Historical land use by domestic grazing revealed by the soil seed bank: a case study from a natural semi-arid grassland of NW Patagonia

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Abstract

Livestock can affect the soil seed bank through various mechanisms associated with changes in vegetation. We sampled vegetation and seed banks of Patagonian grasslands, in areas with different historical use by grazing (exclosure, moderate use and high use) to evaluate to what extent changes in vegetation are reflected in the seed bank. We also evaluated the effect of historical grazing on horizontal spatial seed distribution by sampling under shrubs and in between plant gaps. We focused the study on functional groups, and on the palatable *Poa ligularis*, an indicator of grassland status. In general, the proportional changes in composition and abundance of functional groups produced in grazed sectors (relative to each exclosure) were bigger for the seed bank than for the aboveground vegetation. Impacts on seed bank were led by a decrease (moderate use), or total disappearance (high use) of perennial grasses, results clearly reflected by *P. ligularis*. Although shrubs were represented in vegetation, they were undetected in the seed bank through germination, probably due to the lack of the conditions required for breaking seed dormancy. Intensive grazing produced homogenization in seed spatial distribution. Our work revealed a poor contribution of the seed bank to vegetation regeneration at increasing historical use by grazing. We recommend sampling the seed bank when monitoring the conservation status of grasslands to obtain consistent management guidelines.

Introduction

Several anthropogenic disturbances affect vegetation of natural arid and semi-arid ecosystems. One of the most widespread disturbances based on the extent of its consequences is overgrazing by livestock, an important promoter of desertification (Geist and Lambin, 2004; Millennium Ecosystem Assessment, 2005). Overgrazing can affect system integrity, which is in large part mediated by direct impact of animals on the standing vegetation (Schlesinger *et al.*, 1990; López *et al.*, 2013). It involves loss of vegetation cover (Mofidi *et al.*, 2013), reduction or local disappearance of forage species (Hunt, 2001; Pazos *et al.*, 2007), and species and/or functional group replacement (typically grasses by woody plants) (Bertiller and Bisigato, 1998; Cibils and Borrelli, 2005). These changes may indirectly affect soil seed bank composition, abundance and spatial distribution (Sternberg *et al.*, 2003; Pazos and Bertiller, 2008). In turn, alterations in the seed bank status can affect the expression of standing vegetation, generating a positive feedback on system degradation.

The soil seed bank is constituted by all viable seeds present on or in the soil or associated litter (Simpson *et al.*, 1989) and is considered crucial for the persistence of many species after aboveground community disturbances (Simpson *et al.*, 1989; Fenner and Thompson, 2005). Livestock can affect size and composition of seed bank through various mechanisms associated with changes in vegetation. On one hand, the decrease in plant cover due to intense tissue removal (in particular reproductive tissues) may affect the amount and quality of seed supply (Bullock *et al.*, 1994; Sternberg *et al.*, 2003), with negative consequences on the seed bank recharge of the consumed

plants. Accordingly, seed bank abundance of the most palatable species can be particularly reduced. On the other hand, structural changes in the environment associated with degradation by grazing can affect the quantity or quality of microsites (Kinloch and Friedel, 2005a; López *et al.*, 2013). This may reduce the number of microsites available to retain seeds (Kinloch and Friedel, 2005a), decreasing the possibilities of incorporating them into the soil seed bank. Hence, domestic livestock may blur the patterns of horizontal seed distribution set in the absence of grazing. The effects of grazing on the soil seed bank are not unique for all natural grassland communities (Kinloch and Friedel, 2005b); instead, they can be strongly shaped by the historical grazing intensity and duration and by the species-specific responses to this disturbance.

A large portion of the temperate grasslands of South America is located in the southern tip of the continent, in Patagonia, Argentina. These natural arid and semi-arid systems have been extensively grazed by domestic livestock (mostly sheep) since the late nineteenth century, and grazing has been so generalized that, at present, there are no ungrazed areas (Paruelo *et al.*, 2006; Michelson, 2008). Over time, evidence of desertification associated with grazing has been detected, although the degradation level is variable among vegetation types (Paruelo *et al.*, 2006). In relative terms, sheep impose greater harvesting pressure on vegetation than native herbivores. One of the reasons for this is that sheep are confined to fenced areas, causing repeated stress on the same plants (Paruelo *et al.*, 2006). Many studies have quantified the effects of livestock grazing on native vegetation (e.g. Gittins *et al.*, 2010; Srur *et al.*, 2013); however, soil seed bank responses under different grazing regimes and its relationship to established vegetation have been considerably less explored. This information can be useful as a baseline to monitor rehabilitation potential of overgrazed areas, the resilience of grazed communities to natural disturbances (e.g. droughts and volcanic eruptions), and support actions aimed at increasing the protected areas of the Patagonia temperate grasslands. The latter has been recently proposed by private initiatives (Michelson, 2008) due to the extremely low representation of temperate grasslands ecosystems in national and worldwide protected area systems (Hoekstra *et al.*, 2005; Paruelo *et al.*, 2006).

In this work, we sampled the aboveground vegetation and soil seed bank of natural grasslands of NW Patagonia, in areas with different degrees of historical grazing to evaluate to what extent changes in aboveground vegetation due to different historical use by grazing are reflected in the soil seed bank. We focused our study on functional groups defined by life forms (i.e. shrubs, herbs and perennial grasses) and on the perennial grass *Poa ligularis* Nees ex Steud. This species is the dominant grass of the studied grasslands and has high forage value, so it could be considered a key indicator of domestic grazing impact on grassland status (Velasco and Siffredi, 2009). We also evaluated the effect of grazing history on the horizontal distribution of seeds (considering density and size of seeds) by sampling in two contrasting microsites: leeward to shrubs and gaps (i.e. interpatches areas).

