

Effects of Fire on Seedling Diversity and Plant Reproduction (Sexual vs. Vegetative) in Neotropical Savannas Differing in Tree Density

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ABSTRACT

Little is known about the effects of fire on the structure and species composition of Neotropical savanna seedling communities. Such effects are critical for predicting long-term changes in plant distribution patterns in these ecosystems. We quantified richness and density of seedlings within 144 plots of 1 m² located along a topographic gradient in long-unburned (fire protected since 1983) and recently burned (September 2005) savannas in Brazil. These savannas differ in tree density and canopy cover. Sites along the gradient, however, did not differ in species composition prior to the fire. In recently burned savannas we also evaluated the contribution of vegetative reproduction relative to sexual reproduction by quantifying richness and density of root suckers. Finally, we tested seed tolerance to pulses of high temperatures—similar to those occurring during fires on the soil surface and below—of five dominant savanna tree species. Seedlings were more abundant and diverse in unburned than in burned savannas. Seedling species composition differed among unburned and burned savannas probably reflecting early differences in root: shoot biomass allocation patterns. In recently burned savannas, root suckers were more abundant and diverse than seedlings. Relatively long exposures (>10 min) of temperatures of 90 °C reduced seed germination in all studied species suggesting a negative effect of fire on germination of seeds located at or aboveground level. Because vegetative reproduction contributes more than sexual reproduction in burned environments, frequent fires are likely to cause major shifts in species composition of Neotropical savanna plant communities, favoring clonally produced recruits along tree density/topographic gradients.

Abstract in Portuguese is available in the online version of this article.

Key words: Brazil; cerrado; disturbance; heat shocks; root suckers.

FIRE IS A MAJOR DISTURBANCE FACTOR IN TROPICAL SAVANNAS (Bond & van Wilgen 1996). The Brazilian Cerrado, which contains the largest savanna region in South America (Gottsberger & Silberbauer-Gottsberger 2006), covers approximately two million km², nearly 23 percent of the country (Oliveira & Marquis 2002). The total number of woody species in the Brazilian savannas is about 2000, and individual sites may contain up to 70, or more, woody species per hectare (Haridasan 2008). Many Cerrado savannas burned every 1–3 yr (Hoffmann 2002), although some remain unburned for longer periods. Most savanna species are therefore likely to have evolved with a long history of periodic burning, as natural fires occurred in these savannas long before human-induced ones did (Ledru 2002). However, the recent expansion of human occupation of the Cerrado during recent decades has changed the natural fire regime—both the season and frequency of burning—which has had important consequences for the structure and composition of vegetation (Miranda *et al.* 2002).

Brazilian savannas are characterized by a strong seasonality of precipitation and by well-drained, deep, nutrient-poor soils (Furley 1999). These savannas exhibit consistent changes in tree

canopy cover, tree density, and tree size along shallow topographic gradients of 30–60 m in elevation and a few km in length (Goodland & Pollard 1973). Vegetation structure varies from closed savannas with a relatively high density of tall trees (up to 12 m) in the upper-most portions of the gradient, to open savannas with relatively few small trees in the lowest portions of the topographic gradient. In contrast, herbaceous plants are more abundant in open than in closed savannas (Eiten 2001).

Fires are common during the dry season (April–September), when low relative humidity and abundant dry biomass of herbaceous species increase the risk of fire spread (Coutinho 1990). During the wet season (October–March) the vegetation may be susceptible to burning especially after long rain-free periods (Coutinho 1990). Natural fires also occur during the transition between wet and dry seasons (Ramos-Neto & Pivello 2000). Most savanna species disperse their seeds during the late dry season and early wet season (Salazar *et al.* 2012a) and therefore seeds of many species may encounter fire events during their lifetime. The effects of fire on seed germination and further seedling establishment largely depend on the tolerance to high temperatures to which seeds are exposed during fires (Ribeiro *et al.* 2013). Long exposures to high temperatures are expected to negatively affect seed viability. However, seeds could tolerate short exposures to high temperatures or long exposures to moderate

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temperatures. Certain combinations of temperature / time of exposure could even break dormancy and speed germination (Ribeiro *et al.* 2013). The effect of fire on seed germination of savanna woody species, however, remains unclear. While some studies have shown positive effects of pulses of temperatures ranging from 70 to 100 °C on the germination of several woody species, in particular those with hard seed coats (Heringer 1971), other studies have shown negative or no significant effects of high temperatures on seed germination of savanna tree species (Rizzini 1976, Felipe & Silva 1984, Ribeiro *et al.* 2013).

Although fire is a major determinant of the vegetation structure in Neotropical savannas (Furley 1999), little is known about its effects on seedling establishment, particularly in savannas that differ in tree density and canopy cover. Because of species-specific differences in fire tolerance (Gignoux *et al.* 2009), fire may greatly influence the structure and species composition of seedling communities by constraining post-fire germination and seedling establishment of woody and herbaceous species differentially along tree density gradients in these savannas. Differential post-fire seedling establishment is particularly important because most Neotropical savanna woody species do not form persistent soil seed banks (Salazar *et al.* 2011) and because of high post-dispersal seed removal and short seed longevity (Salazar *et al.* 2012a).

