1   **Title:** Heterogeneity decreases as time since fire increases in a South American
2   grassland

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4   **Running title**: Fire promotes heterogeneity in grassland

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26

27 **Abstract**

28 Questions

29 Disturbances change the fundamental properties of grasslands on different spatio-
30 temporal scales. Uruguay belongs to the Río de la Plata grasslands, and 60% is occupied
31 by native grasslands dominated by perennial species. In plant communities dominated by
32 tall tussock grasses, patchy and asynchronous field-burns are a traditional practice among
33 ranchers. We asked: How do the structural characteristics of vegetation vary in patches
34 with different time since the last fire?

35 Location

36 Grassland of the *Eastern Hills*, Uruguay.

37 Methods

38 We selected 18 grazed sites in order to obtain a spatial chronosequence with four age
39 categories since the last fire: 6, 18, 30, and more than 60 months before sampling. Plant
40 composition, species richness, coverage of each species, bare soil, and standing dead
41 biomass were determined in plots of 25 m^2. We used NMDS and MRPP to determine
42 differences in community composition, and the ANOVA or Kruskal–Wallis test to compare
43 structural variables between patches of different burning ages.

44 Results

45 Patches of different burning age had different species composition. Species richness,
46 Shannon diversity index, evenness, and bare soil decreased, whereas plant coverage,
47 standing dead biomass, and vegetation strata increased as time since the last fire
48 increased.

49 Conclusions

50 Our study confirmed occasional and localized field-burns as major driver of vegetation
51 change and structural diversity in a grazed native grassland dominated by a tall tussock
52 grass. On a larger scale, we observed the coexistence of patches in different successional
53 stages and differences in species composition between patches belonging to early stages.

54   These grasslands require asynchronous burning of patches to generate structural changes

55   that maximize both the spatial and temporal heterogeneity.

56   **Keywords**

59

Introduction

Disturbances are integral and relevant phenomena worldwide which can promote heterogeneity and change fundamental properties on different spatio-temporal scales in grassland ecosystems (Gibson, 2009). Traditionally, disturbances have been considered to be events that trigger the process of plant succession (Johnson & Miyanishi, 2007). Disturbance events generate mosaics of dynamic patches of vegetation with different successional stages (Fuhlendorf, Engle, Kerby, & Hamilton, 2009; Milchunas & Lauenroth, 1993; Pickett & White, 1985). It is widely recognized that fire and grazing are the main agents of disturbance, which determine the plant diversity in open (consumer-controlled) ecosystems, such as native grasslands (Archibald, Hempson, & Lehmann, 2019; Bond & Keeley, 2005; Oesterheld, Loreti, Semmartin, & Paruelo, 1999; Pausas & Bond, 2020). Furthermore, some authors argue that grazing and fire are as important as the climate in controlling some grassland ecosystems (Koerner & Collins, 2013; Pausas & Bond, 2019).

Grazing and fire can temporarily alter the structure in various grasslands worldwide by differentially influencing community dominance, diversity, and resource heterogeneity (Archibald et al., 2019; Bond & Keeley, 2005; Fuhlendorf et al., 2009; Gibson, 2009). In productive sub-humid native grasslands dominated by perennial species, large herbivores generally increase diversity through selective grazing (Lezama et al., 2014), and increase resource heterogeneity through trampling and nutrient deposition (Lezama & Paruelo, 2016; Mikola et al., 2009). Conversely, fire has been considered as a large-scale generalist "herbivore" that homogenizes resources (Bond & Keeley, 2005). In tall grasslands of North America, fire can uniformly remove aboveground biomass, and as fire frequency increases, it promotes species turnover, and a decrease in species diversity (Collins & Calabrese, 2012; Collins, Glenn, & Gibson, 1995; Gibson & Hulbert, 1987). Furthermore, there is a decrease in the abundance of forbs and C_3 grasses, and concomitantly, an increase in the abundance of the fire-tolerant C_4 grasses (Collins, 1992; Collins & Calabrese, 2012; Collins et al., 1995). On the other hand, fire suppression can determine transitions from mesic grasslands to shrublands or woodlands (Ratajczak, Nippert, Briggs, & Blair, 2014). In South Africa, frequent fires result in short and diverse grasslands, weakly dominated by a group of grasses; as the frequency of fires decreases, species richness is reduced, resulting in dominance by a few tall tussock grasses (Kirkman et al., 2014). However, fire-grazing interaction can increase plant diversity even further, both in North America and South Africa (Collins & Barber, 1986; Koerner & Collins, 2013). In the Mediterranean basin, it has been proposed that fire promotes a change in the

interaction between plant populations, from competition for light in long unburned areas to facilitation in recently burned areas, since many species benefit from growing in association with tussock grasses (Incerti et al., 2013). Most species-rich sites in south-eastern Australia have a history of frequent burning (Morgan, 1998). The lack of fire could have negative consequences for local biodiversity by limiting opportunities for the establishment of smaller and shorter-lived species (Zedler, 2007), as well as a loss of legumes, small-seeded species, and species of low stature (Leach & Givnish, 1996).