Materials and methods

Study area and sampled sites

The study was carried out in semi-arid grasslands located in the north-western Patagonia region, Argentina. The area belongs to the Extra-Andean Occidental Patagonia district (Godagnone and Bran, 2009). Climate is semi-arid and temperate, characterized by warm and dry summers, and cool and wet winters. Mean annual precipitation is 268·5 mm, of which 70% falls in autumn and winter. Mean annual temperature is 7·7°C; the mean maximum temperature of the warmest month (January) is 15°C, and the mean minimum temperature of the coldest month (July) is 2·1°C. Although the cold season occurs from April to October, frost probability exists throughout the year. Relief is predominantly flat or convex, with shallow soils (30 cm) of sandy loam texture, and neutral pH (Haplargid type; Godagnone and Bran, 2009). Bedrock is mainly composed of metamorphic and plutonic rocks (Godagnone and Bran, 2009). Vegetation cover ranges 40–60% and is dominated by the perennial tussock grasses *Poa ligularis* Nees ex Steud. (dioecious species), and *Pappostipa speciosa* var. *speciosa* (Trin. and Rupr.) Romasch, and by the shrubs *Mulinum spinosum* (Cav.) Pers., and *Senecio filaginoides* DC (Godagnone and Bran, 2009). These natural systems constitute an area of great forage interest, which has been historically used for animal husbandry (mainly sheep under extensive grazing). The forage biomass production for representative steppes in a good preservation state is estimated in 650 kg dry matter ha^{-1} year^{-1} (López *et al.*, 2010). Most of the perennial grass species have a high forage importance (i.e. plants highly consumed by domestic animals), reproduce strictly from seed and form transient seed banks (see Table 1 for bio-ecological traits). The relative importance of dominant perennial grasses is determined by the grazing history (Godagnone and Bran, 2009).

The study area was affected by the fall of volcanic ash emitted after the eruption of the Puyehue–Cordón Caulle Volcanic Complex (40°30′S–72°12′W) in June

Table I Bio-ecological traits of the perennial grasses from the studied semi-arid grasslands. The forage importance of each species is based on their relative level of consumption by sheep.

Species	Seed weight (mg)[A]	Reproductive strategy[B]	Seed bank[C]	Forage importance[D]
Bromus setifolius J. Presl	5·28	Sexual	Transient[1]	High
Festuca argentina (Speg.) Parodi	2·93	Sexual	Transient[2]	Nil
Festuca pallescens (St.-Yves) Parodi	1·85	Sexual	Transient[3]	High
Hordeum comosum J. Presl	3·68	Sexual	Transient[4]	High
Pappostipa speciosa (Trin. & Rupr.) Romasch.	9·22	Sexual	Transient[5]	Intermediate
Poa lanuginosa Poir.	0·10	Asexual	Persistent[6]	High
Poa ligularis Nees ex Steud.	0·16	Sexual	Persistent[7]	High

Data source: [A]Mean value obtained after weighting sixty batches of ten seeds each; [B,D]Velasco and Siffredi (2009); [C1]Rotundo and Aguiar (2005), [C2–7]Gonzalez (2011).

2011 (National Service of Geology and Mining). A fine-textured tephra formed a 3- to 5-cm-thick layer that in the following months was redistributed among microsites by wind and precipitation (Gaitán *et al.*, 2011). The mineralogical composition of the tephra corresponded to abundant volcanic glass and few aluminosilicates (Cremona *et al.*, 2011). This material made a low to very low contribution of nutrients in an available form to vegetation (Cremona *et al.*, 2011). Vegetation and seed bank samplings were performed ca. 1 year after the eruption, and at that time, grasslands were free of sheep as they had been removed soon after the eruption to protect them from the negative effects of ash.

Sampling was performed in the Campo Anexo Pilcaniyeu of EEA INTA Bariloche (41°01′42″S–70°35′21″W), in two sites (6 km apart) that belong to the same vegetation community, each one having two paired sectors with different grazing histories (stocking rate and time). Based on a recent study (López *et al.*, 2013), the sectors within each site were described as: *Moderate historical use* site: a sector with a moderate stocking rate of 0·3 sheep ha^{-1} year for over 22 years (*Grazed*) and a paired 1·5 ha exclosure against large herbivores built over 22 years ago (*Exclosure*); and *High historical use* site: a sector with a high stocking rate of 0·6–0·7 sheep ha^{-1} year for at least 80 years (*Grazed*) and a paired 1 ha exclosure built over 38 years ago (*Exclosure*). According to the results obtained by López *et al.* (2013), among the sectors described above, the one that withstood the highest grazing pressure represented the most degraded state of the community, with a considerable reduction of key ecosystem processes such as recruitment and water use efficiency. Both exclosures were similar in structural and functional attributes and represented a good condition of the grassland community.

Sampling design

In the spring 2012, we sampled the aboveground soil seed bank (September) and vegetation (December) in the grazed sectors and its paired exclosures. Vegetation was sampled in December to include the summer annuals. We identified and measured cover percentage of all vascular plants species, using a 1 m^2 plot (subdivided in 50 × 50 cm quadrats to increase measurement accuracy) placed randomly 20 times in each sampled sector. To estimate the soil seed bank, we sampled different microsites, considering that recruitment is spatially heterogeneous with most recruitment occurring under shrubs (López, 2011). Then, we randomly collected thirty soil samples (each 7·3 cm in diameter and 3 cm in depth) from shrub microsites (leeward) and thirty soil samples from its closest gaps. We gathered a total of 240 soil samples [30 samples × 2 microsite types (shrubs and gaps) × 4 contrasting grazing histories (two grazed sectors and two exclosures)]. On October 2012, soil samples were sieved to remove organic debris and stones and placed in a glasshouse with uncontrolled conditions for 10 months, until seedling emergence had ceased. Temperature inside the glasshouse was ca. 5 °C above outside temperature. The mean outside monthly temperatures (day/night values) for the studied period in Bariloche are 13/2·3°C (October), 15·7/4°C (November), 19/6·9°C (December), 20·4/7·8°C (January), 19·8/7·7°C (February), 18/5·9°C (March), 14·1/3·7°C (April), 10·3/2·1°C (May), 6·9/–0·4°C (June), 7·4/–1·2°C (July) and 8·6/–0·4°C (August) (Meteorological Station, INTA Bariloche). Pots were watered regularly and seedlings were counted and removed weekly. Plants that could not be early identified were transplanted in separate pots and grown until they could be identified. However, among grass species, only *P. ligularis* was identifiable to species level. To weight

