UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

INSTITUTO DE BIOCIÊNCIAS

PROGRAMA DE PÓS-GRADUAÇÃO EM BOTÂNICA

GRASSLAND MANAGEMENT EFFECTS ON BIODIVERSITY AND ECOSYSTEM FUNCTIONING

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Porto Alegre

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Tese apresentada ao Programa de Pós-Graduação em Botânica como um dos requisitos para a obtenção do grau de Doutor em Botânica pela Universidade Federal do Rio Grande do Sul.

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SUMMARY

ABSTRACT

Grasslands worldwide occupy approximately 40% of Earth terrestrial surface and are habitat for large biodiversity and provider of ecosystem functions, being fire and grazing the key drivers of grassland biodiversity and ecosystem. Despite it, grasslands are one of the most converted ecosystems worldwide, wherein different vegetation management to raise herds lead to changes on plant species composition and ecosystem functioning. Three articles composed this thesis in order to elucidate how these changes are driven by different land uses from livestock perspective. We assessed how plant species composition differs in terms of plant species composition, richness, and ecosystem functions by comparing (i) natural grasslands and cultivated pastures from North and South America; (ii) natural grasslands grazed and ungrazed for 8 eights in Río de la Plata grasslands region (RPG); and (iii) different fire treatments, including fire and grazing interaction, in subtropical grasslands from southern Brazil. Our results showed clear differences among treatments from all chapters, wherein mainly findings showed that (i) conversion of natural grasslands into cultivated pastures, and large grazers exclusion lead to reduced root biomass and higher arthropods herbivory damage, which may increase, respectively, risk to adverse climatic events (e.g. drought) and pest infestation; (ii) reduction on plant species richness in cultivated pastures and in ungrazed areas in comparison to, respectively, natural grasslands and grazed areas from RPG; and (iii) disruption of biotic interactions by reduction on lepidopterans and coleopterans abundances as floral visitors in cultivated pastures. Furthermore, our findings regarding different fire treatments showed the potential of prescribed fires to reduce and increase, respectively, shrubs and C3 grasses coverages while maintaining plant species richness, indicating its use as vegetation management tool in grazing systems.

Keywords: disturbance, fire, grazing, livestock, rangelands.

GENERAL INTRODUCTION

Occupying about 40% of Earth terrestrial surface, grasslands are habitat for large biodiversity. Plants are the main food source for herbivores and pollinators and driver of ecosystem functions (Gaujour et al. 2012). The latter can be defined as the stocks and fluxes of matter and energy over time and space driven by biological activity (Hooper et al. 2005), and grasslands are worldwide recognized as provider of functions such as both above- and below-ground biomass productivity (Reich et al. 2012; Leidinger et al. 2017), floral and nectar resources for pollinators (Orford et al. 2016), carbon sequestration (Steinbeiss et al. 2008, Bai and Cotrufo 2022), and maintenance of water quantity and quality (Bengtsson et al. 2019). Fire and grazing are key drivers of grassland biodiversity and ecosystem functioning (Bond and Keeley 2005, Allred et al. 2011). Fire removes dead biomass, making light incidence more uniform along canopy, altering plant species composition (Vermeire et al. 2018), affecting nutrient cycling (Reinhart et al. 2016), enhancing forage quality and digestibility (Allred et al. 2011), and improving habitat quality for grassland-obligate wildlife species (Engle et al. 2016). On the other hand, grazers change plant communities and vegetation structure by biomass removal and through effects of trampling and deposition of feces/urine (Adler et al. 2001, Liu et al. 2015, Lezama and Paruelo 2016). Fire and grazing, alone or in combination, thus are responsible for the maintenance of the ecological characteristics of grasslands under productive climatic conditions, ultimately also being responsible for the delivery of ecosystem services. However, despite the provision of goods and services, grasslands are one of the most converted ecosystems worldwide (Hoesktra et al. 2005) being transformed into monocultures of crops and trees or into urban areas (Rands et al. 2010; Parr et al. 2014).

In the livestock context, grazing animals, usually domesticated cattle, are being raised in cultivated pastures – that substitute more diverse natural grasslands - for reasons such as the need for forage production during climatically limited periods, to improve yields, or even to simplify grazing management (D'Antonio and Vitousek 1992; Carvalho and Batello 2009). Beyond the objective to simplify grazing management and thus grazing lands, the restriction of fire use as vegetation management tool in Brazil and higher prices of crops (e.g., soybean) also contribute to conversion of natural grasslands (Modernel et al. 2016, Pivello et al. 2021). The primary effect of conversion is the replacement of native plant communities, which are heterogeneous and diverse, by communities dominated by few exotic plant species, leading to reduced species richness and altering biodiversity and ecosystem functioning (Marquard et al., 2013). These changes reflect on ecosystem function. In grazed areas and in comparison to low diverse plant communities, high diverse communities had higher root biomass and lower invertebrate damage (Leidinger et al. 2017); higher abundance of soil invertebrates (Norton et al. 2022) and pollinators (Rosenberger and Conforti 2020). Furthermore, species-poor environments with simplified canopy and roots structures are less resistant and resilient to stress events in comparison to diverse environments (Stockes et al. 2014). Importantly, these changes occur also when traditional use of natural grassland is abandoned. In many grassland systems, the exclusion of large grazers reduces plant species richness as result of dominance of few tall plant species more efficient in light capture (Boldrini & Eggers 1996, Altesor et al. 2006) associated with reduction on root biomass (Altesor et al. 2006, López-Mársico et al. 2015). Overall, there still is a need for studies reporting changes on ecosystem functions in contrasting grasslands uses for livestock purposes to identify best management practices that conserve biodiversity and enhance ecosystem functionality.

One factor that could contribute to grasslands conservation is the reintroduction of fire as vegetation management tool, especially interacting with grazing, which increases landscape heterogeneity by promoting a shifting mosaic with different vegetation patches, being critical for conservation of grassland biodiversity broadly (Fuhlendorf and Engle 2004, Ricketts and Sandercock 2016) and can be strategically applied as a grassland management tool to maintain sustainable livestock production (Powell et al. 2017). Subtropical grasslands in southern Brazil are, due to their ecological features, considered as fire-dependent ecosystem (Pivello et al. 2021). However, fire is not often applied in grasslands situated in the southern part of the state of Rio Grande do Sul state, in contrast to the highland grasslands further north (Overbeck et al. 2007, 2022). Recent studies carried out on fire effects in the subtropical grasslands showed positive effects of fire on communities of plants (Ferreira et al. 2021, da Silva et al. 2020, López-Mársico et al. 2020, Loydi et al. 2020), including stimulation of germination (Cuello et al. 2020), ant diversity (da Silva et al. 2020) and birds (Beal-Neves et al. 2020), and on habitat heterogeneity and diversity (Beal-Neves et al. 2020). These results pointed out the potential of fire to shape vegetation structure and biodiversity in this region, but the interaction among fire and grazing, which is historically the most common disturbance in the region, has not yet been studied.

By identifying how grassland management, especially for livestock production, influences biodiversity and ecosystem functioning, is key to guide policy development for management practices that conserve biodiversity, increase forage production and animal performance, and enhance ecosystem functionality. In this thesis, three articles were developed and presented as distinct chapters:

i. Chapter 1: Biodiversity and ecosystem functions in natural grasslands and cultivated pastures from North and South America. This article was presented during my PhD thesis qualification and submitted to the journal Applied Vegetation Science.

ii. Chapter 2: Exclusion from grazing reduces biodiversity and changes ecosystem functions in Río de la Plata grasslands. This article will be submitted to the journal Applied Vegetation Science.

iii. Chapter 3: Interactive effects of fire and grazing on vegetation structure and plant species composition in subtropical grasslands. This article was submitted to the journal Applied Vegetation Science.

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CHAPTER 1: Biodiversity and ecosystem functions in natural grasslands and cultivated pastures from North and South America[1]

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Abstract

Conversion of natural grasslands into cultivated pastures affects ecosystem productivity and biodiversity. However, which ecosystem functions are affected by conversion and associated ecosystem sustainability implications remain unclear. We therefore assessed climatically contrasting grasslands from North and South America that concomitantly support both biodiversity and livestock production - specifically comparing natural grasslands and cultivated pastures. We measured plant species richness and ecosystem functions covering below- and aboveground productivity, arthropod composition, and herbivory using rapid ecosystem function assessment procedures. Differences in levels of ecosystem productivity and biota were found among treatments, wherein cultivated pastures had reduced root biomass at least in one layer, and greater magnitude of herbivory. Natural grasslands had higher Lepidoptera and Coleoptera invertebrate abundance as floral visitors, whilst Coleoptera and Hemiptera were more abundant in cultivated pastures as soil arthropods. In contrast, plant species richness was not directly linked to any ecosystem function or arthropod abundance variables, suggesting that multiple indicators of biotic heterogeneity across variable spatio-temporal scales are necessary to fully understand the impacts of natural grasslands conversion into cultivated pastures on ecosystem functions and biodiversity. Our findings suggest that conversion of natural grasslands into cultivated pastures can jeopardize ecosystem long term sustainability through drought risk via root biomass some years, reinforcing the need for grazing management that integrate biodiversity conservation with animal production. This could incentivize the prevention of natural area conversion and reward ranchers who sustainably graze natural grasslands.

Keywords: land-use, livestock, management, Pampas, Rangelands

Introduction

Ecosystem functions can be defined as the stocks and fluxes of matter and energy over time and space driven by biological activity (Hooper et al. 2005) or, according to Felipe-Lucia et al. (2020), as ecological processes that indirectly benefit people. Both definitions encompass the generally recognized importance of land-use to maintain ecosystem functionality (Tilman et al. 2014). Nevertheless, land-use changes are occurring globally (Simberloff et al. 2013; Steffen et al. 2015) contributing to biodiversity loss and, consequently, disruption of ecological processes and biotic interactions that affect ecosystem functioning (Díaz et al. 2013). These changes can be observed in many ecosystems worldwide but especially in grasslands, which occupy about 40% of Earth terrestrial surface and provide critical habitat for numerous floral and faunal species. They are important for humans also as many grasslands are used as forage for grazing livestock production, besides other important ecosystem services (Sala and Paruelo 1997; Leidinger et al. 2017; Squires et al. 2018). Nonetheless, grasslands are among the most converted ecosystems (Hoekstra et al. 2005) and continue to be transformed into agriculture, tree plantations, and urban areas (Rands et al. 2010; Parr et al. 2014).

Natural grasslands are usually associated with ecosystem functions including both above- and below-ground biomass productivity (Reich et al. 2012; Leidinger et al. 2017), provision of floral and nectar resources for pollinators (Orford et al. 2016), soil carbon sequestration (Steinbeiss et al. 2008), and resistance to drought (De Keersmaecker et al. 2016). On the other hand, converted grasslands, besides reduction of plant diversity, can be associated with changes in soil chemistry, reduction of floral and nectar resources for pollinators, mammals, reduced structural habitat for birds, and a greater susceptibility to biological invasions (Flynn et al. 2009; Roscher et al. 2009; Laliberte and Tylianakis 2011; Allan et al. 2014). In an agricultural context, natural grasslands

have often been converted to cultivated pastures (i.e., often through the planting of exotic cultivated forage species) for many reasons including developing alternative forages for climatically limited periods, to improve yields, or even to simplify management (D'Antonio and Vitousek 1992; Carvalho and Batello 2009; Sichonany et al. 2016; Lark et al. 2018). However, the replacement of diverse and heterogeneous native plant communities for homogenous exotic forage monocultures (hereafter as cultivated pastures), may jeopardize inherent ecosystem biodiversity and ecosystem functioning (Marquard et al., 2013). Comparing southern Brazil natural grasslands with secondary grasslands both grazed by cattle, Leidinger et al. (2017) found higher values of plant and root biomass, lower invertebrate damage. Furthermore, Norton et al. (2022) in Great Britain grasslands used for livestock grazing, found a positive relationship between abundance of soil invertebrates and plant richness in natural grasslands but reduced soil invertebrate abundance in pastures with lower plant richness. Even though the conservation of natural grasslands is recognized as a pressing issue, surprisingly, the comparative effects are limited for natural and cultivated pastures for pollinators abundance. Rosenberger and Conforti (2020), comparing restored grasslands (prairies) with cultivated pastures (grazed by cattle), found higher abundance of bumble bees in native-dominated areas due increased number of plant species and dicotyledon flower richness. Overall, few studies reported changes on ecosystem functions in contrasting grasslands uses for livestock purposes, indicating a need for multiple ecosystem functions monitoring to identify these changes to support future investments and guide policy development for management practices that conserve biodiversity and enhance ecosystem functionality.

Grassland ecosystems and rangelands in western North America and South America *Campos* are species-rich environments (Pennington et al. 2017; Andrade et al. 2019), used for

livestock grazing (Carvalho and Batello 2009; Holechek et al. 2010). In both hemispheres these ecosystems are being transformed to other land uses, including into cultivated pastures for livestock purposes (Fuhlendorf et al. 2018; Staude et al 2018) but the consequences of these changes on different ecosystem processes have been poorly studied. According to Staude et al. (2018), conversion of natural grasslands into exotic monocultures reduced plant species richness, increased environmental homogenization, and may cause a reduction of ecosystem resilience. Furthermore, natural grasslands play an essential role in conserving multitrophic biodiversity and provision of ecosystem functions, which is jeopardized by land-use intensification (Gossner et al., 2016). Additionally, the understanding of ecosystem functions responses is useful to inform grasslands management aiming to increase animal and vegetation production while maintaining biodiversity (Perrings et al. 2011, Koschke et al. 2012).

Here, we used rapid ecosystem function assessment procedures proposed by Meyer et al. (2015) to compare natural and cultivated grasslands regarding a range of ecosystem function indicators. This includes indicators related to productivity, based on biomass, and indicators related to biotic interactions, based on presence of different invertebrate groups and their effects (Weisser and Siemann 2004, Ramoelo et al. 2015). Also, it has been shown before that higher plant richness improves ecosystem functioning through different species niches, which optimize resource use (Cardinale et al. 2012). We conducted this study in two climatically contrasting regions (Western Rangelands from United States, and *Campos* from southern Brazil) in which livestock production is an important socio-economic contributor to local economies and where grasslands are valued for the provision of habitat to conserve biodiversity but are subjected to different forms of conversion and land use change. We hypothesized that (1) biomass-related variables will be greater in cultivated pastures, (2) floral visitors and soil arthropods will be

reduced in cultivated pastures, (3) herbivory damage will be higher in cultivated pastures, and (4) these specific impacts will each be directly linked to plant species richness being at higher level in more species-rich areas.

Methods

Study sites

The study was conducted in two grassland regions, the western rangelands in the United States (hereafter as *Western Rangelands*; Havstad et al. 2009) and the *Campos*, part of the Rio de la Plata Grassland ecoregion (hereafter as *Campos*; Soriano et al. 1992). The two regions contrast in terms of climate (Table 1). In both regions, grasslands are species-rich (Pennington et al. 2017; Andrade et al. 2019) and are used for livestock grazing (Carvalho and Batello 2009; Holechek et al. 2010). *Western Rangelands* and *Campos* are also habitats for native herbivores (including medium to large herbivores from Antilocapridae (absent in *Campos*), Caviidae, and Cervidae families). In *Western Rangelands*, study sites were located in the states of Idaho (municipality of Picabo) and Wyoming (municipalities of Laramie and Powell). In Picabo and Powell, sites belong to The Nature Conservancy, while Laramie sites are property of the University of Wyoming. All *Campos* sites are located in Rio Grande do Sul state (municipalities of Cacequi, Santa Maria, Santiago and São Francisco de Assis). With the exception of the Santa Maria site, which belongs to Federal University of Santa Maria, they are all on private property. In total, we worked at six paired sites of natural grassland and cultivated pastures (hereafter as blocks) per region (Figure 1, Table 1), totalizing 12 blocks.
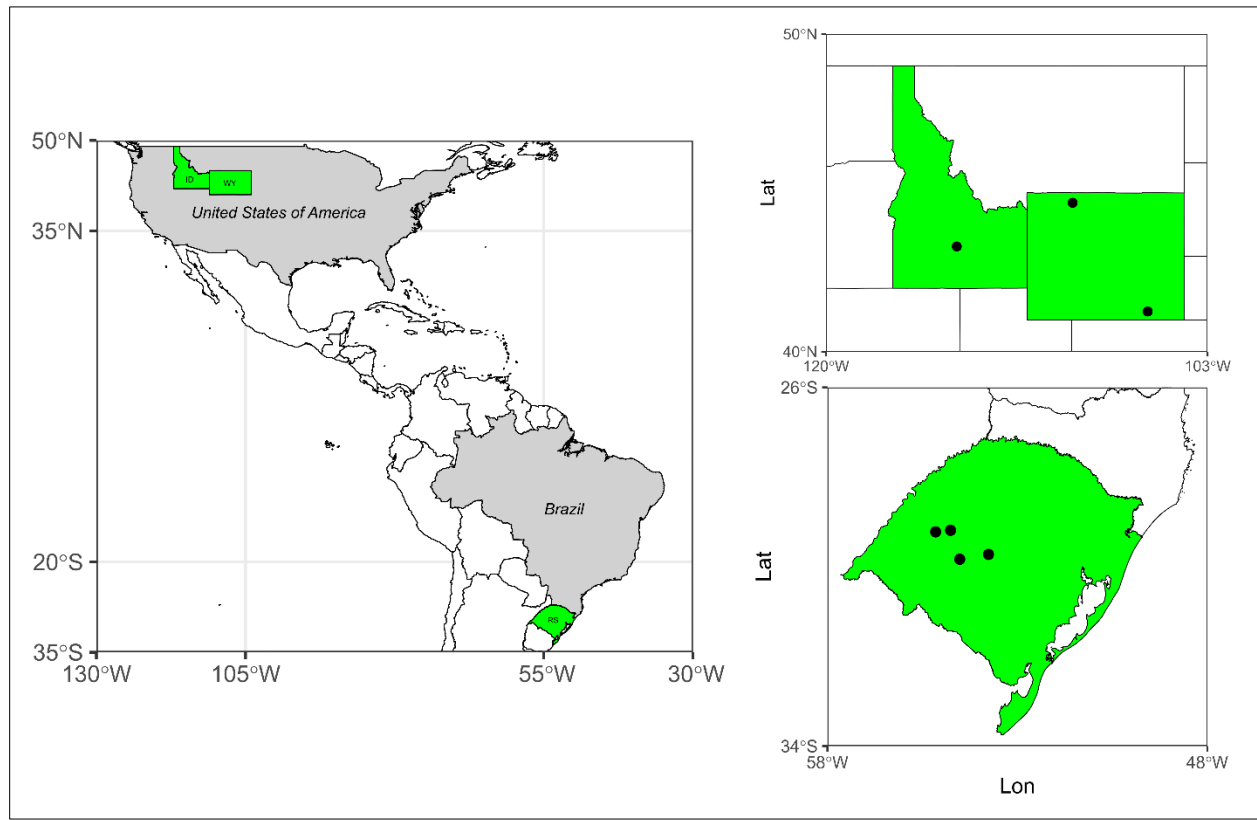
Figure 1. Map of study sites in North and South Americas.

