Effects of Fire Frequency on Seed Sources and Regeneration in Southeastern Amazonia

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The trajectory and recovery time of fire-disturbed forests depend on the capacity of seedlings and resprouts to get established over time. Here, we investigated the mechanisms associated with fire effects on post-fire regeneration in the context of a large-scale fire experiment located in southeastern Amazonia. Specifically, we tested the hypothesis that recurrent understory fires reduce forest regeneration capacity by reducing the recruitment of seedlings, the viability of the soil seed bank, the seed rain, and the resprouts. The experiment was comprised of three 50-ha plots: an unburned control, and two plots that were experimentally burned three (triennially) or six (annually) times between 2004 and 2010. Seedlings represented 2.4 and 0.6% of the seed rain and soil seed bank in the Control and burned treatment plots, respectively. Compared to the control, the triennial burns caused more reduction than the annual ones in seed rain (−42 vs. −10%) and seed bank (−78 vs. −50%). These fire-related reduction in seed rain in both treatments explain most of the low post-fire density of seedlings (0.4 and 0.8 ind/m^2, respectively), compared with the Control (2.9 ind/m^2). The 3-year interval between fires contributed to highest mortality of seeds stored in the soil than in the annual burn treatment, where fires were less severe. However, there were 5.5 times more resprouting in the plot burned triennially compared with the one burned annually. In both treatments, though, the number of resprouts declined over time. In conclusion, seeds from both seed rain and seed bank and resprouting were negatively affected by both fire frequencies, reducing the likelihood of native species recovery by altering the regeneration pathways. This situation is expected to be increasingly common in southeastern Amazon forests, particularly under warmer and dryer climate conditions.

Keywords: deforestation, global climate change, resprout, seed rain, soil seed bank, understory fire

INTRODUCTION

Human activity is the main ignition source for Amazon wildfires. People use fire as a management tool, to clean deforested fields, to manage pasturelands, or in slash-and-burn agricultural systems (Nepstad et al., 2001; Lindenmayer et al., 2009; Brando et al., 2019). Although dense forests can maintain high levels of moisture underneath their canopies, therefore reducing litter flammability,

extreme droughts and forest degradation can disrupt this natural firebreak. When this disruption occurs, management fires often escape into primary forests and cause fire-related tree mortality, especially along forest edges. Wildfires have been widespread over the past decades along the "Arc of deforestation" in Amazonia (Nepstad et al., 2001, 2008; Aragão et al., 2018), where droughts, sources of ignition, and forest degradation are all common. Brazil is the sixth-biggest emitter of greenhouse gases on the planet, with 1.9 billion tons of carbon dioxide emissions in 2018 and 44% was connected to land-use change (Sistema de Estimativas de Emissões e Remoções de Gases de Efeito Estufa (SEEG), 2020). Following climate and land-use changes in the coming decades, wildfires are expected to become even more common (Brando et al., 2020), with important implications for forest carbon dynamics and biodiversity.

Post fire recovery of Amazon forests depends on the capacity of reproductive trees to resist fire and of seedlings and resprouts to get established. On one hand, Amazon tree communities are highly susceptible to fires; their thin bark often exposes cambium cells to damaging levels of fire-related heat (Brando et al., 2012; Staver et al., 2019). While some larger, thicker-barked trees survive low-to-medium intensity fires over the short-term, fire-related wounds to trees' roots, stems, and vascular systems are expected to substantially reduce trees' lifespans and cause prolonged forest degradation (Barlow et al., 2003; Staver et al., 2019). On the other hand, tropical forest communities are resilient to a range of disturbances. This suggests that post-fire regeneration, originated from seed rain, seed bank, and resprouting, can lessen some of the negative ecological effects caused by fires. Therefore, an important knowledge gap is whether and in what extent these fire-disturbed forests can recover. Indeed, fire-disturbed forests are expected to partially or fully recover their structure, diversity, and functions over time (Alencar et al., 2006; Balch et al., 2008; Morton et al., 2013; Brando et al., 2019), but assessments of the effects of understory fires on forest regeneration processes is still scarce. Thus, quantify the regeneration diversity and abundance can shed some light on the capacity of forests to recover some of their fundamental properties after wildfires (Coe et al., 2013; Brando et al., 2019).

Post-fire seedling recruitment—the prevalent regeneration mode of tropical forests—likely compensates some losses in forest diversity associated with fire disturbance (Kennard et al., 2002; Hoffmann et al., 2003; Balch et al., 2013). However, this recovery capacity may decline with death of reproductive trees caused by fire, leading to a decline in seed sources. Although seeds stored in the soil (the seedbank) can enable seedling recruitment, those seeds tend to be short-lived (i.e., <3 years), thus highly dependent on the seed rain, and relatively vulnerable to high-intensity, recurrent fires (Clark et al., 1999; Bond and Midgley, 2003; Wright et al., 2005; Norden et al., 2007; Poorter et al., 2010). However, when seed-to-seedling recruitment failures, resprouts (root, basal, or epicormic resprouting) shows to be another important regeneration source, contributing to post-fire recovery and maintenance of diversity (Poorter et al., 2010). It remains unclear, however, how fire intensity and frequency affect tree resprouting as a recovery mechanism.

Amazon surface fires vary in extent, frequency, and intensity. How these metrics interact has important effects on post-fire trajectories of Amazon forests. When fires occur less frequently but are more intense (Balch et al., 2008), post-fire mortality of large trees tend to increase disproportionally (Brando et al., 2014). Because larger individuals tend to produce more seeds than small ones, high-intensity fires can substantially reduce seed production and associated regeneration. In addition, when fires occur less frequently, we expect more fuel accumulation, higher fuel continuity, and higher burned area—an important predictor of fire-related seedling and sapling mortality (Balch et al., 2011, 2015). Although high fire frequency may also kill reproductive trees and drive changes in seed sources, these fires are likely to be less intense. However, frequent fires can still substantially affect forest regeneration by killing seedlings, resprouts and seeds in the seed bank (Montibeller-Santos, 2014; Cury, 2016). Few studies though have quantified how different fire frequencies and intensity affect tropical forest regeneration.

