The dynamics of three shrub species in a fire-prone temperate savanna: the interplay between the seed bank, seed rain and fire regime

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Abstract A model was developed to assess how the seed rain and fire regime affect seed bank dynamics and seedling establishment of three native shrub species (*Acanthostyles buniifolius*, *Baccharis pingraea* and *Baccharis dracunculifolia*) with different regeneration strategies, in temperate South American savanna. Seed bank and seed rain were quantified for each species under different fire regimes, and their relative roles in regeneration were evaluated. All species had short-term persistent seed banks and high annual variability in seed production. A high proportion of seeds deposited in the seed rain produced seedlings after fire; few entered the soil seed bank. Fire killed a high proportion of the seeds in the soil seed bank. Seedlings derived from the seed rain had a higher probability of surviving for 2 years than seedlings emerging from the soil seed bank. In the absence of fire, establishment depended on germination both from the seed rain and the soil seed bank, whereas with annual fire, establishment was primarily dependent on germination of seeds arriving in the annual seed rain, regardless of species' regeneration strategies. These results help to explain changes in the vegetation of South American temperate savannas as a result of changes in fire regime and grazing management during the last 50 years. By revealing the crucial roles of the soil seed bank and seed rain in regeneration, this study provides vital information for the development of appropriate management practices to control populations of shrub species with different regeneration strategies in South American temperate savannas.

Keywords Asteraceae · Obligate seeders · Persistent seed bank · Sprouters · Transient seed bank

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Introduction

Fire opens up spaces in plant communities that can be occupied either by the re-sprouting of established plants or by the establishment of new plants from seed (Keeley 1981; Whelan 1995; Bond and van Wilgen 1996). Species that can re-colonise open ground by

re-growth are commonly referred to as sprouters, and those without the capacity for sprouting are referred to as obligate seeders. Following fire, sprouters can also re-colonise open ground from seed (Keeley 1981). In general, obligate seeders have more persistent seed banks (i.e. seed banks containing a higher proportion of seeds that are viable for more than a year, Thompson 1993) because their regeneration after fire depends entirely on establishment from seed, whereas species that can re-sprout tend to have more transient seed banks (Zammit and Zedler 1988; Auld et al. 2000, Campbell and Clarke 2006).

Seeds, either from a soil seed bank, or arriving as seed rain dispersed from neighbouring unburnt areas, are essential not only for the immediate regeneration and maintenance of seeder species following fire, but also for the long-term persistence of sprouters, despite their capacity for re-growth (Clarke and Knox 2002; Lamont and Wiens 2003). Although persistent seed banks play a role in the long-term maintenance of species in fire-prone habitats (Whelan 1995; Cohen et al. 2004; Auld and Denham 2006), regular disturbance by fire does not favour the accumulation of large seed banks containing many persistent seeds, because there is insufficient time to replenish such seed banks between successive fires (Lamont et al. 1991; Cohen et al. 2004; Auld and Denham 2006). The annual seed rain is, therefore, expected to be of greater importance for regeneration in habitats with frequent fires (Whelan 1986; Zammit and Zedler 1988; Auld et al. 2000).

In recent decades, anthropogenic activities have increasingly altered the structure of savanna ecosystems in many parts of the world as a result of changing their disturbance regimes, in particular by suppressing fires, eradicating native herbivores and introducing grazing livestock and exotic trees (McPherson 1997; Silva et al. 2001; Cabral et al. 2003; Altesor et al. 2006). In South America, in the temperate savanna of El Palmar National Park (EPNP) in north-east Argentina, three native shrub species—*Acanthostyles buniifolius*, *Baccharis pingraea* and *Baccharis dracunculifolia* (all Asteraceae)—have increased their distributional range and abundance since the National Park was established in 1965. These changes in range and abundance have been associated with changes in management practices, including control of fire, which previously occurred annually or biennially, and complete exclusion of grazing (Goveto 2005). If fire had been completely suppressed, shrub encroachment would have been dominated by fire-sensitive species, as in other South American savannas (Moreira 2000). However, this is not the case in EPNP where, despite efforts to prevent fires, most of the park has been burnt at least once between 1968 and 2000, and some areas have been burnt on as many as 13 occasions (Goveto 2005). The three species have different regeneration strategies after fire (Galíndez et al. 2009a). *A. buniifolius* is a sprouter, *B. dracunculifolia* is an obligate seeder and *B. pingraea* is a facultative sprouter (i.e. it can regenerate both from seeds and from sprouts). We predicted that, regardless of their regeneration strategy, the establishment of all three species in environments where fire is suppressed would show strong dependence both on the annual seed rain and on germination from the soil seed bank, whereas in environments experiencing frequent fires, establishment would depend primarily on the annual seed rain. To test these predictions we quantified the dynamics of the seed bank and seed rain both with and without fire for all three species, and developed a conceptual model to assess how the annual seed rain and fire regime affected their seed bank dynamics and establishment. The results are used both to explain the encroachment of shrubs in these South American temperate savannas and to suggest appropriate management practices for their control.