Table 1. Number of blocks, precipitation, altitude, climate, and GPS coordinates of each study site

Study site (number of blocks)	Mean annual temperature (ºC)	Mean annual precipitation (mm)[1]	Mean 30-day preceding sampling precipitation (mm)[2]	Altitude (m)	Climate[3]	Coordinates (latitude; longitude)
Powell (3)	6.8	172	50	1560	BSk	44.68º; -109.01º
Laramie (2)	9.0	291	24	2200	BSk	41.30º; -105.66º
Picabo (1)	8.0	330	18	1486	Dsb	43.31º; -114.17º
Cacequi (2)	19.2	1630	88[¶] – 50[‡]	149	Cfa	-29.83º; -54.51º
Santa Maria (1)	19.3	1688	102	90	Cfa	-29.72; -53.76º
São Francisco de Assis (1)	18.9	1717	89	180	Cfa	-29.22º; -55.14º
Santiago (2)	14.6	1832	147[¶] – 88[‡]	254	Cfa	-29.18º; -54.75º

[1]30-year precipitation average; data from NOAA (2019) for *Western Rangelands* sites and from INMET (2019) for *Campos* sites

In both regions, natural grasslands are threatened by potential into cultivated pastures and/or to energy use (Fuhlendorf et al. 2018; Staude et al 2018) and grasslands are managed for livestock purposes. Study sites are used for grazing, and grazing intensity is approximately 1 animal unit ha^{-1} in natural areas, and 2.5 animal unit ha^{-1} in cultivated pastures. The exception is Picabo cultivated area, which is used for hay production. Dominant plant species (i.e., species with relative cover higher than 10 %) of each block from natural and cultivated areas are presented in Table 2.

Table 2. Dominant plant species (relative cover, %) and photosynthetic pathway for grasses (C3 or C4) of each treatment in each block in North and South hemispheres.

Study site	Block	Natural area	Cultivated pasture
Powell[1]	1	*Artemisia tridentata* (31.6), *Elymus elymoides* (28.4; C3)	*Bromus inermis* (42.0; C3), *Medicago sativa* (19.1)
Picabo[1]	2	*Juncus arcticus* (33.7), *Carex nebrascencis* (15.5), *Carex simulata* (13.0), *Deschampsia cespitosa* (13.0; C3)	*Medicago sativa* (66.3), *Agropyron intermedium* (18.5, C3)
Laramie[1]	3	*Sporobolus airoides* (39.8; C4), *Poa fendleriana* (27.5; C3)	*Alopecurus pratensis* (74.3; C3), *Agropyron cristatum* (12.3; C3)
Laramie[1]	4	*Hesperostipa comata* (39.0, C3), *Artemisia frigida* (19.6), *Delphinium bicolor* (19.1)	*Agropyron cristatum* (80.9, C3), *Linaria dalmatica* (17.3)
Powell[1]	5	*Artemisia tridentata* (31.6), *Pseudoregneria spicata* (22.4, C3)	*Bromus inermis* (43.4; C3), *Medicago sativa* (28.9)

Powell[1]	6	*Pseudoregneria spicata* (27.9, C3), *Artemisia tridentata* (21.7)	*Medicago sativa* (40.7), *Bromus inermis* (32.2; C3)
Santiago[2]	7	*Andropogon lateralis* (21.4; C4), *Paspalum notatum* (12.3; C4), *Eryngium horridum* (11.7)	*Lolium multiflorum* (59.9; C3)
Santa Maria[2]	8	*Paspalum notatum* (20.2; C4), *Dichondra sericea* (11.8), *Andropogon lateralis* (11.3; C4)	*Lolium multiflorum* (84.9; C3)
Cacequi[2]	9	*Aristida laevis* (31.5; C4), *Paspalum notatum* (20.2; C4), *Aristida jubata* (15.7; C4)	*Brachiaria* spp (64.9; C4)
Cacequi[2]	10	*Aristida laevis* (21.4; C4), *Andropogon lateralis* (16.1; C4), *Elephantopus mollis* (14.3)	*Pennisetum americanum* (75.2; C4), *Digitaria sanguinalis* (21.5; C4)
São Francisco de Assis[2]	11	*Paspalum notatum* (24.2; C4), *Cantinoa mutabilis* (14.5), *Setaria parviflora* (10.2; C4), *Desmodium incanum* (10.2)	*Pennisetum americanum* (37.5; C4), *Digitaria sanguinalis* (33.7; C4)
Santiago[2]	12	*Paspalum notatum* (22.8; C4), *Andropogon lateralis* (19.9; C4), *Desmodium incanum* (10.5)	*Pennisetum americanum* (76.6; C4)

[1]Located in United States of America

[2]Located in Brazil

Sampling scheme

Each site contained a natural grassland area (NG) and a cultivated pasture (CP) (hereafter "treatments"). Areas at each site (minimum area: one hectare) were selected such that treatments had similar soil types, topography, and land-use goal (i.e., livestock production) within each block. Cultivated pastures from blocks 1, 2, 4 and 6 had mechanical irrigation and all CP from both hemispheres received fertilization management. Field surveys were conducted in June-July 2018

(northern hemisphere Spring/Summer) in *Western Rangelands*. In Campos, field surveys were conducted in two different periods in Campos, but primarily October 2018, i.e., southern hemisphere spring, and February 2019, southern hemisphere summer, to take into account peak biomass of C3 grasses and C4 grasses. We sampled ecosystem functions at five systematically distributed points per treatment (as in Leidinger et al. 2017; Figure 2), with a distance of 30 m between points, and following the procedures proposed by Meyer et al. (2017). At each point, we established multiplots (MPs) for assessment of primary and secondary productivity and vegetation structure. Additionally, we installed four arthropod sampling points (ASPs) per site, situated between MPs, to assess arthropod composition.
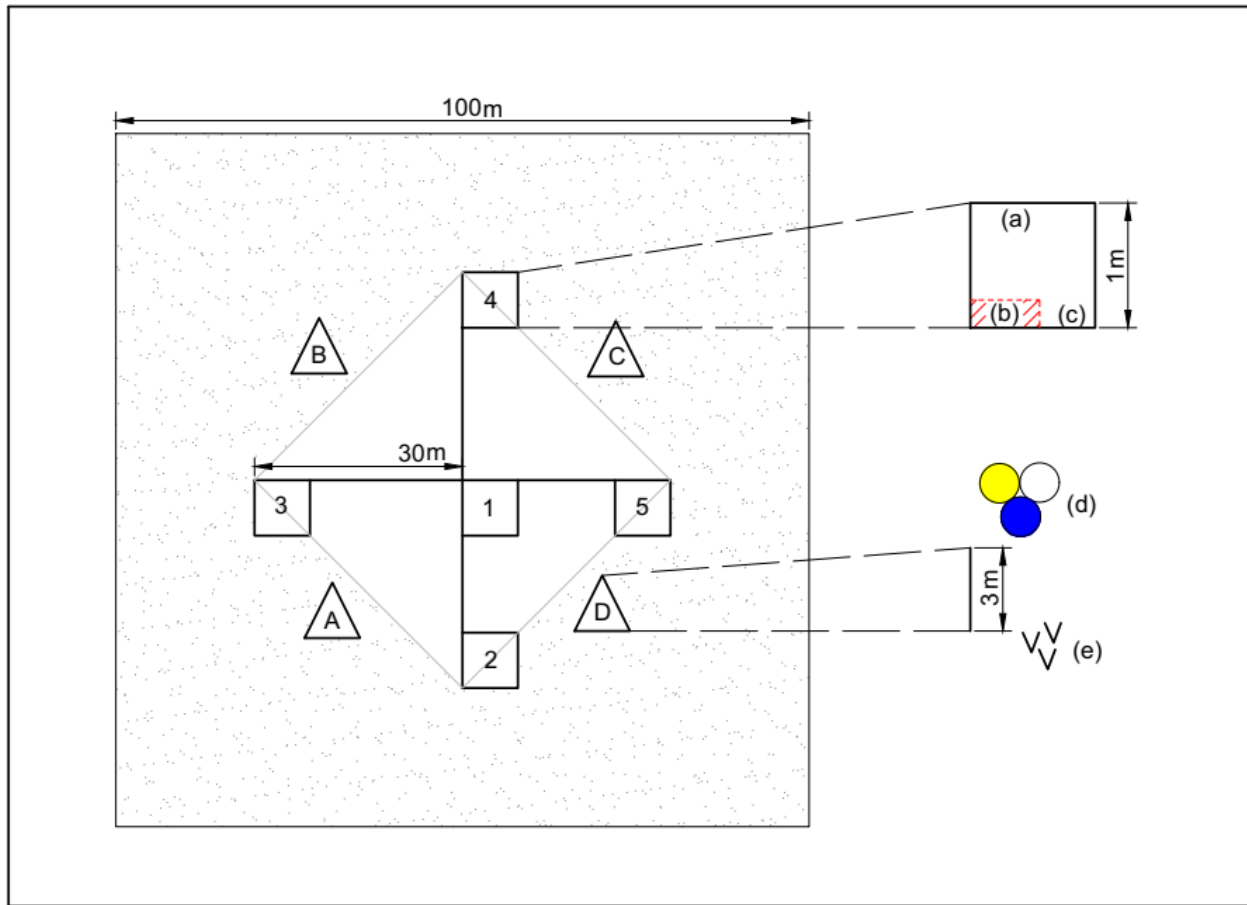
Figure 2. Site sampling scheme with multiplots (1-5) and arthropod sampling point (A-D); multiplot detail with sampling location of vegetation data (a), available forage and herbivory (b), root biomass (c), floral visitors (d), soil arthropods (e).

Vegetation data

Plant species composition data were obtained in 5 quadrats of 1 m² (1m × 1 m) inside each MP. In each quadrat, we identified all vascular plant species to the lowest possible taxonomic level; unknown specimens were collected and later identified using taxonomic literature. Cover of each plant species was estimated using a modified Braun-Blanquet scale (Braun-Blanquet 1979) (i.e.,

< 1 %; 1-5 %; 5-15 %; 15-25 %; 25-50 %; 50-75 %; 75-100 %) also we recorded number of flowering plant species (hereafter as flowers). Species were classified according to functional life form (i.e., cacti, C3 grass, C4 grass, forb, graminoid (incl. grasses, sedges, and rushes), legume, and shrubs (incl. sub-shrubs lignified only at the base) based on Nelson et al. (2015). Additionally, we estimated the percentage of bare soil cover per subplot.

Available forage

Available forage as an approximation of aboveground primary productivity was estimated by sampling peak standing biomass in 20 cm × 50 cm plots in all five MPs. Biomass was cut at ground level, including dead biomass and woody components of vegetation. In MPs 1, 3 and 5, biomass was separated into different forms of functional life form in order to obtain relative contribution of each to total biomass productivity. Samples were dried for 48 h or until dry at 70°C before weighing.

Belowground primary productivity

Standing root biomass (i.e., root biomass including root litter) was evaluated as an indicator of belowground net primary productivity (Ni 2004; Ravenek et al. 2014). Volumetric soil samples of 20 cm depth with 10 cm diameter were collected in MPs 1, 3, and 5 (adapted from Böhm 2012). Samples were split into two 10-cm layers (Root 0-10 cm and Root 10-20 cm) and the sum of both resulted in total root biomass (Root total). Roots were separated by washing the soil samples through a sieve and then dried for 48 hours at 70°C before weighing.

Arthropod composition

Floral visitor abundance was assessed using three pan traps of colors blue, yellow, and white (Nuttman et al. 2011), filled with water and drops of detergent and mounted at vegetation height

at each ASP. Organisms on the soil surface were sampled through pitfall traps comprised of plastic pots (capacity of 50 mL and with 9 cm diameter) filled with 30 mL of alcohol 70% and drops of detergent. They remained open for 24 hours in the field and, at each ASP, three plastic pots were placed 3 meters away. Samples from pan and pitfall traps were preserved in alcohol 70 % and all adult specimens were identified and grouped according to its order (Formicidae individuals were grouped separated from its order Hymenoptera). Treatments inside each block were sampled randomly and, for statistical analysis, we excluded soil mesofauna orders (i.e., mostly microarthropods of 0.2 - 4 mm in body size; Flórián et al. 2019) and rare orders with less than six captured individuals.

Herbivory

The herbivory assessment was restricted to damage caused by invertebrates, as suggested by Meyer et al. (2017), and was quantified by calculating the proportion of damaged leaves according to Souza et al. (2013). A total of 50 leaves were randomly drawn from vegetation samples of MPs 1, 3, and 5. Leaves from each life form were sampled proportional to their estimated relative biomass.

Statistical analyses

We tested the effects of treatments on all response variables by using linear mixed models (LMM). Variables were transformed (log-transformed: variables of arthropods abundance; available forage, number of species flowering and plant species richness; square-root-transformed: root biomass, height of canopy, coverage of functional types and herbivory variables) to improve variance homogeneity and normality of errors that were assessed, respectively, according to Bartlett and Shapiro-Wilk tests. Then, treatments (i.e., natural grasslands and cultivated pastures)

were used as predictor variable (fixed effect), while hemisphere (north or south), block, mean 30-day preceding sampling precipitation, season of sampling (spring or summer), and municipality were considered as random variables. We built models using function *lmer* from the R package *lme4* (Bates et al. 2014). When a significant effect of treatment on any of the response variables was found, Tukey test was used for pairwise comparison among treatments, adjusting the P-values with the Bonferroni correction and setting $\alpha = 0.05$ as probability limit for rejection of null hypothesis, using *glht* function from the *multcomp* package (Hothorn et al. 2008). Additionally, we used a simple linear least squares regression model to determine the relationship between plant species richness and measured ecosystem functions that statistically differed to assess which functions were influenced directly by plant species richness due to its positive relationship with ecosystem functioning (Cardinale et al. 2012; Zirbel et al. 2019). For floral visitors, we ran simple linear regression using number of flowering species and abundance of each floral visitor order as independent and dependent variables. All statistical analyses were performed with R software (R Core Team, 2020).

Results

The number of plants species (richness) and flowering species were higher in natural grasslands than in cultivated pastures, while percentage of bare soil did not differ (Table 3). Available forage biomass and root biomass from ground level to 10 cm depth did not differ between treatments, however for 10-20 cm layer and total root biomass were, respectively, 2.6 and 1.6 times higher in natural grasslands than cultivated pastures (Table 3). Regarding floral visitors, abundance of Lepidoptera and Coleoptera were, respectively, 11% and 30% higher in natural grasslands than in cultivated pastures. On the other hand, soil arthropods from Coleoptera and Hemiptera orders were, respectively, 58% and 23% higher in cultivated pastures than natural grasslands (Table 3).

Arthropod damage (i.e., herbivory) was about 61% higher in cultivated pastures than in natural grasslands.

Table 3. Mean ($\pm$ SE) of structure of vegetation and ecosystem functions variables.

	Natural grasslands	Cultivated pastures
Structure of vegetation		
Richness (1 m^2 plot)	**14.1^a ± 2.1**	**5.3^b ± 0.7**
Flowers (1 m^2 plot)	**5.2^a ± 0.8**	**2.5^b ± 0.4**
Bare soil (%)	13.3 ± 3.4	15.8 ± 4.6
Biomass		
Forage biomass (kg/ha)	1760 ± 293	1850 ± 374
Root 0-10 cm (g/dm^3)	17.0 ± 2.6	12.1 ± 2.6
Root 10-20 cm (g/dm^3)	**6.2^a ± 2.8**	**2.3^b ± 0.5**
Root total (g/dm^3)	**23.2^a ± 4.9**	**14.4^b ± 3.1**
Floral visitors (trap^{-1})		
Coleoptera	**1.9^a ± 0.4**	**1.7^b ± 0.4**
Diptera	4.1 ± 0.8	4.6 ± 0.9
Formicidae	0.5 ± 0.2	0.9 ± 0.3
Hemiptera	1.1 ± 0.2	1.5 ± 0.2
Hymenoptera	5.0 ± 2.1	6.1 ± 1.3
Lepidoptera	**1.3^a ± 0.1**	**1.0^b ± 0.1**
Soil arthropods (trap^{-1})		

Araneae	1.5 ± 0.2	1.3 ± 0.2
Coleoptera	$1.2^b \pm 0.2$	$1.9^a \pm 0.2$
Formicidae	10.7 ± 2.3	16.1 ± 4.3
Hemiptera	$1.3^b \pm 0.1$	$1.6^a \pm 0.1$
Opiliones	1.3 ± 0.5	0.6 ± 0.2
Orthoptera	1.0 ± 0.1	0.9 ± 0.2
Herbivory (%)	$38.0^b \pm 2.5$	$61.7^a \pm 3.3$

[a, b] Different letters represent significant differences between treatments ($P < 0.05$).

Coverage of grass was approximately 25 % higher in cultivated pastures than natural grasslands, while succulent Cactaceae and sedge/rush were only present in natural grasslands (Table 4). Coverage of shrub was about 10.5% in natural grasslands and 0.3% in cultivated pastures, while forb and legume did not differ among treatments(Table 4).

Table 4. Cover (mean ± SE) of life forms per region and treatment

	Natural grasslands	Cultivated pastures
Grass	$60.4^b \pm 5.0$	$75.8^a \pm 5.8$
Cactaceae	$1.1^a \pm 0.05$	$0.0^b \pm 0.0$
Forb	11.6 ± 2.2	10.5 ± 2.5
Sedge/rush	$8.2^a \pm 5.1$	$0.0^b \pm 0.0$
Legume	7.7 ± 3.5	13.5 ± 6.5
Shrub	$10.5^a \pm 3.6$	$0.3^b \pm 0.2$

[a, b] Different letters represent significant differences between treatments ($P < 0.05$).

Linear regression results showed that none of ecosystem functions that differed among treatments were predicted solely by plant species richness (Root 10-20 cm: R^2 = -0.011, P = 0.396; Root total: R^2 = 0.004, P = 0.306; Coleoptera (as floral visitors): R^2 = 0.085, P = 0.091; Lepidoptera (as floral visitors): R^2 = 0.088, P = 0.277; Coleoptera (as soil arthropods): R^2 = -0.045, P = 0.891;; Hemiptera (as soil arthropods): R^2 = -0.036, P = 0.651; and Herbivory: R^2 = 0.002, P = 0.317). Also, no relationship was found between the number of flowering species with abundance of floral visitors from Coleoptera (R^2 = 0.037, P = 0.185) and Lepidoptera (R^2 = -0.017, P = 0.461) orders.