seed bank density values obtained from microsites with a differential amount of accumulated ash (see details in next section), we also measured ash depth in twenty randomly selected shrub microsites (leeward) and its closest gaps in each sampled site. Finally, we classified seeds from the seed bank in two seed mass classes (see below) to evaluate the effect of grazing history on the horizontal distribution of different sized seeds. Seed mass data were obtained by weighing ten or sixty batches of ten fresh seeds per species (depending on seed availability), with the exception of two exotic species *Cerastium arvense* L. and *Sonchus asper* (L.) Hill whose seed weight was obtained from Török *et al.* (2013).

Data analysis

We described the community composition for the sectors with different historical use by grazing in each of two grasslands according to functional groups (perennial grasses, shrubs and herbs), species origin (native or exotic) and relative forage value of perennial grasses. To assess the impact of grazing on standing vegetation and soil seed bank, we compared cover and seed density of total vegetation, perennial grasses, *P. ligularis*, shrubs and herbs between grazed and ungrazed sectors within each site, using *t*-tests or Mann–Whitney *U*-tests (independent samples; nonparametric data). To compare seed bank density between microsites that had different amounts of accumulated ash, we weighted each seed abundance value considering the mean ash accumulated in the microsite from where it came, using the following formula:

Weighted seed density$_{gap(Excl)}=$

$$\frac{Seeds{\cdot}m^{-2}*Mean\ ash\ depth_{gap(Excl)}}{(Ash_{shrub(Excl)}+Ash_{gap(Excl)}+Ash_{shrub(Graz)}+Ash_{gap(Graz)})/4}$$

where each weighted value was the result of dividing the product of the seed density in a sample (expressed in square metres) by the mean ash depth of that microsite, divided by the average ash depth considering all the sectors and microsites from each site. In the formula, the terms 'Excl' and 'Graz' refer to 'exclosure' and 'grazed' sectors, respectively, while 'gap' and 'shrub' refer to the sampled microsites. Weighted values of seed bank abundance (i.e. density and density by seed mass classes) were compared between shrub and gap microsites within each sector using Wilcoxon tests (dependent samples; nonparametric data). Seed size was categorized in two classes: 0–0·5 mg and >3 mg. The intermediate seed mass class was excluded from the analysis as it was not found in the site with a moderate historical use, and was poorly represented

(<10%) in the site with high historical use. Ash depth was compared between microsites within each sector using Wilcoxon tests. The significance level was α = 0·05.

Results

The relative representation of the functional groups differed greatly between vegetation and seed bank (Figure 1). Both sites were dominated by perennial grasses in the vegetation and by herbs in the soil seed bank (Figure 1, Table 2). Shrubs were also present in the vegetation of both sites, but they appeared only in the seed bank of the site with high grazing use (Figure 1).

All the shrub seeds belonged to *S. filaginoides* (Asteraceae). Exotic species were mostly annuals (Table 2) and had minor importance in standing vegetation composition, but a large representation in the seed bank especially in the grazed sectors (Figure 2).

In general, proportional changes produced in grazed sectors (relative to each exclosure) were bigger for the seed bank than for the aboveground vegetation (Figure 1). On one hand, the site with moderate use did not show significant changes in total vegetation, perennial grass and shrub cover between the exclosure and the grazed sectors ($U = 190\cdot5$, $t_{38} = 0\cdot41$, and $U = 160$, respectively; $P > 0\cdot05$ for all cases; Figure 3a, c,e), but cover of *Poa ligularis* and herbs diminished under grazing (*P. ligularis* cover: $U = 110$, $P < 0\cdot05$; herb cover: $U = 61$, $P < 0\cdot001$; Figure 3c,g). The grazed sector had a marked decrease in grass seeds especially in *P. ligularis* (grass seeds: $U = 297$, $P < 0\cdot05$; *P. ligularis* seeds: $U = 282\cdot5$; $P < 0\cdot05$; Figure 3c) and an increase in herb seed density ($U = 215$, $P < 0\cdot001$; Figure 3g). Notably, we did not find any shrub seed in the seed bank of the moderate grazing history site (Figure 3e). On the other hand, in the site with high grazing use, we observed no differences in total vegetation, and perennial grass cover between the exclosure and the grazed sectors ($U = 185\cdot5$, and $U = 150$, respectively; $P > 0\cdot05$ for both cases; Figure 3b,d), but there were notable differences in *P. ligularis*, shrub and herb cover (*P. ligularis* cover: $U = 11\cdot5$, $P < 0\cdot001$; shrub cover: $U = 105\cdot5$, $P < 0\cdot05$; herb cover: $U = 0\cdot00$, $P < 0\cdot001$). In the grazed sector, *P. ligularis* abundance diminished dramatically to almost zero cover (Figure 3d), shrub cover increased by almost 200% compared to the exclosure (Figure 3f), and herb cover was half of that found in the exclosure (Figure 3h). Total seed bank abundance decreased in the grazed sector (Figure 3b) due to the absence of grass seeds, and a decrease of herb seeds (Figure 3d,h). However, herb seed bank density was not statistically different to the exclosure ($U = 392\cdot5$,

Table 2 Mean cover percentage (±SE) of the standing vegetation (VEG) and mean seed bank (SB) density (seeds $m^{-2} \pm$ SE) in exclosed and grazed sectors from grasslands with moderate and high historical use by sheep respectively.

