



Different post-fire stages encompass different plant community compositions in fire-prone grasslands from Southern Brazil[☆]

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ABSTRACT

Fire is among the major forces shaping patterns in nature. Although sometimes it is only acknowledged for its destructive power, it was one of the evolutionary drivers that produced present-day species and biome distributions, being inextricably linked to fire-prone ecosystems such as grasslands. Knowing how fire influences grassland biodiversity is therefore fundamental to conserve it. Here we analyzed the taxonomic and functional structure of grassland plant communities from Southern Brazil under different post-fire successional stages. We sampled 12 sites that were fire-suppressed for varying amounts of time (3 to 300 months), categorized in three post-fire stages: freshly-burnt (FB), intermediate-burnt (IB), and old-burnt (OB). We compared these stages regarding taxonomic composition, species richness, diversity (partitioned in different spatial components), and functional composition (based on plant life forms and metabolism). We sampled 307 plant species from 52 families. Species richness was lower in the OB stage, and did not differ between FB and IB stages. Species composition was markedly different between stages. Forty-five percent of the sampled species were exclusive to one post-fire stage, and only 23% were shared among them. Old-burnt sites share only up to 5% species with other stages and concentrated most of the trees and tussocks. Because long-term fire suppression allowed for the entry of different floristic elements in the community, taxonomic diversity (beta and gamma levels) was higher in old-burnt sites. Our results provide empirical evidence that fire suppression is likely to shift the fire-prone grasslands from Southern Brazil towards different ecosystem states. However, whether this process is leading towards a closed forest system or to a different system with coexisting grassland and forest elements, remains an open question. The sharp floristic differences and exclusive species in the different post-fire stages reinforce the need to include fire in the conservation framework for fire-prone grasslands.

1. Introduction

Fire is one of the major drivers of biodiversity patterns and processes worldwide. It was an important force in the past, dating back as far as the origin of land plants (Bowman et al., 2009) and shaping species and even biome distributions, as well as evolutionary processes (Bond and Keeley, 2005). As a result, present-day open ecosystems such as grasslands are evolutionarily related to and dependent on disturbance regimes that include fire (Veldman et al., 2015). In fact, the role of fire can be more important for vegetation patterns and processes than climatic conditions in some parts of the world (Bond et al., 2003). Despite the common misconception that grasslands are man-made (analogous to a

secondary forest), these fire-prone ecosystems are just as ancient and 'natural' as old-growth forests, harboring high levels of endemism and unique species sets (Veldman et al., 2015).

The general effect of disturbance on plant community structure is to increase overall species richness and diversity by breaking (i.e., reducing) the dominance of one or a restricted set of species that would dominate the community in its absence (Pickett, 1985). This short-term, disturbance-promoted diversity peak is usually associated with the reduction of the dominance of species that thrive without disturbance (notably large C4 tussock grasses) and the opening of the community, allowing for less competitive species to establish. However, it is important to consider not only the immediate post-fire response of

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grassland communities, but also the long-term effects of fire suppression, as well as the intermediate post-fire successional stages that lie in-between. Current evidence indicates that post-fire dynamics in South Brazilian grasslands comprise a short-term diversity peak after a fire event, followed by a return to pre-fire conditions in approximately two years (Overbeck et al., 2005; Overbeck and Pfadenhauer, 2007), and similar dynamics were reported for grazing disturbance in the same region (Ferreira et al., 2018; Ferreira et al., 2020). The return to pre-disturbance conditions is led by the fast growth of highly productive species, which restabilize diversity values through competitive exclusion, while disturbance suppression simultaneously selects for traits that homogenize plant communities in terms of life forms and general habitat structure (Ferreira et al., 2020; Lezama et al., 2014; Overbeck et al., 2005). Although these short-term responses of grasslands to disturbance (or disturbance suppression) are relatively well-established, data on how these ecosystems respond to long-term disturbance suppression as we present here are still scarce.

Although it is correctly assumed that most plants from flammable ecosystems have mechanisms to tolerate fire (Bowman et al., 2014; Pausas et al., 2018), plant species respond differently to fire according to species-specific tolerance mechanisms and local ecosystem properties (Bond and Van Wilgen, 1996). This phenomenon makes it hard to draw broad generalizations on the effects of fire over plant communities (Uys et al., 2004). Knowledge on the response of plant species to fire is still lacking for most ecosystems (Pausas, 2019), especially in highly productive and species-rich grasslands such as those present in Southern Brazil (Overbeck and Pfadenhauer, 2007). Classifying species into life forms is recurrently used as a means to reduce the complexity of such systems in order to detect plant community responses to disturbance considering their functional dimension (Diaz and Cabido, 1997). However, species taxonomic identities should not be neglected, because (i) different species are likely to respond differently to fire, (ii) knowledge on these species-specific responses is incipient, and (iii) general conservation policies, environmental licensing, and delimitation of protected areas rely heavily on lists of endemic and endangered species. To effectively protect these species in fire-prone ecosystems, their relationship with fire disturbance must be better understood. Just like sets of plant life forms respond differently to fire, it is reasonable to assume that different sets of species (i.e., community composition) will follow a similar response pattern.

This study analyses taxonomic and functional descriptors of grassland plant communities under different post-fire successional stages, measured as the amount of time elapsed since the last fire event (more simply, time since fire). We categorized grassland sites as freshly-burnt, intermediate-burnt, and old-burnt. Specifically, we compared post-fire stages regarding the following plant community descriptors: (i) taxonomic species richness, diversity, and composition, and (ii) functional diversity and composition (as the relative contribution of plant life forms). In addition, we evaluated differences in habitat descriptors related to disturbance regimes (overall vegetation height and cover, litter, and bare soil). Based on the premise that fire disturbance is one of the most important drivers of grassland vegetation dynamics (Bond and Parr, 2010; Bowman et al., 2020; Keeley et al., 2005), we hypothesized that these descriptors would respond to the time since fire gradient.

Specifically, we hypothesized that (i) because species that draw benefits shortly after fire events or from long-term disturbance suppression tend to dominate communities in the extremes of the post-disturbance gradient (Ferreira et al., 2020; Overbeck et al., 2005; Pausas, 2019) richness and diversity descriptors will peak in intermediate-burnt sites and show lower values in freshly-burnt and old-burnt sites, fitting a recurrent pattern in disturbance-diversity relationships (Mackey and Currie, 2001); (ii) because species are expected to respond differently to fire (Pausas, 2019), species composition will differ between post-fire stages in a way that different subsets of species will be unique to freshly-burnt sites (fire-specialists) and to old-burnt sites (fire-avoiders), while the remaining species (fire-generalists) will

be shared between stages, and intermediate-burnt sites will share species with both extremes of the gradient (thus increasing overall species richness in that stage, as in the first prediction); and (iii) because processes such as shrub encroachment and forest expansion are favored by lower frequency or absence of fire (Guido et al., 2017; Sühs et al., 2020), lignified species such as shrubs and trees will be more representative in old-burnt sites.