Discussion

Our study suggests clear differences in levels of ecosystem function, productivity, and biotic activity between natural grassland and cultivated pasture. Differently from what we had expected, forage availability was not higher in cultivated pastures than in natural grasslands; however, in interpreting this value it needs to be considered that stocking rates are higher in cultivated pastures and that our data represents only a single temporal data point (i.e., does not account for vegetation development through time relative to management practices). Moreover, in natural grasslands, higher coverage of shrub species could overestimate forage biomass due to its high woody biomass and lignin contents (Hooker et al. 2008). Root biomass was higher in natural grasslands in the 10-20 cm layer and when considered total biomass from ground level to the depth of 20 cm depth. While it has been shown before that root biomass increases with plant richness (Mueller et al. 2013; Ma and Chen 2016), we did not find a direct relation among number of plant species and

root biomass. Patterns of root production can be affected by dominance of certain plant functional groups and even by individual species within functional groups by different root traits that increase vertical root segregation and the proportion of deep root biomass (Garnier and Navas 2012, Muller et al. 2013, Swindon et al. 2019). In this sense, the difference between our treatments regarding root biomass possibly can be attributed to low diversity of plant species, and consequently low variation of root traits, of cultivated pastures, which limits the allocation of roots in deeper soil layers and reduces total amount of root production of plant communities in comparison to natural grasslands. According to Bestelmeyer et al. (2015), reduction of root biomass reveals a potential vulnerability of attempting to optimize productivity especial under adverse conditions like drought periods, especially in species-poor environments with simplified canopy and roots structures that are less resistant and resilient to stress events in comparison to multispecies environments (Stockes et al. 2014), reinforcing the risk of convert natural grasslands into cultivated pastures.

Land-use changes impact arthropod communities through modifications of vegetation structure and plant species composition (Joern and Laws 2013). In our study, abundance of Lepidoptera as floral visitors was reduced in cultivated pastures in comparison to natural grasslands, but it was not related to number of flowering species. This likely was due to larger and more uniform vegetation patches in these areas that reduce habitat preferred by lepidopterans for breeding, foraging, and roosting (Ouin et al. 2004; Flick et al. 2012). This group is particularly sensitive to habitat changes and can be used not only as a biodiversity indicator, but also as an indicator of ecosystem degradation (Nelson 2007; Rader et al. 2016). The abundance of floral visitors from Coleoptera order was also lower in cultivated pastures, but it was not related directly either with plant species richness or number of flowering species. Higher abundance of coleopterans as floral visitors was reported by Sjödin et al. (2008) in species-rich grassland sites

with tall vegetation and higher quantities of biomass due to higher forage sources to herbivorous beetles and more prey for carnivores beetles. Coleoptera is a very heterogeneous order that includes species with different life histories (Sjödin et al. 2008) being difficult the finding for a general explanation for this pattern, however, according to Sayers et al. (2019), higher abundance of coleopterans in flowers may be due to presence of specific flower attributes or plant species from natural grasslands.

The conversion of natural areas into monocultures selects for emerging ground beetles with small average body size and good dispersal ability (Rainio and Niemelä 2003; Hanson et al. 2016) and such selection for and against can lead to dominance of only a few specialist species. In our study, the more heterogeneous habitat in natural grasslands allowed for the existence of a large array of beetles with various feeding habits which may increase diversity of coleopterans in total but lead to lower abundances of any single species. Furthermore, the higher abundances of soil-dwelling Coleoptera in cultivated pastures reinforces the potential use of coleopterans as indicators for habitat change (Byers et al. 2018), especially in no-tillage systems that provide sufficient organic matter and ideal conditions for egg oviposition (Silva et al. 1996). A similar response was observed for hemipterans (as soil arthropods) that were more abundant in cultivated pastures than in natural grasslands but without direct relationship with plant richness differing from Benito et al. (2004) results that showed beneficial effects of lower plant diversity on insect groups with potential to become pests (e.g., hemipterans) in cultivated pastures. Higher abundance in cultivated pastures of our study may be linked with monocultures structure that can be used by hemipterans as an optimal refuge and high-quality source of food (Crotty et al. 2015).

Herbivory damage was more severe in cultivated grasslands which is in accordance with results from Leidinger et al. (2017) who found low levels of herbivory in *South Brazilian* secondary grasslands after agriculture use as forage by cattle and attributed it to higher dominance of grasses in grasslands which are generally less damaged by herbivores that prefer forbs (Gossner et al. 2014). In our study, both treatments were grass dominated, and had similar forb coverage, but nonetheless, cultivated pastures had higher damaged levels from invertebrate herbivores. According to Root (1973), greater forage quality in cultivated pastures could increase herbivory damage when compared to natural grasslands. The inability to establish a more predictive relationship between herbivory and species richness can be attributed to the other unaccounted for ecological processes that act at different landscape scales and have complex interactions (Egorov et al. 2017; Ebeling et al. 2020).

In our study, differences were observed among treatments from variables of vegetation structure, biomass productivity and arthropods abundance, but different from our initial expectation, these responses were not directly predicted by plant species richness, even with a reduction of, approximately, 62% in cultivated pastures compared to natural grasslands. The latter are subdued to conversion into cultivated pastures aiming to increase biomass production for livestock, which was not supported by our findings and reveal a potential risk of natural grasslands conversion due to reduction of root biomass production, possibly reducing the capacity of plant communities to persist under stress events (e.g., drought). The impacts on invertebrates abundance can be also an indicative of ecosystem functionality disruption on converted areas: (1) reduction of floral visitors abundance in cultivated pastures may be due to disappearance of some focal plant species essential to pollinators; and (2) increases of coleopterans and hemipterans abundances in cultivated pastures can be, respectively, an indicative of habitat change and reveal a potential pest

issue. As these responses were not directly related to decreases of plant species richness, we may expect an indirect effect of it: the homogenization of plant communities as consequence of conversion of natural grasslands into cultivate pastures, which could have caused loss of relevant plant functional traits, jeopardizing the provision of some ecosystem functions.

Conclusion

Our cross-hemispherical experiment indicates that cultivated pastures are not analogous to natural grasslands in terms of biodiversity and ecosystem function. Root biomass is associated with plant resource use and had its production reduced in converted areas at least in one layer, which may jeopardize ecosystem long term sustainability through drought risk. Some of the most important floral visitors (i.e., Coleoptera and Lepidoptera individuals) were disfavored by natural grasslands conversion into cultivated pastures, disrupting natural biotic plant-pollinator relations. Differences among treatments regarding soil arthropods abundance can be indicative of habitat changes. Higher levels of invertebrate herbivory can be associated with forage quality of cultivated pastures but can be a risk of future pest infestation. It is important to highlight that none of natural grasslands sites received any kind of management practice aiming to improve vegetal/animal performance (e.g., fertilization, irrigation) which is different from most cultivated pasture sites sampled in our study. This highlights the need for conscientious management of natural grasslands and cultivated pastures contextually to optimize livestock production while maintaining ecosystem functionality. Concomitantly to this, it is necessary to develop grazing management systems that integrate biodiversity conservation indicators with animal production, thus preventing conversion of natural areas which results in losses in biodiversity and changes in ecosystem services. This

ultimately may inform the development of policies that reward ranchers who produce livestock sustainably in natural grasslands, in the *Western Rangelands* and *Campos* regions as well as in other natural grazing regions globally.

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CHAPTER 2: Grazing exclusion reduces biodiversity and disrupts ecosystem functions in Río de la Plata grasslands[1]

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Abstract

While it is generally understood that grazing management can affect biodiversity and ecosystem function of natural grasslands, there is a need to quantify the magnitude and direction of change for ecosystem functions potentially affected by grazing and determine the extent of such changes on ecosystem sustainability. The Río de la Plata grasslands (RPG) of South America are such a biodiverse ecosystem that simultaneously provides forage for livestock production. We therefore assessed the effects of two grazing management treatments, continuous grazing (grazed) and grazing exclusion (ungrazed), in RPG of Brazil on plant species richness, below- and above-ground productivity, and arthropods composition and herbivory as indicators of ecosystem function using rapid ecosystem function assessment procedures. Treatments and sampling were implemented beginning in 2010 across 6 study sites (Aceguá, Alegrete, Bagé, Eldorado do Sul, Lavras, and Santa Maria). Differences in levels of ecosystem productivity and biotic activity were found among treatments when sampled 8 years after the experiment started. The ungrazed treatment had higher aboveground total and dead biomass, reduced root biomass at least in one layer, higher herbivory damages, and higher abundance of dipterans as floral visitors. Grazed grasslands had higher plant species richness. Plant species richness was positively related to root biomass (from ground layer to 10 cm depth, and total biomass from ground level to 20 cm depth) and negatively linked to aboveground dead biomass and herbivory damage, indicating the potential of plant species richness to predict some ecosystem changes, and suggesting the inclusion of variables related to biotic heterogeneity have utility for assessing the impacts of grazing management on ecosystem function and biodiversity. Our findings suggest that grazing exclusion can decrease RPG ecosystem functionality and reduce species niches by vegetation homogenization, thus jeopardizing long term sustainability of the grassland ecosystem. Further

studies should focus on key processes RPG and other grasslands globally in more detail, as a basis for policies that support land users who manage natural grasslands to optimize ecosystem functionality while provisioning or enhancing key ecosystem services.

Introduction

Grasslands occupy approximately 40% of Earth's terrestrial surface and sustain biodiversity and livelihoods via the provisioning of critical ecosystem services such as herbaceous biomass production (Leidinger et al. 2017), nectar and pollen resources for pollinators (Black et al. 2011), water infiltration and storage (Bengtsson et al. 2019), and carbon sequestration (Bai and Cotrufo 2022). Grassland plants and grazing animals are key elements of grassland ecosystems, where plants serve as forage source for domestic and wild herbivores (Squires et al. 2018) and such grazing is one of the main drivers of vegetation dynamics (Koerner and Collins, 2014). Grazing regimes influence plant communities in terms of composition and structure (Bakker et al. 2006) and greatly affect ecosystem functionality (McNaughton 1994). Despite the goods and services provided by grasslands, they are unfortunately one of the most converted ecosystems worldwide (Hoesktra et al. 2005) and in many regions of the world suffer transformation into agronomic monocultures of crops and trees or into urban areas (Rands et al. 2010; Parr et al. 2014). However, how ecosystem functions are affected by changes in plant communities through various management strategies continues to be poorly understood (Kremen 2005).

The Río de la Plata grasslands (RPG) are a grass-dominated ecosystem supporting extensive floral and faunal biodiversity serving as forage resource base for herbivores. Livestock production has been the main economic activity for generations and persists today (Pallares et al. 2005). Currently, land-use changes can lead to rapid losses of RPG natural vegetation (Baeza et al. 2022), altering ecological properties and biotic interactions and impacting ecosystem functioning directly and indirectly (Díaz et al. 2013). According to Modernel et al. (2016), the land-use changes, including the conversion of 2 million hectares of native vegetation from 2000 to 2010 to soybean due to high prices and grazing management with low forage allowance, are the

main drivers of negative impacts on provision of RPG ecosystem services through biodiversity reduction, increased erosion risk, and reduced soil carbon stocks. Given the forage potential of native plant species and the significance of livestock for the rural and regional economy, it is important to understand how grazing regimes impact RPG ecosystem functioning to identify potential incentives for vegetation management that sustainably merge biodiversity maintenance, animal performance, and provision of ecosystem function.

In RPG specifically, grazing mechanistically influences ecological properties of the natural ecosystem by directly removing biomass and indirect effects such as trampling, urine and feces deposition which cumulatively effects plant communities (Lezama & Paruelo 2016) via spatiotemporal plant composition changes (Oesterheld et al., 1999). Areas under high grazing pressure are dominated by prostrate plants that are adapted to frequent defoliation events given their fast-growing potential by way of rapid capture and use of resources (Cruz et al. 2010, Ferreira et al. 2020). On the other hand, suppression of domestic grazers alters plant composition, leading to dominance of fewer more physically dominant tall plant species (i.e., tussock grasses and shrubs) that are more efficient in light capture (Boldrini & Eggers 1996, Altesor et al. 2006, Cruz et al. 2010). Plant species richness has been reported to be higher in grazed areas compared to ungrazed areas (Rusch & Osterheld 1997, Chaneton et al. 2002, Boldrini & Eggers 1996, Altesor et al. 2005, Altesor et al. 2006), which can be indicative of optimized resource availability and use though differential species niches (Cardinale et al. 2012).

Such effects on plant composition influence primary productivity, which is an important mechanism of energy flow (McNaughton et al. 1989) and can vary according to land management in RPG. Yet disagreement in the literature persists as for example, comparing areas continuously

grazed (at least 25 years managed with a moderate stocking rate [i.e., < 0.5 animals per hectare]) to areas excluded from domestic herbivores for 9 years, Altesor et al. (2005) found higher aboveground biomass in grazed *vs.* ungrazed, contrasting with results from Ferreira et al. (2020) that found higher values in ungrazed areas (4 years without grazing from domestic herbivores) than continuously grazed area (ranging from 0.6 to 0.9 animal units per hectare). In both studies, plant composition was a key driver of responses, as implicated by the reduction of cover of prostrate species in ungrazed areas. The main reason for the difference in aboveground biomass appears to be the higher recruitment and production of C4 tussock grasses in ungrazed sites from Ferreira et al. (2020). In terms of below-ground productivity, root biomass was reported to increase under presence of grazing (Altesor et al. 2006, Piñeiro et al. 2009, López-Mársico et al. 2015), especially in the top layer of the soil, due to greater rhizome biomass typically produced by plants with evolutionary adaptations to frequent defoliation events (Cruz et al. 2010). Changes in vegetation cover and structure have been shown to impact, directly or indirectly, other trophic levels in the ecosystem. For instance, Ferreira et al. (2020) reported higher abundance of spiders in ungrazed areas, which may affect invertebrate herbivory rates through herbivore-predator interactions and relative ratios in grasslands (Ebeling et al. 2014). While grazed areas had similar phenological patterns to ungrazed areas (Díaz et al. 1994, Oleques et al. 2019), the more pronounced presence of Asteraceae shrubs that are pollinator-generalists may lead to higher pollen and nectar resource levels for insects in the absence of grazing (Oleques et al. 2019). Few studies in the region, however, integrate data on different species groups or ecosystem functions to evaluate effects of land management (see e.g., Podgaiski et al. 2014 for fire effects on soil processes or Leidinger et al. 2017 for effects of past land use change on several ecosystem processes in the highland grasslands located to the North of the RPG). Except for Oleques et al.

(2019), no studies have directly analyzed the potential of plant species richness as a predictor of ecosystem function, even though species richness is directly and indirectly intertwined with multiple ecosystem properties (Garnier et al. 2016) and influenced by grazing regimes in this region (e.g., Boldrini & Eggers 1996, Altesor et al. 2005).

Here, we used rapid ecosystem function assessment procedures proposed by Meyer et al. (2015) to compare grazed and ungrazed RPG natural grassland for a range of ecosystem function indicators. This includes indicators related to productivity, based on biomass, and indicators related to biotic interactions, based on presence of different invertebrate groups and their effects (Weisser and Siemann 2004, Ramoelo et al. 2015). We also measured plant species richness, a key factor for ecosystem functioning (Cardinale et al. 2012). We conducted this study in the Brazilian portion of the RPG, i.e., the southern part of the South Brazilian grasslands (*Campos Sulinos*, Andrade et al. 2019), in the context of a regional LTER study on grassland management and conservation (Ferreira et al. 2020). We hypothesized that (1) available forage will be higher at ungrazed sites mainly due to higher dominance of erect grasses and shrubs that have taller structures with higher lignin content, (2) root biomass will be higher in grazed areas due to the greater prevalence of such plants adapted to frequent or constant defoliation by grazing animals, (3) herbivory damage will be lower in ungrazed areas due to a vegetation structure that benefits predator abundance and lower forage quality, (4) floral visitors will be more abundant in ungrazed areas due to higher abundance of shrubs from the Asteraceae family, and (5) soil arthropods will be reduced in grazed areas due to changes induced by grazing (i.e., lower vegetation height and trampling) that increase mortality. We hypothesized that ecosystem functions will be directly associated with plant species richness by the mechanism that higher species richness leads to higher productivity and higher abundance of other organismal groups.

Methods

Study sites

The study was conducted in Rio Grande do Sul, the southernmost Brazilian state, in the sub-humid Rio de la Plata grasslands ecoregion. The region's grasslands support extensive biodiversity (Andrade et al. 2019) and have been used for livestock grazing since the introduction of domestic herbivores in the 17th century (Carvalho and Batello 2009). Study sites were located in municipalities of Aceguá, Alegrete, Bagé, Eldorado do Sul, Lavras, and Santa Maria (Fig. 1, Table 1).
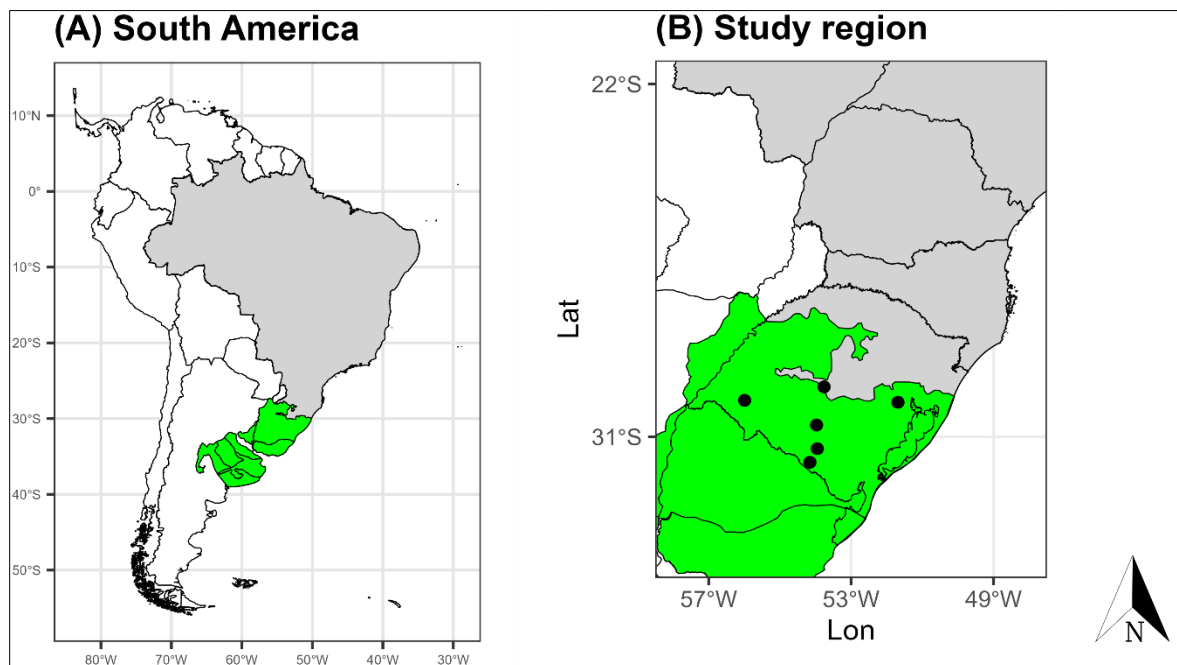
Figure 1. Map of study sites in Rio de la Plata grasslands.