Functional group Family and Species	Seed mass (mg)	Moderate historical use				High historical use			
		Exclosure		Grazed		Exclosure		Grazed	
		VEG	SB	VEG	SB	VEG	SB	VEG	SB
Grasses									
Poaceae									
Bromus setifolius J. Presl		1·90 (0·50)		1·60 (0·70)		0·90 (0·23)		0·15 (0·10)	
Festuca argentina (Speg.) Parodi		1·65 (1·10)		2·18 (1·10)		0·10 (0·10)		5·70 (2·0)	
Festuca pallescens (St.Yves) Parodi						0·30 (0·30)		0·03 (0·03)	
Hordeum commosum J. Presl		0·13 (0·10)		0·13 (0·10)		0·18 (0·07)		0·03 (0·03)	
Pappostipa speciosa (Trin. & Rupr.) Romasch.		2·38 (1·0)		9·80 (2·0)		10·85 (2·20)		18 (3·70)	
Poa lanuginosa Poir.				0·28 (0·20)				0·05 (0·10)	
Poa ligularis Nees ex Steud.	0·16	20·25 (2·70)	143 (40)	11·05 (2·40)	8 (8)	18·98 (2·44)	53 (32)	0·13 (0·10)	
Vulpia sp. C. C. Gmell								0·03 (0·03)	
Perennial grasses*	3·67[†]		53 (26)		30 (14)		68 (27)		
Total grasses		26·31	196	25·04	38	31·31	121	24·12	
Shrubs									
Asteraceae									
Nassauvia glomerulosa (Lag. ex. Lindl.) D. Don						0·88 (0·85)		6·10 (2·30)	
Senecio bracteolatus Hook. & Arn.				0·35 (0·40)				0·45 (0·40)	
Senecio filaginoides D. C.	1·0	2·98 (1·0)		0·85 (0·80)		1·25 (0·64)	8 (8)	2·63 (1·70)	15 (10)
Apiaceae									
Mulinum spinosum (Cav.) Pers.		0·78 (0·60)		3·83 (1·90)		2·43 (0·88)		3·68 (1·80)	
Berberidaceae									
Berberis heterophylla Juss. ex Poir.		0·15 (0·10)						0·50 (0·50)	
Cactaceae									
Maihuenia patagonica (Phil.) Britton et Rose								0·20 (0·20)	
Ephedraceae									
Ephedra ochreata Miers		0·18 (0·10)						0·05 (0·10)	
Fabaceae									
Adesmia obcordata Clos		0·08 (0·04)						0·03 (0·03)	
Adesmia volckmannii Phil.		1·35 (0·80)		2·30 (2·0)		0·13 (0·10)		0·70 (0·70)	
Rosaceae									
Tetraglochin alatum (Gill. ex Hook. & Arn.) Kuntze		0·40 (0·30)		2·85 (1·20)		0·03 (0·03)		0·03 (0·03)	
Total shrubs		5·92		7·88		4·72	8	14·37	15

Table 2 (continued)

Functional group Family and Species	Seed mass (mg)	Moderate historical use				High historical use			
		Exclosure		Grazed		Exclosure		Grazed	
		VEG	SB	VEG	SB	VEG	SB	VEG	SB
Herbs									
Amaryllidaceae									
Rodophiala mendocina (Phil.) Ravenna				0·05 (0·03)		0·28 (0·14)		0·05 (0·03)	
Asteraceae									
Hypochaeris incana (Hook. & Arn.) Macloskie						0·03 (0·03)			
Leucheria achillaeifolia Hook. & Arn.		0·44 (0·10)		0·03 (0·03)		0·94 (0·25)			
Perezia recurvata (Vahl) Less.		0·83 (0·30)		0·33 (0·20)		1·43 (0·57)			
<u>*Sonchus asper*</u>‡ (L.) Hill	0·29		8 (8)						
<u>*Triptilion achilleae*</u> D. C.				0·1 (0·05)		0·03 (0·03)		0·43 (0·20)	
Brassicaceae									
<u>*Draba verna*</u>‡ L.	0·016		91 (28)		75 (37)		75 (35)		60 (32)
Boraginaceae									
<u>*Heliotropium paronychioides*</u> A. D. C.	0·18				8 (8)				
Caryopyllaceae									
Cerastium arvense‡ L.	0·15	0·45 (0·20)		0·08 (0·10)		0·63 (0·17)	23 (17)	0·50 (0·20)	8 (8)
<u>*Holosteum umbellatum*</u>‡ L.	0·09		38 (16)		166 (48)		68 (35)		15 (10)
Euphorbiaceae									
Euphorbia collina Phil.				0·10 (0·10)					
Iridaceae									
Olsynium junceum (E. Mey. ex J. Presl) Goldblatt		1·25 (0·30)		0·15 (0·10)		0·20 (0·20)		0·05 (0·03)	
Sisyrinchium arenarium Poepp.				0·05 (0·03)		0·18 (0·13)		0·03 (0·03)	
Polemoniaceae									
<u>*Microsteris gracilis*</u>‡ (Hook.) Greene	1·41	0·03 (0·02)		0·20 (0·10)			8 (8)		
Rubiaceae									
Oreopulus glacialis (Poepp.) Ricardi		1·13 (0·70)							
Santalaceae									
Arjona tuberosa Cav.		0·23 (0·10)		0·10 (0·10)		0·64 (0·16)		0·28 (0·10)	
Scrophulariaceae									
Calceolaria polyrrhiza Cav.	0·04	0·80 (0·20)	166 (41)	0·35 (0·10)	151 (43)	0·63 (0·28)	45 (25)	0·20 (0·10)	45 (17)
Total herbs		5·16	303	2·79	400	4·99	219	1·57	128
Unid dico			8 (8)		8 (8)		23 (17)	0·03 (0·03)	
Total		37·39	507	35·71	446	41·02	371	40·09	143

Unid dico, Unidentified dicotyledon. Seed weight was only indicated for species found in the seed bank. *Seeds of perennial grasses that were not identified to species level.

