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# Field burn versus fire-related cues: germination from the soil seed bank of a South American temperate grassland

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**Research Paper**

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## Abstract

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

## Introduction

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

Fire is associated with several plant traits, including whole-plant fire responses, serotiny, and fire-stimulated seed germination and flowering (He and Lamont, 2018; Archibald et al., 2019). In fire-prone ecosystems, such as Mediterranean-type shrublands, fire or one of its components (e.g. heat, smoke, ash and charred wood) promotes the germination of soil-stored seeds (Dixon et al., 1995; Keeley and Fotheringham, 1998; Moreira et al., 2010; Keeley et al., 2012). Increasing temperature breaks the physical dormancy of some hard-seeded species (Keeley, 1991; Moreira et al., 2010; Baskin and Baskin, 2014), and plant-derived smoke or aqueous smoke extracts promote germination by breaking physiological dormancy in many plant families (Van Staden et al., 2000; Keeley et al., 2011). Also, fire can indirectly stimulate germination by increasing the incidence of radiation reaching the soil (Keeley and Fotheringham, 2000).

In comparison to Mediterranean ecosystems, much less is known about the response of plants to grassland fires (Pausas and Paula, 2019). The Río de la Plata grasslands are one of the largest areas of natural temperate sub-humid grasslands in the world, covering more than 700,000 km$^2$ distributed across eastern Argentina, the whole of Uruguay and southern Brazil (Soriano et al., 1991). The history of fire in the Río de la Plata grasslands is largely unknown, but palaeoecological data suggest that fire became frequent about 7000–3000 years ago, after the arrival of human populations in the region (Behling et al., 2004; Kaal et al., 2019). Currently, grazing by livestock is the main productive activity, and in certain areas, controlled burns are used to increase primary production and forage quality (Pillar and de Quadros, 1997; Laterra et al., 2003; López-Mársico et al., 2019). Many species that grow in the region are herbaceous plants that exhibit a great variety of subterranean or near-ground bud-bearing structures that allow them to resprout after disturbances such as grazing, drought and fire (Overbeck and Pfadenhauer, 2007; López-Mársico et al., 2019). In Brazilian *campos* grasslands, Overbeck and Pfadenhauer (2007) found that about 70% of the species are able to resprout from belowground organs, and only 1% are obligate seeders.

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

While the evidence accumulated so far indicates an absence of fire-stimulated germination in these grasslands, scaling up individual responses to the community as a whole must be done with caution. The Río de la Plata grasslands is a highly diverse region that hosts nearly 5000 species belonging to 194 families (Andrade et al., 2018), and fire could trigger the germination of many other species that have not yet been tested. In addition, the experimental germination tests oversimplify the overall effect of fire, which is known to cause a multitude of changes in the environment (Keeley and Fotheringham, 2000). On the other hand, certain forbs could be favoured by prescribed burns in Uruguayan grasslands. Grazed communities generally consist of a few matrix-forming $C_4$ perennial grasses, and a diverse array of interstitial grasses and forbs (Rodríguez et al., 2003; Altesor et al., 2005). For grasses, post-fire resprouting may play a major role in their regeneration, but forbs are a phylogenetically heterogeneous group of species (Fernández et al., 2019) and their dynamics seem to be regulated by processes other than grazing (Rodríguez et al., 2003). In this context, we expected that seeder forbs would benefit most from the post-fire environment, contributing to the maintenance of these highly diverse communities.

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

## Materials and methods

### Study area

The site used for collecting soil seed bank samples is a natural grassland of the *Quebrada de los Cuervos* protected landscape, Uruguay (32°55′ S, 54°26′ W; Fig. 1). The site belongs to the geomorphological region Eastern Hills (*Sierras del Este*), which covers 25,000 km$^2$ (14.3% of the country's surface) and is characterized by great heterogeneity in terms of relief, rocky outcrops, slope and soil depth (Baeza et al., 2019). The average annual rainfall in the area is 1318 mm and the average annual temperature is 17°C (INIA-GRAS, 2018). The rainfall is distributed throughout the year, without distinction between dry and wet periods. Grasslands and shrubby grasslands occupy nearly 66% of the region, and grazing by livestock is the main productive activity (Baeza et al., 2019). Three grassland communities are recognized in this region (Lezama et al., 2019): the densely vegetated grasslands (*Eryngium horridum–Juncus capillaceus* community, associated with medium and deep soils; plant cover around 90%), the sparsely vegetated grasslands (*Trachypogon spicatus–Crocanthemum brasiliense* community, associated with shallow soils; plant cover 60–80%) and the tall and densely vegetated grasslands (associated with humid or temporarily inundated soils; plant cover around 95%). The latter occupies the concave zones of the landscape (Gallego, 2013), on moderately drained Argisols with a time-limited perched water table when present (Durán, 1985). Vegetation is distributed in two strata, one low (5 cm), conformed by grasses and perennial herbaceous forbs (e.g. *Paspalum pumilum*, *Chascolytrum poomorphum*, *Eragrostis bahiensis*, *Axonopus fissifolius*, *Dichanthelium sabulorum*, *Centella asiatica*), and one tall (60 cm) defined by the tussock grass *Saccharum angustifolium* (Ness) Trin (mean cover: 60–70%) (Lezama et al., 2019). Tussock grasslands are managed using prescribed burns to improve forage quality for cattle (Royo-Pallarés et al., 2005). The large amount of dry standing dead matter and litter that accumulates over many growing seasons provides the necessary fuel for fire ignition and spread.