We aimed to evaluate seedling establishment of woody and herbaceous species along a tree density gradient in long-unburned (fire protected since 1983) and recently burned (September 2005) Neotropical savannas that differ in tree density and canopy cover, from closed (40–60% canopy cover) to open savannas (~10% canopy cover). Also, we aimed to experimentally test seed tolerance to pulses of high temperatures—similar to those occurring during fires on the soil surface and below—of five dominant savanna tree species.

Although many savanna species reproduce vegetatively via root suckers (Rizzini & Heringer 1962) and sucker production largely increases following fires (Hoffmann 1998), the importance of vegetative reproduction relative to sexual reproduction in burned areas is less known than in long-unburned areas at the community level. In long-unburned areas, sexual reproduction contributes more than vegetative reproduction to population growth (Miyanish & Kellman 1986, Ramos 1990, Matos 1994, Hoffmann 1999, Miranda *et al.* 2002). Thus, we also aimed to evaluate the contribution of vegetative reproduction relative to sexual reproduction in burned savannas by quantifying density and richness of root suckers and seedlings.

Compared with adults, seedlings and juveniles are less likely to resist fire because they may lack thick bark that helps insulate living tissues from high fire temperatures (Miranda *et al.* 2002). Therefore, we expect higher richness and density of seedlings in long-unburned savannas compared with recently burned savannas. We also expect differential seedling species composition between unburned and burned savannas because plants differ greatly in their tolerance to fire and in their capacity to recover subsequently (Gignoux *et al.* 2009). As fire greatly limits seed supply and seedling establishment of fire-sensitive species (Moreira 2000, Hoffmann & Moreira 2002, Nefabas & Gambiza 2007),

but tends to favor the establishment of species that are able to reproduce vegetatively via root suckers (Oliveira & Silva 1993, Hoffmann 1998, Setterfield 2002, Hoffmann & Solbrig 2003), we expect higher richness and abundance of root suckers relative to seedlings in recently burned savannas.

Here, we coupled observational and experimental results to address the following questions: (1) do richness, density, and species composition of seedlings differ among long-unburned and recently burned savannas? (2) Do richness and density of seedlings and root suckers differ in recently burned savannas? (3) How do pulses of high temperatures affect seed germination of savanna trees under controlled experimental conditions?

METHODS

STUDY SITE.—We conducted this study from June 2005 to July 2008 at the Instituto Brasileiro de Geografia e Estatística (IBGE) a 1300-ha reserve located 35 km south of Brasília, Brazil (15°56' S, 47°63' W, 1100 m asl). Mean monthly temperatures range from 19 to 23 °C. Average annual precipitation is ~1500 mm with a pronounced dry season from May to September. The months of June, July, and August are often completely rainless. Average relative humidity during the rainy season is about 80 percent and drops to 55 percent during the dry season when daily minimum relative humidity may reach values as low as 10 percent. The IBGE reserve contains all major physiognomies from closed to very open savannas. Closed savannas are semi-closed woodland (40–60% tree crown cover) with a moderately high (usually 12 m) tree canopy. Because of the shade created by the canopy trees, the herbaceous and semi-woody ground vegetation has a lower cover than in relatively open cerrado vegetation. Intermediate savannas are dominated by trees and shrubs (canopy generally less than 7 m in height), with between 10–40 percent tree crown cover. Open savannas have few short trees and scattered shrubs over a nearly continuous grass layer (the woody layer usually covers less than 10% of the ground).

In June 2005, we established two transects of approximately 1000 m in length in the reserve, spanning three major savanna physiognomies: closed, intermediate, and open savannas. We placed the transects ~2000 m apart in different places of the IBGE reserve, each one along a continuous topographic gradient ranging from 1126 to 1143 m asl in physiognomic forms protected from fire since 1983. Because preliminary species-area curves in closed and open savannas indicated that about 85 percent of the maximum richness of woody plants (>1 m) was attained at 1024 m², we established randomly nine plots of 20 × 20 m along each transect (three per physiognomy).

In September 2005 (end of the dry season) a large accidental fire spreading from nearby reserves burned approximately half the reserve, including one transect we established in June 2005. Prior to the fire, the similarity (Bray–Curtis similarity coefficient) in species richness and abundance of woody plants (>1 m height) between physiognomies from each transect was 0.78, 0.74, and 0.67 for closed, intermediate, and open savannas, respectively. Species composition within each vegetation type did not differ

significantly from each other prior to the fire (ANOSIM $R_{\text{closed}} = 0.2$, $P = 0.54$; $R_{\text{intermediate}} = 0.28$, $P = 0.1$; $R_{\text{open}} = 0.35$, $P = 0.3$), but physiognomies differed in tree basal area and maximum tree height. We re-established the plots in the burned savannas in July 2007 (2 yr after the accidental fire).

