

1 **Field-burn vs. fire-related cues: germination from the soil seed bank of a**
2 **South American temperate grassland**

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4 Running head title: Fire and seed germination in a temperate grassland

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16

17 **Abstract**

18 Fire and grazing are large-scale disturbances that shape the structure and
19 function of open habitats. In temperate grasslands of southern South America,
20 fire is used as a management tool to control tussock grasses and improve
21 forage quality. In this study, we examined if fire and two of its components (heat
22 and smoke) affect germination from the soil seed bank of a temperate grassland
23 in Uruguay. Soil samples were extracted from a recently burned site and from
24 an adjacent area that had not been burned for at least four years. The latter
25 were subjected to four treatments: 1- Heat shock, 2- Smoke, 3- Heat shock and
26 smoke, and 4- Control. The samples were placed in a germination chamber and
27 germination was recorded for 140 days. Field-burn was the treatment that
28 differed most from the control. This treatment produced a significant increase in
29 density and richness of germinants and the germination peak preceded those of
30 the remaining treatments. The three treatments involving fire-related cues did
31 not affect seedling richness and density, but the germination of some individual
32 species was enhanced by some of them, mainly those in which the seeds were
33 smoked. Our results show that fire and its components stimulate the
34 germination of some species of the Río de la Plata grasslands, contrary to what
35 had been observed previously in the region. We also suggest that, unlike
36 Mediterranean-type systems, other fire cues, alone or in combination with heat
37 and smoke, may promote germination after a fire event.

38

39 **Introduction**

40 Fire and grazing are considered large-scale disturbances that shape the
41 structure and function of grasslands (Oesterheld *et al.*, 1999; Pausas and Bond,
42 2019). Grassy biomes are millions of years old (Jacobs *et al.*, 1999), and have
43 been historically associated with large grazing mammals (Coughenour, 1985).
44 In addition, natural fires have occurred in grasslands since the late Miocene,
45 favored by the accumulation of highly flammable dead standing litter (Bond and
46 Parr, 2010; Pausas and Bond, 2019), although their effects have been poorly
47 studied. Grass-fueled fires are characterized by rapid combustion and spread,
48 low fire residence time and low temperature (Zedler, 2007; Archibald *et al.*,
49 2013; Simpson *et al.*, 2016). Fire acts directly on the established vegetation and
50 its consequences consist basically of the removal of biomass, both living and
51 dead (Knapp *et al.*, 1998), the reduction of surface albedo, the increase in soil
52 temperature, and the volatilization and release of nutrients (Gibson, 2009).
53 Nowadays, anthropogenic fires pre-empt natural fires in most parts of the world
54 (Bond and Parr, 2010).

55

56 Fire is associated with several plant traits, including whole-plant fire responses,
57 serotiny, and fire-stimulated seed germination and flowering (He and Lamont,
58 2018; Archibald *et al.*, 2019). In fire-prone ecosystems, such as Mediterranean-
59 type shrublands, fire or one of its components (e.g. heat, smoke, ash, and
60 charred wood) promotes the germination of soil-stored seeds (Dixon *et al.*,
61 1995; Keeley and Fotheringham, 1998; Moreira *et al.*, 2010; Keeley *et al.*,
62 2012). Increasing temperature breaks the physical dormancy of some hard-

63 seeded species (Keeley, 1991; Moreira *et al.*, 2010; Baskin and Baskin, 2014),
64 and plant-derived smoke or aqueous smoke extracts promote germination by
65 breaking physiological dormancy in many plant families (Van Staden *et al.*,
66 2000; Keeley *et al.*, 2011). Also, fire can indirectly stimulate germination by
67 increasing the incidence of radiation reaching the soil (Keeley and
68 Fotheringham, 2000).

