



ELSEVIER

Contents lists available at ScienceDirect

## Global Ecology and Conservation

journal homepage: <http://www.elsevier.com/locate/gecco>

## Original Research Article

# Short term patterns of germination in response to litter clearing and exclosure of large terrestrial vertebrates along an Amazon forest regrowth gradient

Alexander Roldán Arévalo-Sandi <sup>a, b</sup>, Darren Norris <sup>a, b, c, \*</sup><sup>a</sup> Programa de Pós-graduação em Ecologia, Instituto Nacional de Pesquisas da Amazônia (INPA), Manaus, AM, Brazil<sup>b</sup> School of Environmental Sciences, Universidade Federal do Amapá (UNIFAP), Macapá, AP, Brazil<sup>c</sup> Programa de Pós-Graduação em Biodiversidade Tropical, Universidade Federal do Amapá (UNIFAP), Macapá, AP, Brazil

## ARTICLE INFO

## Article history:

Received 15 September 2017

Received in revised form 6 December 2017

Accepted 21 December 2017

## Keywords:

Amazon forest  
Direct seeding  
Forest succession  
Forest regrowth  
Forest restoration  
Germination  
Seed predator  
Seed disperser  
Vertebrate  
Mammal

## ABSTRACT

Efforts to restore tropical forest ecosystem services depend on understanding the barriers to germination of species of economic and cultural interest. Here we use two important non-timber forest product species (NTFP: *Inga capitata* Desv. – Fabaceae and *Euterpe oleracea* Mart. – Arecaeae) to compare germination across a forest regrowth gradient in the northeast Brazilian Amazon. Experimental treatments were used to examine the effects of mid to large-bodied vertebrates and litter cover on seed fate (germination, removal and invertebrate infestation) in 15 lowland sites within small-holder properties. Regrowth stage was classified into three groups, with five sites each of: late second-regrowth forest, early second-regrowth forest and abandoned pasture. We conducted a paired split-plot experiment using experimental plots composed of a vertebrate exclosure versus an open treatment and subplots with and without litter. We used Generalized Linear Mixed-Effects Models (GLMMs) to compare additive and interaction effects of treatments across regrowth stages compared with 15 paired control sites. We found that the effects of regrowth stage and exclosure were species specific and these effects differed between responses (germination, removal and invertebrate infestation). Clearing litter generated a significant effect only for invertebrate infestation, which increased in the cleared plots. Our findings show that seed removal limits germination success for both species across forest regrowth stages, with invertebrate infestation also having important but secondary effects. Increased removal and unfavorable abiotic conditions make direct seeding unviable in pasture sites. We suggest that direct seeding is a viable alternative for the establishment of these widely available NTFP species in late and early regrowth forests.

© 2017 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

## 1. Introduction

One option to revert tropical forest loss is the restoration of degraded forests and deforested landscapes (Chazdon and Guariguata, 2016; Holl, 2017). However, restoration actions are often considered to be economically expensive, especially

\* Corresponding author. School of Environmental Sciences, Universidade Federal do Amapá (UNIFAP), Macapá, AP, Brazil.  
E-mail address: [darren.norris@unifap.br](mailto:darren.norris@unifap.br) (D. Norris).

in developing nations. Faced with limited resources there is increasing interest in accelerating natural regeneration within restoration actions (Chazdon and Guariguata, 2016; Holl, 2017; Meli et al., 2017).

Effective restoration relies on overcoming barriers to native plant regeneration that predominate in the earliest stages of succession (Chazdon and Guariguata, 2016; Holl, 2017). Barriers to regeneration differ with successional stage (Aide et al., 2000; Martínez-Garza and Howe, 2003; Shoo et al., 2016) and common interventions include varied strategies to suppress herbaceous vegetation (e.g. cutting or herbicide treatment), and measures to bolster propagule supply (e.g. direct seeding and artificial bird perches (Shoo and Catterall, 2013; Holl, 2017; Holl et al., 2017)). Direct seeding may be an effective and economic path for regeneration/restoration at both large and local spatial scales (Shoo and Catterall, 2013; Holl, 2017) but is potentially limited in tropical regions by factors such as predators and litter cover (Ganade and Brown, 2002; Shoo and Catterall, 2013; Palma and Laurance, 2015; Chazdon and Guariguata, 2016).

