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Differential seedling establishment of woody plants along a tree density gradient in Neotropical savannas

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Summary

1. Seedling dynamics are crucial for understanding spatial plant distribution patterns, yet little is known about seedling establishment in Neotropical savannas because empirical studies at the community level are scarce.

2. Over 2 years, we studied the recruitment and survival of an initial seedling assemblage and three cohorts of recruits of woody plants within 216 plots of 1 m^2 located along a tree density gradient in the savannas of central Brazil. These savannas differ in tree density and canopy cover, from closed (high canopy cover) to open savannas (low canopy cover), and are located along shallow topographic gradients.

3. We measured community-wide seedling limitation (i.e. proportion of 1-m^2 plots without seedlings of any woody species), photosynthetic photon flux density, litter cover, soil moisture and soil nutrients in each savanna type. Because closed savannas had lower PPFD and higher leaf litter cover than open savannas, we evaluated the effects of light level and litter cover on seedling emergence of nine dominant savanna woody species under controlled conditions in a glasshouse.

4. Density, recruitment and survival of seedlings decreased over time because of mortality in all savanna types, but they were consistently higher in closed than in open savannas. Community-wide seedling limitation was significantly lower in closed (0.16 ± 0.03) than in open (0.30 ± 0.05) savannas.

5. In the glasshouse, high litter cover and very low light levels reduced seedling emergence of most species, suggesting an adaptation to delay seed germination until the wet season when soil water availability is high and leaf litter rapidly decomposes.

6. *Synthesis*: In Neotropical savannas, tree canopy cover facilitates seedling establishment of woody species by reducing stressful environmental conditions. In particular, low irradiance and high litter cover in closed savannas enhance the recruitment and survival of woody seedlings relative to open savannas by reducing soil water deficits and increasing nutrient availability in the upper soil layers. The higher seedling limitation of tree species in open than in closed savannas contributes to maintain relatively different balances between trees and herbaceous plants along topographic gradients in Neotropical savannas and helps to explain spatial distribution patterns of woody species in these ecosystems.

Key-words: canopy cover, Cerrado, determinants of plant community diversity and structure, light level, litter cover, seedling limitation

Introduction

Seedling recruitment is a bottleneck in the population dynamics of many plant species because plants at this stage exhibit high mortality as a consequence of both environmental and competition constraints (Harper 1977). Recruitment reflects not only seed production but also the compound filtering effects of seed dispersal and seedling establishment (Uriarte *et al.* 2005). At local scales, successful plant establishment has to overcome two successive bottlenecks: (i) seed limitation, the absence of recruitment because of limited seed supply and/or limited seed dispersal, and (ii) establishment

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limitation, the absence of recruitment because of limited suitable sites that provide the conditions required for seed germination and seedling establishment (Eriksson & Ehrlén 1992; Clark, Macklin & Wood 1998; Muller-Landau *et al.* 2002).

In contrast to temperate and tropical forests, the role of seed and seedling limitation in Neotropical savannas is unclear because empirical studies at the community level are scarce (Salazar *et al.* 2012). Moreover, reproduction of woody plants by seeds in tropical savannas has long been considered a rare event (Sarmiento 1984). Resprouting after extended drought periods or after fire events is frequently observed in trees and shrubs (Gottsberger & Silberbauer-Gottsberger 2006), and thus, clonal spread has been thought to be the major means of reproduction, despite the fact that most trees do produce seeds and experiments in the laboratory have proved high germination capacity (Melo *et al.* 1998; Felfilli *et al.* 1999). Studies also have shown that seeds can germinate under natural conditions and that tree seedlings eventually develop into adult plants (Franco 2002).

The savannas of central Brazil (Cerrado), the largest tropical ecosystem after the Amazonian rain forest, cover *c.* 2 million km^2, nearly 23% of the country. The total number of woody species in the whole biome is about 2000, and individual sites may contain up to 70 or more woody species per hectare (Haridasan 2008). These savannas are characterized by a strong seasonality of precipitation and by well-drained, deep, nutrient-poor soils (Furley 1999). Vegetation exhibits consistent changes in tree canopy cover, tree density and tree size along shallow topographic gradients of 30–60 m in elevation and a few kilometres in length. 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. Herbaceous plant abundance follows the opposite trend, with higher abundance in open than in closed savannas (Eiten 2001).

Environmental conditions in Neotropical savannas are stressful for the survival and growth of woody seedlings because of the 5-month dry season (Hoffmann 2000), limited availability of soil nutrients (Haridasan 2008) and frequent burning that occurs at 1- to 5-year intervals, mostly during the dry season (Coutinho 1990). Although increases in tree density after fire exclusion have been observed in Brazilian savannas (Moreira 2000), fire by itself cannot explain the low tree densities in open savannas and the consistent variation in tree cover along topographic gradients (Goldstein *et al.* 2008) because the same relative difference between woody and herbaceous plant abundance is maintained along topographic gradients even after decades of fire suppression (Goodland & Pollard 1973; Furley 1999; Moreira 2000), suggesting that other factors also may be involved in tree density variation.

In the absence of fire, light availability (Matlaga & Horvitz 2009), soil water deficits in the upper soil layers (Hoffmann 1996), nutrient availability (Barloto, Bonal & Goldberg 2006; Breen & Richards 2008), leaf litter cover (Molofsky & Augspurger 1992) and grass biomass (Hagenah *et al.* 2009) may constrain the quality and quantity of sites for emergence and establishment of seedlings. These environmental factors may change along tree density/topographic gradients affecting seedling establishment of woody species differentially among savanna types. In particular, low irradiance, low grass cover and high litter cover in closed savannas should enhance the recruitment and survival of woody seedlings relative to open savannas by reducing soil water deficits and increasing nutrient availability in the upper soil layers. Little is known about the establishment of woody seedlings in Neotropical savannas. Specifically, it is not clear whether or not seedling dynamics of woody species are consistent with variations in tree canopy cover across the Cerrado landscape or which environmental factors limit seedling establishment in the absence of fire.

In this study, we investigated the importance of spatial variation in canopy cover and seasonal variation in precipitation on the density, recruitment and survival of seedlings of woody species in Neotropical savannas with no recent history of fires which differ in tree density and canopy cover. We coupled observational and experimental results to address the following questions: (i) Does seedling establishment (density, recruitment and survival) of woody plants differ among closed (40–60% canopy cover), intermediate (< 40% canopy cover) and open (*c.* 10% canopy cover) savannas? (ii) To what extent are closed, intermediate and open savannas seedling-limited? (iii) Given the differences in tree canopy cover among savanna types, how do litter cover and light level affect seedling emergence of savanna woody plants under controlled experimental conditions?