SEEDLING DIVERSITY IN LONG-UNBURNED AND RECENTLY BURNED SAVANNAS.—To quantify seedling density and richness in long-unburned and recently burned savannas, we randomly established eight subplots of 1×1 m within each of the 20×20 m plots located along each transect, for a total of 144 subplots (24 per physiognomy). We established eight subplots of 1×1 m because preliminary species–plot curves in unburned closed and open savannas indicated that about 90 percent of the maximum richness of seedlings was attained using eight 1 m^2 plots (Salazar *et al.* 2012b). We identified and counted all seedlings up to 30 cm tall within each of the 1 m^2 plots located in unburned and burned savannas. We conducted the seedling censuses in July 2007, 2 yr after the accidental fire of September 2005 in the burned savannas; and 2 yr after we established the permanent plots in the unburned savannas. The unburned transect includes seedlings established until July 2007 and corresponds to Transect 2 of Salazar *et al.* 2012b. A survey in the unburned savannas indicated that seedling species composition did not differ significantly since plot establishment (July 2005) to seedling censuses (July 2007) ($R = 0.1$, $P = 0.6$; Fig. S1). Thus, the comparisons between unburned and burned sites highly reflect the changes due to fire in seedling species composition because sites did not differ significantly in species composition before the fire and also because seedling composition did not differ significantly from plot establishment until seedling censuses in the unburned plots.

To evaluate the importance of vegetative reproduction relative to sexual reproduction in burned savannas, we identified and measured the height of root suckers of woody and herbaceous species up to 30 cm tall. Root suckers refer to new stems originating from buried root buds at some distance from the original parent stem, but still connected to it. Sprouts originating from the base of roots or stems (root-crown and basal-stem sprouts, respectively) of adults were considered as a persistent mechanism rather than as a reproductive mechanism for extensive vegetative proliferation or clonal growth (Del Tredici 2001). We distinguished seedlings from suckers by double checking whether they

had distinct primary (tap) roots, lack of underground connection with roots or rhizomes of adult plants, and small size. To check for root connections we followed stems and roots by carefully removing litter and soil layers so we could exclude suckers from seedlings as much as possible. We used herbarium specimens, reference specimens collected in the field, and received help from local expert botanists to identify seedlings and suckers. We did not evaluate root suckers in the unburned transect or before the fire, and consequently the results, even though robust, should be interpreted carefully.

EFFECTS OF HEAT SHOCKS ON SEED GERMINATION OF WOODY SPECIES.—To evaluate seed tolerance of savanna woody species to high temperatures, we exposed seeds to pulses of high temperatures at different times (heat shocks). Seeds were collected from mature fruits of five dominant tree species (Table 1). These species span the entire or a large portion of the topographic gradient (Silva Júnior 2005); belong to a wide range of plant families; had a relative abundance higher than 10 percent in our study sites; and had initial seed viability higher than 50 percent. Despite differences in dispersal season and longevity, seeds of these species may encounter fire events during their lifetime (Table 1). After cleaning, we placed the seeds in 90-mm-diameter glass Petri dishes. We then heated the seeds at 30, 60, and 90 °C in an oven for 5, 10, and 15 min, using a factorial experimental design for a total of nine heat-shock treatments. Treatments simulate ranges of temperature time periods that have been found at depths from 0 to 7 cm depth in savanna soils during fires (Coutinho 1990, Miranda *et al.* 1993) or inside woody fruits of some species (Cirne 2002). We compared the treatments with a control (seeds not exposed to heat). Each treatment consisted of five replicates of 10 seeds, for a total of 500 seeds per species. After being heat shocked, we sowed the seeds in an artificial substrate (mix of 70 percent peat moss and 30 percent vermiculite) in germination trays. We randomly placed the trays on greenhouse benches, under temperature conditions of 25–28 °C, in 30 percent full sunlight, and watered them daily with an automated sprinkler system. We monitor germination (radicle emergence) every 3 d for 60 d.

DATA ANALYSES.—We examined differences in seedling richness and density of woody and herbaceous species in long-unburned and recently burned savannas at the subplot level with two-way

TABLE 1. Seed characteristics of the studied savanna woody species. Dispersal mechanism and fruiting period information from Silva Júnior (2005). Estimated longevity: number of months that seeds remain viable (viability >1%) in dry storage conditions at 20°C (Salazar *et al.* 2011).

Species	Family	Dispersal mechanism	Fruiting period	Mean fresh mass (g)	Longevity (months)
<i>Acosmium dasycarpum</i>	Leguminosae	Wind	March–June	0.039	13.43
<i>Brosimum gaudichaudii</i>	Moraceae	Mammal/bird	July–December	1.211	–
<i>Eriotbeca pubescens</i>	Malvaceae	Wind	August–November	0.120	5.81
<i>Ouatea hexasperma</i>	Ochnaceae	Bird	October–January	0.108	9.01
<i>Solanum lycocarpum</i>	Solanaceae	Mammal/bird/gravity	March–July	0.026	7.72

–; Not determined.