The *Río de la Plata* grasslands, distributed across eastern Argentina, southern Brazil and the entirety of Uruguay are considered one of the most extensive temperate sub-humid grassland areas of the world (Dixon, Faber-Langendoen, Josse, Morrison, & Loucks, 2014; Oyarzabal, Andrade, Pillar, & Paruelo, 2019; Soriano et al., 1991). Particularly, grasslands of Uruguay and southern Brazil belong to a wide subtropical to temperate transition zone (Andrade et al., 2018; Overbeck & Pfadenhauer, 2007). In the Brazilian *Campos*, it has been observed that in areas recently burned, the coverage of graminoids decreases, and species richness can increase or remain the same (Fidelis, Blanco, Müller, Pillar, & Pfadenhauer, 2012; Overbeck, Müller, Pillar, & Pfadenhauer, 2005; Overbeck & Pfadenhauer, 2007). It has been observed that as time since the last fire increases, the standing dead biomass of the dominant tussock grasses accumulates, and prevents the establishment of other species through competitive exclusion, which causes a decrease in species richness (Overbeck et al., 2005). In the Argentinian *flooding Pampa*, the burning of tussock grass *Paspalum quadrifarium* can lead to a change in species composition, an improvement in forage quality, and a short-term increase in the aboveground productivity (Laterra et al., 1998). A fire event opens up space for livestock accessibility and promotes the invasion by exotic species (Juan, Monterroso, Sacido, & Cauhépé, 2000; Laterra, Vignolio, Linares, Giaquinta, & Maceira, 2003; Laterra, 1997). The effects of fire on the structure of tussock grasses depend on the time since fire, and grazing (Laterra et al., 2003).

Experimental evidence has shown that grazing modifies the structure and functioning of the Uruguayan grasslands (Altesor, Oesterheld, Leoni, Lezama, & Rodríguez, 2005; Altesor et al., 2006; López-Mársico, Altesor, Oyarzabal, Baldassini, & Paruelo, 2015; Rodríguez, Leoni, Lezama, & Altesor, 2003). However, fire effects have been poorly studied in these areas. In grasslands of the geomorphological region Eastern Hills (*Sierras del Este*), field-burns can enhance the forage quality of the tussock grass *Saccharum angustifolium* (Panicoideae) and reduce its coverage (Royo Pallarés, Berretta, & Maraschin, 2005) creating open spaces prone to colonization by other species that

129   propagate vegetatively or by seeds (López-Mársico, Farías-Moreira, Lezama, Altesor, &

130   Rodríguez, 2019; Cuello, López-Mársico, & Rodríguez, in press). In addition, it has been

131   observed that the canopy of *S. angustifolium* can reduce the incidence of light reaching the

132   lower plant stratum by up to 83% (López-Mársico et al., 2019). To our knowledge, no

133   studies have been conducted on the community changes as time since the last fire

134   increases. This study aimed to describe the post-fire successional process of a grazed

135   community of native grasslands dominated by *S. angustifolium*. This goal was achieved

136   through a spatial chronosequence of patches with different time since the last fire.

137

138   **Methods**

139   <u>Study area</u>

140   The *Río de la Plata* grasslands cover about 700 000 km^2 between latitude 28º and 38º S,

141   and host about 5000 plant species, represented mostly by the Asteraceae and Poaceae

142   families (Andrade et al., 2018). In Uruguay specifically, 60% of the country is occupied by

143   native original grasslands, where livestock is the main productive activity (Baeza, Rama, &

144   Lezama, 2019), and most of them never has been ploughed or sowed by exotic species.

145   The study area is located in the *Quebrada de los Cuervos* protected landscape (QCPL),

146   and surrounding areas (78 500 ha), which includes grasslands, shrublands, and

147   woodlands (32°55'S, 54°26'W; Figure 1). The management of this area is a combination of

148   livestock production and landscape conservation. The soils are Argisols; the average

149   annual precipitation for the last 10 years before sampling was 1338 mm (distributed

150   throughout the year, without distinction between dry and wet periods), and mean annual

151   temperature for the same period was 17.1°C, with the maximum monthly average of

152   23.2°C in January, and minimum monthly average of 10.8°C in July (INIA-GRASS, 2018).

153   The study area is part of the Eastern Hills, a geomorphological region that encompasses

154   25 000 km^2 (14.3% of the country's surface), of which 66% are native grasslands (Baeza

155   et al., 2019).

156   Three grassland communities are recognized in this region with different physiognomy and

157   environmental characteristics (Lezama, Pereira, Altesor, & Paruelo, 2019). The community

158   II, (sparsely-vegetated grasslands *Trachypogon spicatus-Crocanthemum brasiliense*) is

159   associated with shallow soils, and plant coverage between 60-80%. The community IV

160   (densely-vegetated grasslands *Eryngium horridum-Juncus capillaceus*) is associated with

161   medium and deep soils, and plant coverage near 100%. The community V (tall and

