



## Soil seed bank in a subtropical grassland under different grazing intensities

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### ABSTRACT

Grazing is an important determinant for the composition and structure of grasslands; however, soil seed bank (SSB) response to grazing intensity is poorly investigated. We analyzed SSB richness and density in a subtropical grassland in southern Brazil with different forage offers (low, intermediate, high and very high), that is, contrasting grazing intensities. The SSB was evaluated by the seedling emergence method. We collected ten SSB samples at two layers (0–5 and 5–10 cm) in spring and autumn in each of grazing intensity treatments. We surveyed the established vegetation to assess its similarity with the SSB. Treatment effects were analyzed by Poisson regression while compositional differences were visualized by ordination. We found 103 species in the SSB, of which 71 were also found in established vegetation. We found a positive correlation between SSB density and grazing intensity. High grazing intensity influences patterns of composition and dominance in the SSB, while no strong differences were found among the other treatments. The SSB was characterized by low participation of dominant grasses in the vegetation and the dominance of ruderal species, indicating that recovery from the SSB after total removal of vegetation (severe disturbance) may be limited in grasslands in the region.

**Keywords:** disturbance, grassland management, Pampa, plant community dynamics, recovery potential, resilience, transient seed bank

## Introduction

A remarkable characteristic of old-growth grasslands (that is, ancient, biodiverse grassy ecosystems; Veldman *et al.* 2015) is the high resilience of the plant community to endogenous disturbances such as fire and herbivory (Overbeck *et al.* 2005; Buisson *et al.* 2018). Most and especially the dominant species are able to resprout from below-ground gems (bud bank), allowing for quick vegetation recovery after above-growth biomass removal by disturbances such as fire (Overbeck *et al.* 2005; Fidelis & Blanco 2014) or grazing (Rueda *et al.* 2010). However, the

soil seed bank (SSB) also is important in plant community assembly and vegetation recovery as it contributes to the recruitment of new individuals (Bakker *et al.* 1996). The SSB may be referred to as the “memory” of plant populations and can even preserve genotypes that have been absent from established vegetation for a long time (Harper 1977). Often, the SSB is categorized according to persistence of the seeds in the soil (Bakker *et al.* 1996). Baker (1989) considers time (that is, years) as a metric for classification of seed persistence in the soil. Thompson *et al.* (1997), in contrast, classifies SSB persistence based on the vertical distribution (that is, soil layers) of the seeds in the soil,

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also considering the relation of the SSB to above-ground vegetation composition.

Grazing is an important determinant for the composition and structure of grassland vegetation, in consequence of two processes: consumption of plant biomass and trampling by animals (Kinucan & Smeins 1992; Gasparino *et al.* 2006; Lezama *et al.* 2014). In subtropical grasslands in southern Brazil, differences in grazing intensity promote strong changes in vegetation composition, both considering species identity and functional groups (Cruz *et al.* 2010). Areas with high grazing intensity are usually dominated by stoloniferous and/or rhizomatous grasses and herbs (Adler *et al.* 2001). Caespitose grasses and subshrubs characterize less intensively grazed patches, and longer-term abandonment will lead to dominance of species from these groups, mostly as a result of competition for light (Rodríguez *et al.* 2003; Lezama *et al.* 2014). Cattle has high preference for low-growing and more palatable grasses, thus creating a positive feedback mechanism that ensures the dominance of caespitose species and subshrubs under low grazing intensities (Cruz *et al.* 2010). At an intermediate grazing intensity, vegetation structure becomes more heterogeneous and plants with contrasting habit contribute more equally to the plant composition (Adler *et al.* 2001; Nabinger *et al.* 2009). Intermediate stocking rates (that is, animal units per area unit) in general leads to higher plant species diversity and richness and to higher pasture productivity (Overbeck *et al.* 2007; Nabinger *et al.* 2009; Loydi 2019).

Although the responses of above-ground vegetation to varying grazing intensities are already relatively well known – as sketched above – very little is known about its effects on the soil seed bank (SSB) in subtropical grasslands. Information on size and composition of the SSB under different grazing intensities is important to better understand the potential of vegetation recovery after overgrazing (that is, vegetation under intensive grazing for extended periods, without sufficient recovery periods). SSB studies in grazed grasslands can thus contribute to the planning of ecological restoration (see for example, Buisson *et al.* 2018). However, most soil seed bank studies in subtropical grasslands in South America are limited to the comparison of the SSB in grazed areas to that in ungrazed areas (for example, Marco & Páez 2000; Marquez *et al.* 2002; Haretche & Rodríguez 2006). In general, the SSB studies in grazed grassland in South America find a larger SSB density under grazing when compared to ungrazed areas. Few studies evaluated the effects of different grazing intensities on the SSB and on its similarity to above-ground vegetation, difficulting the interpretation of the role of the SSB in vegetation dynamics (Favreto *et al.* 2000; Marco & Páez 2000; Morici *et al.* 2009).

Here, our aim was to explore the effects of distinct grazing intensities on: (i) SSB species composition, richness and density, (ii) similarity of established vegetation and SSB in terms of floristic composition, (iii) seed bank type

according to seed persistence in the soil, and (iv) functional strategies of species in the SSB. Our hypothesis is that SSB composition varies with grazing intensities, corresponding to changes in vegetation composition. We expect to find a higher similarity between SSB and established vegetation in areas with more intense grazing, due to the high density of species with ruderal character, that is, plants with fast development cycles and high seed production that are commonly present in areas with high disturbance intensity (Grime 1979).

