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UN Decade On Ecosystem Restoration

Why forest fails to recover after repeated wildfires in Amazonian floodplains? Experimental evidence on tree recruitment limitation

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Abstract

1. Amazonian floodplain forests are particularly vulnerable to wildfires that spread during extreme droughts, causing large-scale forest dieback. After a second wildfire, these forests persist trapped with low tree cover and empty seed banks, yet the mechanisms that could explain this arrested succession remain unknown.
2. Here we use a 4-year field experiment to test whether tree recruitment failures in burnt floodplain forests are caused by environmental filtering, limiting early seedling emergence and establishment. We sowed seeds and planted seedlings of six floodplain trees with contrasting life strategies, and tested the roles of environmental filters by comparing tree seedling performances under different habitats (i.e. unburned forest, forest edge with burnt site, forest burnt once and forest burnt twice), and by manipulating soil root mats and herbaceous cover.
3. Our results show that seedling emergence was around 15% across all habitats. In general, seedlings performed best in burnt forests. Seedling growth was highest in forests burnt once, possibly because of high nutrient availability after fire. In forests burnt twice, tree seedlings grew relatively less, as nutrients become limiting due to flood erosion; yet, seedlings survived longer, possibly because of lower competition with sparse, naturally recruiting trees. We found similar patterns for seedlings that emerged in the field from sowed seeds.
4. *Synthesis*. Our experimental evidence suggests that environmental filtering related to soil nutrient limitations may slow down forest recovery after repeated wildfires. Yet, our findings showing that floodplain trees are able to germinate from seeds and establish successfully in twice burnt forests suggest that seed limitation may be the reason why forest recovery fails persistently. A corollary to the problem is that repeatedly burnt forests seem to be trapped by a self-reinforcing feedback, in which low tree cover reduces seed dispersal and consequently seed availability, keeping tree cover low. Overall, our findings indicate that active restoration initiatives based on seeding native tree species may help accelerating the recovery of degraded floodplain forests after repeated wildfires.

KEYWORDS
arrested succession, climate change, extreme drought, igapó, resilience, seed dispersal, seedling establishment, tropical forests

1 | INTRODUCTION

The persistence of tropical forests depends on their ability to recover from disturbances (Barlow et al., 2018; Cole et al., 2014). Climate change has intensified extreme drought events, hurricanes and wildfires, exposing tropical forests to unprecedented disturbance regimes that may compromise forest functioning and resilience (Barlow et al., 2018; Silvério et al., 2019). Wildfires in particular are serious threats to wet tropical forests because these ecosystems have been rarely exposed to fire in evolutionary and historical times (Cochrane, 2003). When tropical forests burn, tree mortality can be high (23%–44%, or up to 90% in floodplain forests), allowing the spread of flammable herbaceous plants that increase the vulnerability to repeated fires (Brando et al., 2014; Cochrane, 2003; Flores et al., 2014; Silvério et al., 2013).

After large-scale disturbances, forest recovery may fail due to several processes that limit tree recruitment, including tree survival and resprouting, seed bank mortality, seed production, seed dispersal, as well as seedling emergence, survival and establishment as adult trees (Chazdon, 2003; Connell & Slatyer, 1977). Overall, forest recovery depends on the capacity of surviving trees to resprout and new trees to recruit. The success of new trees to recruit depends on two main processes; seed limitation and establishment limitation. Seed limitation is the reduction of tree recruitment caused by the lack of seeds (Turnbull et al., 2000), whereas establishment limitation is the reduction of recruitment caused by environmental filtering and mortality agents, such as predation or herbivory acting on young tree seedlings and saplings (Nathan & Muller-Landau, 2000). For instance in forests recently disturbed by severe, large-scale events, tree recruitment often depends on long-distance seed dispersal, which may eventually result in seed limitations (Nathan & Muller-Landau, 2000; Turnbull et al., 2000). Once tree seeds arrive in these burnt forests, they still need to overcome new local environmental conditions that can act as filters for the growth and survival of young seedlings and saplings (Chazdon, 2003; Connell & Slatyer, 1977). The relative importance of these different stages of tree recruitment varies across ecosystems. However, in some cases, multiple recruitment limitations can slow down forest recovery, maintaining a persistently open vegetation state of arrested succession (Acácio et al., 2007; Flores et al., 2016; Holmgren et al., 2000; Veldman & Putz, 2011).

In the Amazon region, wildfires have become more frequent and intense during extreme drought events (Alencar et al., 2015; Brando et al., 2014). Recently, wildfires have penetrated closed-canopy forests throughout the Amazon basin (Alencar et al., 2015; Aragão et al., 2007), causing the most severe impacts on floodplain forests, where field and satellite evidence have revealed massive tree mortality and tree cover loss (Flores et al., 2014, 2017; Nogueira et al., 2019; Resende et al., 2014). In particular, nutrient-poor black water floodplain forests of the Amazon can be surprisingly flammable because they have a root mat that, once dried, can easily burn (dos Santos & Nelson, 2013). Their forest structure is slightly more open, compared to upland (*terra firme*) forests, allowing this material to desiccate faster (Almeida et al., 2016). Root mats are a forest adaptation to the seasonal flooding that protects the organic soil layer of these floodplain forests by effectively recycling nutrients and preventing water erosion (Stark & Jordan, 1978), but they can also spread human-ignited wildfires during extreme drought events (Flores et al., 2014; Goulding et al., 1988).

In floodplain forests of the central Amazon, one single wildfire can kill up to 90% of all trees (Flores et al., 2014; Resende et al., 2014). Because these forests recover slowly after the first fire, they may spend 10–20 years in an open state with high herbaceous cover, vulnerable to reburning (Flores et al., 2016, 2017). As the forest recovers, herbaceous cover eventually disappears, but belowground, fuel slowly builds up as root mats accumulate again, keeping the forest vulnerable to drought-driven fires (Flores et al., 2016). After a second wildfire, floodplain forests may persist in an open vegetation state, with low tree cover, depleted seed banks, high herbaceous cover and eroded soils (Figures 1 and 2; Flores et al., 2016; Flores & Holmgren, 2021a).