Direct seeding has been used as part of restoration actions with non-timber forest products (NTFPs) that are an important source of income to millions of people world-wide (Poffenberger and Singh, 1992; He et al., 2009). Non-timber forest products can potentially contribute towards sustainable development across rural Amazon regions (Richards, 1993). For example, the açai palm (*Euterpe oleracea*) is an important component of local and regional economies across the Amazon (Brondizio et al., 2002). The management and conservation of these important tropical NTFPs depends on a detailed understanding of plant recruitment. The processes that influence recruitment are strongly affected by myriad abiotic (i.e. habitat type, light, soil and nutrients (Guariguata and Ostertag, 2001)) and biotic (i.e. predation (Nepstad et al., 1996; Piironen et al., 2017), fungal infection (Bagchi et al., 2014) and litter effect (Xiong and Nilsson, 1999; Ganade and Brown, 2002)) factors. Therefore, it is necessary to understand how these limiting factors act along a successional gradient and in response to different levels of human disturbance to improve natural regeneration, restoration and agroforestry strategies (Richards, 1993; Shoo and Catterall, 2013; Palma and Laurance, 2015; Chazdon and Guariguata, 2016; Holl, 2017).

The diversity of Amazon forests means it is hard to separate the complex interactions driving recruitment (Guariguata and Ostertag, 2001; Camargo et al., 2002; Chazdon and Guariguata, 2016; Holl, 2017), yet previous studies clearly show how different animal groups can generate important impacts on plant recruitment. Seeds can be preyed upon by both vertebrates and invertebrates (Stoner et al., 2007; Griffiths et al., 2016), which may play key roles in limiting germination and subsequent recruitment. Exclosure experiments have revealed the impact of vertebrates on seed and seedling survival, especially in tropical forests to elucidate how this group contributes to the community structure of tropical forest trees (Asquith et al., 1997; Beck et al., 2013; Paine et al., 2016). Litter effect, is another factor affecting recruitment, and may also contribute to influence different processes by reducing erosion, evapotranspiration, thereby improving microclimatic conditions for seed germination and seedling establishment (Facelli and Pickett, 1991; Xiong and Nilsson, 1999; Ganade and Brown, 2002).

In this study, we evaluated the effects of mid to large-bodied vertebrates and litter cover on seed fate along a forest regrowth gradient of two important NTFPs species (*Euterpe oleracea* and *Inga capitata*). To quantify the effects of these different factors we experimentally assessed short term seed fate (germination, removal and invertebrate infestation) in three forest regrowth stages (late second-regrowth forest, early second-regrowth, and abandoned pasture).