†Mean seed mass of perennial grasses is reported.

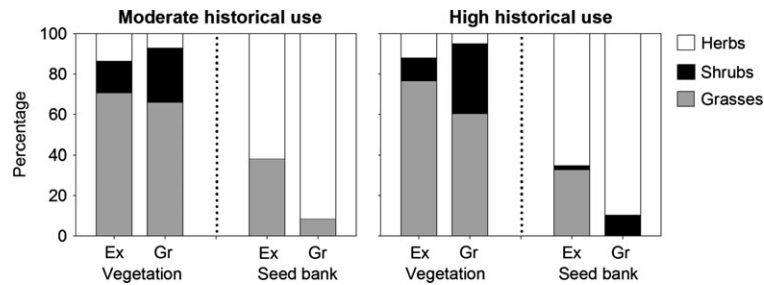‡Exotic species. Annual species were underlined.

Figure 1 Relative representation of the functional groups in the standing vegetation and the seed bank of exclosure (Ex) and grazed (Gr) sectors from grasslands with moderate and high historical grazing pressure. Herbs, shrubs and perennial grasses are indicated in white, black and grey colour respectively.
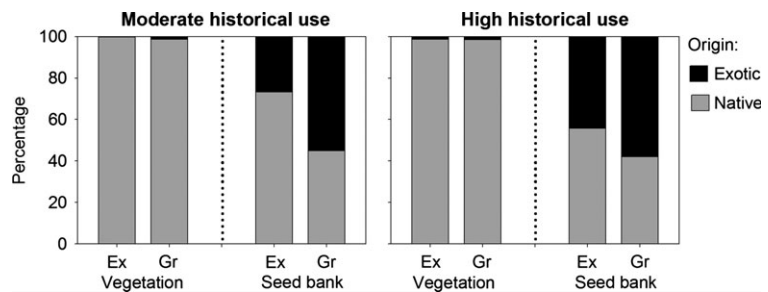
Figure 2 Relative representation of exotic (in black) and native (in grey) species in the standing vegetation and the seed bank of exclosure (Ex) and grazed (Gr) sectors from grasslands with moderate and high historical grazing pressure.

$P > 0.05$; Figure 3h). We found shrub seeds in this site, but in low density in both sectors (7.5 and 15 seeds m^{-2} in the exclosure and grazed sectors – no weighted data, respectively; Figure 3f).

Grazed sectors suffered marked changes in the relative composition of grasses with different forage importance in comparison with the exclosures (Figure 4). This was particularly notable in the sector with high grazing pressure, where grasses with the highest forage importance were non-existent, and grasses of intermediate forage importance were dominant (Figure 4). The category of grasses with nil forage importance comprised only *Festuca argentina* (Speg.) Parodi.

Considering horizontal seed distribution in microsites, in both sites, seed density was greater in leeward of shrubs than in gaps (moderate exclosure: $Z = 3.25$; moderate grazed: $Z = 3.06$; high exclosure: $Z = 2.76$; $P < 0.01$ for all cases), excepting in the grazed sector with a high grazing history where seed density was similar in both microsites ($Z = 0.54$, $P > 0.05$; Figure 5).

There were differences in the horizontal spatial distribution of different sized seeds in most of the studied sectors. Shrubs harboured a greater amount of the smallest seeds than between plant gaps in the site with a moderate historical use (exclosure: $Z = 3.10$; grazed: $Z = 2.98$, $P < 0.01$ for both cases; Figure 6), and also

in the exclosure of the site with a high use ($Z = 2.27$, $P < 0.05$; Figure 6). Only in the exclosure of the moderately used site did shrubs also harbour a larger amount of the bigger seeds than the gaps ($Z = 2.02$, $P < 0.05$; Figure 6). The grazed sector with high use did not contain the bigger seed class nor any *P. ligularis* seeds, and the distribution of the smallest seeds was similar between microsites ($Z = 0.47$, $P > 0.05$; Figure 6).

In both sites, ash depth was greater in the lee of the shrubs than in the interplant gaps (moderate exclosure: $Z = 3.80$; moderate grazed: $Z = 3.91$; high exclosure: $Z = 3.91$; high grazed: $Z = 3.62$; $P < 0.001$ for all cases). Shrubs accumulated an average ash layer of 3–4.4 cm, and gaps accumulated an average ash layer of 0.5–0.8 cm.