Overall, understory fires are expected to be stronger at forest edges, where post-fire reduction in seedling and soil seed bank abundance are likely to be higher, both by direct killing of seeds and seedlings and by reduced seed rain, which in turn is caused by increased tree mortality. The delayed mortality of large trees following fire (Silvério et al., 2018) may also contribute to changes in the forest microclimate and thus strongly influence seed germination and seedling establishment (Clark et al., 1999; Cochrane and Schulze, 1999; Cochrane, 2003; Peres et al., 2003; Wright et al., 2005; Barlow and Peres, 2006; Balch et al., 2015).
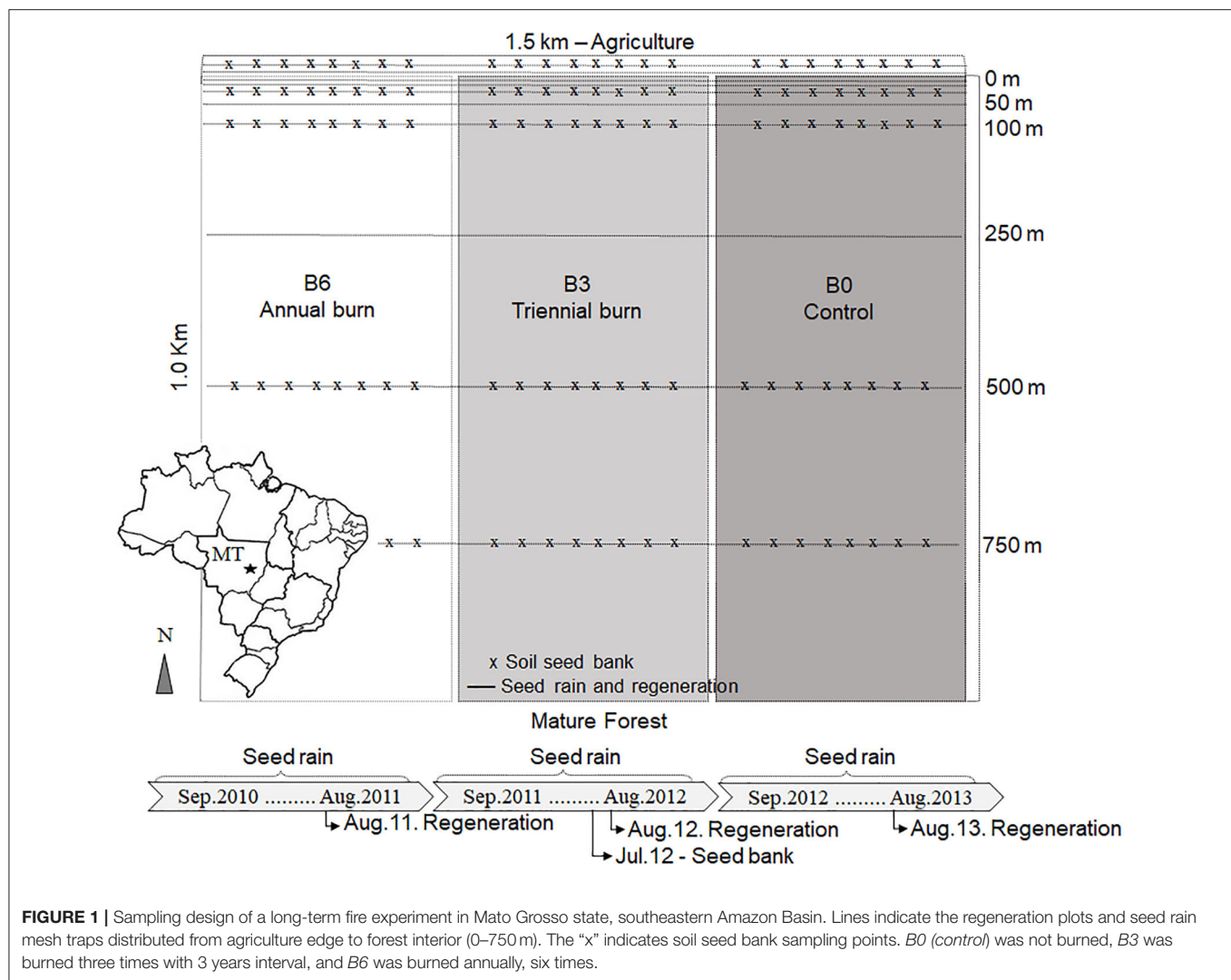
Here, we assessed how changes in fire frequency and edge distance affect forest regeneration and two associated processes: seed rain and storage of seeds in the soil. We studied these processes in a large burning experiment conducted in a transitional forest, between Amazonia and Brazilian Cerrado. Our main predictions are that: (1) fire drives reduction in forest regeneration capacity by reducing the number of regenerating plants and the seed rain and seed bank, with stronger effects where fires are more frequent; (2) forest regeneration processes (seedlings, resprouts, seed rain, and seed bank) are more severely impacted near the forest edges; (3) post fire forest regeneration mode shifts from seedling to resprouting.

MATERIALS AND METHODS

Study Site

Beginning in 2004, a large-scale burning experiment (150-ha) was conducted in a transitional forest, located between Amazon rainforest and Brazilian Cerrado (Savanna), in a privately owned agricultural holding (80,000-ha), in Mato Grosso state, Brazil (13°04′35″ S; 52°23′08″ W; **Figure 1**). The farm was partially cleared (~50%) for pasture in the 1980s and was converted for soybean cultivation between 2003 and 2008.

The soil is classified as dystrophic red-yellow Oxisols by Instituto Brasileiro de Geografia e Estatística (IBGE) (2009), being deep, well-drained and with low natural fertility (Instituto Brasileiro de Geografia e Estatística (IBGE), 2007). According to Köppen's classification, the climate is tropical Aw, with an average annual precipitation of 1,770 mm (data from 2005

FIGURE 1 | Sampling design of a long-term fire experiment in Mato Grosso state, southeastern Amazon Basin. Lines indicate the regeneration plots and seed rain mesh traps distributed from agriculture edge to forest interior (0–750 m). The "x" indicates soil seed bank sampling points. *B0 (control)* was not burned, *B3* was burned three times with 3 years interval, and *B6* was burned annually, six times.

to 2011; Rocha et al., 2014), a rainy season extending from September to April, and a dry season between May and August, when monthly rainfall is typically below 10 mm (Rocha et al., 2014).