69 In comparison to Mediterranean ecosystems, much less is known about the
70 response of plants to grassland fires (Pausas and Paula, 2019). The Río de la
71 Plata grasslands is one of the largest areas of natural temperate subhumid
72 grasslands in the world, covering more than 700,000 km² distributed across
73 eastern Argentina, the whole of Uruguay, and southern Brazil (Soriano *et al.*,
74 1991). The history of fire in the Río de la Plata grasslands is largely unknown,
75 but paleoecological data suggest that fire became frequent about 7,000-3,000
76 years ago, after the arrival of human populations in the region (Behling *et al.*,
77 2004; Kaal *et al.*, 2019). Currently, grazing by livestock is the main productive
78 activity, and in certain areas, controlled burns are used to increase primary
79 production and forage quality (Pillar and de Quadros, 1997; Látterra *et al.*, 2003;
80 López-Mársico *et al.*, 2019). Many species that grow in the region are
81 herbaceous plants that exhibit a great variety of subterranean or near-ground
82 bud-bearing structures that allow them to resprout after disturbances such as
83 grazing, drought, and fire (Overbeck and Pfadenhauer, 2007; López-Mársico *et al.*,
84 2019). In Brazilian *campos* grasslands, Overbeck and Pfadenhauer (2007)
85 found that about 70% of the species are able to resprout from belowground
86 organs, and only 1% are obligate seeders.

87 Despite the importance of belowground bud banks in survival and resprouting
88 after a fire event (Pausas *et al.*, 2018; Pausas and Paula, 2019), plants may
89 also persist and regenerate through recruitment by seeds stored in the soil. For
90 this to happen, species must generate a fire-resistant seed bank, and
91 recruitment of new individuals would be expected to be promoted, as in other
92 fire-prone environments. However, seeds of several species that thrive in the
93 Río de la Plata grasslands are not stimulated to germinate, despite being
94 tolerant, when experimentally exposed to fire-related cues, such as heat-shock
95 and smoke (Overbeck *et al.*, 2006; Fidelis *et al.*, 2010; 2016; López-Mársico *et*
96 *al.*, 2019). Similar results were also reported for herbaceous species from rocky
97 outcrop vegetation of south-eastern Brazil (Le Stradic *et al.*, 2015) and also
98 from Brazilian savannas (Fichino *et al.*, 2016; Ramos *et al.*, 2016; 2019).
99 Accordingly, it was suggested that grassland species of southern South
100 America rely mostly on their ability to resprout by means of subterranean or
101 protected basal buds after a fire event (Overbeck and Pfadenhauer, 2007;
102 López-Mársico *et al.*, 2019).

103 While the evidence accumulated so far indicates an absence of fire-stimulated
104 germination in these grasslands, scaling up individual responses to the
105 community as a whole must be done with caution. The Río de la Plata
106 grasslands is a highly diverse region that hosts nearly 5,000 species belonging
107 to 194 families (Andrade *et al.*, 2018), and fire could trigger the germination of
108 many other species that have not yet been tested. In addition, the experimental
109 germination tests oversimplify the overall effect of fire, which is known to cause
110 a multitude of changes in the environment (Keeley and Fotheringham, 2000).
111 On the other hand, certain forbs could be favored by prescribed burns in

112 Uruguayan grasslands. Grazed communities generally consist of a few matrix-
113 forming C₄ perennial grasses, and a diverse array of interstitial grasses and
114 forbs (Rodríguez *et al.*, 2003; Altesor *et al.*, 2005). For grasses, post-fire
115 resprouting may play a major role in their regeneration, but forbs are a
116 phylogenetically heterogeneous group of species (Fernández *et al.*, 2019) and
117 their dynamics seem to be regulated by processes other than grazing
118 (Rodríguez *et al.*, 2003). In this context, we expected that seeder forbs would
119 benefit most from the post-fire environment, contributing to the maintenance of
120 these highly diverse communities.

121 In this study, we analyze the response of the seed community (i.e. seed bank)
122 to heat-shock, smoke, and their interaction, and compare the effect of these fire
123 signals with the overall effect of fire. Specifically, we aim to answer the following
124 questions: 1) How do fire-related cues affect the germination of a temperate
125 grassland seed bank? and 2) How close are the experimental effects to those of
126 a field fire?

