

Seed rain and soil seed bank compensatory roles on *Nassella tenuis* (Phil.) Barkworth seedling recruitment in ungrazed and grazed sites

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Abstract: In semi-arid lands, vegetation is distributed in shrub patches immersed in a less vegetated interpatch matrix. Grazing affects perennial grass seed bank through a decrease in seed rain and an increase in seed predation and soil compaction. Nevertheless, some species with anchorage mechanisms in their seeds might overcome this, such as *Nassella tenuis* (Phil.) Barkworth. This is an important species in grazing paddocks because it has an intermediate palatability and its relatively tolerant to grazing. These characteristics allow *N. tenuis* to increase its abundance in grazed sites. Our objective was to assess how grazing affects the key palatable species from seeds to seedlings: i.e., seed rain, soil seed bank, and seedling recruitment in different microsites along a windward-leeward transect across shrub canopy. We hypothesized that: (1) the negative effects of grazing on *N. tenuis* fructification are reflected in its seed rain, soil seed bank, and seedling recruitment, especially in interpatches; (2) *Nassella tenuis* seed rain reduction, soil compaction by cattle in grazed sites, and removal of seeds by wind decrease its soil seed bank, especially in microsites exposed to the predominant wind; and (3) the decrease in *N. tenuis* soil seed bank and cover increase in annual species in grazed sites have negative effects on its seedling recruitment, especially in microsites exposed to predominant wind. We placed seed traps, collected soil samples, and monitored seedling recruitment in different locations around shrub canopy to address our hypotheses. Also, we established a manipulative experiment in which we sow *N. tenuis* seeds and followed its recruitment in different microsites. We compared the seed rain, soil seed bank, natural seedling recruitment, and sown seeds recruitment of *N. tenuis* between grazed and ungrazed sites. We analyzed differences between microsites along a windward-leeward transect across shrubs patches. Seed rain and soil seed bank had the same density in patches and interpatches both in ungrazed and grazed sites. But seed rain was higher, and soil seed bank was lower in ungrazed sites than in grazed sites. Almost all under-canopy microsites showed greater soil seed bank abundance and natural seedling recruitment in ungrazed sites. Sown seeds recruitment was the same between grazed and ungrazed sites, but it showed protective effects of shrubs in leeward microsites under grazed sites. As a conclusion, seed rain and soil seed bank are complementary under grazed sites.

Keywords: seed rain; soil seed bank; seedling recruitment; microsites; grazing

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1 Introduction

Arid and semi-arid lands are highly distributed worldwide, representing 40% of terrestrial space (Cherlet et al., 2018). Due to their low precipitation rate, these regions are unfit for cropping activities, and grazing by domestic livestock is their main land use type (Asner et al., 2004). Several studies have demonstrated the impact of livestock grazing on standing vegetation in different arid and semi-arid regions of the world (Allington and Valone, 2014; Hanke et al., 2014; Eldridge et al., 2016), including the negative effects on some aspects of vegetation reproductive potential (Vázquez and Simberloff, 2004; Paruelo et al., 2008; Pol et al., 2014). Protection from shrub as nurse plants may serve to alleviate some of the detrimental effects of grazing on seed dynamics (Milton and Wiegand, 2001; De la Cruz et al., 2009; Badano et al., 2016; Moreno de las Heras et al., 2016; Funk et al., 2019; Val et al., 2020). But researches that examine how seeds dynamics are affected by grazing and shrub protection together are absent. This is a continuous process that includes seed rain, soil seed bank, and seedling recruitment. A better understanding of the effects of grazing on all the stages is needed to forge adequate management practices that lead to the sustainable use for livestock of arid and semi-arid lands.

The vegetation of northeastern Patagonian Monte is composed of shrublands with associated grasses (Roig et al., 2009). Even though evidence shows that grazing affects several vegetation functional types (shrub, herb, grass, and biological soil crusts) in this system, and the greater impact is suffered by palatable grass species (Peter et al., 2013). As a response, more palatable grasses reduce cover or density as grazing intensity increases, meanwhile, less palatable grass species develop strategies to explore gaps left by the latter (Pazos and Bertiller, 2007). In addition, grazing interferes with perennial grass soil seed bank maintenance through a decrease in seed rain, an enhance in seed predation, and an increase of soil compaction that hinders seed burial (Fuls, 1992; Marone et al., 1998; Leder et al., 2015; Leder et al., 2017).