Discussion

In the semi-arid grasslands studied, grazing by domestic animals produced greater relative changes in the soil seed bank than in the vegetation. As was shown in Figure 1, composition and abundance of functional groups in the seed bank were proportionately more affected by livestock, compared to the vegetation, with greater changes in the grassland that had experienced higher grazing pressure for a longer period of time (ca. 80 years). The impact on the seed bank was led by a
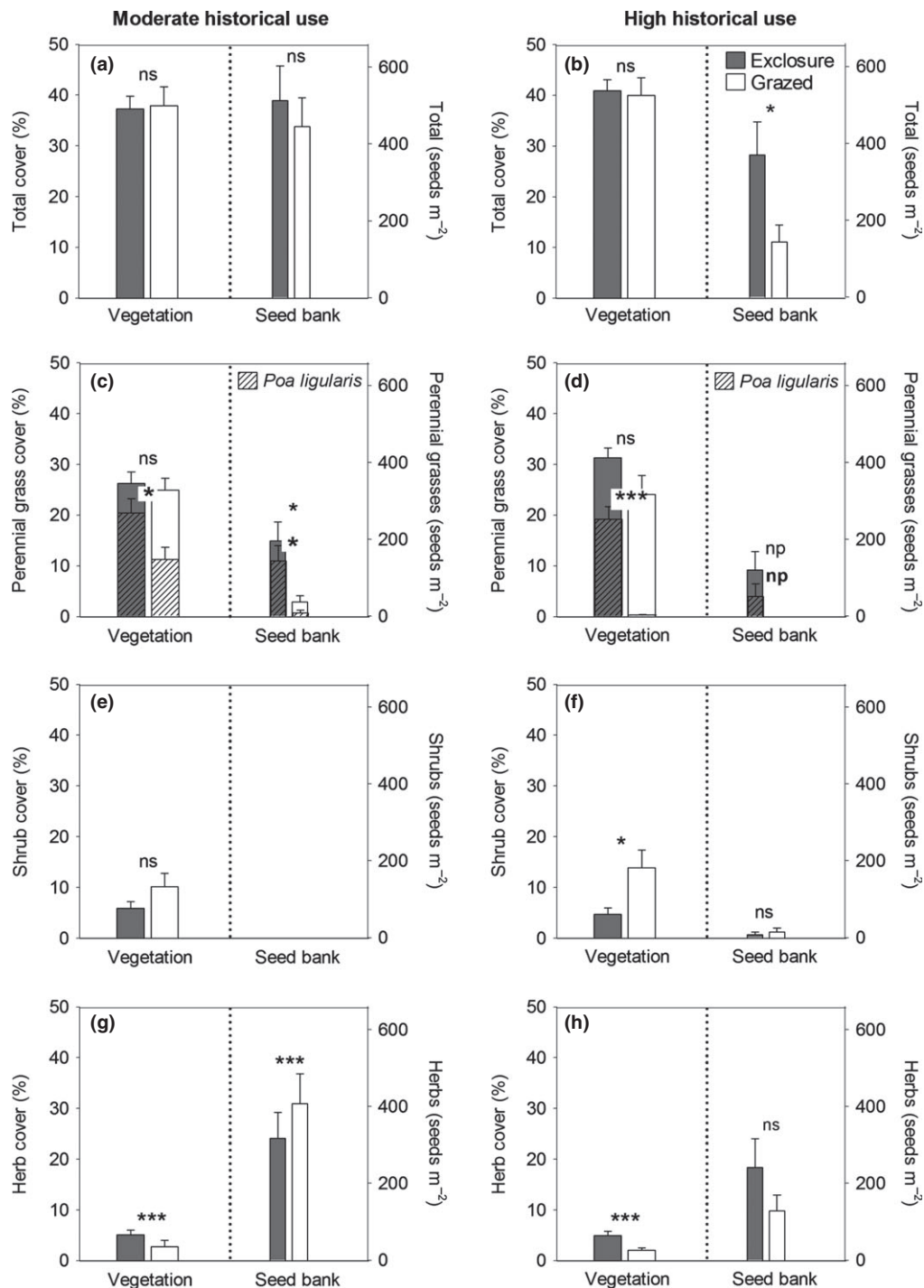
Figure 3 Aboveground vegetation cover (%) and seed bank abundance (seeds m^{-2}) of grasslands with moderate (left column) and high (right column) historical use by grazing in exclosures (grey bars) and grazed sectors (white bars). (a, b) Cover and seed density of total vegetation; (c, d) cover and seed density of perennial grasses; (e, f) cover and seed density of shrubs; and (g, h) cover and seed density of herbs. Symbols in bold indicate the results obtained from *Poa ligularis* comparisons. np: statistical analysis not performed; ns: non-significant differences; * = $P < 0.05$; *** = $P < 0.001$.
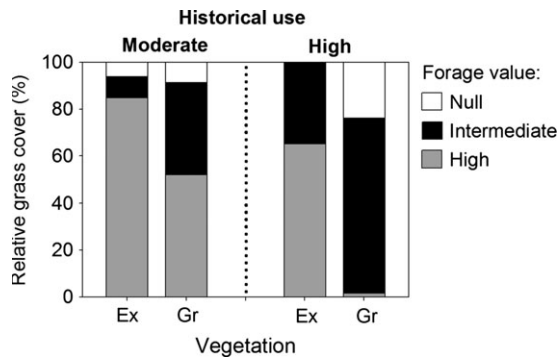
Figure 4 Relative composition (%) of grasses with different forage importance of exclosed (Ex) and grazed (Gr) areas of grasslands with moderate and high historical use by grazing. Grasses with null, intermediate and high forage importance are indicated in white, black and grey colour respectively.

marked decrease (under moderate use), or total disappearance (under high use) of perennial grasses, a result clearly reflected by *Poa ligularis*, the dominant grass in the absence of grazing.

Perennial grasses that dominate perennial grassland communities commonly have a low presence in the seed bank (Rice, 1989). The scarce representation of many perennial grasses can be explained by the relatively big size and flat shape of their seeds which hinders their burial and leads to the formation of transient seed banks (Thompson, 1987; Thompson *et al.*, 1993). As an example, the proportions of seeds in soil of *Pappostipa speciosa* (seed size: 12 mm long and 1 mm wide), a common perennial grass of many areas in Patagonia, have been found to be nil or very low (from <1 to <6·3%, control treatments) in studies performed in different communities (Ghermandi, 1997; Gonzalez and Ghermandi, 2008; Pazos and Bertiller, 2008). Contrary to expectations, perennial grasses from the exclosures studied here made a considerable contribution to the seed bank (30–40%), mostly represented by *P. ligularis* seeds. Unlike most

perennial grass of the community, *P. ligularis* has very small seeds (0·16 mg) which would favour the formation of persistent seed banks and therefore its dominance within the grass fraction of the seed bank.