Materials and methods

STUDY SITE

This study was conducted from June 2005 to July 2008 at the Instituto Brasileiro de Geografía e Estatística (IBGE) reserve, a 1300-ha field station located 35 km south of Brasilia, Brazil (15° 56′ S, 47° 63′ W, altitude 1100 m). Average annual precipitation is about 1500 mm with a pronounced dry season from May to September. The months of June, July and August often are rainless. Average daily relative humidity during the rainy season is about 80% and drops to 55% during the dry season when daily minimum relative humidity may reach values as low as 10% at midday. Mean monthly temperatures ranged from 19 to 23 °C. The soils are very deep and well-drained oxisols. Litter accumulates mainly in the dry season because most trees shed their leaves at this time and decomposition rates are low because of low water potentials in the upper soil layers (Villalobos-Vega *et al.* 2011). The IBGE reserve contains all major savanna types: closed savannas are semi-closed woodlands (40–60% tree crown cover) with a moderately tall (7–12 m) tree canopy and mean leaf area index (LAI) of 1.5. Intermediate savannas are savannas dominated by trees and shrubs (canopy generally < 7 m tall) with 10 to < 40% tree crown cover and mean LAI of 0.8. Open savannas have few short trees and scattered shrubs over a near continuous grass layer (the woody layer usually covers < 10% of the ground) and mean LAI of 0.5 (Salazar *et al.* 2011).

In June 2005, we established three transects of *c.* 1000 m in length, spanning the three major vegetation types (closed, intermediate and open savannas) in different places of the IBGE reserve. We

placed the transects along continuous topographic gradients, each with a different elevation, ranging from 1117 to 1153 metres with a mean difference in elevation across vegetation types of 31.3 m ± 4.5 m, in areas that had been protected from fire for at least 30 years to rule out the confounding effect of fire on sexual reproduction on most Cerrado woody species (Hoffmann 2000) and to eliminate potential effects of different fire frequencies in each study site. Because preliminary species–area curves in closed and open savannas indicated that about 85% of the maximum richness of woody plants (> 1 m) was attained at 1024 m^2, we established randomly nine plots of 20 × 20 m along each transect (three per vegetation type), for a total of 27 permanent plots. A total of 90, 70 and 85 woody species were found in closed, intermediate and open savannas, respectively.

ENVIRONMENTAL CONDITIONS AT THE STUDY SITE

We measured photosynthetic photon flux density (PPDF) and red/far-red wavelength ratios of transmitted light to quantify light quantity and quality in each savanna type, because both metrics of light may affect seed germination. We quantified PPFD by using a portable photosynthetically active radiation sensor wand (AccuPAR LP-80; Decagon Devices, Washington, DC, USA). PPFD was measured 1 m above the soil surface, at midday on clear days at 30 random points in each permanent plot in the three vegetation types, in June 2007 (early dry season)**.** We measured red/far-red wavelength ratios of transmitted light at midday on clear days, in nine randomly selected plots (three per vegetation type) 30 cm above the soil surface, in June 2006. We measured the quality of transmitted light (200–1100 nm) with a portable USB-2000 fiber optic spectrometer connected to a portable computer. We calculated red/far-red ratios by dividing the per cent of transmitted light in the red waveband (600–700 nm) by the per cent of transmitted light in the far-red wavebands (700–800 nm) using OOIBASE 32TM operating software (http://www.oceanoptics.com).

We determined soil nutrient availability, pH, soil gravimetric water content and soil water potential by collecting soil samples of 250 g each with an auger at the centre of each 20 × 20 m plot. We collected soil samples at 0–5 cm, 5–10 cm, 10–25 cm and 25–50 cm. For chemical analyses and water content, a total of 108 soil subsamples (27 plots, four depths) were obtained in July 2005 (mid-dry season). Soil chemical analyses included pH, exchangeable Ca, Mg, Al, K, available P, Fe, Mn, Zn, Cu, total N and organic carbon.

To determine soil gravimetric water content, we placed soil subsamples in tin sample canisters (2–3/8″ diameter × 1–3/4″ height). In the laboratory, canisters were weighted and oven-dried at 65 °C for at least 72 h until they reached a constant mass. We calculated the gravimetric soil water content as follows: ((soil fresh mass − soil dry mass)/soil dry mass) × 100%. To determine soil water potential among vegetation types, we used the filter paper technique developed for Whatman filter paper No. 42 (Deka *et al.* 1995). We collected soil samples in July 2008 (mid-dry season).

SEEDLING DENSITY, RECRUITMENT AND SURVIVAL

To quantify seedling density, recruitment and survival, we established eight subplots of 1 × 1 m within each of the 20 × 20 m plots for a total of 216 plots (72 per vegetation type) because preliminary species-plot curves in closed and open savannas indicated that about 90% of the maximum richness of seedlings was attained using 8 1-m^2 plots (see Fig. S1 in Supporting Information). Seedling plots were established randomly within each 20 × 20 m plot. In July 2006, all seedlings up to 30 cm tall in the plots were tagged with a unique number and identified to morphospecies. We excluded individuals arising through vegetative reproduction. Subsequent censuses took place in March 2007, July 2007, November 2007 and July 2008. During each census, newly recruited seedlings were tagged, and missing seedlings were recorded as 'dead'. Seedlings were identified by comparing seedling specimens growing in the vicinity of the subplots with reference specimens at the IBGE herbarium, help of experts and published literature. The initial seedling assemblage corresponded to the seedlings established before July 2006. Seedlings recruited from July 2006 to March 2007 made up Cohort 1; seedlings recruited from March 2007 to July 2007 made up Cohort 2; and seedlings recruited from July 2007 to November 2007 made up Cohort 3.

SEEDLING LIMITATION

We quantified species-specific seedling limitation for 16 woody species found in the initial seedling assemblage and in each cohort, in all vegetation types according to Clark, Macklin & Wood (1998) and Muller-Landau *et al.* (2002). Seedling limitation was calculated as the proportion of subplots where seedlings of a particular woody species did not occur (seedling limitation = $1 - (r/n)$ where r is the number of subplots where seedlings of a particular woody species occur, and n is the number of 1-m^2 subplots (i.e. eight 1-m^2 subplots in each 20 × 20 m plot). We also quantified community-wide seedling limitation for the initial seedling assemblage and for each cohort as the proportion of 1-m^2 subplots in each 20 × 20 m plot without seedlings of any woody species.