The vegetation is classified as Evergreen Seasonal Forest, with low deciduousness on dry season (Instituto Brasileiro de Geografia e Estatística (IBGE), 2012). Due to the proximity of the Brazilian Cerrado this forest is also known as "transitional forest" (Kunz et al., 2009). The forest has a relatively low canopy height (averaging 20 m) and lower plant diversity in comparison with the wetter forests of the central Amazon (Balch et al., 2008). Furthermore, about 97 species of trees and lianas ≥10 cm diameter at 1.3 m stem diameter breast height (DBH) were identified over the entire 150-ha experimental area (Balch et al., 2011). The nine most common species accounted for 50 percent of the Vegetation Importance Index (Balch et al., 2008, 2011), and more than 74% of tree and shrub species also occur in the adjacent Cerrado vegetation (Cury, 2016).

Experimental Design

The experimental site was located in a forest with no history of logging or previous fires. The experimental area of 1.5 × 1.0 km (150-ha; **Figure 1**) was subdivided into three 50-ha plots: an unburned *Control* (*B0*), a plot burned three times at 3-year interval (*B3;* burned in 2004, 2007, and 2010), and a plot burned six times (*B6*), with annual experimental fires from 2004 to 2010 (except for 2008). The controlled fires were conducted toward the end of the dry season (August) and were carried out by a large team of researchers, students, volunteers, technicians, firefighters, farm workers, among others. To know more about the site description, the study design, aboveground biomass, canopy height, canopy density, and the burning experiment with the ignition technique that were used to burn the plots (see Balch et al., 2008).

Seed Rain

The seed rain was sampled biweekly for 36 months, starting after the last fire event in September 2010 until August 2013.

We collected seed rain using 0.5 m^2 mesh traps ($N = 90$ per 50-ha plot) placed systematically throughout the experimental plots, from the cleared edge to the forest interior (0, 10, 15, 30, 50, 100, 250, 500, and 750 m; **Figure 1**). Traps were suspended ~1 m above the forest floor adjacent to regeneration sub-plots (described below). Number of seeds was quantified, and all seeds were identified to species or at least genera by means of comparison with a reference seed collection.

Seed Bank

Two years after the 2010 fires, 120 soil samples were taken from the burned treatments and the control ($N = 40$ per plot) using a cylinder with 20 cm diameter and 5 cm deep. The samples were collected at 0, 30, 100, 500, and 750 m from forest edge. One soil sample was composed by a mix of two cores taken from <2 m away from the regeneration sub-plot (**Figure 1**). Samples were then stored in polythene bags placed in Styrofoam boxes, in a shaded cover for 1 week. Afterward, the soil was spread in perforated plastic trays, keeping soil layer height up to 3 cm, and placed in a greenhouse watered twice a day. Four control trays containing only sterilized sand were randomly distributed among the samples to control potential contamination from seeds dispersed through the greenhouse. Seedlings were weekly censored for the period of 1 year. All individuals that emerged were counted, identified through comparison with seedlings, juveniles and adult materials previously collected during fieldwork. Non-identified seedlings were transplanted into pots and grown until a larger size to further identification. Six months after the beginning of the experiment, the soil was overturned to promote the germination of seeds that were deeply buried. This method was adapted from Roberts (1981).

Seedlings and Resprouts

The focus of this study was the woody vegetation: therefore, all seedlings, saplings and resprouts of tree, shrub and liana species were recorded in 90 0.5 m^2 sub-plots per 50-ha plot. Sub-plots were distributed systematically adjacent to seed traps, from the edge to the forest interior (**Figure 1**). All plants taller than 5 cm and ≤1 cm DBH (diameter at breast height or 1.30 m above ground) were tagged and identified. The regeneration mode (i.e., individuals originating from seed germination or root, basal, or epicormic resprouts) was also recorded. Whenever needed, small excavations near the plant base were done to confirm the resprouting origin. Censuses were conducted annually in 2011, 2012, and 2013. If a given individual was present in one inventory and not in the next, it was considered dead. Plants that were present in those inventories but lacked living structures and had no resprouting were recorded as dead. Plant species were identified using the botanical collection of IPAM (Amazon Environmental Research Institute) and the Herbarium NX at Mato Grosso State University at Nova Xavantina. Species names followed the Missouri Botanical Garden Tropicos® database (www.tropicos.org; **Table S1**).

Statistical Analysis

To evaluate the degree in which fire frequency (triennially and annually burned) affect species diversity, we analyzed patterns of species richness and abundance using individual-based species accumulation curves for seeds from seed rain, seeds stored in the soil, seedlings and resprouts. Also, structure and composition of communities (i.e., seed rain, seed bank, seedlings and resprouts) were analyzed using non-metric multidimensional scaling (NMDS). The NMDS was generated from a Bray-Curtis dissimilarity matrix and *a posteriori* analysis of similarities (ANOSIM) was used to test whether there are significant differences among burned plots and the control, and to analyze whether structure and composition of seedling assemblages were similar to that in the seed rain and seed bank samples. We also analyzed species abundance by means of Whittaker plots for seeds and regenerating woody plants.

A generalized linear model (GLM) was used to account for differences in the species richness and abundance among edge distances (nine distances for seed rain, seedlings and resprouts; five distances for seed bank), and also among years (2011, 2012, 2013, except for seed bank that was collected only in 2012). GLM was performed using Poisson distribution, indicated for counts with Quasi-Poisson correction when data presented variance higher than average.