Table 1. Mean annual temperature and precipitation, altitude, and GPS coordinates of each study site in the Rio de la Plata grasslands of Brazil.

Study site	Mean annual temperature (ºC)[1]	Mean annual precipitation (mm)[1]	Altitude (m)	Coordinates (latitude; longitude)
Aceguá	17.9	1152	163	31º38'55" S; 54º09'26" W
Alegrete	18.6	1507	189	30º04'08" S; 55º59'27" W
Bagé	17.9	1599	231	31º18'05" S; 53º56'54" W
Eldorado do Sul	18.8	1455	64	30º07'04" S; 51º41'0.6" W
Lavras	17.9	1449	334	30º41'55" S; 53º58'11" W
Santa Maria	19.3	1688	102	29º43'31" S; 53º45'34" W

[1]30-year average; data from INMET (2023)

Experimental design

This study is part of a Long-Term Ecological Research study (LTER/PELD *Campos Sulinos* – CNPq) and we are reporting the results from data obtained in the 8th year of the project, which started in 2010. Two additional sites that are not part of this LTER were used (Eldorado do Sul and Lavras). At each of the six study sites, we used 70 m × 70 m plots with two contrasting treatments (i) continuous grazing (hereafter as "grazed"), and (ii) grazing exclusion (hereafter as "ungrazed") in a blocked design. Continuously grazed areas were situated within larger paddocks maintained under traditional grazing regimes where cattle had continuous and unrestricted access at all times. The mean annual stocking rate was approximately 0.9 animal units per hectare. Areas excluded from domestic herbivore grazing were maintained with permanent fence that restricted access since 2010. No other disturbances (e.g., fire, mowing) took place in any of the sites during the experiment.

Sampling scheme

Field surveys were conducted 8 years after the establishment of the experiment in October-November 2018 (e.g., during the spring season of the southern hemisphere). We sampled ecosystem functions at five systematically distributed points per treatment (similar to Leidinger et al. 2017; Fig. 2), with a distance of 25 m between points, and following the procedures followed by Meyer et al. (2017). At each point, we established multiplots for assessment of primary and secondary productivity and vegetation structure. Additionally, we installed four arthropod sampling points per site, situated between multiplots, to assess arthropod composition.
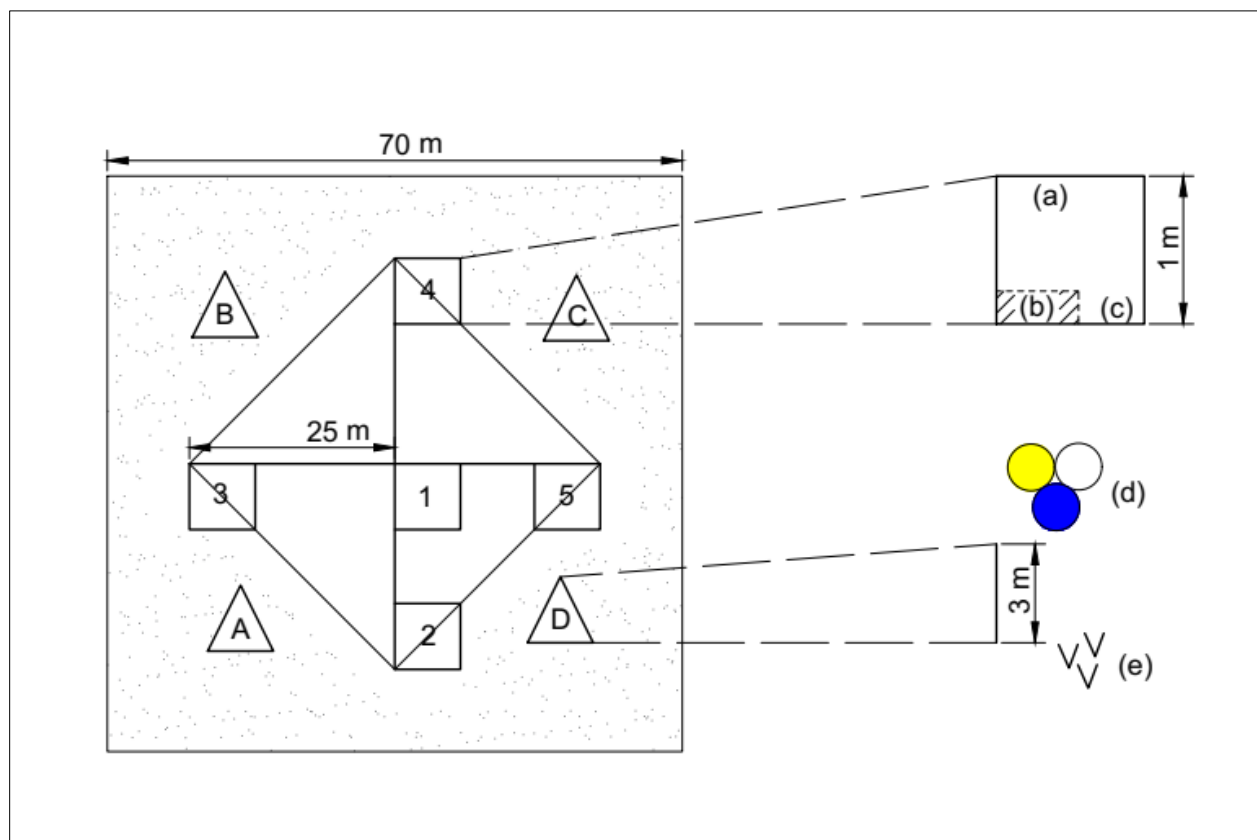
Figure 2. Site sampling scheme with multiplots (multiplot; 1-5) and arthropod sampling point (arthropod sampling point; A-D); MP detail with sampling location of (a) vegetation data, (b) available forage and herbivory, (c) root biomass, (d) floral visitors, and (e) soil arthropods.

Vegetation data

Plant species composition and diversity (i.e., richness, Shannon diversity, and evenness) data were obtained in 5 quadrats of 1 m^2 (1 m × 1 m) inside each multiplot. In each quadrat, we identified all vascular plant species to the lowest possible taxonomic level and any unknown specimens were collected and later identified using taxonomic literature and dichotomous keys. Canopy cover of each plant species was estimated using a modified Braun-Blanquet (1979) scale (i.e., < 1 %; 1-5 %; 5-15 %; 15-25 %; 25-50 %; 50-75 %; 75-100 %). We recorded number of flowering plant species (hereafter as "flowers") and, separately, number of flowering plant species from Asteraceae family. Species were classified according to life form (i.e., prostrate grass, erect grass, forb, sedge/rush, legume, and shrub [incl. sub-shrub lignified only at the base]) following Nelson et al. (2015). Furthermore, in each quadrat, we measured five maximum vegetation heights using a graduate ruler.

Available biomass

Available biomass, as an approximation of aboveground primary productivity, was estimated by sampling peak standing biomass in 20 cm × 50 cm plots in all five multiplots. Biomass was cut at ground level, including dead biomass and woody components of vegetation. In 3 MPs (specifically 1, 3 and 5; see Fig. 2), biomass was separated into different life forms in order to obtain relative

contributions of each to total biomass. Samples were dried for 48 h or until dry at 70 °C before weighing.

Belowground primary productivity

Standing root biomass (i.e., root biomass including root litter) was evaluated as an indicator of belowground net primary productivity (Ni 2004; Ravenek et al. 2014). Volumetric soil samples of 20 cm depth with 10 cm diameter were collected in 3 multiplots (specifically 1, 3 and 5; see Fig. 2) following modified procedures adapted from Böhm (2012). Samples were split into two 10-cm layers by depth (0-10 cm and 10-20 cm) and the sum of both resulted in total root biomass. Roots were separated by washing the soil samples through a sieve and then dried for 48 hours at 70 °C before weighing.

Arthropod composition

Floral visitor abundance was assessed using three pan traps of colors blue, yellow, and white (Nuttman et al. 2011), filled with water and drops of detergent and mounted at vegetation height at each ASP. Organisms on the soil surface were sampled through pitfall traps comprised of plastic pots (capacity of 50 mL and with 9 cm diameter) filled with 30 mL of alcohol 70 % and drops of detergent. They remained open for 24 hours in the field and, at each arthropod sampling point, three plastic pots were placed 3 meters away. Samples from pan and pitfall traps were preserved in alcohol 70 % and all adult specimens were identified and grouped according to its order (except Formicidae individuals were grouped separate from the order Hymenoptera). Treatments inside each block were sampled randomly and, for statistical analysis, we excluded soil mesofauna orders (i.e., mostly microarthropods of 0.2 - 4 mm in body size; Flórián et al. 2019) and rare orders with less than six captured individuals.

Herbivory

The herbivory assessment was restricted to damage caused by invertebrates, as suggested by Meyer et al. (2017), and was quantified by calculating the proportion of damaged leaves according to Souza et al. (2013). A total of 50 leaves were randomly drawn from vegetation samples of multiplots 1, 3, and 5. Leaves from each life form were sampled proportional to their estimated relative biomass.

Statistical analyses

We tested the effects of treatments on all response variables by using linear mixed models (LMM). Variables were transformed (log-transformed: variables of arthropods abundance; biomass, vegetation height, number of species flowering, number of Asteraceae species flowering [as this family was shown previously to drive flower visitor abundances; Oleques et al. 2019], plant species richness; Shannon diversity and evenness; square-root-transformed: root biomass, height of canopy, coverage of functional types, bare soil, litter, and herbivory variables) to improve variance homogeneity and normality of errors that were assessed, respectively, according to Bartlett and Shapiro-Wilk tests. Then, treatments were used as predictor variables (fixed effects), while block was considered as random effect. We built models using function *lmer* from the R package *lme4* (Bates et al. 2014). When a significant effect of treatment on any of the response variables was found, Tukey test was used for pairwise comparison among treatments, setting $\alpha =$ 0.05 as probability limit for rejection of null hypothesis, using *glht* function from the *multcomp* package (Hothorn et al. 2008). Additionally, we used linear regression to determine the strength and significance of relationships between plant species richness and various ecosystem functions that differed among treatments to determine if there was a direct influence of plant species richness due to its positive relationship with ecosystem functionality (Cardinale et al. 2012; Zirbel et al.

2019). For floral visitors that differed among treatments, we ran linear regressions using abundance of each floral visitor order as dependent variable, and number of flowering species, coverage of Asteraceae species and vegetation height as independent variables. Finally, principal component analysis (PCA) was applied to characterize patterns of ecosystem functions supported in different treatments using *vegan* package (Oksanen et. al 2007). All statistical analyses were performed with R software (R Core Team, 2020).

Results

The number of plant species (richness), Shannon diversity and evenness were, respectively, 1.7, 1.2 and 1.02 higher in grazed than ungrazed treatment, similar pattern was observed for number of flowering species, which were 2.1 higher in grazed than ungrazed (Table 3). On the other hand, vegetation height and standing dead biomass were, respectively, 4.2 and 2.6 times higher in ungrazed than grazed (Table 3). Available and dead biomass were, respectively, 1.7 and 4.8 higher in ungrazed than grazed, while green biomass was similar among treatments. Root biomass from 10-20 cm depth did not differ between treatments, however from 0-10 cm layer and total root biomass were, respectively, 2.7 and 2.4 times higher in grazed than ungrazed (Table 3). Regarding floral visitors, there was difference only for the abundance of Diptera, which was 2.3 times higher in ungrazed in comparison to grazed. No differences were found from any soil arthropods orders, and herbivory (i.e., arthropod damage) was about 57% higher in ungrazed treatment than in grazed (Table 3).

Table 3. Mean (± SE) of structure of vegetation and ecosystem functions variables.

	Grazed	Ungrazed

Botanical Richness and Structure		
Species Richness (1 m^2 quadrat)	**22.3^a ± 1.1**	**12.8^b ± 1.9**
Shannon diversity	**3.56^a ± 0.04**	**2.90^b ± 0.09**
Evenness	**1.61^a ± 0.001**	**1.57^b ± 0.008**
Flower Richness (1 m^2 quadrat)	**10.3^a ± 1.7**	**4.8^b ± 1.2**
Height (cm)	**15.5^b ± 3.0**	**65.4^a ± 9.8**
Above- and Below-Ground Biomass		
Total aboveground biomass (kg/ha)	**1285^b ± 267**	**2201^a ± 375**
Aboveground green biomass (kg/ha)	1190 ± 234	1737 ± 276
Aboveground dead biomass (kg/ha)	**96.7^b ± 38.9**	**465^a ± 125**
Root 0-10 cm (g/dm^3)	**18.5^a ± 4.0**	**6.8^b ± 1.5**
Root 10-20 cm (g/dm^3)	1.7 ± 0.4	1.4 ± 0.7
Root total (g/dm^3)	**19.7^a ± 4.3**	**8.3^b ± 2.1**
Invertebrate Floral Visitors (plot^{-1})		
Coleoptera	15.0 ± 5.0	9.0 ± 1.7
Diptera	**33.3^b ± 4.5**	**77.2^a ± 14.1**
Formicidae	7.3 ± 2.3	9.8 ± 5.7
Hemiptera	4.3 ± 2.4	7.3 ± 3.2
Hymenoptera	20.5 ± 5.1	22.0 ± 5.2
Lepidoptera	15.0 ± 5.0	9.0 ± 1.7
Soil Arthropods (plot^{-1})		
Araneae	4.7 ± 1.3	4.7 ± 1.5
Coleoptera	12.5 ± 4.6	10.5 ± 1.8
Formicidae	130.3 ± 30.1	93.7 ± 31.5
Hemiptera	2.5 ± 1.3	3.5 ± 1.5
Herbivory (%)	**35.0^b ± 3.4**	**55.0^a ± 6.7**

[a, b] Different letters represent significant differences between treatments (P < 0.05).

Coverage of erect grasses, forbs, graminoids and legumes did not differ among treatments, except for the coverage of prostrate grasses was higher in grazed with 27.3 % compared to 2.3 % in ungrazed, while shrubs coverage lower in grazed with 1.0% in grazed compared to 25.7 % in ungrazed (Table 4).

Table 4. Coverage (mean ± SE) of life forms per treatment

	Grazed	Ungrazed
Prostrate grasses	**27.3ᵃ ± 7.9**	**2.3ᵇ ± 1.8**
Erect grasses	40.0 ± 6.1	45.2 ± 10.8
Forbs	26.2 ± 4.5	23.8 ± 4.3
Sedges and rushes	1.7 ± 0.7	0.8 ± 0.6
Legumes	3.5 ± 1.3	1.2 ± 0.6
Shrubs	**1.0ᵇ ± 0.5**	**25.7ᵃ ± 9.2**

[a, b] Different letters represent significant differences between treatments (P < 0.05).

Linear regression results revealed that root biomass from 0-10 m, total biomass, dead biomass, and herbivory were all predicted by plant species richness (all P-values < 0.05; Fig. 3), whilst available biomass was not predicted by plant species richness ($R^2 = 0.204$, $P = 0.141$). Plant species richness did not predict abundance of Diptera ($R^2 = 0.118$, $P = 0.276$), number of flowering species ($R^2 = 0.149$, $P = 0.216$), abundance of shrubs ($R^2 = 0.204$, $P = 0.141$), or number of flowering Asteraceae species ($R^2 = 0.246$, $P = 0.101$), however there was a significant positive relation with height of vegetation ($R^2 = 0.537$, $P = 0.007$).
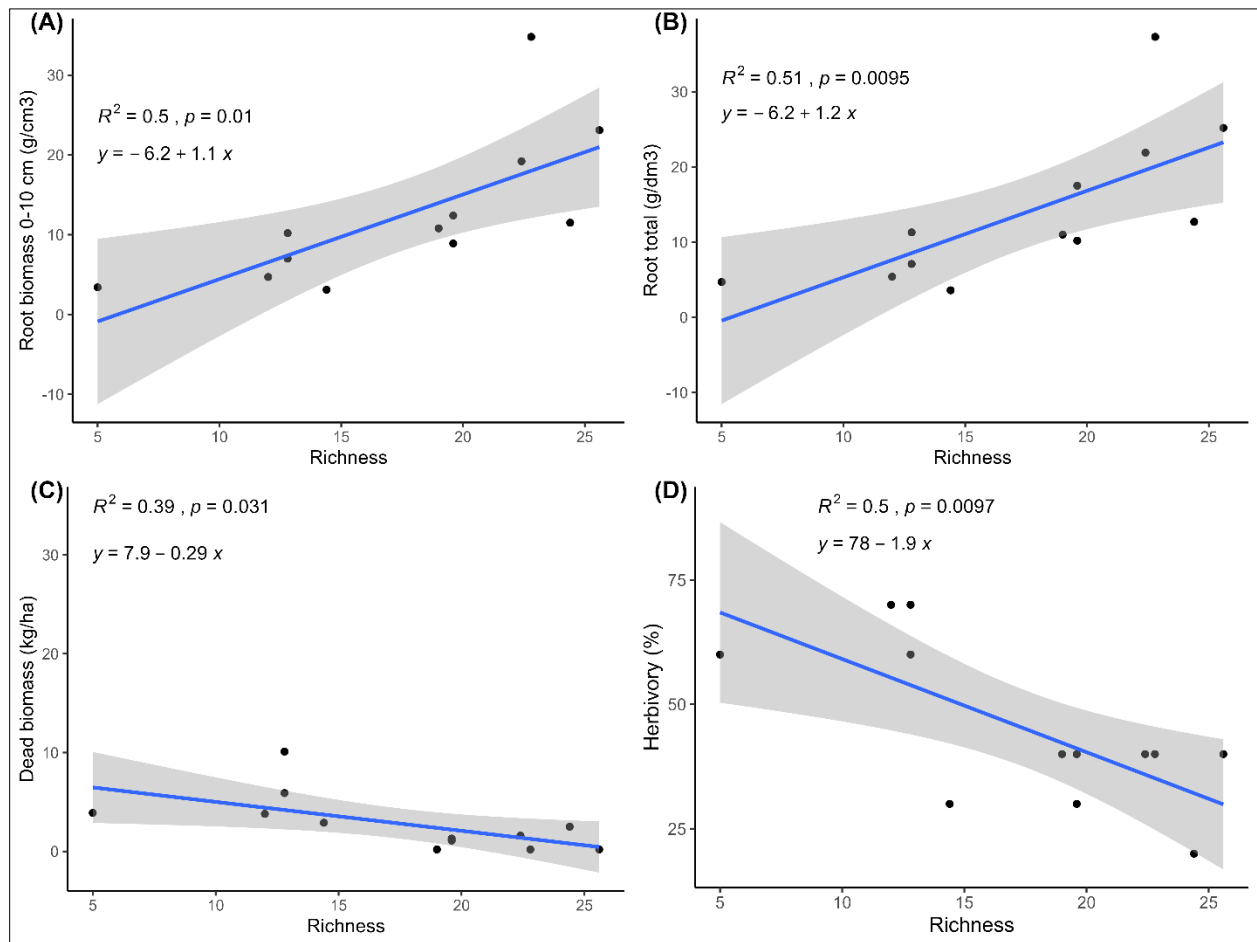
Figure 3. Linear regressions and 95 % confidence intervals for average response of (A) Root biomass from 0 – 10 cm, (B) Root total biomass, (C) Dead biomass, and (D) Herbivory using plant species richness ("Richness") as predictor.