Nassella tenuis (Phil.) Barkworth is a grass species of intermediate palatability because it has short leaves with trichomes (Kröpfl and Villasuso, 2012; Siffredi et al., 2015). Besides, it increases coverage in grazed sites (Fernández et al., 2009), replacing *Poa ligularis* Nees. Ap. Steudel (Peter et al., 2013). In addition to its resistance to grazing (Pazos and Bertiller, 2007), *N. tenuis* fruits have a long spiraled and geniculated awn, and a sharp tip, which together gives them an anchorage and burial mechanism (O'Connor, 1991; Chambers, 2000). This strategy might allow their seeds to get into the soil, even in soils compacted by cattle (Fuls, 1992), and avoid the high seed predation in grazing sites (Marone et al., 1998). These features would enable this species to form its soil seed bank. Even though *N. tenuis* tolerates grazing and is provided of that mechanism of soil anchorage, evidence showed that the compensatory vegetative growth promoted by grazing decreases the species flowering capacity (Kröpfl et al., 2007).

Because *N. tenuis* itself is distributed across almost all arid and semi-arid Argentinian and Chilean regions (Anton and Zuloaga, 2018), and several grass species of Argentina present this type of seed morphology (Amarilla et al., 2017), we considered it as a good model species to assess the impact of grazing over awned seeds dynamics and seedling recruitment. Nevertheless, a recent study proposes that perennial grasses recruitment is negatively affected by the large pulse of annual plants recruitment that occurs at the same time and at a high rate (Leder et al., 2021).

Patch-interpatch vegetation structure in the Monte system promotes a heterogeneous context for soil nutrients, microclimate, seeds, and plants distribution that generates different microsites around dominant shrubs (Aguilar and Sala, 1999; Bisigato et al., 2009). So, searching merely for patch-interpatch differences is a harsh simplification of this complex system (Caballero et al., 2008). We must take into account changes around shrub canopy structure promoted by grazing and the predominantly west wind that might affect microsites surrounding them (Aerts et al., 2006). In this context, in arid ecosystems, the shrub canopy develops an anisotropic growth and presents higher particle deposition at the leeward side (leaves, seeds, small stems, etc.) (Whitford, 2002). So, we must consider a windward-leeward transect that goes through shrub canopies and establish different microsites along the way (Leder et al., 2017). In addition, different

circumstances accompany seed dispersal, burial, and germination processes; both related to animal and abiotic interactions (predation, wind erosion, drought period, etc.). This enhances the importance of studying all stages regarding awned grasses sexual reproduction. With this complex scenario in mind, the objective of this study was to assess how grazing affects a key palatable species from seeds to seedlings: seed rain, soil seed bank, and seedling recruitment in different microsites along a windward-leeward transect across shrub canopy.

Based on previous studies, we established three hypotheses concerning this species seeds and seedlings dynamics: Hypothesis 1: The negative effects of grazing on *N. tenuis* fructification are reflected in its seed rain, soil seed bank, and seedling recruitment, especially in interpatches; Hypothesis 2: *Nassella tenuis* seed rain reduction, soil compaction by cattle in grazed sites, and removal of seeds by wind decrease its soil seed bank, especially in microsites exposed to the predominant wind; and Hypothesis 3: The decrease of *N. tenuis* soil seed bank and increase in annual species cover in grazed sites have negative effects on seedling recruitment, especially in microsites exposed to predominant wind.

2 Materials and methods

2.1 Study area

The study area was located in the Adolfo Alsina (40°40'S, 64°10'W), Río Negro Province, Argentina. The climate belongs to the dry subtemperate, with warm temperatures in the summer (24°C) and mild in the winter (7°C). This region has high variability in rainfall within and between years, with mean annual value of 255 mm (Godagnone and Bran, 2009). During the sampling period, mean monthly precipitation was slightly higher than the mean for the area, but mean temperature followed historical values (Fig. 1). Soils are Aridisols composed by fine loam, and a moderate northwesterly wind occurs throughout the year (12 km/h) (Godagnone and Bran, 2009). Vegetation in the area is a shrubland steppe corresponding to the Monte Phytogeographical Province, South District, North Patagonia (Roig et al., 2009). This community is characterized as a Zygophyllaceae shrubland with *Prosopis* spp. and *Geoffroea decorticans* (Gillies ex Hook. & Arn.) Burkart as the only tree species (Oyarzabal et al., 2018). The shrub layer is composed of *Larrea divaricata* Cav., *Chuquiraga erinacea* D. Don, and *Condalia microphylla* Cav., and is immersed in a winter-growing grasses layer dominated mainly by *Nassella tenuis* and *Poa* spp.