Diversity index of seedlings, resprouts, seed rain and seed bank in the **Table S3** was performed using the Shannon Index, the most commonly used, although it considers equal weight between rare and abundant species; and Simpson's diversity index (D) that provides a good estimate of diversity in small population sizes (Magurran, 1988). We also calculated Pielou's evenness (equitability).
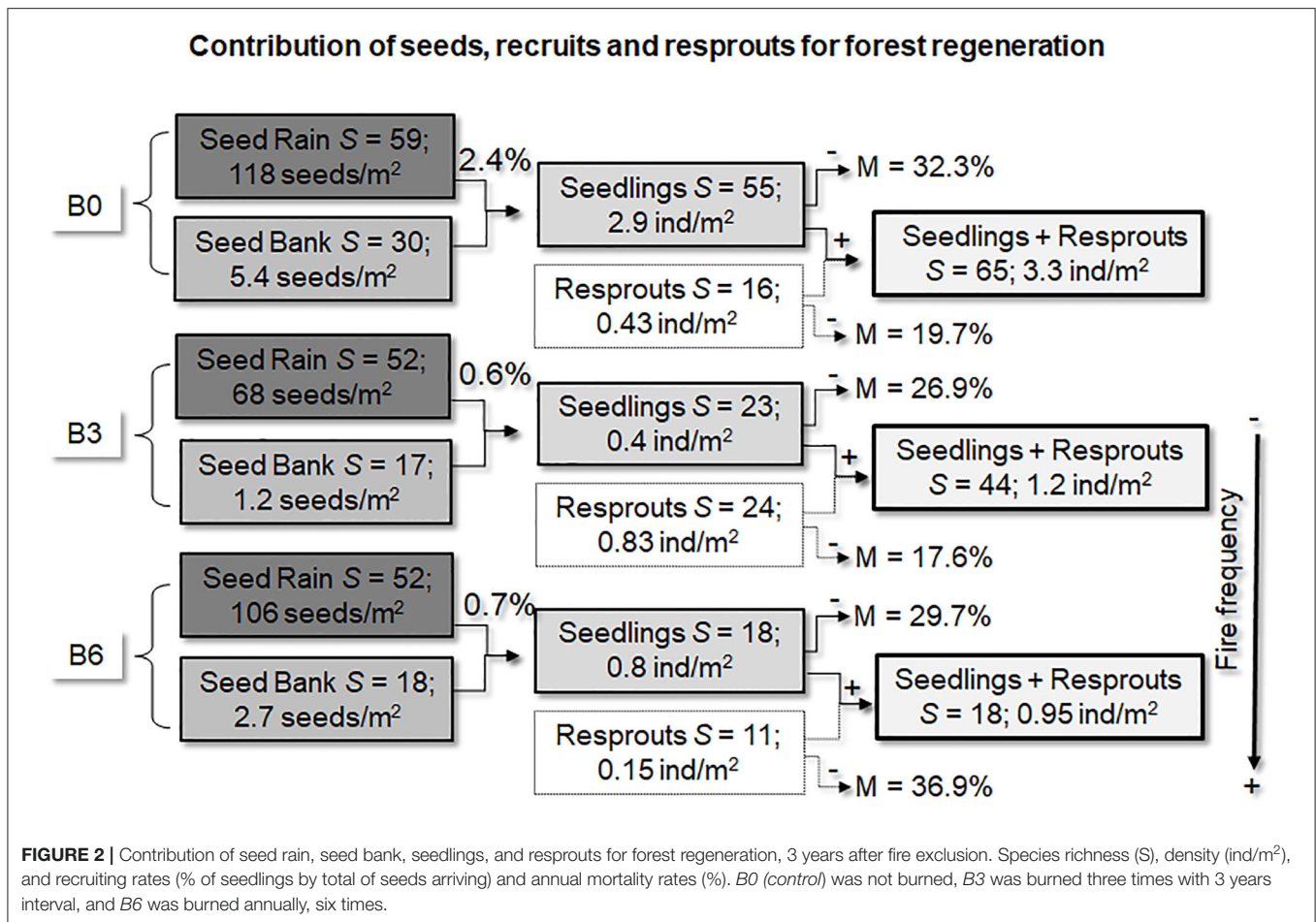
All analyses were performed using R (R Core Team, 2014) and the package Vegan (Oksanen et al., 2013).

RESULTS

Seed Rain

We collected a total of 39,356 seeds spread across 67 species (**Tables S1, S2**): 15,911 (59 species) in *B0*; 9,135 (52 species) in *B3*; and, 14,310 (52 species) in *B6* (**Figure 2**). The seed rain density in the *Control* (118 seeds/m^2) was higher than in *B3* (68 seeds m^2) but similar compared to *B6* (106 seeds m^2; **Figure 2**). Species richness in the seed rain was also significantly higher in the Control than in the burned plots (see the Species Accumulation Curves; **Figure 3A**). In addition, according to our NMDS analysis, the structure and composition of the community in the seed rain were similar between the two burned treatments, and both differed from the *Control* (ANOSIM: $R = 0.07$; $P = 0.001$). *Sloanea eichleri* was the most abundant species in *B3*, accounting for 50% of the total number of seeds in seed rain in this treatment; *Xylopia frutescens* was the most abundant species in *B6* (**Figure 4A**).

Species richness in the seed rain was lower near the edge than in the forest interior in both burned plots, with a stronger effect in *B3* than in *B6* (**Table S4**; **Figure 5A**). In contrast, in the *Control* plot species richness was similar between the forest edge and interior (**Table S4**; **Figure 5A**). Seed rain density was

FIGURE 2 | Contribution of seed rain, seed bank, seedlings, and resprouts for forest regeneration, 3 years after fire exclusion. Species richness (S), density (ind/m^2), and recruiting rates (% of seedlings by total of seeds arriving) and annual mortality rates (%). *B0 (control)* was not burned, *B3* was burned three times with 3 years interval, and *B6* was burned annually, six times.

also lower at the forest edge and up to 500 and 750 m into the forest in *B3* and *B6*, respectively. In the *Control*, edge effects on seed density were observed only in the first 15 m from the forest border. The number of species and density of seeds in the seed rain were constant along the years in both burned treatments and in the Control.

