



# Conservation of species-rich subtropical grasslands: traditional management vs. legal conservation requirements in primary and secondary grasslands

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## ABSTRACT

Land-use change is the main cause of biodiversity losses, and for grasslands includes changes in management. The last 10 years has seen afforestation of traditionally grazed grasslands increase considerably in the understudied Serra do Sudeste region of the Brazilian Pampa, turning the region into a mosaic of tree plantations, natural ecosystems (partly in conservation areas without grazing management) and other land uses. We evaluated grassland plant community structure and composition in conservation areas considering two distinct types of land-use history and compared them to grasslands under traditional management. The study was carried out at 58 sites. Per site, three plots were established to sample the plant composition of the herbaceous and shrub layers. We used ordination techniques and indicator species analysis to describe patterns of community composition. We recorded a total of 516 species, thus confirming the high biodiversity of the region. We detected differences in vegetation structure and composition between primary and secondary grasslands. Our study emphasizes the need to increase conservation efforts in the region and points out that current conservation approaches should be evaluated critically regarding their effects for biodiversity conservation and that adequate grazing management is key for grassland biodiversity conservation.

**Keywords:** biodiversity, conservation, grazing, Pampa, primary grassland, secondary grassland, species richness, subtropical grasslands, vegetation management

## Introduction

The strongest driver of biodiversity loss in the world is land-use change (Sala *et al.* 2000; Millennium Ecosystem Assessment 2005). The modification of natural landscapes into areas for agricultural production has led to widespread destruction of habitats and to fragmentation of previously continuous habitat into smaller and more isolated fragments. Habitat fragmentation exposes remnants of natural vegetation to edge effects and constrains dispersal

between them, with negative consequences for population dynamics and community composition (Fahrig 2003; Hanski *et al.* 2013; Damschen *et al.* 2014; Haddad *et al.* 2015). In the specific case of grasslands, land-use change is not restricted to the complete replacement of the original vegetation by other land uses but can also be related to changes in management intensity of grasslands (Koch *et al.* 2016). Contrasts in land-use intensity and the specific management history of remaining fragments induce variation in habitat quality and select different species combinations (Freschet *et al.* 2013; Allan *et al.* 2015; Newbold *et al.* 2015). Changes in

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management of grassland – which can vary from overgrazing to abandonment – can thus lead to large changes in vegetation structure and composition. For example, in South American subtropical grasslands, heavy grazing usually leads to relatively homogeneous vegetation with rather low species richness, while abandonment causes dominance of tall-growing tussock grasses (e.g. Boldrini & Eggers 1996; Lezama *et al.* 2014; Modernel *et al.* 2016). In the long term, absence of grazing may lead to shrub encroachment and, in some cases, to the substitution of grasslands by forest vegetation (e.g. Oliveira & Pillar 2004).

In southern Brazil, Eucalypt plantations have expanded greatly in the past ten years (Torchelsen *et al.* 2018). In some regions of the country, e.g. in parts of the Pampa grassland region, entire farms are transformed into Eucalypt plantations. However, some parts of these former farms are not planted, as Brazilian legislation (Lei 12.651/2012) requires the establishment of Permanent Preservation Areas (Portuguese acronym: APP) and Legal Reserves (RL). APPs are established around springs and water bodies (with APP width depending on width of the water body), on steep hillslopes and on tops of hills and mountains. RLs are a part of the rural property (20% in the Pampa biome, with the possibility to include APP areas in the calculation) where natural vegetation cannot be removed, and only sustainable use is possible (see Brancalion *et al.* 2016 and Metzger *et al.* 2019 for details and discussion). In some cases, areas that had been used for agriculture previous to tree planting are declared as RL. In the context of Eucalypt plantations, this usually means the development of secondary grasslands which can differ considerably from primary grasslands in terms of species composition, including a higher proportion of exotic, and sometimes invasive, species (Koch *et al.* 2016). APPs are usually not under grazing management, in contrast to RL areas. However, in the latter, traditional management is mostly abandoned in the context of Eucalypt plantations. Often cattle, usually from neighboring properties, still is present in the areas, but generally at low and not controlled stocking rates; additionally, grazing usually occurs without otherwise common management practices such as periodic removal of shrubs or other undesired species. Previous work has shown that low grazing intensity leads to dominance of tall-growing grasses and shrubs and to diversity losses (e.g. Lezama *et al.* 2014; Koch *et al.* 2016). While the substitution of natural grasslands by other land uses has been quantified for grasslands in southern Brazil (Andrade *et al.* 2015), quantification of effects of changed management within grazed grasslands is more difficult (Koch *et al.* 2016) and has not been undertaken for grasslands in the Brazilian Pampa region.

In Brazil's southernmost state Rio Grande do Sul (RS), companies of the tree plantation sector maintain 525 thousand hectares of land, mostly RL and APP, that have not been planted and are considered conservation areas. This is almost equivalent to the sum of the existing

protected areas in the state of RS, which illustrates the high relevance of these areas for biodiversity conservation, even more so considering the rapid land-use change in the region (Oliveira *et al.* 2017) and the lack of an adequate conservation policy of grasslands (Overbeck *et al.* 2015). The challenge is to implement, in these areas, management that contributes to the maintenance of biodiversity within severely altered landscapes. The Serra do Sudeste region, situated in the southeastern part of the state, is especially affected by Eucalypt plantations (Gautreau & Vélez 2011). At the same time, the region is poorly studied regarding plant species composition and conservation value, even though it is considered a high-priority region for conservation (MMA 2000).