Although previous observational evidence suggests that low nutrient availability and competition with herbaceous plants may contribute to tree recruitment failures in repeatedly burnt floodplain forests (Flores et al., 2016; Flores & Holmgren, 2021a), the actual mechanisms that explain the lack of tree establishment remain poorly understood. Soil erosion reduces nutrient availability potentially reducing tree growth rates (Flores et al., 2020), whereas herbaceous plants may reduce tree growth especially in the early phases of tree establishment by limiting seedling emergence and by competing with young seedlings (Hoffmann et al., 2004). Both drivers, nutrient availability and herbaceous cover, ultimately limit forest recovery and therefore increase the risk of reburning (Cochrane, 2003). Moreover, the fact that seed banks remain empty for at least a decade in twice burnt floodplain forests (Figure 2; Flores et al., 2016) may be an indication that dispersal

FIGURE 1 Amazonian floodplain forests of the Rio Negro degraded by wildfires. (A) Front view of the unburnt forest from the river channel. (B) Unburnt forest from inside. (C) Forest burnt once 3 years earlier, with no signs of recovery. (D) Same forest as in (C), burnt once 7 years before the picture, now with massive tree recruitment. (E) Forest burnt a second time 3 years earlier, with no signs of recovery. (F) Same forest as in (E), burnt for the second time 7 years before the picture, still with no signs of recovery. Note how the different habitats (B–F) changed from the start to the end of the experiment. Credits: (A, C, E) Bernardo M. Flores, and (B, D, F) Peter van der Sleen

interactions with frugivores have been disrupted (Hawes et al., 2020), which may also contribute to limit forest recovery. In such cases, forest recovery may depend upon the arrival of tree seeds from distant sources (Chazdon, 2003; Guevara et al., 1986; Nathan & Muller-Landau, 2000; Turner et al., 1998), often dispersed by animals that can be very sensitive to open disturbed areas (Barlow & Peres, 2004; Barlow et al., 2016; Hawes et al., 2020; Ritter et al., 2012).

Here we experimentally tested whether environmental filtering contributes to slow down tree establishment in burnt floodplain forests of the Rio Negro, in the Central Amazon region. More specifically, we test the hypothesis that repeatedly burnt forests persist in an open state under arrested succession because tree seeds that arrive are not able to emerge and establish due to environmental conditions (specifically, the absence of a canopy and root mat layers, nutrient limitations and competition with herbaceous plants). Supported by previous findings showing that seed banks remain empty in repeatedly burnt forests, and that these forests fail to recover persistently (Figure 2; Flores et al., 2016; Flores & Holmgren, 2021a), we further discuss the potential role of different processes that might limit tree recruitment. We studied different habitats of this floodplain landscape (Figure 1; Figure S1), including unburnt forest, forest edge with burnt sites, forest burnt once and forest burnt twice. In each habitat, we added seeds and planted seedlings of six floodplain tree species with contrasting life strategies, and monitored seedling emergence, growth and survival for 4 years. We also manipulated environmental conditions, to assess the relative importance of resource availability and competition with herbaceous plants. Based on the experimental evidence, we discuss the most promising restoration strategies for fostering the regeneration of Amazonian floodplain forests degraded by wildfires.

2 | MATERIALS AND METHODS

2.1 | Study system

We studied the floodplain forests of the middle Rio Negro, Central Amazonia (Figure 1; Figure S1). In this region, floodplains cover 4,100 km^2 (Latrubesse & Franzinelli, 2005), and due to their biological value, the whole area was recently incorporated into the Ramsar Convention (Rio Negro site, https://rsis.ramsar.org/ris/2335). In addition to forests, patches of white-sand savanna are found scattered

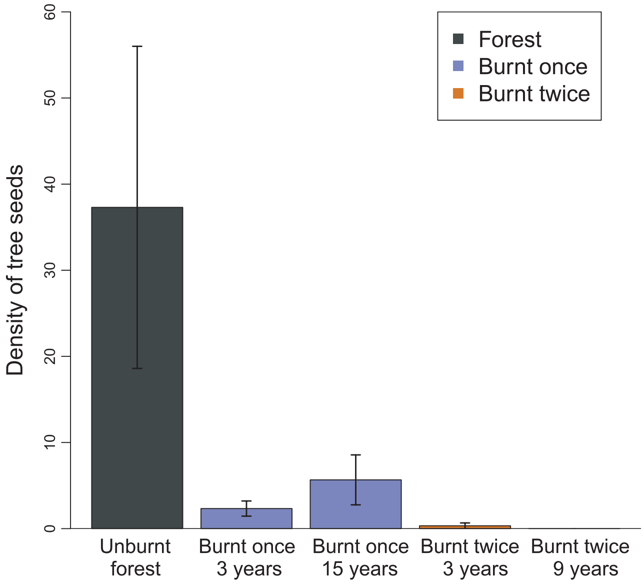

FIGURE 2 Density of tree seeds in the seed banks of floodplain forests in the study region, before and after wildfires. Compared to unburnt forests, forests burnt once have less seeds, but with time, they are able to recover their seed banks (see increase from 3 to 15 years after fire). In contrast, forests burnt twice remain with empty seed banks for at least 9 years after fire. Density of tree seeds in these different habitats were collected in cores of 10 × 15 × 5 cm (length × width × depth), including the surface litter and root mat, from three distinct sites representing each habitat (adapted from Flores et al., 2016)

across the floodplain landscape, with distinct flora and fauna (Adeney et al., 2016). In this region, flooding is associated with the acid (pH 3.5–4.0) and nutrient-poor black water rivers that expand laterally during the rainy season, forming ecosystems known locally as igapó (Junk et al., 2015). The annual flood-pulse of 5.5 m in amplitude inundates the floodplain landscape from 5 to 11 months annually (lowest in January and highest in July). In the region, mean annual precipitation is 2,400 mm and well distributed, with montlhy rainfall over 100 mm throughout the year (based on daily measures since 1967, *Agência Nacional de Águas*—https://www.ana.gov.br/). Although the soil of these floodplains is naturally poor in nutrients, forests can grow up to 30 m tall, and attain 300 Mg/ha of biomass (Junk et al., 2015).

2.2 | Experimental design

We selected two experimental sites based on the fire history of the middle Rio Negro floodplain landscape from 1973 through 2013 (Flores et al., 2016). One site had burnt once (0.8218°S, 63.0062°W, Figure S2), and the other site had burnt twice (0.8157°S, 63.2726°W, Figure S3). Both sites had 3 years since the last burn, and had similar flooding regimes (172 days/year for the once burnt, 165 days/year for the twice burnt). In the field, we experimentally tested whether environmental filtering could help explain the persistent recruitment failure of forest tree species reported for burnt floodplain forests (Flores & Holmgren, 2021a; Flores et al., 2016). We manipulated seed arrival and emergence by sowing seeds and planting seedlings of floodplain trees and assessing whether they could establish. We assessed environmental filters by comparing seedling emergence (germination), growth and survival across contrasting forest habitats (i.e. unburnt forest, forest edge with once burnt forest, forest burnt once and forest burnt twice) and manipulated resource conditions (with/without root mat) and competition with herbaceous plants (with/without herbs).