EFFECT OF LITTER COVER ON SEEDLING EMERGENCE

To quantify litter cover among vegetation types, we took litter samples from each permanent plot at three random points, during the beginning of the dry season (May 2006). All litter contained in squares of 25 cm × 25 cm × 5 cm was collected and oven-dried at 65 °C until it reached a constant mass. In the field, we collected mature seeds from several individuals of nine woody species: *Acosmium dasycarpum* (Vogel) Yakov. (Fabaceae), *Brosimum gaudichaudii* Trécul. (Moraceae), *Eriotheca pubescences* Mart & Zucc. (Malvaceae), *Guapira graciliflora* (Mart. Ex J.A. Schmidt.) Lundell. (Nyctaginaceae), *Guapira noxia* (Netto) Lundell (Nyctaginaceae), *Miconia ferruginata* DC. (Melastomataceae), *Ouratea hexasperma* (A.St.Hil.) Baill. (Ochnaceae), *Palicourea rigida* Humb., Bonpl. & Kunth.(Rubiaceae) and *Solanum lycocarpum* A.St.Hil (Solanaceae). These species span all or a large portion of the entire topographic gradient (Silva Júnior 2005), differ in their dispersal season and mode of dispersal, belong to a wide range of plant families and growth forms from shrubs to trees, had a relative abundance higher than 10% in our study sites and had an initial seed viability higher than 50%.

To examine the effect of litter cover on seedling emergence of each species, we used an experimental design with three levels of litter cover and five replicates of 20 seeds each under glasshouse conditions. Litter was collected from random plots of each vegetation type. A bulked sample of oven-dried mixed litter from different species was used to cover the trays. Seeds were sown in plastic trays (25 cm × 30 cm × 4 cm) filled with an artificial substrate (mix of 70% peat moss and 30% vermiculite) for a total of 15 trays per species. Five trays were covered with 25 g of dry litter (2 g cm^{-2}), five trays were covered with 50 g of dry litter (4 g cm^{-2}), and five control trays received no litter. Litter cover levels were consistent with field conditions in closed and open savannas at the beginning of the

dry season. However, seeds may experience different litter cover levels in the field because leaf litter fall is highly seasonal. The trays were randomly distributed on the glasshouse benches, under temperature conditions of 25–28 °C, 32% full sunlight, and watered daily with an automated sprinkler system. Seedling emergence, defined as emergence of cotyledons, was monitored every 3 days up to 4–5 weeks (depending on the species).

EFFECT OF LIGHT LEVEL ON SEEDLING EMERGENCE

To examine the effect of light level on seedling emergence, we used a factorial experimental design with four light levels and five replicates of 20 seeds each under glasshouse conditions. Seeds were collected in the field from several individuals of each of the nine species mentioned above. Seeds were sown in plastic trays (25 cm × 30 cm × 4 cm) filled with an artificial substrate (mix of 70% peat moss and 30% vermiculite) for a total of 20 trays per species. Five trays were covered with 'shade cloth' to provide 16% full sunlight (262 μmol m^{-2} s^{-1}), 5 trays were covered with 'shade cloth' to provide 20% full sunlight (350 μmol m^{-2} s^{-1}), 5 trays were covered with aluminium foil to produce 0% full sunlight (0.5 μmol m^{-2} s^{-1}), and five trays were not covered and received 32% full sunlight (525 μmol m^{-2} s^{-1}). These light levels are below the light saturation values for photosynthesis of the studied woody species (results not shown). To allow for water and oxygen diffusion, several 1-mm holes were opened in the aluminium foil. Trays were randomly distributed on the glasshouse benches, under temperature conditions of 25–28 °C, and were watered daily with an automated sprinkler system to field capacity. Seedling emergence, defined as emergence of cotyledons, was monitored every 3 days for 4–5 weeks (depending on the species).

DATA ANALYSIS

The effect of vegetation type and time on seedling density and richness of each cohort was analysed with a mixed model approach for repeated measures (Pinheiro & Bates 2000) with vegetation as fixed factor and time as a repeat random factor. Mixed effects models are useful in cases where temporal pseudoreplication in the seedling variables measured violates the crucial assumption of independence of errors. 'Transect' also was included as random factor. Because the response variable (seedling density) departed from a normal distribution, a Poisson error distribution structure was used. To estimate the model parameters, we used a generalized linear mixed model (GLMM). Minimum adequate models were obtained by deleting non-significant terms ($P > 0.05$) from the full model consisting of all variables and their interactions. The model selection procedure was based on a penalized likelihood measure of the goodness-of-fit, the Akaike Information Criterion (AIC). The effect of vegetation type (fixed effect) and time (random effect) on the richness/density ratio of recruited seedlings also was analysed with GLMM, but using a normal error distribution structure. We calculated richness/density ratios of recruited seedlings to remove the effect of density on richness. These statistical analyses were performed in R version 2.91 (http://www.R-project.org). We used the package 'LME4' for GLMMs. We used Kaplan–Meier survival analysis (Fox 2001) to analyse the effects of vegetation type on survival of seedlings. We estimated cohort survivorship ('survival function') and the probability of surviving the first 8 months and the probability of surviving to the end of the study period for each cohort within each vegetation type. We chose 8 months to compare the survival of seedlings across cohorts within a similar time interval. Pairwise comparisons of the survival functions of seedlings between vegetation types for 8 months and to the end of the study were made with a log-rank test (Krebs1999).

Differences in community-wide seedling limitation measured as the proportion of sites (1-m^2 plots) without seedlings of any woody species, as well as the specific seedling limitation of 16 woody species among vegetation types in each cohort, were examined using non-parametric Kruskal–Wallis tests. The data from the three transects of each vegetation type were pooled. Differences in PFFD, red/far-red ratios and soil nutrients among vegetation types were examined with one-way ANOVAS. Tukey's HSD post hoc tests were used for multiple comparisons of means. Differences in soil gravimetric water content and soil water potential among vegetation types and collection depth were examined with two-way ANOVAS with vegetation type and soil depth as fixed factors. Differences in seedling emergence under different litter cover and under different light levels among vegetation types and species were examined with two-way ANOVAS with vegetation type as fixed factor and species as random factor. For each species, separate one-way ANOVA tests were performed to examine the effect of litter cover and light level on seedling emergence. Tukey's HSD post hoc tests were used for multiple comparisons of means. These analyses were performed using the program JMP 7 (SAS, Cary, NC, USA).