In this study, we present an analysis of composition and structure of plant communities in primary and secondary grasslands in conservation areas established in the context of Eucalypt plantations, with the overall aim of assessing conservation status of these areas. Our references are primary grassland subjected to traditional grazing management, *i.e.* an intermediate grazing level that corresponds to good conservation state (see Koch *et al.* 2016). We hypothesized that areas without formal management in the context of afforestation areas would differ from reference grasslands in terms of floristic composition and structure, as low grazing intensity implies in higher dominance of tall-growing species, as summarized above. Specifically, we expected to find 1) higher abundance of woody species (both grassland shrubs and pioneer forest species) in these areas that are still grazed, but are not under traditional management (primary grasslands in conservation areas; PGCA) in comparison to primary grassland subjected to the traditional management (PGTM); 2) lower species richness in areas where traditional management had been abandoned (PGCA), in consequence of lower grazing pressure, and 3) higher importance of exotic species in areas where conservation areas included secondary grasslands that established spontaneously on former agricultural land (secondary grassland in conservation areas; SGCA).

## Materials and methods

### Study region

Our study region comprises the southern part of the Serra do Sudeste mountain range in the extreme south of Brazil, between the municipalities of Bagé, Jaguarão, Caçapava do Sul and Pelotas (total area of approx. 15.000 km<sup>2</sup>; Fig. 1). The region is a conservation priority area due to high levels of endemism, including of herbaceous plant species (MMA 2000). In terms of geology, the region is characterized by dominance of granitic and magmatic formations. Climate is Cfa according to the Köppen classification (Alvares *et al.* 2013): temperate, with cold winters and hot summers, without rainy or dry seasons. The average temperature of the



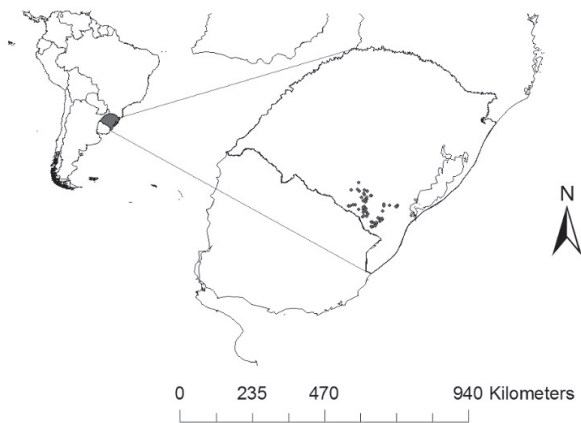
coldest month is above 11.3 °C. The topography is slightly undulated to strongly accentuated (altitudes from 30 to 430m a. s.) and soils are poor in nutrients, ranging from deep to shallow soils, depending on topographic situation (Streck *et al.* 2008). Natural vegetation cover is formed by forest-grassland mosaics, with forests occurring mainly along river valleys. In comparison to other regions of Rio Grande do Sul state, the region still contains a large proportion of

primary grassland (Andrade *et al.* 2015), however, in the past decade, there has been a fast expansion of exotic tree plantations, mainly Eucalypt (Gautreau & Véléz 2011).

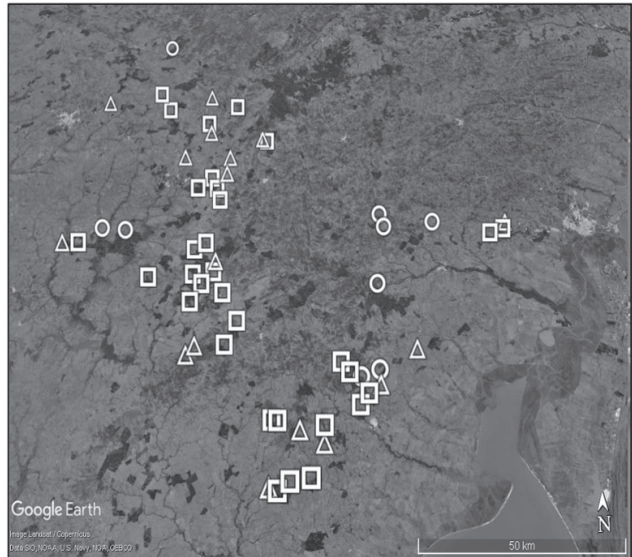
### Sampling design and data collection

The study was conducted at a total of fifty-eight sites which included three distinct types of grasslands with

