



## ECOSYSTEMS

# Reference values and drivers of diversity for South Brazilian grassland plant communities

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**Abstract:** The South Brazilian grasslands (*Campos Sulinos*) form the dominant vegetation in southern Brazil. They are species-rich ecosystems that occur under distinct geomorphological and climatic conditions but spatial variation of plant species diversity remains understudied. Here, we present a detailed description of plant communities across the region. Our data were obtained in 1080 plots, representing well-preserved grasslands in different ecological systems. Apart from describing alpha and beta diversity, we investigated the relations of plant communities with environmental features. We identified 759 plant species and found clear differences in community composition across the region. Northern and Southern highland grasslands, humid and dry coastal grasslands and the mesic Pampa grassland were clearly distinct, related to climatic and edaphic features. While species abundance distribution was markedly uneven, local species richness was high, above 20 species/m<sup>2</sup>, especially in the highlands and in mesic Pampa sites, on shallow soils. The predominant component of beta diversity was species turnover, which suggests that a network of well-conserved grasslands distributed across the region would be the best strategy to protect plant diversity. Our results establish regionalized reference values for richness and diversity that can be useful for initiatives of restoration and conservation of these grasslands.

**Key words:** Beta diversity, conservation, environmental gradient, species richness, turnover.

## INTRODUCTION

Open ecosystems such as grasslands often still are mistakenly considered as deforested areas (Silveira et al. 2020) or as potential sites for restoration through tree plantations (Veldman et al. 2015a). In contrast, recent research has clearly established that many tropical and subtropical grasslands are, in fact, old-growth ecosystems that harbor high biodiversity, economic and cultural values (Parr et al. 2014, Veldman et al. 2015b). Natural grasslands in Brazil are threatened by rapid land use conversion. For example, 24% of original grassland area in Brazil's Pampa biome was converted to other

uses between 1985 and 2018 (Souza et al. 2020) and in some regions less than 20% of the original grassland cover remain. Even more alarming, 40% of the original vegetation cover of the Cerrado was lost by 2002 (Sano et al. 2010). At the basis of this conservation problem, along with the communication gap between science and stakeholders (Azevedo-Santos et al. 2017), is our limited knowledge about the biodiversity of these ecosystems (Oliveira et al. 2017), jeopardizing conservation of natural resources (Bini et al. 2006).

Particularly relevant for improving biodiversity knowledge is quantitative

vegetation sampling, which is complementary to the recording of floristic lists. Only quantitative data allows to understand the processes behind community assembly, for instance by considering patterns of species co-occurrence, relationships between vegetation and environmental factors, and how plant species themselves influence the local environment (HilleRisLambers et al. 2012, Weiher & Keddy 2004). Understanding diversity changes across space (i.e., beta diversity) is essential to unveil the factors driving community structure, including deterministic processes (such as environmental filtering, species interactions) and neutral processes (such as random extinctions and ecological drift) (Chase & Myers 2011). Environmental filters are often considered as drivers of plant community assembly (Laliberté et al. 2014). Disentangling relations between different drivers not only allows us to interpret current vegetation dynamics, but also to develop scenarios for the future, for example, in the face of climate change, and to reveal specific habitat characteristics that need to be protected or restored.

Data from quantitative sampling may be also relevant for environmental planning (Magnusson et al. 2005). It allows to decompose beta diversity patterns into their turnover and nestedness components, a crucial step to guide conservation (Socolar et al. 2016). When beta diversity is mainly due to species substitution from one site to the next (turnover), the best conservation option is to target multiple sites. However, when it is due to species loss from a richer set to a poorer one (nestedness), it may be more efficient to target the richest site. Importantly, both relations of environmental factors with biodiversity patterns and effective conservation strategies vary with spatial scale (Bini et al. 2006).

The *Campos Sulinos* region (hereafter South Brazilian grasslands), located in the

southernmost part of Brazil, include the Pampa grasslands, in the southern half of Rio Grande do Sul state, and the highland grasslands in the southernmost part of the Atlantic Forest (IBGE 2019, Overbeck et al. 2007). The number of plant community studies in the region has increased over the last 25 years, but with a clear bias to few areas, primarily those situated closer to research institutions (see Boldrini & Overbeck 2015). Only recently the first comprehensive study on grasslands for the entire region was published (Andrade et al. 2019). This study used plant community data obtained in standardized sampling at 156 sites to investigate patterns of species spatial distribution associated with climatic and edaphic factors. It provided an important first step towards knowledge of grassland plant communities in the region as a whole. It also established, for the first time, a classification of South Brazilian grasslands based on quantitative data, confirmed floristic differences between highland grassland, mesic and humid Pampa grasslands, and listed indicator species for each grassland type. However, Andrade et al. (2019) did not focus on a more detailed analysis of the influence of soil features on species distribution, the sampling sites were placed on coarse spatial resolution soil maps (scale of 1:5,000,000) and no data on local edaphic characteristics was used in their study.

Given that most parts of the globe are influenced by human activities, it is important – apart from the obvious and urgent need to reduce pressure on the environment and on biodiversity in general – to study biological communities and their ecological determinants in well-conserved regions in order to obtain reference data for conservation and restoration purposes. A study conducted in the region has shown that even grassland areas in regions with an intermediate degree of habitat loss (areas

with more than 50% of natural grasslands) are affected by land-use change: suppression of grasslands leads to species losses and homogenization of remnant plant communities (Staude et al. 2018). Furthermore, conservation through sustainable use (Boavista et al. 2019) and active restoration (Thomas et al. 2019) are increasingly relevant topics in the region but are still in need of conceptual underpinning and field evidence, also to support restoration or conservation goals (e.g., Prach et al. 2019).

Here, we use field data collected in the PPBio *Campos Sulinos* project to investigate and discuss diversity patterns of the South Brazilian grasslands plant communities. The Brazilian Research Program on Biodiversity (acronym PPBio, from *Programa de Pesquisa em Biodiversidade*), established in the context of the Convention on Biological Diversity, includes a total of fifteen sites in the South Brazilian grassland region in which grassland plant communities, forest tree communities, amphibians, birds and fishes were sampled (e.g. Dala-Corte et al. 2016, Fontana et al. 2018, Madalozzo et al. 2017). Our first objective was to explore patterns of spatial distribution of plant species. We expected to confirm the patterns observed by Andrade et al. (2019), with three major grassland groups (highland, mesic Pampa and humid Pampa grasslands). Secondly, we searched for relations between the observed patterns of species distribution and environmental and spatial filters, using locally obtained soil data and climatic variables. Due to the large extent of the entire gradient (660 km) and differences in altitude (from the sea level to more than 900 m.a.s.l.), we expected to find strong effects of spatially structured climatic filters shaping species distribution patterns in the South Brazilian grasslands, especially related to temperature and precipitation.

As a third goal, we present values of grassland plant community descriptors, such as species richness and diversity, at different spatial scales. Our aim is that these values may be used as reference, or practical targets, to achieve in grassland conservation and restoration initiatives. They include soft (easy to access) and popular indexes, such as species richness and Shannon diversity, but also more ecologically meaningful indexes, such as beta diversity and its components (turnover and nestedness). So far, this kind of information has rarely been available at a scale beyond the local plot (i.e., sampling unit). Since studies often vary in terms of sampling scale, and statistical methods that allow to compare species richness at different spatial scales are seldom applied outside the scientific community, we expect that presenting these descriptive metrics at different spatial scales will help stakeholders to better evaluate South Brazilian grasslands conservation status and restoration success.