However, the relative contribution of *P. ligularis* (a dioecious species) to the seed bank greatly diminished in grazed sectors probably due to an impoverishment in seed soil recharge, mediated in part by sex-related mechanisms. In the Patagonian steppe of SW Chubut, Graff *et al.* (2013) found male-biased ratios of this species with increasing grazing intensity derived from a female-biased consumption by domestic herbivores. This biased consumption was a result of a greater distance of females than males from unpalatable competitors, which made the former more visible and exposed to be browsed. This mechanism could explain why, although the moderately grazed sector had half the cover of *P. ligularis* compared to the neighbouring exclosure (11 vs 20% respectively), it had eighteen-fold fewer seeds of this species in the seed bank (8 seeds m^{-2} vs 143 seeds m^{-2}; Table 2). In the same way, micro-environments with low nutrient and water availability in arid steppes of NE Patagonia were better tolerated by male than female individuals of *P. ligularis* (Bertiller *et al.*, 2000, 2002). Accordingly, the reduction in quantity of *P. ligularis* seeds observed in our study could be to a large extent a consequence of sex-biased responses to higher foraging pressure and environmental degradation at increasing grazing intensities.

The studied grassland sites were composed of five to ten shrub species (Table 2), but we only found one shrub species, *S. filaginoides* (Asteraceae), in the seed bank of the site with high grazing pressure. *Senecio filaginoides*, similar to the native *S. bracteolatus* Hook. & Arn. (Gonzalez and Ghermandi, 2012), appears to have no dormant seeds (transient seed bank). Apparently, the method used to estimate the seed bank (seedling emergence) failed in detecting shrub species with dormant seeds. As an illustration, empirical evidence indicates that seeds of *M. spinosum* (one of the dominant shrubs) have morphophysiological dor-
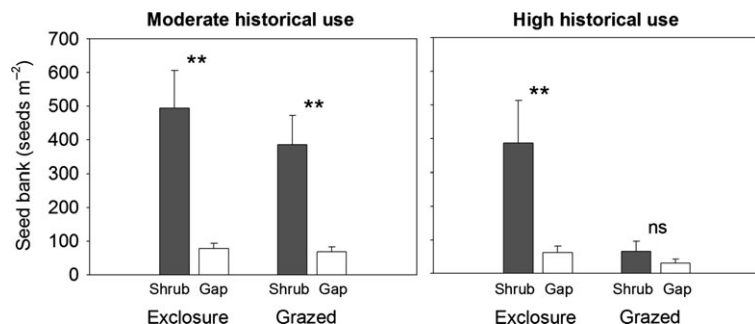
Figure 5 Seed bank density (seeds m^{-2}; weighted values) leeward of shrubs (grey bars) and in interplant gap microsites (white bars) in exclosed and grazed sectors of grasslands with moderate and high historical use by grazing. ns: non-significant differences; ** = P < 0·01.
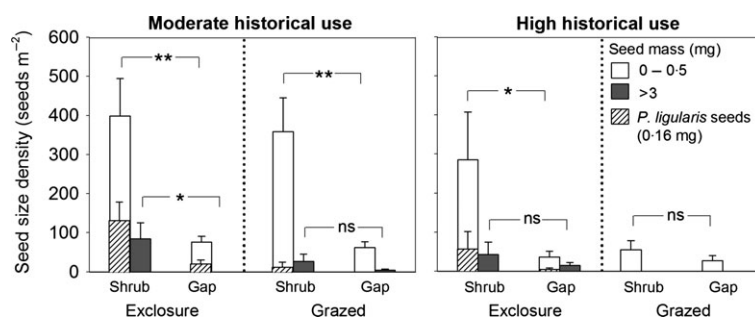
Figure 6 Seed size density (seeds m^{-2}; weighted values) leeward of shrubs and in gap microsites in the exclosure and the grazed area of grasslands with moderate and high historical use by grazing. White bars indicate density of seeds weighing up to 0·5 mg, and black bars indicate density of seeds heavier than 3 mg. *Poa ligularis* values are showed with dashed bars within the smallest seed size category. ns: non-significant differences; * = $P < 0.05$; ** = $P < 0.01$.

mancy (Gonzalez, 2011), a kind of dormancy that has been reported in other species from the Apiaceae family (Baskin and Baskin, 1998; Finch-Savage and Leubner-Metzger, 2006). Seed germination of this species requires time for the embryo to grow and a dormancy-breaking treatment (Finch-Savage and Leubner-Metzger, 2006). In early studies, dormancy of *M. spinosum* seeds was broken after 2 years of seed burial in field or after long periods of laboratory treatments that included cool stratification (Soriano, 1960). No germination occurred after 6 months of seed burial (Soriano, 1960), and germination was nil (Bran, 1982) or low in absence of cold stratification under laboratory conditions (Gonzalez and Ghermandi, 2012). In the present study, it is probable that seeds of *M. spinosum* (and other shrub species) could not be detected through germination due to the absence of the conditions required to promote the breakdown of seed dormancy. This hypothesis acquires relevance when it is considered that in a grassland where *M. spinosum* had a cover similar to that registered in the exclosures studied here, Fernández *et al.* (2002) retrieved 644 seeds m^{-2} of this species from soil samples gathered from gap edges. Unlike our study, they separated seeds manually from soil. Although studies on ecobiology of Patagonian species are increasing, there remains a lack of knowledge of germination ecophysiology for most of the species. Among many other applications, this information can be valuable to understanding soil seed bank responses of grasslands impacted by different land uses.