Forest habitats have distinct seed banks (Figure 2, Flores et al., 2016). Unburnt forests have intact seed banks with plenty seeds of many species (Flores et al., 2016). We do not have information for seed banks in the forest edge, but even if they were reduced during the fire, the presence of trees probably contributed to their fast recovery (Guevara et al., 1986). When these forests are burnt for the first time, they lose most of their seed banks, but with time, seed banks are able to recover. In contrast, when they are burnt a second time, seed banks become persistently empty, with no signs of recovery for at least 9 years.

Forest habitats have also distinct environmental filters that could reduce tree recruitment (Table 1). In the unburnt forest, closed canopies block solar irradiance shading the understory, and root mats contribute to retain nutrients in the superficial soil (Figure 1B; Table 1). In the forest edge near once burnt sites, solar irradiance is higher than inside the forest, while root mats are still present. In the forest burnt once, at the start of the experiment, 100% of the trees had been killed and most of the root mats were consumed by the wildfire, herbaceous plants covered ~72% of the area, and topsoils were enriched by nutrients released during combustion (Figure 1C; Table 1). However, conditions in the forest burnt once changed drastically during the course of the experiment, as young trees were recruited densely (Figure 1D). In the forest burnt twice, at the start of the experiment, trees were practically absent, as well as root mats, herbaceous plants covered 100% of the area, and soil nutrients were limiting, compared to the other habitats (Figure 1E; Table 1). These harsh conditions in the forest burnt twice persisted during the course of the experiment (Figure 1F).

For Experiment 1, we used a split-plot factorial design to assess the effects of forest canopy and root mats on seedling performances. In the unburnt forest, we established 10 blocks separated at least 30 m from each other, with two paired plots each (of 4 × 4 m); one pair in the forest interior and another in the forest edge, at the border with the forest burnt once (Figures S1a and S2). For each pair of plots, we randomized which plot would have the root mat (and litter layer) removed, and which would retain it, as our controls. We removed the root mat with a hoe, a manipulation that was still visible after 4 years. In this experimental set up, we tested how contrasting root mat conditions affect seedling emergence and establishment under the closed canopy and edge of the forest.

For Experiment 2, we used a second split-plot factorial design to assess the effects of herbaceous cover on tree seedling recruitment in forests burned once and twice. We established 10 pairs of plots

TABLE 1 Environmental conditions in unburnt forests and recently burnt forests in the study landscape, including both experimental areas

Habitat	No. burns	Last burn (years)	Tree basal area (m^2/ha)	Recovery rate (m^2 ha^{-1} year^{-1})	Herbaceous cover (%)	Root mat thickness (cm)	Soil P (mg/kg)	Soil total P (mg/kg)	Soil carbon (g/kg)	Soil nitrogen (g/kg)
Unburnt forest	0	—	29.7 (±7.5)	—	0 (±0)	7.6 (±0.9)	24.9 (±6.1)	247.1 (±104.3)	50.4 (±19.7)	3.9 (±1.7)
Burnt once	1	3	0 (±0)	0.83 (±0.1)	72 (±43)	0.6 (±1.2)	29.9 (±13.8)	341.9 (±136.2)	56.3 (±18.3)	4.6 (±1.3)
Burnt twice	2	3	0.3 (±0.4)	0.42 (±0.5)	100 (±0)	0.1 (±0.2)	14.4 (±4.6)	164.4 (±173.5)	36.8 (±6.1)	3.0 (±0.7)

Note: Means and CIs estimated from three sites for each habitat. Vegetation structural data and forest recovery rates (of tree basal area) adapted from Flores et al. (2016). Soil data from top soil layer (0–20 cm) adapted from Flores and Holmgren (2021).

(4 × 4 m) in the forest burnt once with at least 30 m distance from each other and randomized which plot would have the herbaceous cover removed, and which one would retain it (Figures S1b and S2). We established 10 comparable experimental pairs of plots in the forest burnt twice, with and without herbaceous cover (Figure S3). Herbaceous cover (including herbs and graminoid vegetation) was present in all plots, and we removed it with a hoe in the treatment plots at the start of the experiment. Although herbaceous cover regrew sparsely over time, treatment plots with removed herbaceous covered were still distinct from control plots after 4 years.

Overall, in these two field experiments, we established 80 plots, with 20 plots in each habitat, of which 10 were under manipulation treatment and 10 were controls. Each plot of 4 × 4 m was divided into nine equal quadrants (Figure S1). In six of those quadrants, we planted tree seedlings, with each species per quadrant in randomized positions. The other quadrants were used for inserting tree seeds, with species in the same order in which we planted the tree seedlings. Seedling and seed quadrants of each species were mapped to be precisely monitored in the next years.

2.3 | Tree species: Selection, seed collection and seedling preparation

We selected six tree species with distinctive functional traits that occur in our study system (Table 2). In our previous study (Flores & Holmgren, 2021a), we inventoried multiple sites including forests, burnt sites and white-sand savannas. Based on information about the relative abundances of 172 tree species, we identified these six that were relatively common in at least one of these habitats (see Figure S4). *Hevea spruceana* is typical of unburnt forest habitats. *Handroanthus serratifolius* is typical of white-sand savanna habitats, but it may also occur in the burnt forest. *Macrolobium acaciifolium* and *Eschweilera tenuifolia* are common in burnt forests, but also occur in unburnt forests. *Duroia eriopila* and *Buchenavia oxycarpa* are generalist species that occur in all habitats. Species *Macrolobium acaciifolium* and *Eschweilera tenuifolia* are long living pioneers that require abundant light conditions to recruit, but once they reach the canopy they can live up to 500 years or more (Resende et al., 2020; Schöngart et al., 2005).

