive species with a minimal effect on natives: a field experiment using enclosures. *Biol. Invasions* **18**, 2943–50.
- Cavagnaro F. P. & Golluscio R. A. (2017) Structural anti-herbivore defense reduction of two Patagonian spiny shrubs in response to long time exclusion of large herbivores. *J. Arid Environ.*, **142**, 36–40.
- Chara J., Reyes E., Peri P., Otte J., Arce E. & Schneider F. (2018) *Silvopastoral Systems and their Contribution to Improved Resource Use and Sustainable Development Goals: Evidence from Latin America*. CIPAV, Cali.
- Chauchard S., Pille G. & Carcaillet C. (2006) Large herbivores control the invasive potential of nonnative Austrian black pine in a mixed deciduous Mediterranean forest. *Can. J. For. Res.* **36**, 1047–53.
- Chytrý M., Jarošík V., Pyšek P. et al. (2008) Separating habitat invasibility by alien plants from the actual level of invasion. *Ecology* **89**, 1541–53.
- Cingolani A. M., Posse G. & Collantes M. B. (2005) Plant functional traits, herbivore selectivity and response to sheep grazing in Patagonian steppe grasslands. *J. Appl. Ecol.* **42**, 50–9.
- Clauss M., Kaiser T. & Hummel J. (2008) The morphophysiological adaptations of browsing and grazing mammals. In: *The Ecology of Browsing and Grazing* (eds I. J. Gordon & H. Prins) pp. 46–88. Springer, Berlin, Heidelberg.
- Coronato F. (2010) *Le rôle de l'élevage ovin dans la construction du territoire de la Patagonie*. L'Institut des Sciences et Industries du Vivant et de l'Environnement (Agro Paris Tech), Paris.
- Crawley M. J. (2013) Mixed-effect models. In: *The R Book* (ed. John Wiley & Sons) pp. 681–714. Wiley, Chichester.
- Crozier E. R. & Ledgard N. J. (1990) Palatability of wilding conifers and control by simulated sheep browsing. In: *Alternatives to the Chemical Control of Weeds. Proceedings of International Conference, Rotorua, July 1989. Bulletin No 155* (eds C. Basset, L. J. Whitehouse & J. A. Zabkiewicz) pp. 139–43. Ministry of Forestry, Forest Research Institute, Christchurch.
- Danell K. & Bergström R. (2003) Mammalian herbivory in terrestrial environments. In: *Plant – Animal Interactions* (eds C. M. Herrera & O. Pellmyr) pp. 107–31. Plant – Animal Interactions. An Evolutionary Approach, Blackwell Publishing.
- de Villalobos A., Zalba S. M. & Peláez D. V. (2011) *Pinus halepensis* invasion in mountain pampean grassland: effects of feral horses grazing on seedling establishment. *Environ. Res.* **111**, 953–9.
- Elton C. S. (1958) *The Ecology of Invasions by Plants and Animals*. Methuen, London.
- Essl F., Milasowszky N. & Dirnböck T. (2011) Plant invasions in temperate forests: resistance or ephemeral phenomenon? *Basic Appl. Ecol.* **12**, 1–9.
- Essl F., Mang T. & Moser D. (2012) Ancient and recent alien species in temperate forests: steady state and time lags. *Biol. Invasions* **14**, 1331–42.
- Facelli J. M. & Temby A. M. (2002) Multiple effects of shrubs on annual plant communities in arid lands of South Australia. *Austral Ecol.* **27**, 422–32.
- Fridley J. D., Stachowicz J. J., Naeem S. et al. (2007) The invasion paradox: reconciling pattern and process in species invasions. *Ecology* **88**, 3–17.
- Frost R. A. & Launchbaugh K. L. (2003) Grazing for rangeland weed management a new look at an old tool. *Rangelands* **25**, 43–7.
- Golluscio R. A., Bottaro H., Rodano D. R. et al. (2009) Divergencias en la estimación de receptividad ganadera en el noroeste de la Patagonia: diferencias concep-

- tuales y consecuencias prácticas. *Ecol. Austral* **19**, 3–18.
- Gómez J. M., Hódar J. A., Zamora R., Castro J. & García D. (2001) Ungulate damage on Scots pines in Mediterranean environments: effects of association with shrubs. *Can. J. Bot.* **79**, 739–46.