Materials and methods

Study area

The study was carried out in EPNP (31° 55′ S, 58° 17″ W), an 8,500 ha area of protected habitat located in the Entre Ríos province of Argentina. The climate is temperate (mean annual temperature 19.0 ± 3.8 °C), and wet (mean annual precipitation 1,346 ± 291 mm), with 58 % of the rain falling during the warm season (October–April). From 2000 to 2010, precipitation was higher than this long-term average (mean 1,445 ± 63 mm), with 46 % of the rain falling during the autumn (March–June) and 30 % falling during spring (September–December). The study area was located on well-drained, fine sandy loam soils with vegetation dominated by shrubs characteristic of warm regions, including members of the genera *Acanthostyles, Baccharis*, *Eupatorium* and *Vernonia* and by grasses including *Panicum prionitis*, *Paspalum quadrifarium*, *Andropogon lateralis* and *Axonopus sufultus*. Biomass

is higher in patches with less shrub cover (Goveto 2005). The study area was subjected to accidental fires in summer 1992 (in December) and 1997 (in March). Part of the area (referred to below as the burnt site) was also burnt during an intense wildfire in January 2000, whereas the remainder (referred to below as the unburnt site) has not been burnt since 1997.

Study species

The study species are perennial shrubs that grow up to 3 m in height. All are native to Argentina, Brazil, Uruguay, Paraguay and Bolivia (Zuloaga and Morrone 1999). *Acanthostyles buniifolius* (=*Eupatorium buniifolium*) is hermaphroditic whereas *B. pingraea* (=*B. medullosa*) and *B. dracunculifolia* are dioecious. Flowering typically occurs from December to March. Several achenes (hereafter referred to as seeds) are produced per capitulum, and dispersed by wind between February and May (Burkart 1974). All three species produce seeds with small mass and size (0.06–0.1 mg and ≤1 mm, Galíndez et al. 2009b). The life-span of *B. dracunculifolia* is approximately 5 years, whereas *B. pingraea* and *A. buniifolius* can live for around 10 and 20 years, respectively.

Types of seed bank and germination requirements

To determine the type of seed bank (i.e. transient or persistent) accumulated by each species, 30 plastic trays (20 × 20 cm in area and 5 cm in depth), each containing sterilized soil, were placed at randomly chosen locations within the burnt site just before seed dispersal in January 2000, so that they received dispersing seeds in that year both from plants that had re-sprouted after fire and from plants growing in nearby unburnt habitat. *B.pingraea* sprouted and produced seeds on the burnt site in 2000, whereas *A. buniifolius* sprouted but did not produce seeds until 2001. *B. dracunculifolia* did not produce seeds on the burnt site until 2002. All three species produced seeds on the unburnt site in 2000, 2001 and 2002. The size of trays used for these samples (20 × 20 × 5 cm—i.e. 2,000 cm^3 of soil) was also used in several other parts of the study, and is referred to below as a sampling unit. Seeds were prevented from dispersing onto the surface of these soil samples for the next 2 years by covering them with fine nylon mesh (0.07 × 0.07 mm). No seeds remain on parent plants after dispersal ends in

May, so that in each of these years, the filters were removed at the end of seed dispersal. Thus, the only seeds of the study species that accumulated in these soil samples were those dispersed in early 2000. Seedlings that emerged from these soil samples were identified and counted every month from January 2000 until December 2002, and then removed. No seedlings emerged after December 2002.

Germination requirements and longevity of seeds of the three species were determined in the laboratory, using seeds collected in March 2000. Four replicates of 25 seeds per treatment per species were sown in Petri dishes on the surface of 1 % agar, and incubated under either constant (25 °C) or alternating (30/20 °C) temperature regimes under either an 8 h light/16 h dark photoperiod regime, or in complete darkness. Germination was recorded daily for 30 days in the light/dark treatments. For the dark treatment the Petri dishes were wrapped in aluminium foil, and germination was recorded once, after 30 days. For all species, germination was defined as having occurred when the emergent radical was ≥1 mm in length.

To evaluate seed longevity, seeds collected in 2000 were stored in paper bags under laboratory conditions (15–25 °C), and their germination tested after 1 and 2 years. Because of the results obtained from the experimental examination of germination requirements (see below), seed germination was only evaluated under alternating temperature, and under the light/dark regime, as described above.

Seed bank dynamics model

The dynamics of seeds in the seed bank were examined, and the effects of different fire regimes on seedling establishment were modelled for each species. The phases and transitions considered in the model are illustrated in Fig. 1, and estimation of values for each of the input variables is explained below. All values in the model were quantified in terms of seed or seedling densities m^{-2}.

The number of *germinable seeds produced* (A in Fig. 1) was estimated from the mean number of capitula produced by 20 randomly selected reproductive individuals of each species at each of the study sites. The total number of flowering branches was counted for each individual, and three branches were randomly selected, harvested and taken to the laboratory to determine the number of capitula produced. Thirty