162 densely-vegetated grasslands *Chascolytrum poomorphum, Paspalum pumilum*) is
163 associated with humid or temporarily inundated soils, and plant coverage near 100%. The
164 latter occupies concave zones of the landscape, and the vegetation physiognomy consist
165 of two strata, one low, conformed by grasses and perennial herbaceous forbs (*e.g.*
166 *Paspalum pumilum, Chascolytrum poomorphum, Eragrostis bahiensis, Axonopus*
167 *fissifolius, Dichanthelium sabulorum, Centella asiatica*), and one tall (60 cm), dominated by
168 *S. angustifolium*, a tussock grass featuring a landscape appearance of monospecific
169 patches. This tussock grass accumulates a large amount of standing dead biomass
170 because it has little grazing value. Therefore, these zones have historically been burned
171 by livestock ranchers to promote the resprouting of *S. angustifolium*, and improve forage
172 for domestic livestock (López-Mársico, Lezama, & Altesor, 2019; Royo Pallarés et al.,
173 2005). The field-burns realized by livestock ranchers are asynchronous depending upon
174 the accumulation of standing dead material, which occurs between approximately 3 to 5
175 years without fire. Fire spreads quickly creating irregular patches (Bruzzone, López-
176 Mársico, & Gallego, unpublished data) because the accumulation of combustible material
177 is distributed heterogeneously. That is due, on the one hand, to burns in different years,
178 and on the other hand, to the existence of patches of shallow soils that prevent the growth
179 of *S. angustifolium*. In the QCPL and surrounding areas, about 500 burned patches have
180 been registered in three different years, from 0.1 to 8 ha (Bruzzone et al., unpublished
181 data). In the QCPL specifically, field-burns are allowed in some areas between autumn
182 and spring, with the endorsement of the *Sistema Nacional de Áreas Protegidas* authorities
183 (Bianco & Lapetina, 2013).

184 <u>Sampling sites</u>

185 All study sites belong to the community V (according to Lezama et al., 2019) and were
186 burned always in winter-spring. The selection of the sampling sites was done in three
187 steps to obtain a spatial chronosequence with four categories of time since the last fire:
188 approximately 6, 18, 30, and more than 60 months before floristic sampling performed in
189 February of 2016. Firstly, through interviews with livestock ranchers in the study area,
190 zones that had not been burned for at least five years (burned more than 60 months
191 before sampling) were selected. Secondly, from the image bank available on Google
192 Earth, sites which had been burned in 2013 (approximately 30 months before sampling)
193 were selected. Finally, through field-trips, other sites which had been burned in 2014
194 (approximately 18 months before sampling), and 2015 (approximately 6 months before
195 sampling) were selected. Patches within the same paddock, which belonged to the same
196 burning age category, were discarded. Once approximately 10 sites (larger than 1 ha) of

197 each burning age category were located, a maximum of 5 sites were randomly selected.
198 One of the burning age categories (30 months before sampling) had very small burned
199 areas and therefore only three sites were included. The minimum distance between the
200 different patches was 500 m. The 18 sites selected were georeferenced at the time of
201 floristic sampling, and each site was characterized according to the position and slope in
202 the landscape.

203 In each site, species composition, richness, and coverage of each species, were
204 determined according to the Braun Blanquet method in a 25 m^2 plot. Plants that could not
205 be identified in the field were herborized and identified in the laboratory. Regarding
206 taxonomic nomenclature, all species were carefully reviewed in online databases
207 TROPICOS (http://www.tropicos.org, last access: 02/03/2020) and Instituto de Botánica
208 Darwinion (http://www.darwin.edu.ar/Proyectos/FloraArgentina/Especies.asp, last access,
209 02/03/2020). The species pool was classified into six plant groups (Figure 2). In our
210 classification, the position of the buds (Raunkiaer's criterion) was joined with information
211 obtained from other studies of the Río de la Plata grasslands (Altesor et al., 2019, 2005;
212 Overbeck & Pfadenhauer, 2007). First, we separated the woody species from the
213 herbaceous ones. Woody species were categorized as shrubs and sub-shrubs, and
214 herbaceous species were categorized as annuals and perennials. The annual species
215 include both dicots and monocots. The perennials were subdivided into tall tussock
216 grasses, monocotyledons, and dicotyledons. The tall tussock grasses were distinguished
217 from the other monocotyledons in having the greatest coverage in this plant community,
218 and supplying the fuel for fire propagation. From the same 25 m^2 plots the percentages of
219 coverage of each plant group, standing dead biomass, and bare soil were recorded.
220 Additionally, the percentage of each vegetation stratum was recorded. The strata were
221 defined based on the height of the vegetation. The basal stratum was determined by
222 species up to 15 cm high (mostly monocots), the second stratum was determined by
223 species up to 100 cm high (mostly tussock grasses), and the third stratum was determined
224 by species greater than 100 cm high (shrubs).