## Materials and methods

### *Study area and experimental treatments*

The work was developed at a natural grassland site at the Estação Experimental Agronômica of the Universidade Federal do Rio Grande do Sul (30°05'S 51°40'W), located in the Central Depression of Rio Grande do Sul (Fedrigo *et al.* 2018). Climate is subtropical (Köppen's cfa; Peel *et al.* 2007). Average annual precipitation is 1.445 mm and is well distributed during the year, but water deficits events can occur from November to March (that is, during Southern Hemisphere spring and summer; Bergamaschi *et al.* 2003). Grasslands in the region are species-rich and dominated by perennial C4 grasses, but C3 grasses are also present. Poaceae, Fabaceae, Cyperaceae, Rubiaceae and Apiaceae are the principal plant families (Andrade *et al.* 2019).

Our research setting was a long-term grazing (that is, 24 years) experiment with different grazing intensities defined by different forage offers (FO). The treatments are daily forage allowances (that is, forage offer) of four, eight, 12, and 16 kg of dry matter mass (DM) per 100 kg of animal live weight (LW), where 4 % represents the highest grazing intensity (low forage allowance) and 16 % the lowest grazing intensity (high forage allowance). Animal stocking rates are adjusted monthly in order to keep forage offer constant throughout the year. Forage offer is defined and determined regularly based on the weight of forage dry matter per unit area (paddock) and the number of animal units at a specific time (for details see Cruz *et al.* 2010; Fischer *et al.* 2019). The experiment was designed in blocks with two replicates, with similar relief conditions, totaling eight experimental units (that is, two paddocks for each treatment). Paddock size is approximately 3 to 5 ha. For SSB analysis and for sampling of established vegetation, we used five permanent plots (1 m<sup>2</sup>) in each paddock (totaling ten plots per treatment). The minimum distance between plots was 50 m and the distance to any fence (adjacent treatments) was 20 m. Humid depressions were excluded.

### *Soil sampling for seed bank analysis*

We collected soil in spring (October 2012) and autumn (March 2013); this allowed us to consider seasonal



differences, caused for example by phenological differences or dormancy patterns of the grassland species. We collected soil with a manual auger (diameter: 5 cm, length: 10 cm) at the five permanent plots per paddock, using four points per plot. The soil samples were split into two layers: upper (0-5 cm) and lower (5-10 cm) to analyze the vertical distribution of seeds in the soil and to classify the species in the SSB regarding their permanence in the soil, following Thompson *et al.* (1997). At each sampling date, the four samples collected in each layer in the field were combined to one sample per plot, totaling 20 samples per treatment (10 per layer). The soil was allowed to dry at ambient temperature for one week.

### *Germination and seedling count*

For the SSB analysis, we used the seedling emergence method (Roberts 1981). The experiment was performed in a greenhouse at the Departamento de Plantas Forrageiras e Agrometeorologia of the Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil, in ambient temperature condition and with regular watering. For each sampling plot, 50% of the total volume of soil collected (for each layer) was mixed with the same volume of vermiculite (Favreto & Medeiros 2006), to increase the moisture holding capacity in the sample. The samples were distributed in aluminum trays (capacity 700 ml volume), forming a soil layer of approximately 2 cm. To monitor contamination from the seed rain, trays with sterile soil were distributed at random among the trays with collected soil; no seedling emergence was observed in any of the trays with sterile soil. Germination of plants was observed for one year for all samples and emergent seedlings. Emergent seedlings were identified (using appropriate botanical keys and taxonomic literature), counted and removed weekly. For species that were not identified at the seedling stage, at least one individual was transplanted into a separate container until it reached its reproductive stage and then was identified, using dichotomous keys and taxonomic literature.

### *Established vegetation survey*

To analyze the similarity between the seed bank and the established vegetation, a survey of the vegetation was realized in spring of 2012. At the points where the soil had been collected for the seed bank study, the vegetation was surveyed in plots of 1 m<sup>2</sup>. All species present were identified and had their absolute cover estimated on the Londo (1976) decimal scale. Species that were not identified in situ were collected for later identification, using dichotomous keys and taxonomic literature. Species names were verified through the website Flora do Brasil 2020 em construção (2020). Classification into families follows APG IV (2016).

### *Data analysis*

Number of seedlings per plot (sampling unit) and layer was converted into number of seedlings per m<sup>2</sup>, for each sampling date. For this conversion, we used the equation

$$S = \frac{1}{(2 * Ac)} * ns$$

where S= seedlings/m<sup>2</sup>, Ac= area of soil sample ( $\pi * r^2$ ) and ns= number of germinated seedlings per plot. Circle area of the soil sampling (Ac) was multiplied by two because we used 50 % of the collected soil that comes from four samples per plot, corresponding to the area of two samples (Baum *et al.* 2013); see *Soil sampling for seed bank*. We categorized the species found in the SSB and established vegetation according to the following functional attributes: life cycle (perennial and non-perennial, that is, annual and/or biannual; Burkart 1969), ruderal life strategy (following the concept of Grime (1979) for ruderal species, using personal observations and knowledge of botanists and agronomist in the region) and growth forms (caespitose graminoids, prostrate graminoids, erect forbs, prostrate forbs, rosulate forbs, subshrubs; Setubal 2010). We used the categories proposed by Thompson *et al.* (1997) to categorize species in the SSB according to their persistence in the soil (transient, short-term persistent or long-term persistent). This classification considers the distribution of the seeds in the different soil layers and their occurrence (or not) in the established vegetation. The transient seed bank is composed of species present in the established vegetation and in the upper soil layer. The short-term persistent seed bank is formed by species whose seeds present greater abundance in the upper soil layer and are also present in the lower soil layer, but in a smaller amount compared to the upper layer. The long-term seed bank is formed by species that present greater or equal abundance in the lower soil layer in relation to the upper layer.