127 **Materials and methods**

128 *Study area*

129 The site used for collecting soil seed bank samples is a natural grassland of the
130 *Quebrada de los Cuervos* protected landscape, Uruguay (32°55' S, 54°26' W;
131 Fig. 1). The site belongs to the geomorphological region Eastern Hills (*Sierras*
132 *del Este*), which covers 25,000 km² (14.3% of the country's surface) and is
133 characterized by great heterogeneity in terms of relief, rocky outcrops, slope,
134 and soil depth (Baeza *et al.*, 2019). The average annual rainfall in the area is

135 1,318 mm and the average annual temperature is 17°C (INIA-GRAS, 2018). The
136 rainfall is distributed throughout the year, without distinction between dry and
137 wet periods. Grasslands and shrubby grasslands occupy nearly 66% of the
138 region, and grazing by livestock is the main productive activity (Baeza *et al.*,
139 2019). Three grassland communities are recognized in this region (Lezama *et*
140 *al.*, 2019): the densely-vegetated grasslands (*Eryngium horridum-Juncus*
141 *capillaceus* community, associated with medium and deep soils; plant cover
142 around 90%), the sparsely-vegetated grasslands (*Trachypogon spicatus-*
143 *Crocantemum brasiliense* community, associated with shallow soils; plant
144 cover 60-80%) and the tall and densely-vegetated grasslands (associated with
145 humid or temporarily inundated soils; plant cover around 95%). The latter
146 occupies the concave zones of the landscape (Gallego, 2013), on moderately
147 drained Argisols with a time-limited perched water table when present (Durán,
148 1985). Vegetation is distributed in two strata, one low (5 cm), conformed by
149 grasses and perennial herbaceous forbs (e.g. *Paspalum pumilum*,
150 *Chascolytrum poomorphum*, *Eragrostis bahiensis*, *Axonopus fissifolius*,
151 *Dichantheium sabulorum*, *Centella asiática*), and one tall (60 cm) defined by
152 the tussock grass *Saccharum angustifolium* (Ness) Trin (mean cover: 60-70%)
153 (Lezama *et al.*, 2019). Tussock grasslands are managed using prescribed burns
154 to improve forage quality for cattle (Royo-Pallarés *et al.*, 2005). The large
155 amount of dry standing dead matter and litter that accumulates over many
156 growing seasons provides the necessary fuel for fire ignition and spread.

157

158 *Soil seed bank samples and treatments*

159 The sampling was carried out in the austral spring (October 2017), before the
160 dispersion of seeds of most species, in order to sample the seeds with longer
161 persistence in the soil. About 75% of the species of the community are warm-
162 season growers that set seeds during the austral summer (December-March).
163 On the other hand, cool-season grasses and some winter forbs set seeds
164 during November-December. Only a minor fraction of winter forbs set seeds
165 before the sampling date (Rosengurtt, 1979).

166 In total, 40 samples were collected, 8 of them from a recently burned site (two
167 hours before sampling), and the remaining 32 from an adjacent site that had not
168 been burned for at least four years. Sampling points were spaced every 2
169 meters along a transect, avoiding the area below the canopy of *S.*
170 *angustifolium*. Soil cores were taken with a core sampler of 8 cm in diameter,
171 inserted 5 cm into the soil. Each soil sample was sectioned lengthwise, and one
172 of the halves was discarded. The other half (126 cm³) was stored in a plastic
173 bag and transported to the laboratory.

174 Samples were air dried for two days. Subsequently, the soil was disaggregated
175 and all plant debris was removed. Soil samples were spread evenly in
176 aluminum trays (11x15x5 cm) containing a 1-cm deep layer of sterile sand. The
177 32 samples collected from the unburned site were subjected to four treatments
178 (eight samples per treatment): 1- Heat shock of 100°C (H), 2- Smoke (S), 3-
179 Heat shock and smoke (HS), and 4- Control (C; no heat shock and no smoke).
180 A fifth treatment consisted of the samples taken from the burned site (B). Heat
181 shock treatment was applied to the samples for a period of 5 minutes using a
182 preheated oven. The selected temperature is within the range of temperature