We collected tree seeds during the high water season of 2013 (between May and July), when most floodplain trees reproduce (Kubitzki & Ziburski, 1994; Parolin et al., 2004) and approximately 6 months before establishing the field experiments. Seeds were collected from at least five different individuals of each species in floodplain forests of the study landscape. For the species *Macrolobium acaciifolium*, *Buchenavia oxycarpa*, *Eschweilera tenuifolia* and *Handroanthus serratifolius*, seeds were removed directly from the canopy. For species *Hevea spruceana* and *Duroia eriopila*, seeds were collected floating in the water below their mother trees. For each species, we collected around 1,000 seeds and maintained them inside mesh bags, under shallow water and beneath the floodplain forest canopy. This method protects seeds from predators and breaks

TABLE 2 List of tree species used in the experiment, with their main habitats and traits

Local name	Species name	Family	Main habitat	Species traits (mean ± CI)			
				AG height (cm)	BG depth (cm)	Root:shoot ratio	Seed mass (g)
Seringueira	*Hevea spruceana* (Benth.) Müll. Arg.	Euphorbiaceae	Forest	77.5 ± 3.2	28 ± 4.6	0.44 ± 0.07	2.6 ± 0.3
Cuãium	*Duroia eriopila* L. f.	Rubiaceae	Burnt	6.1 ± 1.5	9.1 ± 1.9	0.35 ± 0.09	0.02 ± 0.004
Arapari	*Macrolobium acaciifolium* (Benth.) Benth.	Fabaceae	Burnt	56.7 ± 12.3	36.8 ± 3.6	0.51 ± 0.06	3.1 ± 0.4
Tanimbuca	*Buchenavia oxycarpa* (Mart.) Eichler	Combretaceae	Generalist	12.9 ± 3.7	15.8 ± 2.8	0.45 ± 0.08	0.3 ± 0.04
Macacarecuia	*Eschweilera tenuifolia* (O. Berg) Miers	Lecythidaceae	Burnt	32.6 ± 6.5	25.6 ± 3.0	0.34 ± 0.04	2.1 ± 0.3
Capitari (ipê)	*Handroanthus serratifolius* (Vahl) S.O. Grose	Bignoniaceae	Savanna	13.8 ± 2.4	31.7 ± 3.8	1.77 ± 0.24	0.03 ± 0.007

Note: Traits were measured in a randomized selection of seeds ($N = 20$) and seedlings ($N = 10$) at the start of experiment (3 months after germinating in the shade-house). Species names according to Flora do Brasil 2020. Main habitat according to species natural relative abundances in the study system (Figure S4; Flores & Holmgren, 2021a). AG and BG refer to above- and below-ground plant structures.

their dormancy, allowing seeds to germinate when floodwaters recede (Kubitzki & Ziburski, 1994; Parolin et al., 2004). In September 2013, we sowed 500 seeds of each species in individual seedling bags of 15 × 23 cm that were kept watered in a shade-house, so that seedlings could germinate and grow until the start of the next dry season. The shade-house had 70% of the natural irradiance and seedlings were irrigated at the end of each day. The remaining seeds were maintained below water to be sowed at the start of the experiment, together with the seedlings produced.

In the shade-house, all species had seedling emergence rates above 64% (320 out of 500 planted seeds), except for *Hevea spruceana* with only 40% of seedling emergence. *Eschweilera tenuifolia* had the highest seedling emergence rate of 96%. Seedling emergence is the combined result of both germination success and early growth of the seedlings above the soil surface. In January 2014, we planted four seedlings and five seeds of each species in each plot, for a total of 320 seedlings and 400 seeds per species, or 4,320 individuals. For *Hevea spruceana*, we planted 196 seedlings in a lower number of field plots because this species had a lower germination rate. At the time of planting, we measured the height of each individual seedling. After 1 month, 1 year, and 4 years, we monitored all individuals to assess seedling emergence, growth and survival. We also measured growth and survival of the individuals that emerged from seeds inserted in the field.

Before implementing the field experiments in January 2014, we randomly selected 20 seeds and 10 seedlings of each species to measure their traits (Table 2). All seedlings had the exact same age of 3.5 months. We dried all seeds and seedlings, and then measured seed mass, above and below-ground biomass of seedlings, root and shoot length of seedlings, as well as leaf mass. We also calculated root:shoot ratios as below-ground biomass divided by above-ground biomass (including wood and leaf). These traits are known to correlate with plant resource acquisition and performance under various environmental conditions (Hoffmann & Franco, 2003; Howe & Smallwood, 1982; Leishman et al., 2000; Mokany et al., 2006; Moles & Westoby, 2006).

2.4 | Statistical analyses

We monitored the field experiments 1 month, 1 year and 4 years after planting. For each monitoring period, we estimated seedling emergence, growth and survival for each species. Seedling emergence was calculated as the percentage of the five sowed seeds of each species that emerged aboveground in each plot (0%–100%). Seedling growth between monitoring periods was calculated as the mean stem vertical growth among the four planted seedlings of each species in each plot. Seedling survival was calculated as the percentage of the four planted seedlings of each species that had survived in each plot (0%–100%). For seedlings that emerged in the field from sowed seeds, we calculated growth and survival in the same way. Over the four experimental years, we lost some of our plots due to disturbances, such as strong currents during the high water season, or large tree falls. As a result, the numbers of plots representing ‘unburnt forest’ and ‘forest edge’ habitats were 16 after 1 month, 14 after 1 year and 13 after 4 years. For ‘burnt once’ habitats, the numbers of plots were 12 after 1 month, 12 after 1 year and 7 after 4 years. For ‘burnt twice’ habitats, we maintained 20 plots during the entire 4 years of monitoring.

We did all analyses using R v.3.6.1 (R Core Team, 2019), and prepared our figures using R software base plots, and the ‘ggplot2’ package (Wickham, 2016). We used linear mixed models (LMM) from the R package ‘lme4’ (Bates et al., 2015), using the function ‘lmer’ with ‘Gaussian’ error distribution for seedling growth, and the function ‘glmer’ with ‘binomial’ error distribution for seedling emergence and survival, due to the binary nature of these variables.

First, we compared seedling performances across the four forest habitats (i.e. unburnt forest, edge, burnt once and burnt twice) considering only control plots, where we did not manipulate local environmental conditions, and with all six species together. For each response variable (i.e emergence, growth, survival), we used models with 'habitat' as fixed factor, and 'species' and 'block' as random factors (e.g. lmer(*y* ~ habitat + (1|block) + (1|species)). We included 'species' as a random factor to test for a general effect of the predictors and assess differences among the four habitats, without making distinctions among species. We assessed the residual plots from each model to control for normality.

We then used the same approach to analyse the effects of root mat and herbaceous cover (in Experiments 1 and 2 respectively), but with separate models for each species, so that we could assess more subtle differences in species responses to environmental filters. By testing species separately, we were able to explore how their responses differed across environmental conditions. For Experiment 1, to assess seedling performances under contrasting irradiance conditions (unburnt forest vs. forest edge habitats) and root mat (removed vs. present), we used models with 'habitat' and 'rootmat' as fixed factors with interaction, and included as a random factor the pair of 'plots' nested within 'blocks' (e.g. lmer(*y* ~ habitat × rootmat + (1|block/plots)). For Experiment 2, to assess seedling performances in burnt once and twice forest habitats, and with contrasting herbaceous cover (removed vs. present), we used models with 'habitat' and 'herb' as fixed factors with interaction, and included as a random factor the pair of 'plots' nested within 'blocks' (e.g. lmer(*y* ~ habitat × herb + (1|block/plots)). We also assessed the residual plots from each model to control for normality; we log transformed seedling growth for *D. eriopila* and *H. serratifolius*. To estimate chi-square and *p*-values for fixed effects from our LMMs (via lme4::glmer), we used the 'ANOVA' function (R Core Team, 2019) that provides deviance tables for comparing nested models with a likelihood-ratio test.