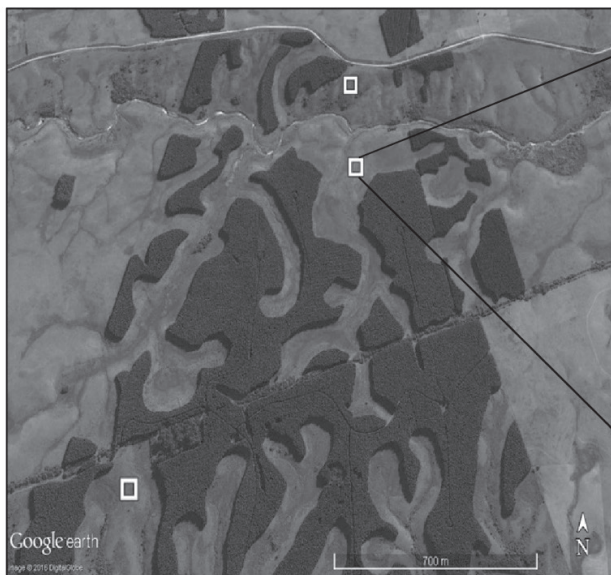
(A) Location of the study region in southern Brazil



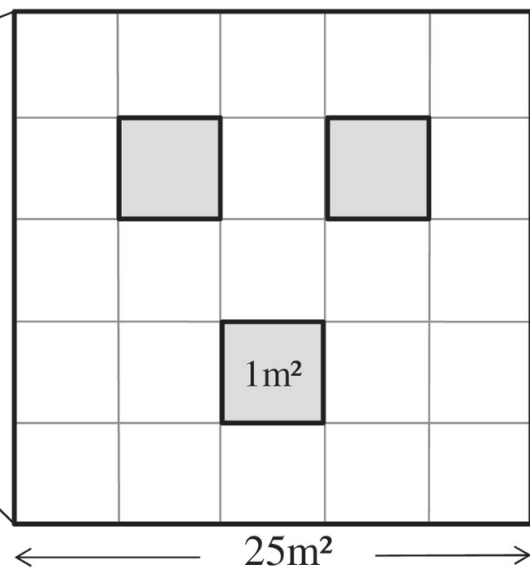
(B) Distribution of 58 study sites within the study region



(C) Sampling design on each sample unit



(D) Sampling design on each site



**Figure 1.** (A) Location of the study region in southern Brazil; (B) distribution of 58 study sites throughout the study region (background map©Google Earth 2015): □ PGCA = primary grasslands in conservation areas; ○ SGCA = secondary grassland in conservation areas; △ PGTM = primary grassland subjected to the traditionally management; (C) sampling design at each of the 1 sample unit containing three 25 m<sup>2</sup> study sites (plots); and (D) sampling design on each plot (25 m<sup>2</sup>) containing three randomly selected subplots of 1 m<sup>2</sup>.

contrasting land-use histories and management intensities: 1) primary grasslands in conservation areas (PGCA; n=31) without formal management (*i.e.* varying, but usually rather low cattle stocking rates) and long history of livestock grazing, located within or close to the Eucalypt plantations; 2) secondary grassland in conservation areas (SGCA; n=7), recovering from conversion to arable land with grazing at variable stocking rates, located within or close to the Eucalypt plantations; 3) primary grassland subjected to the traditional management (PGTM; n=20) of the region (extensive livestock: cattle average 0.5-1 animals per hectare). We consider as “conservation areas” those areas in the context of the Eucalypt plantation where no trees were planted, *i.e.* mostly, but not exclusively, APP and RL areas. Fieldwork was conducted in spring and summer of 2013 and 2014. The Eucalypt plantations had been established seven - eight years (2006) before our sampling, and tree height varied from 8 to 12 m.

### Vegetation data

At each site, we randomly allocated three plots of 25 m<sup>2</sup>, with a distance of at least 1km from each other. For site selection, we used a buffer of 30 m to native forest, Eucalypt plantations, roads and any other type of land use besides natural grasslands. All sites were located in dry grasslands (humid grasslands or wetlands were not included). In these 25 m<sup>2</sup> plots, we identified the average height and abundance of tree, shrub and sub-shrub species (woody species). Additionally, we recorded all species listed on the Red List of endangered species in RS state (Rio Grande do Sul 2014) and all species endemic to the Pampa biome (according to Andrade *et al.* 2018). In each 25 m<sup>2</sup> plot, we randomly allocated three subplots of 1 m<sup>2</sup> where we identified all vascular plant species and estimated their cover according to the Londo (1976) scale. Additionally, we recorded vegetation height (measured at 5 points), percentage of plant litter, percentage of dead biomass on plants, manure and exposed soil. Vegetation parameters were calculated according to Mueller-Dombois & Ellenberg (1974): relative cover (RC), relative frequency (RF), and importance value index (IVI). Species were classified regarding their origin (native/exotic; Rolim *et al.* 2014) and degree of threat was checked in the current Red List for the state (Decreto Estadual n° 52.109/2014).