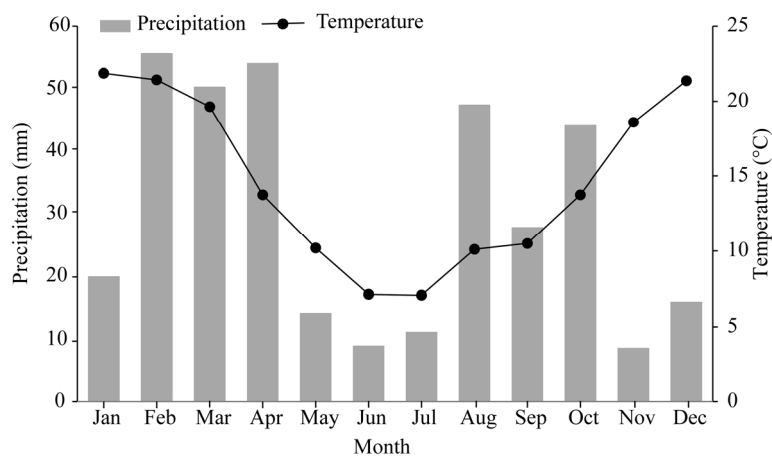


Fig.1 Mean monthly precipitation and temperature during the sampling period

2.2 Sampling design

The study area was a 30 km×10 km unit of Monte vegetation and included two ranches. We selected six paddocks. Three 600 hm² paddocks had a long history of continuous sheep-grazing at the usual rate for the region (0.18 sheep/hm²), and three 1-hm² paddocks were excluded from

grazing by a wire fence for, at least, 10 years. Grazed and ungrazed paddocks were separated by a buffer zone of, at least, 50 m. On each paddock, we used different sampling methods to estimate *N. tenuis* seed rain (SR), soil seed bank (SSB), natural seedling recruitment (NSR), and sown seed recruitment (SSR). To study differences between microsites, we randomly selected shrubby patches with a radius greater than 1 m and nearby interpatches on each paddock. We selected patches with similar size, shape, and specific composition separated for, at least, 10 m.

In each patch, 6 microsites were marked. For the purpose of our study, a microsite was a small soil surface located in a specific position around the shrub patch according to the wind direction. Then, microsites had particular characteristics regarding the predominant wind and the protection of shrub canopy. Then, we selected 6 microsites in each patch along a transect that crossed shrubs patches through the center and followed the predominant wind direction (west-to-east and windward-to-leeward). Microsites were named as follows: windward interpatch (outside the shrub canopy), windward border (at the border of the shrub canopy), windward mid patch (in the middle distance between the border and the trunk), leeward mid patch, leeward border, and leeward interpatch.

2.3 Seed rain sampling

We randomly selected 5 shrubs with a radius greater than 1 m. We placed one seed trap under their canopy and another in its adjacent interpatch, with a minimum separation of 1 m between them. Seed traps consist of 30 cm×30 cm synthetic fabric (wadding) of 1.5 cm thick and were fixed to the ground with an iron frame and nails. We considered that this type of trap was appropriate due to the anchorage mechanism of *N. tenuis* seeds and the density of the fabric.

Seed traps were placed in September 2010 (early spring) and were replaced every two months until August 2011 (late winter). Removed seed traps were placed into paper bags and kept in a freezer (−18°C) for 3 d to prevent seed predation by insects trapped in the wadding. *Nassella tenuis* seeds were manually removed from the wadding and counted. We included in the analysis only potentially viable *N. tenuis*. Those were seeds that resisted the application of a light pressure used with a tweezer, as probed by other authors (Bertiller and Aloia, 1997; Mayor et al., 2003; Tuesca et al., 2004).