In addition, we tested whether the species traits 'seed mass' and 'root:shoot ratio' (Table 2) were good predictors of seedling performance (i.e. emergence, growth, survival) in control plots across the four forest habitats (i.e. unburnt forest, edge, burnt once and burnt twice). For this, we used simple Pearson correlation analyses with the mean trait and performance values for each species in each habitat.

3 | RESULTS

3.1 | Tree seedling emergence, growth and survival across habitats

We found that, considering the six species together across habitats, 15%–20% of all seeds emerged (Figure 3A), mostly within the first year (Figure S5). Seedling emergence rates did not vary between habitats, but they did vary between species ($p < 0.001$; Figure 4A; Table S1), with the long-living pioneer species *Eschweilera tenuifolia* and *Macrolobium acaciifolium* showing the highest mean seedling emergences of 58% and 24% respectively. *Duroia eriopila* and *Buchenavia oxycarpa* had lower emergences of 13% and 9% respectively. *Hevea spruceana* (forest species) and *Handroanthus serratifolius* (white-sand savanna species) practically did not germinate in the field.

Average seedling growth, considering the six species together, varied between habitats ($p < 0.001$; Figure 3B). Growth was highest in the forest burnt once, followed by forest burnt twice, forest edge and unburnt forest with the lowest growth rates. The same pattern was found for seedlings that emerged from seeds sowed in the field (Figure S6a). Growth in the unburnt forest was very low and sometimes negative when individuals lost part of their stems and resprouted from the base. On average, all species grew well in burnt forests. The long-living pioneer *Macrolobium acaciifolium* had

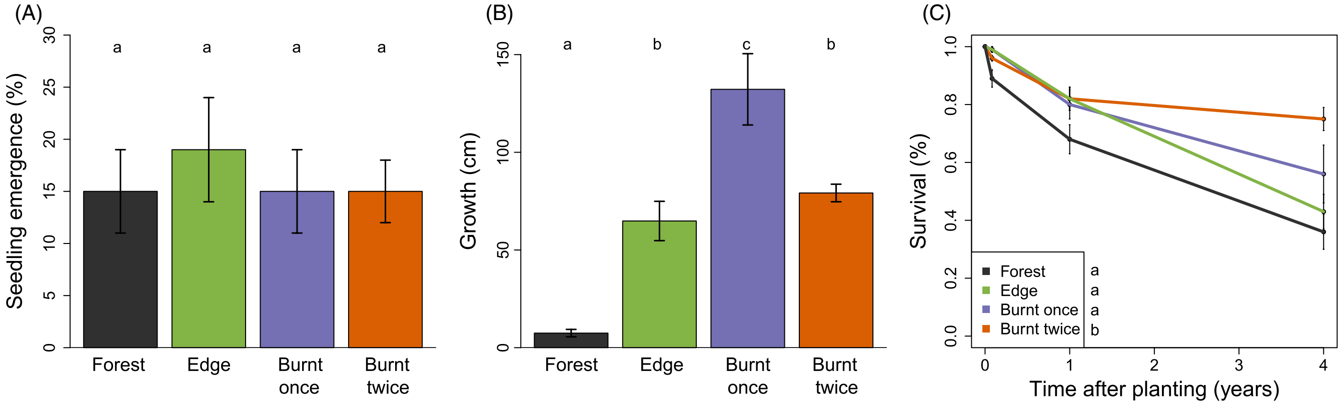

FIGURE 3 Performances of seeds and seedlings planted in the four forest habitats: forest (unburnt), edge (of forest with burnt once), burnt once and burnt twice. (A) Percentage of seedling emergence from inserted seeds. (B) Growth of planted seedlings during the 4 years. (C) Survival of planted seedlings during the 4 years. Values represent estimated means and standard errors, including all six species together. Small letters indicate significant differences based on LMM, with $p < 0.05$. Growth and survival patterns were remarkably similar for the seedlings that emerged from seeds sowed in the field (Figure S6)