### Data analysis

For all analyses, we pooled the plot data to the site level. General patterns of species composition were explored by Principal Coordinate Analysis (PCoA), using species' mean cover per site, for both the herbaceous layer and the woody species. Only species with IVI above 1 % were included in the ordination analysis of the herbaceous layer. Treatments were compared by randomization tests regarding, separately,

vegetation height, total vegetation cover, bare soil, litter and manure. We used Euclidian distance for univariate analyses and Chord distance for multivariate analyses, with 999 permutations, and  $\alpha = 0.05$  as probability limit for rejection of the null hypothesis. These analyses were conducted using the software MULTIV (available at: <http://ecoqua.ecologia.ufrgs.br/>). We applied Benjamini-Hochberg correction (Benjamini & Hochberg 1995) to control for false discoveries due to multiple comparisons (critical value for the false discovery rate: 0.05). To evaluate the preference of species for the different site categories and their combinations, we applied indicator species analysis (Dufrene & Legendre 1997) for those species with IVI higher than 1 %, using the function ‘*indicspecies*’ of the R package ‘*multipatt*’, based on the ‘correlation index (r)’ (Cáceres *et al.* 2010). In addition, linear regression was used to test the effect of the average height of the vegetation and the abundance of shrubs/trees on species richness in the herbaceous layer. For this, data were log-transformed to obtain normality.

## Results

Overall, 516 plant species were identified in the sampling of the herbaceous stratum at the 58 grassland sites (Tab. 1). The most important families in terms of species numbers were Poaceae (109 species), Asteraceae (102 species), Fabaceae (36 species), and Cyperaceae (33 species), together constituting 53 % of all species. In the 174 sampled plots of 25 m<sup>2</sup>, twenty-eight species that are included in the list of endangered species were recorded: 26 were found in PGCA and 13 in PGTM; in SGCA, no endangered species were recorded (Tab. S1 in supplementary material). Species richness (Fig. 2A) on the site and plot level was higher ( $p = 0.02$ ) in reference grasslands (PGTM) than in secondary conservation grasslands (SGCA), while no differences were found with primary grasslands in conservation areas. SGCA sites showed a mean value of 21 species in subplots of 1 m<sup>2</sup> and 75 species the 25 m<sup>2</sup> plots (that is, combined data from the three 1 m<sup>2</sup> subplots within each 25 m<sup>2</sup> plot) while PGCA sites had mean values of 31 and 109 species and PGTM sites of 34 and 102 species in subplots of 1 m<sup>2</sup> and plots of 25 m<sup>2</sup>, respectively. The woody species with highest cover values recorded in 25 m<sup>2</sup> plots were *Baccharis crispa*, *Acanthostyles buniifolius*, *Baccharis riograndensis*, and *Baccharis dracunculifolia*. The number of shrub individuals differed between SGCA and PGTM, but not between PGTM and PGCA ( $p > 0.05$ ; Fig. 2B). The abundance of shrubs in 25 m<sup>2</sup> plots showed a negative effect on the richness of herbaceous community in 1m<sup>2</sup> subplots ( $R^2 = 0.30$ ;  $p < 0.01$ ), and sites with the highest vegetation height had lower species richness ( $R^2 = 0.24$ ;  $p < 0.01$ ).

Of the species sampled in the herbaceous layer, 29 were exotic, and total cover of exotic species – including invasive species such as *Cynodon dactylon*, *Eragrostis plana* and *Cirsium vulgare* – was higher in PGCA and SGCA when



compared to PGTM ( $p < 0.01$ ). The PCoA ordination reflected compositional differences in herbaceous communities between PGTM and SGCA, but with considerable overlap between PGCA and the other two types of grassland (Fig. 3A). The first axis separated sites with high cover of *Paspalum notatum* (correlation to the axis: -0.96) and *Axonopus affinis* (-0.50), on the left side of the figure, from areas with high cover of the exotic and invasive grass *Cynodon dactylon* (0.61) and the shrubs *Baccharis dracunculifolia* (0.54) and *Acanthostyles buniifolius* (0.52), on the right side. Along the second axis, the species with the highest correlation were the grasses *Axonopus suffultus* (0.93), *Danthonia cirrata* (0.69), *Piptochaetium stipoides* (0.61), *Schizachyrium tenerum* (0.59) and *Aristida venustula* (0.52), all associated to PGCA plots. The first and second axes of the PCoA based on woody species composition (Fig. 3B) together accounted for 46% of the variation in the data. The first axis separated sites with high cover of *Acanthostyles buniifolius* (correlation to the axis: 0.85), *Baccharis dracunculifolia* (0.59) and *Sida rhombifolia* (0.54) from sites with high cover of *Baccharis riograndensis* (-0.68). The second axis explained 19% of the variation of the data, species with the highest correlation to the axis were *Baccharis crispa* (0.83) and *Baccharis ochracea* (0.62).

Both using grassland composition data (all species with  $IVI > 1$ ) and data of the woody species sampled in the 25m<sup>2</sup> plots, PGTM differed from the other two grassland types in multivariate randomization tests. The average number of woody plants was higher for SGCA and PGCA and lower than in PGTM. Vegetation cover, vegetation height, litter and bare soil differed between treatments ( $p < 0.05$ ; Tab. 1).