Results

ENVIRONMENTAL CONDITIONS

Photosynthetic photon flux density was about three times lower in closed than in intermediate or open savannas (one-way ANOVA, $F_{2,272} = 402.92$, $P < 0.0001$; Fig. 1a). Red/far-red ratios of transmitted light also were lower in closed than in

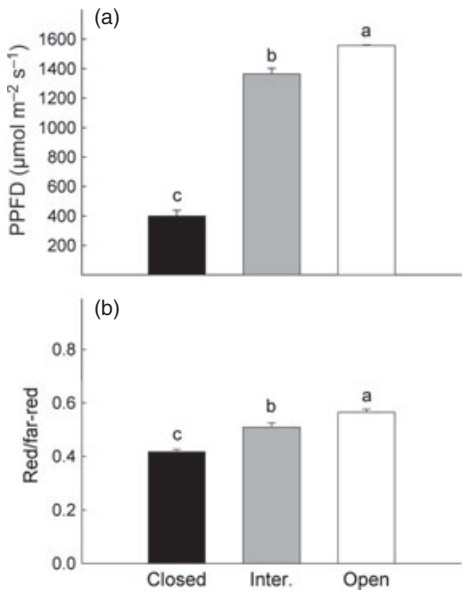

Fig. 1. Photosynthetic photon flux density (PPFD) (a), red/far-red wavelength ratios (b) in closed, intermediate (Inter.) and open savannas. Red/far-red ratios and PPFDs were measured at 30 cm and 1 m, respectively, above the soil surface. Bars are means ± SE. Bars with the same letter do not differ significantly ($P < 0.05$) among vegetation types according to Tukey's HSD post hoc test ($n = 90$ for PPFD. $n = 25$ for red/far-red).

intermediate or open savannas (one-way ANOVA, $F_{2,65} = 35.13$, $P < 0.0001$; Fig. 1b). Soil concentrations of most nutrients were similar among the three vegetation types (See Table S1 in Supporting Information). Soil pH was lower in closed than in intermediate or open savannas, but soil aluminium concentration showed the reverse pattern (Table S1). Soil water potential did not differ among vegetation types (two-way ANOVA, $F_{\text{Veg } 2,96} = 1.03$; $P = 0.36$; $F_{\text{Depth } 3,96} = 41.18$; $P < 0.0001$; $F_{\text{Veg} \times \text{Depth } 6,96} = 0.15$; $P = 0.98$); however, it was significantly lower (more negative) in the upper parts of the soil profile (5–10 cm) than in deeper layers (25–50 cm) along the gradient (Fig. S2). Likewise, mean gravimetric water content in all vegetation types significantly increased with soil depth (two-way ANOVA, $F_{\text{Veg } 2,96} = 1.50$; $P = 0.23$; $F_{\text{Depth } 3,96} = 211.91$; $P < 0.0001$; $F_{\text{Veg} \times \text{Depth } 6,96} = 1.14$; $P = 0.35$), but did not differ among vegetation types (Fig. S2).

SEEDLING DENSITY, RECRUITMENT AND SURVIVAL

Density (number of seedlings per m^2) of the initial seedling assemblage and the three cohorts studied decreased significantly over time in all vegetation types (Fig. 2, See Table S2 for statistical test results). Seedling density was significantly lower in open than in closed savannas in the three cohorts, but not in the initial seedling assemblage (Table S2). Richness (number of species m^{-2}) and density of new recruits also decreased over time, in particular between the first and second censuses (results not shown), and were higher in closed than in open savannas (See Table S3 for statistical test results). In closed savannas, mean richness across cohorts ranged from 2.30 to 0.94, while in open savannas, it ranged from 1.86 to 0.55.

For the initial seedling assemblage, the probability of surviving the first 8 months of the study period was higher in open than in closed or intermediate savannas (log-rank test $\chi^2_{\text{closed vs. open}} = 6.03$, d.f. = 1, $P < 0.01$; $\chi^2_{\text{intermediate vs. open}} = 10.24$, d.f. = 1, $P = 0.001$; Fig. 3a). However, the probability of surviving the 24 months of study period for these seedlings did not differ among vegetation types (log-rank test $\chi^2 = 1.81$, d.f. = 2, $P = 0.40$; Fig. 3a). In contrast, the probability of surviving the 8 and 16 months of the study period for seedlings in Cohort 1 was significantly higher in closed and intermediate savannas than in open savannas (log-rank test$_{[8\ \text{months}]}$ $\chi^2_{\text{closed vs. open}} = 20.32$, d.f. = 1, $P < 0.0001$; $\chi^2_{\text{intermediate vs. open}} = 12.88$, d.f. = 1, $P = 0.0003$; log-rank test$_{[16\ \text{months}]}$ $\chi^2_{\text{closed vs. open}} = 22.50$, d.f. = 1, $P < 0.0001$; $\chi^2_{\text{intermediate vs. open}} = 24.87$, d.f. = 1, $P < 0.0001$; Fig. 3b). Although not significant, the probability of surviving the 8 and 12 months of the study period for seedlings in Cohort 2 was consistently higher in closed than in open savannas (Fig. 3c). The same pattern was observed for the seedling survivorship in Cohort 3 during the first 8 months of the study period (Fig. 3d).

SEEDLING LIMITATION

Community seedling limitation (proportion of 1-m^2 subplots without woody seedlings) was significantly lower in closed

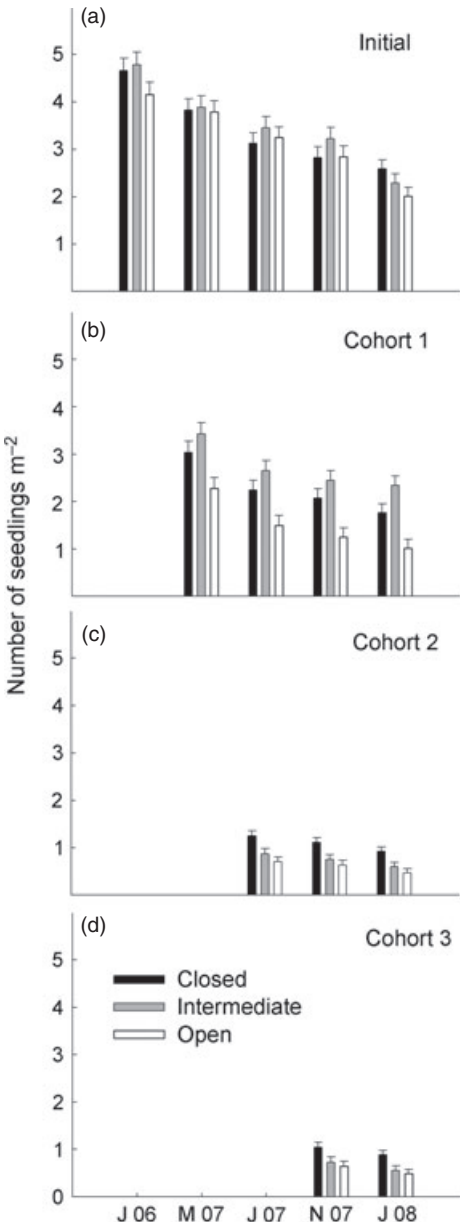

Fig. 2. Number of seedlings m^{-2} (density) in the (a) initial seedling assemblage, (b) Cohort 1, (c) Cohort 2 and (d) Cohort 3 in closed, intermediate and open savannas during each census period: J 06 (July 2006), M 07 (March 2007), J 07 (July 2007), N 07 (November 2007) and J 08 (July 2008). Bars are means ± SE ($n = 72$) during each census period.