2. Material and methods

2.1. Study sites and sampling design

Data were collected in grassland sites from Southern Brazil, in the Brazilian Pampa biome, which is inserted in the larger region known as Río de la Plata Grasslands, a diverse mosaic of temperate grassland ecosystems that covers ca. 700,000 km² in eastern Argentina, Southern Brazil, and Uruguay (Soriano, 1992). Within their Brazilian distribution, they are threatened ecosystems that had lost 60% of their original cover (ca. 175,000 km²) by 2002, with most of the remnants being classified as 'degraded' (Andrade et al., 2015). Although grassland landscapes dominate most of this region, grassland-forest mosaics are common (Andrade et al., 2018). They are remnants of the cooler and drier climate of the last glacial period, submitted to frequent fire events since the early Holocene (Behling and Pillar, 2008). Current evidence suggests that fire and grazing disturbances not only maintain their high levels of diversity, but also hinder processes related to the reduction of grassland areas, such as shrub encroachment and forest expansion (Boavista et al., 2019; Guido et al., 2017; Sühs et al., 2020, 2018).

Sampling was conducted from September 2016 to March 2017 in the metropolitan region of Porto Alegre, Rio Grande do Sul, Brazil (30°07'S, 51°09'W). Climate is subtropical humid with no dry season (Köppen's Cfa), with mean annual temperature and precipitation ranging from 18 to 20 °C and from 1300 to 1500 mm, respectively (Alvares et al., 2013). Soil is usually poor in nutrients, with shallow A horizon and frequent rock outcrops (Streck et al., 2008). We sampled 12 grassland sites distributed in four hills (three sites per hill), all of which were in protected areas: Morro do Osso Natural Park, Saint'Hilaire Municipal Park, São Pedro Wildlife Refuge, and Itapuã State Park. Sampling sites are inserted in a chain of granitic hills that runs through a large, urbanized area, representing the last remnants of original vegetation cover in the region. In these hills, grasslands mostly cover hilltops and northern slopes, while forests (Atlantic rainforest) cover southern slopes and follow river courses (Setubal and Boldrini, 2012). Although all study sites are inserted in protected areas (therefore, none of them are subjected to grazing), there is no formal land management policy for grassland ecosystems, which are periodically submitted to accidental or deliberate (illegal) fire events. Fire frequency is variable and directly influenced by the urbanization level in the landscape matrix (Beal-Neves et al., 2020b), producing a mosaic of grasslands under different post-fire successional stages.

We selected 12 grassland sites with different time elapsed since the last fire event, which was estimated using satellite images and validated with managers and official records of protected areas. We grouped sites into three post-fire successional stages, according to time elapsed since the last fire event: freshly-burnt (FB, four sites, 3–17 months), intermediate-burnt (IB, five sites, 19–120 months) and old-burnt (OB, three sites, >300 months; Appendix B, Fig. A, C). We tested the consistency of the classification into post-fire stages (i.e., if each category comprised discrete plant communities) with cluster analysis (details below). With this chronosequence, we used space as a surrogate of time to capture different points in the trajectory of grassland plant communities after the reset promoted by fire. In each site, we defined a one-hectare circular plot, in which plant community sampling was carried out. Plots were preferentially centered in the grassland area within each site, with a minimum distance of 200 m from forest edges, placed in north-oriented slopes in most cases, avoiding steep inclinations as well

as deeper and moister soils. See [Beal-Neves et al. \(2020b\)](#) for detailed information on the sampling sites.

2.2. Plant community and habitat structure sampling

To perform the quali-quantitative sampling of grassland vegetation, ten one-square-meter quadrats were systematically distributed to evenly sample each 1-ha circular plot. We avoided areas with large trees (which occur scattered in the grassland matrix) but included typical grassland shrubs. We identified all plant species present in each quadrat (including tree saplings and juveniles), and used Londo's decimal scale to estimate their cover ([Londo, 1976](#)). In addition, in each quadrat, we measured vegetation height (cm) in five points using a sward stick, and estimated the cover of litter, bare soil, and overall vegetation (using Londo's scale as well). Vegetation height measurements comprised the maximum vegetation height in each point (i.e., not discriminating between species or life forms), considering the highest live part of the plant that touched the sward stick (i.e., not considering the standing dead biomass of large tussocks). Plant community data were pooled in matrix W_q of 1 m² quadrats described by plant species cover, from which we derived matrix W_p of 1-ha plots described by plant species cover. All collected plant specimens were deposited in the Museum of Science and Technology of Pontifícia Universidade Católica do Rio Grande do Sul (MCT-PUCRS).

2.3. Plant functional traits

We used an approach based on plant functional traits to describe the functional dimension of the plant community and assess its variation across post-fire successional stages. We classified all plant species into life forms, following the classification proposed by [Ferreira et al., \(2020\)](#). This classification, which is a modification of Raunkiaer's system, is based on plant architecture, lignification, and strategy of habitat occupation, which provides a description of grassland vegetation structure with life form categories that are responsive to shifts in disturbances. We collapsed finer categories from the original classification into coarser ones and added a 'tree' category. Our final list comprised 10 life forms: tussocks, prostrated plants, rosettes, herbaceous forbs, geophytes, therophytes, succulents, lignified forbs, shrubs, and trees. In addition, we classified graminoid species (grasses and sedges) according to metabolic pathway (C3 or C4). Metabolic information and life form category for species that were not present in the original classification were obtained from the literature (Appendix A, Table A.2). Plant life forms and graminoid metabolic pathway (nominal traits) were expanded into binary traits and summarized in matrix B of plant species described by traits.

2.4. Statistical analysis

We used a multivariate approach to explore differences in plant community structure and species composition. We tested for differences in composition between post-fire stages with multivariate analysis of variance with permutation ([Anderson, 2001](#)). We used nonmetric multidimensional scaling (NMDS) to plot sites and species. These analyses we carried out with packages 'vegan' ([Oksanen et al., 2020](#)) and 'RVAideMemoire' ([Hervé, 2020](#)), based on Bray-Curtis dissimilarities ([Legendre and Legendre, 2012](#)) between sampling units of Matrix W_p , after transformation with the Hellinger method ([Legendre and Gallagher, 2001](#)). In addition, we performed a cluster analysis of matrix W_p to validate our *a priori* classification in post-fire stages (i.e., to test if communities from the same stage showed higher similarity among themselves than with communities from a different stage). Cluster analysis was carried out with package 'factoextra' ([Alboukadel and Mundt, 2020](#)), based on Bray-Curtis dissimilarities and Ward's clustering criterion ([Legendre and Legendre, 2012](#)). We tested for the optimal number of clusters using gap statistics ([Tibshirani et al., 2001](#)). Cluster analysis resulted in three optimal clusters, which coincided with

our *a priori* classification (Appendix B, Fig. D), leading us to use it in all further analyses.

We tested the strength and significance of the relationship between species occurrence and each post-fire stage with indicator species analysis ([De Cáceres et al., 2012](#)). In this analysis, we evaluated the association between species from matrix W_p and a vector of site-group memberships (post-fire stages), using the 'IndVal' index to estimate the strength of association. The significance of the association between species and stages was estimated with permutational analysis, generating a *p*-value corrected for multiple testing for each species-group pair. Among the significant relationships, we selected the five species with the highest IndVal for each post-fire stage to include in the results and highlighted their location in the ordination space of the NMDS.