## MATERIALS AND METHODS

### Study region

The South Brazilian grasslands span over the three southernmost states of Brazil, under subtropical climate, with the proportion of grasslands in the landscape increasing towards south. The climate in the region ranges from Cfa, at lower altitudes, to Cfb at altitudes above 600 m (Alvares et al. 2013). Precipitation is well distributed along the year, without a dry season, ranging from 1,000 mm to 2,200 mm, with decreasing values towards the southern part of the region (Alvares et al. 2013). However, recent climate series (years 2006 to 2016) indicate high precipitation variability: the average monthly precipitation in the driest year was 86 mm in Jaguarão municipality (coordinates 32°14' S, 53°46' W), and 341 mm in the wettest year in

Alegrete municipality (coordinates 29° 46' S, 55° 23' W).

Different types of geological substrate occur in the study region: igneous volcanic rocks (basalt) in the northern part, igneous plutonic and metamorphic rocks (granite) in the south, and sedimentary material mainly in the coastal region (for details on geology and soils see Andrade et al. 2019). Most grasslands in the region are under grazing by domestic livestock, and fire is commonly used as a management tool mainly in the highland grasslands (Andrade et al. 2015, Overbeck et al. 2007). Fire and grazing are known to influence vegetation structure and composition in the region (e.g., Boavista et al. 2019, Koch et al. 2016, Overbeck et al. 2018). Under moderate intensity or frequency, they are considered key processes for maintenance of the characteristics of natural systems, as in other productive grassland systems around the world (Lezama et al. 2014).

### Sampling design and procedures

PPBio sites were established in the different ecological systems defined for Rio Grande do Sul state by H. Hasenack et al. (unpublished data) and, additionally, in Santa Catarina and Paraná states. The classification of ecological systems aims at presenting a mesoscale biophysical characterization of the landscape. Ecological systems were defined based on a combination of topographic variables (altitude and slope; EMBRAPA 2013, IBGE 2019) and soil functional classes (soil map from SAA/RS-IBGE/SC (2013) reclassified according to soil hydromorphism, fertility and depth). While the variables used to differentiate the ecological systems do not include explicit quantitative vegetation data, a description of typical plant species in the different systems was made based on expert knowledge (Boldrini et al. 2009). The resultant

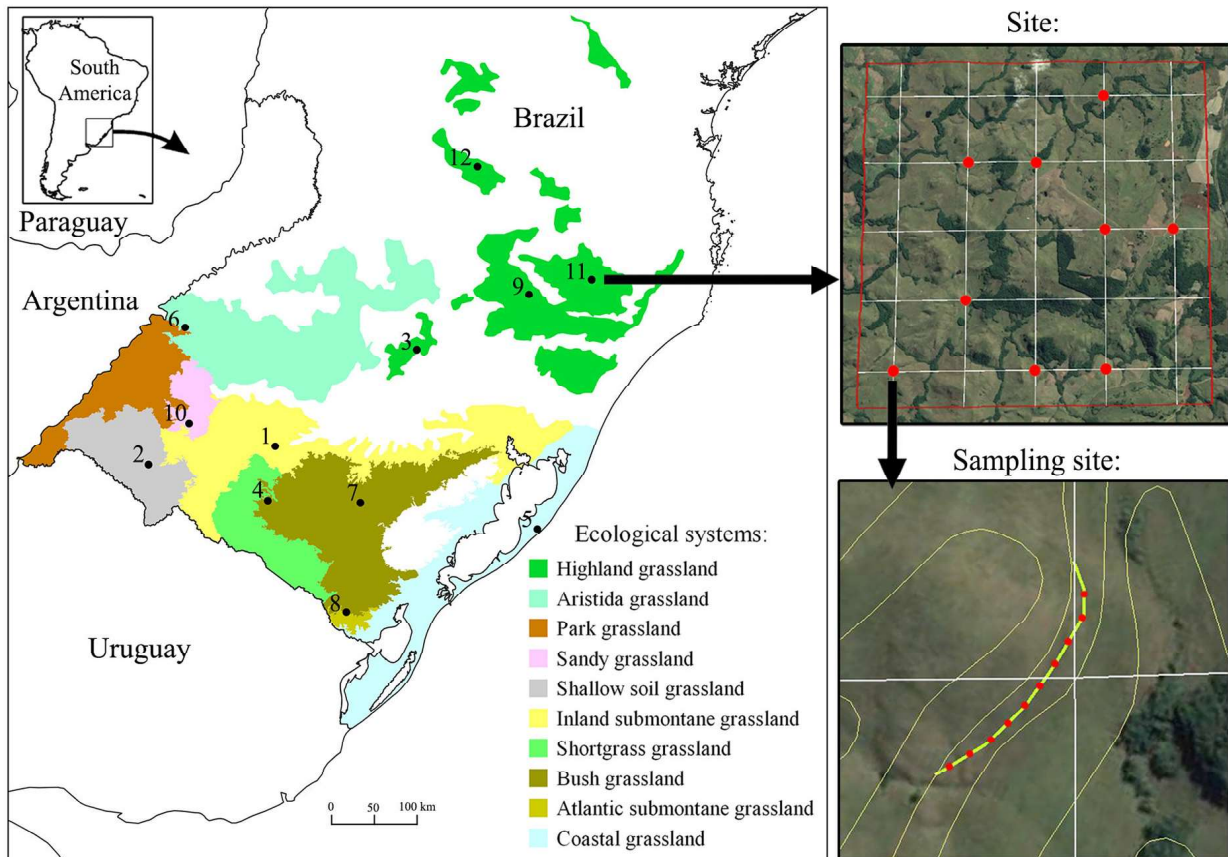
map presents ten ecological systems where grasslands are the dominant vegetation type.

Grassland vegetation sampling was conducted at eight sites in the Pampa grasslands and four sites in the highland grasslands (Figure 1), with one site in each ecological system. For site selection, areas with low degree of conversion to other land uses were chosen. In the *Aristida* grassland ecological system, where land-use change is especially strong (Andrade et al. 2015), it was not possible to find areas matching this requirement, therefore it was not included in our study. At each site, a 5 x 5 km grid of five vertical lines and five horizontal lines was drawn on the map with the orientation angle set to better encompass grasslands remnants. Among the 25 intersection points of the grid lines, nine were randomly chosen to place a 250 m long transect (totalling 108 transects across the 12 grids). At each transect we placed 10 plots (1 m x 1 m), equally distanced following the isocline to reduce local heterogeneity. In each plot, the cover of each vascular plant species was estimated.

Field sampling was conducted during spring and summer in 2014, 2015, and 2016, and plant communities at each site were sampled only once. All vascular plants had their taxonomic identity verified with specific literature. Nomenclature follows the Brazilian Floristic List (Flora do Brasil 2020). Plants that could not be identified to the species level corresponded to 4% of total vegetation cover and were excluded from statistical analysis.