183 used by Overbeck *et al.* (2006) and López-Mársico *et al.* (2019), which is
184 supposed to cover the thermal conditions potentially encountered by seeds
185 during fire in the Río de la Plata grasslands. The time of exposure is close to
186 the maximum residence time of the fire at soil surface (330s) reported by Fidelis
187 *et al.* (2010) for experimentally burned plots -excluded from burning for 6 years-
188 in Brazilian *campos* grasslands. For the smoke treatment, we used a bee
189 smoker connected to a plastic chamber (60×40×30 cm), where the samples
190 were placed and fumigated with smoke derived from burning 50 g of dry
191 biomass of *S. angustifolium*. Once the chamber became saturated with smoke,
192 it was left sealed for the same exposure time as the heat-shock treatment (5
193 minutes). The smoke methodology is an adaptation of the method proposed by
194 Dixon *et al.* (1995), and it was used by López-Mársico *et al.* (2019) for individual
195 seeds collected at our study site.

196 The soil seed bank was estimated using the seedling emergence method
197 (Roberts, 1981; Simpson *et al.*, 1989). Trays were placed in a germination
198 chamber with a 12/12 h light/dark and 25°/20°C regime, simulating the
199 environmental conditions of eastern Uruguay during spring (INIA-GRAS, 2018).
200 The trays were regularly watered, and rotated weekly to expose the samples to
201 the same radiation conditions. Emerging seedlings were identified and
202 removed. Unidentified seedlings were transplanted to a separate pot, and
203 grown until they were identifiable. All species were carefully revised, regarding
204 taxonomic nomenclature, in online databases TROPICOS
205 (<http://www.tropicos.org>, last access: 18/04/2020) and Instituto de Botánica
206 Darwinion (<http://www.darwin.edu.ar/Proyectos/FloraArgentina/Especies.asp>,
207 last access, 18/04/2020). The trays were monitored for 140 days, after which

208 few seedlings emerged. One of the samples of the HS treatment was discarded
209 because it presented a disproportionate number of seedlings compared to the
210 rest of the trays.

211 *Data analysis*

212 Density (number of seedlings/m²) and richness of emerged seedlings (total and
213 discriminated in dicotyledons and monocotyledons) were calculated for each
214 treatment. The comparison of the variables among treatments was carried out
215 through a one-way ANOVA, followed by Tukey's post hoc test ($\alpha = 0.05$). Prior
216 to analysis, total and dicot density data were log-transformed, as they did not
217 meet the assumptions of normality and/or homoscedasticity. A chi-square test
218 was performed to investigate whether the proportion of monocots and dicots
219 varied in the different treatments. All analyses were performed with the software
220 InfoStat (Di Rienzo *et al.*, 2016).

221

222 **Results**

223 Throughout the experiment a total of 625 seedlings emerged, of which 425 were
224 dicots and 200 were monocots. Of the total, 525 could be identified at the genus
225 or species level, while the remaining 100 (63 dicots and 37 monocots) died
226 before identification. Overall species richness was 54 (31 dicots and 23
227 monocots). The most species-rich plant families were Asteraceae (15 spp),
228 Poaceae (12 spp) and Cyperaceae (6 spp). The remaining species belong to 12
229 plant families (Supplementary Table 1).

230 *Effect of treatments on seedling density and richness*

231 Field-burn treatment (B) significantly increased the emergence of seedlings
232 from the soil seed bank by 161%, as compared with the control (C). Heat (H),
233 smoke (S), and heat plus smoke (HS) treatments did not significantly affect total
234 seedling density, although the number of seedlings in S and in HS were 64%
235 and 31% greater than in control respectively, and did not differ from B. The
236 density of dicots and monocots followed the same trend as the total density:
237 treatment B was the only treatment that significantly enhanced seedling
238 emergence, compared to the control (Fig. 2). The monocot/dicot ratio was
239 32/68% and there were no significant differences among treatments ($\chi^2 = 0.526$;
240 $gl = 4$; $p = 0.97$).