We compared plant species richness between post-fire successional stages using sample-coverage based rarefaction and extrapolation ([Chao et al., 2014](#)). This approach considers the 'coverage deficit' (i.e., the probability that a newly sampled individual belongs to an unsampled species), rendering less biased estimation of richness, especially when comparing different communities that have potentially different distributions of rare species. We adjusted extrapolated curves based on species frequencies separately for 1m² quadrats and 1-hectare plots, extrapolating to twice the original sampling effort for the first and thrice for the latter. We generated 95% confidence intervals based on 9999 permutations for each curve. We assumed that species richness between a given pair of post-fire stages was significantly different when there was no overlap between confidence intervals. These analyses were carried out with the 'iNEXT' package ([Hsieh et al., 2016](#)). We compared taxonomic plant species diversity between post-fire stages using an additive spatial diversity partitioning approach ([Baselga, 2012](#)), in which we considered two spatial grains: 1m² quadrats (alpha; matrix W_q) and 1-hectare plots (gamma; matrix W_p). With this approach, we estimated overall beta diversity (Sorensen dissimilarity), as well as the relative contribution of its underlying components: spatial turnover (Simpson's dissimilarity) and nestedness (nestedness-resultant proportion of Sorensen dissimilarity). Diversity partitioning analyses were performed with package 'betapart' ([Baselga and Orme, 2012](#)).

To evaluate differences in the dominance of functional traits between post-fire stages, we scaled-up individual species trait information to the community level by computing community-weighted mean (CWM) traits. Scaled-up trait information was summarized in matrix T of sites described by mean trait values, obtained with matrix multiplication $T = W_p B$ ([Pillar et al., 2009](#)). In this case, CWM traits represent the varying proportions of each life form category and metabolic pathway of graminoids in each community. In addition, we calculated plant functional diversity (Rao entropy) for each site, based on community and trait information from matrices B and W_p and on trait dissimilarities between species computed with Gower distances ([Podani, 1999](#)). In this framework, functional diversity represents how well distributed is the dominance of life forms or metabolic pathways in each site. We used permutational tests to compare post-fire successional stages regarding species taxonomic diversity (alpha, beta, and gamma components), functional diversity, and habitat descriptors. Permutational analyses were performed with package 'lmPerm' ([Wheeler and Torchiano, 2016](#)), considering 9999 random iterations in each test. For significant differences, we performed pairwise post-hoc permutational tests, correcting pairwise *p*-values with method *fdr* ([Benjamini and Hochberg, 1995](#)). Finally, we used the multilevel method (MLM3) proposed by [Ter Braak \(2019\)](#) to test the response of CWM traits to the post-fire gradient. All analyses were carried out in the R environment ([R Core Team, 2021](#)), considering the probabilistic threshold of $\alpha = 0.05$ to reject the null hypothesis and including site as a random factor when applicable.

3. Results

3.1. Plant species richness, diversity, and habitat structure

We sampled 307 plant species, distributed in 52 botanical families (see Table A1 in Appendix A). Only six taxa could not be identified to the species level (<2% of the total plant species). The most representative families in the freshly-burnt (FB) and intermediate-burnt (IB) sites were Asteraceae, Poaceae, Fabaceae, and Cyperaceae. Old-burnt (OB) sites showed a slightly different pattern, with Poaceae overtaking the place of Asteraceae as the richest family (Table 1). We found four exotic species distributed in the three categories of post-fire successional stages (*Cerastium glomeratum* [exclusive to IB sites], *Drymaria cordata* [IB], *Pinus elliottii* [IB, OB], and *Veronica* sp. [FB]). Eleven species are included in the regional list of extinction-threatened plants (SEMA, 2014), categorized as Vulnerable (5), Endangered (3), and Critically Endangered (3), according to IUCN criteria (IUCN, 2012). Interestingly, three threatened species were only found in FB sites (*Eugenia dimorpha*, *Mandevilla coccinea*, *Oxypetalum muticum*), while another three species were exclusive to OB sites (*Butia odorata*, *Dyckia choristaminea*, *Dyckia maritima*). Intermediate-burnt sites presented the lower number of threatened species, and no species were exclusive to this post-fire stage (Table 2).

Total plant species richness was lower in old-burnt (OB) sites in comparison with freshly-burnt (FB) and intermediate-burnt (IB) sites (i.e., confidence intervals did not overlap, irrespective of sampling effort), although it did not differ between FB and IB sites (i.e., confidence intervals overlap). This pattern was recovered by coverage-based rarefaction analysis considering both sampling scales (i.e., 1-square meter quadrats and 1-hectare plots; Fig. 1A,B). Sample coverage was overall high, capturing at least 80% of species richness in all time since fire categories and at both sampling scales (Fig. 1C,D; see interpolated values). However, sample coverage was lower (and notably variable between post fire categories) in 1-hectare plots, reaching 100% coverage for all categories only when the number of sampling units was extrapolated three times (Fig. 1D). Nevertheless, the general patterns of species richness held true when samples were standardized by their coverage (Fig. 1E,F).

Taxonomic diversity showed different patterns of response to time

Table 1

Distribution of plant species richness ($N = 307$) in families ($N = 52$) collected in 12 grassland sites from Southern Brazil under different post-fire successional stages. FB = freshly-burnt (3–17 months), IB = intermediate-burnt (19–120 months), OB = old-burnt (>300 months).

Family	Number of species			
	Total	FB	IB	OB
Asteraceae	67	48	46	27
Poaceae	53	39	43	31
Fabaceae	31	18	23	9
Cyperaceae	15	11	10	7
Rubiaceae	13	11	9	7
Apiaceae	9	6	7	6
Apocynaceae	8	6	1	4
Euphorbiaceae	8	5	6	4
Iridaceae	8	3	7	4
Malvaceae	7	4	6	0
Orchidaceae	7	1	3	4
Polygalaceae	7	4	7	1
Verbenaceae	7	5	6	2
Lamiaceae	5	2	4	1
Acanthaceae	4	1	3	1
Convolvulaceae	4	3	3	2
Myrtaceae	4	3	2	2
Bromeliaceae	3	1	1	2
Caryophyllaceae	3	1	2	0
Oxalidaceae	3	3	2	0
Plantaginaceae	3	2	2	0
Other families	38	17	23	22

Table 2

Extinction-threatened species collected in 12 grassland sites from Southern Brazil under different post-fire successional stages. FB = freshly-burnt (3–17 months), IB = intermediate-burnt (19–120 months), OB = old-burnt (>300 months). Threat category follows IUCN (IUCN, 2012) criteria, being CR = Critically Endangered, EN = Endangered, and VU = Vulnerable.

Species	Threat	Family	Life form	Post-fire stage		
				FB	IB	OB
<i>Aspilia pascaloides</i>	CR	Asteraceae	herbaceous forb	1	1	0
<i>Butia odorata</i>	EN	Arecaceae	tree	0	0	1
<i>Dyckia choristaminea</i>	EN	Bromeliaceae	succulent	0	0	1
<i>Dyckia maritima</i>	VU	Bromeliaceae	succulent	0	0	1
<i>Dyckia remotiflora</i>	VU	Bromeliaceae	succulent	1	1	0
<i>Eugenia dimorpha</i>	VU	Myrtaceae	shrub	1	0	0
<i>Funarium flavum</i>	CR	Apocynaceae	lignified forb	1	0	1
<i>Mandevilla coccinea</i>	VU	Apocynaceae	lignified forb	1	0	0
<i>Moquiniastrum cinereum</i>	EN	Asteraceae	lignified forb	0	1	1
<i>Oxypetalum muticum</i>	CR	Apocynaceae	lignified forb	1	0	0
<i>Parodia ottonis</i>	VU	Cactaceae	succulent	1	0	1

since fire according to the spatial level (Fig. 2A–C). Alpha diversity (mean values for 1-square-meter quadrats) did not differ between time since fire categories ($p = 0.580$), whereas gamma diversity (1-hectare plots) and beta diversity were higher in OB sites, but did not differ between FB and IB sites ($p = 0.506$). Conversely, OB sites showed lower functional diversity when compared to IB sites, but neither OB sites nor IB sites differed from FB sites ($p = 0.398$ and 0.457 , respectively; Fig. 1D).