Fig. 1 Conceptual model of the seed bank and seedling establishment dynamics. *Numbers* next to the *arrows* refer to calculations of the proportions of the seeds or seedlings making the transition between the stages linked by the *arrows*. See text for a full description of each of these calculations
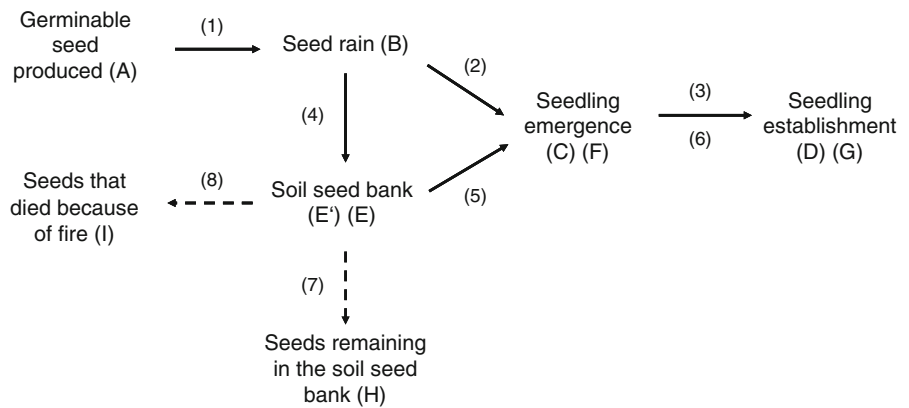
capitula were selected at random from these branches, and the seeds from each one were placed in separate Petri dishes, on filter paper soaked in distilled water, and incubated at 30/20 °C in an 8 h light/16 h dark regime. The total number of germinable seeds produced m^{-2} by each species was estimated as the mean number of germinated seeds per capitulum × the mean number of capitula per branch × the total number of flowering branches per reproductive individual × the density of reproductive individuals of the species.

To estimate densities of seeds arriving as *seed rain, and of seedlings emerging and seedlings establishing* from the seed rain (B, C and D in Fig. 1, respectively), 240 plastic trays, each containing a sampling unit of sterilized soil, were placed randomly within the burnt site before seed dispersal commenced in January 2000. Once seed dispersal had finished (May 2000), 120 of these trays were transferred to a greenhouse with a temperature of 25 ± 5 °C, an 8 h light/16 h dark cycle and weekly watering. The number of seeds arriving as *germinable seed rain* was estimated indirectly to be equal to the number of seedlings that emerged from these trays (see Cohen et al. 2004) until emergence ceased in June 2001. The other 120 trays remained in the field, and *seedling emergence* was recorded every month until seed dispersal began in the following year (January 2001). *Seedling establishment* was estimated as the proportion of these seedlings that survived in the field over 2 years (June 2000–June 2002).

To estimate densities of seeds in the *soil seed bank*, and densities of s*eedlings emerging* and *seedlings establishing* m^{-2} from the soil seed bank (E, F and G in Fig. 1, respectively), the surfaces of 240 sampling units were marked out before seed dispersal began in January 2000 in randomly chosen locations in the burnt site.

Before dispersal began, 120 of these sampling units were selected at random and excavated to a depth of 5 cm. Each was placed in a plastic tray and transferred to a greenhouse where the *germinable soil seed bank* was estimated indirectly by the number of emerging seedlings recorded each month until emergence ceased. Seed input into the 120 sampling units that remained in the field was prevented by covering them with fine mesh during seed dispersal (January–May 2000 and January–May 2001) and *seedling emergence* was recorded every month in each of these sampling units until December 2001, after which no more seedlings emerged. *Seedling establishment* was estimated as the proportion of emerged seedlings that survived in the field over 2 years (December 2000–December 2002). The number of *germinable seeds remaining in the soil seed bank* (H in Fig. 1) was estimated as the difference between (E) and (F) over 2 years (December 2000–2002). The number of *seeds that died because of fire* (I in Fig. 1) was estimated as the difference between E and E', where E' is the number of seeds that emerged from the soil seed bank at the unburnt site, determined using the same methods as in the burnt site).

The probabilities of transitions 1–8 (Fig. 1) between the phases in the model were estimated as follows: transition 1 = B/A; transition 2 = C/B; transition 3 = D/C; transition 4 = 1—transition 2; transition 5 = F/E; transition 6 = G/F; transition 7 = 1—transition 5; and transition 8 = I/E'.

Seed bank dynamics: the role of the seed rain and the fire regime

To examine how seed bank dynamics and seedling establishment of each of the three species were
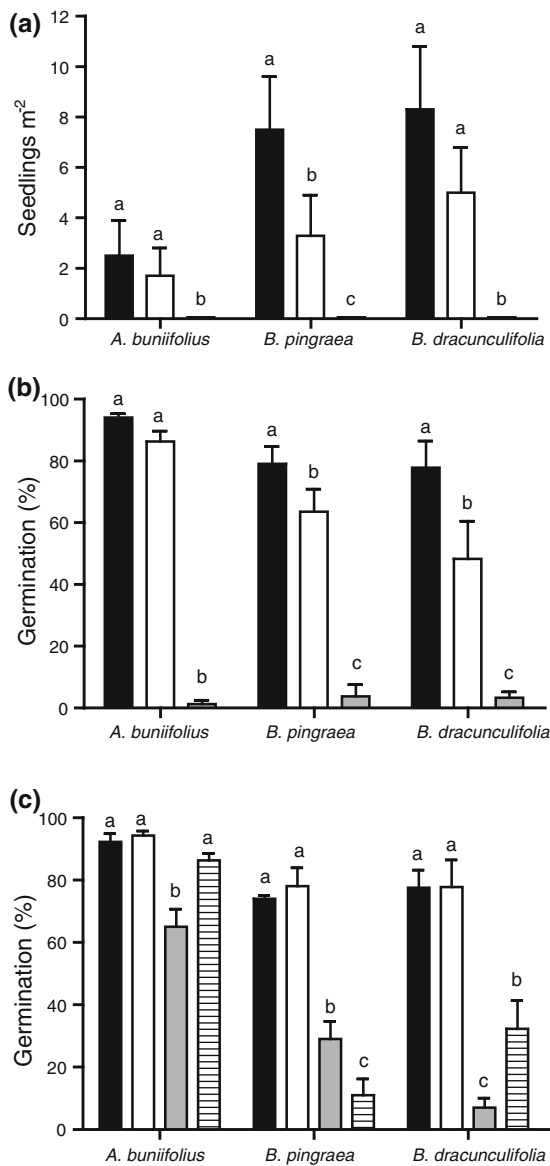
Fig. 2 **a** Mean (±SE) number of seedlings m^{-2} emerging at the burnt site from seeds dispersed in 2000 (■), from seeds remaining in the soil seed bank for 1 year (□), and from seeds remaining in the soil seed bank for 2 years (▦), for the three study species. **b** Mean (±SE) seed germination percentages of fresh seeds (■), seeds stored for 1 year under laboratory conditions (□) and seeds stored for 2 years under laboratory conditions (▦), for the three study species. **c** Mean (±SE) seed germination percentages under different light and temperature conditions (■: 25 °C with light, □: 20/30 °C with light, ▦: 25 °C without light, ≡: 20/30 °C without light) for the three study species. *Different letters* above the *bars* indicate significant differences ($P < 0.05$ or greater) between numbers of seedlings that emerged for each species after different periods of time, or for percentage germination of each species when subjected to different incubation conditions. These statistical comparisons are only made on results within species