### Soil seed bank samples and treatments

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

In total, 40 samples were collected, 8 of them from a recently burned site (2 h before sampling) and the remaining 32 from an adjacent site that had not been burned for at least 4 years. Sampling points were spaced every 2 m along a transect, avoiding the area below the canopy of *S. angustifolium*. Soil cores were taken with a core sampler of 8 cm in diameter, inserted 5 cm into the soil. Each soil sample was sectioned lengthwise, and one of the halves was discarded. The other half (126 cm$^3$) was stored in a plastic bag and transported to the laboratory.

Samples were air-dried for 2 d. Subsequently, the soil was disaggregated and all plant debris was removed. Soil samples were spread evenly in aluminium trays (11 × 15 × 5 cm) containing a 1-cm deep layer of sterile sand. The 32 samples collected from the unburned site were subjected to four treatments (eight samples per treatment): (1) heat shock of 100°C (H), (2) smoke (S), (3) heat shock and smoke (HS) and (4) control (C; no heat shock and no smoke). A fifth treatment consisted of the samples taken from the burned site (B). Heat-shock treatment was applied to the samples for a period of 5 min using a preheated oven. The selected


**Fig. 1.** Geographic location of the geomorphological region Eastern Hills (Sierras del Este), Uruguay. The grey area denotes the limit of the region, and the star shows the location of soil collection. About 65% of the region is covered by natural grasslands.

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

The soil seed bank was estimated using the seedling emergence method (Roberts, 1981; Simpson et al., 1989). Trays were placed in a germination chamber with a 12/12 h light/dark and 25/20°C regime, simulating the environmental conditions of eastern Uruguay during spring (INIA-GRAS, 2018). The trays were regularly watered and rotated weekly to expose the samples to the same radiation conditions. Emerging seedlings were identified and removed. Unidentified seedlings were transplanted to a separate pot and grown until they were identifiable. All species were carefully revised, regarding taxonomic nomenclature, in online databases TROPICOS (http://www.tropicos.org, last accessed 18 April 2020) and Instituto de Botánica Darwinion (http://www.darwin.edu.ar/Proyectos/FloraArgentina/Especies.asp, last accessed 18 April 2020). The trays were monitored for 140 days, after which few seedlings emerged. One of the samples of the HS treatment was discarded because it presented a disproportionate number of seedlings compared to the rest of the trays.

### Data analysis

Density (number of seedlings per $m^2$) and richness of emerged seedlings (total and discriminated in dicotyledons and monocotyledons) were calculated for each treatment. The comparison of the variables among treatments was carried out through a one-way ANOVA, followed by Tukey's *post hoc* test ($\alpha = 0.05$). Prior to analysis, total and dicot density data were log-transformed, as they did not meet the assumptions of normality and/or homoscedasticity. A chi-square test was performed to investigate whether the proportion of monocots and dicots varied in the different treatments. All analyses were performed with the software InfoStat (Di Rienzo et al., 2016).

## Results

Throughout the experiment a total of 625 seedlings emerged, of which 425 were dicots and 200 were monocots. Of the total, 525 could be identified at the genus or species level, while the