Vegetation structure was markedly variable between time since fire categories (Fig. 2E–H). Intermediate-burnt sites showed the highest average vegetation height, followed by FB and OB sites (Fig. 2E). Overall vegetation cover also peaked in IB sites, with FB showing the lowest values (Fig. 2F). Freshly-burnt sites showed the lowest percentage of litter (Fig. 2G). The percentage of bare soil decreased as time since fire increased, with sharp differences between the extremes: 22% on average in freshly-burnt grasslands and 5% in old-burnt grasslands.

3.2. Plant species taxonomic composition

The three post-fire successional stages presented different plant community compositions (Fig. 3A; MANOVA sum of squares = 0.831, $F = 2.578$, $p = 0.002$; $p < 0.05$ for all permutational pairwise comparisons). Moreover, 140 species (46%) were found in only one of the post-fire stages, and only 72 species (23%) were shared by the three stages (Fig. 3B). Old-burnt sites showed the clearer separation in the ordination space (Fig. 3A), but the lower number of exclusive species (Fig. 3B). Freshly-burnt and intermediately-burnt sites shared more common species between themselves (22%) than with OB sites (3 and 5%, respectively).

The analysis of indicator species selected sets of species with different compositions of botanical families and life forms for each post-fire successional stage (Table 3). For FB sites, indicator species included a common prostrated species (*Richardia grandiflora*) and lignified species that invest in underground storage organs (*Calea uniflora* and *Disynaphia ligulifolia*), whereas IB sites included lignified species without such underground organs (*Desmanthus virgatus*). Notably, species selected as indicators of OB sites included a tree (*Dodonaea viscosa*) and a C4 grass that dominates large areas with broad and tall tussocks (*Saccharum angustifolium*), but no prostrated species.

3.3. Plant life forms

The relative contribution (community-weighted mean traits; CWM) of plant life forms in grassland plant communities varied according to

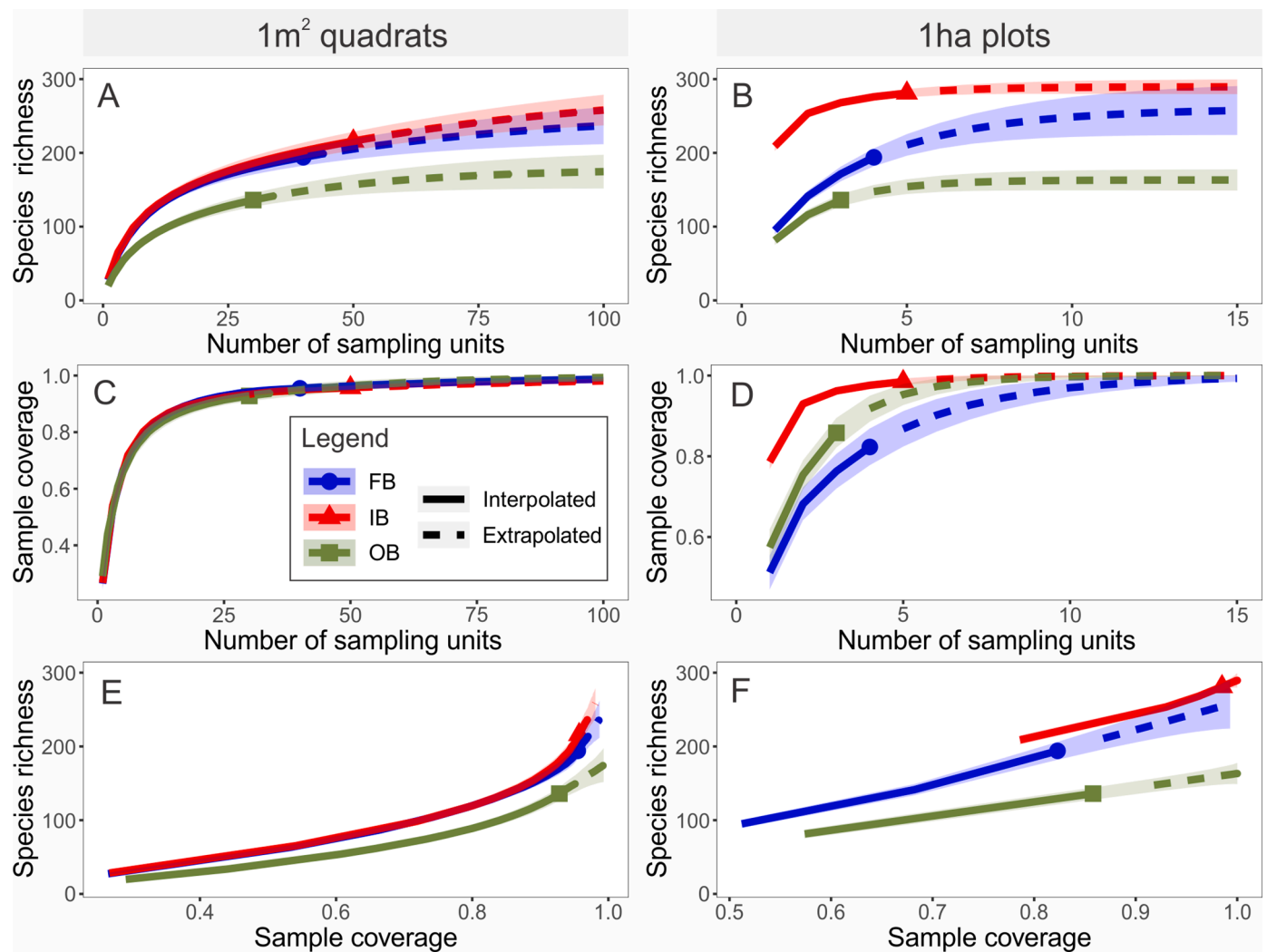


Fig. 1. Rarefaction and extrapolation curves of plant species richness ($N = 307$) collected in 12 grassland sites from Southern Brazil under different post-fire successional stages. FB = freshly-burnt (3–17 months), IB = intermediate-burnt (19–120 months), OB = old-burnt (>300 months). Separate curves for one-hectare plots ($N = 12$) and one-square-meter quadrats ($N = 120$). A–B. Sample-size-based rarefaction. C–D. Sample completeness. E–F. Coverage-based rarefaction. Confidence intervals that show no overlap represent statistically significant differences between post-fire stages.

time since fire category (Fig. 4). Intermediate-burnt sites presented a significantly higher contribution of prostrated and rosetted species, as well as species with C3 metabolism, but the lowest contribution of shrubs. Interestingly, freshly-burnt and old-burnt sites presented similar patterns for some life forms, such as the low contribution of prostrated plants and the high contribution of shrubs. Succulent plants and trees predominated in OB sites, which also presented the lowest contributions of rosettes, but higher contribution of C3 plants in comparison with FB sites. There were no significant differences between post-fire stages considering CWM values for herbaceous forbs, geophytes, therophytes, and C4 plants. Considering only the species richness in each life form (i. e., without cover values of each species, included in CWM calculations; Table 4), therophytes were absent from OB sites, while geophytes and herbaceous forbs presented more species (absolute numbers only) in IB sites. Most tree species were restricted to OB sites (Table 4): *Agarista eucalyptoides* (Ericaceae), *Butia odorata* (Arecaceae), *Calliandra tweedii* (Fabaceae), *Myrcia palustris* (Myrtaceae), and *Myrsine guianensis* (Primulaceae).