affected by seed rain and fire regime, we used the transitional values generated above and ran a model, in which the *germinable seeds produced* phase was modified according to two management scenarios, namely *without fire*, in which it was assumed that fire occurred only in 2000, and *with annual fires*, in which it was assumed that fire occurred each year from 2000 to 2002 inclusive, with neighbouring unburnt areas remaining as sources of seeds. *Germinable seeds produced* in 2001 and 2002 were estimated following the methods used to determine seed production in 2000.

Since seed longevity does not exceed 2 years for any of the species (see Results), the model only considered seeds present in the soil seed bank in 2001 and 2002, and those arriving by dispersal in the previous years (2000 and 2001) as sources for recruitment of new plants. Seeds that did not germinate in 2000, 2001 or 2002 were considered to be dead.

Statistical analysis

The proportion of seeds that germinated in the laboratory under different light and temperature conditions was compared by ANOVA, after arcsine$\sqrt{prop}$ transformation to normalise the data, with light, temperature and their interaction as main effects. A Tukey comparison of means test was used to determine significance of differences between treatments when ANOVA results were significant. The number of germinable seeds produced in 2000, 2001 and 2002 was compared using Kruskal–Wallis tests (InfoStat 2005).

Results

Types of seed bank and germination requirements

For all three species, seedlings emerged both from recently dispersed seeds and from seeds that had been in the soil seed bank for up to 1 year. Virtually no seedlings emerged from seeds dispersed 2 years previously for any of the species (Fig. 2a). For all species, freshly harvested seeds, and seeds stored for a year under laboratory conditions, showed higher germination percentages (50–90 %) than seeds that had been stored for 2 years (<4 %, Fig 2b).
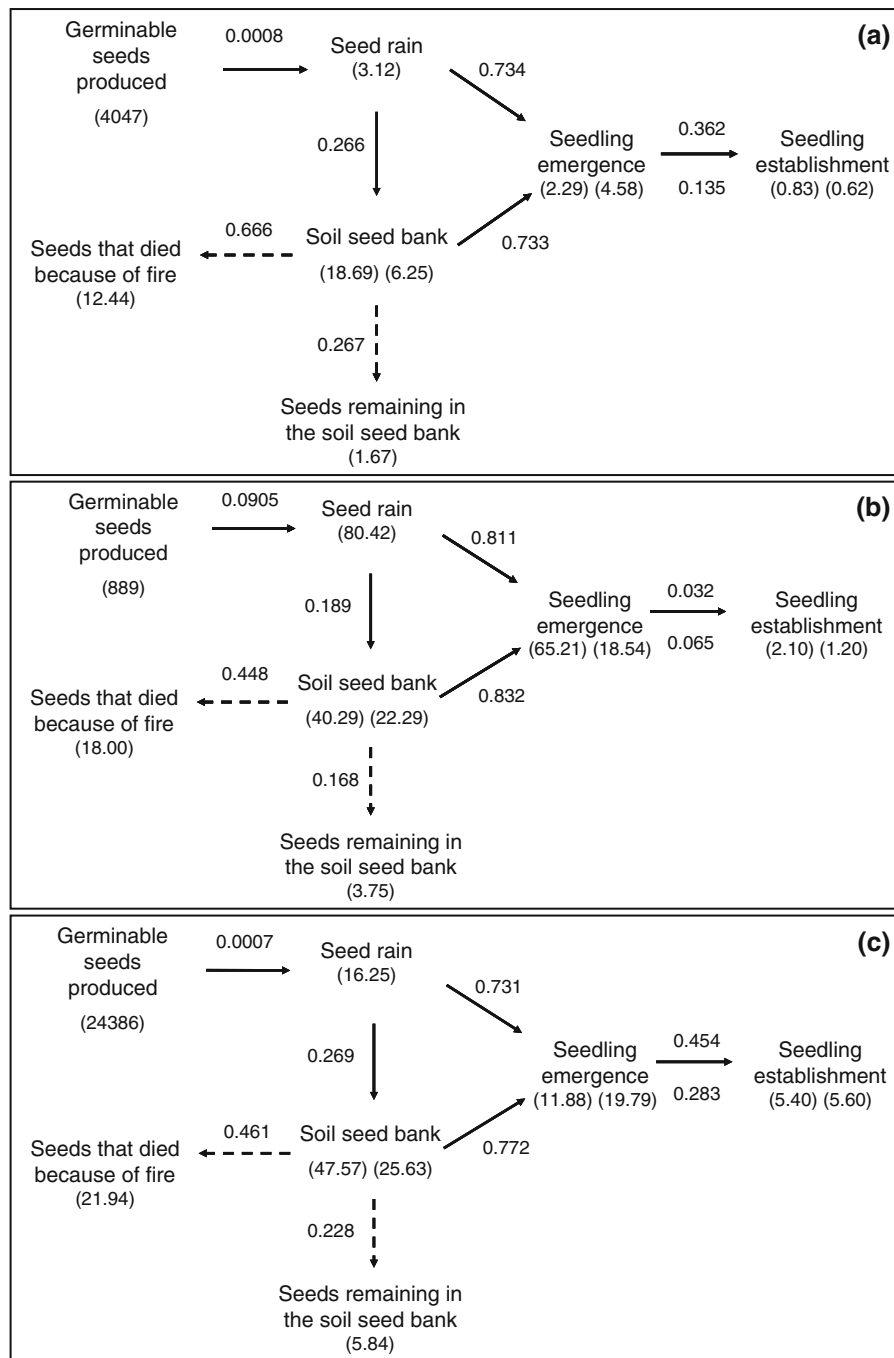
Fig. 3 Seed bank and seedling establishment dynamics of **a** *A. buniifolius,* **b** *B. pingraea* and **c** *B. dracunculifolia,* after the fire of 2000 at the burnt site. The values in *parentheses* are numbers m^{-2} and the values next to the *arrows* are the proportions of the seeds or seedlings making the transition between the stages linked by the *arrows*