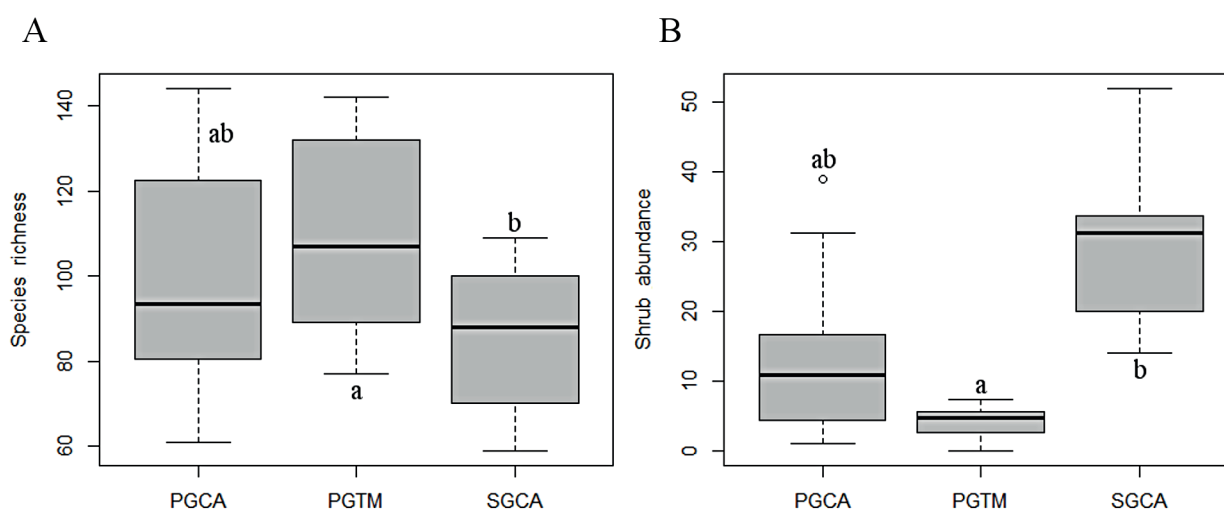
From 39 species ( $IVI > 1\%$ ) tested in indicator species analysis, 21 species were selected ( $p < 0.05$ ), 14 species associated with one group and seven species associated with two groups. These seven species were indicative for the combination of reference grasslands under traditional

management and grasslands within afforestation areas (PGTM and PGCA), and six of them had correlation intensity values above 40% (all with  $p < 0.01$ ): *Oxalis eriocarpa* ( $r=0.53$ ), *Piptochaetium stipoides* ( $r=0.46$ ), *Evolvulus sericeus* ( $r=0.44$ ), *Mnesithea selleana* ( $r=0.43$ ), *Aristida venustula* ( $r=0.41$ ), and *Aspilia montevidensis* ( $r=0.40$ ;  $p=0.01$ ). Five species were indicative for SGCA, one of them the invasive exotic grass *Cynodon dactylon* ( $r=0.61$ ), also the species with the highest correlation value. The other species (also all with  $p < 0.01$ ) were the shrub *Baccharis dracunculifolia* ( $r=0.50$ ) and the herbaceous species *Sisyrinchium micranthum* ( $r=0.49$ ), *Hypoxis decumbens* ( $r=0.46$ ), and *Eryngium horridum* ( $r=0.40$ ). For PGCA, three indicator species were found ( $p < 0.01$ ): *Danthonia cirrata* ( $r=0.46$ ), *Paspalum plicatulum* ( $r=0.43$ ), and *Axonopus suffultus* ( $r=0.40$ ). For PGTM, a total of five species were selected and showed correlation values above 40% ( $p < 0.01$ ), such as *Richardia humistrata* ( $r=0.53$ ), *Paspalum notatum* ( $r=0.53$ ), *Eragrostis neesii* ( $r=0.46$ ), *Dichondra sericea* ( $r=0.44$ ), and *Steinchisma hians* ( $r=0.40$ ). The complete list of species selected by the indicator species analysis is presented in the appendix (Tab. S2 in supplementary material).

## Discussion

### High plant species richness in an under-surveyed region

Our study is the first to comprehensively conduct vegetation sampling in grasslands in the Serra do Sudeste, with a total of 58 sites distributed in a region of 15.000 km<sup>2</sup>. When considering the total percentage of remaining natural vegetation, the Serra do Sudeste is one of the best-preserved areas of the Brazilian Pampa biome (Andrade *et al.* 2015). Due to shallow and poor soils and an accentuated topography in large parts of the region, suitability for the