ANOVAs with fire and savanna physiognomy as fixed factors. We previously transformed the data to fulfill the assumptions of normality by taking the root square and adding 0.5 to each original value. We used Tukey HSD *post-hoc* tests for multiple comparisons of pairs of means. We also examined differences in richness and density of seedlings and suckers in recently burned savannas with two-way ANOVAs with physiognomy and reproductive strategy as fixed factors.

We used non-metric multidimensional scaling (NMDS) ordinations to examine patterns of species composition in unburned and burned savannas at the plot/community level (combining all subplots within each plot from each physiognomy). NMDS is a robust method for analyzing community data (Clarke & Warwick 2001). We performed the ordinations with PRIMER v5 (PRIMER-E Ltd, Plymouth, U.K.). The purpose of the ordinations was to represent the sites as points in a two-dimensional space, such that the relative distances among points reflect the relative dissimilarities of the sites. Similarities between every pair of plots from each site were calculated using Bray–Curtis similarity coefficient, after square-root transforming species abundances of every entry in the similarity matrix. We used an analog of the two-way-ANOVA (ANOSIM two-way crossed layout) to analyze the similarities in species composition among unburned and recently burned savannas under the null hypothesis that there are no differences between unburned and burned savannas allowing for physiognomy differences. ANOSIM routines are permutation/randomization tests that assess differences between average ranks between and within groups. Values on a scale of 0 (indistinguishable) to 1 (all similarities within groups are less than any similarity

between groups) reflect multiple pair-wise comparisons with groups well separated ($R > 0.75$), groups overlapping but differing ($R > 0.5$), or barely separated at all ($R < 0.25$). To examine the change in species composition due to fire in each physiognomy we used Spearman rank correlation coefficients (ρ) between all the elements of pairs of similarity matrices constructed with the abundance of each species before and after the fire. Values of ρ close to 1 indicate high similarity between matrices, whereas values close to 0 indicate low similarity between matrices.

We used failure time analysis (Kaplan–Meier model, Fox 2001) to examine differences in the relative rates of seed germination among heat-shock treatments in each species. We used log-rank (Mantel–Cox) tests (Krebs 1999) to perform pair-wise comparisons of the survival functions constructed with the number of seeds germinated in each heat-shock treatment during the study period. We performed these analyses with the program PASW Statistics 18 (SPSS, 2009).

RESULTS

We recorded a total of 56 woody species and 20 herbaceous species of seedlings (Table S1). Richness and density of woody seedlings were higher in unburned than in burned savannas (richness $F_{\text{fire } 1138} = 7.43$, $P = 0.007$; density $F_{\text{fire } 1138} = 7.33$, $p = 0.008$). The effect of fire on woody seedlings, however, was striking in closed savannas (Fig. 1; $F_{\text{richness (fire} \times \text{vegetation)} 2138} = 3.12$, $P = 0.05$; $F_{\text{density (fire} \times \text{veg)} 2138} = 5.74$, $P = 0.004$). Similarly, richness and density of herbaceous seedlings were higher in

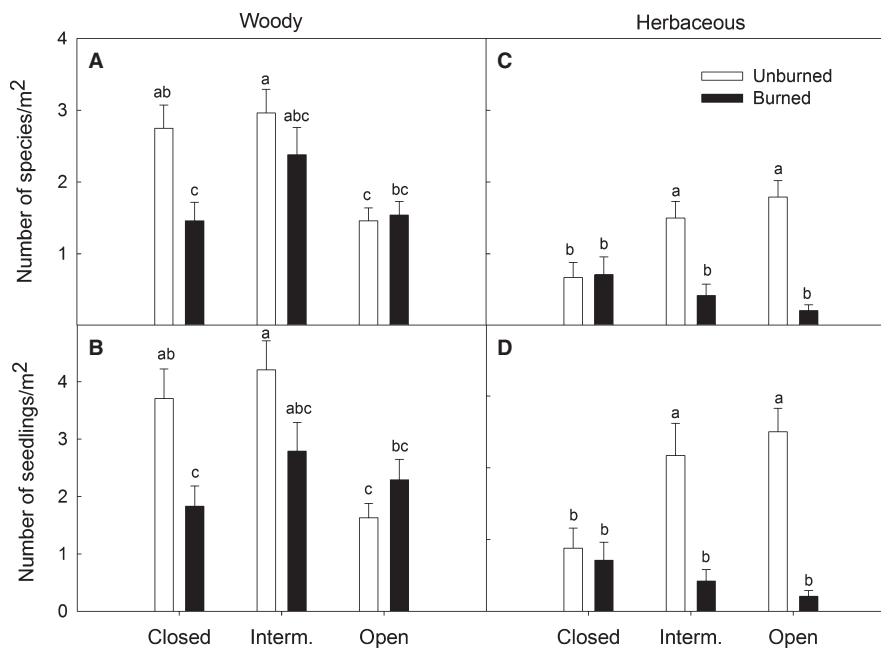


FIGURE 1. Number of species/m² of woody (A) and herbaceous (C) seedlings, and number of seedlings/m² of woody (B) and herbaceous (D) species in long-unburned and recently burned closed, intermediate (Interm.) and open savannas. Bars are means + SE ($N = 24$); bars topped with the same letter do not differ significantly ($P < 0.05$) among savanna physiognomies according to Tukey HSD *post-hoc* test.