4. Discussion

In this paper, our goal was to evaluate how fire affects grassland plant communities by measuring community descriptors in sites under

different post-fire successional stages. As expected, plant species richness was lower in old-burnt (OB) sites. In the absence of disturbances (or when disturbance is too frequent/intense), sets of species are benefitted, showing increased dominance and may outcompete co-occurring species, which ultimately reduces the overall number of species (Durigan, 2020; Ferreira et al., 2020; Overbeck et al., 2005). However, species richness did not peak in intermediate-burnt (IB) sites as we had hypothesized. Rather, freshly-burnt (FB) and IB sites showed similar species richness. These results suggest that the short-term increases in plant species richness promoted by fire may in fact last longer as community dynamics proceed without new fire events. Although we did find higher absolute richness values for IB sites when considering the coarser spatial scale of one-hectare plots (see Fig. 1B, interpolated values and most of the extrapolation curve), the difference between IB and FB stages disappears when richness is standardized by sample coverage (Fig. 1F). Old-burnt grasslands presented higher gamma (one-hectare plots) and beta diversity, whereas there was no difference regarding alpha diversity (i.e., mean diversity values based on 1 m² quadrats) between post-fire stages. A study carried out in Argentinian grasslands found a similar response pattern to grazing disturbance: grazing exclusion promoted reduction in plant diversity at the local scale, but increased it when larger areas were considered (Chaneton and Facelli, 1991). Species richness in disturbance-prone grasslands is simultaneously

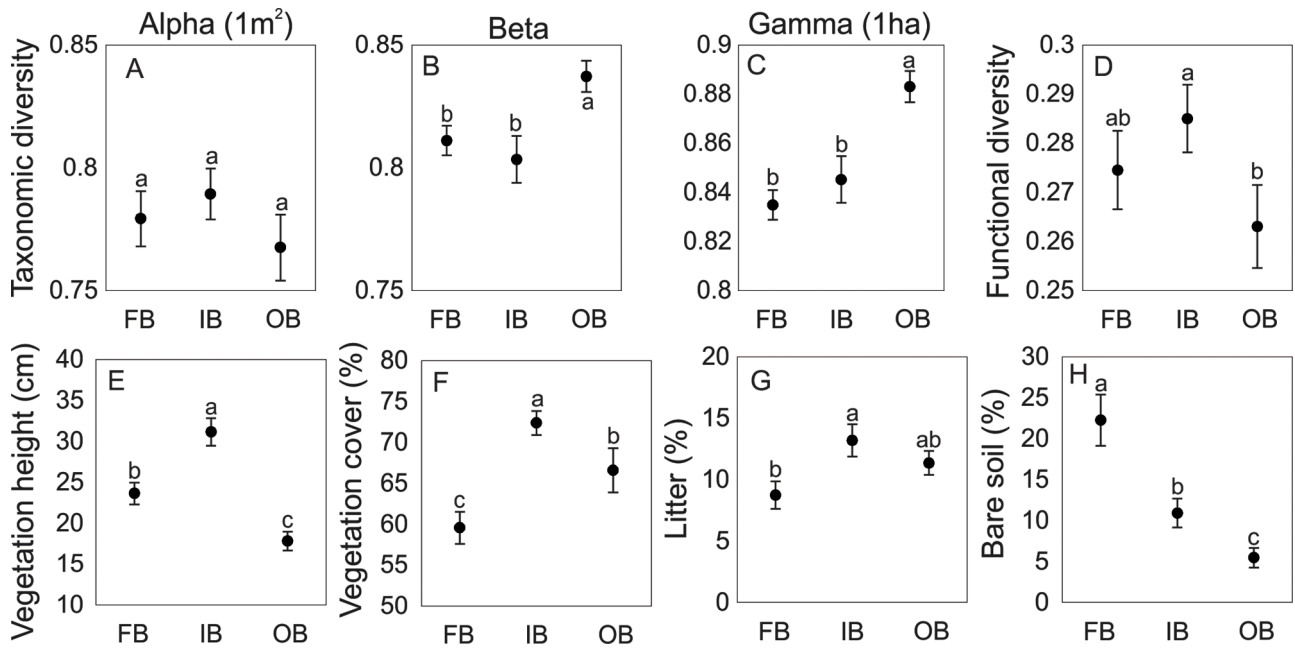


Fig. 2. Plant diversity and habitat structure variables collected in 12 grassland sites from Southern Brazil under different post-fire successional stages. FB = freshly-burnt (3–17 months), IB = intermediate-burnt (19–120 months), OB = old-burnt (>300 months). A–C. Taxonomic diversity measured in different spatial grains. A. alpha diversity (Simpson index). B. Beta diversity (Sorensen dissimilarity). C. Gamma diversity (Simpson index). D. Functional diversity (Rao entropy). E–H. Habitat structure variables; mean values based on 120 sampling quadrats. E. Mean vegetation height. F. Mean vegetation cover. G. Mean percentage of litter. H. Mean percentage of bare soil. Different letters indicate statistically significant differences (fdr-corrected pairwise permutational $p < 0.05$).

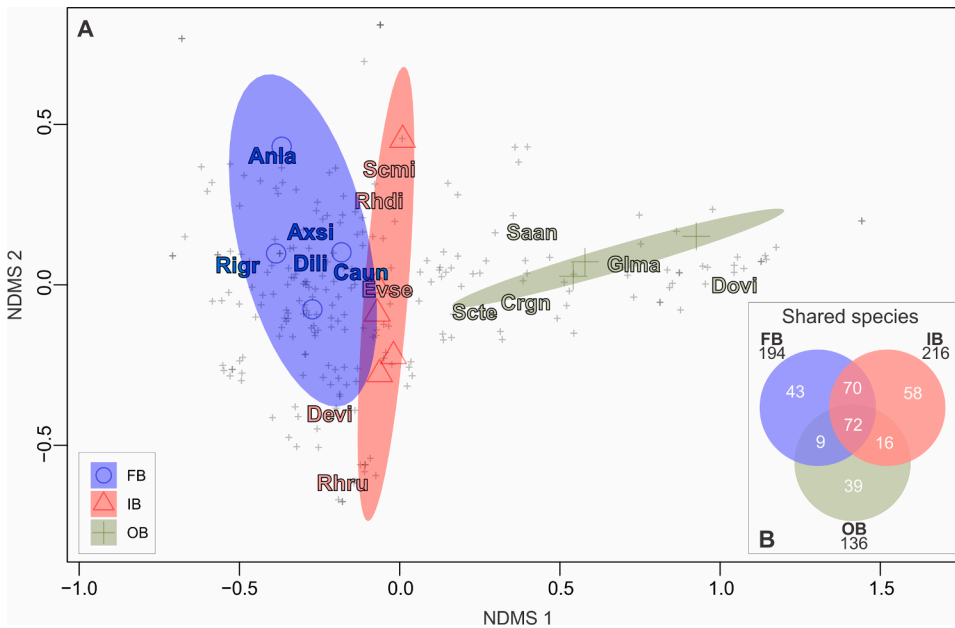


Fig. 3. A. Ordination scatterplot obtained from a NMDS analysis of plant communities collected in 12 grassland sites from Southern Brazil under different post-fire successional stages. FB = freshly-burnt (3–17 months), IB = intermediate-burnt (19–120 months), OB = old-burnt (>300 months). Confidence ellipses (95%) plotted for each stage. Most species plotted as small crosses; indicator species for each post-fire stage plotted with acronyms (see Table 3 for full indicator species information). B. Number of shared and exclusive species (inside circles) and total number of species (outside circles) for post-fire stages.

determined by local factors, related to dispersal and establishment, and by the species pool considering larger spatial scales (Olf and Ritchie, 1998). In that sense, although disturbance suppression tends to reduce species richness at the local scale by modulating interspecific interactions as discussed above, it may also promote the addition of disturbance-intolerant species to the species pool, potentially increasing species richness at larger scales.