225 Data analysis

226 We used Non-Metric Dimensional Scaling (NMDS) with a distance matrix based on the
227 Sørensen's index calculated from quantitative data, to identify the main floristic gradients.
228 Only species that were present in more than 10% of the sites (at least two out of 18) were
229 included in the multivariate analysis since infrequent species of random occurrence can
230 generate spurious results (McCune & Mefford, 2011). The matrix used in the analyses

231   contained 125 species and 18 observations. Percent of variation in the original data that
232   was explained by the ordination was calculated through Pearson r^2, correlating the
233   distance between the plot scores in ordination space and the distance in the original matrix
234   (McCune & Mefford, 2011). To explore the relationship between the ordination and plant
235   structural variables, bare soil, and standing dead biomass, we computed correlations
236   between the scores of each plot in the first two axes of the NMDS ordination and the
237   corresponding values for each variable, using Pearson´s *r* correlation coefficient. On the
238   other hand, *IndVal* calculated from species cover-abundance data (transformed to
239   percentage values) were used to identify diagnostic species for each time since the last
240   fire. The differences in community composition between patches of different time since the
241   last fire were tested using the Multiresponse permutation procedure (MRPP). MRPP
242   provides a statistic *δ*, which is the weighted mean within-groups distance and is associated
243   with a *p*-value that indicates the likelihood of getting a *δ* equal or smaller than that
244   observed by chance (McCune & Mefford, 2011). These statistical analyses were
245   performed in the PCORD package (version 6.19).

246   We compared mean species richness, Shannon diversity index, evenness (Shannon
247   diversity index divided by the natural logarithm of species richness), and total plant
248   coverage among four different categories of time since the last fire using a one-way
249   ANOVA. Means that were significantly different at $p < 0.05$ were separated by Tukey post
250   hoc. Data for bare soil, standing dead biomass, species richness, and coverage of the
251   plant groups, among four different categories of time since the last fire, did not meet
252   ANOVA assumptions and were analyzed using a Kruskal–Wallis test. All these statistical
253   analyses were performed in Infostat v 2016 (Di Rienzo et al., 2016).

254   **Results**

255   <u>Species composition and heterogeneity</u>

256   A total of 168 species of vascular plants belonging to 39 families were recorded in all
257   sampling sites (Appendix S1). Most species (73%) belonged to two plant groups
258   (monocots and dicots), while the other four plant groups comprised the remaining 27% of
259   the species. The two dominant plant families were Poaceae (46 species) and Asteraceae
260   (42 species). Within the Poaceae, the main genera were: *Paspalum* (7 species), *Eragrostis*
261   (5 species), *Nasella* (4 species) and *Chascolytrum* (4 species), while within the Asteraceae
262   the main genera were: *Baccharis* (8 species) and *Senecio* (3 species). In contrast, 22 plant
263   families were represented by a single species.

264 Patches of different time since last fire had different species composition (MRPP:
265 Observed δ = 0.37; p = 0.0001). A two-axis ordination with a final stress value of
266 6.63 emerged as the best solution from the NMDS analysis. The two axes extracted
267 represented 76.3% of the variation (Figure 3). The first axis showed a clear temporal
268 pattern, and was positively correlated with species richness, bare soil, and dicots
269 coverage, and negatively correlated with total plant coverage, standing dead biomass, and
270 coverage of two plant groups: tall tussock grasses and shrubs. The second axis reflected
271 spatial differences among recently burned sites, and was negatively correlated with
272 annuals coverage.

273 The indicator species were determined for each category of time since the last fire
274 (Appendix S2). In the sites burned approximately 6 months before sampling, the indicator
275 species were two grasses (*Steinchisma hians* and *Aristida laevis*) and four dicots (*Senecio*
276 *selloi*, *Solidago chilensis*, *Stenachaenium megapotamicum,* and *Plantago myosuros*). In
277 the sites burned approximately 18 months before sampling, the indicator species were two
278 dicots (*Pterocaulon balansae* and *Stemodia verticillata*) and one shrub (*Baccharis spicata*).
279 In the sites burned approximately 30 months before sampling, the indicator species were
280 two grasses (*Eragrostis lugens* and *Schizachyrium microstachyum*) and one sub-shrub
281 (*Baccharis trimera*). In the sites burned more than 60 months before sampling, the
282 indicator species was only the tall tussock grass dominant in the study site (*Saccharum*
283 *angustifolium*).

284 <u>Species richness, diversity and evenness</u>

285 Species richness, Shannon diversity index, and evenness tended to decrease as time
286 since the last fire increased (Figure 4; Table 1; Appendix S2). Species richness in the sites
287 burned 6 and 18 months before sampling were 30% higher than in the sites burned more
288 than 60 months before sampling, whereas the sites burned 30 months before sampling did
289 not differ from the sites of the other burning ages (F = 3.5; p = 0.04; Figure 4). However,
290 the species richness of the different plant groups showed no difference in the time elapsed
291 since the last fire (Table 2; Appendix S2). The diversity in the sites burned 6 and 18
292 months before sampling were between 25% and 60% higher than in the sites burned 30
293 and more than 60 months before sampling (F = 11.5; p < 0.001; Table 1). The evenness in
294 the sites burned 6 months before sampling was 48% and 63% higher than in the sites
295 burned 30 and more than 60 months before sampling respectively, whereas the sites
296 burned 18 months before sampling did not differ from the sites of the other burning ages (F
297 = 4.1; p = 0.03; Table 1).