In our multivariate analyses using PCA, axes 1 and 2 explained, respectively explained 37.3 % and 17.0 % of total variation of sampling units, and two distinct groups were formed mainly along axis 1, according to ellipses formed around the centroids of grazing and exclusion treatments (Fig. 4). The ellipse of grazed treatment was positioned distinctly in multidimensional space to the right of the zero line on the positive portion of axis 1 and was associated with plant species

richness, Shannon diversity, evenness, number of flowering species, root biomass from 0-10 cm depth, and root total biomass. The ellipse of the ungrazed treatment did not overlap the grazed treatment ellipse and was positioned in multidimensional space to the left of the zero line on the negative portion of the same axis and was associated with vegetation height, herbivory, and aboveground biomass (i.e., total, green and dead). The variation along axis 2 was very similar among treatments.
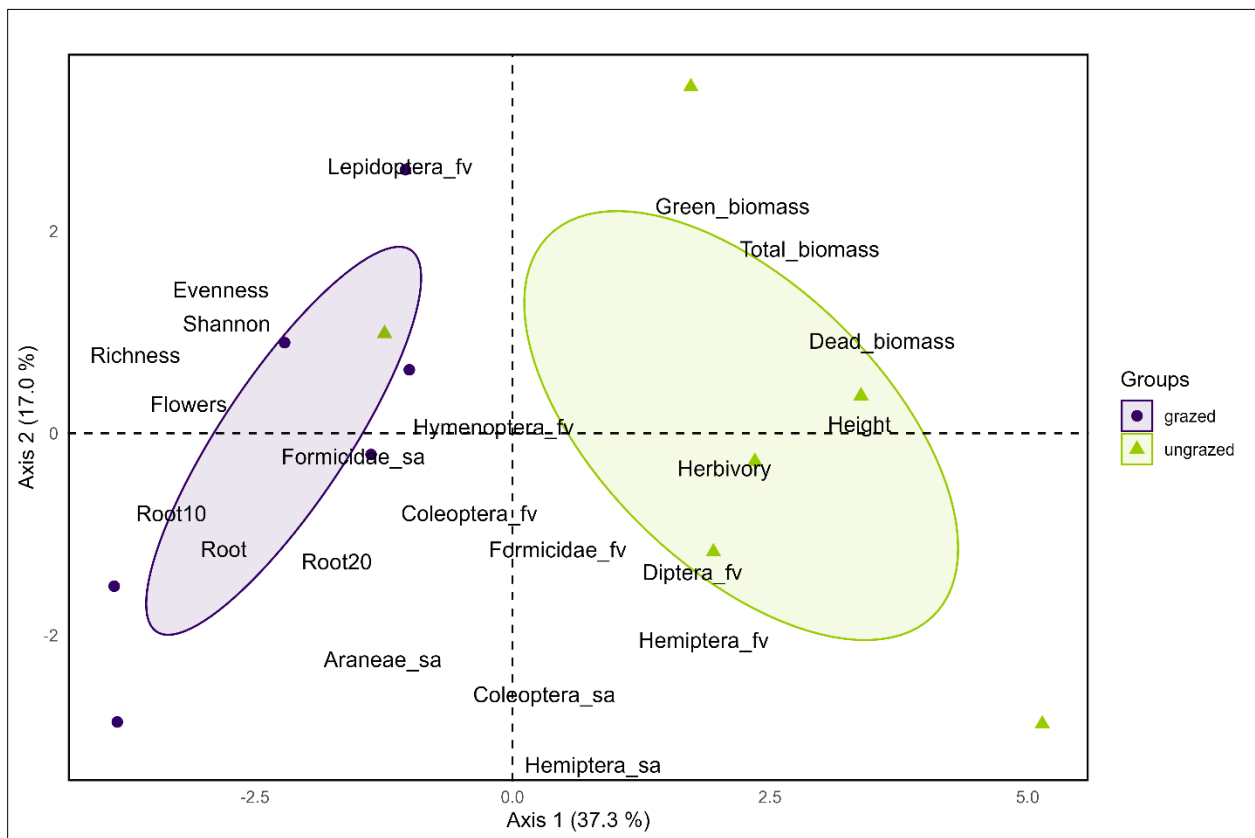
Figure 4. Principal component analysis (PCA) of the set of ecosystem functions, plant species richness ("Richness"), Shannon diversity ("Shannon"), evenness ("Evenness), number of flowering species ("Flowers"), total aboveground biomass ("Total_biomass"), green biomass

("Green_biomass"), dead biomass ("Dead_biomass"), and vegetation height ("Height") of grazed areas (filled circles) and ungrazed areas (filled tringles). Legends: [-sa]Soil arthropods, [-fv]Floral visitors. *Ellipses* indicate 95% confidence intervals around the centroids of the treatments (grazed and ungrazed).

Discussion

This experiment reveals distinct empirical evidence of differential effects of grazing exclusion on ecosystem productivity and biotic activity Río de la Plata grasslands of Brazil. As hypothesized, total aboveground biomass was higher in ungrazed than in grazed treatment where shrub coverage increased significantly and coverage of low-growing prostrate grasses was concomitantly reduced (Table 4). Regarding root biomass, in ungrazed treatment, there were lower values of root total and 0-10 cm depth layer biomass driven possibly by reduction of prostrate grasses (Table 4), which invest more in belowground biomass through the development of rhizomes as a mechanism to persist under frequent defoliation (Fedrigo et al. 2022). A similar result was reported by López-Mársico (2023) where they showed that grazing exclusion increased amounts of standing dead biomass, decreased coverage of total live plants, and caused a root biomass reduction. However, in our study, no reduction of living biomass was observed in ungrazed (Table 3), reinforcing that changes of root biomass were due to higher coverage of shrubs in ungrazed treatment (Table 4). Furthermore, there was a positive relationship between plant species and root biomass variables (Fig. 3 A-B), which can be associated with enhanced plasticity in root allocation and optimized resource utilization by niche complementarity, resulting in higher root biomass production in the grazed treatment (Tilman et al. 2001, Muller et al. 2013).

Modifications on vegetation structure and plant species composition though grazing management may change arthropod communities (van Klink et al. 2015). Regarding floral visitors abundance, our results showed differences only for dipterans, which were more abundant in the ungrazed treatment. However, this was not directly related to shrub abundance or abundance of flowering Asteraceae species, but a response from vegetation height, which was 4.2 times higher in ungrazed than grazed treatment (Table 3). Our findings are similar to those from Ryder et al. (2005), who reported reduction of microhabitats exploited by many dipteran families in areas with lower vegetation height as an inherent consequence of large herbivore grazing. Furthermore, some dipterans can benefit from higher food provision during juvenile stages, which are facilitated in environments that combine greater vegetation physical dominance through height and litter accumulation (Sjödin et al. 2008). Thus, our results suggest that, rather than floral and plant species richness variables, dipteran abundance may be driven by environmental conditions during the earlier life stages that are influenced by changes of vertical structure of vegetation as influenced by grazing or grazing exclusion.

Soil arthropods can be affected by large grazers through unintentional ingestion, trampling, provision of resources for certain groups (e.g., dung feeders, scavengers), but also by changing litter and plant characteristics (van Klink et al. 2015, Perrin et al. 2020). However, we did not find any differences among the two treatments (Table 3), nor any direct relation of any order with plant species richness. Plant species richness is reported to be a poor predictor of arthropod abundance, whilst plant classification according to stature can be a better predictor (van Klink et al. 2015). In our study, we classified specimens only to order, which could limit the resolution of insights from our study in this regard. Furthermore, some responses may only be detectable based on other functional classification criteria (e.g., size, feeding behavior, life stage). Anderson et al. (2012)

found increased levels of dung favoring coprophagous beetles under the presence of grazing cattle, while Van Noordwijk et al. (2012) reported reduction of immobile life stages of ectophagous beetle species in short vegetation through lower vegetation height that limited escape possibilities from grazing activities. Trampling by domestic herbivores was reported as a direct source of mortality of large dung beetles (Negro et al. 2011, Perrin et al. 2020) and soil-diggers (Perrin et al. 2020), while small dung-dwellers are favored by highly grazed habitats (Perrin et al. 2020). These studies indicate that grazing has differential effects on soil arthropods across the diversity of evolutionary adaptations and that their detection may require not only taxonomic but also functional classification.

Herbivory damage by arthropods was more severe in ungrazed treatment (Table 3), which contrasts with our initial hypothesis, and had a negative relation with plant species richness (Figure 3). According to Gómez and Gonzalez-Megías (2002), cattle and insects may compete for plant resources, which may reduce abundance of herbivorous insects under grazing (Vandegehuchte et al. 2017). As no differences were found among treatments for available green biomass in our study (Table 3), competition for plant resources seems not to be the primary driver of greater herbivory in ungrazed treatment. Rather, it may be due to the direct effects of grazer presence and unintentional predation of arthropods during grazing activities that shifted the assemblage toward generalist invertebrate herbivores in ungrazed areas (Torma et al. 2023), corroborating results from Root (1973), who observed lower herbivory damage in plant species rich communities as result of lower specialist densities. Furthermore, grazing exclusion decreased prostrate grass coverage, which belongs to a family reported to be less damaged by arthropods (Gossner et al. 2014), structuring plant communities more susceptible to herbivory damage.

Differences among treatment were found for structure of vegetation, biomass, floral visitors, and herbivory damage variables. Plant species richness was approximately 74 % higher in grazed than ungrazed treatment, but in contrast to our initial hypotheses only a few variables (i.e., aboveground dead biomass, root biomass from 0-10 cm depth, total root biomass (i.e., from 0-20 cm depth, and herbivory) were directly predicted by it. Large grazer removal can jeopardize the biodiversity supporting role of these natural grasslands and agricultural value due to increases of aboveground dead biomass and shrubs (which have lower forage value for domestic livestock). Furthermore, plant communities from grazed treatment had higher number of species and root biomass, which may be indicative of greater drought resilience possibly via stabilization effects of plant species richness with respect to soil respiration (Burri et al. 2018) and by the presence of species that display advanced adaptive strategies to compensate for jeopardized ecophysiological functions of soil moisture limitations (Mariotte et al. 2015). The opposite responses found in ungrazed treatment may be indicative that the facilitated plant communities have constrained capacity to rapidly return to high productivity under drought events limited which could be considered a type of resilience to stress or disturbance. While soil arthropod abundance was similar between treatments, higher abundance of dipterans as floral visitors in ungrazed treatment was indicative of vertical structure changes of vegetation driven by dominance of fewer but taller plants (erect grasses and shrubs). Greater herbivory damage by arthropods may be due to a shift towards more generalist herbivores as a consequence of grazer removal and vegetation homogenization. As most of ecosystem functions responses were not directly related to plant species richness, we may expect that exclusion of cattle grazing decreased plant species richness causing the loss of functional diversity that led to vegetation homogenization, which ultimately affected ecosystem functionality.

Conclusion

Our experiment indicates that exclusion of cattle grazing affects biodiversity and ecosystem functioning Río de la Plata grasslands. The evolutionary role of large herbivore grazing is clearly important for sustaining plant species richness and root biomass and may facilitate landscape heterogeneity in this ecoregion which could be important for biodiversity and resilience to drought events. Greater abundance of dipterans as floral visitors in ungrazed treatment indicated a clear change in the vertical structure of vegetation, which was homogenized by dominance of fewer but taller plant species, indicating changes to plant-pollinator interactions. Soil arthropod abundance was similar among treatments, but it can be attributed to limitations of our taxonomic identification, which highlight the need for functional classification of specimens in order to detect changes related to grazing management in future studies. Greater herbivory damage by arthropods in ungrazed treatment was negatively associated with plant species richness, suggesting a direct effect of grazer's presence by unintentional predation of arthropods. Our findings highlight the importance of long-term ecological research with grazing to elucidate changes on biodiversity and ecosystem functionality across spatiotemporal scales. Exclusion of cattle grazing, which is likely not a feasible long-term feasible management strategy on private land in regards to rural economies and associated vulnerable pastoralist people, also does not benefit grassland biodiversity as shown by Boldrini & Eggers (1996) for plants, Vizentin-Bugoni et al. (2015) and Lorenzón et al. (2023) for birds. Such cascading effects as presented here for the first time to our knowledge, suggest a range of different ecosystem processes of productivity and biotic activity are at risk. Future studies should quantify key ecosystem process more directly (e.g., biomass pools and fluxes, carbon sequestration, water regulation) which will have additional implications for climatic change. This

will be critical to further develop recommendations and policies that sustain rural pastoralist communities, biodiversity, and ecosystem services simultaneously.

Acknowledgements

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Supplemental file 1 – Plant species list with family and life form

Species	Family	Life form
Justicia guaranitica	Acanthaceae	Forb
Ruellia brevicaulis	Acanthaceae	Forb
Stenandrium diphyllum	Acanthaceae	Forb
Pfaffia tuberosa	Amaranthaceae	Forb
Zephyranthes mesochloa	Amaryllidaceae	Forb
Schinus polygamus	Anacardiaceae	Tree
Centella asiatica	Apiaceae	Forb
Cyclospermum leptophyllum	Apiaceae	Forb
Eryngium ciliatum	Apiaceae	Forb
Eryngium eburneum	Apiaceae	Forb
Eryngium elegans	Apiaceae	Forb
Eryngium horridum	Apiaceae	Forb
Eryngium nudicaule	Apiaceae	Forb
Eryngium sanguisorba	Apiaceae	Forb
Oxypetalum solanoides	Apocynaceae	Forb
Hydrocotyle exigua	Araliaceae	Forb
Aristolochia sessilifolia	Aristolochiaceae	Forb
Acanthostyles buniifolium	Asteraceae	Shrub
Acmella bellidioides	Asteraceae	Forb
Aspilia montevidensis	Asteraceae	Forb
Austroeupatorium linearifolium	Asteraceae	Shrub
Baccharis articulata	Asteraceae	Forb
Baccharis coridifolia	Asteraceae	Forb
Baccharis crispa	Asteraceae	Forb
Baccharis dracunculifolia	Asteraceae	Shrub
Baccharis riograndensis	Asteraceae	Forb
Calea uniflora	Asteraceae	Forb
Chaptalia exscapa	Asteraceae	Forb
Chevreulia acuminata	Asteraceae	Forb
Chevreulia sarmentosa	Asteraceae	Forb
Conyza bonariensis	Asteraceae	Forb
Elephantopus mollis	Asteraceae	Forb
Facelis retusa	Asteraceae	Forb
Gamochaeta americana	Asteraceae	Forb
Gamochaeta coarctata	Asteraceae	Forb
Hypochaeris radicata	Asteraceae	Forb
Orthopappus angustifolius	Asteraceae	Forb
Pterocaulon alopecuroides	Asteraceae	Forb
Pterocaulon polypterum	Asteraceae	Forb
Senecio brasiliensis	Asteraceae	Shrub
Senecio heterotrichius	Asteraceae	Shrub

Senecio selloi	Asteraceae	Shrub
Solidago chilensis	Asteraceae	Forb
Soliva pterosperma	Asteraceae	Forb
Vernonanthura discolor	Asteraceae	Forb
Vernonanthura nudiflora	Asteraceae	Forb
Cerastium glomeratum	Caryophyllaceae	Forb
Maytenus muelleri	Celastraceae	Tree
Commelina erecta	Commelinaceae	Forb
Dichondra sericea	Convolvulaceae	Forb
Evolvulus sericeus	Convolvulaceae	Forb
Bulbostylis capillaceus	Cyperaceae	Sedge/rush
Carex phalaroides	Cyperaceae	Sedge/rush
Carex sororia	Cyperaceae	Sedge/rush
Cyperus aggregatus	Cyperaceae	Sedge/rush
Fimbristylis dichotoma	Cyperaceae	Sedge/rush
Kyllinga brevifolia	Cyperaceae	Sedge/rush
Kyllinga odorata	Cyperaceae	Sedge/rush
Rhynchospora setigera	Cyperaceae	Sedge/rush
Euphorbia selloi	Euphorbiaceae	Forb
Tragia volubilis	Euphorbiaceae	Forb
Hypoxis decumbens	Hypoxidaceae	Forb
Herbertia lahue	Iridaceae	Forb
Sisyrinchium micranthum	Iridaceae	Forb
Juncus microcephalus	Juncaceae	Sedge/rush
Salvia ovalifolia	Lamiaceae	Forb
Scutellaria racemosa	Lamiaceae	Forb
Adesmia incana	Leguminosae	Legume
Aeschynomene falcata	Leguminosae	Legume
Desmanthus virgatus	Leguminosae	Legume
Desmodium incanum	Leguminosae	Legume
Eriosema tacuaremboense	Leguminosae	Legume
Rhynchosia corylifolia	Leguminosae	Legume
Stylosanthes leiocarpa	Leguminosae	Legume
Trifolium polymorphum	Leguminosae	Legume
Cliococca selaginoides	Linaceae	Forb
Cuphea glutinosa	Lythraceae	Forb
Krapovickasia flavescens	Malvaceae	Forb
Sida rhombifolia	Malvaceae	Forb
Tibouchina gracilis	Melastomataceae	Forb
Campomanesia aurea	Myrtaceae	Shrub
Oxalis brasiliensis	Oxalidaceae	Forb
Oxalis eriocarpa	Oxalidaceae	Forb
Oxalis lasiopetala	Oxalidaceae	Forb
Plantago tomentosa	Plantaginaceae	Forb
Agrostis montevidensis	Poaceae	Erect grass