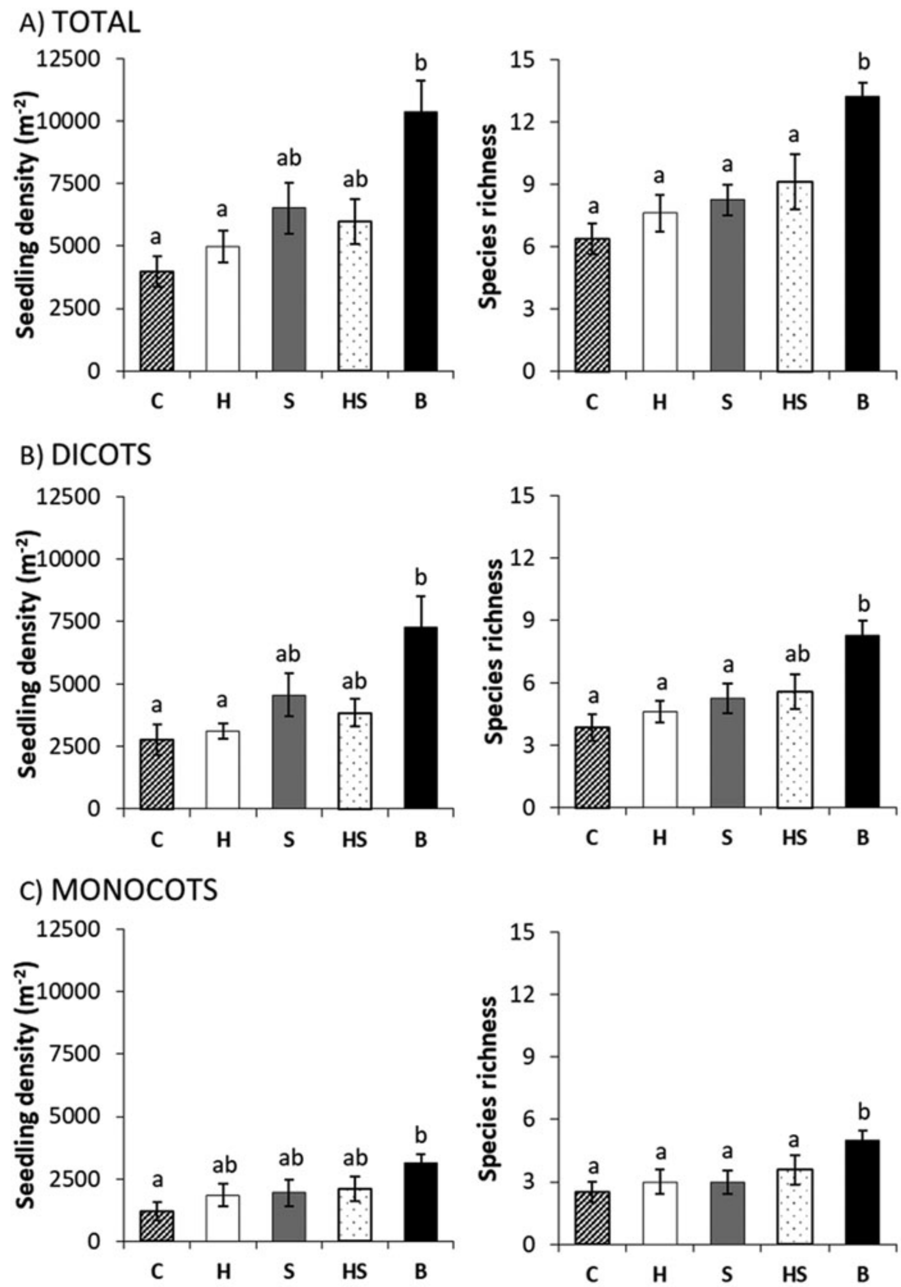


**Fig. 2.** Seedling density and species richness of the soil seed bank of an Uruguayan temperate grassland, in response to five different fire-related treatments: (A) total; (B) dicots and (C) monocots. Values are means (±SE; *n* = 8). Different letters indicate significant differences between treatments ($P < 0.05$) in a post-ANOVA Tukey test. B = field burn; H = heat shock; S = smoke; HS = heat shock and smoke; C = control.

remaining 100 (63 dicots and 37 monocots) died before identification. Overall species richness was 54 (31 dicots and 23 monocots). The most species-rich plant families were Asteraceae (15 spp.), Poaceae (12 spp.) and Cyperaceae (6 spp.). The remaining species belong to 12 plant families (Supplementary Table S1).

### *Effect of treatments on seedling density and richness*

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

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

### *Germination timing*

Seedlings emerged earlier in B, reaching a maximum in the first and second week of the experiment. In the remaining treatments, including the control, the maximum germination peak occurred between the second and third week of the experiment (Fig. 3).

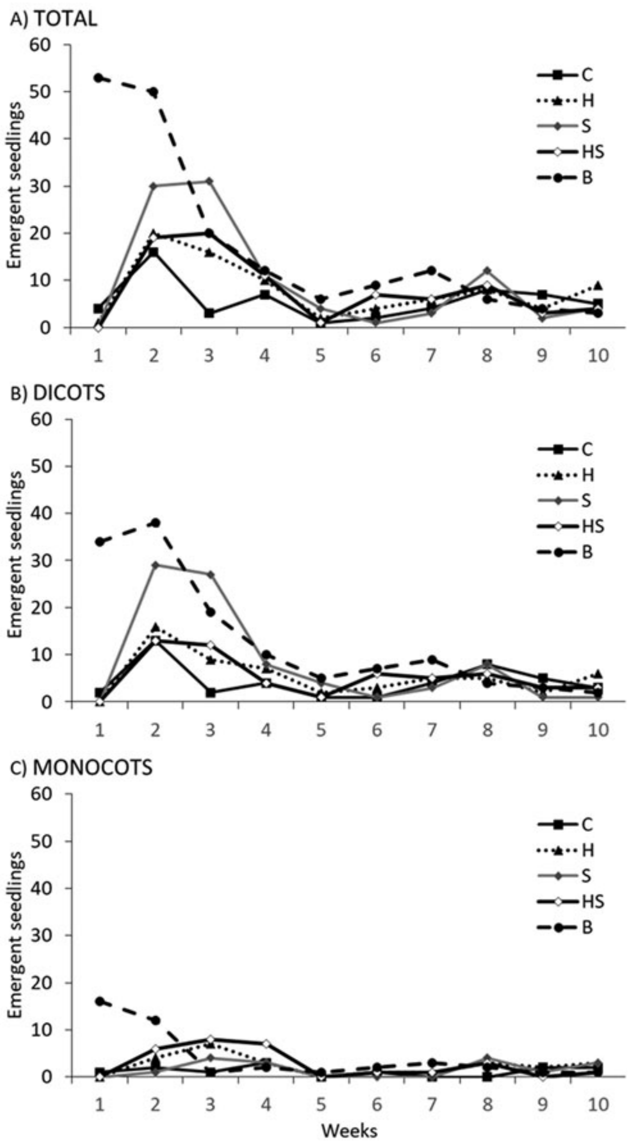


**Fig. 3.** Number of weekly germinants which emerged from the soil seed bank of an Uruguayan temperate grassland, in response to five different fire-related treatments: (A) total; (B) dicots and (C) monocots. Only the first 10 weeks of the total 20 weeks that the experiment lasted is shown. B = field burn; H = heat shock; S = smoke; HS = heat shock and smoke; C = control.