- Higgins S. I. & Richardson D. M. (1998) Pine invasions in the southern hemisphere: modelling interactions between organism, environment and disturbance. *Plant Ecol.* **135**, 79–93.
- Hjältén J., Danell K. & Lundberg P. (1993) Herbivore avoidance by association: vole and hare utilization of woody plants. *Oikos* **68**, 125–31.
- Hobbs R. J. & Huenneke L. F. (1992) Disturbance, diversity, and invasion: implications for conservation. *Ecosyst. Manag.* **6**, 324–37.
- Honkanen T., Haukioja E. & Suomela J. (1994) Effects of simulated defoliation and debudding on needle and shoot growth in Scots pine (*Pinus sylvestris*): implications of plant source/sink relationships for plant-herbivore studies. *Funct. Ecol.* **8**, 631–9.
- Huntly N. (1991) Herbivores and the dynamics of communities and ecosystems. *Annu. Rev. Ecol. Syst.* **22**, 477–503.
- Iannone B. V., Potter K. M., Hamil K. A. D. *et al.* (2016) Evidence of biotic resistance to invasions in forests of the Eastern USA. *Landsc. Ecol.* **31**, 85–99.
- Keane R. M. & Crawley M. J. (2002) Exotic plant invasions and the enemy release hypothesis. *Trends Ecol. Evol.* **17**, 164–70.
- Knapp L. B., Fownes J. H. & Harrington R. A. (2008) Variable effects of large mammal herbivory on three non-native versus three native woody plants. *For. Ecol. Manage.* **255**, 92–8.
- Kuijper D. P. J., Cromsigt J. P. G. M., Jedrzejewska B. *et al.* (2010) Bottom-up versus top-down control of tree regeneration in the Białowieża Primeval Forest, Poland. *J. Ecol.* **98**, 888–99.
- Kurtz D. B., Asch F., Giese M., Hulsebusch C., Goldfarb M. C. & Casco J. F. (2016) High impact grazing as a management tool to optimize biomass growth in northern Argentinean grassland. *Ecol. Indic.* **63**, 100–9.
- Lalampaa P. K., Wasonga O. V., Rubenstein D. I. & Njoka J. T. (2016) Effects of holistic grazing management on milk production, weight gain, and visitation to grazing areas by livestock and wildlife in Laikipia County, Kenya. *Ecol. Process.* **5**, 17.
- León R. J. C., Bran D., Collantes M., Paruelo J. M. & Soriano A. (1998) Grandes unidades de vegetación de la Patagonia extra andina. *Ecol. Austral* **8**, 125–44.
- Levine J. M. (2000) Species diversity and biological invasions: relating local process to community pattern. *Science* **288**, 852–4.
- Levine J. M., Adler P. B. & Yelenik S. G. (2004) A meta-analysis of biotic resistance to exotic plant invasions. *Ecol. Lett.* **7**, 975–89.
- Lockwood J. L., Hoopes M. F. & Marchetti M. P. (2013a) An introduction to invasion ecology. In: *Invasion Ecology* (eds J. L. Lockwood, M. F. Hoopes & M. P. Marchetti) pp. 1–23. Blackwell Publishing, Chichester.
- Lockwood J. L., Hoopes M. F. & Marchetti M. P. (2013b) Disturbance. In: *Invasion Ecology* (eds J. L. Lockwood, M. F. Hoopes & M. P. Marchetti) pp. 99–128. Blackwell Publishing, Chichester.
- Lonsdale W. M. (1999) Global patterns of plants invasions and the concept of invasibility. *Ecology* **80**, 1522–36.
- Maron J. L. & Vila M. (2001) When do herbivores affect plant invasion? Evidence for the natural enemies and biotic resistance hypotheses. *Oikos* **95**, 361–73.
- Martin P. H., Canham C. D. & Marks P. L. (2009) Why forests appear resistant to exotic plant invasions: intentional introductions, stand dynamics, and the role of shade tolerance. *Front. Ecol. Environ.* **7**, 142–9.
- McAlpine K. G., Howell C. J. & Wotton D. M. (2016) Effects of tree control method, seed addition, and introduced mammal exclusion on seedling establishment in an invasive *Pinus contorta* forest. *N. Z. J. Ecol.* **40**, 302–9.