2.4 Microsites selection and characterization

To analyze microsites heterogeneity, we randomly selected five shrubs with at least a 1-m radius on each paddock. Then, we identified 6 microsites as described in the sampling design.

On each patch, we took two perpendicular measurements of its width to estimate patch cover, its height, and the distance between microsites. We measured wind speed at opposite microsites at the same time using two portable anemometers placed at 10 cm from the soil surface. We captured ten wind blows on each paired microsite at the same time. Also, we measured the basal cover of three functional groups: biological soil crust, perennial, and annual plants along a 1-m transect at each microsite (perpendicular to the east-to-west central transect). Cover of each functional group was measured as the proportion of the transect intercepted by the base of all individuals of all the species of a functional group.

2.5 SSB

We randomly selected 5 shrubs and established the position of 6 microsites as described above. We took one SSB sample on each microsite using an open-ended soil corer (5 cm in depth and 10 cm in diameter). Samples included litter. Previous studies in similar systems suggested that most of SSB is located at 3 cm deep (Caballero et al., 2008). Samples were taken at the beginning of the autumn (April 2013) after perennial grasses seed rain (Leder et al., 2015) and before its recruitment (Leder et al., 2021). All samples were washed and sieved with a 500-μm sieve, dried on a stove at 50°C, and observed under a stereoscopic microscope. Potentially viable *N. tenuis* seeds were separated and counted. Seeds that resisted the application of a light pressure were considered potentially viable (Bertiller and Aloia, 1997; Mayor et al., 2003; Tuesca et al., 2004).

Because the sampling design for the seed rain was different, we generated the same data design for SSB and NSR doing the media between the two interpatch samples and the media between the four under-canopy samples. Doing so allowed us to analyze the effect of seed rain on SSB.

2.6 NSR

To assess NSR, we randomly selected 5 shrubs and established the position of 6 microsites. Previous to the autumn rains (April 2013), all *N. tenuis* individuals were removed from each microsite. A year later (March 2014), we placed a grid with 10×10 squares of 4 cm² on each microsite to count the number of squares where at least one *N. tenuis* seedling was established as a proxy for recruitment density (seedlings/m²).

2.7 SSR

To test if seedling recruitment differences were a direct consequence of seed bank characteristics, we performed a manipulative experiment sowing a known number of *N. tenuis* seeds. We randomly selected 5 shrubs and established the position of 6 microsites. On each microsite, the first 10 cm of soil was removed in a 30 cm×30 cm plot and replaced with a soil and sand mix (2:1) that was previously sieved to extract propagules. Before the autumn rains (March 2015), 40 seeds of *N. tenuis* were sown on each microsite. Seeds were evenly spread and then covered with the soil mix previously mentioned. We considered the number of seeds sown as appropriate according to the species germination capacity (53.75%) published by Peter et al. (2016) for the same area. Sowed seeds were harvested from different mother plants in the previous fructification season (summer in 2014–2015). A year later (April 2016), the number of seedlings recruited from sown seeds was estimated using a grid with the method previously described for natural seedling recruitment.

2.8 Statistical analysis

We used one-way analysis of variance (ANOVA) to assess the effects of microsites on functional groups cover, SR, SSB, NSR, and SSR. When homogeneity of variance was not met, the nonparametric Kruskal–Wallis test was used. We used *t*-tests to analyze differences between analogous microsites in grazed and ungrazed treatments. Wind speed between opposite microsites, and all grazed and ungrazed sites were tested with Student *t*-tests. All statistical analyses were performed using InfoStat software (Di Rienzo et al., 2011).

3 Results

3.1 Microsites characterization

Patches of grazed sites had taller shrubs ($t = -2.277$, $P = 0.031$) with greater cover ($t = -3.466$, $P = 0.002$) and also larger distances between microsites compared with those of ungrazed sites.

We recorded between two- and six-times higher wind speeds in windward microsites than in leeward microsites, regardless of the treatment (Fig. 2a). All microsites in ungrazed sites had higher perennial grasses and mosses cover than grazed ones, meanwhile, grazed sites showed a higher annual plants cover (Fig. 2b). We also analyzed differences in functional groups cover generating a mean value with data from all the microsites for each treatment, and we found that annual plants cover was three times higher than perennial grass cover in grazed sites (Fig. 2c). Bare soil proportion was smaller in middle microsites than in less protected microsites (Fig. 2b and c).