Seed Bank

In soil seed bank samples, we recorded 2,330 viable seeds from 34 species (**Tables S1**, **S2**): 1,352 in the *Control* (30 species; 538 seeds/m^2); 310 in *B3* (17 species; 123 seeds/m^2); and 668 in *B6* (18 species; 266 seeds/m^2; **Figure 2**). Species accumulation curve showed that species richness was higher in the *Control* than in *B6*, but similar between *B6* and *B3* (**Figure 3B**). The structure and composition of the soil seed bank community was also affected by the experimental fires, with significant differences between the *Control* and burned plots (ANOSIM: $R = 0.33$; $P = 0.001$). *Miconia* was the most abundant genus in the seed bank, with its five species contributing 84, 92, and 95% of the total seeds in the seed bank in the *Control*, *B3* and *B6*, respectively (**Figure 4B**). Surprisingly, despite being the most abundant species in the seed bank, no species from the genus *Miconia* was found among the regeneration.
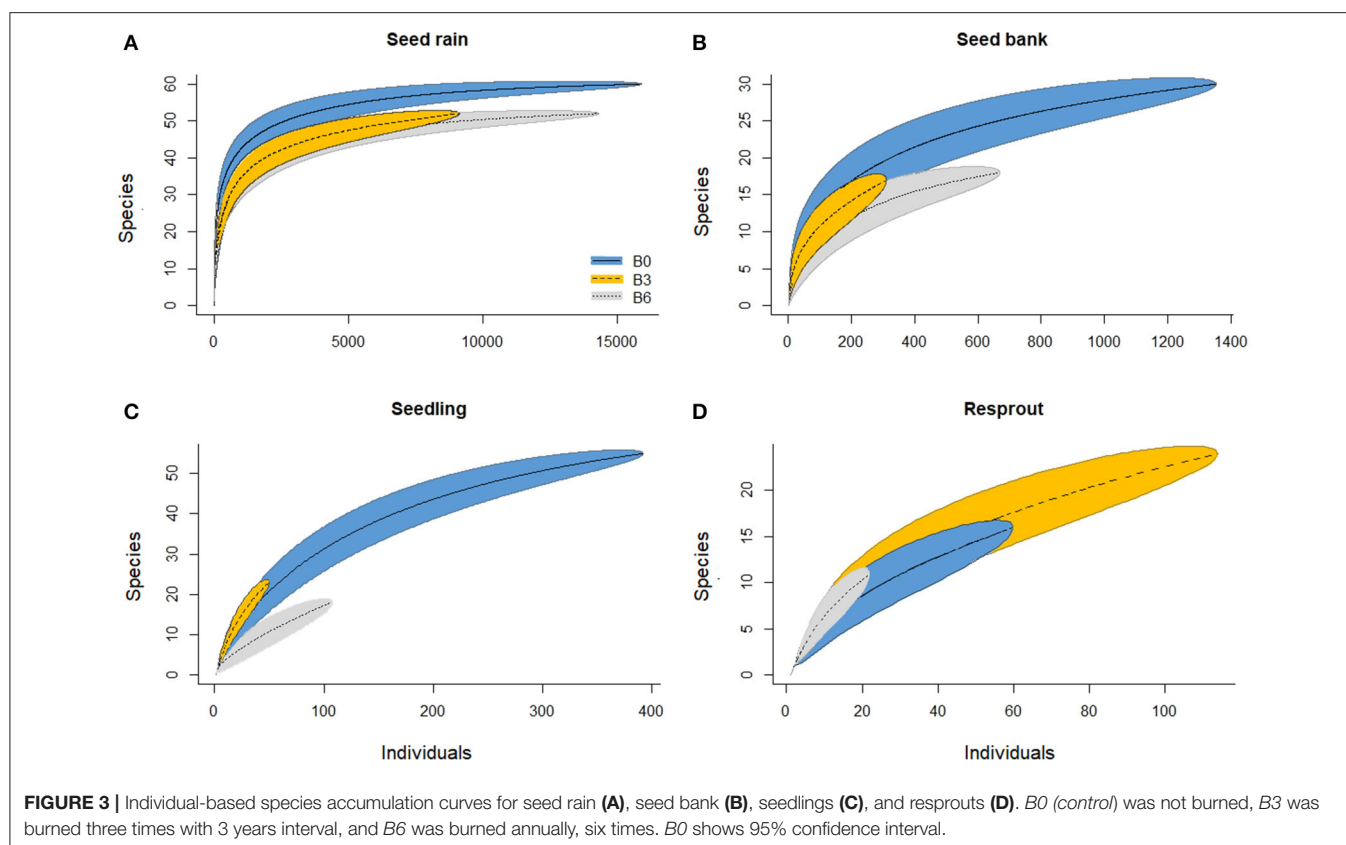
Edge distance influenced the species richness and abundance of seeds in the seed bank in both burned treatments. Compared with the forest interior, we observed lower species richness in the seed bank samples in *B3* and *B6* up to 500 and 100 m (**Table S4**; **Figure 5B**), respectively. Also, the abundance of seeds was reduced up to 500 m from edge in *B3* (GLM, $P < 0.004$), and along all distances in *B6*, presenting no significant differences between 0 and 750 m.

Summarizing, we observed a large reduction in seed availability in both fire treatments. The species richness and the number of seeds of both seed sources (seed rain and seed bank) were reduced, for example, decreased 45 and 13% of seeds, respectively, in *B3* and *B6*, compared with *B0* (**Figure 2**).

Seedlings and Resprouts

Overall, we registered 114 species in the four sampling components (seed rain, seed bank, seedlings, and resprouts; **Table S1**). From this total, only four species were represented in all components (*Amaioua guianensis*, *Doliocarpus spatulifolius*, *Solanum mauritianum*, and *Tachigali vulgaris*); and 55 in only one component (**Table S1**). These results suggest that regeneration occurred via multiple pathways.

The sampled regeneration (seedlings and resprouts) comprised 72 species and 739 individuals. From all species,

FIGURE 3 | Individual-based species accumulation curves for seed rain **(A)**, seed bank **(B)**, seedlings **(C)**, and resprouts **(D)**. *B0 (control)* was not burned, *B3* was burned three times with 3 years interval, and *B6* was burned annually, six times. *B0* shows 95% confidence interval.

65 were found in the *Control*, 44 in *B3*, and 18 in *B6* (**Figure 2**). Summing the seedlings and resprouts together, the density of these small individuals was higher in the *Control* (3.3 ind/m^2) than in *B3* (1.2 ind/m^2) and *B6* (0.9 ind/m^2; **Figure 2**). Evaluating only seedlings, the species accumulation curves indicate that species richness also decreased with increasing fire frequency (55 species in the *Control*; 23 in *B3*; and 18 in *B6* (**Figure 3C**). However, the density of seedlings was 87% lower in *B3* (49 individuals; 0.4 ind/m^2) and 73% lower in *B6* (106 individuals; 0.8 ind/m^2), compared with *Control* (391 individuals; 2.9 ind/m^2; **Figure 2**).

Interestingly, seeds in the seed rain (from 2011 to 2013) and soil seed bank samples (2012), only 2.4% resulted in new seedlings in the *Control*, vs. 0.6–0.7% in the burned plots (**Figure 2**). Also, our analysis showed that the seedlings samples were more similar to the seed rain samples [ANOSIM; $R = 0.35$ (*B3*) and 0.36 (*B6*)] than to the seed bank [ANOSIM; $R = 0.41$ (*B3*) and 0.64 (*B6*)] in both burned plots (**Figure 6**).

B3 had two times more species and 5.5 times more live stems of resprouts than *B6* (**Figures 2**, **3D**). This is important to explain why the sum of regeneration stems (i.e., counting seedlings and resprouts) presented more than twice the number of species and individuals in *B3* than in *B6*. *Pyrostegia venusta*, the most abundant liana in *B3*, was present both as seedlings and resprouts (**Figures 4C,D**).