The role of disturbances as drivers of biodiversity patterns and processes has been studied for a long time in ecology, and diversity-disturbance relationships (DDR) can be variable. Peaked or hump-shaped DDRs (i.e., higher diversity values under intermediate

disturbance frequency or intensity) are more common when the disturbance in question is considered to be intrinsic to the study system (Mackey and Currie, 2001), which would be the case for fire disturbance in most grasslands (Bond and Keeley, 2005). Although peaked DDRs are indeed very commonly reported for grazing disturbance in grasslands (Cingolani et al., 2005), fire effects over similar systems can be different (Harrison et al., 2003; Spasojevic et al., 2010). Previous works have reported short-term diversity peaks after fire disturbance in south Brazilian grasslands (Overbeck et al., 2005), but information on the effects of long periods without fire is scarce. A recent work focused on the influence of fire on plant and bird communities from south Brazilian

Table 3

Indicator species of three post-fire successional stages for plant communities collected in 12 grassland sites from Southern Brazil. 'IndVal' represents the strength of association of each pairwise species-stage relationship. All species were significantly related to the respective stage ($P < 0.05$).

Post-fire stage/species	Family	Acronym	IndVal	Life Form	Metabolism (graminoid species)
Freshly-burnt					
<i>Andropogon lateralis</i>	Poaceae	Anla	0.665	tussock	C4
<i>Axonopus siccus</i>	Poaceae	Axsi	0.645	tussock	C4
<i>Richardia grandiflora</i>	Rubiaceae	Rigr	0.594	prostrated	–
<i>Disynaphia ligulifolia</i>	Asteraceae	Dili	0.569	shrub	–
<i>Calea uniflora</i>	Asteraceae	Caun	0.556	lignified forb	–
Intermediate-burnt					
<i>Rhynchospora rugosa</i>	Cyperaceae	Rhru	0.616	tussock	C3
<i>Evolvulus sericeus</i>	Convolvulaceae	Evse	0.599	prostrated	–
<i>Desmanthus virgatus</i>	Fabaceae	Devi	0.576	lignified forb	–
<i>Rhynchosia diversifolia</i>	Fabaceae	Rhdi	0.543	prostrated	–
<i>Schizachyrium microstachyum</i>	Poaceae	Scmi	0.529	tussock	C4
Old-burnt					
<i>Dodonaea viscosa</i>	Sapindaceae	Dovi	0.850	tree	–
<i>Schizachyrium tenerum</i>	Poaceae	Scte	0.775	tussock	C4
<i>Glechon marifolia</i>	Lamiaceae	Glma	0.730	lignified forb	–
<i>Saccharum angustifolium</i>	Poaceae	Saan	0.640	tussock	C4
<i>Croton gnaphalii</i>	Euphorbiaceae	Crgrn	0.610	shrub	–

grasslands found a peaked DDR for several plant community descriptors, although the post-fire successional gradient was shorter (up to 180 months without fire) in comparison with the data we presented here (Beal-Neves et al., 2020a). The unexpectedly higher values of taxonomic diversity in old-burnt grasslands we report here suggest that the long-term fire suppression (at least 25 years) may be driving these communities towards a different ecosystem state, in which different floristic elements that are unable to establish under higher fire frequencies increase diversity. This is corroborated by the extremely low number of species that old-burnt sites shared with the other post-fire stages (3 to 5%). Although OB sites still retain the floristic composition and vegetation structure of a grassland, it seems plausible to assume that disturbance suppression is leading them to a different ecosystem state, which would be a forest in the study region. This composition and structure, with coexisting grassland and forest elements, could be interpreted as an intermediary state in that process. It has been suggested that the distribution of forests in subtropical South America (under temperate climate) is in part determined by fire and grazing disturbances (Bernardi et al., 2016). Fire can be the major driver of vegetation patterns in several ecosystems (Bond et al., 2005; Keeley et al., 2012), in part because it maintains alternative vegetation states under certain climatic conditions (Pausas, 2015). Therefore, it is reasonable to assume that fire suppression is likely to shift the fire-prone grasslands from Southern Brazil towards forest states. However, we need long-term experimental studies that monitor this transition to clarify the role of fire in the process, and to detect possible intermediary states between typical grassland e forest states.

Based on satellite images, Sühs et al. (2020) concluded that south Brazilian grasslands can be lost (i.e., can shift towards different vegetation types) due to disturbance suppression within 30 years. Indeed, our data suggest that grasslands may be starting to shift towards a different system within this fire suppression timespan. Post-fire stages presented discrete plant community compositions, with several unique species. Plant species that have evolved in fire-prone ecosystems have mechanisms to cope with fire disturbance (Bond and Keeley, 2005; Bowman et al., 2014). However, these mechanisms can be remarkably variable between species (Overbeck and Pfadenhauer, 2007; Pausas, 2019), raising the need for fire ecology to understand how fire affects different organisms to unravel broader fire effects over ecosystem patterns and processes (McLauchlan et al., 2020). For example, a given set of species may be benefited immediately after a fire event by breakdown of seed dormancy, or simply by not having germination viability affected by fire (Fidelis et al., 2016; Le Stradic et al., 2015; Overbeck et al., 2006). However, species that rely on such mechanisms, especially those that depend on fire to break seed dormancy, will be negatively affected

by the absence of fire, and are likely to disappear from grasslands that have been fire-suppressed for long periods. Similarly, species that resprout quickly after fire from underground storage organs (Canadell and Zedler, 1995; Pausas et al., 2018), have fire-induced blooming (Bastian, 2020; Fidelis and Blanco, 2014; Haddad and Valio, 1993), or fast resource-acquisition and growth strategies, should harvest immediate benefits after fire events, but are likely to face diminishing returns as time without fire increases, like the two indicator species of our freshly-burnt sites that have resprouting underground organs (*Calea uniflora* and *Disynaphia ligulifolia*). While these fire-specialist species predominate or are exclusively found in freshly-burnt grasslands, a different set of fire-avoider species assume their role in fire-excluded (i. e., old-burnt) grasslands, whereas fire-generalists are found throughout the post-fire gradient. For example, although the tree *Dodonaea viscosa* was much more representative in the OB stage, to the point of being selected as one of its indicator species, it was also present (but less frequent and abundant) in FB and IB stages. Differently, *Myrcia palustris* is a tree species that was exclusive to OB sites. These findings corroborate previous suggestions that *D. viscosa* is a generalist species found throughout the post-fire successional mosaic in the sampling region, while *M. palustris* is more commonly found in forest-grassland transitional zones (Müller et al., 2007). Although our sampling design and plot size only enabled the detection of tree seedlings and saplings within the grassland matrix (i.e., far from forest borders), the presence of this species is further evidence that OB sites may be transitioning towards a different ecosystem state, which will likely have more exclusive tree species if fire-suppression is maintained, configuring the classic example of successional dynamics via species substitution.