3.2 SR, SSB, and NSR in patches and interpatches

There were no differences in SR and SSB of *N. tenuis* between patches and interpatches neither in ungrazed nor in grazed sites (Fig. 3). Ungrazed sites had a lower SR but a higher SSB of *N. tenuis* in patches (Fig. 3). At interpatch level, grazed sites showed a higher SR than that of ungrazed sites, but a lower SSB (Fig. 3).

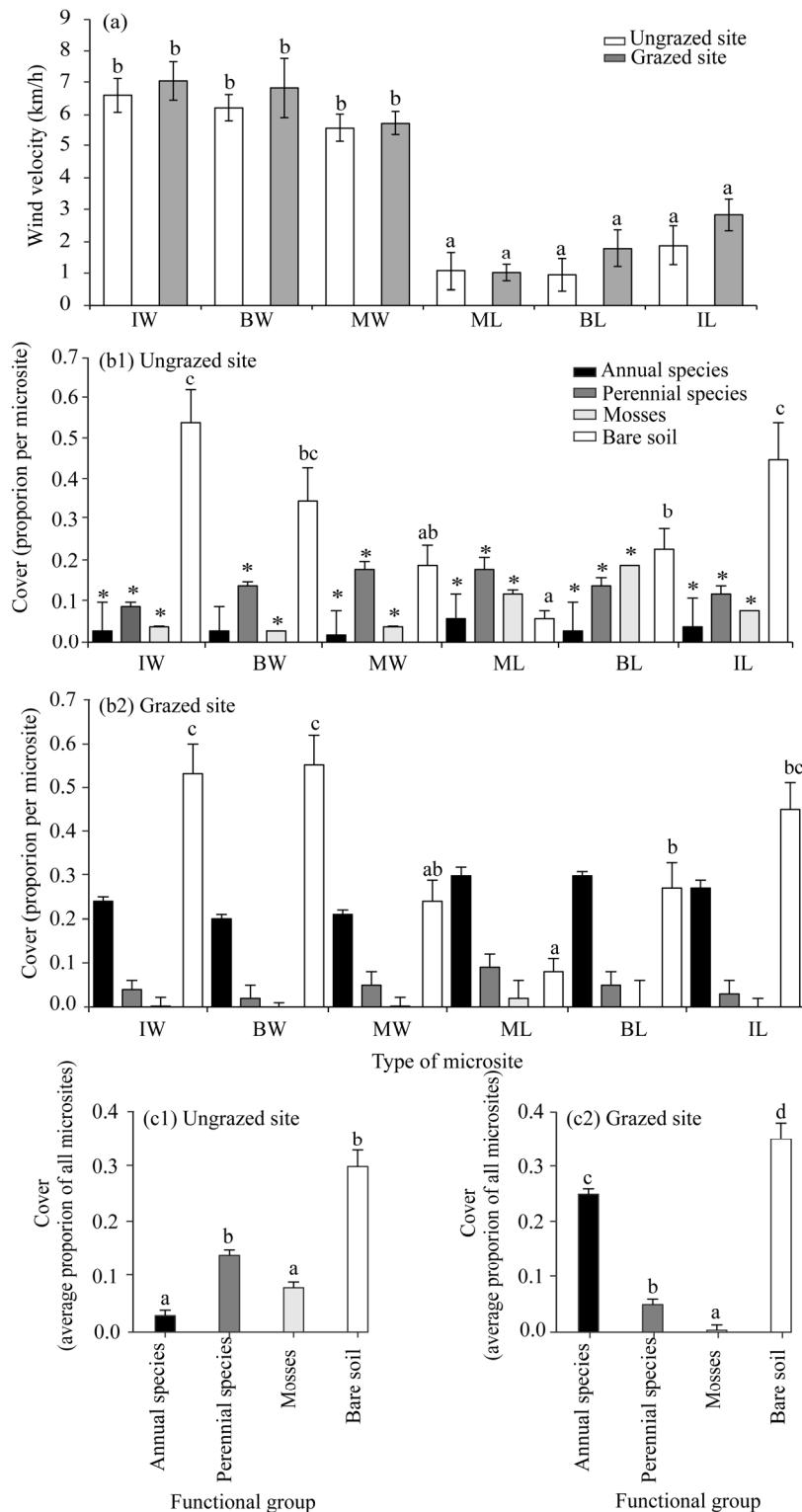


Fig. 2 Wind velocity (a) and vegetation cover (b and c) among six microsites between grazed and ungrazed sites. Stars mean significant differences ($P < 0.05$) between ungrazed and grazed sites, and different lowercase letters indicate significant differences ($P < 0.05$) in vegetation cover between microsites (b) or functional groups (c). IW, windward interpatch; BW, windward border; MW, windward mid patch; ML, leeward mid patch; BL, leeward border; IL, leeward interpatch.