Andropogon lateralis	Poaceae	Erect grass
Andropogon macrothrix	Poaceae	Erect grass
Andropogon selloanus	Poaceae	Erect grass
Andropogon ternatus	Poaceae	Erect grass
Anthaenantia lanata	Poaceae	Erect grass
Aristida circinalis	Poaceae	Erect grass
Aristida jubata	Poaceae	Erect grass
Aristida laevis	Poaceae	Erect grass
Aristida murina	Poaceae	Erect grass
Aristida venustula	Poaceae	Erect grass
Axonopus affinis	Poaceae	Prostrate grass
Axonopus argentinus	Poaceae	Erect grass
Bothriochloa laguroides	Poaceae	Erect grass
Briza minor	Poaceae	Erect grass
Bromus auleticus	Poaceae	Erect grass
Calamagrostis viridiflavescens	Poaceae	Erect grass
Chascolytrum lamarckianum	Poaceae	Erect grass
Chascolytrum rufum	Poaceae	Erect grass
Chascolytrum subaristatum	Poaceae	Erect grass
Danthonia cirrata	Poaceae	Erect grass
Danthonia montevidensis	Poaceae	Erect grass
Dichanthelium sabulorum	Poaceae	Prostrate grass
Eleusine tristachya	Poaceae	Erect grass
Eragrostis airoides	Poaceae	Erect grass
Eragrostis lugens	Poaceae	Erect grass
Eragrostis plana	Poaceae	Erect grass
Ichnanthus procurrens	Poaceae	Erect grass
Melica rigida	Poaceae	Erect grass
Mnesithea selloana	Poaceae	Erect grass
Paspalum compressifolium	Poaceae	Erect grass
Paspalum dilatatum	Poaceae	Prostrate grass
Paspalum notatum	Poaceae	Prostrate grass
Paspalum plicatulum	Poaceae	Erect grass
Paspalum pumilum	Poaceae	Prostrate grass
Paspalum quadrifarium	Poaceae	Erect grass
Paspalum umbrosum	Poaceae	Erect grass
Paspalum urvillei	Poaceae	Erect grass
Piptochaetium bicolor	Poaceae	Erect grass
Piptochaetium lasianthum	Poaceae	Erect grass
Piptochaetium lasiantum	Poaceae	Erect grass
Piptochaetium montevidense	Poaceae	Erect grass
Piptochaetium stipoides	Poaceae	Erect grass
Poa bonariensis	Poaceae	Erect grass
Saccharum angustifolium	Poaceae	Erect grass
Schizachyrium spicatum	Poaceae	Erect grass

Schyzachirium microstachyum	Poaceae	Erect grass
Setaria parviflora	Poaceae	Erect grass
Setaria vaginata	Poaceae	Erect grass
Sporobolus indicus	Poaceae	Erect grass
Steinchisma hians	Poaceae	Erect grass
Stipa setigera	Poaceae	Erect grass
Stipa tenuiculmis	Poaceae	Erect grass
Trachypogon montufarii	Poaceae	Erect grass
Vulpia bromoides	Poaceae	Erect grass
Polygala adenophylla	Polygalaceae	Forb
Polygala australis	Polygalaceae	Forb
Polygala linoides	Polygalaceae	Forb
Polygala pumila	Polygalaceae	Forb
Polygonum persicaria	Polygonaceae	Forb
Anagallis arvensis	Primulaceae	Forb
Borreria dasycephala	Rubiaceae	Forb
Borreria verticillata	Rubiaceae	Forb
Galianthe fastigiata	Rubiaceae	Forb
Galium hirtum	Rubiaceae	Forb
Galium richardianum	Rubiaceae	Forb
Richardia brasiliensis	Rubiaceae	Forb
Richardia humistrata	Rubiaceae	Forb
Piriqueta selloi	Turneraceae	Forb
Glandularia peruviana	Verbenaceae	Forb
Glandularia selloi	Verbenaceae	Forb
Verbena ephedroides	Verbenaceae	Forb
Verbena montevidensis	Verbenaceae	Forb
Verbena rigida	Verbenaceae	Forb

CHAPTER 3: Interactive effects of fire and grazing on vegetation structure and plant species composition in subtropical grasslands[1]

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Abstract

Fire and grazing, and their interaction, are the main drivers of vegetation structure and plant species composition in many grasslands globally. However, for subtropical grasslands in southern Brazil, the interactive effects of fire and grazing on botanical characteristics remain relatively unstudied, even though this ecosystem is recognized as fire-dependent with critical importance for livestock grazing. Beginning in 2017 on a privately owned ranch, we established an experiment with three treatments: fire only (F), grazing only (G), and fire and grazing (F+G). Grazing was continuous, and prescribed fires applied annually in winter. We assessed vegetation structure and plant species composition during spring (early November) and summer (early March) of 2017, 2018, 2019 and 2020 (only summer season). Differences in vegetation structure were found among treatments, wherein bare soil, height of canopy and available forage were higher in F, and lower but similar in F+G and G. Plant species richness was equal among treatments, but coverage of C3 grasses was higher in F treatment than other treatments, and shrub coverage was lower in F and F+G in comparison to G. In F+G treatments, available biomass for subsequent burning was limiting resulting in lower fuel continuity, reduced fire spread, and ultimately less total area burned in the subsequent years. Our findings suggest that interaction of fire and grazing can be a useful vegetation management tool to maintain plant richness, while decrease coverage of shrubs which are potentially toxic to herds and limit growth and availability of more preferred forage plants. As a low-cost technique, fire can be adopted by ranchers as a management practice to improve forage quality for livestock while concurrently preserving native biodiversity of these grasslands.

Keywords: disturbance, ecosystem management, functional groups, prescribed fire, pyrodiversity

Introduction

Globally, fire and grazing are key drivers, often interacting, of grassland species diversity (Bond and Keeley 2005) and facilitate grassland ecosystem structure and functioning (Allred et al. 2011). Fire can alter plant species composition (Vermeire et al. 2018), affect nutrient cycling (Reinhart et al. 2016), enhance forage quality and digestibility (Allred et al. 2011), and improve habitat quality for grassland-obligate wildlife species (Engle et al. 2016). Herbivores alter vegetation structure through direct (*i.e.*, grazing) and indirect (e.g., trampling, deposition of feces and urine) effects, altering both plant species composition and the physical structure of vegetation (Adler et al. 2001, Liu et al. 2015, Lezama and Paruelo 2016). Both disturbances have independent effects, but their interaction increases landscape heterogeneity by promoting a shifting mosaic with different vegetation patches, which is suggested to be critical for conservation of grassland biodiversity broadly (Fuhlendorf and Engle 2004, Ricketts and Sandercock 2016) and can be strategically applied as a grassland management tool to maintain sustainable livestock production (Powell et al. 2017).

The interaction between fire and grazing is driven mainly by canopy openness, which decreases plants competition for light, water, and nutrients (Everson and Everson 1987). This resource alteration is facilitated by focal grazing due to herbivore's preference for burned patches over areas not recently burned due to greater protein, digestibility, and palatability of the emerging plants (Archibald et al. 2005). This interaction affects the vegetation structure and plant composition due to different effects on plant functional groups (Fuhlendorf et al. 2009), such as favoring forbs (Fuhlendorf and Engle 2004, Vermeire et al. 2004), legumes (Spasojevic et al. 2010), whilst limiting growth of shrubs (Ansley et al. 2010). Grass responses are variable though where the fire and grazing interaction can be beneficial for rhizomatous grasses (Pfeiffer and

Steuter 1994, Neary et al. 1999) and C4 grasses (Limb et al. 2011a), but may decrease the coverage of tallgrasses (Fuhlendorf and Engle 2004, Vermeire et al. 2004) and C3 grasses (Anderson et al. 1970). However, these responses are dynamic and can vary spatiotemporally according to climate, fire (e.g., frequency, intensity, season of burning), grazing regimes (e.g., stocking rate, animal type and category), and soil related variables (e.g., Brockway et al. 2002, Govender et al. 2006, Fuhlendorf et al. 2009), which requires knowledge about particular ecosystem characteristics to assess the effects of fire and grazing interaction on plant communities.

Grasslands are one of the most endangered ecosystems around the world (Hoekstra et al. 2005) and are mainly used as forage source to livestock production (Squires et al. 2018), which reinforces the urgent need to develop management tools consistent with historical disturbance regimes and aiming to preserve biodiversity inherent from these ecosystems while also optimizing animal performance (Limb et al. 2011b, Powell et al. 2017). The fire and grazing interaction can be advantageous to livestock management by maintaining forage resources (Spiess et al. 2020), creating spatial heterogeneity that will provide a buffer against livestock productivity loss, stabilize annual cattle gains, and mitigate drought effects (Allred et al. 2014). However, due to implementation of other techniques (e.g., mowing) and cultural aversion to accept fire as an agent of grasslands maintenance, its use has been limited (Bowman et al. 2009) jeopardizing natural grasslands conservation that employ the ecological interaction as a management strategy. However, several questions remain regarding effects of fire and grazing interactions on vegetation composition and structure over temporal scales, and this knowledge can provide support for interactive management that comprise benefits from this interaction.

The subtropical grasslands in southern Brazil, a region considered as fire-dependent ecosystem (Pivello et al. 2021), even though currently mostly under grazing and without much use

of fire, are species rich grasslands (Andrade et al. 2019) with high relevance as a forage source for domestic livestock (Carvalho and Batello 2009). The interactive effects of fire and grazing have been poorly studied in this ecosystem so far, despite the potential of facilitating land management and benefiting biodiversity. Fire in these grasslands is known to have occurred in the past millennia (Behling et al. 2005). Today, fire is rarely used, in contrast to the highland grasslands just north of the subtropical grasslands in Rio Grande do Sul state where it is a common management tool for reducing accumulated biomass and promote resprouting at the end of the winter (Overbeck et al. 2007, 2018). Recent studies carried out in the subtropical grasslands region showed positive effects of fire on communities of plants (Ferreira et al. 2021, da Silva et al. 2020, López-Mársico et al. 2020, Loydi et al. 2020), including stimulation of germination (Cuello et al. 2020), ants (da Silva et al. 2020) and birds (Beal-Neves et al. 2020), and on habitat heterogeneity and diversity (Beal-Neves et al. 2020). This highlights the potential benefits of fire as a tool of vegetation management. However, the interaction among fire and grazing, which is historically the most common disturbance in the region, has not yet been studied.

We installed a long-term fire and grazing experiment in Santiago, Rio Grande do Sul state, to assess effects of fire, grazing and their interaction on plant community composition and structure. Over the course of four years, we applied three prescribed fires and evaluated effects on vegetation structure, available biomass, plant species richness, and composition. We hypothesized that, when compared to areas managed with solely fire or grazing, areas managed with fire and grazing, due to higher grazing pressure after fire (Fuhlendorf and Engle 2004, Allred et al. 2011), will have (i) higher soil exposure and lower height of canopy, and (ii) lower available biomass, (iii) reduced plant richness, (iv) lower coverage of legumes, erect grasses and shrubs, (v) higher coverage of prostrate grasses and forbs, (vi) higher and lower coverage of, respectively, C4 and

C3 grasses. We also expect these effects will be observed along the studied temporal scale, indicating the interactive effect of fire and grazing through time.

Methods

Study site

The study was conducted in a private property (Fazenda São Lourenço) located in the municipality of Santiago, Rio Grande do Sul (RS) state (Figure 1-A). The grassland is part of the Río de la Plata region (Soriano et al. 1992) and is used for livestock grazing. Annually there are approximately 180 cows and 145 calves, with average weight of 390 and 150 kg, respectively. These cattle are maintained in a 183 hectares area managed at a stocking rate of approximately 1.1 animal unit (UA) per hectare which is reflective of common grazing management applied to natural grasslands in this region. The altitude is 254 m above sea level and climate is humid subtropical (Kottek et al. 2006) with mean annual temperature and precipitation of 14.6 ℃ and 1832 mm, respectively (INMET 2020).
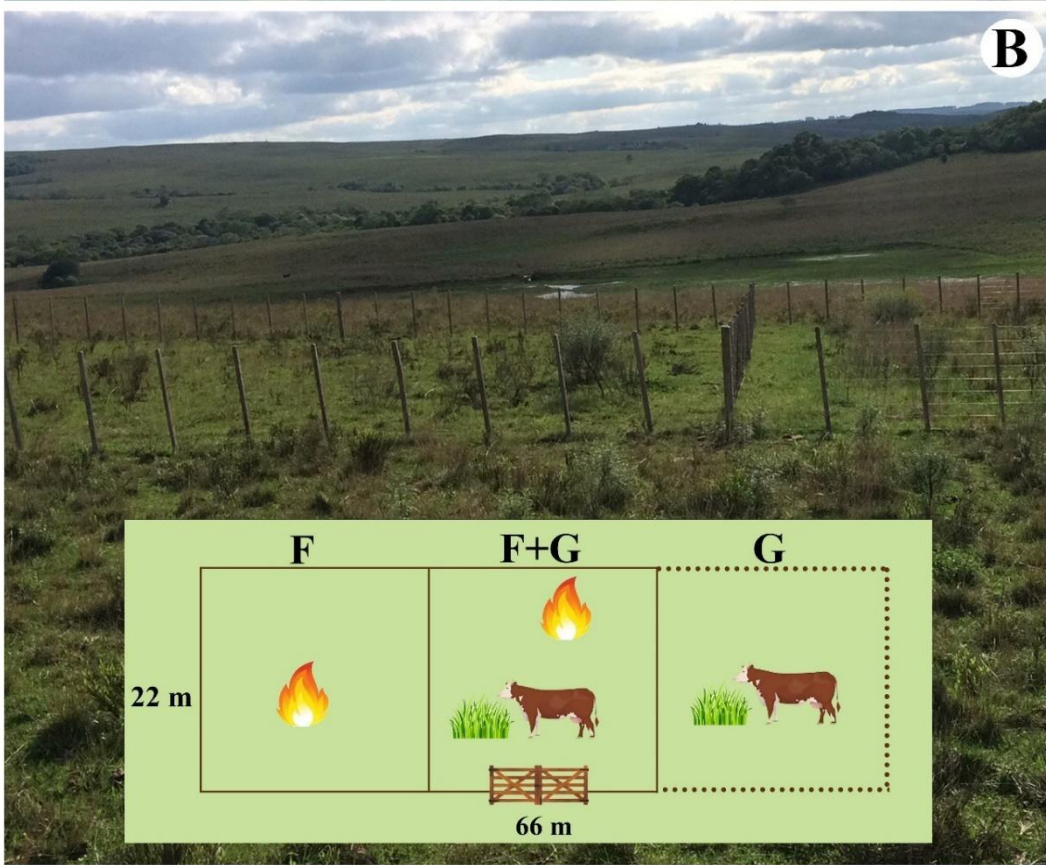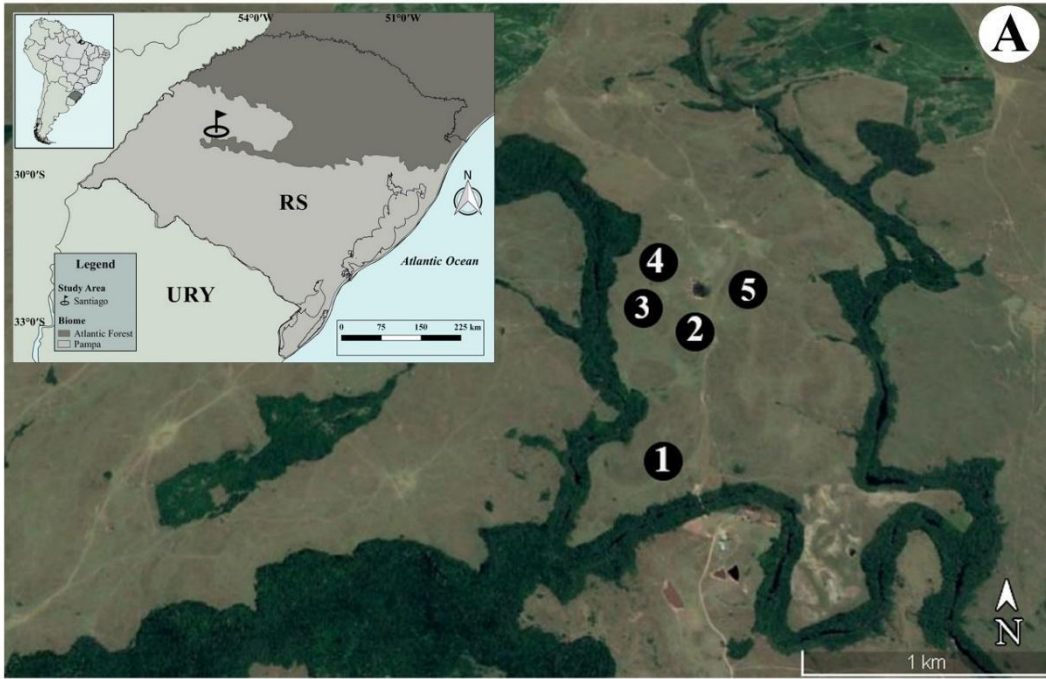
Figure 1 – (A) Study site location and distribution of blocks (#1-5) over the area; (B) Landscape view and scheme of an experimental block where treatments are F = fire only, F+G = fire and grazing, and G = grazing only.

Study design

In March 2017, five blocks of 1,452 m^2 (22 m × 66 m) were established in a natural grassland area (Figure 1b), with a mean distance of 590 m between blocks (ranging from 100 to 1100 m). Each block was subdivided into three plots of 484 m^2 (22 m × 22 m), in which treatments were randomly assigned (Figure 1-B). The three different treatments were: (1) fire only or annually burned and ungrazed (hereafter as "F"), (2) fire and grazing or annually burned and grazed (hereafter as "F+G"), and (3) grazing only or non-burned and grazed (hereafter as "G"). The G treatment is reflective of the most common current management regime in the area. From March to August 2017, treatments F and F+G were kept fenced to allow biomass accumulation for the first prescribed fire, which was conducted during the traditional winter burning season in the region typically in August (Brunel et al 2021, INPE 2022). Immediately after the prescribed fire, fences from F+G were opened to allow cattle entrance and remained open until late April – early May 2018 and 2019 when they were closed again until the following prescribed fire. Vegetation surveys were conducted during spring (early November) and summer (early March) of 2017, 2018, 2019 and 2020 (only summer season).