### Predictive variables

We obtained edaphic and climatic data for all 108 transects. Soil samples were collected at three points per transect, to a depth of 30 cm whenever possible (but never less than 15 cm) and were mixed into one composite soil sample per transect. The collected soil was analysed



**Figure 1.** Spatial distribution of the 12 sampled sites in the South Brazilian grasslands in the different ecological systems (based on H. Hasenack et al., unpublished data). Numbers correspond to the location of sites in the following states and municipalities: Rio Grande do Sul (RS) state: 1 São Gabriel (sgb), 2 Quaraí (qua), 3 Soledade (sol), 4 Lavras do Sul (lav), 5 Tavares (tav), 6 Santo Antônio das Missões (sam), 7 Santana da Boa Vista (sbv), 8 Jaguarão (jag), 9 Vacaria (vac), 10 Alegrete (ale); Santa Catarina (SC) state: 11 Painel (pai); and Paraná (PR) state: 12 Palmas (pal). At right, example of a site with nine randomly selected points to place the transects and a detail of a transect with the ten plots (1 m x 1 m) represented by the red dots.

following protocols presented by Tedesco et al. (1995). We considered the following edaphic variables (see Table I for measurement units and analytical method of soil variables): percentage of clay, coarse sand, fine sand and silt, organic matter content, pH, phosphorus, potassium, nitrogen, aluminium, calcium and magnesium contents, cation exchange capacity, base saturation and aluminium saturation. The complete data can be found in Supplementary Material (Tables SII and SIII).

Climatic data were compiled from 33 meteorological stations of the *Instituto Nacional de Meteorologia* (INMET) within the region, for a

ten-year time series (2006 to 2016). Data were interpolated through inverse distance weighting and extracted to the transects coordinates with Qgis software (version 3.4.10). We used the following variables: maximum daily temperature, minimum daily temperature, minimum daily air humidity, maximum monthly precipitation, and minimum monthly precipitation.

To account for the influence of the nested sampling design (i.e., transects within sites), as well as to evaluate which environmental variables were spatially structured, we added spatial variables to our analysis. We extracted ordination axes of a principal coordinate

**Table I. Environmental, spatial variables (and analytical method) and their correlation with grassland plant communities from South Brazilian grasslands. Only variables selected as significantly correlated ( $p < 0.05$ ) with the species composition variance are shown. For complete set of explanatory variables see supplementary material.**

Code	Variable	Unit	Method of analysis	R <sup>2</sup>	AIC	p
<i>Environmental:</i>						
Al	Exchangeable aluminium	cmol <sub>c</sub> /dm <sup>3</sup>	Extracted with KCl 1mol L <sup>-1</sup>	0.12	208.38	0.002
minT	Minimum temperature	°C	Extracted from INMET database	0.05	203.00	0.002
Clay	Clay	%	Densimeter method	0.03	199.36	0.002
minAH	Minimum air humidity	%	Extracted from INMET database	0.03	196.22	0.002
minP	Minimum precipitation	mm	Extracted from INMET database	0.02	193.54	0.002
BSat	Base saturation	%	Bases extracted with ammonium acetate	0.02	187.99	0.002
Ca	Exchangeable calcium	cmol <sub>c</sub> /dm <sup>3</sup>	Same as Al	0.02	190.87	0.002
pH	pH	-	In water	0.02	189.37	0.002
maxT	Maximum temperature	°C	Extracted from INMET database	0.02	185.08	0.002
OM	Organic matter	%	Humid digestion	0.02	186.59	0.002
Csand	Coarse sand	%	Granulometry from 0.2 to 2 mm	0.01	184.30	0.004
<i>Spatial:</i>						
MEM1	1 <sup>st</sup> ordination axis	-	PCoA based on spatial coordinates	0.14	205.43	0.002
MEM2	2 <sup>nd</sup> ordination axis	-	PCoA based on spatial coordinates	0.03	186.78	0.002
MEM3	3 <sup>rd</sup> ordination axis	-	PCoA based on spatial coordinates	0.03	183.10	0.002
MEM4	4 <sup>th</sup> ordination axis	-	PCoA based on spatial coordinates	0.07	196.70	0.002
MEM5	5 <sup>th</sup> ordination axis	-	PCoA based on spatial coordinates	0.03	193.46	0.002
MEM6	6 <sup>th</sup> ordination axis	-	PCoA based on spatial coordinates	0.02	181.27	0.002
MEM12	12 <sup>th</sup> ordination axis	-	PCoA based on spatial coordinates	0.02	179.58	0.002
MEM13	13 <sup>th</sup> ordination axis	-	PCoA based on spatial coordinates	0.03	190.23	0.002

analysis based on central spatial coordinates of the transects (Moran's Eigenvector Maps - MEM, Borcard et al. 2011). To produce the MEMs, the matrix of distance among pairs of coordinates was truncated at the smallest distance that kept all points connected, i.e. 221.2 km.

We did not include grazing intensity proxies in our analysis since we considered grazing levels to be similar across the region. All study sites

were under traditional grazing management, which for South Brazilian grasslands means high grazing intensity (Carvalho & Batello 2009), indicated by the overall low vegetation height (average 15.8 cm, standard deviation  $\pm$  13.2 cm).

### Data analysis

Using transects described by plant species composition, we performed hierarchical

clustering, based on UPGMA after Jaccard-based pairwise species turnover comparing all pairs of transects (Baselga 2012). Consistency of groups was tested with approximately unbiased p-values obtained via 999 multiscale bootstrap resampling (Shimodaira 2004). Groups with  $p < 0.05$  were considered consistent. The dendrogram was cut at the height of 0.75 resulting in five consistent and ecological meaningful groups. Approximately unbiased p-values for all dendrogram nodes are shown in the Figure S2.

To elucidate the relations of plant community patterns and soil and climate characteristics, we performed redundancy analysis (RDA) based on the Hellinger-transformed matrix of relative species cover per transect (Legendre & Gallagher 2001). We also performed variance partitioning (Borcard et al. 2011) to verify how much of compositional variance was related only to the environment (climate and soil), only to space (MEM) and to the shared effect of environment and space. To avoid inflation in both procedures, we first removed all environmental variables that had a collinearity factor greater than 10 (Oksanen et al. 2017). Collinearity was detected using a variance inflation factor calculated for each environmental explanatory variable using the  $r^2$  value of the regression of that variable against all other explanatory variables. The remaining variables were submitted to forward selection of predictive variables. This procedure adds and drops variables in a model, aiming to maximize  $R^2$  at every step, the procedure stops when the  $R^2$  starts to decrease, or when the  $R^2$  of the scope is exceeded ( $R^2$  with all explanatory variables = 0.34), or when the p-value threshold ( $p > 0.05$ ) is exceeded (Blanchet et al. 2008). For a graphical representation of the selected spatial variables see Figure S1.

We calculated indexes of species richness (S) at the site, transect and plot levels. Shannon

diversity ( $H'$ ) and its expression by the effective number of species (Jost 2006) were calculated for each plot; we present average levels per site. The effective number of species, was calculated through the exponential of  $H'$ , this estimates how many species with equitable abundances would be required to obtain the same value of  $H'$  (Jost 2006, Magurran 1988). To further describe plant species abundance relationships in the communities, we also calculated the evenness index (E) at the plot level, which expresses the ratio between observed diversity and maximum diversity (i.e., if all species were equally abundant in communities) (Magurran 1988).