Germination of seeds of all species was affected by treatment (Fig. 2c). For *A. buniifolius* (the sprouter species) and *B. dracunculifolia* (the obligate seeder species) germination was affected by the interaction between light and temperature ($F_{1, 12} = 5.4$, $P = 0.038$ and $F_{1, 12} = 6.2$, $P = 0.028$, respectively). For *A. buniifolius* the lowest germination occurred in darkness at constant temperature (65 %), whereas for

B. dracunculifolia, the lowest percentages were recorded in darkness at constant and alternating temperatures (7 and 30 %, respectively). For *B. pingraea* (the facultative sprouter), only light affected germination ($F_{1, 12} = 126.4$, $P < 0.0001$). Germination was higher in light (on average 76 %) than in dark treatments (on average 26 %).

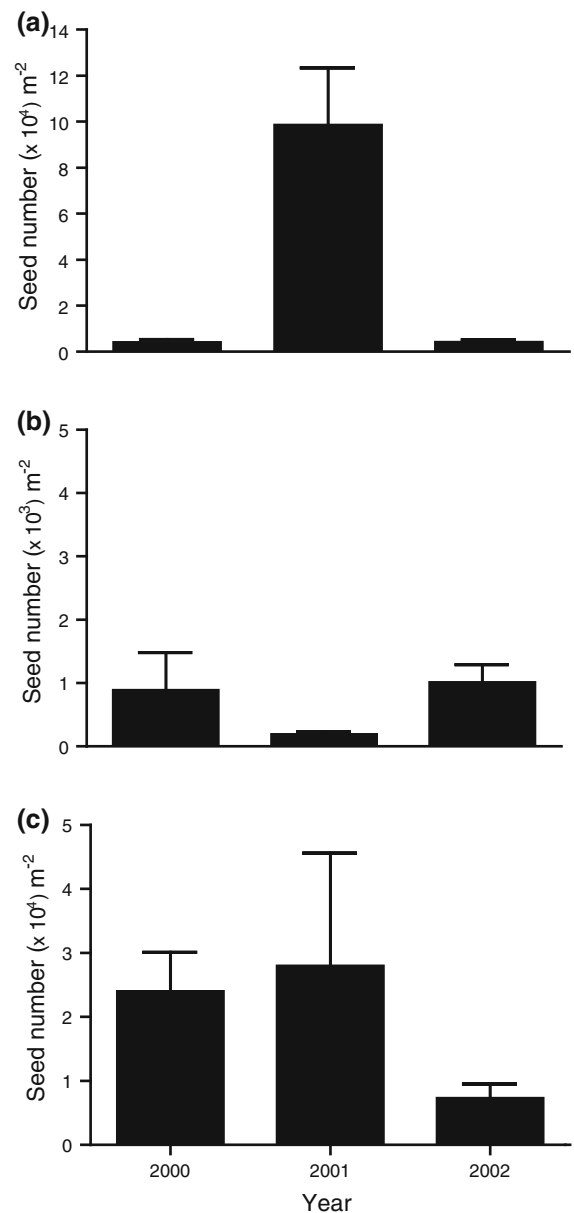Seed bank dynamics model

For all three species, the seed rain produced seedlings immediately after dispersal, in autumn (March–June), whereas seedlings arising from the soil seed bank emerged during the spring months (September–December).