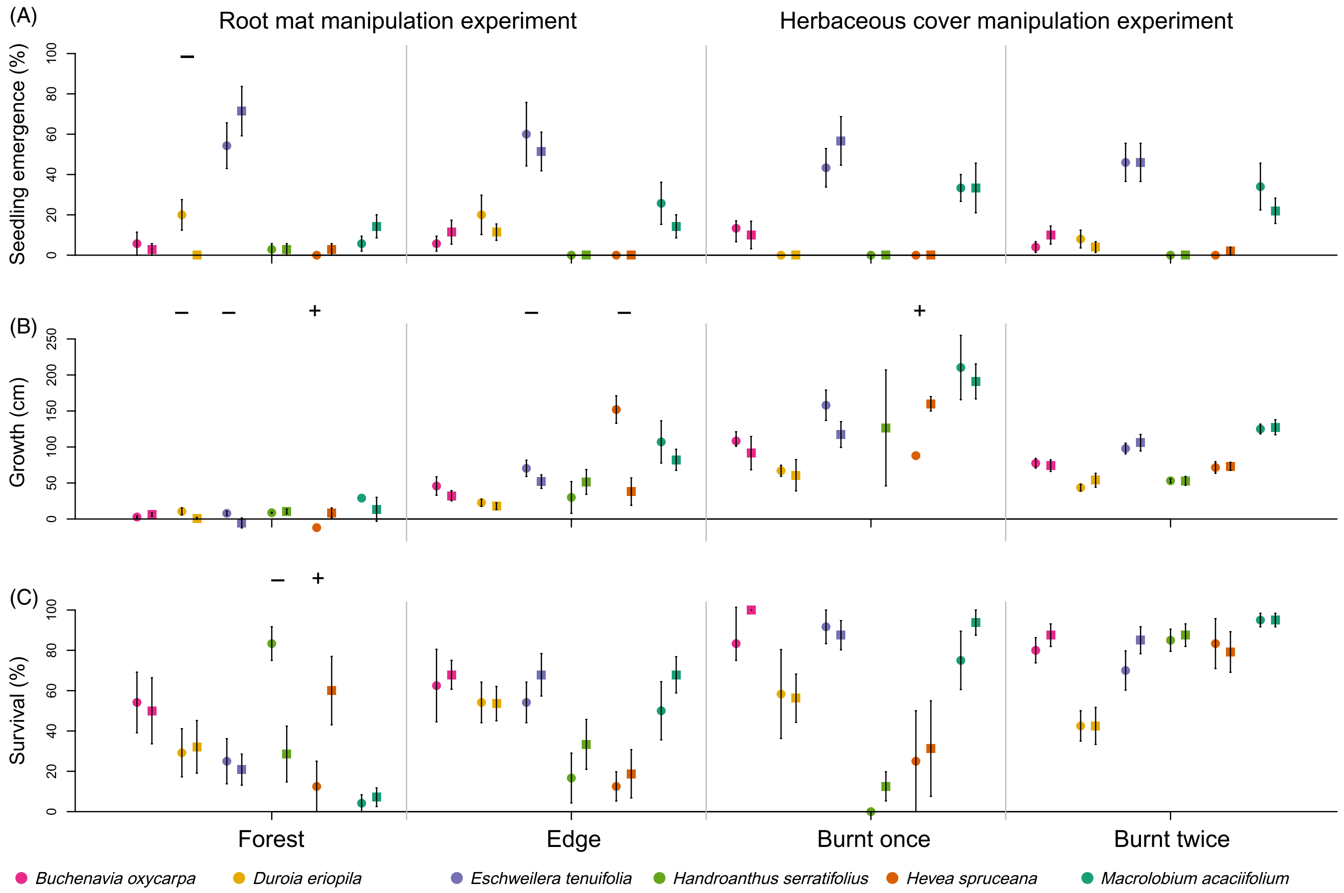

FIGURE 4 Effects of root mat and herbaceous cover manipulation on the performances of planted tree seeds and seedlings in the four habitats: forest, forest edge, forest burnt once and forest burnt twice. (A) Percentage of seedling emergence. (B) Seedling growth in 4 years since planting in experimental sites. (C) Seedling survival up to 4 years since planting. Circles (●) indicate control plots and squares (■) indicate manipulated plots. Values represent estimated means and standard errors. Positive and negative symbols on top of each panel indicate significant manipulation effects based on LMM (Table S1). Colours indicate the species identities (legend at the bottom)

the highest mean growth of 121 cm in 4 years ($p < 0.001$; Figure 4B; Table S1), reaching up to 3 m tall in one plot.

Seedling survival after 4 years was high in all four habitats, considering the six species together (Figure 3C). Survival was highest in the forest burnt twice with 80% survival ($p < 0.001$), followed by forest burnt once, edge and unburnt forest with the lowest values of 40% survival. The same pattern was found for seedlings that emerged from seeds sowed in the field (Figure S6b). Survival varied more between species than between habitats (Figure 4C), with the pioneers typical of burnt sites *Eschweilera tenuifolia* (62% survival) and *Macrolobium acaciifolium* (63% survival), as well as the generalist species *Buchenavia oxycarpa* (73% survival) having the highest mean survival rates (Table S1). The white-sand savanna species *Handroanthus serratifolius* had the highest survival in the unburnt forest but did not survive in the forest burnt once. In contrast, the long-living pioneers *Macrolobium acaciifolium* and *Eschweilera tenuifolia* had their lowest survival values in the unburnt forest and highest in burnt forests. A similar pattern was observed for the forest species *Hevea spruceana*, which survived better in the forest burnt twice than in the unburnt forest.

3.2 | Experiment 1: Assessing the effects of root mats in the unburnt forest

Root mat removal in the unburnt forest and forest edge habitats had no effect on seedling emergence of most species, with the exception of *Duroia eriopila* that emerged less in the forest (Figure 4A; Table S1). Root mat removal influenced seedling growth differently across species (Figure 4B; Table S1). Although most species did not change their growth rates with root mat removal, *Hevea spruceana* grew slightly more when root mat was removed in the unburnt forest, but much less when it was removed at the edge. *Duroia eriopila* grew slightly less in the unburnt forest when root mat was removed, but this manipulation had no effect at the edge. *Eschweilera tenuifolia* grew slightly less in both the unburnt forest and forest edge when root mat was removed. Root mat removal had no effects on seedling survival of most species (Table S1; Figure 4C), with the exceptions of *Handroanthus serratifolius* that survived much less and *Hevea spruceana* that survived much more when root mat was removed in the unburnt forest.

3.3 | Experiment 2: Assessing the effects of herbaceous cover in burnt forests

Herbaceous cover removal in forests burnt once and twice had no effect on seedling emergence, growth or survival of any species (Table S1; Figure 4A,B). One single exception was *Hevea spruceana* that grew more in the forest burnt once when herbaceous cover was removed.

3.4 | Correlating seedling performances to their morphological traits

Differences in seedling emergence between species were not correlated to their seed masses (Figure S7a) and root:shoot ratios (Figure S8a). Nonetheless, the two species with highest seedling emergence rates (i.e. *M. acaciifolium* and *E. tenuifolia*) have the first and third highest seed masses. Seedlings initial growth investments on biomass (both above and below ground) during their first 3 months of life in the shade-house were positively correlated with their seed masses (Figure S9). In the field, after 4 years, species differences in seedling growth were also positively correlated with their seed masses, but only in the forest edge ($r = 0.87$, $p = 0.02$) and in the forest burnt twice ($r = 0.81$, $p = 0.05$; Figure S7b). We found no correlations between species growth rates and their root:shoot ratios; neither during their initial growth in the shade-house (Figure S9), nor after 4 years in any of the habitats or experimental conditions in the field (Figure S8b). Species differences in seedling survival were negatively correlated with their seed masses in unburnt forest habitats only ($r = 0.81$, $p = 0.05$; Figure S7c). Again, we found no correlations between species survival rates and their root:shoot ratios in any of the habitats or experimental conditions in the field (Figure S8c).

4 | DISCUSSION

Our experimental results assessing tree seedling emergence, growth and survival provide strong evidence that the causes explaining why Amazonian floodplain forests fail to recover after repeated wildfires (Flores et al., 2016; Flores & Holmgren, 2021a) are not related to changes in environmental conditions. Overall, seeds and seedlings that we planted in burnt sites, germinated as well as they did in the unburnt forest. In fact, most seedlings grew and survived better in burnt forests than they did in the unburnt forest. In forest burnt once, seedlings benefited from abundant light and soil nutrients (Table 1), which allowed them to grow faster than seedlings in the unburned forest, forest edge and forest burned twice. This finding reveals why floodplain forests are still capable of recovering after a single wildfire (Flores et al., 2016, see Figure 1D). In forest burnt twice, seedlings grew relatively less than in forest burnt once, probably because of the nutrient-limited soil (Table 1), but survived relatively more, suggesting a higher chance of establishing as adults.