We explored patterns of spatial heterogeneity in species composition by calculating beta diversity and its components, turnover and nestedness. We used the Jaccard-based multiple-site dissimilarity index ( $\beta$ -jac) to calculate beta diversity, turnover and nestedness (Baselga 2012), comparing species composition among the nine transects at each site and thus obtaining one value representative of the heterogeneity per site.

All analyses were performed in the R environment. Package 'iNEXT' and function 'ChaoShannon' were applied to calculate metrics of Shannon diversity and effective number of species (Chao et al. 2014). Package 'betapart' was used to calculate beta diversity, turnover and nestedness (Baselga & Orme 2012). From the package 'vegan' (Oksanen et al. 2017), we used functions 'vif.cca' to verify for collinearity in explanatory variables, 'ordistep' to perform forward selection of explanatory variables, 'rda' to run redundancy analysis and 'varpart' to run variance partitioning analysis. Hierarchical Clustering analysis was performed with the 'pvclust' package, function 'pvclust' (Suzuki & Shimodaira 2015).

## RESULTS

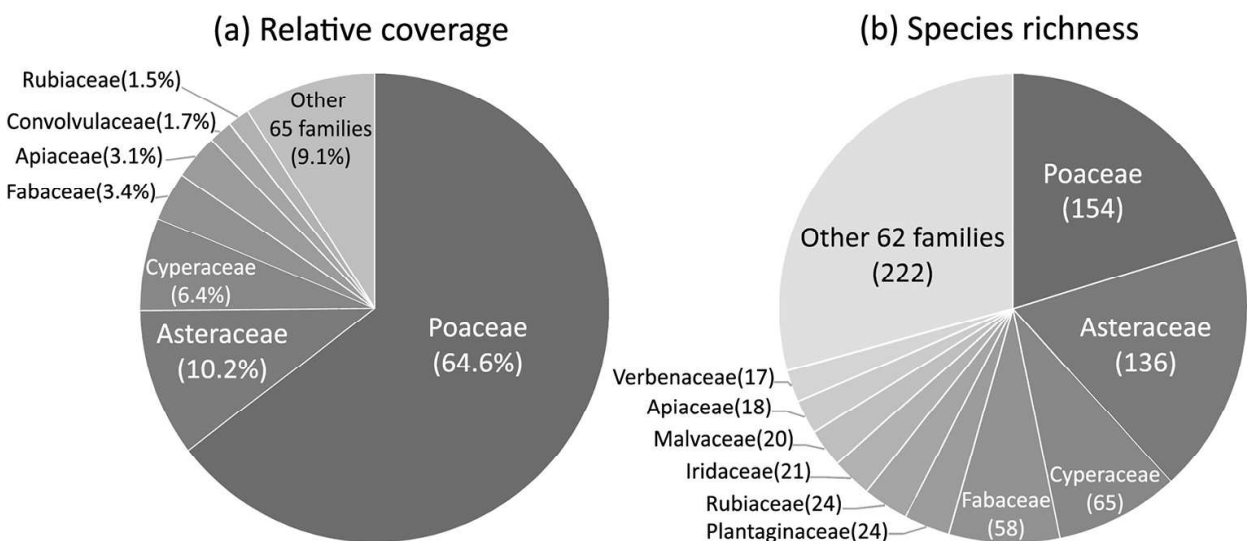
At the twelve sites (total of 108 transects and 1080 plots), 759 plant species from 72 families were found (see Table SI for complete species list). The most species-rich families were Poaceae (154 species, Figure 2b), Asteraceae (136), Cyperaceae (65), and Fabaceae (58). Considering species cover, Poaceae stood out as the most important family (64.6%, Figure 2a).

Cluster analysis showed five consistent groups with distinct species composition (Figure 3). Two distinct groups were at the highland grassland region: southern highland grassland (SHG), comprising sites from Paniel, Soledade and Vacaria, and northern highland grassland (NHG), represented by the Palmas site, the northernmost site of our sampled gradient. The Pampa region was subdivided in three different groups: mesic Pampa grassland (MPG), the largest group, represented by seven sites (Alegrete, Jaguarão, Lavras do Sul, Quaraí, Santana da Boa Vista, Santo Antônio das Missões and São Gabriel) and two groups in the coastal region coastal Pampa grassland group 1 (CG1) five out of the nine transects from Tavares site;

and coastal Pampa grassland group 2 (CG2) four transects from Tavares site.

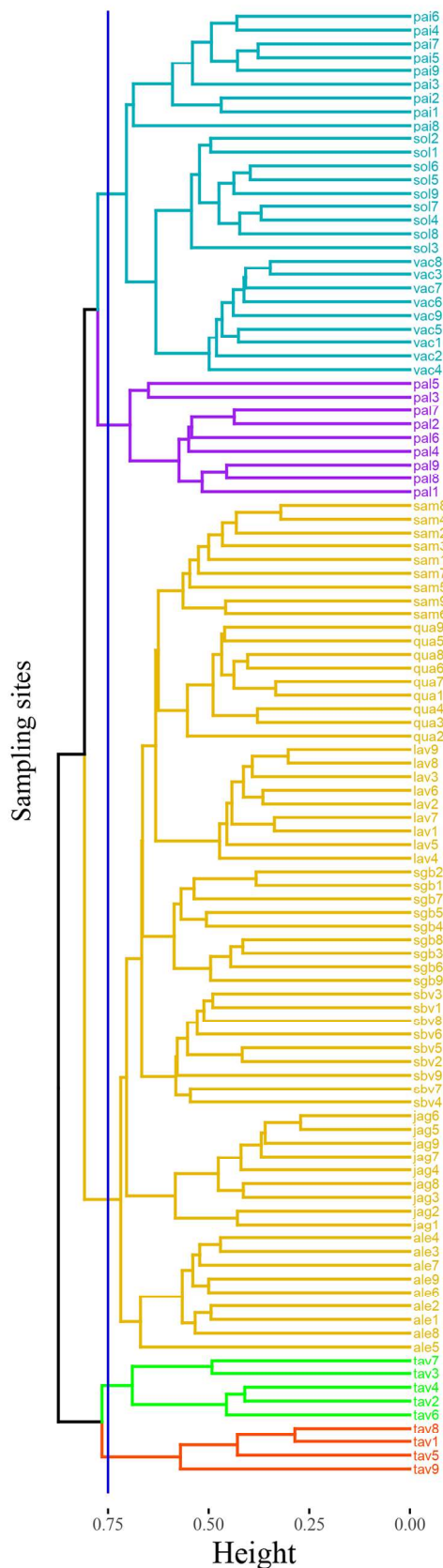
Among the 759 plant species found, almost half (308) were shared by Pampa and highland grasslands. The Pampa grasslands (comprising MPG, CG1 and CG2) presented the higher number of exclusive species (258) compared to the highland grasslands (SHG and NHG, 193 species). Species richness varied from 119 species at the site in Tavares to 262 species at the Soledade site (Table II). At all but two sites, equivalent number of species had a value of less than half of the mean species richness per plot, which indicates high dominance by few species at this scale in the South Brazilian grasslands (Table II).