When looking at distance from forest edge, both burned treatments exhibited reduced species richness of seedlings along

all distances. A different pattern was registered in the *Control*, which increased gradually the number of species beginning at 30 m from the edge (**Figure 5C**). Resprouting species richness had no difference in the *Control* regardless of edge distance. Contrasting, *B3* increased the number of species with regrowth of stems in all distances (**Figure 5D**).

We noticed that in the second year after fires (2012) the seedling species richness increased in *B3*, as well as the density in *B3* and *B6* (**Figure 7A**). In 2012, only two species accounted for 90% of all seedlings recruited in *B6* treatment, with *Pouteria ramiflora* alone accounting for 66.5% and *Amaioua guianensis* for 23.5%. In contrast, resprouts showed a gradual reduction through the years in both burned plots, losing species and density in the third year after fire exclusion in 2013 (**Figure 7B**). The *Control* had no resprouting species and density variation through the years (**Figures 7A,B**). Finally, seedling mortality was similar among plots, around 29% per plot per year. On the other hand, resprout mortality was similar between Control (19.7%) and B3 plot (17.6%), but higher in *B6* (36.9%; **Figure 2**).

DISCUSSION

Experimental fires caused substantial reduction in the abundance and diversity of seedlings in both fire treatments by reducing the seed rain, killing seedlings and impoverishing the seed bank. We also observed decreasing post-fire resprouting due complex
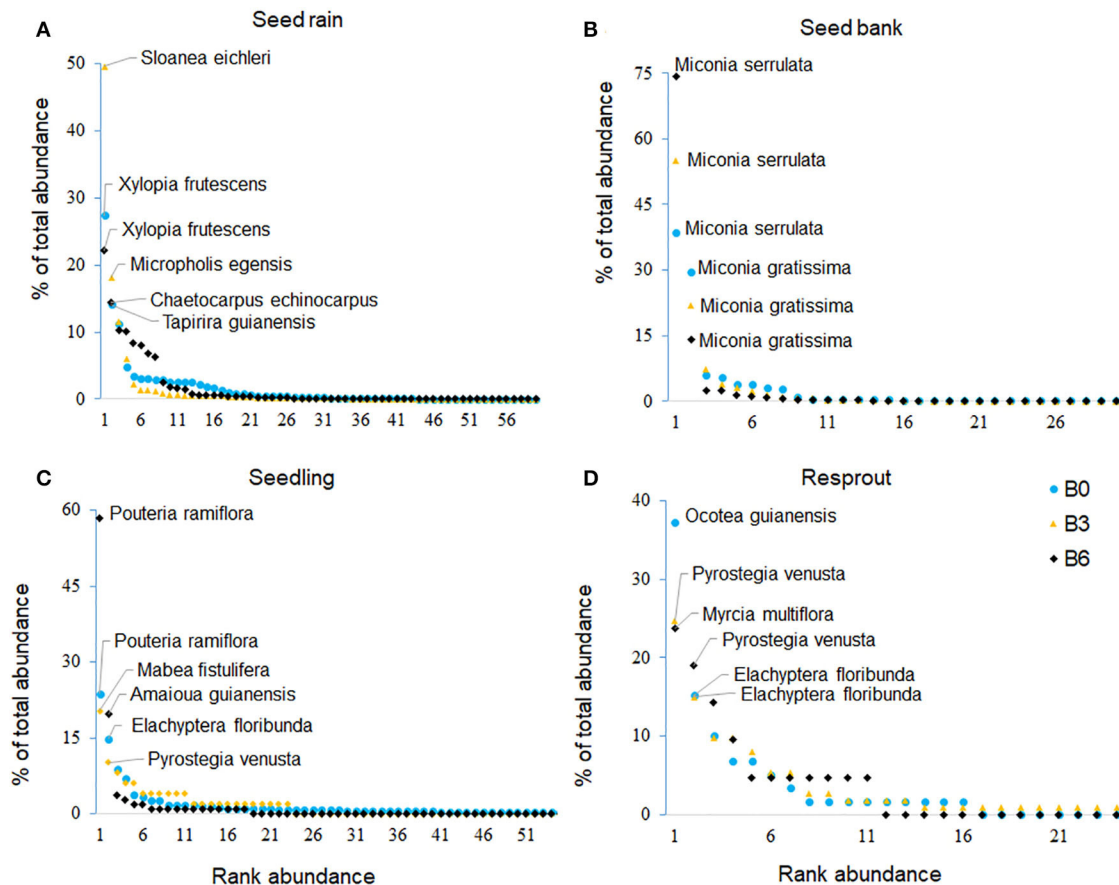
FIGURE 4 | Whittaker's plot (rank-relative abundance diagram) of woody species (trees and lianas) sampled from the seed rain **(A)**, seed bank **(B)**, seedlings **(C),** and resprouts **(D)**. The two more abundant species by treatment were identified in the plot. *B0 (control)* was not burned, *B3* was burned three times with 3 years interval, and *B6* was burned annually, six times.

fire effects. These results suggest that recurrent fires potentially threaten all regeneration modes, reducing forest postfire recovery ability. The differences in regeneration patterns along forest edge-to-interior gradients also suggest that forest fragmentation, by creating more edges, can further reduce post-fire forest regeneration (Silvério et al., 2018). Along these highly flammable edges, high-intensity fires can cause substantial reduction in seedlings, resprouts, seed rain, and seed bank. Although forest regeneration may recover over longer time spans (Brando et al., 2014; Balch et al., 2015), it is unclear how the observed changes in regeneration associated with fire will influence future plant communities.

The first hypothesis about reduction in forest regeneration capacity by reducing the number of regenerating plants and the seed rain and seed bank in the treatment burned six times annually was partially corroborated. The most likely explanation for this result is differences in fire intensity between the two treatments. The longer fire interval in the treatment burned three times triennially, allowed increased fire intensity and severity during the droughts of 2007 and 2010 (Brando et al., 2014; Balch et al., 2015). As a result, both seed sources (seed rain

and seed bank) and the pool of resprouting stems experienced sharper declines, especially at the forest edges, where the fires were even more intense (Kennard et al., 2002; Barlow and Peres, 2006; Brando et al., 2014; Montibeller-Santos, 2014; Ribeiro and Borghetti, 2014). Furthermore, post-fire tree mortality was lower in the high fire frequency plot (Brando et al., 2019). This helps to explain why the seedling assemblage was more similar with the seed rain than with the soil seed bank community. Thus, corroborating Barlow and Peres (2006), local fruit production represents an important source of viable seeds for post-fire forest recovery, at least during the earliest stages of regeneration.