We evaluated differences of density and species richness among treatments by Poisson regression (suitable for modeling variables involving count data) and a post-hoc test (Tukey's) on the R platform (R Development Core Team 2019), using packages "vegan" (Oksanen *et al.* 2018) and "multcomp" (Hothorn *et al.* 2008). We run the analyses twice, considering the two SSB layers separately and combined, but separately for each sampling date. To visualize differences in composition and abundance of the SSB in the four treatments (considering the two layers together), we conducted a principal coordinate analysis (PCoA) based on chord distances, using the program MULTIV (Pillar 2006). The similarity of vegetation and SSB for each treatment was evaluated by help of the Sørensen similarity index Qs (Zuur *et al.* 2007). For variance analysis of Sørensen values per plot among treatments we performed Linear regression (lm) and a post-hoc test (Tukey's) on the R platform (R



Development Core Team 2019), using packages “*vegan*” (Oksanen *et al.* 2018) and “*multcomp*” (Hothorn *et al.* 2008).

## Results

### Characteristics of the soil seed bank

We registered a total of 103 taxa in the SSB, distributed in 22 botanical families. Asteraceae (total of 8,032 seedlings/m<sup>2</sup>), Poaceae (4,095 seedlings/m<sup>2</sup>), Cyperaceae (3,195 seedlings/m<sup>2</sup>) and Hypoxidaceae (2,095 seedlings/m<sup>2</sup>) were the families with highest density (Tab. S1 in supplementary material). According to the classification by Thompson *et al.* (1997), 74 % of the species found in soil from the spring sampling were transient, 14 % were short-term persistent and 12 % were long-term persistent. In the autumn sampling, 77 % of the species were transient, 13 % of the species were short-term persistent and 10 % of the species were long-term persistent (Tab. S1 in supplementary material). Caespitose graminoids (grasses, sedges, rushes) accounted for 33 % of species, rosulate forbs for 17% and erect forbs for 15 %. The group of perennial plants corresponded to 73 % of the species, and ruderal plants represented 42 % of the species in SSB samplings in both seasons.

In the spring soil seed bank sampling, we found 83 species, and Asteraceae was the most abundant family (total of 6,995 seedlings/m<sup>2</sup>). In the autumn SSB sampling, we registered 84 species, and Poaceae was the most abundant family (total of 1,777 seedlings/m<sup>2</sup>). We observed the opposite regarding species richness per family: Poaceae showed the highest richness in the spring SSB samples, while Asteraceae was the family with highest richness in autumn SSB samples. *Gamochaeta coarctata* (Asteraceae), *Hypoxis decumbens* (Hypoxidaceae), *Hydrocotyle exigua* (Araliaceae), *Piptochaetium montevidense* (Poaceae) and *Sisyrinchium micranthum* (Iridaceae) were the most abundant species in the two seasons.

Poisson regression revealed significantly higher values of seedling density and richness in the upper layers of the SSB, for both seasons (Tab. 1B).

In the spring SSB, considering the two soil layers and all grazing intensity treatments, we recorded a total of 2,578 seedlings (16,420 seedlings/m<sup>2</sup>), with 67 % in the upper layer. The treatment with high grazing intensity (4 % FO) showed higher seedling density compared to the other three treatments (Fig. 1A, Tab. 1); no significant differences were found among the treatments with intermediate and low grazing intensities (8 %, 12 % and 16 % FO; Fig. 1A, Tab. 1). Regarding richness, we only found significant differences between the 4 % treatment, the highest grazing intensity treatment and the 12 % treatment (low grazing intensity; Fig. 2A, Tab. 1). In the autumn SSB, we sampled a total of 1.526 seedlings (9,720 seedlings/m<sup>2</sup>), also considering both soil layers and all grazing intensity treatments, where 75 % emerging seedlings were found in the upper layer. Again, the upper layer presented significantly higher seedling density and species richness than the lower layer. Comparisons of seedling density between the grazing intensity treatments, considering both layers together, evidenced significant differences only among the 12 % treatment (low grazing intensity) and the 4 % (high grazing intensity) and 8 % (intermediate grazing intensity) treatments (Tab. 1). For richness, we did not find differences among treatments considering the layers together (Tab. 1).

In the PCoA of the SSB composition of the spring and autumn samples, grazing intensity treatments were not separated clearly. The explanation of the ordination axes was weak, both for the ordination of spring (axis 1: 19.7 %, axis 2: 13.2 %, Fig. 3) and autumn SSB (axis 1: 12.8 %, axis 2: 14.9 %, figure not shown due to unclear and overlapping groups). However, the ordination showed that the experimental units in the 4% treatment (high grazing intensity) were separated from the other grazing intensity treatments, for both seasons. The species with highest correlation

**Table 1.** Poisson regression results indicating variances between treatments, considering the layers separately (A) and together (B). Signif. codes: 0 ‘\*\*\*’, 0.001 ‘\*\*’, 0.01 ‘\*’, 0.05 ‘.’.

Treatments (% FO)	Spring				Autumn			
	Density		Richness		Density		Richness	
	z-value	p-value	z-value	p-value	z-value	p-value	z-value	p-value
<b>A. (layers separately)</b>								
4% upper - 4% lower	9.900	< 0.001 ***	4.363	< 0.001 ***	12.055	< 0.001 ***	6.426	< 0.001 ***
8% upper - 8% lower	7.991	< 0.001 ***	3.204	0.02885 *	6.026	< 0.001 ***	5.087	< 0.001 ***
12% upper - 12% lower	8.578	< 0.001 ***	4.290	< 0.001 ***	8.668	< 0.001 ***	4.761	< 0.001 ***
16% upper - 16% lower	3.204	0.02885 *	3878	0.00246 **	10.237	< 0.001 ***	5.845	< 0.001 ***
<b>B. (layers together)</b>								
8% - 4%	-4.913	<0.001 ***	-1.456	0.46403	0.348	0.98552	0.206	0.997
12% - 4%	-7.265	<0.001 ***	-3.245	0.00628 **	-3.098	0.01012 *	-1.676	0.336
16% - 4%	-6.688	<0.001 ***	-1.730	0.30751	-1.143	0.66285	0.052	1.000
12% - 8%	-2.409	0.0754	-1.805	0.27047	-3.442	0.00329 **	-1.881	0.236
16% - 8%	-1.814	0.2660	-0.276	0.99268	-1.490	0.44332	-0.155	0.999
16% - 12%	0.597	0.9328	1.531	0.41840	1.964	0.20154	1.728	0.309

Note. Parameter estimates of Poisson model.