At all sites, the sum of the cover of the five most abundant species added up to more than 40% of total vegetation cover (Table III). A large fraction of these abundant species occurred throughout most parts of the South Brazilian grasslands. A notable exception were the two grassland groups in the coastal region, characterized by the dominance of species that are absent in the other sites, such as *Axonopus* sp. and *Paspalum vaginatum* (dominant species



**Figure 2.** Plant families contributing the most to (a) the total relative cover and (b) number of species found in the South Brazilian grasslands.





**Figure 3.** Hierarchical clustering analysis, based on UPGMA method and using Jaccard-based pairwise species turnover as dissimilarity metric, showing the separation of South Brazilian grasslands in five groups, top to bottom: Southern highland grassland (in Portuguese *campos de altitude do sul*), Northern highland grassland (*campos de altitude do norte*), Mesic Pampa grassland (*campos mésicos do Pampa*), Coastal Pampa grassland group 1 (*campos costeiros do Pampa grupo 1*) and Coastal Pampa grassland group 2 (*campos costeiros do Pampa grupo 2*). Group consistency was tested with approximately unbiased p-values obtained via 999 multiscale bootstrap resampling, groups with  $p < 0.05$  were considered consistent (see for dendrogram with all p-values).

at CG1 and CG2, respectively). Considering this list of 27 plant species with highest cover values per site, 19 were grasses, five belonging to the genus *Paspalum Schizachyrium tenerum* was very important at the NHG and SHG groups, representing the dominant species in three out of the four highland grassland sites (Vacaria, Painel and Palmas). *Paspalum notatum* and *Andropogon lateralis* were the most important plant species in terms of cover in the MPG sites.

Accordingly, the RDA analysis showed the separation of NHG and SHG dominated by *S. tenerum* (the positive portion of RDA1 axis in Figure 4) from MPG sites dominated by *A. lateralis* (the negative portion of RDA1 axis). *P. notatum* was also characteristic of the MPG sites, but positively correlated with the second RDA axis (RDA2, Figure 4). The RDA also showed a strong relation between vegetation and soil pH, with the highest concentration of aluminum, i.e., acid soils, in SHG sites (see Table I, aluminum has the highest  $R^2$  and AIC values). Climatic variables were also important to explain species composition patterns. Increased maximum monthly precipitation was particularly related with highland sites (NHG and SHG), whereas higher values of minimum temperature were associated to the coastal area, CG1 and CG2 (Figure 4). Indeed, the model

with environmental variables explained 34% of species composition variance (Table IV). However, the spatial variables had almost the same importance ( $R^2 = 0.35$ ). By controlling for the spatial influence in environmental variables, the model explanation decreased ( $R^2 = 0.12$ ), indicating that environmental variables along the South Brazilian grassland region are highly spatially structured (Table IV).

Although most abundant species were well distributed along the entire gradient, spatial heterogeneity per site was generally high, especially at the coastal grassland site ('tav' in Figure 5). At all sites, the greater part of beta

diversity was due to species substitution across transects (the turnover component in Figure 5).

## DISCUSSION

Around the world, grassland vegetation has been neglected in terms of science and conservation (Overbeck et al. 2015, Veldman et al. 2015a,b). The results we present here contribute to a detailed characterization of still rather intact grassland landscapes in terms of species richness and dominance patterns in the plant community; our results thus can serve to establish regional reference values for grassland conservation or

**Table II. Richness and diversity metrics of vegetation in South Brazilian grasslands. Richness (S) given for the three sampling scales: site 25 km<sup>2</sup>, transect 250 m<sup>2</sup> and plot 1 m<sup>2</sup>. Shannon diversity (H'), effective number of species and equability (E) were calculated for each plot, values presented are average and standard deviation for the 90 plots at each site, except for Tavares site where two groups of grasslands could be differentiated: Coastal Pampa grassland group 1 with 50 plots and Coastal Pampa grassland group 2 with 40 plots. \*All plots from Tavares were grouped to calculate the metrics at the site level in order to preserve the 25 km<sup>2</sup> scale.**

Municipality	Ecological system	Site (spp/25 km <sup>2</sup> )	Samp. site (spp/250 m <sup>2</sup> )	Plot (spp/1 m <sup>2</sup> )	H' (nats/ind)	Equivalent S	E	Hill numbers (q=1)
Alegrete (ale)	Sandy grassland	175	70.33 (±4.72)	21.8 (± 4.61)	2.06 (± 0.31)	8.27 (± 2.76)	0.67 (± 0.07)	87.17 (± 2.49)
Jaguarão (jag)	Atlantic submontane grassland	196	83.33 (±18.28)	22.94 (± 5.15)	2.53 (± 0.3)	13.17 (± 4.18)	0.77 (± 0.05)	111.56 (± 2.21)
Lavras do Sul (lav)	Shortgrass grassland	197	87.77 (± 14.24)	24.45 (± 8.19)	2.36 (± 0.44)	11.62 (± 4.9)	0.72 (± 0.07)	97.45 (± 2.11)
Quaraí (qua)	Shallow soil grassland	252	111.55 (± 18)	30.5 (± 9.84)	2.51 (± 0.41)	13.32 (± 5.24)	0.74 (± 0.06)	140.304 (± 2.7)
Santo Antônio das Missões (sam)	Park grassland	196	84.22 (± 13.8)	25.48 (± 8.7)	2.23 (± 0.44)	10.24 (± 4.42)	0.7 (± 0.08)	105.83 (± 2.19)
Santana da Boa Vista (sbv)	Bush grassland	247	96.88 (± 14.53)	26.85 (± 6.33)	2.38 (± 0.32)	11.50 (± 3.97)	0.73 (± 0.08)	121.61 (± 2.77)
São Gabriel (sgb)	Inland submontane grassland	225	85.55 (± 11.41)	25.41 (± 6.15)	2.34 (± 0.32)	10.95 (± 3.62)	0.72 (± 0.06)	110.17 (± 2.99)
Tavares (tav)	Coastal grassland	119	30.77 (± 14.26)	10.41 (± 4.65)	1.56 (± 0.55)	5.40 (± 2.39)	0.69 (± 0.09)	63.14 (± 2.42)
Painel (pai)	Highland grassland	256	97.44 (± 10.52)	26.44 (± 6.15)	2.44 (± 0.33)	12.08 (± 3.61)	0.75 (± 0.07)	121.72 (± 2.71)
Palmas (pal)	Highland grassland	244	87 (± 8.8)	22.91 (± 5.85)	2.32 (± 0.35)	10.89 (± 4)	0.74 (± 0.06)	126.64 (± 3.36)
Soledade (sol)	Highland grassland	262	105.55 (± 20.01)	31.4 (± 6.9)	2.52 (± 0.38)	13.42 (± 5.37)	0.73 (± 0.07)	120.397 (± 3.04)
Vacaria (vac)	Highland grassland	218	98.77 (± 9.01)	30.85 (± 8.29)	2.52 (± 0.36)	13.28 (± 4.44)	0.74 (± 0.05)	103.526 (± 2.21)

restoration. The total number of 759 species in our data set represents roughly one fourth of the 3.000 plant species estimated for the South Brazilian grasslands (Overbeck et al. 2007). For the Pampa as a whole, considering