Only ungrazed patches showed differences between SR and SSB of *N. tenuis*, with higher seeds stored in the seed bank than supplied by SR sampled that year (Fig. 3). Interpatches and grazed patches did not show differences between the number of seeds in SSB and the amount supplied by SR during the sampling period. In summary, grazed sites received a higher seed supply than ungrazed sites, but their SSB is lower. NSR did not show significant differences between grazing treatments, nor patches, and interpatches, but it was lower than SSB in ungrazed patches (Fig. 3).

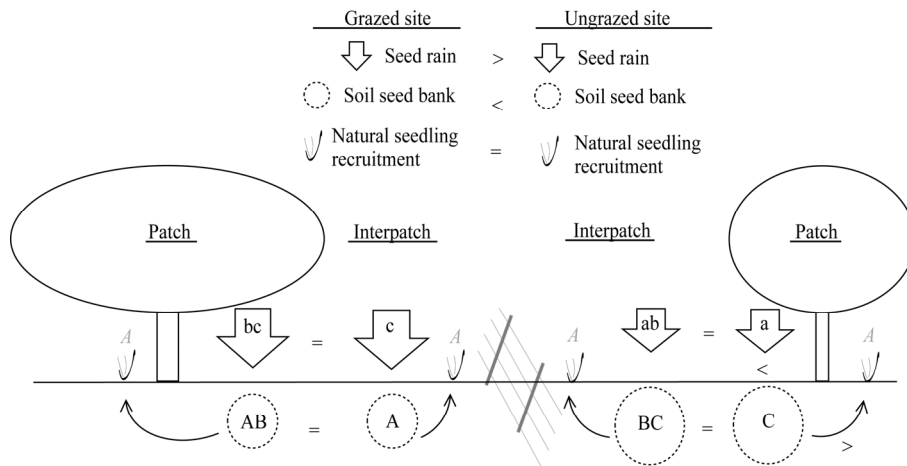


Fig. 3 *Nassella tenuis* seed rain, soil seed bank, and natural seedling recruitment between grazed and ungrazed sites, and between patches and interpatches. Different letters show statistically significant differences ($P < 0.05$) between the combinations of treatment (grazed and ungrazed) and position (patch and interpatch). Lowercase letters: seed rain; capital normal letters: soil seed bank, capital italic letters: seedling recruitment.

3.3 SSB and NSR along a windward-leeward transect

Under *N. tenuis* canopy, microsites showed greater SSB and NSR in ungrazed sites, except at the leeward border (Table 1). SSB was higher than NSR in under-canopy leeward microsites of ungrazed sites ($t = 2.500$, $P < 0.05$).

Table 1 Soil seed bank (SSB), natural seedling recruitment (NSR), and sown seeds recruitment (SSR) of *N. tenuis* under different microsites and treatments

Index	Site	Windward interpatch	Windward border	Windward mid patch	Leeward mid patch	Leeward border	Leeward interpatch
SSB	U	518.1±211.9	390.7±119.0 ^a	424.6±150.6 ^a	925.7±283.0 ^a	424.6±177.0	229.0±82.7
	G	135.9±85.7	17.0±11.6 ^b	127.4±68.1 ^b	178.3±80.4^b	144.4±69.1	119.0±75.1
NSR	U	37.04±14.85	51.85±14.60	71.11±19.30	34.81±7.96	59.26±15.85	76.30±31.70
	G	37.04±19.78	49.63±26.00	22.22±10.34	8.89±4.75	21.48±10.37	44.40±13.40
SSR	U	4.44±2.37 ^A	8.88±3.63 ^A	5.93±5.19 ^{AB}	13.33±4.63 ^B	21.47±8.36 ^{AB}	4.44±1.81 ^A
	G	2.96±1.31 ^{AB}	2.96±1.31 ^{AB}	15.55±9.50 ^A	19.99±5.34 ^B	11.10±4.60 ^B	8.14±3.98 ^{AB}

Note: Different lowercase letters within the same column indicate significant differences between ungrazed and grazed paired microsites at $P < 0.05$ level. Different uppercase letters show significant differences among microsites in ungrazed and grazed sites at $P < 0.05$ level. Bold numbers indicate significant differences between SSB and NSR in that particular microsite and grazing treatment at $P < 0.05$ level. U, ungrazed site; G, grazed site. Mean±SE.