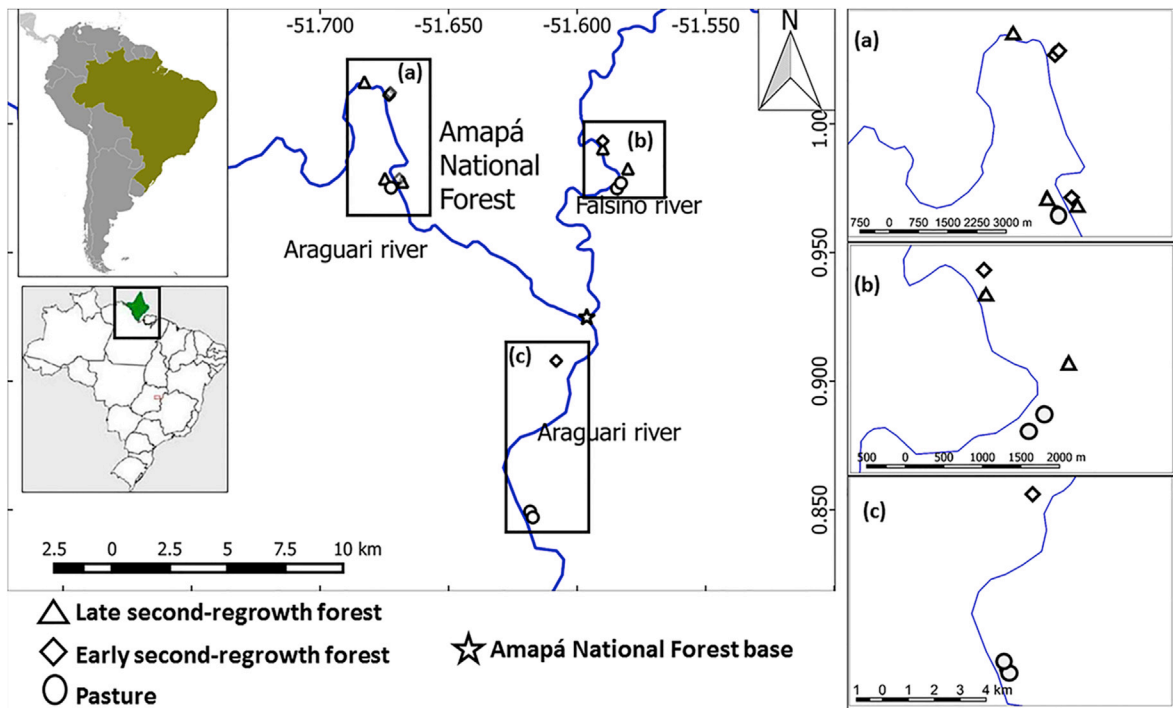
## 2. Material and methods

### 2.1. Study area and sampling units

The study was conducted in 15 private properties surrounding the Amapá National Forest (Floresta Nacional Amapá—hereafter ANF). ANF is a sustainable-use protected area, of approximately 460,000 ha (ICMBIO, 2014), located on the pre-Cambrian Guianan shield craton at the base of the Tumucumaque Uplands, in the northeast Brazilian Amazon (0°55'29"N, 51°35'45"W, Fig. 1). The regional phytophysionomies consist of evergreen tropical rainforest vegetation (Gond et al., 2011), predominantly never flooded “*terra-firme*” forest, with some areas of flooded forest, bamboo and rocky outcrops (ICMBIO, 2014). The regional climate is classified by Köppen-Geiger as Am (Equatorial monsoon (Kotteck et al., 2006)) with annual rainfall ranging from 2200 mm to 2500 mm during the last five years (2012–2016, (ANA, 2017), S1 Fig). During the months with highest precipitation levels (February to April), rainfall may reach 500 mm/month. The dry season (September to November) is characterized by total precipitation below 150 mm/month ((ANA, 2017), S1 Fig).

Data collection was conducted in 15 *terra-firme* sites located in private small-holder properties that were selected on the basis of differences in land-use histories (Norris and Michalski, 2013) and forest regrowth/regeneration stage (Fig. 1). All sites were close (110–554 m) to rivers (100–200 m wide) that are navigable by motorized boats, but due to riverbank formation the sites are never flooded.

Based on the land-use history the 15 sites were grouped into three regrowth stage classes: late second-regrowth forest (N = 5, most recent human disturbance between 20 and 25 years), early second-regrowth (N = 5, most recent human disturbance between 1 and 5 years), and abandoned pasture (N = 5, pasture areas dominated by grasses and herbs but that had never been used to raise livestock i.e. forest was cleared and grass planted in anticipation of cattle that were never bought). Each of the 15 sites was paired with a nearby (60–150 m) control site i.e. 20–30 m tall *terra-firme* forest without a history of mechanized timber extraction. To reduce the possible confounding influence of edge effects, all regrowth and control sites were established at 30 m from the nearest control-regrowth habitat edge (S2 Fig).



**Fig. 1.** Map of the study area around the Amapá National Forest. Different shaped points show the location of different forest regrowth stages in the small holder properties close to rivers (solid blue lines). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