(0.16 ± 0.03) than in open (0.30 ± 0.05) or intermediate savannas (0.32 ± 0.05) (Kruskal–Wallis test; $H = 5.83$, d.f. = 2, $P = 0.04$). Community seedling limitation increased significantly with subsequent cohorts (Kruskal–Wallis test, $H = 43.84$, d.f. = 3, $P < 0.001$) from 0.07 in the initial seedling assemblage to 0.12 in Cohort 1, 0.35 in Cohort 2 and 0.48 in Cohort 3. Through the entire study period, the number of new recruits in each plot significantly decreased as the number of pre-existing seedlings increased in closed, but not in intermediate or open savannas (Fig. 4). In all three vegetation types, specific seedling limitation for most of the 16

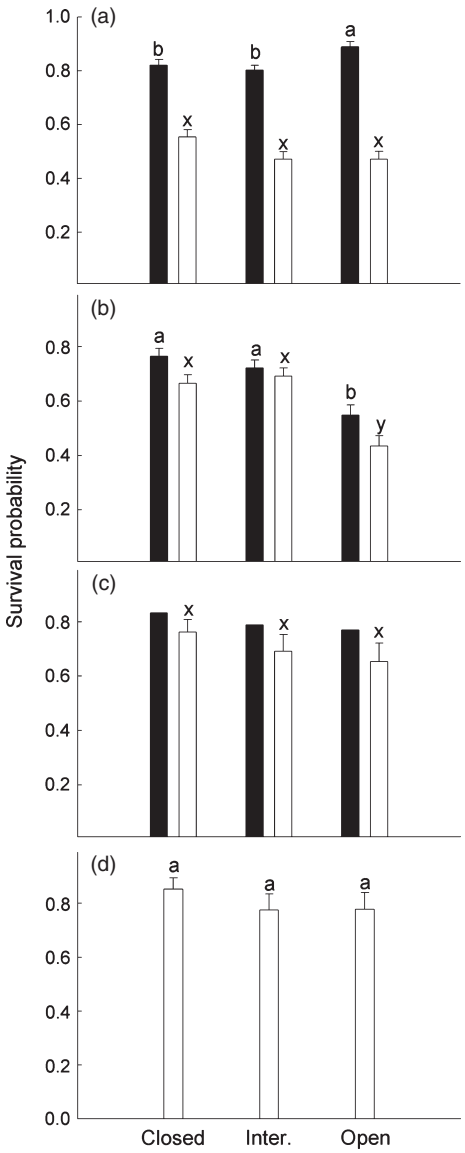

Fig. 3. Mean (±SE) survival probability (estimated by the Kaplan–Meier product-limit method) at 8 months (white bars) and at the end of the study period (black bars) for (a) the initial seedling assemblage, (b) Cohort 1, (c) Cohort 2 and (d) Cohort 3 in closed, intermediate (Inter.) and open savannas. Study period for initial seedling assemblage was 24 months, for Cohort 1 was 16 months, for Cohort 2 was 12 months, and for Cohort 3 was 8 months. Bars of the same colour topped with different letters differ significantly at $P < 0.05$ (pairwise log-rank test comparisons). Survival probability at the end of 8 months' study of Cohort 2 was extrapolated from fitted linear equations (closed: $y = 1 - 0.0007x$, $r^2 = 1$; intermediate: $y = 0.98 - 0.0008x$, $r^2 = 0.97$; open: $y = 1.02 - 0.001x$, $r^2 = 0.98$), and therefore, standard errors are not shown.

woody species selected for individual studies was higher than 80% (Table 1). Significant differences among the three vegetation types were found for some, but not for all species within each cohort (Table 1). Mean seedling limitation across woody plants of Cohort 2 and Cohort 3 was significantly higher in open than in intermediate or closed savannas (Kruskal–Wallis test, $H_{cohort2} = 14.83$, d.f. = 2, $P = 0.0006$, $H_{cohort3} = 9.27$, d.f. = 2, $P = 0.01$; Table 1).

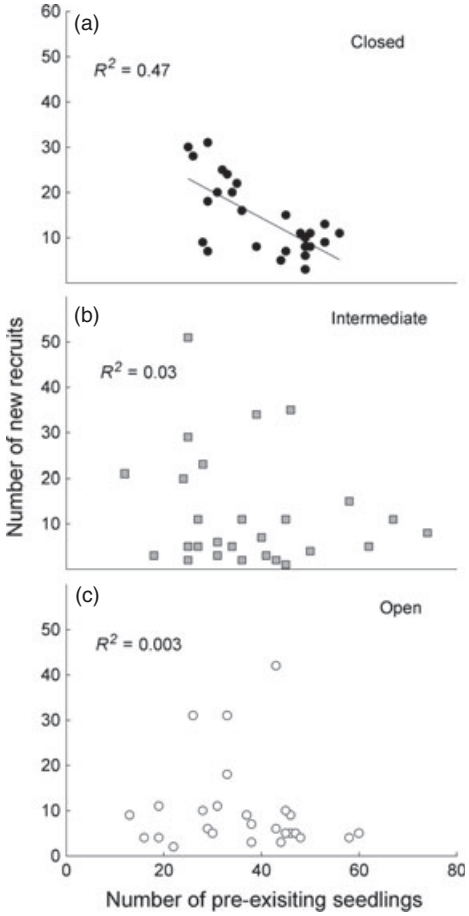

Fig. 4. Total number of new recruits as a function of number of pre-existing seedlings in each plot (8 m^2) located in closed (a), intermediate (b) and open savannas (c) for the entire study period. Significant linear functions were found for closed ($y = 37.45 - 0.58x$, $P < 0.0001$), but not for intermediate ($y = 17.96 - 0.15x$, $P = 0.37$) or for open savannas ($y = 11.08 - 0.04x$, $P = 0.80$).

SEEDLING EMERGENCE UNDER DIFFERENT LITTER COVER AND LIGHT LEVELS

In the field, litter cover collected at the beginning of the dry season was significantly higher in closed than in intermediate or open savannas (Fig. 5, insert). In the glasshouse experiment, seedling emergence differed among species ranging from 2% in *G. graciliflora* with 4 g cm^{-2} of litter and *E. pubescens* with no litter to 88% in *B. gaudichaudii* with no litter (Fig. 5). Litter cover, species and their interaction had a significant effect on seedling emergence (two-way ANOVA, $F_{litter\ 2,103} = 7.98$, $P = 0.0006$; $F_{species\ 8,103} = 40.81$, $P < 0.0001$; $F_{litter \times species\ 16,103} = 2.63$, $P = 0.002$), indicating that the effect of litter cover depended on the species. Seedling emergence of *B. gaudichaudii*, *P. rigida*, *G. noxia* and *A. dasycarpum* was significantly lower with high litter cover (4 g cm^{-2}) than with no litter cover or low litter cover (2 g cm^{-2}) (Fig. 5). Emergence of *G. graciflora*, *E. pubscens* and *O. hexosperma* seedlings was also consistently low with high litter cover.