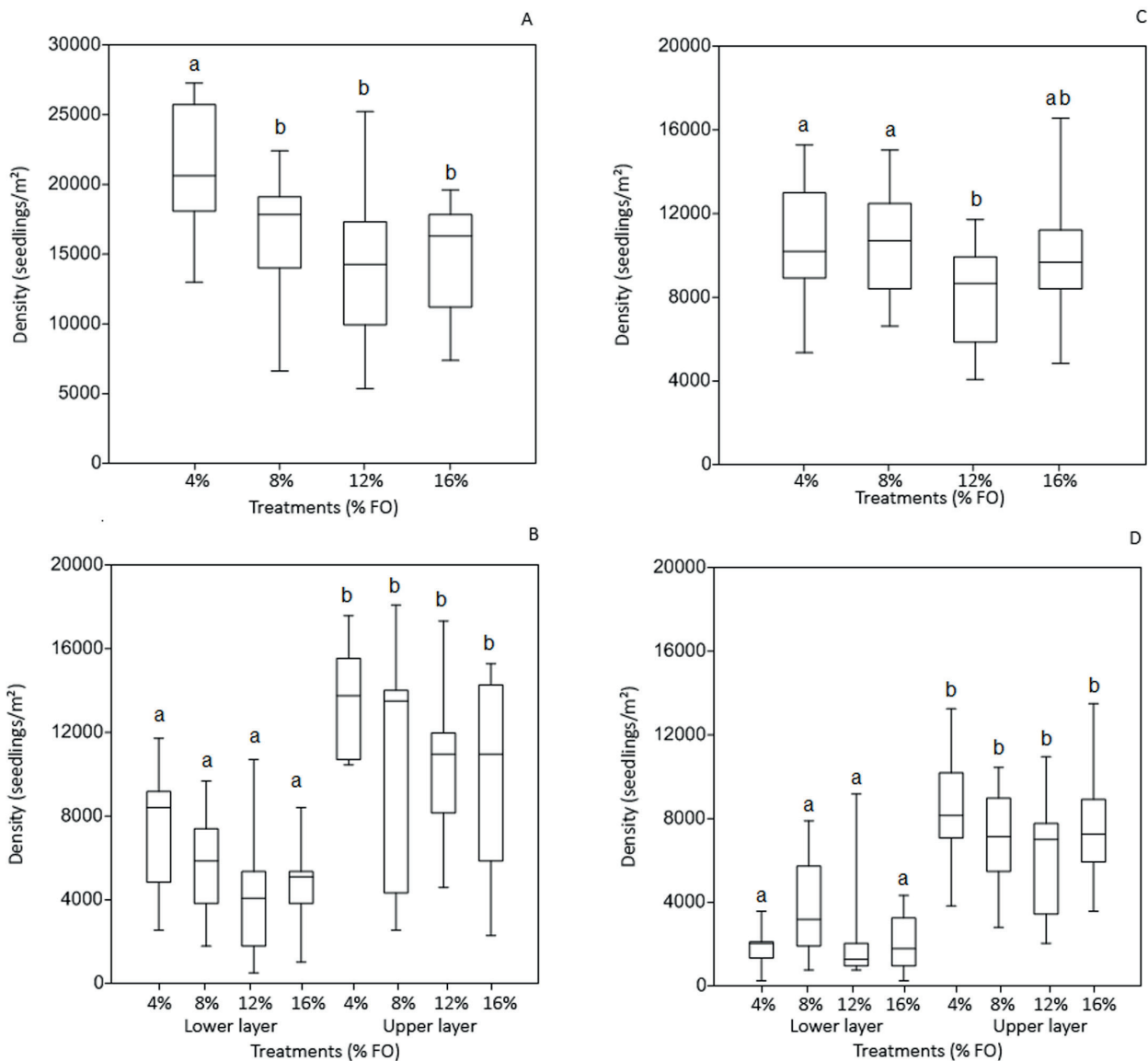
coefficients to the first axis in the spring sampling (Fig. 3), that is, species associated to high grazing intensity, are ruderal plants that have high investment in seed production, common in areas with high grazing pressure, such as *Cyperus aggregatus* (Cyperaceae), *Galium ssp.* (Rubiaceae) and *Gamochaeta ssp.* (Asteraceae) (see the list of species cited in the caption in Fig. 3).

For both sampling seasons we found predominance of species with a transient seed bank. The treatment with high grazing intensity (4% FO) consistently presented the highest percentage of species with transient seed bank (spring: 77%; autumn: 80%) and lower percentage of species with long-term persistent SSB (spring: 10%; autumn: 5%) compared to the other treatments. The treatment with the

highest percentage of species with long-term persistent SSB (spring: 15%; autumn: 13%) was the intermediate grazing intensity (8% FO) treatment. Finally, we found the highest percentage of species with short-term persistent SSB (spring: 16%; autumn: 14%) in the treatment with the lowest grazing intensity (16% FO).