For *A. buniifolius* (Fig. 3a), 0.08 % of the germinable seeds produced in 2000 reached the soil in the form of seed rain, of which 73 % produced seedlings and the rest entered the soil seed bank. 36 % of the seedlings that emerged from the seed rain in 2000 were still alive in 2002 (0.83 plants m^{-2}). Of the seeds that were already present in the soil seed bank before seed dispersal, 67 % were killed by fire in 2000. Of the remaining seeds, 73 % produced seedlings and 13 % of these were still alive in 2002 (0.62 plants m^{-2}). Seeds that did not germinate from the seed bank that existed prior to seed dispersal remained in the soil seed bank (1.67 seeds m^{-2}).

For *B. pingraea* (Fig. 3b), 9 % of the germinable seeds produced in 2000 were added to the soil as seed rain, of which 81 % produced seedlings in the same year. Only 3 % of these seedlings were still alive in 2002 (2.10 plants m^{-2}). Of the seeds that were present in the soil seed bank before 2000, 45 % were destroyed by the fire in that year. Of the remaining seeds, 83 % produced seedlings and 6 % of these were still alive in 2002 (1.20 plants m^{-2}). The 17 % of seeds that did not germinate remained in the seed bank (3.75 seeds m^{-2}).

For *B. dracunculifolia* (Fig. 3c), 0.07 % of the germinable seeds produced in 2000 reached the soil as seed rain, and 73 % of these seeds produced seedlings in 2000. 45 % of these seedlings were still alive in 2002 (5.40 seedlings m^{-2}). Of the seeds recorded in the soil seed bank prior to 2000, 46 % were killed by the fire in that year. Of the remaining seeds, 77 % produced seedlings in 2000 and 28 % of these seedlings were still alive in 2002 (5.60 seedlings m^{-2}). The 23 % of seeds that did not germinate remained in the seed bank (5.84 seeds m^{-2}).

Fig. 4 Mean (±SE) number of germinable seeds produced m^{-2} by **a** *A. buniifolius*, **b** *B. pingraea* and **c** *B. dracunculifolia,* in each of the studied years at the burnt and unburnt sites. Note differences in scale on the y-axis

Seed bank dynamics: the role of the seed rain and the effect of fire regime

The number of germinable seeds produced m^{-2} showed significant variation between years for all three species (*A. buniifolius*: *Kruskal–Wallis* = 54.3, $P < 0.0001$; *B. pingraea*: KW = 10.7, $P = 0.004$, *B.*
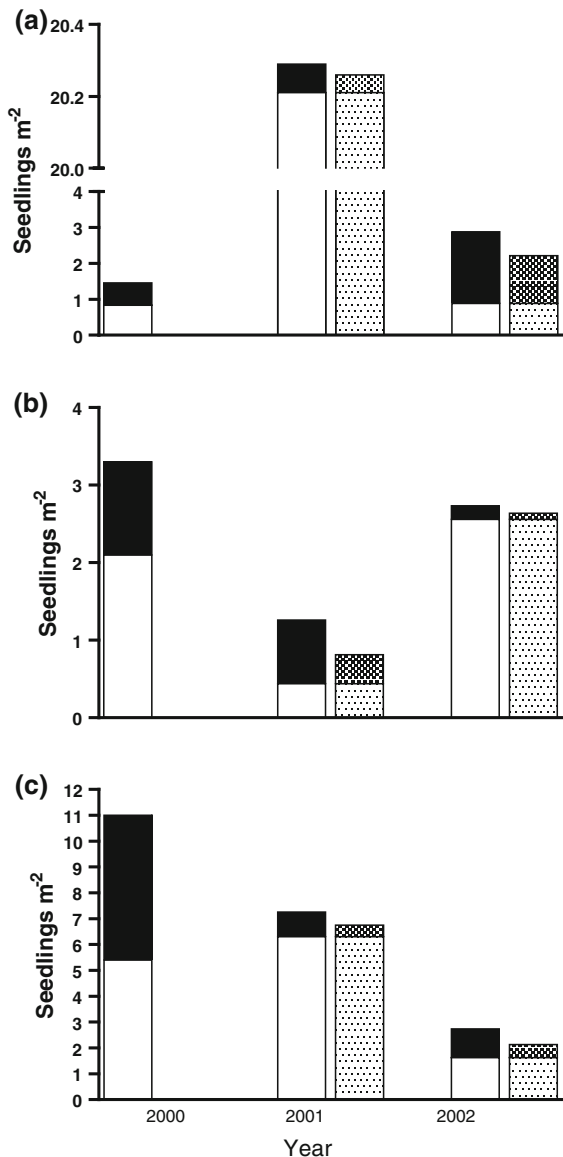
Fig. 5 Seedling establishment (seedlings m^{-2}) from the seed rain (□) and from the soil seed bank (■) in the *without fire* scenario, and from the seed rain (⬚) and from the soil seed bank (▨) in the *annual fire* scenario, for each year, of **a** *A. buniifolius*, **b** *B. pingraea* and **c** *B. dracunculifolia*

dracunculifolia: $KW = 20.88$, $P < 0.0001$, Fig. 4). For *A. buniifolius*, seed production was on average 23 times higher in 2001 (98,746 germinable seeds m^{-2}) than in the other 2 years (Fig. 4a). For *B. pingraea* it was on average 5.3 times higher in 2000 and 2002 (1,085 germinable seeds m^{-2}) than in 2001 (Fig. 4b), and for *B. dracunculifolia*, seed production was on

average 3.6 times higher in 2000 and 2001 (24,386 germinable seeds m^{-2}) than in 2002 (Fig. 4c).