Vegetation data

Plant species composition was measured in 5 permanently marked quadrats of 1 m^2 (1 m × 1 m) inside each plot. In each quadrat, we identified all vascular plant species to the lowest possible taxonomic level; unknown specimens were collected and later identified using taxonomic

literature. Cover of each plant species was estimated using a modified Braun-Blanquet scale (Braun-Blanquet 1979) (i.e., < 1 %; 1-5 %; 5-15 %; 15-25 %; 25-50 %; 50-75 %; 75-100 %). Species were classified according to functional groups (i.e., erect grasses, prostrate grasses, forbs, legumes, and shrubs, incl. sub-shrubs lignified only at the base). Grasses were also classified by photosynthetic pathway of either C3 (cool-season) or C4 (warm-season). Graminoids, excluding species from Poaceae family, were listed but not considered in our analyses due to their low relative coverage along all managements (< 1 %). Additionally, we estimated the percentage of bare soil and measured canopy height at five points in each quadrat using a graduate ruler. Finally, as an indicator of fire management potential, after each prescribed fire we estimated the proportion of the area burned in each quadrat.

Available forage and fuel biomass

Available forage (an approximation of aboveground primary productivity) and fuel biomass were estimated, respectively, during vegetation surveys and right before prescribed fire. For these purposes, we sampled standing biomass in five 20 cm × 50 cm quadrats in each plot, cutting all biomass, including dead biomass and woody components of vegetation, inside each quadrat at ground level. Samples were then dried for 48 h at 70 °C and weighed.

Statistical analyses

Variables were transformed (log-transformed: plant species richness; square-root-transformed: available forage, fuel biomass; arcsine-transformed: all others) and their average per block, season and number of fire events were calculated for further statistical analyses. The first vegetation survey (carried out before experiment establishment) was used only to assess differences for variable responses among treatments prior the experiment installation. We performed all our data analyses in R (R Development CoreTeam 2017) using 'car' (Fox and Weisberg 2018), 'lme4'

(Bates et al 2014) and 'MuMIn' (Barton 2017) packages. We used linear mixed models (LMM) pooling together all collected data to evaluate the response of vegetation (i.e., structure, biomass, and plant composition) to different factors. For this purpose, treatment (F, F+G, G), season of sampling (spring, summer), and growing season (i.e., 1 for spring 2017 to summer 2018, 2 for spring 2018 to summer 2019, and 3 for spring 2019 to summer 2020) were considered the fixed effects, and block (1 to 5) the random effect in the model (without interaction term). To assess differences among burned area and fuel biomass, we used LMM considering these variables as response, and treatment (F and F+G) and block (1 to 5) as, respectively, fixed and random effects. For all analyses, the residual plots did not reveal any deviations from homoscedasticity (Bartlett test) or normality (Shapiro-Wilk test). We examined the effects of fixed effects on each response variable using Wald test and, when differences were found for fixed effects, specific contrasts using Tukey HSD post-hoc comparisons were performed and $\alpha = 0.05$.

Results

The vegetation structure, before the administration of any treatments (i.e., summer 2017), was similar among treatments (Table 1), however it varied in different ways after the first experimental treatment (Figure 2). Bare soil, height of canopy and available forage were higher in F treatment than others. Season effect was observed for bare soil, which had higher values in spring; while growing season effects were observed for height and available forage, being higher in the second year in comparison to the first year, however no differences were among first and third, and second and third year.

Table 1 – Mean (± standard error) of bare soil, height of canopy and available forage before experiment establishment in each treatment (Fire, F; Fire + Grazing, F+G; Grazing, G) of the fire-grazing experiment in Santiago, RS, Brazil.

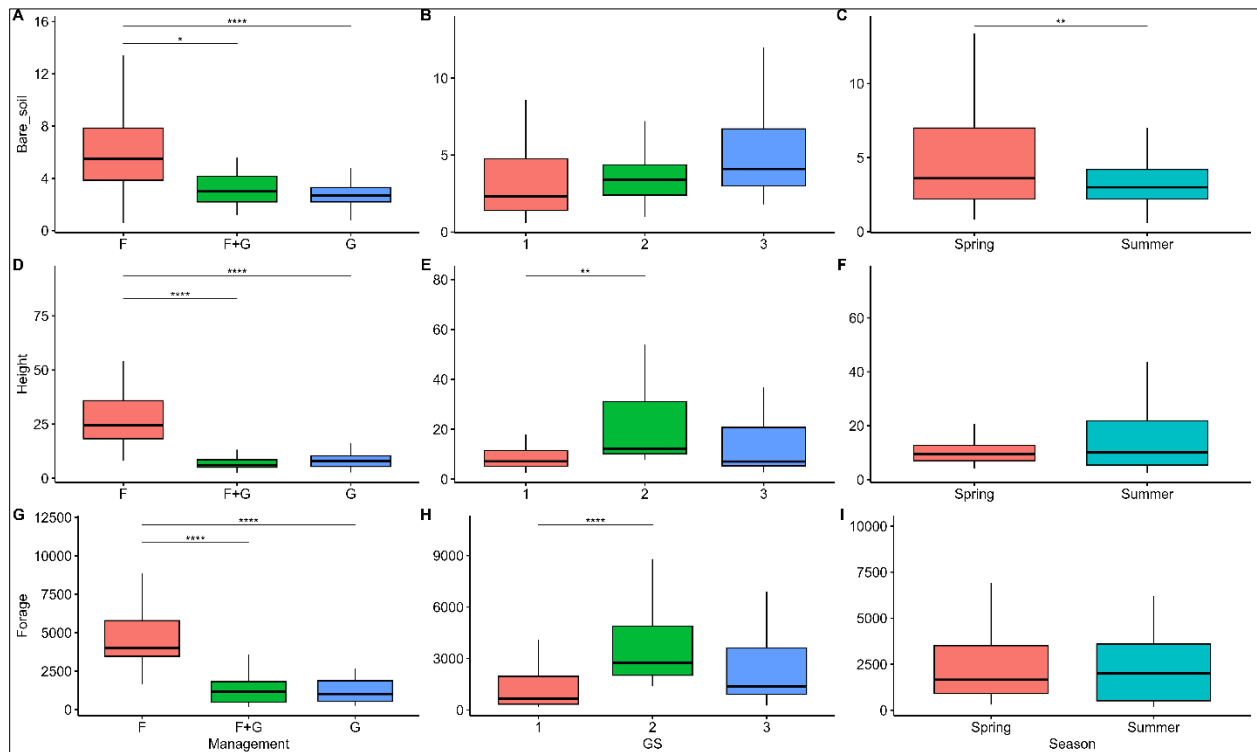| Sampling period		Treatment		
		F	F+G	G
Summer-2017				
	Bare soil (%)	1.3 ± 0.4	1.0 ± 0.2	1.4 ± 0.3
	Height (cm)	16.5 ± 1.2	18.2 ± 2.5	15.9 ± 2.2
	Available forage (kg of dry matter ha^{-1})	2484 ± 134	2674 ± 277	2419 ± 248

Figure 2 – Boxplots showing the effect of treatments, number of fire events, and seasons on bare soil (%; A-C), height (cm; D-F), and available forage (kg of dry matter ha^{-1}; G-I) of the fire-grazing experiment in Santiago, RS, Brazil. Boxplots represent median and 1st and 3rd quantile. The variables plotted on the y-axis are with original values. Asterisks represent statistically significant differences (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.001$) between fixed effects.

Plant species richness was similar among treatments but was higher in 2nd year in comparison to 3rd year of prescribed fire, and higher during the spring. The coverage of C3 grasses was higher in F and lesser but equal in F+G and G treatments (Figure 3). Furthermore, C3 grasses coverage was higher during the spring than summer. Coverage of C4 differed among F+G and F treatments, being lesser in the latter, while value from G treatment was equal to other treatments. The effect of season was also observed, being higher in summer than spring. No differences were

found for forbs regarding treatment, growing season, and season, and Leguminosae had only difference among 1st and 3rd years of prescribed fire. The coverage of shrubs was only affected by treatment, being lower in those with fire presence than in control.
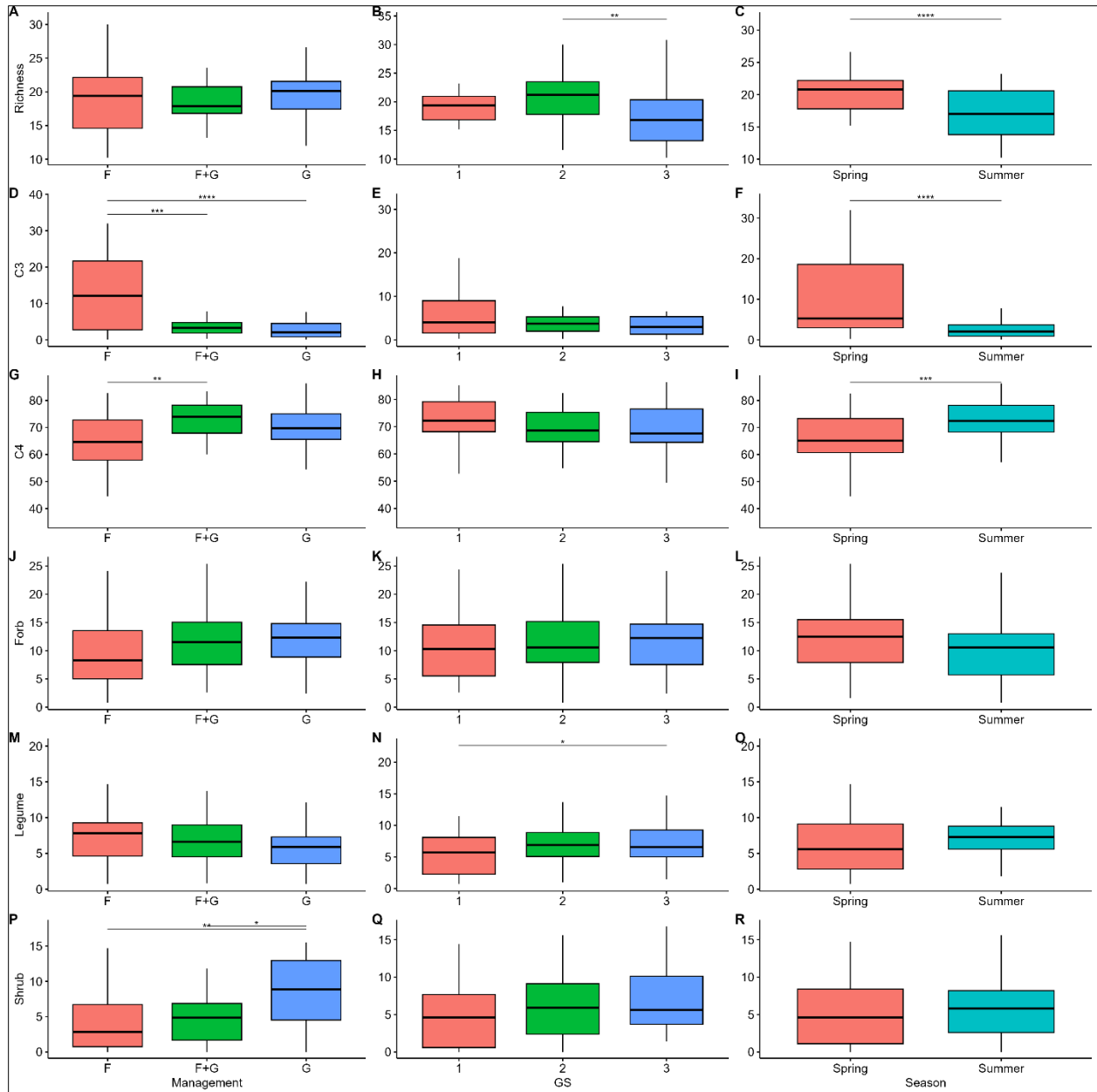

Figure 3 - Boxplots showing the effect of treatments, number of fire events, and seasons on plant richness (A-C), coverage of C3 grasses (D-F), C4 grasses (G-I), forb (J-L), Leguminosae (M-O),

and shrub (P-R) of the fire-grazing experiment in Santiago, RS, Brazil. Boxplots represent median and 1st and 3rd quantile. The variables plotted on the y-axis are with original values. Asterisks represent statistically significant differences (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.001$) between fixed effects. Measure unit: richness (species $plot^{-1}$) all others (%).

Cover of prostrate and erect grasses were, respectively, lesser and higher in F than F+G and G treatments (Figure 4). There was only one difference among the first and second year of prescribed fires, which the latter had lower coverage of prostrate grasses, but no differences were observed among first and third, and second and third year of prescribed fire (Fig. 4B). There was no seasonal effect regarding the coverage of both types of grasses (Fig. 4, C and E).

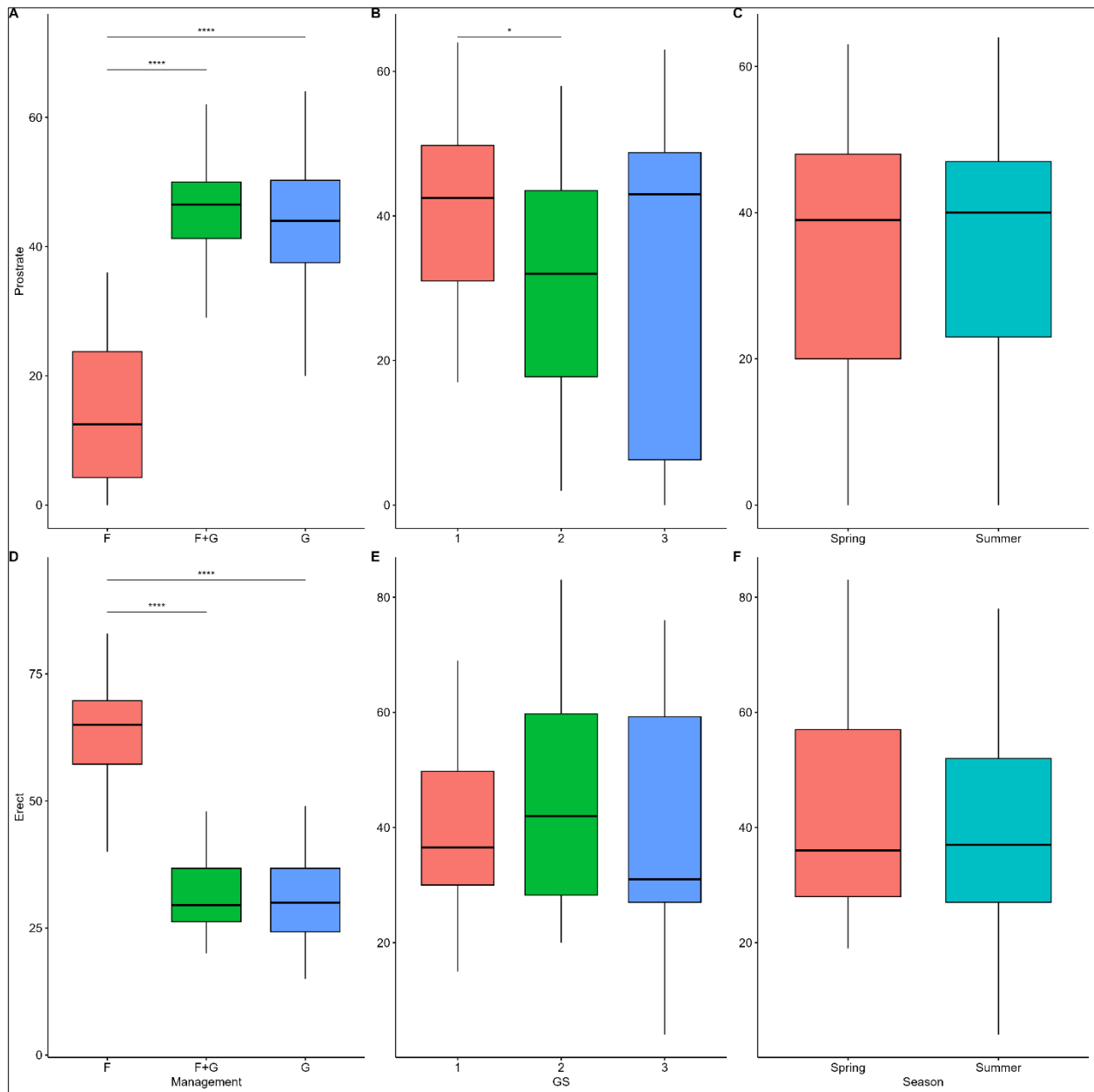Figure 4 – Boxplots showing the effect of treatments, number of fire events, and seasons on prostrate (A-C) and erect (D-F) grasses coverage (%) of the fire-grazing experiment in Santiago, RS, Brazil. Boxplots represent median and 1st and 3rd quantile. The variables plotted on the y-axis are with original values. Asterisks represent statistically significant differences (* $p < 0.05$, **** $p < 0.001$) between fixed effects.

Before the first prescribed fire, in 2017, fuel biomass did not differ among F and F+G managements, but, before the second and third prescribed fires, it was, approximately, 75 % and 90 % higher in F management in 2018 and 2019, respectively (Table 2). The burned area was similar between F and F+G treatments only in the first prescribed fire (2017), but, in 2018 and 2019, there was more than 90 % of burned area in F management, while F+G treatment had value close to 8 % (Figure 5).

Table 2 – Mean (± standard error) of fuel biomass before prescribed fires (2017, 2018 and 2019) and management (Fire, F; Fire + Grazing, F+G) of the fire-grazing experiment in Santiago, RS, Brazil.

	2017		2018		2019	
	F	F+G	F	F+G	F	F+G
Fuel biomass (kg of dry matter ha^{-1})	5640 ± 816	4720 ± 338	**7058^a ± 592**	**1092^b ± 88**	**5500^a ± 626**	**1160^b ± 335**

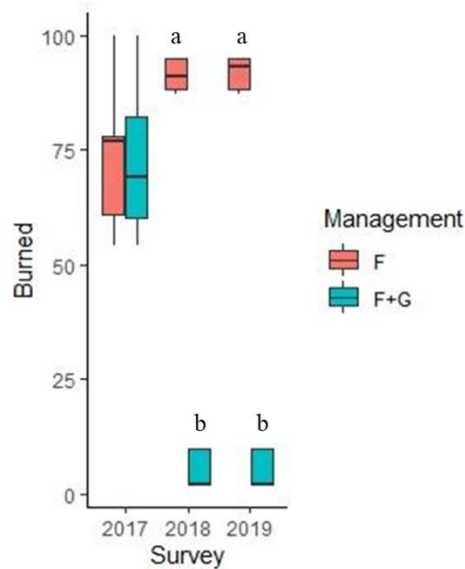Different letters represent statistically significant differences (at $\alpha = 0.05$) between managements

Figure 5 – Mean (± standard error) of burned area (%) after prescribed fires in each treatment (F and F+G) of the fire-grazing experiment in Santiago, RS, Brazil. Different lowercase letters represent statistically significant differences among treatments, per year (p < 0.05).