299   The total coverage of plants in the sites burned 6 months before sampling was between 14

300   % and 21% lower than in the sites of the other burning ages (F = 7.5; $p < 0.01$; Figure 4;

301   Appendix S2). The tall tussock grasses coverage showed a sustained increase from

302   30.2%, in the sites burned 6 months before sampling, to 79.2% in the sites burned more

303   than 60 months before sampling (H = 14.5; $p < 0.01$; Table 2). The monocots coverage

304   was marginally lower in the sites burned more than 60 months before sampling than in the

305   sites of the other burning ages (H = 6.47; $p = 0.09$; Table 2). The sub-shrubs coverage

306   was marginally higher in the sites burned 30 months before sampling than in the sites of

307   the other burning ages (H = 7.37; $p = 0.06$; Table 2). The coverage of the three remaining

308   plant groups did not show a different dynamic across time since the last fire (Table 2). The

309   bare soil was maximum in the sites burned 6 months before sampling and it decreased as

310   the time since the last fire increased (H = 14.2; $p < 0.01$; Table 1; Appendix S2).

311   Conversely, the standing dead biomass accumulation was minimum in the sites burned 6

312   months before sampling and maximum in the sites burned more than 60 months before

313   sampling (H = 13.2; $p < 0.01$; Table 1; Appendix S2). On the other hand, the sites burned

314   more than 60 months before sampling had three vegetation strata (1- monocots and

315   dicots, 2- tall tussock grasses and sub-shrubs and 3- shrubs), while the other burning ages

316   had only the first two strata (Table 1; Appendix S2).

Discussion

318   This study provides evidence about the successional process triggered by field-burns in a

319   native grassland dominated by a tall tussock grass and under livestock grazing. Two main

320   observations result from this work: temporal heterogeneity on a small patch scale, and

321   spatio-temporal heterogeneity on a large scale. On a patch scale, fire removes

322   aboveground biomass of the dominant species (*S. angustifolium*) and causes a release of

323   resources, such as space and light (López-Mársico et al., 2019; Royo Pallarés et al.,

324   2005). The bare soil gaps that appeared are quickly colonized by herbaceous monocots

325   and dicots, and we observed that species richness, diversity index and evenness

326   decreased as time since the last fire increased. There was also a species turnover, and an

327   accumulation of standing dead biomass as time since the last fire increased. On a large

328   scale, asynchronous field-burns generate a mosaic of patches of different ages. In

329   addition, differences in species composition between patches belonging to the same stage

330   decreased as time since the last fire increased.

331

According to the structural changes in the vegetation through spatial chronosequence, we propose a model that represents a patch-scale cycle (Figure 5), based on the Pyric-Herbivory model (Fuhlendorf et al., 2009). The cycle starts with a burning event and goes through different stages as time since the last fire increases. After a burning event, areas of bare soil are prone to be colonized by different species which explains the aforementioned marked turnover of species, as in other grasslands worldwide (Kirkman et al., 2014; Koerner & Collins, 2013; Overbeck & Pfadenhauer, 2007). The existence of a large regional species pool, which is locally variable, leads to a high variability among patches (Appendix S1). The tall tussock *S. angustifolium* resprouts, thus improving the forage quality and promoting their consumption by livestock. It has been demonstrated that after fire, herbivores are attracted to burned areas (Fuhlendorf & Engle, 2004; Laterra et al., 2003). In Uruguayan grasslands, grazing drastically increases the local diversity (Altesor et al., 2005; Rodríguez et al., 2003). The selective herbivory on recently burned patches could explain why it is that species richness does not decrease until at least 30 months have passed since the fire event. After that time, the forage quality of *S. angustifolium* decreases and their consumption is avoided, resulting in an increase of their coverage. The patch-scale cycle ends when *S. angustifolium* becomes dominant and a large amount of standing dead biomass accumulates again; the species richness decreases and the patches homogenize. Under these conditions, after approximately five years since the last fire, livestock ranchers usually burn the patch again which restarts the cycle.

On a large scale, two processes promote spatial heterogeneity: the temporal dynamics generated by asynchronous burning, and the differential colonization of recently burned patches. The successional processes, which occur simultaneously, generate dynamic mosaics of vegetation patches of different stages. The ordination shows that differences in species composition, as well as in other structural variables of the plant community, occur as time since the last fire increases. In addition, the habitat patchiness generated by occasional and localized burns may be increased by selective grazing (Adler, Raff, & Lauenroth, 2001), which in turn, may promote a heterogeneous mosaic of shifting patches (Fuhlendorf et al., 2009). The other process is mediated by differences in spatial colonization, a sort of rescue-effect (Brown & Kodric-Brown, 1977). Fire removes aboveground biomass of competitively dominant species, and bare soil patches are colonized through vegetative propagation from neighboring sites, by germination of a bank or seed rain, or through resprouting from a bud bank (López-Mársico et al., 2019; Cuello et al., in press). These multiple colonization events could explain various spatial dynamics in

patches of the same burning age. The high number of indicator species found in the recently burned patches reflects the spatial heterogeneity at this stage of plant succession.