unburned than in burned savannas (richness $F_{\text{fire } 1138} = 54.95$, $P < 0.0001$; density $F_{\text{fire } 1138} = 45.47$, $P < 0.0001$) and the effect of fire on herbaceous seedlings also differed across savanna physiognomies ($F_{\text{richness (fire} \times \text{vegetation)} 2138} = 8.55$, $P = 0.0003$, $F_{\text{fire} \times \text{vegetation } 2138} = 9.65$, $P = 0.0001$; Fig. 1).

Root suckers of woody species were more abundant and diverse than woody seedlings in recently burned savannas (Fig. 2). Richness of root suckers of herbaceous species was higher than richness of seedlings of herbaceous species in burned closed and open savannas, but not in burned intermediate savannas (Fig. 2). Density of root suckers of herbaceous species was higher than density of seedlings of herbaceous species, particularly in burned open savannas (Fig. 2). Root suckers of woody species were taller than seedlings particularly in recently burned closed and intermediate savannas (Table 2).

Seedling species composition changed before and after the fire in each physiognomy ($\rho_{\text{closed}} = 0.2$, $P = 0.01$; $\rho_{\text{intermediate}} = 0.5$, $P = 0.01$; $\rho_{\text{open}} = 0.2$, $P = 0.01$). As a result, the overall seedling species composition differed significantly among long-unburned and recently burned savannas ($R = 0.85$, $P = 0.001$; Fig. 3A). Physiognomies partially overlapped in species composition, but were statistically distinguishable ($R = 0.54$, $P = 0.001$). Species composition of woody seedlings also differed among unburned and recently burned savannas (Fig. 3B). Unburned and burned plots overlapped in their woody species composition, but separated well in the two-dimensional space ($R = 0.62$, $P = 0.004$). Species composition of herbaceous seedlings also differed significantly among long-unburned and recently burned savannas (Fig. 3C). Unburned and burned plots separated well in the two-dimensional space ($R = 0.72$, $P = 0.001$).

EFFECTS OF HEAT SHOCKS ON SEED GERMINATION OF WOODY SPECIES.—Heat-shock treatments significantly affected seed germination in the five studied species ($\chi^2_{A. asycarpum} = 141.35$, $df = 9$; $P < 0.0001$; $\chi^2_{B. gandichandii} = 121.93$, $df = 9$, $P < 0.0001$; $\chi^2_{E. pubescens} = 234.6$, $df = 9$, $P < 0.0001$; $\chi^2_{O. hexasperma} = 212.1$, $df = 9$; $P < 0.0001$; $\chi^2_{Solanum} = 415.7$, $df = 9$; $P < 0.0001$). Although species differed in their germination response to treatments, heat shocks of 90 °C for 10 and 15 min reduced seed germination in all studied species (Fig. 4). Heat-shock treatments of 30 °C during 5 and 10 min increased significantly seed germination in *Ourotea hexasperma* and *Solanum lycocarpum* (Fig. 4). However, heat-shock treatments of 30 and 60 °C for 5, 10, or 15 min did not enhance seed germination of the remaining studied species (Fig. 4).

DISCUSSION

Seedlings of woody and herbaceous species were more abundant and diverse in long-unburned savannas than in recently burned savannas, suggesting that fire constrained post-burn seedling establishment of savanna species, particularly in species lacking fire-protective mechanisms (Hoffmann 1998). In frequently burned intermediate Brazilian savannas, late dry season fires consumed almost all fruit production of common trees (Ramos 2004). Although seeds of some species may survive the passage of fire by forming transient soil seed banks (Sato 2003), most Neotropical savanna woody species do not form persistent soil seed banks (Salazar *et al.* 2011) because of high post-dispersal seed removal and short seed longevity (Salazar *et al.* 2012a). Thus, the lower density and richness of seedlings in burned