241 Species richness (total and by taxonomic group) was significantly increased in
242 treatment B, compared to the control. Species richness was not affected by
243 heat, smoke, or heat plus smoke treatments (Fig. 2). The monocot/dicot ratio
244 was 39/61%, having no significant differences among treatments ($\chi^2 = 0.655$; gl
245 $= 4$; $p = 0.96$).

246 *Germination timing*

247 Seedlings emerged earlier in B, reaching a maximum in the first and second
248 week of the experiment. In the remaining treatments, including the control, the
249 maximum germination peak occurred between the second and third week of the
250 experiment (Fig. 3). The smoke treatment produced the second highest
251 germination peak, mainly due to the germination of dicots (Fig. 3b). After ten
252 weeks, nearly 80% of the total number of individuals recorded had already
253 germinated.

254 *Species composition*

255 The most abundant species in each taxonomic group (relative density >3%,
256 considering all treatments as a whole) accounted for 80% of all emerging
257 seedlings. Dominant dicots germinated in all treatments, but germination was
258 especially enhanced in B. The germination behavior of dominant monocots was
259 more heterogeneous (Fig. 4). Species exclusive to a single treatment were rare
260 (most with 1 or 2 individuals) and were mainly recorded in B (5 dicots and 7
261 monocots) (Supplementary Table 1).

262 **Discussion**

263 This study provides evidence of positive effects of fire on seed germination in a
264 temperate grassland of southern South America. Of all the treatments analyzed,
265 field-burn was the treatment that differed most from the control. Field-burn
266 promoted a significant increase in the density and richness of germinants,
267 without affecting the monocot/dicot ratio. In addition, the germination peak in
268 this treatment preceded the peaks of the remaining treatments. On the other
269 hand, fire-related cues (heat shock, smoke) did not affect seedling species
270 richness and density, though the germination of some individual species was
271 enhanced by some of the treatments, mainly those where the seeds were
272 smoked. These results complement and challenge the conclusions of other
273 studies carried out on several grasslands of the region, in which it was found
274 that the effects of heat and smoke had neutral or negative effects on the
275 germination of some species (e.g. Overbeck *et al.*, 2006; Fidelis *et al.*, 2010;
276 2016; López-Mársico *et al.*, 2019). This led researchers to suggest that species
277 from these grasslands rely on their ability to resprout by means of subterranean

278 or protected basal buds after a fire event, rather than on seed recruitment.
279 Regeneration from seeds would be possible if seeds were to tolerate (or
280 survive) a burning event (Overbeck and Pfadenhauer, 2007; Fidelis *et al.*, 2016;
281 López-Mársico *et al.*, 2019), and our results show that several species of this
282 grassland are stimulated to germinate by burning.

283 Treatments involving fire-related cues (heat, smoke, and heat plus smoke) were
284 far from emulating the effects of a real field-burn. Heat and smoke are the most
285 commonly used fire signals in seed germination trials (*e.g.* Dayamba *et al.*,
286 2008; Reyes and Trabaud, 2009; Moreira *et al.*, 2010; Fichino *et al.*, 2016; Kin
287 *et al.*, 2016; Ramos *et al.*, 2016; 2019; Tavşanoğlu *et al.*, 2017; Arcamone and
288 Jaureguiberry, 2018). In fire-prone ecosystems, such as Mediterranean-type
289 shrublands, these signals stimulate the germination of many species, and
290 largely explain the recruitment of new individuals from seeds (Brown, 1993;
291 Dixon *et al.*, 1995; Keeley and Fotheringham, 1998; Paula and Pausas, 2008;
292 Moreira *et al.*, 2010). However, structurally distinct plant communities with
293 historically different fire regimes may be expected to have different responses to
294 fire-related germination cues (Maikano *et al.*, 2018). Our results suggest that
295 other fire-related cues, alone or in combination with heat and smoke, triggered
296 germination in the field-burn treatment. For example, it has been observed that
297 germination can be stimulated by ash -the mineral remain of burned plants- that
298 provide rich mineral nutrition (Ne'eman *et al.*, 2009), by burned plant remains
299 (Wicklow, 1977; Keeley and Nitzberg, 1984) or by burned soil (Blank and
300 Young, 1998). Also, it has been shown that certain trace gases from smoke
301 (*e.g.* nitrogen oxides) induce germination of chaparral species (Keeley and