In the glasshouse, emergence of seedlings across light levels differed among species ranging from 0% in *G. graciliflora*, *G. noxia*, *M. ferruginata* and *P. rigida* with 0% sunlight to

Table 1. Seedling limitation (proportion of subplots of 1 m^2 without seedlings of a particular species) for 16 woody species in closed, intermediate and open savannas in each cohort of recruits. A value of one indicates maximum limitation because seedlings of that particular species were not found in any of the subplots (see methods for details). Values are means ± SE (n = 9) per species. Bold rows indicate species that differ significantly among vegetation types. For these species, means with the same letter do not differ significantly according to Kruskal–Wallis pairwise comparisons ($P < 0.05$). Mean seedling limitations across species are also indicated

	Closed	Intermediate	Open
Cohort 1			
Aspidosperma macrocarpon	0.92 ± 0.04	1.00 ± 0.00	0.98 ± 0.02
Calliandra dysantha	0.87 ± 0.05	0.82 ± 0.06	0.85 ± 0.06
Campomanesia pubescens	**0.99 ± 0.01a**	**0.82 ± 0.06b**	**0.68 ± 0.08b**
Casearia altiplanensis	0.86 ± 0.05	0.80 ± 0.06	0.75 ± 0.06
Chamaecrista orbiculata	0.93 ± 0.02	0.94 ± 0.03	0.92 ± 0.02
Dalbergia miscolobium	0.88 ± 0.03	0.96 ± 0.02	0.93 ± 0.02
Erythroxylum campestre	0.63 ± 0.08	0.74 ± 0.05	0.71 ± 0.06
Eugenia bracteata	0.94 ± 0.02	0.82 ± 0.06	0.90 ± 0.03
Maprounea brasiliensis	0.99 ± 0.01	1.00 ± 0.00	0.96 ± 0.03
Miconia albicans	**0.68 ± 0.06b**	**0.81 ± 0.04ab**	**0.88 ± 0.06a**
Miconia fallax	**0.67 ± 0.05b**	**0.93 ± 0.04a**	**0.90 ± 0.04a**
Myrsine guianensis	**0.81 ± 0.05b**	**0.50 ± 0.83c**	**0.96 ± 0.02a**
Ouratea floribunda	0.86 ± 0.03	0.89 ± 0.04	0.85 ± 0.04
Roupala montana	0.86 ± 0.03	0.89 ± 0.04	0.90 ± 0.05
Rourea induta	**0.90 ± 0.03b**	**0.99 ± 0.01a**	**0.96 ± 0.02ab**
Styrax ferrugineus	0.89 ± 0.03	0.96 ± 0.02	0.97 ± 0.02
Mean	0.85 ± 0.16	0.87 ± 0.18	0.88 ± 0.15
Cohort 2			
Aspidosperma macrocarpon	0.97 ± 0.02	1.00 ± 0.00	1.00 ± 0.00
Calliandra dysantha	0.79 ± 0.05	0.86 ± 0.04	0.86 ± 0.03
Campomanesia pubescens	0.89 ± 0.04	0.79 ± 0.09	0.86 ± 0.03
Casearia altiplanensis	0.82 ± 0.04	0.90 ± 0.06	0.90 ± 0.06
Chamaecrista orbiculata	0.79 ± 0.05	0.90 ± 0.04	0.90 ± 0.08
Dalbergia miscolobium	**0.85 ± 0.05b**	**0.944 ± 0.03ab**	**1.00 ± 0.00a**
Erythroxylum campestre	0.81 ± 0.06	0.86 ± 0.04	0.90 ± 0.05
Eugenia bracteata	**0.96 ± 0.02a**	**0.76 ± 0.08b**	**0.96 ± 0.03a**
Maprounea brasiliensis	0.94 ± 0.02	0.99 ± 0.01	0.93 ± 0.04
Miconia albicans	0.89 ± 0.03	0.94 ± 0.03	0.90 ± 0.03
Miconia fallax	0.93 ± 0.03	0.96 ± 0.04	1.00 ± 0.00
Myrsine guianensis	**0.88 ± 0.05b**	**0.83 ± 0.06b**	**0.99 ± 0.01a**
Ouratea floribunda	**0.96 ± 0.03a**	**0.83 ± 0.04b**	**0.97 ± 0.02a**
Roupala montana	**0.99 ± 0.01a**	**0.92 ± 0.03b**	**1.00 ± 0.00a**
Rourea induta	**0.92 ± 0.03b**	**1.00 ± 0.00a**	**0.97 ± 0.02a**
Styrax ferrugineus	0.96 ± 0.02	0.97 ± 0.02	0.99 ± 0.01
Mean	**0.90 ± 0.12b**	**0.90 ± 0.14b**	**0.94 ± 0.11a**
Cohort 3			
Aspidosperma macrocarpon	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
Calliandra dysantha	0.96 ± 0.02	0.93 ± 0.05	1.00 ± 0.00
Campomanesia pubescens	1.00 ± 0.00	0.99 ± 0.02	0.97 ± 0.02
Casearia altiplanensis	**0.79 ± 0.04b**	**0.99 ± 0.01a**	**0.97 ± 0.02a**
Chamaecrista orbiculata	0.99 ± 0.01	1.00 ± 0.00	0.99 ± 0.01
Dalbergia miscolobium	**0.92 ± 0.04b**	**0.99 ± 0.01ab**	**1.00 ± 0.00a**
Erythroxylum campestre	0.93 ± 0.03	0.86 ± 0.05	0.89 ± 0.04
Eugenia bracteata	1.00 ± 0.00	0.96 ± 0.02	0.97 ± 0.02
Maprounea brasiliensis	0.94 ± 0.03	0.99 ± 0.01	0.97 ± 0.02
Miconia albicans	**0.92 ± 0.03b**	**0.99 ± 0.01a**	**0.99 ± 0.01a**
Miconia fallax	0.90 ± 0.04	0.97 ± 0.02	0.97 ± 0.02
Myrsine guianensis	0.99 ± 0.01	0.94 ± 0.02	0.99 ± 0.01
Ouratea floribunda	0.93 ± 0.02b	1.00 ± 0.00a	1.00 ± 0.00a
Roupala montana	0.97 ± 0.02	0.92 ± 0.04	0.94 ± 0.02
Rourea induta	0.96 ± 0.03	1.00 ± 0.00	0.99 ± 0.01
Styrax ferrugineus	1.00 ± 0.00	0.99 ± 0.01	1.00 ± 0.00
Mean	**0.95 ± 0.09b**	**0.97 ± 0.07**	**0.98 ± 0.05a**