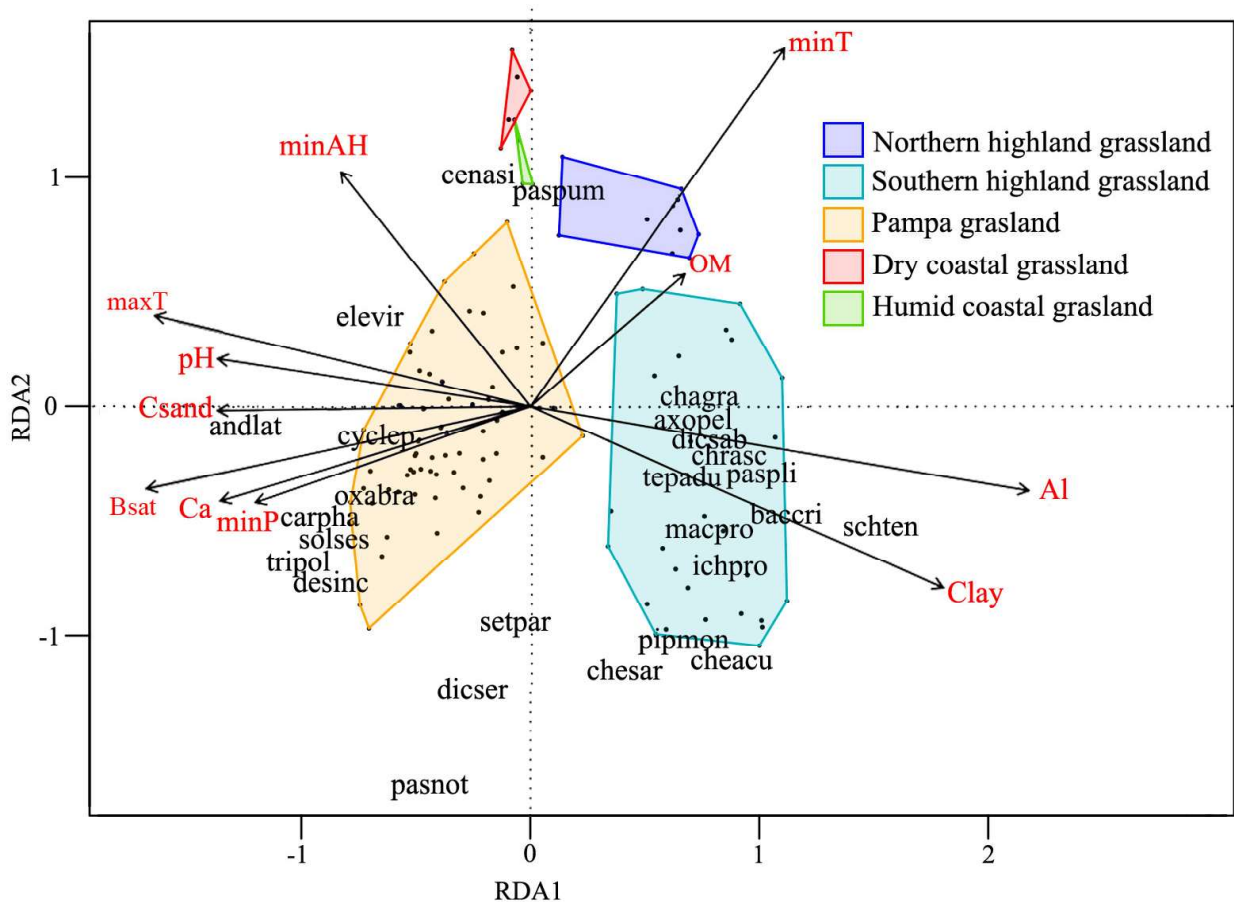
all physiognomies existent in the region, 2.150 plant species have been confirmed (Andrade et al. 2018), and our sampling with 566 species at Pampa sites thus also presents one fourth of the species from this region. However, it is important

**Table III. Relative cover of the five most abundant species occurring at each site. When one species is top five abundant for a given site its relative cover value is given for all sites where it was present. Acronyms for sites and grassland groups are given in Table II. Cover values in bold indicate the species with highest abundance per site.**

Species	Sites											
	ale	jag	lav	qua	sam	sbv	sgb	tav	sol	vac	pai	pal
<i>Agenium villosum</i> (Nees) Pilg.	-	<0.1	-	-	-	-	-	-	1.1	1.2	4.3	-
<i>Andropogon lateralis</i> Nees	<b>33.7</b>	6.3	4.7	10.9	<b>22.6</b>	6.6	12.4	3.7	<0.1	-	0.5	4.3
<i>Andropogon macrothrix</i> Trin.	-	-	-	0.1	-	<0.1	-	-	0.1	<0.1	1.3	6.7
<i>Axonopus affinis</i> Chase	1.8	9	11.1	2.5	2.1	7.8	6.8	2.2	1.6	2.6	3.4	4.8
<i>Axonopus</i> sp.	-	-	-	-	-	-	-	12	-	-	-	-
<i>Baccharis crispa</i> Spreng.	0.3	1.8	1.6	0.4	-	2.1	1.6	-	4.3	3.1	3.1	1
<i>Centella asiatica</i> (L.) Urb.	0.1	3.5	-	-	<0.1	0.1	5.4	1.4	-	-	-	0.4
<i>Dichondra sericea</i> Sw.	2.8	1.2	2	1	1.4	2.2	1.1	0.3	1.7	1.2	0.5	0.2
<i>Eleocharis viridans</i> Kük. ex Osten	-	1.3	3.5	2.5	2.7	0.9	3	6.4	-	-	-	0.8
<i>Elionurus muticus</i> (Spreng.) Kuntze	-	-	-	-	-	-	-	-	0.1	-	-	4.9
<i>Eryngium horridum</i> Malme	-	0.3	1	0.4	0.1	4.2	0.5	-	6.7	-	-	0.1
<i>Ischaemum minus</i> J. Presl	-	0.2	-	-	-	-	<0.1	5.6	-	-	-	-
<i>Panicum aquaticum</i> Poir.	-	-	<0.1	<0.1	-	-	<0.1	5.5	-	-	-	<0.1
<i>Paspalum indecorum</i> Mez	-	-	-	5.2	4.9	-	-	-	-	-	-	-
<i>Paspalum notatum</i> Flüggé	20.5	<b>15.2</b>	<b>17.2</b>	<b>11.5</b>	18.8	<b>18.9</b>	<b>14.1</b>	0.4	<b>22.8</b>	16.6	3.2	0.7
<i>Paspalum plicatulum</i> Michx.	0.5	2.9	0.3	1.1	0.7	0.5	<0.1	-	2.1	3.5	7.8	1.7
<i>Paspalum pumilum</i> Nees	0.3	6.6	0.9	-	-	0.1	1.2	4.4	0.3	-	4.8	2.1
<i>Paspalum vaginatum</i> Sw.	-	-	-	-	-	-	-	<b>17.2</b>	-	-	-	-
<i>Piptochaetium montevidense</i> (Spreng.) Parodi	2.7	3.2	1.9	1.7	0.7	5.5	1.7	-	7.6	7.8	5.7	7.1
<i>Saccharum angustifolium</i> (Nees) Trin.	-	0.1	6.2	-	0.1	-	-	-	1.7	-	2.3	2.3
<i>Schizachyrium tenerum</i> Nees	0.1	1.2	0.2	0.3	-	0.8	-	-	4.2	<b>17.1</b>	<b>13.8</b>	<b>10.2</b>
<i>Steinchisma hians</i> (Elliott) Nash	1.5	2.7	0.9	4.8	3.4	0.1	2.1	0.2	0.9	0.9	0.2	0.2
<i>Trifolium polymorphum</i> Poir.	-	0.2	5.9	0.6	1.1	0.6	0.5	-	-	-	-	-
<i>Vernonanthura nudiflora</i> (Less.) H. Rob.	0.4	-	-	0.3	-	0.2	3.5	0.2	-	0.1	1.2	<0.1
Total:	64.7	55.7	57.4	43.3	58.6	50.6	53.9	59.5	55.2	54.1	52.1	47.5

to recognize that our study was conducted mostly in rather homogenous grazed areas of mesic grasslands that dominate the landscapes (with the exception for the coastal region, where humid grasslands cover considerable areas). Thus, we did not include azonal environments that are found inserted in the grassland matrix, such as rock outcrops or sand depositions.