302 Fotheringham, 1997). Further studies testing other fire signals are needed to
303 support this hypothesis.

304

305 On the other hand, it is also possible that the intensity and duration of heat and
306 smoke applied in our study do not accurately simulate the conditions of a
307 prescribed burn. In particular, the entire process of treating seeds with smoke is
308 highly variable in the literature. Variations include application techniques
309 (smoke fumigation, smoke water, ash/charate, karrikinolide), type of plant
310 material, dilution of the aqueous smoke solution, and exposure time (5 minutes
311 to 24 hours, depending on the application technique), among others. In our
312 experiment, we tried to simulate the low-fire residence time of grass-fueled fires.
313 It is possible that 5-min exposure was not enough to stimulate the germination
314 of all the species, but we tried to be cautious as plant-derived smoke can both
315 stimulate and inhibit germination depending on exposure time (van Staden *et*
316 *al.*, 2000). For example, Keeley and Fotheringham (1998) found that several
317 species of the Californian chaparral enhanced seed germination after 5-min
318 exposure and reduced germination for exposures of 8-min or more. Also, the
319 application technique may affect the results. Carthey *et al.* (2018) found that
320 tests using smoke fumigation (as in our experiment) were significantly more
321 likely to result in enhanced germination than tests using smoke water or
322 ash/charate. Finally, it has been suggested that there are multiple compounds
323 in smoke that stimulate germination, in addition to the non-specific karrikines
324 (Keeley and Pausas 2018). These chemicals could be species-specific, so
325 burning a mixture of species (representative of the diversity of the community)

326 rather than a single one could increase the number of species stimulated to
327 germinate.

328 Field-burn was the only treatment that promoted a significant increase in
329 species richness of monocots and dicots germinants. Twelve exclusive species
330 were registered in this treatment, albeit with very low abundances (1 or 2
331 individuals). Fire frees up space and other resources (e.g. light, inorganic
332 nutrients) and clears environments of competitors, leaving them ready to be
333 colonized (Overbeck *et al.*, 2005; Keeley and Fotheringham, 2000). These
334 spaces can be occupied by competitively subordinated species, which take
335 advantage of the gaps left temporarily by the dominants (Overbeck *et al.*, 2005).
336 Enhanced richness of germinants due to burning or its simulation has been
337 observed in different ecosystems, such as forests (Enright *et al.*, 1997; Read *et al.*,
338 2000; Maikano *et al.*, 2018), shrublands (Figueroa *et al.*, 2009), and prairies
339 (Ren and Bai, 2016).

340 Seedlings began to emerge at least one week earlier in the field-burn treatment,
341 compared to the other treatments. Smoke seems to be partly responsible for
342 this advance, since the peak of germination of this treatment was the closest to
343 that of the field-burn. Similarly, in other studies where the effects of heat and
344 smoke on soil seed bank germination were evaluated, it was found that
345 treatments with smoke promoted earlier germination (Read *et al.*, 2000;
346 Ghebrehiwot *et al.*, 2012). Early germination could be an advantage for
347 opportunistic species growing in environments with highly unpredictable
348 disturbances, such as prescribed burns, given that the first plants to germinate

349 would be those that take advantage of the resources and space made available
350 by fire (Le Stradic *et al.*, 2015; Carthey *et al.*, 2018; López-Mársico *et al.*, 2019).