4.1 | The role of soil root mats

Although the presence of a root mat is very important for nutrient retention in these floodplains forests (dos Santos & Nelson, 2013; Stark & Jordan, 1978), in most cases, root mat removal had not effects on tree seedling establishment (Figure 4; Table S1). For a few species, however, root mat removal had negative effects, particularly on seedling growth (Figure 4B). *Hevea spruceana* was a surprising exception because it survived more when root mat was removed in the unburnt forest, its preferred habitat (Figure S4). These unexpected results could be explained by a combination of reasons. Perhaps early seedling establishment does not depend too strongly on soil nutrient availability, but more on seed reserves and irradiance (Howe & Smallwood, 1982; Leishman et al., 2000; Moles & Westoby, 2006). There could be also confounding effects of experimental manipulations. When we removed the root mats and the litter layer, we may have affected seedling performance in ways unrelated to nutrient acquisition. For instance, in tropical secondary forests of Panamá, a field experiment found that litter removal reduced the survival of fast-growing tree species by exposing them to herbivores (Dalling & Hubbell, 2002). This was perhaps the reason why, when root mat was removed in our experiment, *Hevea spruceana* grew much less in the forest edge.

4.2 | The role of herbaceous plants

When tree seeds arrive in burnt sites, they need to overcome local environmental filters to germinate and establish. In sites burnt twice, where soil erosion had already reduced nutrient availability (Table 1), potentially increasing plant competition for nutrients, we expected that the removal of herbaceous plants would allow seedlings to grow more and survive longer, as demonstrated for other tropical forests (Hoffmann et al., 2004). Yet, contrary to our expectations, the removal of herbaceous plants did not affect seedling performances, with the exception of *Hevea spruceana* that grew better when herbaceous cover was removed in the forest burnt once. Although the effect of removing herbaceous cover slowly fades away with time, the legacy effect was still clearly visible after 4 years in the manipulated plots, where herbaceous cover was lower, compared to the controls (see Figure S10). A plausible explanation is that the native herbaceous plants in those Amazonian floodplains are not as competitive as the invasive grasses that often dominate other burnt tropical forests with high biomass (D'Antonio & Vitousek, 1992; Hoffmann et al., 2004; Silvério et al., 2013; Veldman & Putz, 2011).

4.3 | The potential role of seed limitation

Our experiments have shown that tree seeds are perfectly capable of germinating and establishing new seedlings in the twice burnt forest (Figure 3). Although our experiment did not include control treatments, in which no seeds were added (as suggested by Turnbull et al., 2000), previous observational data have shown that twice burnt floodplain forests persist with empty seed banks and open vegetation (Flores et al., 2016). These two lines of evidence suggest that seed limitation is likely the most important mechanism explaining the arrested succession of repeatedly burnt floodplain forests; probably as a result of insufficient seed production due to high tree mortality, coupled with lack of seed dispersal from unburned forests.

Usually, disturbed forests of the Amazon are swiftly covered by a high density of young recruiting trees that attract animal dispersers, such as birds and bats (Hawes et al., 2020). In our floodplain study system, however, repeatedly burnt forests often persist with very low tree cover; consequently with limited perches for bats and birds, and a hostile habitat for many animals. Floodplain trees are known for having various dispersal agents, including water currents during the inundation season, birds, mammals and fish (Kubitzki & Ziburski, 1994; Parolin et al., 2013). The open vegetation structure of twice burnt forests, however, may influence these interactions. For example the speed of water currents is larger when trees are absent reducing the probability of floating seeds to be trapped in the canopy and sink (Antunes et al., 2019). Also, in other tropical forests, open burned sites are usually unattractive for important animal dispersers that search for fruits, seeds and shelter, which may also be the case in the burnt floodplain forest (Barlow et al., 2016; Barlow & Peres, 2004; Ritter et al., 2012). Large animals, such as tapirs, may travel long distances and disperse seeds across open habitats, but they are more likely to drop those seeds in a distant forest habitat (Fragoso et al., 2003). Many frugivore birds are also capable of moving across open burnt floodplain forests (Ritter et al., 2012), such as the generalist species *Amazona amazonica* (Psittacidae) and *Patagioena cayennensis* (Columbidae), yet the absence of trees in those habitats does not offer them much food. Another possibility is that seeds do arrive in the open burnt forest, but they are quickly predated. Yet, with scarce food and shelter, it seems unlikely that seed predation is causing seed limitation in repeatedly burnt forests.

4.4 | The role of functional traits: Seed mass and root:shoot ratio

In Amazonian floodplains, tree seedlings often grow fast to avoid having their canopies submerged during the high water season (Parolin, 2000). Particularly in the nutrient-poor blackwater floodplains, species with high seed mass have an advantage during their initial growth (Parolin, 2000, see also Figure S9). Indeed, we found that in sites burnt twice, where nutrients are scarce, species with higher seed mass grew significantly faster (Figure S7b), whereas in sites burnt once, all species seem to be taking advantage of abundant light and soil nutrients to grow fast. Interestingly, although seed mass is known to enhance seedling survival in shaded habitats (Leishman et al., 2000; Moles & Westoby, 2006), we found that it reduced survival in the unburnt forest, possibly due to indirect effects of seed predators (Barnett et al., 2012; Kubitzki & Ziburski, 1994). For instance, *M. acaciifolium* and *H. spruceana* seeds are often eaten by frugivore fish when they swim in the flooded forest during the high water season (Kubitzki & Ziburski, 1994; Parolin et al., 2013). In the low water season, terrestrial vertebrates such as monkeys feed on *E. tenuifolia* seedlings within forest habitats (Barnett et al., 2012). Hence, herbivory, together with shaded conditions, may have been the reasons for why both tree species had their lowest survival in the forest (Figure S7c). Although root:shoot ratio did not affect overall seedling performances, the typical white-sand savanna tree *H. serratifolius* was the second best survivor in twice burnt sites, possibly because of its high root:shoot ratio, allowing seedlings to obtain more resources from the nutrient-limited soil (Hoffmann & Franco, 2003; Mokany et al., 2006).

4.5 | Feedbacks in burnt floodplain forests

When wildfires destroy a tropical forest, they disrupt important feedbacks in the ecosystem and create opportunities for new ones to emerge, pushing the ecosystem into an entirely different way of functioning (Cochrane, 2003; Flores & Holmgren, 2021a; Flores et al., 2020; Scheffer et al., 2001; van Nes et al., 2016). The existing evidence combined for these floodplain forests suggests that a positive feedback between tree cover and seed availability may contribute to trap burnt forests in an open vegetation state (Figure 5).