Discussion

Our study reports different responses of vegetation structure and botanical composition among treatments across the three years of fire and/or grazing applications. It is known from other studies that recently burned areas are preferred by grazers due to higher forage quality (Allred et al. 2011) and access (Laterra et al. 2003). This increases grazing pressure at burned sites – the so-called 'magnet effect' (Archibald et al. 2005) – and reduces probability of fires in the near future through reduced flammable biomass accumulation (Limb et al. 2016). However, our results from subtropical grasslands in southern Brazil showed a similar vegetation structure among F+G and G treatments, indicating that native plant species have capacity to rapidly recolonize open areas after fire and grazing events. This response is in line with Cuello et al. (2020) who observed germination

stimulated by fire in native plant species from the same ecoregion, reinforcing their potential to recover in open areas after fire. Furthermore, the maintenance of prostrate grasses in F+G treatment may also have contributed to plants re-establishment after fire given the capacity of these grasses to persist under frequent defoliation (Cruz et al. 2010). On the other hand, in F treatment, we observed higher soil exposure, height of canopy and available forage that may be due to a plant compositional shift, wherein erect grasses became dominant over prostrate grasses (Fig. 4) facilitating biomass accumulation (Fig. 2) and, consequently, promoting more intense fire events, resulting in higher soil exposure. According to Simpson et al. (2016), greater amounts of accumulated biomass lead to higher fire intensity, reducing plants photosynthetic tissues (Kauffman et al. 1994) and creating open spaces in the burned area (Vermeire and Russell 2018, Rodrigues et al. 2021). Available forage biomass was higher in F treatment due to absence of defoliation events by cattle, which provided conditions for biomass accumulation between fire events (Table 2). Comparing F+G to G, there was no reduction of forage (Fig. 2) likely because interactive effects of fire and grazing are scale dependent (Collins et al. 2006). The size of area coupled with the number of herbivores influences animal density and smaller grazed or burned areas will have higher animal density and ultimately greater grazing pressure (Allred et al. 2011), but, in a landscape perspective, grazers will also move between various patches with different biomass availability to meet their nutritional requirements. Furthermore, burning during the cool season, when most plants are dormant in our study region, and plants resistance to disturbances can explain the lack of forage reduction (Brockway et al. 2002).

The richness of plant species did not differ among treatments, which can be attributed to the capacity of grassland plants to persist under both fire and grazing events in these subtropical grasslands in southern Brazil. Both factors have occurred historically in the region and are

considered to be the main determinant of grasslands in this region where climate allows for plant succession from grassland to forest in the absence of disturbance (Pillar and Quadros 1997, Overbeck et al. 2007, Overbeck et al. 2016). Differences were observed when considering coverage of different functional groups, a useful approach to detect the response of plant communities to disturbances (Diaz and Cabido 1997). The higher coverage of C3 grasses in F treatment could be due to the combined effects of winter fire events, which lead to production of new C3 grass sprouts with higher specific leaf area (Ripley et al. 2015) and to rapid coverage of burned areas by rapid nutrient capture and use, and of cattle exclusion, which provided conditions for these grasses to complete their phenological cycle, i.e., reproduce, spread seeds and increase their seed bank. In the F+G treatments, coverage of C3 grasses was not higher than in the G treatment, likely due to consumption of the palatable C3 grass foliage by cattle. In mesic grasslands in North America, frequent fire events tend to reduce spatial variation of vegetation by enhancing the coverage of a low number of highly common C4 grass species (Collins 1992), but this response was not observed in our experiment, with three fire events in consecutive years. It possibly may be due to the analyzed time scale or to the presence of two different growth habits grasses – erect and prostrate – with the same photosynthetic pathway (C4) that are adapted to fire (Trindade and Rocha 2001) and to grazing (Cruz et al. 2010), respectively, ensuring the dominance of C4 grasses along the vegetation. The interactive effect of fire and grazing benefits forbs through reduction of competition due to higher soil exposure and reducing the physical dominance of tall grasses (Fuhlendorf and Engle 2004; Vermeire et al. 2004), however the rapid return of vegetation to pre-fire conditions did not provide these conditions. A similar response was found for legumes, which are one of the most valuable forage species for livestock due to their high content of crude protein (Jacobo et al. 2006) and, according to Spasojevic et al. (2010), fire events increase light

availability, benefiting legumes that have underground storage organs, nonetheless did not vary among treatments. In our study, grazed treatments (F+G and G) have low vegetation height (Fig. 2), which ensure light availability, while in F, wherein light can be a limiting factor, legumes (especially the main species – *Desmodium incanum* (Sw.) DC.; Appendix A) may be favored by presence of underground storage organs (Alemán et al. 2014).

Shrubs usually are a non-desired plant functional group in livestock systems on natural grasslands as they have a higher lignin content and a capacity to encroach, which reduces cover of other plant species and, consequently, jeopardizes animal performance (Limb et al. 2010, 2011). Moreover, some shrub species are toxic for grazing animals (Tokarnia et al. 2002). In our study region, fire was widely used by ranchers in the past to control shrub encroachment, stimulate plant regrowth, control ectoparasites (e.g., ticks), but fire application as vegetation management tool was reduced in the recent past but has not disappeared entirely (INPE 2022). Our results showed the potential of fire for managing shrub encroachment through lower coverage of shrubs (mainly *Baccharis crispa* and *Senecio heterotrichius*) in F and F+G treatments in comparison to G. In F, there was a direct fire effect through annual fires that limit the competitive potential of shrubs (facilitated by biomass accumulation) by burning the majority of the area (Heisler et al. 2004); while the interactive effects of fire and grazing in F+G may have limited shrub coverage by reducing ramet densities and photosynthesis rates, especially after first prescribed fire, wherein the mean burned area was 73 %. Similar response regarding interactive effects of fire and grazing on shrubs were observed by O'Connor et al. (2020), which also suggested a potential for long-term mortality of shrubs due to this interaction.

Regarding growth habit of grasses, in F treatment, we observed a clear shift in terms of habit of grasses: erect grasses (including tussocks) became dominant over prostrate grasses (Fig.

4). This response is associated with the traits of erect grasses that promote flammability and fire tolerance (Simpson et al. 2016). Some species may even that have higher tannin content as strategy to avoid herbivory damage and retard decomposition, ensuring accumulation of fuel biomass load that, consequently, increase possibility of fire (Everson et al. 1988, Ripley et al. 2015). In our case, F areas were dominated by *Andropogon lateralis* Nees (Appendix A), a tall erect tussock-forming and fire-adapted species (Trindade and Rocha 2001). The tall canopy from erect grasses in this treatment concomitantly leads to the reduction of prostrate grasses (Fig. 4). According to Pausas et al. (2018), bud protection is the essential requirement to grass resprouting and erect grasses are associated with belowground bud bank regrowth that confers resprouting advantage after fire events (Coughenour 1985). Contrasting, prostrate grasses are more grazing tolerant, which make them more associated with continuous leaf elongation from their base (Coughenour 1985), suggesting that annual fires in areas excluded from herbivory lead to a reduction of these species. Deviating from our initial hypotheses, fire coupled with grazing did not increase nor decrease, respectively, coverage of prostrate and erect grasses, suggesting the total burned area from 2nd to 3rd (Fig. 5) years was too low to cause changes on the abundance of grasses growth types. This, in turn, appears to be a consequence of grazing in that stocking rates were high enough as not to allow for the accumulation of plant material in summer that would then serve as fuel for a burn in winter – a forage-fuel paradox that influences the grazing-fire probabilities of a patch in the landscape (Fuhlendorf and Engle 2004). In fact, vegetation hardly burned in years 2 and 3 in the F+G treatments.

According to Pimm (1984), resilience is the amount of time required to return to a state, following disturbance, which approximates the pre-disturbance state, and our results indicated native plant species potential to rapidly reestablish even under frequent defoliation. Furthermore,

fire and grazing are distinct factors having different effects on vegetation structure and botanical composition, which create a window of opportunity by using their interaction as a vegetation management tool. The maintenance of plant richness coupled with shrub reduction are important findings in this region because the use of fire as management tool is not expensive and was widely used by past generations of ranch managers which could facilitate the re-emergence of rancher adoption. Future studies regarding the interactive effects of fire and grazing should include different seasons of burning (e.g., burning in the beginning of summer) and different livestock scenarios (e.g., different animal species and/or stocking rates) in order to identify additional potential applications of this ecological management tool for natural grasslands in South America.

Conclusion

Our experiment is the first one to assess interactive effects of fire and grazing in subtropical grasslands in southern Brazil, a fire-prone ecosystem that critical for both biodiversity and livestock production. The ability of native plant species to persist with singular and interactive aboveground disturbances in our study may explain the finding that grazing of recently burned areas did not negatively affect vegetation structure nor richness of plants. Fuel biomass for future fires, under our study conditions, was so low in F+G areas that, in the second and third year of the experiment, the total proportion of burned area was only 8 %, reinforcing the role of grazers as fuel consumers that restrict fire effects in vegetation patches (e.g., tussocks, shrubs). Yet this has implications for wildfire management and potential use to strategically prevent undesirable fire events. From a livestock production perspective, the use of prescribed fire can be applied to reduce shrubs that are an undesirable plant functional group for cattle due to their toxic potential and competition with more desirable species for forage. In areas managed solely with fire, the most

pronounced results were a clear turnover in grass dominance (e.g., erect grasses dominant over prostrate grasses), and an increase in C3 grasses which has utility for grazing management (e.g., selecting areas for prescribed fire application in winter, followed by spring deferment to grazing in the beginning of summer) ultimately attempting to increase coverage of these grass for optimal forage quality, reduced production costs, and enhancing native plant diversity. The use of fire as a vegetation management tool, and at times coupled with grazing, can be a useful approach to ally biodiversity conservation with maintenance of livestock activity on these natural grasslands.

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Appendix A

List of main species, functional group, growth habit (only for grasses) and coverage (%) from F,

F+G and G treatments of the fire-grazing experiment in Santiago, RS, Brazil.

Species	Functional group	Growth habit	F	F+G	G
Andropogon lateralis	C4 grass	Erect	23.3	21.7	20.8
Aristida jubata	C4 grass	Erect	1.4	0.8	0.1
Aspilia montevidensis	Forb		1.8	1.1	0.3
Axonopus affinis	C4 grass	Prostrate	2.7	7.8	13.3
Axonopus argentinus	C4 grass	Erect	1.9	0.3	0.3
Baccharis coridifolia	Shrub		0.3	1.2	0.2
Baccharis crispa	Shrub		0.1	0.8	4.2
Calamagrostis viridiflavescens	C3 grass	Erect	1.9	0.6	0.0
Chascolytrum subaristatum	C3 grass	Erect	4.5	1.6	0.2
Chevreulia acuminata	Forb		0.2	0.9	0.7
Desmodium incanum	Legume		6.5	6.3	5.3
Dichondra sericea	Forb		0.7	2.8	4.4
Elephanthopus mollis	Forb		0.4	0.5	0.9
Eryngium horridum	Forb		1.3	2.5	1.6
Gamochaeta americana	Forb		0.0	0.4	0.8
Ichnanthus procurrens	C3 grass	Erect	0.7	1.5	0.4
Mnesithea selloana	C4 grass	Erect	4.1	2.2	1.0
Paspalum compressifolium	C4 grass	Erect	3.2	0.3	0.1
Paspalum dilatatum	C4 grass	Prostrate	0.1	0.1	0.5
Paspalum notatum	C4 grass	Prostrate	11.7	29.5	29.4
Paspalum plicatulum	C4 grass	Erect	4.7	1.1	1.3
Paspalum umbrosum	C4 grass	Erect	4.0	2.4	2.1
Piptochaetium montevidense	C3 grass	Erect	1.2	0.9	1.1
Richardia humistrata	Forb		0.1	0.7	0.8
Schizachyrium microstachyum	C4 grass	Erect	3.9	0.5	0.3
Schizachyrium tenerum	C4 grass	Erect	1.5	0.0	0.0
Senecio heterotrichius	Shrub		0.0	0.1	0.8
Setaria parviflora	C4 grass	Erect	0.9	0.8	0.9
Steinchisma hians	C3 grass	Erect	2.3	1.1	0.7
Vernonanthura chamaedrys	Shrub		2.2	3.0	2.7
Vernonanthura tweedieana	Shrub		1.1	0.2	0.0

FINAL CONSIDERATIONS

Our study brings a light on how grasslands managements change plant species composition and ecosystem functions in broader (Chapter I), regional (Chapter II) and local (Chapter III) perspectives. The comparison among different grassland forage uses from North and South America, regarding the amount of assessed ecosystem functioning variables, was never carried out until now. Also, our study showed changes on plant species composition and ecosystem functions in a long-term ecological research study with eight years of large grazers exclusion, which is a novelty from subtropical grasslands from southern Brazil; and our study regarding fire and grazing interaction is the first one carried out in South America grasslands and with promising applications of fire in natural grasslands used as forage source for herds.

It was possible to observe clear differences among natural grasslands and cultivated pastures used as forage source for herds in *Western Rangelands* and *Campos* regions. Results indicated jeopardized ecosystem long term sustainability through drought risk via root biomass reduction; disrupted natural biotic plant-pollinator relations via reduced abundance of the main floral visitors (Coleoptera and Lepidoptera); indicative of habitat changes through soil arthropods abundance changes; and future pest infestation given the higher levels of invertebrate herbivory. It was possible to point out the need for grazing management that integrates biodiversity conservation with animal performance as way to prevent natural grasslands conversion. Furthermore, the development of policies to reward ranchers who produce livestock sustainably in natural grasslands may be crucial for natural grasslands conservation. Future studies should be carried out by having field surveys at different times along the year in order to detect possible variations on ecosystem functions and biodiversity.

In the Río de la Plata grasslands, the exclusion of cattle grazing affected biodiversity and ecosystem functioning, wherein the evolutionary role of large herbivore grazing is clearly important for sustaining plant species richness and root biomass and may facilitate landscape heterogeneity in this ecoregion which could be important for biodiversity and resilience to drought events. Greater abundance of dipterans as floral visitors in ungrazed treatment indicated a clear change in the vertical structure of vegetation, which was homogenized by dominance of fewer but taller plant species, indicating changes to plant-pollinator interactions. Greater herbivory damage by arthropods in ungrazed treatment was negatively associated with plant species richness, suggesting a direct effect of grazer's presence by unintentional predation of arthropods. Our findings highlight the importance of long-term ecological research with grazing to elucidate changes on biodiversity and ecosystem functionality across spatiotemporal scales. Exclusion of cattle grazing, which is likely not a feasible long-term feasible management strategy on private land regarding economic aspects also does not benefit grassland biodiversity. Such cascading effects as presented here for the first time to our knowledge, suggest a range of different ecosystem processes of productivity and biotic activity are at risk. Future studies should quantify key ecosystem process more directly (e.g., biomass pools and fluxes, carbon sequestration, water regulation) which will have additional implications for climatic change. This will be critical to further develop recommendations and policies that sustain rural pastoralist communities, biodiversity, and ecosystem services simultaneously.

Fire and grazing interaction was assessed for the first time in subtropical grasslands from southern Brazil through our experiment in Santiago city, Rio Grande do Sul state. The region is a fire-prone ecosystem that is critical for both biodiversity and livestock production. The ability of native plant species to persist with singular and interactive aboveground disturbances in our study

may explain the finding that grazing of recently burned areas did not negatively affect vegetation structure nor richness of plants. Fuel biomass for future fires, under our study conditions, was so low in F+G areas that, in the second and third year of the experiment, the total proportion of burned area was only 8 %, reinforcing the role of grazers as fuel consumers that restrict fire effects in vegetation patches (e.g., tussocks, shrubs). Yet this has implications for wildfire management and potential use to strategically prevent undesirable fire events. From a livestock production perspective, the use of prescribed fire can be applied to reduce shrubs that are an undesirable plant functional group for cattle due to their toxic potential and competition with more desirable species for forage. In areas managed solely with fire, the most pronounced results were a clear turnover in grass dominance (e.g., erect grasses dominant over prostrate grasses), and an increase in C3 grasses which has utility for grazing management (e.g., selecting areas for prescribed fire application in winter, followed by spring deferment to grazing in the beginning of summer) ultimately attempting to increase coverage of these grass for optimal forage quality, reduced production costs, and enhancing native plant diversity. The use of fire as a vegetation management tool, and at times coupled with grazing, can be a useful approach to ally biodiversity conservation with maintenance of livestock activity on these natural grasslands. Future studies should assess the effects of different burning seasons on plant communities and forage quality to allow for the use of prescribed fires as way to increase forage quality for herds while maintain biodiversity from these grasslands.

Overall, this thesis contributes to knowledge on grassland management effects on biodiversity and ecological processes in grassland. Such knowledge is crucial for management of natural and human-modified ecosystems in a world of global changes with high pressure on natural resources that nonetheless will continue to be the basis for human quality of life. Especially

management effects on ecosystem functioning and services have been poorly studied in South Brazilian grasslands, and clearly more studies should be developed.

Other activities carried out during the PhD

Co-authorship in articles

Brunel, M., Rammig, A., Furquim, F., Overbeck, G., Barbosa, H. M., Thonicke, K., & Rolinski, S. (2021). When do farmers burn pasture in Brazil: a model-based approach to determine burning date. *Rangeland Ecology & Management*, *79*, 110-125.

Overbeck, G. E., Scasta, J. D., Furquim, F. F., Boldrini, I. I., & Weir, J. R. (2018). The South Brazilian grasslands–A South American tallgrass prairie? Parallels and implications of fire dependency. *Perspectives in Ecology and Conservation*, *16*(1), 24-30.

Co-authorship in book chapter

Fidelis, A., Schmidt, I. B., Furquim, F. F., & Overbeck, G. E. (2022). Burning in the Pampa and Cerrado in Brazil. In: Weir, J. & Scasta, J.D. (eds.): *Global Application of Prescribed Fire*.

Oral presentation of proceeding in international congress

Furquim F. F., Scasta, J. D., Overbeck, G. E.. Ecotourism and botanical conservation: a case study of regionally endemic cushion plants in exposed calcareous outcroppings in Park County, Wyoming, USA. In: 61st Annual Symposium of the International Association for Vegetation Science, Bozeman, Montana, 2018.