3.4 SSR along a windward-leeward transect

There were no significant differences in SSR between grazed and ungrazed sites. Nevertheless, in grazed sites, leeward mid patch (a highly protected microsite) showed a higher SSR than windward border, and both interpatches microsites (more unprotected microsites) (Table 1). Ungrazed sites showed similar results, with a higher SSR in protected microsites and lower in the most exposed ones.

4 Discussion

4.1 Grazing effects on patch vegetation cover

The negative effects of cattle on perennial grasses and mosses were greatly studied in similar systems (Peter et al., 2013; Funk et al., 2018), and also its positive effects on annual plants development (Beever et al., 2006) agree with our findings. Even though the bare soil surface in patches was similar between grazing treatments, the functional group that 'covered' the rest of the surface was singularly different. Contrasting with ungrazed sites, soil was covered by annual winter species in grazed sites. As a consequence, soil is severely exposed to drought in summer, and in the worst scenario, prone to wind erosion. And the lack of coverage during the hottest and driest season represents a tough microenvironment for a seedling to establish. The increase in shrub size in combination with the dominance of annual plants cover (in detriment of perennial herbs and grasses) is clear evidence of land degradation processes associated with grazing (Beever et al., 2006; Allington and Valone, 2014).

4.2 SR, SSB, and NSR in patches and interpatches

Previous studies showed that SR of perennial grasses decreases with grazing pressure (Leder et al., 2015), but SR of *N. tenuis* was higher in grazed patches. These results disagree with our first hypothesis and show that generalizations based on functional groups are not always appropriate.

In our study region, shrub patches seem to act as seed traps, as proposed in other arid systems (Badano et al., 2016; Val et al., 2020). In this sense, SR and SSB results reinforce the idea of the important effect of secondary dispersal, because ungrazed patches received the lowest amount of SR, but seeds that reach soil surface are buried or secondarily dispersed from interpatches and accumulated underneath shrub canopy. Meanwhile, grazed sites received a higher SR, but seeds are not being incorporated into SSB, which was in agreement with our first hypothesis. Similar results regarding the negative effects of grazing on SSB were registered in other semi-arid systems of the world (O'Connor and Pickett, 1992; Chambers, 2000; DeFalco et al., 2009; Sanou et al., 2018; Val et al., 2020). Along with the possible detriment in seed production as a response to grazing (O'Connor, 1991; Kröpfl et al., 2007; Pol et al., 2014), previous studies in similar systems relate the scarce SSB to an increase in shrub cover on grazed sites (Moreno-de las Heras et al., 2016). So, it is to be determined if the positive seed trap effect that shrubs have is not offset by a negative effect on perennial grasses. This could be explained by the higher seed predation under grazed sites, which diminishes the number of seeds available to enter into SSB (Marone et al., 1998; Wilby and Shachak, 2000) or difficulties for seed burial related to soil compaction by cattle (Fuls, 1992). As a result, SSB of *N. tenuis* is higher under patches in ungrazed sites, compared with grazed ones.

Starting from SSB of different sizes, we were expecting differences in seedling recruitment between grazing treatments and sample positions. The lack of differences could be related to a sparse effect among seedlings. We performed monthly samplings and some seedlings could have emerged and died during that period as a product of competition with other seedlings without our knowledge. If so, it might be a maximum number of seedlings that are able to establish, and it was reached in all treatments and positions. Evidence of this is the fact that SSB under ungrazed patches was the highest, but the number of seedlings recruiting from it was lower concerning the amount of seeds stored, and the same as for the other treatment combinations.