94% in *B. gaudichaudii* with 32% full sunlight (Fig. 6). Light level, species, and their interaction had a significant effect on seedling emergence (two-way ANOVA, $F_{\text{light}\ 3,132} = 20.35$, $P < 0.0001$; $F_{\text{species}\ 8,132} = 31.98$, $P < 0.0001$; $F_{\text{light} \times \text{species}\ 24,132} = 4.82$, $P = 0.002$), indicating that the effect of light level depended on the species. Seedling

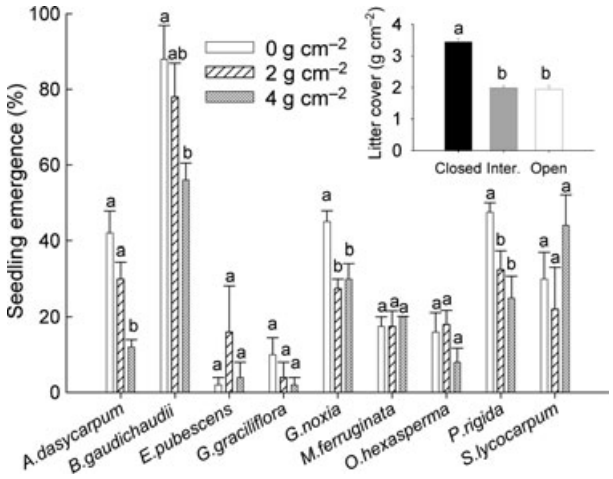

Fig. 5. Seedling emergence (%) of nine Cerrado woody species under different litter cover levels (0 g control, 2 g cm^{-2}, 4 g cm^{-2}) 4–5 weeks after sowing. Bars are means ± SE (n = 5). Insert: surface leaf litter cover collected in the field in closed, intermediate (Inter.) and open savannas at the beginning of the dry season. Bars are means ± SE (n = 27). Bars topped with the same letter do not differ significantly ($P < 0.05$) among litter cover levels according to Tukey's HSD post hoc test.

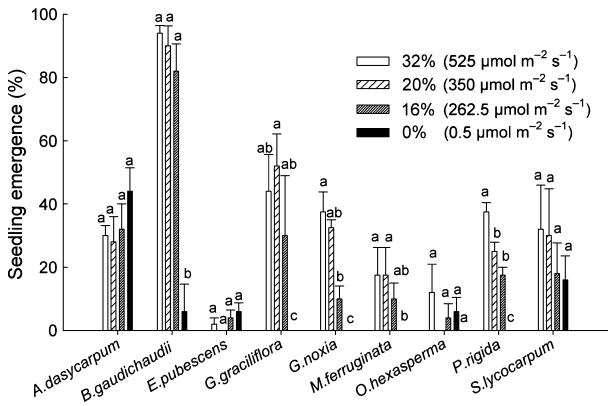

Fig. 6. Seedling emergence (%) of nine Cerrado woody species under different light levels (32% full sunlight, 20% full sunlight, 16% full sunlight and 0% full sunlight) 4–5 weeks after seed sowing. Bars are means ± SE (n = 5); bars topped with the same letter do not differ significantly ($P < 0.05$) among light levels according to Tukey's HSD post hoc test.

emergence of *B. gaudichaudii*, *P. rigida*, *M. ferrugianta*, *G. graciliflora* and *G. noxia* with 0% sunlight (100% shade) was significantly lower than emergence of seedlings with 32%, 20% and 16% full sunlight, suggesting a negative effect of shade on seedling emergence.

Discussion

Density, recruitment and survival of all three cohorts of recruits were consistently higher in closed than in open savannas, suggesting a positive effect of canopy cover on seedling establishment. Our results agree with studies that found higher establishment of tropical savanna trees under relatively high canopy cover compared with open savannas and grasslands (Hoffmann 1996; Sassaki & Felippe 1999; but see Kanegae, Braz & Franco 2000). Canopy cover may facilitate the seedling establishment by mitigating stressful environmental conditions caused by high irradiance, temperature and soil water deficits during germination or shortly thereafter when seedlings are most sensitive to drought (Hoffmann 1996; Gottsberger & Silberbauer-Gottsberger 2006). High irradiance greatly enhances photoinhibition, increases soil and leaf temperature and decreases leaf stomatal conductance of savanna woody plants (Bucci *et al.* 2005). As grass densities are lower in closed than in open savannas (Eiten 2001), the higher density and survival of woody seedlings in closed than in open savannas also could be facilitated by a low competition with herbaceous species. Because most herbaceous plants have relatively shallow roots, during droughts the upper soil layers in closed savannas maintain relatively higher water potentials than soils in open savannas (Bucci *et al.* 2008). Removal or clipping of grasses increased establishment, survival and growth of tropical savanna tree seedlings (Williams *et al.* 2005; Hagenah *et al.* 2009). Thus, differences in grass biomass across vegetation types may result in lower water deficits for seedlings in closed than in open savannas.

Although we did not find significant differences in soil nutrient concentrations among vegetation types during the dry season, long-term litter manipulation experiments in Neotropical savannas found higher rates of litter decomposition and soil nutrient release in litter-added plots than in litter-removed plots (Villalobos-Vega *et al.* 2011). Thus, the high litter cover in closed savannas could further enhance seedling survival by providing large amounts of nutrients into the upper soil profile, particularly at the beginning of the wet season when litter decomposition rates increase (Villalobos-Vega *et al.* 2011).

In our study, seedling density significantly decreased over time in all cohorts in the three vegetation types as a result of mortality. Mean seedling survival across cohorts was higher than 60% in all vegetation types. Hoffmann (2000) and Kanegae, Braz & Franco (2000) found similar survival values for 1-year-old seedlings of several savanna species. The high survival of woody seedlings in all cohorts, particularly during the first 8 months of the study period, could be the result of fast root development and fast build-up of energy reserves on swollen root systems during early seedling establishment. Many savanna species typically have deep taproots that develop quickly at the seedling stage (Oliveira & Silva 1993) and play an important role in seedling drought resistance by allowing access to deep soil water during the dry season (Hoffmann & Franco 2003). During most of the wet season, soil water potential remains near 0 MPa in all vegetation types (Bucci *et al.* 2008). However, short dry spells during the wet season may greatly affect seedling survival across vegetation types by increasing soil water deficits in the upper soil layers (Kanegae, Braz & Franco 2000). In our study, soil gravimetric water content at 50 cm depth across vegetation types was about 40% during the dry season of 2005, and mean soil water potential at 50 cm depth ranged from −0.17 to −0.22 MPa during the dry season of 2008 (Fig. S2). These

results suggest that deep-rooted seedlings would have access to relatively moist soil at 50 cm depth during the dry season which might ensure seedling survival across vegetation types.