*Established vegetation survey and comparison with soil seed bank*

We found 162 species, distributed in 35 families, in the established vegetation survey, conducted in spring 2012 (all four treatments, 10 sampling units each). *Andropogon lateralis* (Poaceae), *Paspalum notatum* (Poaceae), *Eryngium*



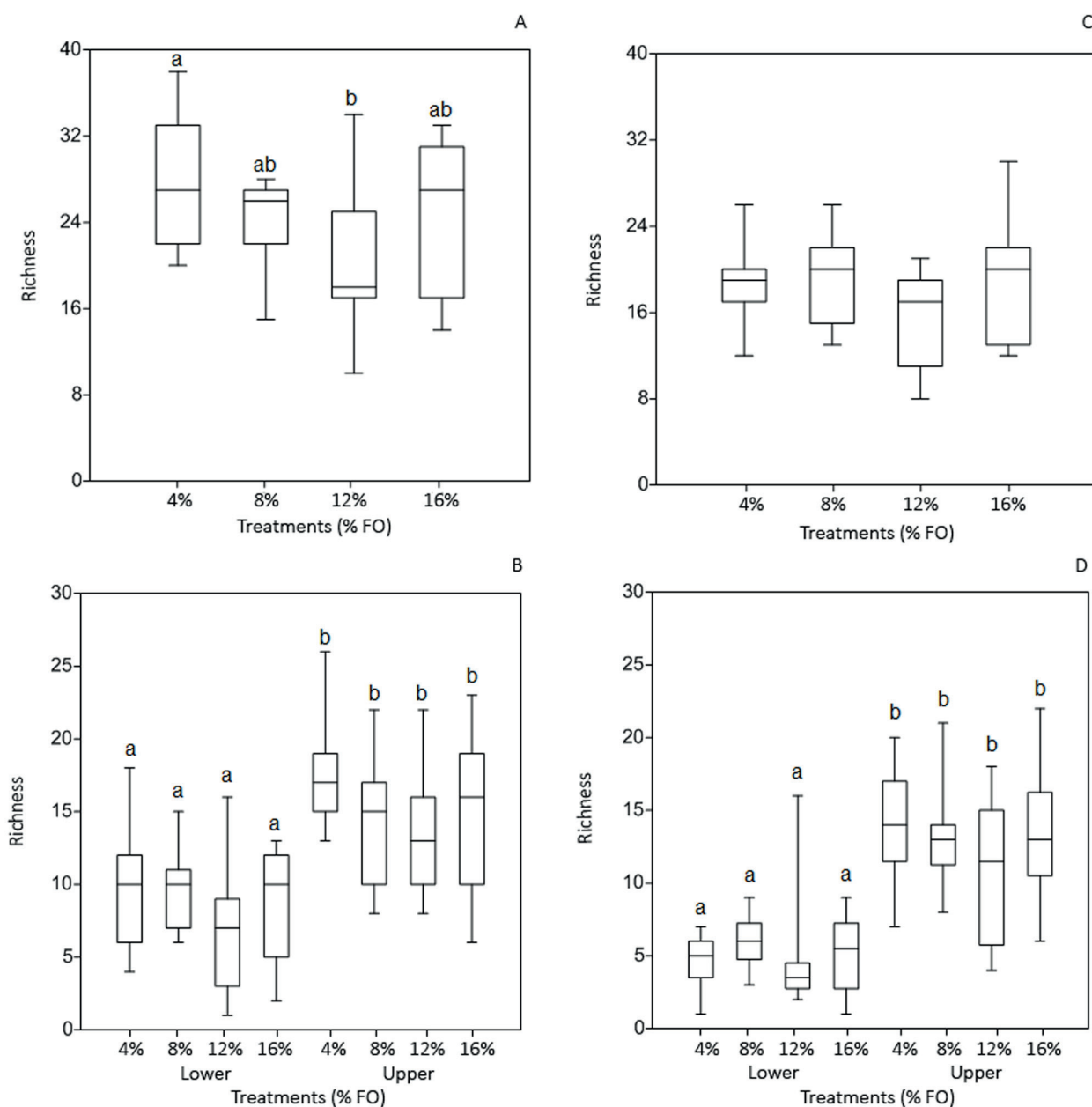
**Figure 1.** Seedling density (seedlings/m<sup>2</sup>) in the soil from subtropical grasslands under contrasting grazing intensities in southern Brazil: (A) total of two layers together in spring sampling; (B) lower/upper layer in spring sampling; (C) total of two layers together in autumn sampling; (D) lower/upper layer in autumn sampling. Different letters indicate significant differences between treatments (p-value < 0.05).



*horridum* (Apiaceae) and *Piptochaetium montevidense* (Poaceae) had highest cover.

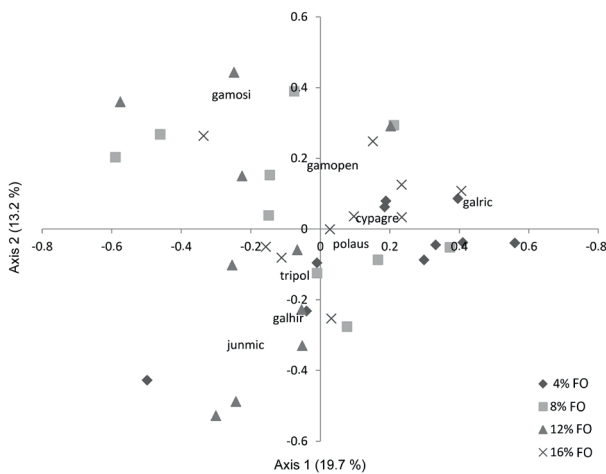
We found 71 species to occur both in the SSB and the established vegetation (Tab. S2 in supplementary material). The SSB contained 34 species not found in vegetation survey (Tab. 2). The Sørensen similarity index ( $Q_s$ ) showed similar values for the SSB composition between all grazing treatments for both spring and for the autumn samples. We found higher similarity values (that is,  $Q_s$  values per treatment) between SSB and established vegetation in the treatment with high grazing pressure (4% FO) compared

to the other treatments, in both seasons samples (Tab. 2). In spring, the treatment with the lowest grazing intensity (16% FO) showed the highest similarity among the SSB and the established vegetation, considering the  $Q_s$  values per plot between treatments (Tab. 2). Through linear regression analysis of  $Q_s$  values per plot, we found, for the spring data, significant differences between the treatment 12% (low grazing intensity) with the treatments 8% (intermediate grazing) ( $p$ -value= 0.0164) and 16% (lowest grazing intensity) ( $p$ -value= 0.0302). For the soil collected in autumn, we did not find significant differences.