Our prediction that post-fire forest regeneration was lower at the forest edge was soundly confirmed. Our results showed that seedlings and seed sources (seed rain and seed bank) were lower within the first 100 m from the agricultural field neighboring the treatment plots. These patterns probably result from the increased dry plant material and lower humidity along forest edges. These characteristics can trigger intense and severe fires (Brando et al., 2014) causing death of large fruiting trees (Barlow and Peres, 2006) and reduction of seeds stored in the soil (Melo et al., 2007). Moreover, three
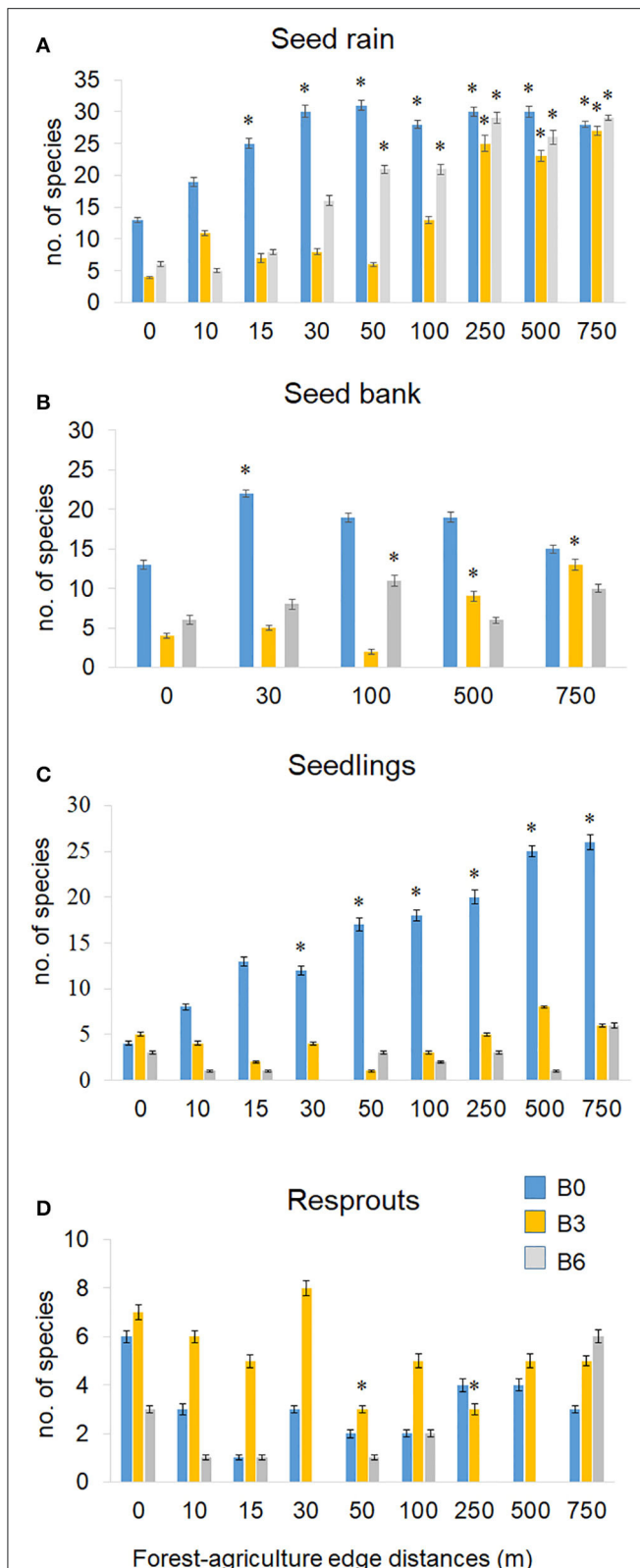
FIGURE 5 | was not burned, *B3* was burned three times with 3 years interval, and *B6* was burned annually, six times. Standard error bars. Asterisks indicate significant differences between distances (10–750 m) with the edge (0 m; GLM; *P* < 0.05).

FIGURE 5 | Number of woody species sampled from seed rain **(A)**, seed bank **(B)**, seedlings **(C)**, and resprouts **(D)** by distance from forest edge. *B0 (control)* *(Continued)*

interdependent processes could also contribute to these results: reduced seed-dispersing animal abundance near the edge (Peres et al., 2003); increased seed predation and increased seedling herbivory (Carvalho et al., 2012; Massad et al., 2013); and seed dehydration (Daws et al., 2005).

Our prediction that regeneration mode is more dependent on resprouting than seedling germination received strong support. We noted that resprouts increased in density just after fire (2011) in the plot burned three times, particularly near the forest edge. As a result, there was increased species diversity in that treatment compared with the plot burned six times. As reported in previous studies, vegetative growth is an adaptive trait allowing response to disturbance and therefore, is a key process for rapid forest recovery (Hoffmann et al., 2003; Poorter et al., 2010). However, we observed that resprouting stem density and species richness declined over time in both burned treatments, especially where fires were more frequent. The possible explanation for this pattern is that the repeated fire events killed the resprouting bank or reduced the capacity of plants to allocate non-structural carbohydrate storage for vegetative growth (Hoffmann et al., 2003). This is compatible with the two-fold greater mortality in the plot burned six times annually (37%) than in the plot burned three times triennially (17.6%) and suggest a major limitation for the recovery of transitional forests after the occurrence of fires.