The smoke treatment produced the second highest germination peak, mainly due to the germination of dicots (Fig. 3B). After 10 weeks, nearly 80% of the total number of individuals recorded had already germinated.

### *Species composition*

The most abundant species in each taxonomic group (relative density >3%, considering all treatments as a whole) accounted for 80% of all emerging seedlings. Dominant dicots germinated in all treatments, but germination was especially enhanced in B. The germination behaviour of dominant monocots was more heterogeneous (Fig. 4). Species exclusive to a single treatment were rare (most with one or two individuals) and were mainly recorded in B (five dicots and seven monocots) (Supplementary Table S1).

## Discussion

This study provides evidence of positive effects of fire on seed germination in a temperate grassland of southern South America. Of all the treatments analysed, field burn was the treatment that differed most from the control. Field burn promoted a significant increase in the density and richness of germinants, without affecting the monocot/dicot ratio. In addition, the germination peak in this treatment preceded the peaks of the remaining treatments. On the other hand, fire-related cues (heat shock and smoke) did not affect seedling species richness and density, though the germination of some individual species was enhanced by some of the treatments, mainly those where the seeds were smoked. These results complement and challenge the conclusions of other studies carried out on several grasslands of the region, in which it was found that the effects of heat and smoke had neutral or negative effects on the germination of some species (e.g. Overbeck et al., 2006; Fidelis et al., 2010, 2016; López-Mársico et al., 2019). This led researchers to suggest that species from these grasslands rely on their ability to resprout by means of subterranean or protected basal buds after a fire event, rather than on seed recruitment. Regeneration from seeds would be possible if seeds were to tolerate (or survive) a burning event (Overbeck and Pfadenhauer, 2007; Fidelis et al., 2016; López-Mársico et al., 2019), and our results show that several species of this grassland are stimulated to germinate by burning.

Treatments involving fire-related cues (heat, smoke and heat and smoke) were far from emulating the effects of a real field burn. Heat and smoke are the most commonly used fire signals in seed germination trials (e.g. Dayamba et al., 2008; Reyes and Trabaud, 2009; Moreira et al., 2010; Fichino et al., 2016; Kin et al., 2016; Ramos et al., 2016, 2019; Tavşanoğlu et al., 2017; Arcamone and Jaureguiberry, 2018). In fire-prone ecosystems, such as Mediterranean-type shrublands, these signals stimulate the germination of many species and largely explain the recruitment of new individuals from seeds (Brown, 1993; Dixon et al., 1995; Keeley and Fotheringham, 1998; Paula and Pausas, 2008; Moreira et al., 2010). However, structurally distinct plant communities with historically different fire regimes may be expected to have different responses to fire-related germination cues (Maikano et al., 2018). Our results suggest that other fire-related cues, alone or in combination with heat and smoke, triggered germination in the field burn treatment. For example, it has been observed that germination can be stimulated by ash – the mineral remain of burned plants – that provide rich mineral nutrition (Ne'eman et al., 2009), by burned plant remains (Wicklow, 1977; Keeley and Nitzberg, 1984) or by burned soil (Blank and Young, 1998). Also, it has been shown that certain trace gases from smoke (e.g. nitrogen oxides) induce the germination of chaparral species (Keeley and Fotheringham, 1997). Further studies testing other fire signals are needed to support this hypothesis.

On the other hand, it is also possible that the intensity and duration of heat and smoke applied in our study do not accurately simulate the conditions of a prescribed burn. In particular, the entire process of treating seeds with smoke is highly variable in the literature. Variations include application techniques (smoke fumigation, smoke water, ash/charate and karrikinolide), type of plant material, dilution of the aqueous smoke solution, and exposure time (5 min to 24 h, depending on the application technique), among others. In our experiment, we tried to simulate the low fire residence time of grass-fueled fires. It is possible that 5-min exposure was not enough to stimulate the germination of all the species,