**Figure 2.** Seedling richness in the soil from subtropical grasslands under contrasting grazing intensities in southern Brazil: (A) total of two layers together in spring sampling; (B) lower/upper layer in spring sampling; (C) total of two layers together in autumn sampling; (D) lower/upper layer in autumn sampling. Different letters indicate significant differences between variables ( $p$ -value < 0.05).

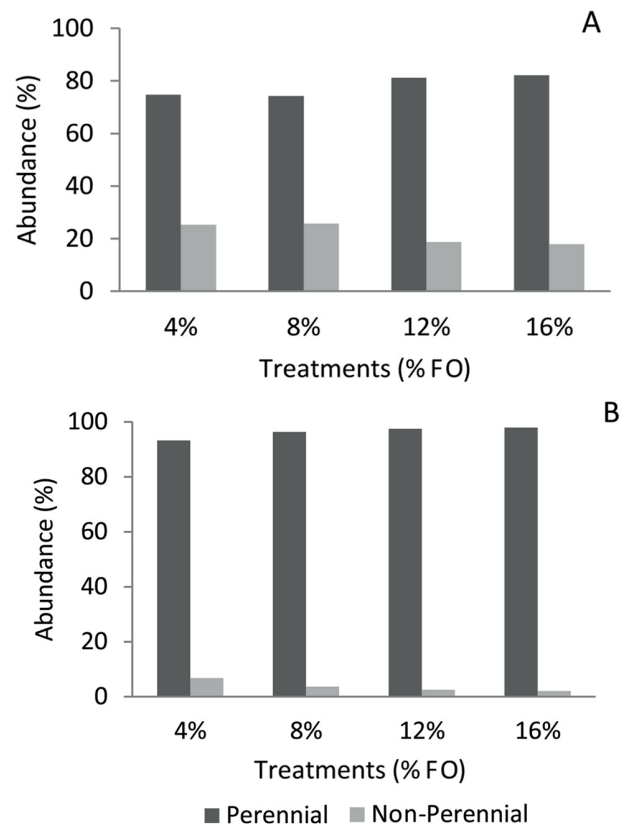
Regarding life cycle, most species that in the SSB and in established vegetation were perennials (Fig. 4). However, if we consider seed density, non-perennials had a much greater importance in the SSB compared to established vegetation (Fig. 4). Caespitose graminoids gradually increased its occurrence with the decrease in grazing intensity, both in the SSB as in vegetation. However, the contribution, considering abundance, of caespitose graminoids was much more pronounced in the vegetation. The percentage of prostrate grass species was lower compared to other growth forms, both in the vegetation and the SSB samples. For established vegetation, we observed that the prostrate grasses gradually decreased, across treatments, towards low grazing intensity. We also observed that the percentage of erect forbs and prostrate forbs decreased from high grazing intensity (4 % treatment) to lowest grazing intensity (16 % treatment), both in the SSB samples and the vegetation. The occurrence of rosulate species was higher in the SSB in comparison to the established vegetation and decreased in the treatments with lower grazing intensity. The SSB and vegetation showed low abundance of subshrubs, with similar values among treatments (Fig. 5).



**Figure 3.** Ordination diagram (PCoA) of spring soil seed bank composition of subtropical grasslands under contrasting grazing intensities in southern Brazil. Symbols represent the different forage offers (% FO). The four points represent each of the different treatments. The set of letters represents the abbreviated names of the species with highest correlation coefficients (cypagre: *Cyperus aggregatus*; galhir: *Galium hirtum*; galric: *Galium richardianum*; gamopen: *Gamochoeta pensylvanica*; gamosi: *Gamochoeta simplicicaulis*; junmic: *Juncus microcephalus*; polaus: *Polygala australis*; tripol: *Trifolium polymorphum*).

**Table 2.** Number of exclusive and shared species in established vegetation and soil seed bank. Sørensen similarity index results (for each treatment and for each sampling plot) in spring and autumn samplings. FO: Forage offer.

Seasons samplings	Spring				Autumn			
	Treatments (% FO)	4 %	8 %	12 %	16 %	4 %	8 %	12 %
Shared species between established vegetation and SSB	30	30	25	26	29	29	25	22
Number of species exclusively in established vegetation	63	73	68	63	60	66	62	62
Number of species exclusively in SSB	37	22	23	28	21	22	27	24
Sørensen (for each treatment)	40.00 %	38.71 %	35.46 %	36.36 %	41.73 %	39.73 %	35.97 %	33.85 %
Sørensen (for each plot)	18.16 %	23.40 %	13.34 %	23.94 %	21.63 %	18.19 %	16.77 %	20.18 %