We observed that, when no field-burns are performed for more than 60 months, the tussock grasses group became dominant, and replaced the other monocots. Although species richness of monocots was not affected by the time since the last fire, they remained stable until 30 months since last fire, and then declined by less than half in the oldest burned sites. It is likely that, the light reduction results in a competitive effect on the monocots. A study in South African grasslands showed a decline in grasses richness as fire frequency decreased (Kirkman et al., 2014). In our study, total species richness decreased as time since the last fire increased, though no single plant group can explain this decline. Furthermore, we observed that a third plant stratum of the shrub *Acanthostyles buniifolius* appeared in sites that had been burned more than 60 months before sampling. This species is widely distributed in Uruguayan grasslands (Altesor et al., 2005, 2006; Fernández & Altesor, 2019; Fernández, Lezama, & Rodríguez, 2019; Fernández, Texeira, & Altesor, 2014) with no apparent dependence on fire. In some mesic grasslands, encroachment results from overgrazing and reduced fire frequency (Gibson, 2009; Ratajczak et al., 2014). However, in Uruguayan grasslands, encroachment has been observed both in ungrazed, and in unburned sites (Altesor et al., 2006; Bernardi, Holmgren, Arim, & Scheffer, 2016).

Implications

In this study, we registered some consequences which burning, as part of the traditional management practice among livestock ranchers, has on the vegetation structure. Prescribed burns, carried out at different times and places, are conducted to improve forage quality and decrease the coverage of the tussock grass *S. angustifolium*. Our results show that this management practice could prevent homogenization of vegetation by maintaining diversity, both on a small, and on a large scale. Similar results have been observed in studies where biomass is only removed by domestic livestock (Ferreira et al., 2020; Rodríguez et al., 2003). Other authors have recently shown that, in the same plant community, burned areas under livestock grazing are more productive than unburned and ungrazed areas (Gallego, Paruelo, Baeza, & Altesor, 2020). Therefore, we propose that these grasslands, dominated by a tall tussock grass, require asynchronous burning of patches in order to generate structural changes, which maximize the spatial and temporal heterogeneity, as well as the aboveground productivity.

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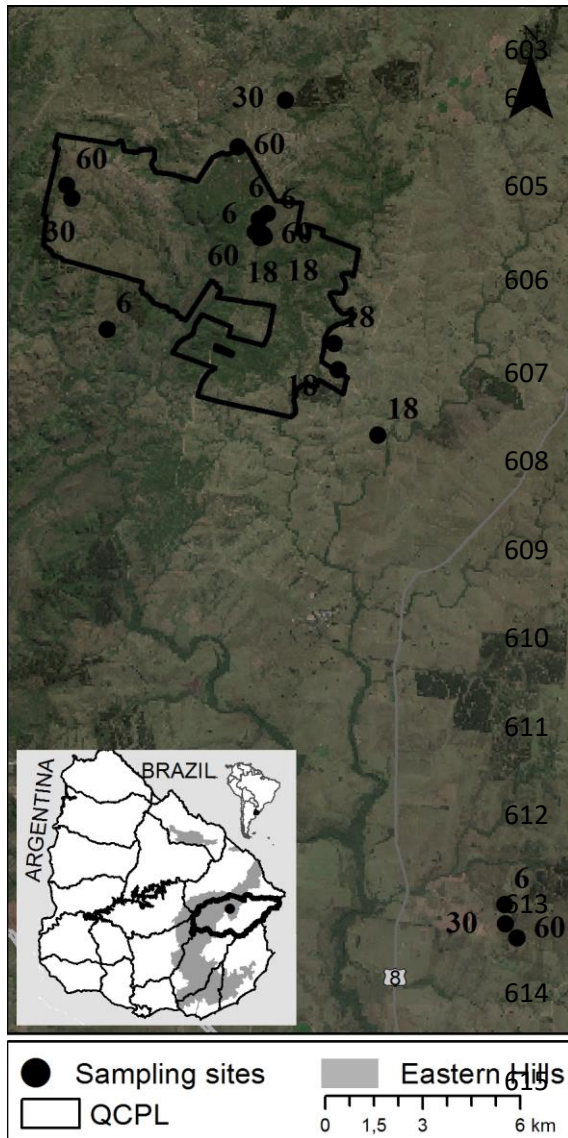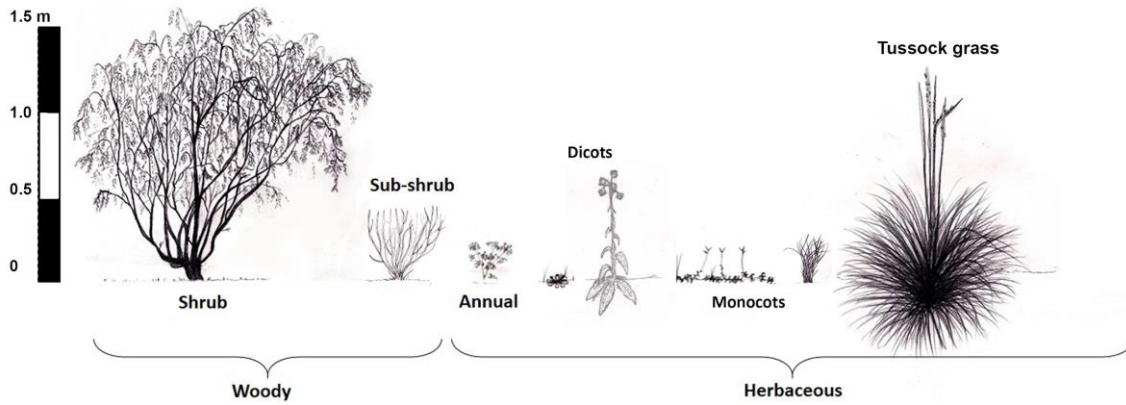
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Figure 1: Location of 18 sampling sites with burning age in *Quebrada de los Cuervos*
protected landscape (QCPL) and surrounding areas. The map of Uruguay denotes the
limit of the Eastern Hills geomorphological region in gray.