Because it is assumed that fire-suppressed grasslands undergo processes of shrub encroachment and forest expansion (Schinestock et al., 2019; Sühs et al., 2020), we hypothesized that lignified life forms would benefit from the long-term fire-suppression in the OB stage. Although the old-burnt stage concentrated most tree species considering both absolute richness and CWM values, it also shared some unexpected similarities with the freshly-burnt stage regarding the functional dimension. For example, FB and OB stages showed similar contributions of shrubs, lignified forbs, and prostrated species. Although shrubs can benefit from fire-suppression to the point of dominating communities (i. e., shrub encroachment), they can also benefit from fire events through fire-induced flowering, which can produce subsequent advantages such as increased population size and overall fitness (Coutinho, 1976; Pilon et al., 2018) and could allow the species to persist longer in the disturbance-suppressed community. It seems that this mechanism can be extended to other lignified life forms: we found the lignified forb *Lantana montevidensis* in all post-fire stages, although the species

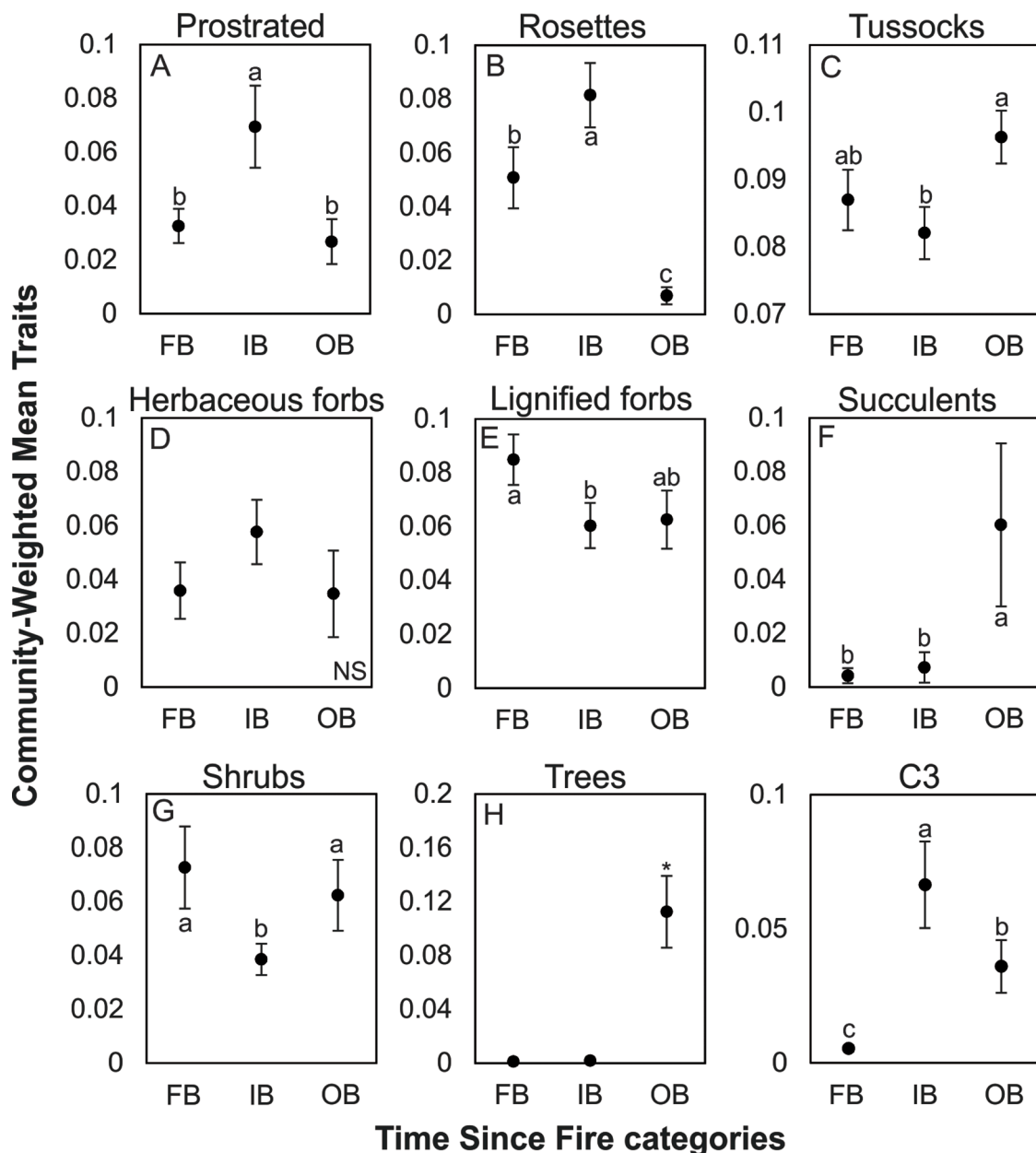


Fig. 4. Relative contribution of plant life forms (A–H) and C3 metabolic pathway (I) measured as community-weighted mean trait values for plant communities collected in 12 grassland sites from Southern Brazil under different post-fire successional stages. FB = freshly-burnt (3–17 months), IB = intermediate-burnt (19–120 months), OB = old-burnt (>300 months). Different letters indicate statistically significant differences (fdr-corrected pairwise permutational $p < 0.05$).

benefits from fire-induced flowering mechanisms (Haddad and Valio, 1993). Lignified forb species from fire-prone grasslands may have higher population densities, increased individual size, and more reproductive structures in freshly-burnt areas, although these benefits can be quickly lost (ca. two years) in the absence of fire (Da Silva et al., 2020).

The response of C3 species to fire suppression showed a ‘peaked’ pattern, i.e., the contribution of C3 species was higher in intermediate-burnt sites. The reduced contribution of these species in FB sites was probably related to their low tolerance to fire and slower recovery after fire events in comparison with C4 grasses (Ripley et al., 2015). However, the mechanism behind the lower contribution of C3 grasses in OB sites is probably different, and related to the poor competitive ability of these species under management suppression (e.g., Ferreira et al., 2020; Ripley et al., 2015). Although fire-suppressed grassland communities such as the North American tallgrass prairies may show increased contribution of C3 species (e.g., Collins et al., 1998; Kahmen and

Poschlod, 2008), the response of temperate South American grasslands may be different, mostly due to different climatic conditions, broader gradients of C3/C4 relative contributions, and evolutionary history with disturbances (Bond et al., 2005), but also because different lineages within the same photosynthetic pathway may show different responses to fire (Ripley et al., 2015).

One of the indicator species of old-burnt grasslands was *Saccharum angustifolium*, an example of highly productive C4 grass, although we found no statistically significant difference in the overall contribution of C4 plants between post-fire stages. It has been reported that *S. angustifolium* can dominate Uruguayan grasslands with up to 70% of mean cover value (Lezama et al., 2019). Although this species is usually avoided by grazing animals when better forage sources are available, this is evidence that *S. angustifolium* can be dominant not only in grasslands under relaxed or suppressed management, but also in areas submitted to continuous grazing and prescribed fires to improve forage

Table 4

Total number of species ($N = 307$) distributed in different life form categories and metabolic pathways (only graminoids) from plant communities collected in 12 grassland sites from Southern Brazil under different post-fire successional stages. FB = freshly-burnt (3–17 months), IB = intermediate-burnt (19–120 months), OB = old-burnt (>300 months). For each post-fire stage, total species number of each category is followed by absolute number and percentage of exclusive species within that given life form.