Water-limited environments display a heterogeneous spatial structure, characterized by vegetated patches interspaced by open areas (Aguiar and Sala, 1999). This heterogeneity is also reflected in the spatial distribution of seeds, whose density is usually greater in vegetated microsites compared with the interplant patch areas (Guo *et al.*, 1998; Kinloch and Friedel, 2005a). In this study, this spatial pattern was observed in the exclosures and in the area that had withstood moderate historical use by sheep. However, intensive grazing produced homogenization in the horizontal spatial distribution of seeds and also in their size structure. The sector with high historical use experienced a substantial decrease of seeds leeward to shrubs, and such densities were similar to seed densities found in gaps and kept only the smaller seed mass class. The reduction in seed entrapment capacity of shrubs in relation to the exclosure could have been determined by a decline in patch connectivity and structural heterogeneity of the community (López *et al.*, 2013) and by a change in the dominant shrub species. A reduced ability of shrubs to trap seeds could be due to differences in size and architecture between the shrub species that dominated the highly grazed sector and its neighbouring exclosure. In the highly grazed area, the short shrub *Nassauvia glomerulosa* (Lag. ex Lindl.) D. Don (5–80 cm high) dominated and, the exclosure was dominated by *M. spinosum*, a spherical cushion shrub that can reach large dimensions (30–300 cm high and 30–300 cm diameter; Table 2).

The studied area was affected by deposits of volcanic ash which was differentially distributed between microsites. Tephra deposits can affect plant functional groups differently, causing greater impact on species that lack underground organs, like annual herbs (del Moral and Grishin, 1999). The absence of annual species from vegetation (mostly exotic species; see Table 2) was probably a consequence of the volcanic ash deposits. The disappearance of annuals after the Puyehue eruption was also observed in other grasslands of the region (Ghermandi and Gonzalez, 2012). In a recent work, Ghermandi and Gonzalez (2012) adjudicated the suppression of annuals to the deposition of a thick ash layer that represented an obstacle to the emergence of the small seedlings produced by annual species. However,

the authors suggested that the disappearance of this functional group from the vegetation would be temporal as they form persistent soil seed banks. In our study, annuals were absent from the vegetation but they were present in great abundance in the seed bank, which is in accordance with the suggestions made by Ghermandi and Gonzalez (2012). Additionally, in semi-arid grasslands of the region, annual plants are typically found in gap microsites (Ghermandi and Gonzalez, 2009); thus, greater ash accumulation leeward of shrubs than in gaps would not mean a major impact on this functional group.

The sector with high historical use had an almost exclusive predominance of the smallest seed mass class (with the exception of *S. filaginoides* seeds; Table 2), mainly represented by the exotic annual herbs *Draba verna* L. (0·016 mg) and *Holosteum umbellatum* L. (0·09 mg), the exotic perennial herb *C. arvense* (0·145 mg), and by the native perennial herb *Calceolaria polyrrhiza* (0·04 mg). The small seed size of these species favours the formation of persistent soil seed banks (Thompson, 1987; Thompson *et al.*, 1993), which ensures their persistence in spite of prolonged and severe disturbance in the environment as it seems was produced by sheep overstocking in the area.

Interestingly, neither total vegetation nor grass cover varied between ungrazed and grazed sectors, independent of their historical use. However, as grazing pressure increased, there were changes in relative abundance of functional groups and also in grass composition. While changes in the relative abundance of functional groups were determined by shrub encroachment and to a lesser extent by diminishing herb cover, changes in grass composition were driven by the replacement of high forage importance grasses by others of intermediate or null value. Shrub encroachment as well as the presence of grasses with little or nil forage importance is indicative of system degradation due to overgrazing. These results lead us to stress how inadequate total or perennial grass cover is as an indicator of the impact produced by domestic animals at the community level, and the importance of determining grass species identities and relative contributions to vegetation when comparing the conservation status of differently grazed areas. Furthermore, the study of species-specific responses to grazing is of importance as generalizations based on species associations cannot often be made (Orre *et al.*, 2005; Franzese and Ghermandi, 2012).

Conclusions

Overall, the results obtained in our work revealed the small size of the native species seed reservoir, changes in seed size structure and seed spatial arrangement, and the poor functionality of the seed bank under increasing domestic grazing pressure. These results would allow us to suggest that in temperate semi-arid grasslands that have endured historically heavy grazing, it would be not possible to rely on the soil seed bank to recover those species that were totally or nearly extinguished from the standing vegetation. Although our work is a study case (with one replicate of each grazing level), we consider that it makes a helpful contribution to the general understanding of the ecological mechanisms that can shape plant communities subjected to different historical use by domestic grazers.

Considering the increase in land degradation in Patagonia (Geist and Lambin, 2004), conservation efforts should be advocated that preserve soil seed bank integrity of grasslands with low or moderate historical grazing use. Management should be aimed at reducing grazing pressure on communities, particularly on palatable species. The generation of knowledge on the germination ecophysiology of native species should be a priority as this information could be used as a baseline to develop *ex situ* forage production alternatives (e.g. through the implementation of innovative methods such as hydroponic crops) or plant nurseries focused on the production of propagules for grassland restoration programmes. These actions, together with a diversification of the economy (e.g. agro-tourism), should be particularly encouraged to mitigate the impact of livestock on temperate natural grasslands of Patagonia. As a final point, because the soil seed bank showed a high sensitivity to grazing, we highlight the importance of sampling when monitoring the conservation status of grasslands, to achieve more knowledge and therefore potentially improve management of these ecosystems.

Acknowledgments

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