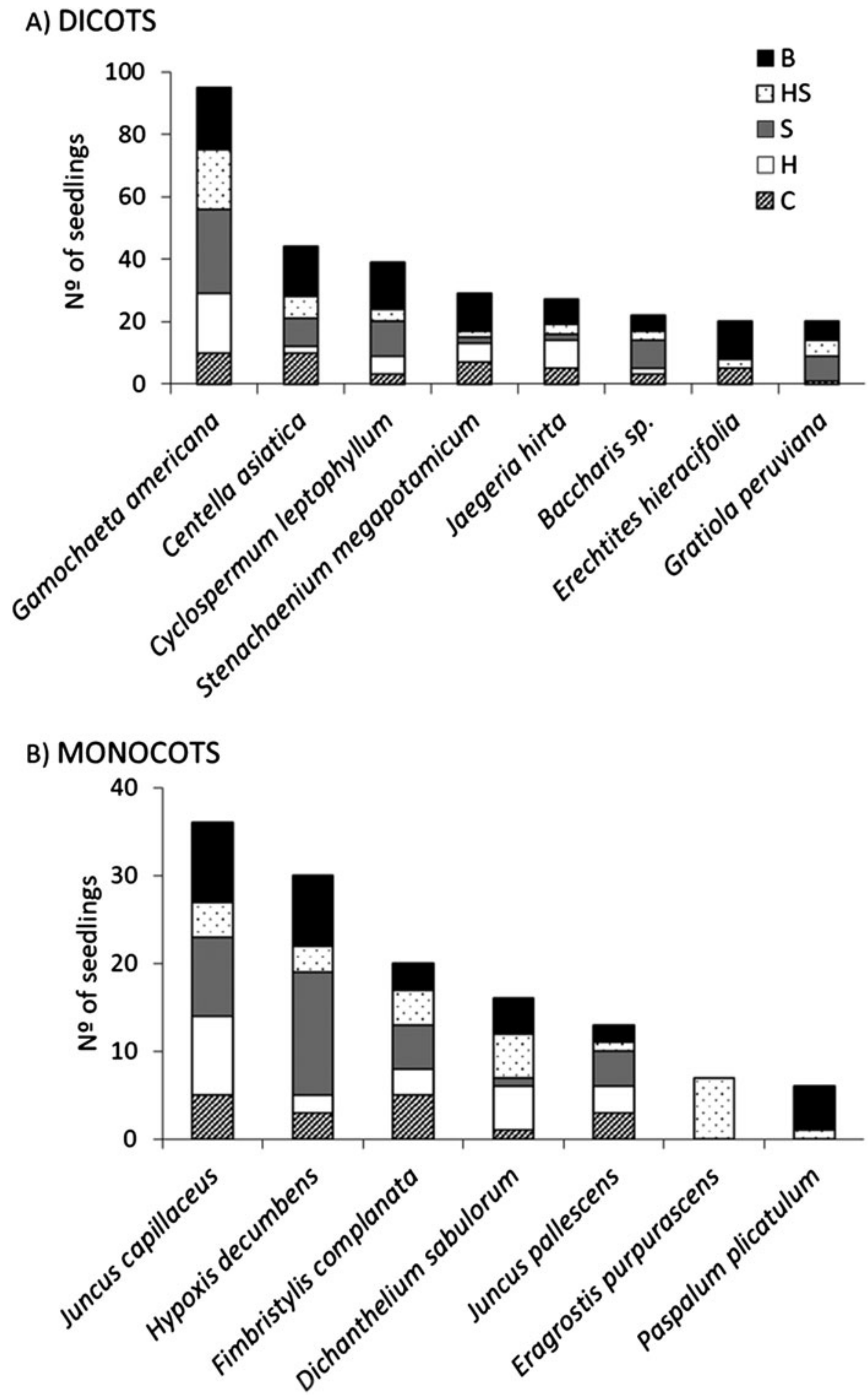


**Fig. 4.** Number of seedlings of the dominant (DR > 3%) dicots (A) and monocots (B) which emerged from the soil seed bank of an Uruguayan temperate grassland, in response to five different fire-related treatments. B = field burn; H = heat shock; S = smoke; HS = heat shock and smoke; C = control.

but we tried to be cautious as plant-derived smoke can both stimulate and inhibit germination depending on exposure time (van Staden et al., 2000). For example, Keeley and Fotheringham (1998) found that several species of the Californian chaparral enhanced seed germination after 5-min exposure and reduced germination for exposures of 8-min or more. Also, the application technique may affect the results. Carthey et al. (2018) found that tests using smoke fumigation (as in our experiment) were significantly more likely to result in enhanced germination than tests using smoke water or ash/charate. Finally, it has been suggested that there are multiple compounds in smoke that stimulate germination, in addition to the non-specific karrikines (Keeley and Pausas 2018). These chemicals could be species-specific, so burning a mixture of species (representative of the diversity of the community) rather than a single one could increase the number of species stimulated to germinate.

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

Seedlings began to emerge at least 1 week earlier in the field burn treatment, compared to the other treatments. Smoke seems to be partly responsible for this advance since the peak of germination of this treatment was the closest to that of the field burn. Similarly, in other studies where the effects of heat and smoke on soil seed bank germination were evaluated, it was found that treatments with smoke promoted earlier germination (Read et al., 2000; Ghebrehiwot et al., 2012). Early germination could be an advantage for opportunistic species growing in environments with highly unpredictable disturbances, such as prescribed burns, given that the first plants to germinate would be those that take advantage of the resources and space made available by fire (Le Stradic et al., 2015; Carthey et al., 2018; López-Mársico et al., 2019).

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

Our study contributes to the knowledge of the effects of fire on recruitment by seeds in a temperate sub-humid grassland of South America. By comparing the effect of fire as a whole with the effect of two of its components, we found that fire produces a postburn flush of seedlings, not entirely attributable to heat or smoke. However, all germination trials reported in the region have been directed to evaluate the effect of these fire-direct signals. The lack of response observed so far may be due to the fact that in this system, prescribed burns spread rapidly and are not very intense (Simpson et al., 2016), which most likely minimizes the effect of heat and smoke. Future research efforts are needed to quantify the net contribution of other fire signals to the germination of seeds of South American grassland species.

**Supplementary material.** To view supplementary material for this article, please visit: https://doi.org/10.1017/S0960258520000288.

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## References

**Altesor A, Oesterheld M, Leoni E, Lezama F and Rodríguez C** (2005) Effect of grazing on community structure and productivity of a Uruguayan grassland. *Plant Ecology* **179**, 83–91.

**Andrade BO, Marchesi E, Burkart S, Setubal RB, Lezama F, Perelman S, Schneider AA, Trevisan R, Overbeck GE and Boldrini II** (2018) Vascular plant species richness and distribution in the Río de la Plata grasslands. *Botanical Journal of the Linnean Society* **188**, 250–256.