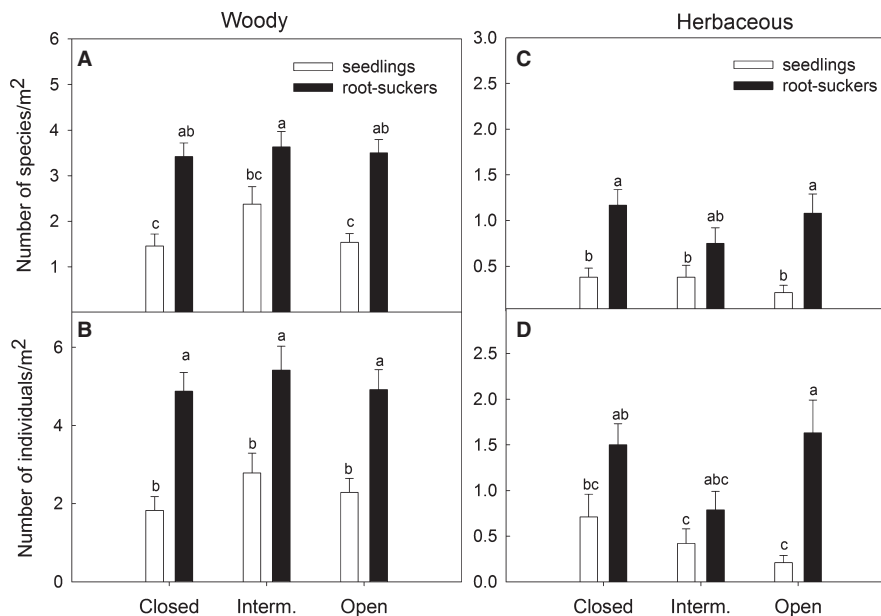


FIGURE 2. Number of species/m² of woody (A) and herbaceous (C) seedlings and root suckers, and number of individuals/m² of woody (B) and herbaceous (D) seedlings and root suckers in recently burned closed, intermediate (Interm.), and open savannas. Bars are means + SE ($N = 24$); bars topped with the same letter do not differ significantly ($P < 0.05$) among savanna physiognomies according to Tukey HSD *post-boc* test.

TABLE 2. Comparison of seedling and root-sucker height above soil surface of woody and herbaceous species in recently burned (closed, intermediate, and open) savannas. Measurements were taken in July 2007 after the accidental fire that occurred in September 2005. Bold rows indicate significant differences in height ($P < 0.05$) between seedlings and root suckers according to t tests.

Habit	Vegetation type	Seedling height (cm) \pm SE	Root-sucker height (cm) \pm SE	t	df	P
Woody	Closed	12.08 \pm 0.62	16.58 \pm 0.94	3.97	158	<0.0001
	Interm.	12.78 \pm 0.75	15.95 \pm 0.58	3.37	194	0.0009
	Open	14.75 \pm 1.05	15.86 \pm 0.58	1.32	164	0.19
Herbs	Closed	19.25 \pm 1.81	16.06 \pm 1.20	1.49	51	0.14
	Interm.	15.85 \pm 2.34	16.58 \pm 1.61	0.30	27	0.76
	Open	13.66 \pm 3.94	16.84 \pm 1.20	0.96	41	0.34

savannas, compared with long-unburned savannas found in this study, suggest that many Neotropical savanna species may lack fire-protective mechanisms for their seeds, which is unexpected due to savanna plants having evolved with fire as a major selective pressure.

In our study, seedling density of woody species decreased largely in burned closed savannas, whereas seedling density of herbaceous species decreased largely in burned open savannas. The high amount of accumulated grass biomass in open savannas likely resulted in a differential effect of fire between plant functional groups across vegetation types because surface fires mostly burn the herbaceous layer of the vegetation (Moreira 2000). The great proportion of herbaceous biomass that serves as fuel for fire expansion, particularly in open savannas (Castro & Kauffman 1998), could help to explain the larger reduction in herbaceous recruits in open than in closed burned savannas.

Richness and density of root suckers in recently burned savannas were significantly higher than richness and density of seedlings. Seedlings, which rely on few seed reserves and their ability to acquire new carbon from photosynthesis, may initially be at a disadvantage in harsh environments compared with plants that develop asexually and receive resources from their parents (Matlaga & Horvitz 2009). Suckers grow much faster than seedlings and can quickly reoccupy their own sites (Bond & Midgley 2001). In our study, root suckers were taller than seedlings, particularly root suckers of woody species in burned closed and intermediate savannas. Hoffmann (1998) found larger size and greater fire tolerance in root suckers relative to seedlings in several savanna woody species.

The ability to resprout largely relies on stored reserves that are protected from disturbance effects (Bell *et al.* 1996). Because building up sufficient reserves takes time, newly germinated seedlings will be unable to resprout if they have not accumulated enough reserves (Gignoux *et al.* 2009) which would explain why in recently burned savannas root suckers were more abundant and taller than seedlings. Thus, vegetative suckers in burned savannas are more likely to contribute to future population growth than seedlings because they may survive frequent burning and reach sexual maturity earlier (Hoffmann 1998). Our results do not allow us to make a clear statement about the contribution of vegetative reproduction via root suckers in unburned savannas

because we did not evaluate root suckers in the unburned transect or before the fire. Despite this caveat, the differential contribution of vegetative versus sexual reproduction in burned areas suggests that frequent fires affect the structure and species composition of savanna plant communities by constraining post-fire seedling establishment, particularly of sensitive tree species (Tomlinson *et al.* 2012). Indeed, in our study, seedling species composition largely changed before and after the fire in each savanna physiognomy. Species composition also differed significantly between frequently burned and single-burned intermediate savannas (results not shown).