In the *without fire* scenario, the model predicted that seedling establishment would be higher for all species in years in which seed production and seed rain were also higher (Fig. 5). For *A. buniifolius* and *B. pingraea,* seedling establishment in years with low seed rain depended mainly on recruitment of seedlings from the seed bank (Fig. 5a, b). In contrast, for *B. dracunculifolia*, seedling establishment also depended on seed rain in years with low seed production (Fig. 5c). In the *annual fire* scenario, the effect of fire was more evident on all species in years in which seedling establishment was lower. The magnitude of this effect was greater in years in which seedling recruitment derived mainly from the seed bank rather than from the seed rain (e.g. *A. buniifolius* in 2002 and *B. pingraea* in 2001).

Discussion

Types of seed bank and germination requirements

The type of seed bank exhibited by species characteristic of fire-prone environments is often reported as being predictable from life-history traits. For example, obligate seeders often have persistent seed banks, since their regeneration after fire depends exclusively on germination from seeds, whereas many species that resprout after fire have transient seed banks (Keeley 1987; Zammit and Zedler 1988; Whelan 1995; Auld et al. 2000,). Our results show that, regardless of regeneration strategy (sprouter, facultative sprouter and obligate seeder, respectively), seeds of the three species studied here—*A. buniifolius*, *B. pingraea* and *B. dracunculifolia*—can remain viable in the soil for up to 2 years, i.e. they have short-term persistent seed banks (Thompson 1993). Auld et al. (2000) obtained similar results for obligate seeder and sprouter species in fire-prone communities of south-eastern Australia, suggesting that even for sprouter species, short-term persistent seed banks contribute to population persistence.

We also found that a high proportion of the seeds of the three species studied here germinate in conditions of high light. Such conditions pertain after fire (Galíndez 2008, 2009b). All three species also have small seeds. Other studies have identified small seed size, and a requirement for light to germinate, as characteristic of species with persistent soil seed banks

(Thompson et al. 1993; Auld et al. 2000; Jankowska-Blaszczuk and Daws 2007; Ortega-Baes et al. 2010; Flores et al. 2011).

Seed bank dynamics model

Some shrub species in fire-prone environments can produce many more seeds in a single good year than are stored in the soil, suggesting that many of the seeds produced each year are lost, either by being transported elsewhere or by being destroyed in situ (Keeley 1977). Our results support these observations. All three species produced many seeds after fire, but <10 % of the seeds produced were incorporated into the soil, probably either because they were dispersed elsewhere because of the absence of standing biomass forming a physical barrier to seed movement after fire, or possibly because of post-dispersal predation. Nevertheless, a high proportion of the seeds of all species that reached the soil germinated immediately after dispersal. In contrast, a number of other studies in fire-prone plant communities have shown that newly emerging seedlings are derived primarily from the soil seed bank rather than from seeds dispersed from neighbouring areas (Keeley and Zedler 1978; Moreno and Oechel 1992; Alexander and Schrag 2003). However, Oliveira (1998) obtained similar results to those reported here, with seeds of many wind-dispersed woody species of savanna habitats germinating immediately after dispersal. The three species studied here are all members of the Asteraceae, and once they reach sexual maturity they produce large numbers of wind-dispersed seeds every year. High seed production and wide dispersal, together with high levels of germination under the open conditions experienced following fire, are all characteristics that would promote success in fire-prone habitats.

Studies in fire-prone environments have shown that, even in low intensity fires, temperatures are high enough to kill most seeds on the soil surface, and survival and germination of seeds in the soil seed bank depends on their depth of burial (Zammit and Zedler 1988; Auld and Denham 2006). This study showed that many of the seeds in the soil seed bank were killed by fire, but a high proportion of seeds buried at depths of ≤1 cm are able to produce seedlings if they survive fire (G. Galíndez, personal observation), presumably because germination is stimulated by factors including increased soil temperature, increased light and nutrient

availability and/or removal of established plants (Keeley et al. 1985; Vilà and Lloret 2000; Ooi et al. 2006). Thus, the impact of fire on the sizes of the seed banks of the study species would be especially severe when the seed rain in the previous year has been small or non-existent. This would be the case when fire has been intense and/or it has covered a large area.

The probability of survival of seedlings in the field, as measured over 2 years, was low for all three species and it differed between seedlings that emerged from the seed rain and seedlings emerging from the soil seed bank. For *A. buniifolius* and *B. dracunculifolia*, seedlings germinating from the seed rain had higher survival than those germinating from the seed bank, whereas for *B. pingraea*, seedlings germinating from the seed rain had lower survival. This may be because germination of *A. buniifolius* and *B. dracunculifolia* occurred mainly in March–April, when temperature and rainfall are relatively high, whereas for *B. pingraea*, most seedlings emerged in May–June when conditions are colder and drier. García-Nuñez et al. (2001) reported that germination at the beginning of the summer is beneficial in savanna habitats with marked seasonality, because it maximises the length of the first growing period. Despite the differences in proportional survival, seedlings derived from the seed rain and seed bank yielded similar densities of surviving plants after 2 years in all three species.

Although all three species had short-term persistent seed banks, the observed densities of seeds and seedlings differed for each species at each phase of the model. In particular, the sprouter *A. buniifolius* produced significantly fewer seeds and seedlings than either the facultative sprouter *B. pingraea* or the obligate seeder *B. dracunculifolia*. Other studies have also shown that sprouters produce fewer seeds, smaller seed banks, and almost always have fewer seedlings and lower seedling survival than non-sprouters (e.g. Bond and van Wilgen 1996).