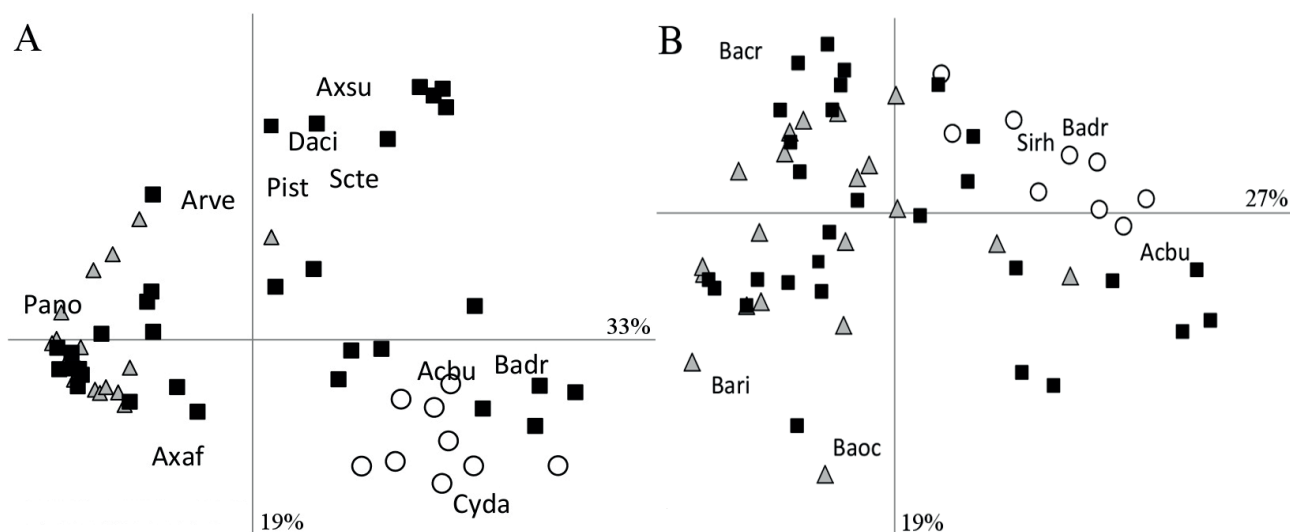
**Figure 2.** Randomization test of the variation in species richness (A) and average shrub abundance (B) among the fifty eight sampled sites (mean values and 25 quartiles). Different letters represent significant differences between treatments ( $p < 0.05$ ).

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cultivation of annual crops is low. However, in recent years, the region has been intensely occupied by Eucalypt plantations which, under the favourable climatic conditions found here, are not demanding in terms of soil. Additionally, the land value in the region is lower than in other parts of the state. While tree plantations themselves reduce the proportion of natural grassland in the region, some grasslands are maintained due to legal requirements as well as differences in site conditions, which offers, at least in theory, opportunities for conservation.

With our sampling, we recorded approximately 1/4 of the total number of grasslands plants known for the Brazilian Pampa grasslands (Boldrini *et al.* unpublished data, see also Andrade *et al.* 2018). The occurrence of the high number of 24 species endemic for the Brazilian Pampa in our sampling likely is related to the fact that the study sites are situated in the geologically oldest region in the Pampa (Hopper 2009;

Bossi & Gaucher 2014; Andrade *et al.* 2019). However, up to now, the region has been neglected by scientific research on vegetation patterns and by conservation actions. For instance, information is still too scanty to develop a classification of distinct plant communities (see *e.g.* Andrade *et al.* 2019 for discussion), no protected areas exist in the region, and recently land use change has been high, causing fragmentation of grasslands with its known negative effects on biodiversity and ecosystem services (Andrade *et al.* 2015; Koch *et al.* 2016; Modernel *et al.* 2016; Staude *et al.* 2018). Legal obligations for establishment of APP and RL – if managed in a way to conserve grassland biodiversity – are important, but are only one approach for conservation that needs to be complemented by other approaches that are more effective in conservation of priority areas and prevention of fragmentation, especially if we aim to meet the Aichi Biodiversity Targets (CBD 2010).



**Figure 3.** Principal coordinate ordination diagram, based on chord distance, showing the first two axes. Symbols represent the sites. Letters represent the initials of the genus and the epithet of species with high correlations to the axes ( $\text{corr.} > 0.5$ ). **(A)** PCoA using grassland species data. **(B)** PCoA using shrub and sub-shrubs species data. In both figures, only species with high correlations ( $\text{corr.} > 0.5$ ) to the axes are shown. **(A)** – Arla: *Aristida venustula*, Axaf: *Axonopus affinis*, Axsu: *Axonopus suffultus*, Brdr: *Baccharis dracunculifolia*, Cyda: *Cynodon dactylon*, Daci: *Danthonia cirrata*, Acbu: *Acanthostyles buniifolius*, Pano: *Paspalum notatum*, Pist: *Piptochaetium stipoides*; Scte: *Schizachyrium tenerum*. **(B)** – Bacr: *Baccharis crispa*; Bari: *Baccharis riograndensis*; Baoc: *Baccharis ochracea*; Acbu: *Acanthostyles buniifolius*; Sirh: *Sida rhombifolia*. ■ PGCA = primary grasslands in conservation areas, ○ SGCA = secondary grassland in conservation areas, △ PGTM = primary grassland subjected to the traditional management.

**Table 1.** Differences in number of individuals and cover of species and species groups (exotic species) as well as parameters indicating vegetation structure between treatments. Different letters represent significant differences between treatments, after randomization testing and correction for multiple comparisons. PGCA = primary grasslands in conservation areas, SGCA = secondary grassland in conservation areas, PGTM = primary grassland subjected to the traditional management.