**Arcamone JR and Jaureguiberry P** (2018) Germination response of common annual and perennial forbs to heat shock and smoke treatments in the Chaco Serrano, Central Argentina. *Austral Ecology* **43**, 567–577.

**Archibald S, Lehmann CER, Gómez-Dans JL and Bradstock RA** (2013) Defining pyromes and global syndromes of fire regimes. *Proceedings of the National Academy of Sciences of the United States of America* **110**, 6442–6447.

**Archibald S, Hempson GP and Lehmann CER** (2019) A unified framework for plant life-history strategies shaped by fire and herbivory. *New Phytologist* **224**, 1490–1503.

**Baeza S, Rama G and Lezama F** (2019) Cartografía de los pastizales naturales en las regiones geomorfológicas de Uruguay predominantemente ganaderas. Ampliación y actualización, pp. 27–48 *in* Altesor A; López-Mársico L and Paruelo JM (Eds) *Bases ecológicas y tecnológicas para el manejo de pastizales II*. Montevideo, INIA.

**Baskin CC and Baskin JM** (2014) *Seeds: ecology, biogeography and evolution of dormancy and germination*. San Diego, Elsevier Academic Press.

**Behling H, Pillar VD, Orlóci L and Bauermann SG** (2004) Late Quaternary Araucaria forest, grassland (Campos), fire and climate dynamics, studied by high resolution pollen, charcoal and multivariate analysis of the Cambara do Sul core in southern Brazil. *Palaeogeography. Palaeoclimatology and Palaeoecology* **203**, 277–297.

**Blank RR and Young JA** (1998) Heated substrate and smoke: influence on seed emergence and plant growth. *Journal of Range Management* **51**, 577–583.

**Bond WJ and Parr CL** (2010) Beyond the forest edge: ecology, diversity and conservation of the grassy biomes. *Biological Conservation* **143**, 2395–2404.

**Bossuyt B and Honnay O** (2008) Heat shock increases the reliability of a temperate calcareous grassland seed bank study. *Plant Ecology* **199**, 1–7.

**Brown NAC** (1993) Promotion of germination of fynbos seeds by plant-derived smoke. *New Phytologist* **123**, 575–583.

**Carthey AJ, Tims A, Geedicke I and Leishman MR** (2018) Broad-scale patterns in smoke-responsive germination from the south-eastern Australian flora. *Journal of Vegetation Science* **29**, 737–745.

**Coughenour MB** (1985) Graminoid responses to grazing by large herbivores: adaptations, exaptations, and interacting processes. *Annals of the Missouri Botanical Garden* **72**, 852–863.

**D'Angela E, Facelli JM and Jacobo E** (1988) The role of the permanent soil seed bank in early stages of a post-agricultural succession in the Inland Pampa. *Argentina Vegetation* **74**, 39–45.

**Dayamba SD, Tigabu M, Sawadogo L and Oden PC** (2008) Seed germination of herbaceous and woody species of the Sudanian savanna-woodland in response to heat shock and smoke. *Forest Ecology and Management* **256**, 462–470.

**Di Rienzo JA, Casanoves F, Balzarini MG, Gonzalez L, Tablada M and Robledo CW** (2016) Grupo InfoStat, *FCA*. Argentina, Universidad Nacional de Córdoba. Available at: http://www.infostat.com.ar/.

**Dixon KW, Roche S and Pate JS** (1995) The promotive effect of smoke derived from burnt native vegetation on seed-germination of Western Australian plants. *Oecologia* **101**, 185–192.

**Durán A** (1985) *Los suelos del Uruguay*. Montevideo, Hemisferio Sur.

**Enright NJ, Goldblum D, Ata P and Ashton DH** (1997) The independent effects of heat, smoke and ash on emergence of seedlings from the soil