These environments often present a specific flora (Porembski & Barthlott 2000, Trindade et al. 2008) and are characterized by the presence of species-rich genera, such as *Parodia* (Cactaceae: 26 species in the region, Larocca & Zappi 2015) and *Dyckia* (Bromeliaceae: 29 species in the region, Forzza et al. 2015) on rock outcrops.



**Figure 4.** Redundancy analysis (RDA) based on the relative frequency of plant species in 108 transects across the South Brazilian grasslands and predictive (edaphic and climatic) variables. Environmental variables explained 34% of species variance. Cluster analysis supported five groups of distinct plant species composition represented by the polygons (see also Figure 3). Acronyms for the environmental variables are given in Table I. For better visualization only species highly correlated ( $r^2 > 0.25$ ) with RDA axis were plotted. Species acronyms are: andlat *Andropogon lateralis*, axopel *Axonopus pellitus*, baccri *Baccharis crispa*, cenasi *Centella asiatica*, cheacu *Chevreulia acuminata*, chesar *Chevreulia sarmentosa*, chrasc *Chrysolaena ascendens*, desinc *Desmodium incanum*, dicsab *Dichantheium sabulorum*, dicser *Dichondra sericea*, carpha *Carex phalaroides*, chagra *Chaetogastra gracilis*, cyclep *Cyclosperrum leptophyllum*, elevir *Eleocharis viridans*, ichpro *Ichnanthus procurrens*, macpro *Macroptilium prostratum*, oxabra *Oxalis brasiliensis*, pasnot *Paspalum notatum*, paspum *Paspalum pumilum*, paspli *Paspalum plicatulum*, pipmon *Piptochaetium montevidense*, setpar *Setaria parvifolia*, schten *Schizachyrium tenerum*, solses *Soliva sessilis*, tepadu *Tephrosia adunca*, tripol *Trifolium polymorphum*.

The high importance of Poaceae and Asteraceae in the South Brazilian grasslands in terms of species richness has been shown previously. Interestingly, however, Fabaceae, shown in many studies to be the third family in terms of species number (Andrade et al. 2016, Ferreira et al. 2010), was replaced by Cyperaceae in our study, a family previously shown to have high importance in grasslands in the coastal region (Bonilha et al. 2017, Ferreira & Setubal 2009, Menezes et al. 2015). Until now, the high richness of Cyperaceae in the South Brazilian grasslands might have been underestimated due to the lack of taxonomic treatment for species-rich genera. Improved knowledge of Cyperaceae species, including the description of new species, may now reveal more accurately the real importance of this plant group to the South Brazilian grasslands flora (e.g. Hefler & Longhi-Wagner 2012, Silva-Filho et al. 2017, Trevisan & Boldrini 2008).

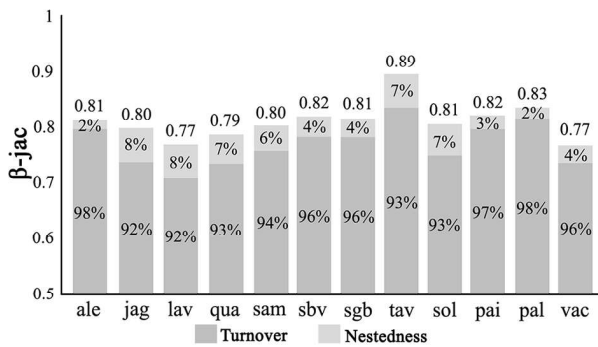
### Environment-vegetation relations reflecting grasslands groups

As to the climatic variables, maximum monthly precipitation and minimum daily temperature were related to community composition patterns indicated by plant species composition in the South Brazilian grasslands (see Figure 4). The highland region had higher values of maximum monthly precipitation, the higher precipitation in this region is one of the factors causing forest expansion over grasslands (Müller et al. 2012). In contrast, some regions in the southern half of Rio Grande do Sul State (in the Pampa, comprising MPG sites), present historical records of hydric deficits, especially in years of La Niña-Southern Oscillation events (Cordeiro et al. 2018). Higher values of minimum temperature were associated to the coastal grasslands, indicating high climatic stability, expected in the coastal region.

Soil features also separated Pampa grasslands from highland grasslands. The concentrations of exchangeable aluminum ( $Al^{+3}$ )

**Table IV. Summary of the variation partitioning of species composition in the South Brazilian grasslands. Environmental and spatial features used as predictive variables are given in Table I. Explanation factors are accepted as significant with  $p < 0.05$ , 'n.t.' accounts for non-testable fractions.**

Effect of the main variable	Explained variation ( $R^2$ )	Df	p
<i>Total effect</i>			
Environment + space	0.47	19	0.001
<i>Partial effects</i>			
Environment	0.33	11	0.001
Environment [space]	0.13	11	0.001
Space	0.34	8	0.001
Space [environment]	0.14	8	0.001
<i>Shared effect</i>			
Environment + space	0.20	-	n.t.
Residuals	0.53	-	n.t.



**Figure 5. Beta diversity (numbers above bars), based on Jaccard-Index, decomposed into turnover and nestedness contribution at the twelve grassland sites in the South Brazilian grasslands.**

and differences in soil granulometry were the principal variables associated to this distinction (Table I). The soils in the highland region are formed by volcanic rocks (basalt, rhyolite, rhyodacite), leading to high aluminum content. As aluminum has low mobility and is easily bounded by organic matter (Li & Johnson 2016), we could observe both components in high proportion in the highland region.

It is important to consider the shared effect of space and environment when we discuss the role of environmental drivers shaping grassland community patterns. Both environmental features and space (i.e., dispersal limitation) are potentially filters of community assembly acting at different spatial scales (Menezes et al. 2016). Climatic variables are intrinsically spatially structured at broader scales (Bell et al. 1993), which makes it difficult to discern between the effects of climate and other spatially structured environmental or biotic factors, such as dispersal limitation, on community composition. Soil properties, in contrast, are usually more influential at local spatial scales (Menezes et al. 2016).