351 Contrary to our expectation, none of the treatments affected the monocot/dicot
352 ratio. In all treatments, the dicots accounted for the highest percentage of
353 germinants, close to 70%. This proportion is very similar to that found in other
354 seed bank studies carried out in Uruguayan grasslands under grazing
355 (Haretche and Rodríguez, 2006; Gallego *et al.*, 2018), which was the initial
356 condition of the study area, before the application of the treatments. High
357 densities of dicots are common in grassland seed banks, and species that are
358 hardly observed in the vegetation are frequently found in the soil samples
359 (Roberts, 1981; D'Angela *et al.*, 1988; Milberg, 1992). The dominant dicot in all
360 treatments was *Gamochaeta americana*, a native small perennial Asteraceae,
361 of medium to low frequency in vegetational surveys (unpublished data). Within
362 the monocots, *Juncus capillaceus* was the most abundant species in most
363 treatments. The species of this genus produce a large amount of small, long-
364 lived seeds, forming large, persistent soil seed banks (Lunt, 1997; Bossuyt and
365 Honnay, 2008). High densities of *Juncus* spp. in seed banks are not exclusive
366 to grassland soils. In a survey from very different ecosystems, Bossuyt and
367 Honnay (2008) found that *Juncus* spp. were present in more than 50% of the
368 soil seed banks analyzed, and were among the five most abundant species.

369 Our study contributes to the knowledge of the effects of fire on recruitment by
370 seeds in a temperate subhumid grassland of South America. By comparing the
371 effect of fire as a whole with the effect of two of its components, we found that
372 fire produces a postburn flush of seedlings, not entirely attributable to heat or

373 smoke. However, all germination trials reported in the region have been
374 directed to evaluate the effect of these fire-direct signals. The lack of response
375 observed so far may be due to the fact that in this system, prescribed burns
376 spread rapidly and are not very intense (Simpson *et al.*, 2016), which most likely
377 minimizes the effect of heat and smoke. Future research efforts are needed to
378 quantify the net contribution of other fire signals to the germination of seeds of
379 South American grassland species.

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390 **Conflict of interest**

391 None

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623 Figure 1. Geographic location of the geomorphological region Eastern Hills
624 (Sierras del Este), Uruguay. The gray area denotes the limit of the region, and
625 the star shows the location of soil collection. About 65% of the region is covered
626 by natural grasslands.

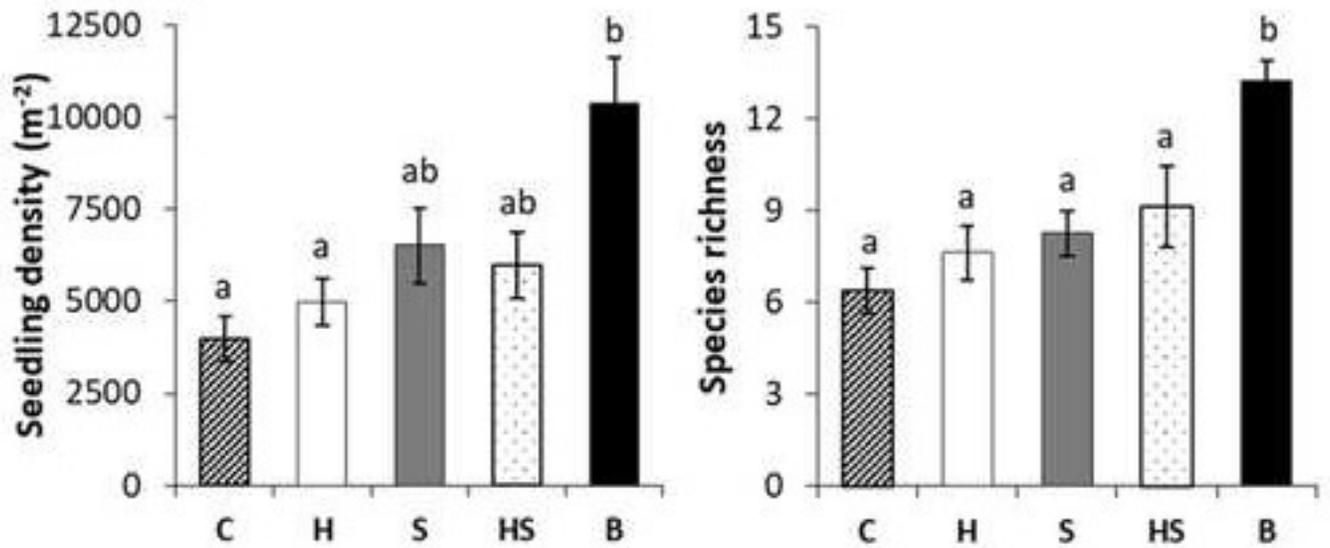
627 Figure 2. Seedling density and species richness of the soil seed bank of a
628 Uruguayan temperate grassland, in response to five different fire-related
629 treatments; (A) Total; (B) Dicots and (C) Monocots. Values are means (\pm SE; n
630 = 8). Different letters indicate significant differences between treatments ($P <$
631 0.05) in a post-ANOVA Tukey test. B=field-burn; H=heat shock; S=smoke; HS=
632 heat shock and smoke; C=control.