Seed bank dynamics: the role of the seed rain and the influence of fire regime

Annual seed production fluctuated several-fold in all three species. Galíndez et al. (2009a) reported that all three species allocate a higher proportion of their aerial biomass to seed production in years in which they first reproduce than in later years of life. Higher reproductive effort has been reported to be

advantageous in environments where the timing of fire is unpredictable (Parker and Kelly 1989; Auld and Denham 2006). For *A. buniifolius* and *B. pingraea*, high seed production in 2001 and 2000, respectively, was associated with the first reproduction of re-sprouted plants in the burnt area, and for *B. dracunculifolia*, high seed production in 2000 was associated with the first reproduction of 3-year-old plants in the unburnt site that had previously been burnt in 1997. As the obligate seeder *B. dracunculifolia* generally takes 3 years to achieve sexual maturity, the presence of a persistent soil seed bank and/or a continuous input of seeds from neighbouring areas, is vital for the persistence of this species in savanna ecosystems with unpredictable occurrence of fire. In contrast, *A. buniifolius* and *B. pingraea* are also able to persist because of their ability to re-sprout following fire.

Early establishment is less dependent on annual seed rain in species with persistent seed banks than in those with transient seed banks (Auld et al. 2000). However, germination, and death of seeds due to loss of viability or fire (Whelan 1995; Hyatt and Casper 2000), leads to a requirement for the addition of seed rain to the soil every year to maintain the seed bank and contribute to the establishment of new plants, as suggested by the model. When the model was run without fire, seedling establishment depended both on the number of seeds deposited on the soil surface in a given year and on the number of seeds remaining in the seed bank from the previous year. In contrast, with the combination of relatively small-scale annual fires (i.e. <50 ha in area, as currently occurs in the Park), and unburnt areas remaining to provide a source of seed rain, establishment depended much more on seed rain than on the seed bank remaining after fire. This suggests that if no reproducing plants remain as seed sources in neighbouring unburnt areas following fire, establishment of new plants will be virtually dependent on germination from the seed bank. Moreover, if there is no seed rain for two or more consecutive years, the seed banks of these species will be completely depleted, resulting in no seedling emergence. Therefore, seed rain is essential both for maintenance of their seed banks and for the long-term persistence of their growing populations.

Consequently, when these savannas were subject to annual fires these shrub species were either excluded or had low population densities (Martinez Crovetto and Piccinini 1951). In the case of the seeder,

B. dracunculifolia, the high frequency of fire prevented completion of the life cycle since it needs 2–3 years to achieve sexual maturity. In the case of the sprouting species, *A. buniifolium* and *B. pingraea*, the high frequency of fire is likely to have limited the capacity to store carbohydrates in their lignotubers. Therefore, persistence of all three species in this community would have been strongly dependent on seed rain from neighbouring areas. When the intervals between consecutive fires became longer than 3 years, and smaller areas were burnt, seeds of these shrubs were able to colonise unburnt areas and establish much denser populations, which also contributed seeds to the seed bank. This was important for all species, but especially for the relatively short-lived *B. dracunculifolia*, with its dependence on establishment from seed.

These results explain the changes that have been observed in the vegetation of South American temperate savannas during the last 50 years. When these habitats were subject to frequent fires (i.e. every 1–2 years) the shrub species studied here occurred at low densities, and their persistence was strongly dependent on the germination of seeds arriving as seed rain from neighbouring unburnt areas. As fire became less frequent, these shrubs increased in density and in the area they occupied, as a result of the establishment of seedlings emerging both from the seed rain and the seed bank. By revealing the crucial role of these two sources of seed in contributing to the population dynamics of shrub species with different regeneration strategies, this study provides vital information on which management practices for conservation of the temperate savannas of South America can be based. Implementation of a management regime that only restores frequent and small-scale (e.g. <50 ha) fires will not be effective in controlling shrub encroachment, because populations can be sustained by seeds arriving as seed rain from nearby unburnt habitat. Very large-scale fire is almost impossible to apply as a management tool at EPNP because of technical and practical difficulties; consequently unburnt areas always remain that are seed sources for these species. Current management to prevent the encroachment of woody species in many temperate savannas includes prescribed fires in combination with other actions including mechanical and chemical control, and grazing by livestock (Redhead et al. 2012; Brudvig et al. 2011). However, grazing has been prevented at EPNP since this protected area was

created in 1965, even though it would contribute significantly to the control of shrub encroachment. In support of this contention, Galíndez (2008) reported that *B. pingraea* and *B. dracunculifolia* are not present in areas where both burning and grazing occur. We therefore recommend the application of both frequent burning and grazing, to restore the savanna vegetation to its former condition.

Conclusion

Acanthostyles buniifolius, *B. pingraea* and *B. dracunculifolia* possess short-term persistent seed banks and high annual variability in seed production. In environments without fire, establishment depends strongly on germination both from the seed rain and the soil seed bank, whereas in environments subjected to annual fires, establishment is primarily dependent on germination of seeds arriving in the annual seed rain, regardless of species' regeneration strategies. These results help to explain changes observed in the vegetation of these South American temperate savannas during the last 50 years, revealing the crucial role of the soil seed bank and seed rain in maintaining populations of shrub species with different regeneration strategies and providing valuable information for the design of management practices for their control.

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