Overall, the results presented here corroborate Andrade et al. (2019), separating the South Brazilian grasslands in three groups of grasslands (highland grassland, mesic Pampa grassland and coastal Pampa grassland) based

on distinct species composition, edaphic and climatic characteristics. Our two groups of highland sites, NHG and SHG, had the same dominant plant species (*S. tenerum* and *P. notatum*), and the distinction in species composition seems to be related with a slightly higher content of aluminium and organic matter in NHG group (NHG: Al = 3.8, OM = 7.2; and SHG: Al = 3.1, OM = 5.2, average values, see table SII). In coastal Pampa grasslands the dominant species in CG2 (*P. vaginatum* and *S. secundatum*) had low coverage, or were absent, in CG1. Considering edaphic differences, in CG2 we found higher base saturation (over 50% in all transects, characterizing eutrophic soils) than in CG1, also acid elements were absent or indetectable (aluminium mostly) in CG2. In fact, transects belonging to the CG2 group were located closer to the Lagoa do Peixe lagoon, where the proximity to the water body may influence soil chemical composition due to more frequent flooding events, shallower water table (Kozłowski 1984) and possible influence of seasonally higher salinity. More scale-refined analysis would help to better disentangle the factors that drive changes in plant community composition within this region.

Classification of the South Brazilian grasslands historically had been based on coarse physiognomic descriptors, such as 'shrubby or dirty grassland' (*campos arbustivos ou sujos*, sensu Lindman 1974) or considering very broadly defined environmental features, such as by using terms like 'dry grasslands' (*campos secos*, sensu Rambo 1942). Andrade et al. (2019) provided the first attempt to classify grasslands based on plant community composition, and our results agree with their classification. Nonetheless, we must recognize that data availability is still too limited for a comprehensive fine-scale classification based on species composition that would be needed

to define grassland habitats of specifically high conservation value or threat status.

A classification of landscapes based primarily on geomorphological variables, on the other hand, such as the classification by Hasenack H et al. (unpublished data) that was used for definition of our study sites (see Figure 1) is useful for the description of different environments and regions. However, geomorphological features may not be directly related to species composition patterns. In fact, the classification of floristic regions, based on plant species composition, has not matched previous classifications in other regions of Brazil as well (Cantidio & Souza 2019, Silva & Souza 2018, Silva-Souza & Souza 2020). It is important to underline that conservation and restoration planning at local or regional scales require larger data sets about plant community and that quantitative field sampling is essential.

### **Evenness, richness, and diversity patterns**

Species abundance distribution at our sites was remarkably uneven. The cover sums of the five most abundant species per site represented over 40% of vegetation cover in all sites, despite the high species richness (average  $S$  in sites was 215) (Table III). All studied sites were under traditional grazing management that also shapes grassland community composition and structure. Farmers usually maintain rather high stocking rates, which can even lead to overgrazing (Carvalho & Batello 2009). This process possibly enhances dominance of few species that are adapted to high grazing pressure (Sosinski & Pillar 2004) and may also lead to a certain homogenization of plant communities, as found in general for biotic communities under land use intensification (Gossner et al. 2016). Lack of disturbance, i.e., exclusion from grazing or fire, on the other hand, has been shown to lead to biodiversity loss once few taller species

become dominant. On the long term, it may lead to the substitution of natural grasslands by shrub- or tree-dominated ecosystems (Koch et al. 2016). Here, working on areas with cattle grazing throughout, we do not expect strong interference in the overall structure of grassland communities due to management as found in Andrade et al. (2019), where one ungrazed site clearly differed from the other sites. However, it would be interesting to further investigate this in future studies to better define optimum grazing levels (or fire frequencies, for that matter, see e.g., Overbeck et al. 2018) from both conservation and production perspectives.

While dominant species are widespread among regions (Table III), rarer species differ more, even within sites. This was highlighted by the high beta diversity observed, with a greater contribution of species substitution (turnover) among transects (Figure 5). In fact, a recent meta-analysis has shown that turnover seems to be the dominant pattern over a broad range of ecosystems and organisms (Soininen et al. 2018), while nestedness patterns are restricted to extreme climates in high latitudes (Dobrovolski et al. 2012). Concerning plant species conservation, high beta diversity due to turnover means that the best way to protect the most of biodiversity is defining a network of well-conserved grasslands distributed over regions along the entire environmental gradient. The Brazilian Native Vegetation Protection Law (Law 12.651/2012) obliges rural properties to maintain or restore native vegetation up to 20% of their total area as Legal Reserve for the conservation of biodiversity and ecosystem services (see Metzger et al. 2019). While our data indicate that the distribution of protected grassland remnants in space – as favored by the Legal Reserve – will be beneficial for conservation of plant diversity, other studies point negative effects of fragmentation (Staude et al. 2018)

which would occur if grasslands were restricted to Legal Reserves. More studies on the relevance of scale and grain for conservation purposes are needed. Moreover, conservation requirements may differ among groups of organisms, since beta diversity, turnover and nestedness show specific patterns for organisms at different trophic levels and dispersal capabilities (e.g., Soininen et al. 2018).

### **Establishing reference values for conservation and restoration**

The species richness values we found at three spatial scales (sites, transects, plots) are informative for a pragmatic definition of reference values for conservation and restoration of the major ecological systems in the South Brazilian grasslands. Although ecological restoration of converted or degraded areas may not achieve the levels of richness and diversity of natural areas, it is important to set clear goals for restoration projects. Grasslands still represent a small portion of areas under restoration in Brazil (Guerra et al. 2020) and determining when a grassland is successfully restored is yet to be discussed (but see Wortley et al. 2013). Species richness, for instance, can be obtained relatively easily and already is a piece of valuable information for conservation (Menezes et al. 2018, Wilson et al. 2012) that should also be useful for restoration purposes.

As shown by equivalent richness and evenness values, South Brazilian grasslands can present highly uneven species abundance distribution, with high dominance of Poaceae species, especially *Andropogon lateralis*, *Paspalum notatum* and *Schizachyrium tenerum*. However, to evaluate grassland conservation status or define restoration targets, it does not appear to be sufficient to consider only composition of dominant species, since South Brazilian grassland have high species richness

that needs to be considered. In fact, during the field sampling of our study, the 'record' value of 56 species in one grassland plot was registered at the Quaraí site, on shallow soil (Menezes et al. 2018). Thus, for conservation and restoration purposes, overall compositional patterns, dominant species, and species richness should be simultaneously used as references.

Future scenarios point out to continued pressure from agricultural expansion on natural ecosystems in southern Brazil (Dobrovolski et al. 2011), resulting in additional biodiversity losses (e.g., Staude et al. 2018). Only the implementation of effective conservation measures can avoid more severe transformation of natural habitats, preserving important ecosystem services (Metzger et al. 2019). We suggest that environmental agencies should establish clear criteria for environmental licensing and restoration/conservation monitoring based on field information as presented here, and that these criteria should be periodically updated to include more recent data. We further urge to continue with standardized vegetation sampling in the region, in order to improve the information basis both for science and conservation.

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## SUPPLEMENTARY MATERIAL

### Tables SI-SIII

### Figures S1-S2

#### How to cite

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### Author contributions

LSM performed field work, plant species identification, leading data analysis and manuscript writing. CVE, DBL, GHMS, RT and IIB contributed with field work and plant species identification. EVM contributed with sampling design conception, environmental data acquisition and contacted land-owners. HH contributed with sampling design conception and environmental data acquisition. VDP contributed with the conception of the study. GEO contributed with the conception of the study, field work and manuscript writing. All authors reviewed the manuscript.