- Mobæk R., Myrsetrud A., Egil Loe L., Holand Ø. & Austrheim G. (2009) Density dependent and temporal variability in habitat selection by a large herbivore; an experimental approach. *Oikos* **118**, 209–18.
- Moe S. R., Loe L. E., Jessen M. & Okullo P. (2016) Effects of mammalian herbivores and termites on the performance of native and exotic plantation tree seedlings. *J. Appl. Ecol.* **53**, 323–31.
- Naeem S., Knops J. M., Tilman D., Howe K. M., Kennedy T. & Gale S. (2000) Plant diversity increases resistance to invasion in the absence of covarying extrinsic factors. *Oikos* **91**, 97–108.
- Nuñez M. A. & Medley K. A. (2011) Pine invasions: climate predicts invasion success; something else predicts failure. *Divers. Distrib.* **17**, 703–13.
- Nuñez M. A., Relva M. A. & Simberloff D. (2008a) Enemy release or invasional meltdown? Deer preference for exotic and native trees on Isla Victoria, Argentina. *Austral Ecol.* **33**, 317–23.
- Nuñez M. A., Simberloff D. & Relva M. A. (2008b) Seed predation as a barrier to alien conifer invasions. *Biol. Invasions* **10**, 1389–98.
- Nuñez M. A., Hayward J., Horton T. R. *et al.* (2013) Exotic mammals disperse exotic fungi that promote invasion by exotic trees. *PLoS One* **8**, 1–6.
- Nuñez M. A., Chiuffo M., Torres A. *et al.* (2017) Ecology and management of invasive Pinaceae around the world: progress and challenges. *Biol. Invasions* **19**, 3099–120.
- Nunez-Mir G. C., Liebhold A. M., Guo Q. *et al.* (2017) Biotic resistance to exotic invasions: its role in forest ecosystems, confounding artifacts, and future directions. *Biol. Invasions* **19**, 3287–99.
- Oduor A. M. O., Gómez J. M. & Strauss S. Y. (2010) Exotic vertebrate and invertebrate herbivores differ in their impacts on native and exotic plants: a meta-analysis. *Biol. Invasions* **12**, 407–19.
- Osem Y., Lavi A. & Rosenfeld A. (2011) Colonization of *Pinus halepensis* in Mediterranean habitats: consequences of afforestation, grazing and fire. *Biol. Invasions* **13**, 485–98.
- Paramidani M., Doffigny C. & Codesal P. (2014) Estudio inicial de Pastizales Ea. “Fortín Chacabuco”. Buenos Aires. Ovis XXI S.A. pp 28.
- Parker J., Burkepile D. & Hay M. (2006) Opposing effects of native and exotic herbivores on plant invasions. *Science* **311**, 1459–61.
- Pauchard A., Escudero A., García R. A. *et al.* (2016) Pine invasions in treeless environments: dispersal overruns microsite heterogeneity. *Ecol. Evol.* **6**, 447–59.
- Pietrzykowski E., McArthur C., Fitzgerald H. & Goodwin A. N. (2003) Influence of patch characteristics on browsing of tree seedlings by mammalian herbivores. *J. Appl. Ecol.* **40**, 458–69.

- Posse G., Anchorena J. & Collantes M. B. (1996) Seasonal diets of sheep in the steppe region of Tierra del Fuego, Argentina. *J. Range Manag.* **49**, 24–30.
- R Development Core Team (2018) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna.
- Rejmánek M. & Richardson D. M. (1996) What attributes make some plant species more invasive? *Ecology* **77**, 1655–61.
- Relva M. A., Nuñez M. A. & Simberloff D. (2010) Introduced deer reduce native plant cover and facilitate invasion of non-native tree species: evidence for invasional meltdown. *Biol. Invasions* **12**, 303–11.
- Richardson D. M. (1998) Forestry trees as invasive aliens. *Conserv. Biol.* **12**, 18–26.
- Richardson D. M. & Pyšek P. (2006) Plant invasions: merging the concepts of species invasiveness and community invasibility. *Prog. Phys. Geogr.* **30**, 409–31.
- Richardson D. M., Williams P. A. & Hobbs R. J. (1994) Pine invasions in the Southern Hemisphere: determinants of spread and invadability. *J. Biogeogr.* **21**, 511–27.