seed bank of a healthy *Eucalyptus* woodland in Grampians (Gariwerd) National Park, western Victoria. *Australian Journal of Ecology* **22**, 81–88.
**Fernández G, Lezama F and Rodríguez C** (2019) Decoupling facilitative effects in a temperate subhumid grassland: photosynthetic metabolism matters. *Plant Ecology & Diversity* **12**, 63–73.
**Fichino BS, Dombroski JRG, Pivello VRA and Fidelis A** (2016) Does fire trigger seed germination in the Neotropical Savannas? Experimental tests with six Cerrado species. *Biotropica* **48**, 181–187.
**Fidelis A, Delgado-Cartay MD, Blanco CC, Muller SC, Pillar VD and Pfadenhauer J** (2010) Fire intensity and severity in Brazilian campos grasslands. *Interciencia* **35**, 739–745.
**Fidelis A, Daibes LF and Martins AR** (2016) To resist or to germinate? The effect of fire on legume seeds in Brazilian subtropical grasslands. *Acta Botanica Brasilica* **30**, 147–151.
**Figueroa JA, Cavieres LA, Gómez-González S, Molina-Montenegro M and Jaksic FM** (2009) Do heat and smoke increase emergence of exotic and native plants in the matorral of central Chile? *Acta Oecologica* **35**, 335–340.
**Gallego F** (2013) *Servicios ecosistémicos del pastizal: el seguimiento de un área protegida como sistema de referencia*. Dissertation, Universidad de la República, Montevideo.
**Gallego F, López-Mársico L, Tommasino A, Casás M, Haretche F, Rodríguez C and Altesor A** (2018) Efectos de la actividad forestal sobre el suelo, la vegetación y el banco de semillas en Sierras del Este, Uruguay, p. 527 *in* Proceedings from the XXVIII Reunión Argentina de Ecología, November 2018, Mar del Plata, Argentina.
**Ghebrehiwot H, Kulkarni M, Kirkman K and Van Staden J** (2012) Smoke and heat: influence on seedling emergence from the germinable soil seed bank of mesic grassland in South Africa. *Plant Growth Regulation* **66**, 119–127.
**Gibson DJ** (2009) *Grasses and grassland ecology*. New York, Oxford University Press.
**Haretche F and Rodríguez C** (2006) Banco de semillas de un pastizal uruguayo bajo diferentes condiciones de pastoreo. *Ecología Austral* **16**, 105–113.
**He T and Lamont BB** (2018) Baptism by fire: the pivotal role of ancient conflagrations in evolution of the Earth's flora. *National Science Review* **5**, 237–254.
**INIA-GRAS** (2018) *Banco de datos agroclimáticos 1965–2018*. Uruguay, Instituto Nacional de Investigación Agropecuaria, Estación experimental Treinta y Tres. Available at: http://www.inia.uy/gras/Clima/Banco-datos-agroclimatico.
**Jacobs BF, Kingston DJ and Jacobs LL** (1999) The origin of grass-dominated ecosystems. *Annals of the Missouri Botanical Garden* **86**, 590–643.
**Kaal J, Gianotti C, del Puerto L, Criado-Boado F and Rivas M** (2019) Molecular features of organic matter in anthropogenic earthen mounds, canals and lagoons in the Pago Lindo archaeological complex (Tacuarembó, Uruguayan lowlands) are controlled by pedogenetic processes and fire practices. *Journal of Archaeological Science: Reports* **26**, 101900.
**Keeley JE** (1991) Seed germination and life history syndromes in the California chaparral. *The Botanical Review* **57**, 81–116.
**Keeley JE and Fotheringham CJ** (1997) Trace gas emissions and smoke-induced seed germination. *Science* **276**, 1248–1250.
**Keeley JE and Fotheringham CJ** (1998) Smoke-induced seed germination in California chaparral. *Ecology* **79**, 2320–2336.
**Keeley JE and Fotheringham CJ** (2000) Role of fire in regeneration from seed, pp. 311–330 *in* Fenner M (Ed.) *Seeds: the ecology of regeneration in plant communities*, Wallingford, CAB International.
**Keeley JE and Nitzberg ME** (1984) Role of charred wood in the germination of the chaparral herbs *Emmenanthe penduliflora* (Hydrophyllaceae) and *Eriophyllum confertiflorum* (Asteraceae). *Madroño* **31**, 208–218.
**Keeley JE and Pausas JG** (2018) Evolution of 'smoke' induced seed germination in pyroendemic plants. *South African Journal of Botany* **115**, 251–255.
**Keeley JE, Pausas JG, Rundel PW, Bond WJ and Bradstock RA** (2011) Fire as an evolutionary pressure shaping plant traits. *Trends in Plant Science* **16**, 406–411.
**Keeley JE, Bond WJ, Bradstock RA, Pausas JG and Rundel PW** (2012) *Fire in Mediterranean ecosystems: ecology, evolution and management*. Cambridge, Cambridge University Press.
**Kin AG, Suárez CE, Chirino CC, Ávila PL and Morici EF** (2016) Impact of heat on seed germination of three perennial grasses in the semiarid region in Central Argentina. *Australian Journal of Botany* **64**, 451–455.
**Knapp AK, Briggs JM, Hartnett DC and Collins SL** (1998) *Grassland dynamics: long-term ecological research in tallgrass prairie*. New York, Oxford University Press.
**Laterra P, Vignolio OR, Linares MP, Giaquinta A and Maceira N** (2003) Cumulative effects of fire on a tussock pampa grassland. *Journal of Vegetation Science* **14**, 43–54.
**Le Stradic S, Silveira FA, Buisson E, Cazelles K, Carvalho V and Fernandes GW** (2015) Diversity of germination strategies and seed dormancy in herbaceous species of campo rupestre grasslands. *Austral Ecology* **40**, 537–546.
**Lezama F, Pereira M, Altesor A and Paruelo JM** (2019) Grasslands of Uruguay: a floristic based description of their heterogeneity. *Phytocoenologia* **49**, 211–229.
**López-Mársico L, Farías-Moreira L, Lezama F, Altesor A and Rodríguez C** (2019) Light intensity triggers different germination responses to fire-related cues in temperate grassland species. *Folia Geobotanica* **54**, 53–63.
**Lunt ID** (1997) Germinable soil seed banks of anthropogenic native grasslands and grassy forest remnants in temperate south-eastern Australia. *Plant Ecology* **130**, 21–34.
**Maikano GN, Cohn J and Di Stefano J** (2018) Are germination cues for soil-stored seed banks different in structurally different fire-prone communities? *Austral Ecology* **43**, 89–101.
**Milberg P** (1992) Seed bank in a 35-year-old experiment with different treatments of a semi-natural grassland. *Acta Oecologica* **13**, 743–752.
**Moreira B, Tormo J, Estrelles E and Pausas JG** (2010) Disentangling the role of heat and smoke as germination cues in Mediterranean Basin flora. *Annals of Botany* **105**, 627–635.
**Ne'eman G, Ne'eman R, Keith DA and Whelan RJ** (2009) Does post-fire plant regeneration mode affect the germination response to fire-related cues? *Oecologia* **159**, 483–492.
**Oesterheld M, Loreti J, Semmartin M and Paruelo JM** (1999) Grazing, fire, and climate effects on primary productivity of grasslands and savannas, pp. 287–306 *in* Walker LR (Ed.) *Ecosystems of disturbed ground*. Amsterdam, Elsevier.
**Overbeck GE and Pfadenhauer J** (2007) Adaptive strategies in burned subtropical grassland in southern Brazil. *Flora* **202**, 27–49.
**Overbeck GE, Müller SC, Pillar VD and Pfadenhauer J** (2005) Fine scale post fire dynamics in southern Brazilian subtropical grassland. *Journal of Vegetation Science* **16**, 655–664.
**Overbeck GE, Müller SC, Pillar VD and Pfadenhauer J** (2006) No heat-stimulated germination found in herbaceous species from burned subtropical grassland. *Plant Ecology* **184**, 237–243.
**Paula S and Pausas JG** (2008) Burning seeds: germinative response to heat treatments in relation to resprouting ability. *Journal of Ecology* **96**, 543–552.
**Pausas JG and Bond WJ** (2019) Humboldt and the reinvention of nature. *Journal of Ecology* **107**, 1031–1037.
**Pausas JG and Paula S** (2019) Grasses and fire: the importance of hiding buds: a response to Moore et al. (2019) 'Effects of drought and fire on resprouting capacity of 52 temperate Australian perennial native grasses'. *New Phytologist* **226**, 957–959.
**Pausas JG, Lamont BB, Paula S, Appezzato-da-Glória B and Fidelis A** (2018) Unearthing belowground bud banks in fire-prone ecosystems. *New Phytologist* **217**, 1435–1448.
**Pillar VD and de Quadros FLF** (1997) Grassland-forest boundaries in southern Brazil. *Coenoses* **12**, 9–26.
**Ramos DM, Liaffa ABS, Diniz P, Munhoz CBR, Ooi MKJ, Borghetti F and Valls JFM** (2016) Seed tolerance to heating is better predicted by seed dormancy than by habitat type in Neotropical savanna grasses. *International Journal of Wildland Fire* **25**, 1273–1280.
**Ramos DM, Valls JFM, Borghetti F F and Ooi M** (2019) Fire cues trigger germination and stimulate seedling growth of grass species from Brazilian savannas. *American Journal of Botany* **106**, 1–12.
**Read T, Bellairs S, Mulligan D and Lamb D** (2000) Smoke and heat effects on soil seed bank germination for the re-establishment of a native forest community in New South Wales. *Austral Ecology* **25**, 48–57.
**Ren L and Bai Y** (2016) Smoke and ash effects on seedling emergence from germinable soil seed bank in fescue prairie. *Rangeland Ecology & Management* **69**, 499–507.e3.