The difference in seedling species composition between unburned and burned sites suggests that species which have the ability to resprout may dominate frequently burned savannas because they largely invest in carbohydrate reserves (Hoffmann *et al.* 2004), root biomass (Hoffmann & Franco 2003), and bark (Hoffmann 2012), whereas species that largely invest in leaves, stems, and growth may dominate unburned savannas (Rossatto *et al.* 2009, Gotsch *et al.* 2010). Thus, the pattern of carbon allocation at early stages to either reserves or to growth could be a major determinant for plant persistence in burned or unburned sites. Our results are consistent with these observations because seedlings of most tree species that exhibit relatively fast growth rates, such as *Aspidosperma macrocarpon*, *Byrsonima verbascifolia*, *Dalbergia miscolobium*, and *Guapira cf. noxia* (Moreira & Klink 2000), were restricted to unburned savannas (Table S1). In contrast, seedlings of shrub and subshrub species associated with high early allocation of reserves to roots (Ressel *et al.* 2004) were mostly present in recently burned savannas (Table S1).

In our study, seeds tolerated pulses of high temperatures up to 90 °C for 5 min, but exposures of longer than 10 min reduced germination in all the studied species. These results suggest a negative effect of high temperatures (>90 °C) during relatively long periods on seed germination of tree species, particularly species that disperse seeds in the early wet season such as *E. pubescens* and *O. hexasperma*. Therefore, it is likely that species that disperse seeds in the wet season mainly avoid fire events and therefore are not selected to tolerate high temperatures. Our results agree with those reported by Rizzini (1976), Felipe and Silva (1984), and Ribeiro *et al.* (2013) where heat shocks of 100 °C for 10 min inhibited or reduced germination

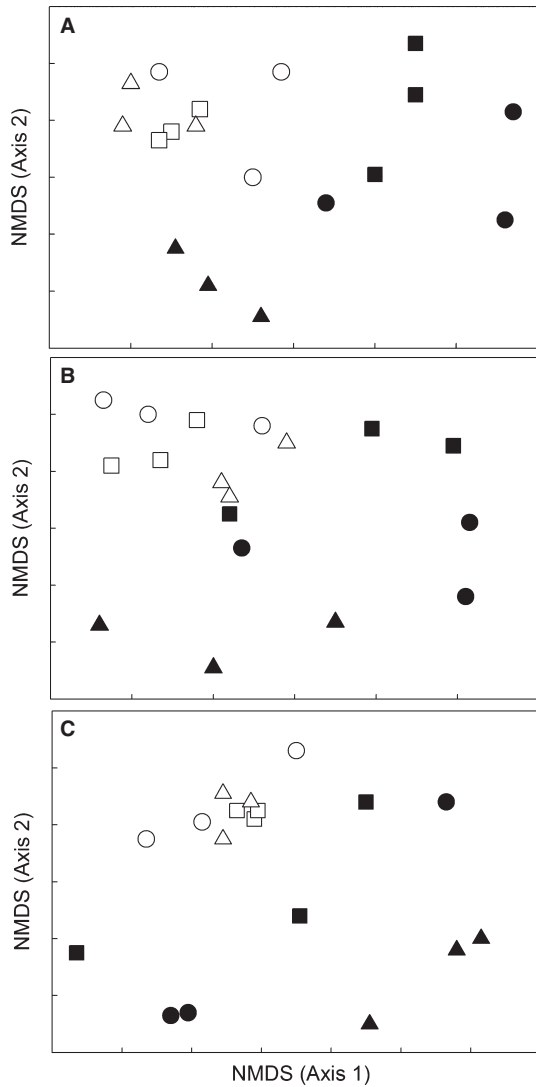


FIGURE 3. Non-metric multidimensional scaling (NMDS) ordination for the overall composition of seedlings of woody and herbaceous species (A), for the composition of woody species (B), and for the composition of herbaceous species (C) in each of the 20 × 20 m plots located in long-unburned savannas (white points) and recently burned savannas (black points). Circles: closed savannas, squares: intermediate savannas, triangles: open savannas. Points that are close together represent sites with similar species composition; points that are far apart correspond to sites with very different species composition.

of many savanna woody species, but disagree with those of Heringer 1971, who reported positive effects of pulses of high temperatures ranging from 70 to 100 °C on the germination of several woody species.