The future trajectory of burned forests strongly depends on how species regenerate in response to one or more fires. According to the prediction that pioneer species would prevail in the soil seed bank and in the regeneration after fires (Garwood, 1989; Montibeller-Santos, 2014), we expected to find some similarity between soil seed bank and seedlings. The high abundance of *Miconia* sp. in the soil seed bank could indicate a high potential for this species to dominate the early succession after the fires (Dalling et al., 1998; Filho et al., 2005). However, we observed no seedlings of this genus in both burned treatments. Moreover, the seed rain was a driver for the first pulse of regeneration, with several species being present in the seed rain and as seedlings (e.g., *Amaioua guianensis*, *Pouteria ramiflora*). We speculate that there are at least three explanations for this result. First, species-specific traits may promote rapid post-fire germination and could be an advantage for some desiccation-sensitive species coming from seed rain, with higher seed nutrient reserve (e.g., *Ocotea* sp., *Pouteria ramiflora*, *Mabea fistulifera*, *Virola sebifera*, *Sacoglottis guianensis*), allowing reduction in seed exposure to predation and desiccation (Daws et al., 2005). Second, seedlings with small seeds (e.g., *Miconia* spp.) may be outcompeted, particularly by fast-spreading invasive grasses near to the forest edge (Snyder and Chesson, 2003; Reid and Holl, 2013; Silvério et al., 2013; Balch et al., 2015).
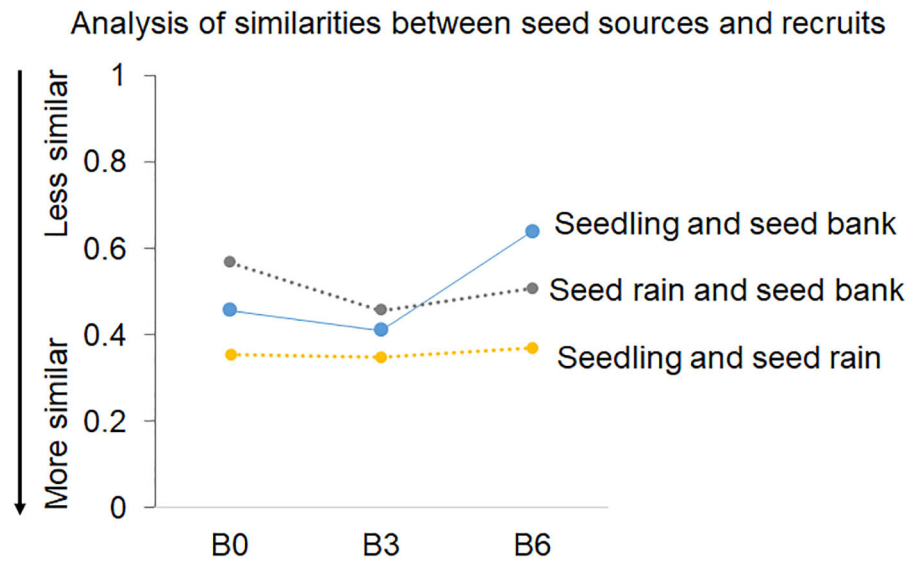
FIGURE 6 | Analysis of similarities (ANOSIM), based on Non-metric multidimensional scaling (NMDS–Bray-Curtis similarity index) comparing the similarities of the structure and species composition of seed source samples (seed rain and seed bank) and seedlings. *B0 (control)* was not burned, *B3* was burned three times with 3 years interval, and *B6* was burned annually, six times.
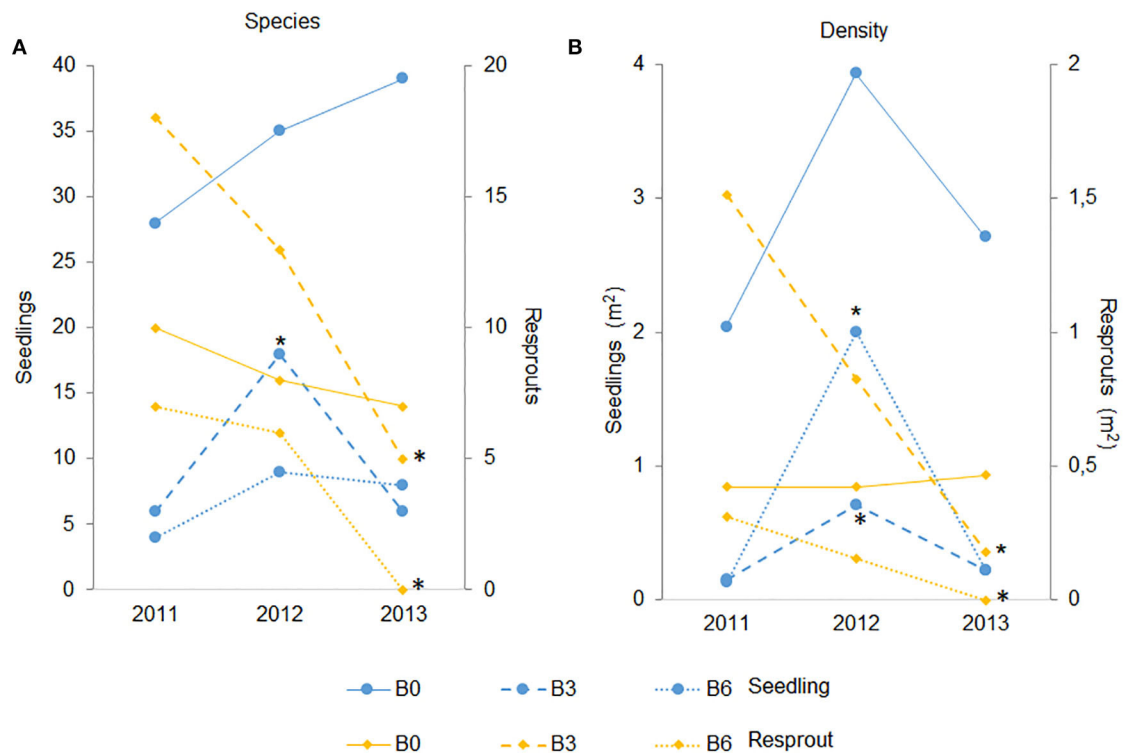

FIGURE 7 | Temporal assessment (2011, 2012, 2013) of seedlings **(A)** and resprouts **(B)** density and species richness. *B0 (control)* was not burned, *B3* was burned three times with 3 years interval, and *B6* was burned annually, six times. Asterisks indicate differences with the first year ($P < 0.05$).

Our results show that recurrent fires can have lasting effects on potential trajectories of tropical forests. Not only fires have major direct effects on the diversity and structure of tropical forests, but also on the capacity of these forests to recover its diversity. We showed that frequent fires can drive reduction in both abundance and species richness of seed rain, seed bank, seedlings, and resprouts, and that this reduction is stronger at forest edges. Monitoring burned areas is essential to understand how the biodiversity will change in the future. Specially now when the deregulation in the current Brazilian environmental legislation will lead to the acceleration in deforestation, forest fragmentation and occurrence of fires.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

AUTHOR CONTRIBUTIONS

PB and JB conceived the burning experiment. RC, JT, and PB conceived the sampling design. RC, CM-S, and PB carried out the experiment in the field. RC wrote the manuscript with support from JB, PB, and JT. All authors contributed to the final version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/ffgc.2020.00082/full#supplementary-material

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