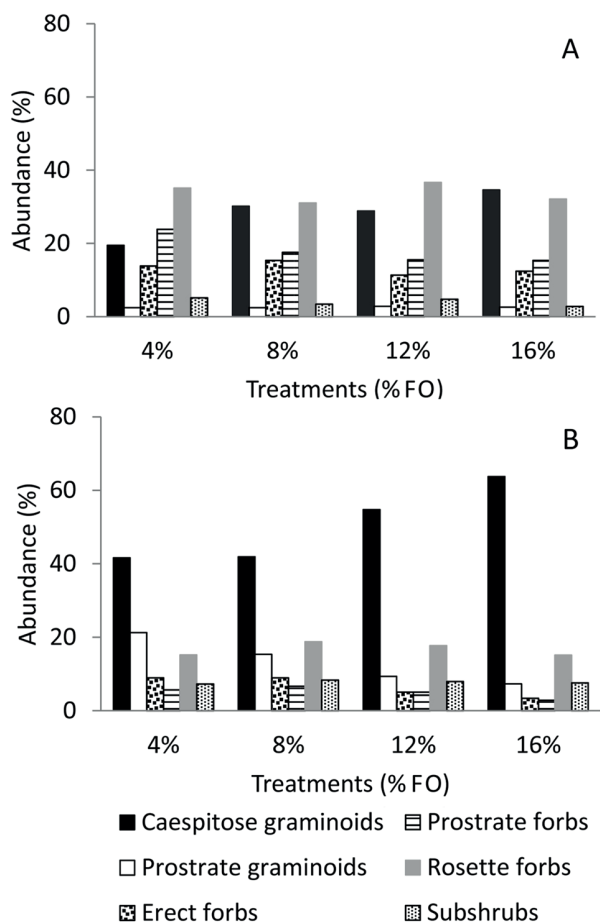


**Figure 4.** Percentage abundance according to life cycle between treatments (% FO). (A) soil seed bank; (B) established vegetation.

## Discussion

### Composition, density and richness of the SSB under different grazing intensities

In this study, SSB density was greater in the treatment with high grazing intensity (4 % FO) compared to the other treatments, similar to what has been demonstrated by Marco & Páez (2000) and Morici *et al.* (2009) in studies conducted in other subtropical grasslands in South America. The high seed density under intensive grazing is a result of the higher percentage of ruderal species, plants with high seed production and frequently present in areas with high disturbance intensity (Grime 1979), such as areas with high grazing intensity. Intense cattle trampling creates more areas with open soil, favoring the



**Figure 5.** Percentage abundance according to growth forms between treatments (% FO). (A) soil seed bank; (B) established vegetation.

colonization by ruderal species (Bullock & Marriott 2000). In the area with high grazing intensity (4% FO), we found, in the SSB, high densities of several plant species with a ruderal life strategy, such as *Hypoxis decumbens* (Hypoxidaceae), *Hydrocotyle exigua* (Araliaceae) and Asteraceae species, principally rosulate species such as *Gamochoeta* spp., *Chevreulia* spp. and *Chaptalia* spp. These species indicate overgrazing in this area (Fedrigo *et al.* 2018). In contrast, cattle are more selectively under higher forage allowance, that is, at lower grazing intensities (Nabinger *et al.* 2009). In the low grazing-intensity treatments (12% and 16% FO), plants with higher palatability are actively selected by animals. This behavior favors the presence of less palatable plants in the vegetation, causing the formation of large tussocks of caespitose grasses and, consequently, high cover values of these species (for example, *Andropogon lateralis* and *Schizachyrium tenerum*, Poaceae). With the reduction of grazing intensity, forb species decrease and caespitose grasses increase in cover, together with subshrubs and unpalatable species such as *Eryngium* spp. (Apiaceae), and vegetation becomes more heterogeneous (Boldrini & Eggers 1996; McIvor *et al.* 2005; Overbeck *et al.* 2007; Nabinger *et al.* 2009). The lower consumption of caespitose and others unpalatable species

in treatments with low grazing intensity leads to a greater accumulation of dry biomass, which could hinder the entry of seeds into the soil (Marco & Páez 2000), despite the lower compaction by trampling. Additionally, the contribution of the species that form a larger SSB (ruderal species, as discussed above) is lower in communities with low grazing intensity. Nonetheless, ruderal species were more important in the SSB than in established vegetation even under low grazing intensities.

#### *SSB characteristics in areas with different grazing intensities compared to other SSB studies*

In surveys with similar total sampling effort as in our study (similar number of samples and same sampling depth) conducted in wet grasslands in southern Brazil, Garcia (2005) and Vieira *et al.* (2015) found similar values for SSB species richness (104 species and 114 species, respectively), but much higher mean values for SSB density (57,001 seedlings/m<sup>2</sup> and 61,796 seedlings/m<sup>2</sup>, respectively). Additionally, Cyperaceae and Juncaceae, whose species produce large amounts of seeds, had a much higher importance in these studies when compared to the mesic grasslands evaluated by us.

In general, SSB richness and density in our study are in accordance with previous results for mesic and dry grasslands of South America, such as those studied by Marquez *et al.* (2002), Funes *et al.* (2001; 2003) and Haretche & Rodríguez (2006) (total average of 780-10,000 seeds/m<sup>2</sup>). Skoglund (1992), in a review study, indicated low seed density values in dry tropical ecosystems (for example, in Savannas: average of 3,000-5,500 seeds/m<sup>2</sup>). Certainly, ecological processes acting at each site – for example, fire, or grazing intensity, as studied here – and functional characteristics of the established vegetation (for example, life cycle, growth forms, photosynthetic pathways, seed germination rate) are also important in structuring SSB patterns, but the contrast between areas under drier and more humid soil conditions is evident in the literature, even though number of studies still is low.