**Reyes O and Trabaud L** (2009) Germination behaviour of 14 Mediterranean species in relation to fire factors: smoke and heat. *Plant Ecology* **202**, 113–121.

**Roberts HA** (1981) Seed banks in soils. *Advances in Applied Biology* **6**, 1–56.

**Rodríguez C, Leoni E, Lezama F and Altesor A** (2003) Temporal trends in species composition and plant traits in natural grasslands of Uruguay. *Journal of Vegetation Science* **14**, 433–440.

**Rosengurtt B** (1979) *Tablas de comportamiento de las especies de plantas de campos naturales en el Uruguay*. Montevideo, División Publicaciones y Ediciones de la Universidad de la República.

**Royo-Pallarés O, Berretta E and Maraschin G** (2005) The South American Campos ecosystem, pp. 171–219 *in* Suttie J; Reynolds SG and Batello C (Eds) *Grasslands of the world*. Rome, FAO.

**Simpson RL, Leek M and Parker V** (1989) Seed banks: general concepts and methodological issues, pp. 3–8 *in* Leek MA; Parker VT and Simpson RL (Eds) *Ecology of soil seed banks*. San Diego, CA, Academic Press.

**Simpson KJ, Ripley BS, Christin P-A, Belcher CM, Lehmann CER, Thomas GH and Osborne CP** (2016) Determinants of flammability in savanna grass species. *Journal of Ecology* **104**, 138–148.

**Soriano A, León RJC, Sala OE, Lavado RS, Deregibus VA, Cauhépé MA, Scaglia OA, Velázquez CA and Lemcoff JH** (1991) Rio de la Plata grasslands, pp. 367–407 *in* Coupland R (Ed.) *Natural grasslands: introduction and western hemisphere*. Amsterdam, Elsevier.

**Tavşanoğlu Ç, Çatav ŞS and Özüdoğru B** (2017) Fire-related germination and early seedling growth in 21 herbaceous species in Central Anatolian steppe. *Journal of Arid Environments* **122**, 109–116.

**Van Staden J, Brown NA, Jäger AK and Johnson TA** (2000) Smoke as a germination cue. *Plant Species Biology* **15**, 167–178.

**Wicklow DT** (1977) Germination response in *Emmenanthe penduliflora* (Hydrophyllaceae). *Ecology* **58**, 201–205.

**Zedler PH** (2007) Fire effects on grasslands, pp. 397–439 *in* Johnson EA and Miyanishi K (Eds) *Plant disturbance ecology: the process and the response*. Berkeley, CA, California Academic Press.