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Figure 2: Classification of the species pool into six plant groups. The species were first divided into woody (shrubs and sub-shrubs) and herbaceous. The latter were divided into annuals and perennials (dicots, monocots and tussock grasses). The annual species and the little dicots are enlarged X 2 due to the small size.

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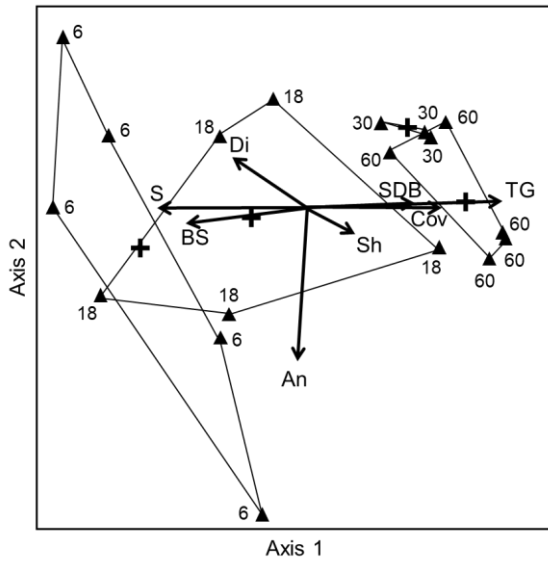
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632 Figure 3: Configuration of (frequency) samples of species composition in a two-
633 dimensional Non-Metric Multidimensional Scaling (NMDS) representation of the Bray-
634 Curtis distances. Sampling plots (▲), boundaries (—) and centroids (+) of each plot group
635 are indicated. The relationship between ordination scores and direction of explanatory
636 variables are indicated (→). Species richness (S), plant coverage (Cov), bare soil (BS),
637 standing dead biomass (SDB), coverage of the plant groups: Tussock grasses (TG),
638 Dicots (Di), Shrubs (Sh) and Annuals (An). Only the variables from the secondary matrix,
639 with an r^2 value larger than 0.2, were plotted.
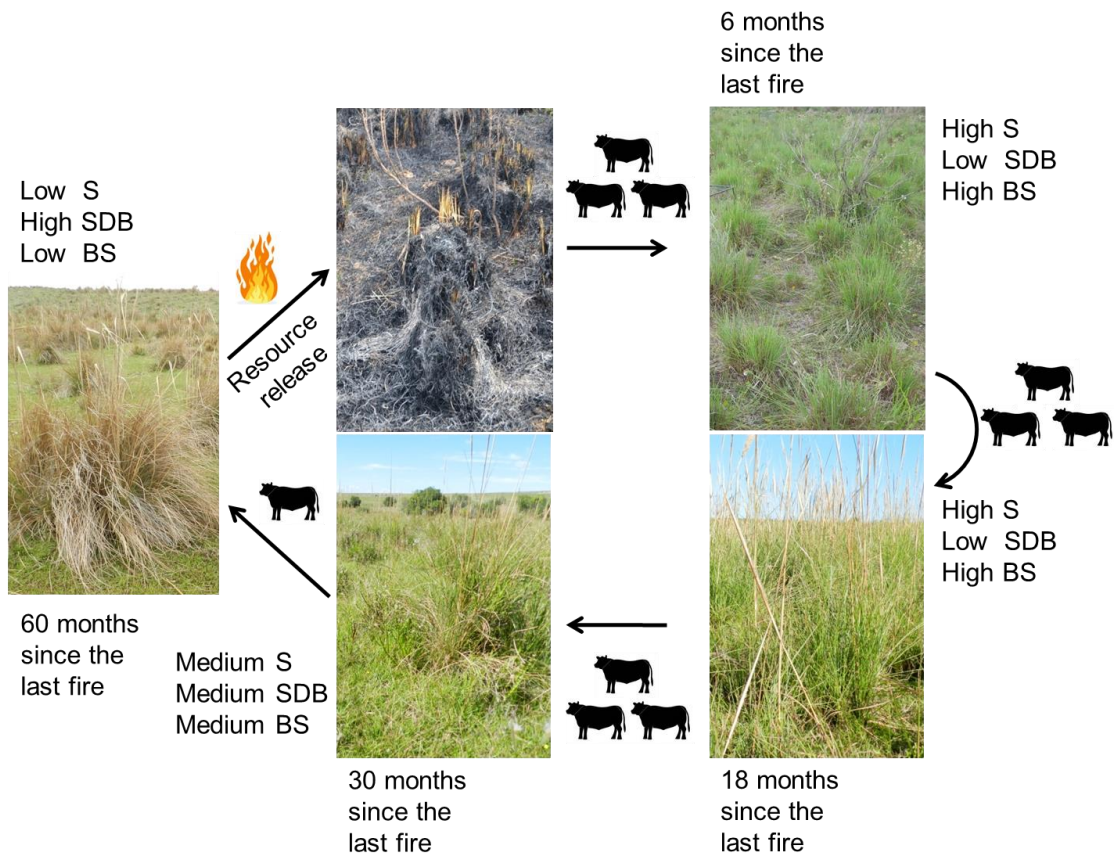
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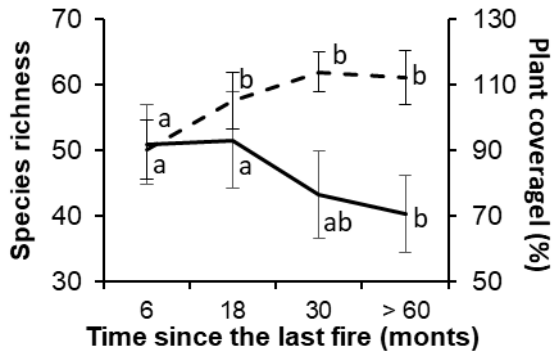
644 Figure 4: Mean species richness ± SD (———) and total plant coverage ± SD (‑ ‑ ‑) at the
645 four categories of time since the last fire in a grassland of the Eastern Hills, Uruguay.
646 Different letters show significant differences among sites with different time since the last
647 fire at $p < 0.05$.