Life form	Number of species			
	Total	FB	IB	OB
Lignified forb	98	62 (16/16%)	67(22/22%)	36 (10/10%)
Tussock	63	47 (8/12%)	50 (10/15%)	36 (5/7%)
Shrub	41	23 (7/17%)	27 (5/12%)	21 (7/17%)
Prostrated	29	20 (2/6%)	25 (6/20%)	10 (2/6%)
Rosette	24	19 (4/16%)	17 (2/8%)	9 (3/12%)
Herbaceous forb	20	11 (1/5%)	14 (5/25%)	8 (3/15%)
Geophyte	12	6 (3/25%)	8 (3/25%)	4 (1/8%)
Tree	8	1 (0)	3 (1/12%)	7 (5/62%)
Therophyte	6	2 (2/33%)	4 (4/66%)	0
Succulent	5	2 (0)	1 (0)	4 (3/60%)
Metabolic pathway (graminoids only)				
C3	18	9 (1/5%)	16 (7/38%)	9 (1/5%)
C4	45	37 (8/17%)	33 (4/8%)	26 (4/8%)
Data not available	5	4	4	3

quality, such as the Uruguayan grasslands (Royo Pallarés et al., 2005). Moreover, there is recent evidence that fire and fire-related cues can stimulate the germination of this (and several other) typical grassland species (Cuello et al., 2020). While highly productive C4 grasses such as *S. angustifolium* can be dominant when submitted to disturbances by grazing and fire, they can also benefit from disturbance suppression by outcompeting other grassland species (e.g., Ferreira et al., 2020, and our results). However, the presence of pioneer tree species in old-burnt grasslands that we reported here indicates that the advantage that highly productive C4 grasses have in the absence of disturbance is likely to be lost when these trees grow to the point of significantly reducing solar incidence in the lower vegetation stratum (Bond et al., 2005). The amount of time that this process will take, and whether it is still possible to return this system to a previous state with a fire event, are still open questions.

Shrubs and tussocks were not drastically suppressed in freshly-burnt sites, while prostrated species were. This pattern could be at least partly related to our sampling design, because we did not sample sites immediately after fire events (when some of the most well-reported community changes take place, as discussed above). Plants with densely grouped leaves such as some tussock species, or with perennating buds above the grassland flammable layer such as shrubs can survive fire events, while most prostrated species are likely to have their above-ground biomass completely suppressed by fire (Overbeck and Pfadenhauer, 2007). The contribution of prostrated species increased in the IB stage and decreased sharply in the OB stage. Although growing closer to the ground can be an extremely effective adaptation to grazing (Ferreira et al., 2020), it does not seem to be advantageous neither in the immediate post-fire dynamics (probably because prostrated grassland species are usually slow-growing) nor in old-burnt sites (where the competitive exclusion mentioned above takes place). Rosettes responded similarly to prostrated species, and probably through similar mechanisms that involve competition. Geophytes, plants that are periodically reduced to belowground organs, were rare in the OB stage. A notable exception was *Binpinnula* sp., an extinction-threatened orchid

geophyte exclusive to OB sites (we could not determine the threat category because we were unable to determine the species, but all native species from this genus are included in regional redlists). Although geophytes are likely present but 'hidden' in the underground bud bank (Fidelis et al., 2014; Pausas et al., 2018), the amount of time that they can remain in that state until the next fire event remains unknown. Finally, it is worth mentioning that the high number of exclusive species in each post-fire stage was reflected in species turnover within certain life form categories. For example: more than 30% of the shrubs present in FB and OB sites were exclusive to each stage (Table 4), despite the overall similarity in the relative contribution of this life form between the stages (Fig. 4G). Simple and easily-accessed 'soft traits' such as life form categories are useful tools that simplify the enormous taxonomic complexity of grassland systems, allowing for the detection of biodiversity responses to disturbance and other relevant processes such as climate change (Diaz and Cabido, 1997). However, the taxonomic information embedded in life forms must also be taken into account, because these responses can be species-specific as well. Considering how phylogenetic similarities between species influence the response of life forms to disturbance is a promising approach.

It is worth mentioning that factors other than the time elapsed since the last fire event could have influenced the patterns we report here. For example, although we controlled as many environmental factors as possible by selecting sampling sites under similar conditions (e.g., distance from forest border, slope orientation and inclination, avoiding water-saturated areas), and by including site as a random variable in data analyses, we did not take soil variables into account. In addition, each post-fire successional stage we explored here comprised a broad range of time, notably the IB stage. The lack of soil data, as well as the amplitude of time since fire within categories, could have blurred more detailed patterns, especially those that can be detected in a fine spatial scale and in the early post-fire succession. These are limitations inherent to our data and should be considered in the interpretation of our results. However, we emphasize that we aimed to detect broader patterns, i.e., the effects of long-term disturbance suppression, rather than the already well-reported short-term responses of plant communities to disturbances.

Our results have important implications for the conservation of grassland plant communities from Southern Brazil. Because plant species composition was markedly different between post-fire stages, conservation efforts must include fire disturbance to maximize conservation results and encompass a representative species set of the grasslands from the region, at least in systems without formal management with grazing animals. Forty five percent of the species we sampled were exclusively found in one of the post-fire stages. Although this high percentage of exclusive species could be partly related to sampling effort in each site, extinction-threatened species followed a similar pattern, with seven out of 11 species (63%) being exclusive to one post-fire stage. Disturbance suppression is a threat to open ecosystems such as grasslands (Abreu et al., 2017; Overbeck et al., 2015), but fire is seldom considered as a management tool in Brazil (Pillar et al., 2010). Detailed protocols to guide the use of fire in management are still lacking for Brazilian non-forest ecosystems (Durigan and Ratter, 2016; Overbeck et al., 2018). Therefore, it is imperative that scientists, decision-makers, and managers of protected areas focus their complementary knowledge and experiences towards this end. Fire should not be viewed only as a destructive force, but as a fundamental process that has influenced most ecosystems for millennia (He and Lamont, 2018; Pausas and Bond, 2020). While our results contribute to the mounting bulk of evidence that a set of species from South Brazilian grasslands depend on disturbances such as fire, they also indicate that long-term fire-suppression encompasses a different species set, also with unique elements. Although this fire-suppressed community is likely to become increasingly different from the 'typical' grassland found in their more frequently-burned counterparts, we showed that the long-term suppression may allow for the entry of different floristic elements that increase overall diversity.

However, management suppression in large areas for long timespans would likely lead to loss of grassland landscapes and biodiversity. Therefore, maintaining fire-suppressed grassland patches in a managed landscape seems to be important not only to maximize conservation efforts (by promoting habitat heterogeneity), but also to improve the understanding of forest-grassland dynamics in the region. South Brazilian grasslands have been heavily converted over the last decades (Souza et al., 2020). The most promising path to maintain these areas with their original cover is to generate, compile, and deliver information on best management practices, rather than outlawing the use of fire as a management tool. Finally, the frequency of large-scale fires is likely to increase in the next decades, due to direct human influence (Balch et al., 2017) and climate change (Liu and Wimberly, 2016; Schoennagel et al., 2017). Improving the knowledge on the relationships between fire and biodiversity is essential to project future scenarios of conservation and land use.

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CRediT authorship contribution statement

Pedro Maria Abreu Ferreira: Conceptualization, Methodology, Formal analysis, Writing – original draft, Visualization, Supervision, Project administration. **Cleusa Vogel Ely:** Data curation, Writing – review & editing. **Mariana Beal-Neves:** Conceptualization, Data curation, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.flora.2021.151937.

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