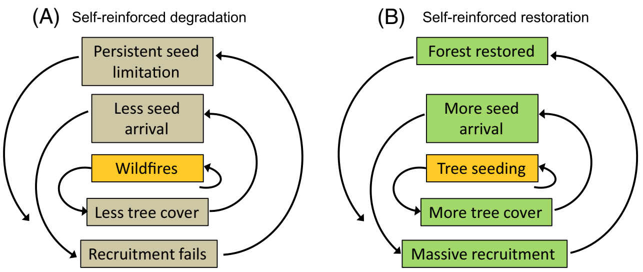

FIGURE 5 Positive feedbacks between tree cover and seed availability may influence the dynamics of Amazonian floodplain forests. Normally, tree cover attracts frugivore animals (fish, birds, mammals) and increases canopy trapping of floating seeds in the high water season. As a result, seeds arrive continuously. (A) When wildfires reduce tree cover severely, seed arrival may be disrupted, reducing seed availability and causing tree recruitment to fail persistently. (B) Active seeding of native tree species in repeatedly burnt sites should increase tree cover, triggering a self-reinforced recovery of the tree-seed feedback that restores the forest

Usually forest trees attract animal dispersers and their canopies trap floating seeds in the high water season, increasing the arrival of tree seeds that can germinate and establish (Antunes et al., 2019; Hawes et al., 2020; Parolin et al., 2013). However, when wildfires reduce tree cover repeatedly, these interactions are lost, thus reducing seed availability and limiting tree recruitment (Figure 5A).

In addition, other positive feedbacks may contribute to arrest forest recovery in these floodplains. For instance low tree cover in burnt sites is expected to accelerate flood erosion, reducing soil fertility and tree growth rates; hence contributing to maintain a low tree cover state (Flores et al., 2020). The classical fire feedback may also play a role here, as low tree cover increases herbaceous cover (Flores et al., 2016), increasing ecosystem flammability and the probability that repeated fires will maintain tree cover low (Grady & Hoffmann, 2012; Murphy & Bowman, 2012). Combined, these mechanisms seem to be the reason why floodplain forests are so fragile, compared to other Amazonian forests (Flores et al., 2017).

4.6 | Managing feedbacks to restore burnt floodplain forests

Forest wetlands are critical for biodiversity conservation and climate mitigation (Strassburg et al., 2020), and restoring these degraded floodplain forests in the Amazon should be a top priority. Managing wildfires is certainly an important measure, as we have shown that after a second burn, floodplain forests fail to recover persistently (Flores et al., 2016; Flores & Holmgren, 2021a). Hence, forests burnt once in the floodplain landscape need to be well protected from wildfires, to prevent recurrent fires and allow their natural recovery through passive restoration pathways. In contrast, reburnt forests will likely depend on active restoration to recover forest structure, diversity and functioning. Seed limitation seems to be the main mechanism limiting tree recruitment after recurrent fires, implying that massive seeding initiatives with native tree species should help contribute to restore these degraded forests (Figure 5B).

Although the six tree species used in our experiments do not represent the diversity of this study system (Junk et al., 2015), they represent an important subset of the dominant species and functional groups (Flores & Holmgren, 2021a). In particular, two of those species featured as promising candidates for restoration initiatives, *Macrolobium acaciifolium* (Fabaceae) and *Eschweilera tenuifolia* (Lecythidaceae); both with high emergence, growth and survival in twice burnt sites. In our study, *E. tenuifolia* was the species with highest emergence success, with 58% emergence across all habitats in the field, and 96% emergence in the shade-house. Both species also have high seed masses, a trait that increased seedling growth rates and hence could accelerate forest recovery (Figure S7b); as previously shown in upland forests of the central Amazon degraded by pasture (Camargo et al., 2002). Both species are long-living pioneers well adapted to burnt sites (Figure S4), implying that their roles in ecosystem functioning may last for centuries. For instance *M. acaciifolium* can live up to 500 years (Schöngart et al., 2005) and *E. tenuifolia* up to 820 years (Resende et al., 2020). Nonetheless, precisely because of their longevities and dominance, these species must not be used alone in seeding initiatives; but instead, they should be combined with other native tree species, to restore diverse forest communities that sustain the biodiversity, functioning and resilience of floodplain ecosystems.

Restoration experiences have been unequally distributed across Brazilian biomes and practically inexistent in floodplain forests of the Amazon and Pantanal (Guerra et al., 2020), apart from small-scale planting efforts conducted by local farmers (McGrath et al., 2005). Our findings from two burnt forests in this Amazonian landscape provide experimental evidence of the main mechanisms likely arresting floodplain forest succession. Our results suggest that seeding could be a successful active restoration strategy and is likely less costly and more effective than planting, but it should be done responsibly, by engaging local communities to capitalize from their ancient ecological knowledge of these systems. It should be combined with the protection of floodplain ecosystems from overfishing (Anderson et al., 2011) and overhunting (Peres et al., 2016) to maintain critical seed dispersers, as well as from the construction of river dams to preserve the hydrological conditions that shaped floodplain biodiversity (Castello & Macedo, 2016; Resende et al., 2020). Hence, the restoration of degraded Amazonian floodplain forests must be integrated with other conservation actions to keep the ecosystem within a safe boundary in the face of current and future perturbations (Scheffer et al., 2015).

5 | CONCLUSIONS

Previous observational evidence of natural recovery in these Amazonian floodplain forests shows that after a second wildfire, burned forests persist in an open state with low tree cover and empty seed banks (Flores et al., 2016), that slowly transitions towards a white-sand savanna state (Flores & Holmgren, 2021a). Our field experiments have now confirmed that when forests are repeatedly burnt, tree growth rates slow down due to soil nutrient limitations. Yet, the fact that our experimental seeds and seedlings germinated and survived well in these burnt sites suggests that the main bottleneck for forest recovery seems to be seed limitation. Our findings indicate that active seeding of native forest species could help boost the restoration of floodplain forests degraded by wildfires.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHORS' CONTRIBUTIONS

B.M.F. and M.H. conceived and designed the experiment; B.M.F. monitored the experiment and conducted the statistical analyses; B.M.F. and M.H. interpreted the results; B.M.F. wrote the manuscript and M.H. contributed substantially to revise the whole text.

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DATA AVAILABILITY STATEMENT

Data available from the Dryad Digital Repository https://doi.org/10.5061/dryad.nk98sf7tx (Flores & Holmgren, 2021b).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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