The fact that grazed interpatches had the same recruitment compared with ungrazed patches is odd. The combination of high SR and low SSB on these spots could indicate that most seeds might germinate almost directly from SR, with a short period of anchorage to the soil without forming a stable and persistent SSB. This could represent an important negative consequence of grazing because the species lacks a functional SSB to recover from after intense disturbances on the interpatches. Badano et al. (2016) in a Mexican desert ecosystem and Montecinos-Navarro et al. (2019) in a Mediterranean plant community suggested facilitation effects of shrubs on the plants growing beneath them, this could explain why grazed patches recruit the same amount of seedlings as ungrazed patches. In summary, a negative effect of grazing on seedling recruitment was not found, as proposed for semi-arid South African shrublands (Milton and Wiegand, 2001).

4.3 SSB and NSR along a windward-leeward transect

Overall results show a general pattern. Under-canopy, microsites are more disturbed under grazed sites, especially at the windward side of the shrub. In these microsites, the combination of scarce vegetation cover and high predominant wind exposure might promote conditions that negatively affect SSB and NSR. This causes windward under-canopy microsites to have lower SSB and NSR under grazed sites, compared with these microsites in ungrazed sites, as proposed in our second and third hypotheses. In previous section, we established the importance of secondary dispersal on the formation of SSB. The microsites analysis showed that, as described in other semi-arid systems, seeds are probably dispersed by wind and accumulate at the leeward side of the shrubs (Giladi et al., 2013). This might explain the lack of differences between grazed and ungrazed sites in these protected microsites. On the other hand, SSB results showed a similar pattern as that proposed by Leder et al. (2017) for perennial grasses in general, proving that *N. tenuis* could be used as a model species for its functional group on SSB analysis.

Differences in NSR followed the same pattern as SSB, as another probe of the protective effect of shrub canopy in undisturbed sites. But even though grazed sites had a lower NSR than ungrazed sites, if we analyze its recruitment in the light of its scarce SSB, seeds recruited represent 24% of buried seeds. Meanwhile, in ungrazed sites, 12% of buried seeds reach the establishment. This might indicate that conditions that favor SSB formation or maintenance do not necessarily encourage seedling recruitment. Another possible explanation might be that seeds accumulate in the seed bank at a high rate but do not receive the appropriate germination stimuli and become senescent, so we might overestimate seed bank abundance when it is not fully functional. This could be tested by analyzing the germinable SSB in future studies.

4.4 SSR along a windward-leeward transect

These differences found in SSB and NSR between grazed and ungrazed sites in certain microsites are not found in the results of SSR. So, starting from a standardized seed bank, the opportunities for seedling emergence and establishment are apparently the same between grazing treatments. Microsites, on the other hand, drive different results on seedling establishment. Results obtained from sowed seeds showed a protected effect provided by shrubs that enhance seedling recruitment, unlike other similar studies (Bisigato and Bertiller, 2004; Busso et al., 2012). Protected leeward microsites have more suitable conditions for seedling establishment than unprotected interpatches and windward microsites, regardless of grazing treatment. This indicates that differences between grazed and ungrazed sites previously described are mainly related to the effect of grazing on the formation or maintenance of SSB. Differences in SSB are then reflected on seedling recruitment. So, once seeds reached the soil surface, the final fate of the seedling is determined by the microsite in which is going to anchor and bury. *Nassella tenuis* reproduction under grazed sites is seriously compromised. Even though a large number of seeds are provided by SR, few end up in the seed bank, and fewer reach establishment as seedlings slowly deplete seed bank abundance.

5 Conclusions

Our study, from seeds to seedling, showed that SR and SSB of *N. tenuis* seem to play complementary roles under grazed sites. The first provides a large number of seeds to compensate for seed loss under grazed sites, and the latter offers a big seed reservoir that awaits germination stimuli in undisturbed sites. We establish the importance of a microsite approach for seeds and seedling studies, as some differences between grazing treatments were microsite-dependent. There is evidence of certain protection promoted by shrub canopies on seedling establishment. It will be interesting to perform further studies to identify which under-canopy characteristics encourage seedling recruitment, and if they are affected by cattle activity. *Nassella tenuis*, a species with a large distribution in the Monte system, could be used as a model species for SSB studies, but not for SR studies, as it showed the opposite behavior compared with perennial grasses as a functional group (perhaps because of its different anchorage mechanism).

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