In our study, the number of perennial species was considerably higher compared to non-perennials, both in vegetation and in the SSB. This result also is in agreement with those from other studies conducted in South American grasslands (Boccanelli & Lewis 1994; Marquez *et al.* 2002; Maia *et al.* 2004; Garcia 2005; Feldman *et al.* 2007). Nevertheless, non-perennial species has a considerable participation in the SSB when compared to established vegetation; likely, these species depend on the SSB for long-term preservation of their populations in the plant community. As previously discussed, most of these non-perennial species are rather ruderal, such as *Lysimachia minima* (Primulaceae), *Conyza bonariensis* and *Gamochoeta simplicicaulis* (Asteraceae), among others. Overall, annual species decreased with the reduction of grazing (Fig. 4), both in SSB and in vegetation, as also found by Rodríguez *et al.* (2003).



### *Relations between SSB sampling periods and SSB type*

In some SSB studies (for example, Funes *et al.* 2003; Ferreira *et al.* 2008; Scott & Morgan 2012), the highest seed densities occur in autumn, when the seeds are included into the soil after dispersal of propagules developed in spring and summer. However, in our study, we did not observe such a pattern: total seedling density was considerably lower in autumn compared to spring. Possibly, the seeds released by plants during the summer were not incorporated into the SSB until collection of soil at the end of March, that is, early autumn. Alternatively, seed production was low during this period, which can be a consequence of relatively lower precipitation during the summer months: it is well known that environmental factors, such as rainfall, can affect seed dispersal (Dukes *et al.* 2005; White *et al.* 2012). In particular, rainy periods can increase plant yield and consequently seed abundance and/or seed germination (Gutiérrez & Merseve 2003; Pol *et al.* 2014). Phenological studies are still rare for our system (for example, Oleques *et al.* 2017), and information about seed production in vegetation is missing for South Brazilian grasslands.

The method we used to classify seed persistence in the soil was based on two distinct sampling dates: this helps to take into account that some species may present dormancy. Importantly, for species with dormancy, the classification of species into seed bank types requires information on timing of seed dispersal (that is, entry of propagules in the SSB) and of seed germination (that is, exit of propagules in the SSB), as proposed by Walck *et al.* (2005). However, this kind of data is still scarce for the region of the Campos Sulinos and should be collected in future studies. In our study, we observed that the SSB is mostly composed of species with a transient seed bank (that is, species that occur only in established vegetation or in the upper soil layer) in both seasons and all treatments. Funes *et al.* (2001) and Marquez *et al.* (2002) also found a predominance of species with transient SSB in both grazed and ungrazed areas. Moreover, the percentage of long/short-persistent species increased in the SSB as grazing intensity decreased: consequently, species with transient SSB decreased with increasing grazing intensity. Probably, the predominance of transient SSB revealed in our study is due to the fact that several of these species are non-perennial, have a ruderal character and show higher density in treatments with greater grazing intensity (for example, 4 % FO), that is, sites with more intense activity of cattle.

### *Relation of SSB with established vegetation*

The Sørensen similarity values calculated between SSB samples and the established vegetation overall were similar among grazing intensity treatments. As in other studies (Lunt 1997; Friend *et al.* 1997; Ghermandi 1997; Funes *et al.* 2001; 2003; Haretche & Rodríguez 2006; Feldman

*et al.* 2007), we observed that the predominant species in the established vegetation at our study site, such as the grasses *Paspalum notatum* and *Andropogon lateralis* (Poaceae) (see also Fedrigo *et al.* 2018), were not present in the SSB or appeared only in very low numbers. Grazing can reduce the presence of reproductive structures of the plants, causing a decrease in the amount of seeds produced and thus reducing the seed bank availability in the soil (Pol *et al.* 2014) and this effect should be stronger under higher grazing intensity, with the expectation of very low-growing species. The lack of dominant grasses in the SSB has been pointed out in other studies conducted in the region (for example, Friend *et al.* 1997; Maia *et al.* 2004; Haretche & Rodríguez 2006; Vieira *et al.* 2015). These species, adapted to grazing and/or fire, are long-lived, that is, mortality should be low, and thus there seems to be no necessity for them to form a large soil seed bank (Medeiros 2000). However, this also means that after more severe disturbances that imply in destruction of rhizomes, such as conversion to arable land, these species will not reestablish easily. *Piptochaetium montevidense* (Poaceae) was an exception; this short grass was one of the species with the highest importance in vegetation and was abundant in the SSB – however, it is a species with a ruderal/opportunistic character, that is, large seed production (Heringer & Jacques 2002).

### *Conclusions and implications*

Our study evidenced high richness and dominance of ruderal species in the SSB in the treatment with the highest intensity of grazing (4 % FO), but few differences among the other treatments. Although the grazing experiment site where our study was conducted only has two replicates, we can conclude that grazing intensity in general does not have a very large impact on the SSB, in contrast to our initial hypothesis - unless it is very high. A grazing land with a forage offer of only 4 % can be considered as overgrazed (Fedrigo *et al.* 2018), and we show here that this is also evidenced in the SSB.

We also observed, in all treatments, the absence or low participation of the species that present high abundance in the established vegetation, such as the dominant grasses that form the vegetation matrix of the studied grasslands. Our results corroborate studies that showed the limitations of SSB in the original recovery of vegetation, principally after more severe disturbances (for example, implementation of other land uses, as monocultures), as also discussed by D'Angela *et al.* (1988) and Vieira *et al.* (2015). This means that the regeneration of the grassland community after severe disturbances will depend heavily on the dispersal of exogenous propagules rather than on the germination of seeds stored in the SSB (D'Angela *et al.* 1988). Consequently, active seed introduction may be necessary for the restoration of degraded grassland (Buisson *et al.* 2018; Thomas *et al.* 2019).



Long-term SSB studies often are difficult in terms of time, physical space, financial and human resources. Nonetheless, studies about soil seed bank, bud bank, seed rain and their role in vegetation dynamics – including population dynamics of specific plant species – are needed to better assess the vegetation patterns in relation to grassland management and to develop adequate restoration strategy for degraded grasslands.

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