	PGTM	SGCA	PGCA	Corrected P value
Shrub abundance (ind.)	4 ( $\pm 1.8$ ) <sup>a</sup>	30 ( $\pm 11$ ) <sup>b</sup>	12 ( $\pm 10$ ) <sup>ab</sup>	0.023
Shrub species richness (species)	2.6 ( $\pm 1.6$ ) <sup>a</sup>	6.3 ( $\pm 3.8$ ) <sup>b</sup>	9.6 ( $\pm 4.1$ ) <sup>c</sup>	0.001
Exotic species (%)	0.1 ( $\pm 0.0$ ) <sup>b</sup>	0.6 ( $\pm 0.0$ ) <sup>a</sup>	0.1 ( $\pm 0.0$ ) <sup>b</sup>	0.001
Vegetation height (cm)	12 ( $\pm 6$ ) <sup>a</sup>	52 ( $\pm 29$ ) <sup>b</sup>	30 ( $\pm 18$ ) <sup>c</sup>	0.016
Litter cover (%)	4 ( $\pm 2$ ) <sup>a</sup>	10 ( $\pm 5$ ) <sup>b</sup>	8 ( $\pm 3$ ) <sup>b</sup>	0.027
Dead biomass on plants (%)	3 ( $\pm 1.6$ ) <sup>a</sup>	6 ( $\pm 3$ ) <sup>b</sup>	7 ( $\pm 5$ ) <sup>b</sup>	0.049
Bare soil cover (%)	4 ( $\pm 5$ ) <sup>b</sup>	14 ( $\pm 7$ ) <sup>a</sup>	8 ( $\pm 10$ ) <sup>ab</sup>	0.083
Manure cover (%)	2 ( $\pm 3$ ) <sup>a</sup>	0.5 ( $\pm 1$ ) <sup>b</sup>	0.3 ( $\pm 2$ ) <sup>b</sup>	0.002



### *Historical use and diversity in secondary grassland*

Our data shows that land-use history directly influences grassland structure and species composition, as has been found in other studies (Alrababah *et al.* 2007; Koch *et al.*, 2016; Modernel *et al.* 2016). For the study region, re-establishment of grasslands after other land uses seems possible, but these secondary grasslands differ from primary grasslands in terms of composition and structure (Koch *et al.* 2016; Torchelsen *et al.* 2018). In general, secondary grasslands on sites with former agricultural use are characterized by nutrient concentrations in the soil that differ from those of primary grasslands, promoting changes in vegetation development (Céspedes-Payret *et al.* 2012; Andrade *et al.* 2015; Vink *et al.* 2016). In our case, secondary grasslands showed lower total species richness and a species composition that differed from that of traditionally managed grasslands. No endangered species were found in SGCA, which shows the impact of land-use change and the low potential recovery of populations of many of these species in secondary grasslands. Cover and number of exotic species in SGCA, on the other hand, was higher than that found in PGCA and PGTM, principally due to the presence of three problematic invasive species, *Ulex europaeus*, *Cynodon dactylon*, and *Eragrostis plana*. Our results underline that without proper management, or possibly active restoration efforts, secondary grasslands will likely remain distinct from natural grasslands (see also Koch *et al.* 2016; Torchelsen *et al.* 2018). The presence of exotic plants is especially problematic, as these species may here establish large populations that then constitute source populations for dispersal into native grasslands in the region (León-Cordero *et al.* 2016 a; b).

### *Grasslands in preservation areas without effective management differ in plant diversity and species composition from traditionally grazed areas*

A conspicuous result of our study is the heterogeneity of the grasslands in conservation areas, in terms both of composition of the herbaceous layer and of the woody species component. This can be explained by three factors: first of all, the sites considered here as conservation areas include sites with distinct site conditions. For instance, the species related to PGCA areas along the second axis of the ordination analysis are mostly indicative of shallow soils and rather low and open grasslands. Even though our traditionally managed sites also include some heterogeneity, it is likely that the bias to more extreme sites is higher within the PGCA category, as the decision of where Eucalypt is not planted is influenced by both legal obligation, in the case of the APPs, and of selection of sites where plantings likely are less productive (or more difficult to work with) due to topographic and soil conditions, for example in the case of RL. Secondly, PGCA sites differ in grazing management