Considerable variation in temperature has been recorded during savanna fires, ranging from 85 °C above soil surface (Miranda *et al.* 1993) to 74, 47, 33, and 25 °C in the soil at depths of 0, 1, 2, and 5 cm, respectively (Coutinho 1990). During a fire in the IBGE reserve, pulses of heat generated by the flames penetrated slowly into the soil with maximum temperatures

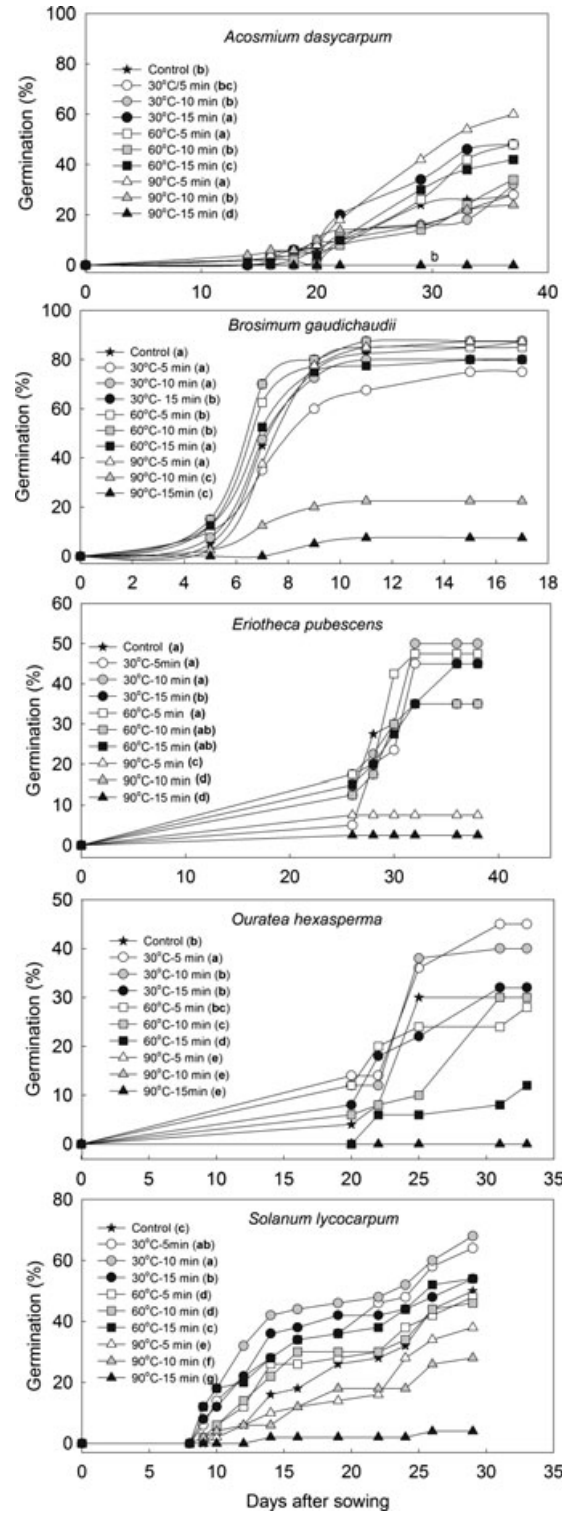


FIGURE 4. Cumulative germination curves of seeds of five savanna woody species under nine heat-shock treatments (30, 60, and 90 °C for 5, 10, and 15 min each) plus control (seeds not exposed to heat shocks). Curves are means of five replicates of 10 seeds each. SE is not shown to aid clarity. Treatments in legend with the same letter do not differ significantly ($P > 0.05$) according to pair-wise log-rank (Mantel-Cox) tests.

(29–38 °C) at 2 cm occurring 5–15 min after the passage of fire (Miranda *et al.* 1993). Our results suggest that seeds buried in the soil or near the soil surface are more likely to tolerate short-term soil heating than seeds that are at or aboveground level. Therefore, low germination of seeds located at or aboveground level is expected in many woody species after the passage of fires, once they have experienced long exposures of temperatures higher than 90 °C, which also explain the low abundance and richness of tree seedlings in recently burned savannas.

In conclusion, in Neotropical savannas fire constrains post-burn seedling establishment. During fires, exposures of high temperatures (>90 °C) for long periods negatively affect seed germination. As a result, species composition of seedlings differed between unburned and burned savannas, with seedlings of most tree species restricted to unburned closed savannas. Differential patterns of seedling species composition between burned and unburned savannas may reflect early differences in root: shoot biomass allocation patterns. In burned savannas, vegetative reproduction contributes relatively more than sexual reproduction. Thus, frequent fires are likely to cause major shifts in species composition of Neotropical savanna plant communities, potentially favoring clonally produced recruits along tree density/topographic gradients.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

FIGURE S1. Non-metric multidimensional scaling ordination for the composition of seedlings of woody and herbaceous species in each of the 20 × 20 m plots located in long-unburned savannas since plot establishment in July 2005 to seedling censuses in July 2007.

TABLE S1. *Species of savanna seedlings sampled in the IBGE reserve, central Brazil, in long-unburned and recently burned savannas differing in tree density.*

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