- Richardson D. M., Wilgen B. W. & Nuñez M. A. (2007) Alien conifer invasions in South America: short fuse burning? *Biol. Invasions* **10**, 573–7.
- Rundel P. W., Dickie I. A. & Richardson D. M. (2014) Tree invasions into treeless areas: mechanisms and ecosystem processes. *Biol. Invasions* **16**, 663–75.
- Sarasola M. M., Rusch V. E., Schlichter T. M. *et al.* (2006) Invasión de coníferas forestales en áreas de estepa y bosques de ciprés de la cordillera en la Región Andino Patagónica. *Ecol. Austral* **16**, 143–56.
- Shea K. & Chesson P. (2002) Community ecology theory as a framework for biological invasions. *Trends Ecol. Evol.* **17**, 170–6.
- Siffredi G. L., Boggio F., Giorgetti H., Ayesa J. A., Kropfl A. & Alvarez J. (2013) *Guía para la evaluación de Pastizales. Para las áreas ecológicas de Sierras y Mesetas Occidentales y de Monte de Patagonia Norte*. INTA, Bariloche.
- Simberloff D. & Von Holle B. (1999) Positive interactions of nonindigenous species: invasional meltdown? *Biol. Invasions* **1**, 21–32.
- Simberloff D., Nuñez M. A., Ledgard N. J. *et al.* (2010) Spread and impact of introduced conifers in South America: lessons from other southern hemisphere regions. *Austral Ecol.* **35**, 489–504.
- Skaug H., Fournier D., Nielsen A., Magnusson A. & Bolker B. (2012) Generalized linear mixed models using “AD model builder” **27**, 233–49.
- Smit C., Den Ouden J. & Müller-Schärer H. (2006) Unpalatable plants facilitate tree sapling survival in wooded pastures. *J. Appl. Ecol.* **43**, 305–12.
- Squibb R. C., Provenza F. D. & Balph D. F. (1990) Effect of age of exposure on consumption of a shrub by sheep. *J. Anim. Sci.* **68**, 987–97.
- Taylor K. T., Maxwell B. D., Pauchard A. *et al.* (2016) Drivers of plant invasion vary globally: evidence from pine invasions within six ecoregions. *Glob. Ecol. Biogeogr.* **25**, 96–106.
- Texeira M. & Paruelo J. M. (2006) Demography, population dynamics and sustainability of the Patagonian sheep flocks. *Agric. Syst.* **87**, 123–46.
- Vila M., Pino J. & Font X. (2007) Regional assessment of plant invasions across different habitat types. *J. Veg. Sci.* **18**, 35–42.
- Villagra E. S., Pelliza A., Willems P., Siffredi G. & Bonvissuto G. L. (2013) What do domestic livestock eat in northern Patagonian rangelands? *Anim. Prod. Sci.* **53**, 360–7.
- Walker J. W., Hemenway K. G., Hatfield P. G. & Glimp H. A. (1992) Training lambs to be weed eaters: studies with leafy spurge. *J. Range Manag.* **45**, 245–9.
- Wickham H. (2009) ggplot2. *Elegant Graph Data Anal.*
- Zamora Nasca L. B., Relva M. A. & Nuñez M. A. (2018) Ungulates can control tree invasions: experimental evidence from nonnative conifers and sheep herbivory. *Biol. Invasions* **20**, 583–91.
- Zamora R., Gómez J. M., Hódar J. A., Castro J. & García D. (2001) Effect of browsing by ungulates on sapling growth of Scots pine in a mediterranean environment: consequences for forest regeneration. *For. Ecol. Manag.* **144**, 33–42.
- Zuloaga F., Morrone O. & Belgrano M. J. (2008) *Catálogo de las plantas vasculares del cono sur (Argentina, sur de Brasil, Chile, Paraguay y Uruguay)*. Missouri Botanical Garden Press, St. Louis.
- Zuur A. F., Ieno E. N., Walker N. J., Saveliev A. A. & Smith G. M. (2009) *Mixed Effects Models and Extensions in Ecology with R*. Springer, Berlin.

SUPPORTING INFORMATION

Additional supporting information may/can be found online in the supporting information tab for this article.

Appendix S1. Details of the list of species on each community type under study.