and grazing history. Some sites are still grazed at low intensity, and without additional management practices (such as periodic mowing to reduce the shrub component in grasslands). Others are in the process of spontaneous succession after long periods with livestock grazing. At these sites, grasslands are dominated by tall-growing tussock grasses and present higher importance of woody species, mostly grassland shrubs; both of these factors reduce species richness (Overbeck *et al.* 2005; Lezama *et al.* 2014), evidenced here by the negative correlations between vegetation height and species richness and between the abundance of woody species and vegetation richness. Concerning the woody species, this relation is mainly influenced by shrubs like *Baccharis dracunculifolia* and *Acanthostyles buniifolius*, that is, grassland shrubs whose abundance is controlled when grasslands are under traditional management. These results are also in line with a recent study on effects of land management for highland grasslands in southern Brazil (Koch *et al.* 2016) and with studies from other grassland systems that showed a decline in species number (Hinman & Brewer 2007; Klimeš *et al.* 2013) or marked changes in species composition (Uys *et al.* 2004; Loydi *et al.* 2012) when fire or grazing were excluded. The accumulation of litter observed in PGCA and SGCA of our study and in other regions after reduction of management intensity (Enyedi *et al.* 2008) can additionally reduce the number of plant species locally (Morgan & Lunt 1999). Further, afforestation around the grasslands, as well as the establishment of shrubs in the absence of management, have been shown to have marked consequences for microclimatic conditions, *i.e.* reduced radiation, air temperature, connectivity between fragments and wind speed, affecting composition and biodiversity (Saraiva & Souza 2012; Souza *et al.* 2013). These effects act in synergy and lead to decreased species richness with plantation age after grassland around afforestation (Bremer & Farley 2010). Thirdly, it needs to be recognized that the presented processes need time: the speed of succession will at a given site will depend on the initial conditions of the vegetation, on the specific abiotic features that govern productivity, and on the presence of vegetation patches that can serve as seed sources for species from different species groups. Clearly, after only seven to eight years, we still cannot expect any dramatic changes as they have been evidenced in grasslands abandoned for longer periods (*e.g.* Overbeck *et al.* 2005). Differences between grassland types under different management thus are a consequence of interacting factors and processes. On a regional scale, this certainly contributes to diversity and thus may be considered efficient for conservation, likely not only for plant species (evaluated in this study) but also for other species groups that depend on grassland structure (see Fontana *et al.* 2016; Overbeck *et al.* 2016). However, longer-term studies are necessary, as plant diversity can be expected to be reduced in grasslands where effectively no more management occurs over longer periods.



### *The need to discuss effectiveness of APP and RL for grassland conservation*

Permanent Preservation Areas (APPs) are areas set aside for the protection of water resources, landscape, geological stability, biodiversity, the genetic flow of animals and plants, protection of soil, and to contribute to the well-being of human populations (Lei 12.651/2012). Even though APPs are placed at sites with specific conditions regarding topography and presence of water bodies, they thus are to be multifunctional in their conservation objectives. The Legal Reserve (RL), on the other hand, aims at preserving natural vegetation while also allowing human use. The important question to which point both conservation approaches are effective for conservation of grassland vegetation is not the main issue of this paper, but our results do allow some comments on the matter. As grasslands in subtropical and tropical regions have evolved with the presence of disturbances such as fire and grazing (Oosterheld *et al.* 1999; Lezama *et al.* 2014; Veldman *et al.* 2015), their conservation requires strategies that include the presence of disturbances. This also offers opportunities for sustainable use and economic benefits, *i.e.* allows for conservation that considers the needs of the local population, a point much focused on in the current conservation debate (*e.g.* Kareiva & Marvier 2012). In Brazil, this is accepted for RL areas, but not much applied in case of APPs, where usually no management takes place. If we consider a landscape where natural vegetation is mostly formed by grasslands and where conversion of grasslands to other land uses is high, it seems reasonable that conservation should give priority to the maintenance of the original vegetation types and not per se exclude disturbances or management that will cause successional processes. Furthermore, shrub encroachment due to absence of management in former grassland sites now in APP may lead to changes in ecosystem processes, such as carbon sequestration in the soil (Jackson *et al.* 2002), water infiltration into the soil (Farley *et al.* 2005) and habitat suitability for other species groups. These aspects should also be considered when making decisions on conservation approaches (Overbeck *et al.* 2016), such as the inclusion or not of management. This is even more important in a region with fast land-use change and the inexistence of protected areas, such as in the Pampa biome, the biome with the highest Conservation Risk Index of all Brazilian biomes (Overbeck *et al.* 2015). Further studies are needed to evaluate, based on empirical evidence, *i.e.* monitoring data, the actual contribution of APP and RL to the proposed conservation objectives, as well as a debate on what these objectives should be this species-rich and unique region of southern Brazil.

### *Conclusion*

While our study evidenced an overall remarkably high plant species diversity, it also showed that grassland

remnants differ in terms of structure and composition in consequence of past land-use and in relation to present management. Secondary grasslands, that is, grasslands with a history of agricultural use, show greater divergence from reference systems than primary grasslands where traditional use has been abandoned. The presence of invasive exotic species, more dominant in secondary grasslands, contributes to losses of typical grassland diversity. Grasslands with a long history of grazing but today with low or now grazing at all proved to be better preserved, but their future is uncertain at sites where no more management takes place. Species richness in the herbaceous layer was not lower in grasslands in conservation areas when compared to those under traditional management. However, the high competitive ability of tussock grasses, shrubs and invasive exotic species are a threat to most of the rare, endangered and endemic species found in the region where no proper management takes place. In the long run, low grazing intensity and even more so the absence of grazing may be detrimental for biodiversity maintenance. Consequently, abandonment of human interference has long-term consequences for composition and species richness and cannot be considered suitable for conservation of grasslands, as also discussed for the highland grasslands of Rio Grande do Sul (Pillar & Velez 2010; Overbeck *et al.* 2016). Current conservation strategies and actions should be critically evaluated, ideally based on evidences from long-term monitoring.

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