633 Figure 3. Number of weekly germinants which emerged from the soil seed bank
634 of a Uruguayan temperate grassland, in response to five different fire-related
635 treatments; (A) Total; (B) Dicots and (C) Monocots. Only the first 10 weeks of
636 the total 20 weeks the experiment lasted are shown. B=field-burn; H=heat
637 shock; S=smoke; HS= heat shock and smoke; C=control.

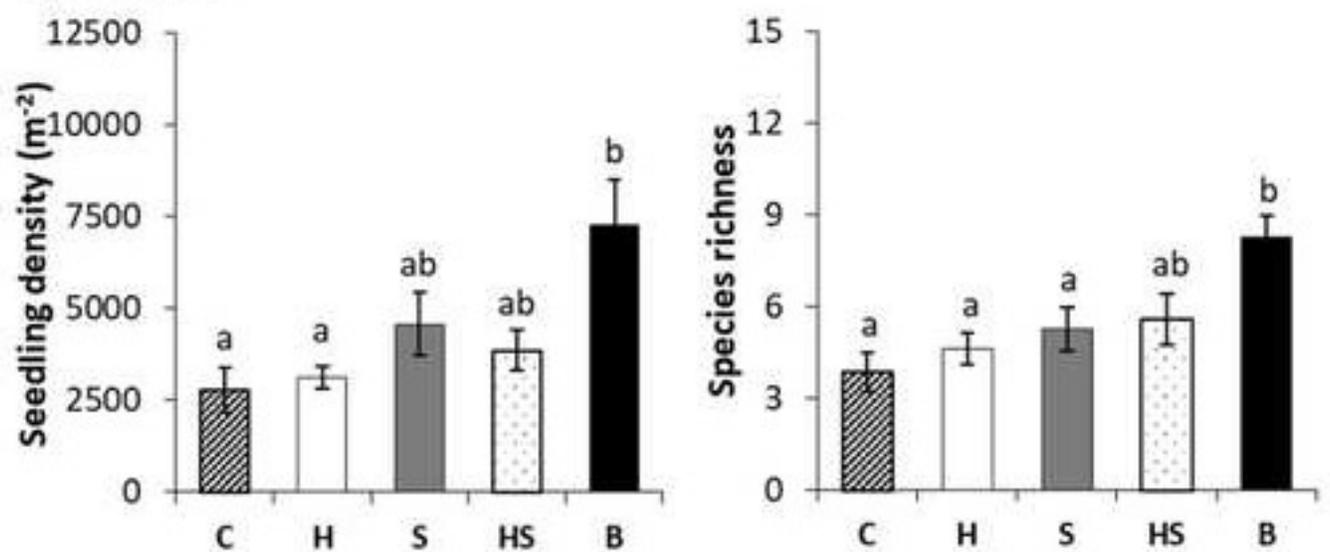
638 Figure 4. Number of seedlings of the dominant ($DR > 3\%$) dicots (A) and
639 monocots (B) which emerged from the soil seed bank of a Uruguayan
640 temperate grassland, in response to five different fire-related treatments.
641 B=field-burn; H=heat shock; S=smoke; HS= heat shock and smoke; C=control.



A) TOTAL



B) DICOTS



C) MONOCOTS