In our study, recruitment decreased significantly over time in all savannas, but it was consistently lower in open than in closed savannas. As a result, community-wide seedling limitation (proportion of 1-m^2 subplots without seedlings of any woody species) and mean specific seedling limitation values were higher in open than in closed savannas, suggesting that open savannas are more limited in seedlings of woody species than closed savannas. Recruitment limitation reflects a combination of seed limitation (i.e. failure of seeds to arrive at all suitable sites) and seedling establishment limitation (i.e. failure of seedlings to establish because of limited suitable sites). Seed limitation of many Cerrado woody species is higher in open than in closed savannas (Salazar *et al.* 2012**).** Our results suggest that in addition to seed limitation, seedling establishment limitation might also constrain the recruitment of Neotropical savanna woody species, particularly in open savannas. The high seedling limitation in open savannas might help to maintain their relatively low tree densities because tree abundance could not increase as quickly if seedlings do not establish in all suitable sites (Muller-Landau *et al.* 2002). High seedling limitation of woody plants also might contribute to maintain species diversity and tree–grass coexistence in Neotropical savanna ecosystems by slowing competitive dynamics of dominant species as in tropical moist forests (Hubbell, Foster & Obrien 1999) and grasslands (Eriksson & Ehrlén 1992).

The differences in irradiance, light quality and litter cover among vegetation types indicate substantial environmental heterogeneity from open to closed savannas. Such heterogeneity in the environment could result in a differential availability of optimal microsites for seedling establishment and for determining the relative performance of each species, as has been found in tropical forests (Uriarte *et al.* 2010). Indeed, through the entire study period, the total number of new recruits in each plot significantly decreased as the number of pre-existing seedlings increased in closed, but not in intermediate or open savannas, suggesting that other factors than seedling density might constrain recruitment in more open savannas. Combining long-term demographic data with fine-scale quantification of environmental variables will help in assessing the relative importance of each environmental variable in determining seedling density, diversity and survival in each vegetation type.

In our glasshouse study, most woody species had low seedling emergence when they were covered with large quantities of litter. Our results are consistent with studies performed on seedlings of several tropical forest species (Molofsky & Augspurger 1992; Dalling & Hubbell 2002; Baeten *et al.* 2009) which have found negative effects of litter cover on seedling emergence. Litter can be a physical barrier for the emergence of seedlings, in particular, those derived from small seeds that lack the reserves required to grow sufficiently fast to penetrate the litter or reach the soil through the litter layer (Vázquez-Yanes & Orozco-Segovia 1993). In addition to litter acting as a physical barrier, litter cover could have reduced seedling emergence by modifying other microenvironmental conditions such as light levels around the seeds (Scarpa & Valio 2008). Seedling emergence of most studied woody species was strongly inhibited under 0% full sunlight, suggesting that similar to tropical forest species, seeds of some savanna species may be positively photoblastic and require light to germinate (Carreira & Zaidan 2007). Although the light levels we used in the glasshouse experiment are below the maximum values found in the field during the mid-dry season, seeds may experience low-light conditions in the field because there is a large variation in daily light levels even during the wet season when light intensity decreases (Kanegae, Braz & Franco 2000).

In the field, light enriched in far-red and litter cover were higher in closed than in open savannas. Both the differences in the quality of transmitted light and the differences in leaf litter cover may highly affect seedling establishment of woody species along tree density gradients in Neotropical savannas because germination and seedling growth of species with positively photoblastic seeds are particularly affected by changes in light spectra (Valio & Scarpa 2001). At a temporal scale, the low seedling emergence of most woody species under high litter cover and very low light level suggests an adaptation to delay seed germination and seedling growth at the onset of the wet season when soil water does not limit plant development (Salazar *et al.* 2011) and litter cover decreases because of rapid decomposition (Villalobos-Vega *et al.* 2011**).** Thus, after the beginning of the wet season, with ample soil water and low litter on the soil surface, there is a window of opportunity for seed germination and seedling growth of savanna woody species.

The results of our study could be useful for conservation and restoration practices. For instance, protecting closed savannas could ensure the long-term survival of natural or reintroduced plant populations because they provide appropriate microsite conditions for seedling recruitment and survival. Conservation practices are critical because rapid conversion of natural vegetation into agricultural lands, high fire incidence and invasion by alien species may greatly affect successional processes in Neotropical savannas. Because the savannas we studied have been protected from fire for a relatively long period of time, successional processes in our study might not be as important as changes resulting from species-specific demographic processes in each vegetation type. However, as global climate change intensifies, long-term demographic studies are highly needed to better understand successional processes of woody plants in Neotropical savannas.

In conclusion, our study shows that in Neotropical savannas tree canopy cover facilitates seedling establishment of woody species by reducing stressful environmental conditions. In particular, low irradiance, low grass biomass and high litter cover in closed savannas enhance the recruitment and survival of woody seedlings relative to open savannas by reducing soil water deficits and increasing nutrient availability in the upper soil layers. The higher seedling limitation of tree species in

open than in closed savannas contributes to maintain relative different balances between trees and herbaceous plants along topographic gradients in Neotropical savannas and helps to explain spatial distribution patterns of woody species in these ecosystems. At a temporal scale, high litter cover and low light levels significantly reduced seedling emergence of most woody species under control conditions, suggesting an adaptation to delay seedling emergence until the wet season when litter rapidly decomposes and soil water availability is high across all vegetation types.

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Supporting Information

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

Figure S1. Number of species of Cerrado woody seedlings as a function of the number of 1-m^2 plots in closed and open savannas.

Figure S2. Soil water potential (a) and soil gravimetric water content (b) in closed, intermediate and open savannas at 5, 10, 25 and 50 cm depth from the surface.

Table S1. Soil nutrient concentrations and pH in closed, intermediate and open savannas. Samples were taken during the dry season at 0–5, 5–10, 10–25 and 25–50 cm soil depth.

Table S2. Effects of vegetation type (V) and time on density (#seedlings m^{-2}) of the initial seedling assemblage and three cohorts of recruits.

Table S3. Effects of vegetation type (V), time and their interaction on richness (#species m^{-2}), density (#seedlings m^{-2}) and the richness/density ratio of newly recruited seedlings across time.

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