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Figure 5: Diagram showing a cycle of structural changes in a native grassland dominated by tall tussock grasses under livestock grazing, after a field-burn event in Eastern Hills, Uruguay, based on Pyric-Herbivory model proposed by Fuhlendorf et al., (2009). The patch-scale cycle starts when the biomass and standing dead biomass of *S. angustifolium* is burned and the grazing pressure increases. Areas of bare soil are colonized by different species, promoting an increase in species richness within the first 6 months after the fire. The plants of *S. angustifolium* keep resprouting and standing dead biomass begins to accumulate. The forage quality of *S. angustifolium* decreases and their consumption is avoided, resulting in an increase of their coverage. The cycle ends when *S. angustifolium* becomes dominant again, and a large amount of standing dead biomass is accumulated; the species richness and bare soil decreases. Under these conditions, the livestock ranchers burn again and the cycle is restarted. The number of cows represents the grazing pressure in each moment of the cycle. S = species richness, SDB = standing dead biomass, and BS = bare soil.

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Table 1: Mean values ± SD of five structural variables in sites of four different categories of time since the last fire. Shannon diversity index (H), evenness (E), bare soil (BS), standing dead biomass (SDB) and strata number (SN). Different letters indicate significant differences ($p<0.05$) between the four categories of time since the last fire.

Structural Variables	Time since the last fire (months)			
	6	18	30	>60
H	1.87 ± 0.20 a	1.65 ± 0.34a	1.33 ± 0.10 b	1.17 ± 0.06 b
E	0.13 ± 0.03 a	0.10 ± 0.03 ab	0.09 ± 0.02 b	0.08 ± 0.01 b
BS	15.0 ± 7.1 a	4.4 ± 3.7 a	0 b	0 b
SDB	0 a	1 ± 2.2 ab	5 ± 0 bc	15 ± 7.1 c
SN	2	2	2	3

Table 2: Mean coverage and species richness of the six plant groups ± SD, in four different categories of time since the last fire. Different letters indicate significant differences ($p<0.1$) between times since the last fire.

Plant groups	Coverage				Species richness			
	Time since the last fire (months)							
	6	18	30	>60	6	18	30	>60
Tussock grasses	30.2 ± 7.4c	51.0 ± 9.6bc	73.3 ± 7.6ab	79.2 ± 7.8a	1.2 ± 0.4	1.2 ± 0.4	1.0 ± 0	1.2 ± 0.4
Monocots	29.5 ± 17.8a	34.3 ± 20.7a	29.7 ± 16.1a	12.1 ± 5.9b	20.2 ± 3.7	20.2 ± 3.3	18.3 ± 2.9	19.2 ± 3.6
Dicots	15.9 ± 18.1	10.7 ± 8.2	3.5 ± 3.1	6.6 ± 5.8	18.4 ± 4.2	16.8 ± 4.6	14.0 ± 8.7	11.8 ± 4.0
Annuals	8.4 ± 10.4	1.3 ± 2.2	0.3 ± 0.1	1.4 ± 2.2	5.2 ± 1.1	5.4 ± 0.9	4.0 ± 2.0	2.8 ± 1.9
Sub-shrubs	1.4 ± 0.8a	1.0 ± 0.9a	5.8 ± 0.6b	1.6 ± 2.1a	3.8 ± 1.1	5.0 ± 1.9	3.3 ± 0.6	2.8 ± 1.3
Shrubs	5.1 ± 4.7	7.1 ± 10.8	1.4 ± 1.5	11.5 ± 9.2	2.2 ±0.4	3.0 ± 1.4	2.7 ± 2.1	2.6 ± 1.1

Supporting information to the paper

López-Mársico, L. et al. Heterogeneity decreases as time since fire increases in a South

American grassland. *Applied Vegetation Science.*

Appendix S1: List of species sampled in a Uruguayan grassland.

Appendix S2: List of original data and results of analyses performed