## 2.2. Seed species

We used two native NTFPs seed species: the “super berry” *Euterpe oleracea* Mart. – Arecaeae and the nitrogen fixing *Inga capitata*. Desv. – Fabaceae (hereafter açai and ingá respectively). Açai is one of the most valuable wild fruit species across Amazonia (Smith, 2015), with the açai production chain estimated to generate over US\$ 1 billion in net revenues annually (Brondízio, 2008). Ingá present economic potential in reforestation, phytotherapy, energy production and as a food source (Pritchard et al., 1995; Bilía et al., 2003). These species were selected as they lack dormancy, germinate rapidly (on average 15 and 45 days for ingá and açai, respectively) and are well known to local communities (Pritchard et al., 1995; Pennington, 1997; Queiroz and Mochiutti, 2012; Smith, 2015). Both species are therefore good candidates as early stage pioneers for natural regeneration and restoration (He et al., 2009; Holl, 2017; Meli et al., 2017). Adult açai bear up to eight fruit bunches. The 11–15 mm berries form green clusters during the immature stages and when fully ripe they become deep purple, the size of a grape (Pompeu et al., 2009), and each berry has one large 7–10 mm seed (Sabbe et al., 2009). Ingá produce pods holding 6–8 seeds (Pennington, 1997). The 17 mm long, bean shaped seeds are enveloped by a fleshy and sugary sarcotesta (Pennington, 1997), which makes the fruits and seeds attractive to many vertebrate dispersers and predators (Bilía et al., 2003; Parolin et al., 2013). Vertebrates are the principal dispersal agents of both species, with birds thought to be primarily responsible for the natural dispersal of açai (Moegenburg and Levey, 2003), whereas ingá is dispersed by a more diverse range of dispersers from lizards to birds (Parolin et al., 2013).

## 2.3. Experimental design

We assess seed fate (germination, removal, and insect infestation) of açai and ingá in three classes of regrowth stage (late second-regrowth forest, early second-regrowth, and abandoned pasture). To assess the effects of mid- and large-bodied vertebrates on seed fate we used a paired design of semipermeable enclosure and open plots. The enclosure plots excluded any activity of medium and large-bodied (>1 kg) terrestrial vertebrates, allowing us to estimate the impact of these animals on germination when compared with the open plots (access for all animals). The enclosure plot fences (2.5 × 2.5 m in area and 1.2 m in height, S2 Fig) were constructed with galvanized wire mesh (1 by 1 cm), firmly attached to wooden stakes. Small vertebrates (<1 Kg, e.g. *Proechimys* spp.), invertebrates and birds were not excluded by the wire mesh, which was raised to leave a 5 cm gap above ground level. The unfenced plots were located no more than 5 m from their paired enclosure plots, with their four corners marked with wooden stakes (S2 Fig).

To examine the effect of litter cover we used a split plot design, with the enclosure/unfenced plots subdivided into two  $0.5 \times 0.5$  m subplots (S2 Fig). We left a 0.5-m-wide buffer zone around each subplot, which minimized the effects of seeds deposited in the subplots by birds perching on the stakes and allowed us to walk around subplots without trampling. To assess the plant litter effect the litter from one subplot was removed, exposing bare soil and the litter in the other subplot was left intact (Ganade and Brown, 2002). Collected seeds were then sown directly onto each subplot (S2 Fig). The seed samples were collected 7–13 days prior to sowing. Seeds were collected from three representative healthy adults of each species and manually selected to obtain apparently undamaged and viable seeds. To simulate post-dispersal patterns, we removed the pulp (epicarp and mesocarp) from all seeds prior to placement; we then mixed and randomly selected seeds for use in the experiment. The seeds were distributed in alternating species rows of six seeds, ( $N = 24$  seeds per subplot, 12 açaí and 12 ingá) and each seed was tagged with a stick (S2 Fig). Providing a total of 2880 seeds (1440 of each species), distributed equally (balanced design) across 30 sites (15 regrowth and 15 paired controls) with an enclosure and open plot within each site, and each plot with bare soil and intact litter subplots.

#### 2.4. Data collection

Data were collected during the wet-dry season transition (May to August 2016), with total monthly rainfall ranging from 80 to 171 mm (S1 Fig). This period corresponds to the peak/end of fruiting of lowland Amazon forests in the study region (Steege and Persaud, 1991), and as such was chosen to provide suitable climatic conditions for the germination of both NTFP species. The experimental plots were monitored once per month (mean interval between samples = 33 days, range = 29–38 days), for two months (mean 65 days, range = 64–68 days), a sufficient time for recording seed fate of both species. Seeds were individually marked and during each visit, we recorded the condition of each seed, which was categorized into nine classes: (1) *germinated*—with radicle emission, (2) *intact*—without any visible damage, (3) *invertebrate predation*—when seeds exhibited physical damage (e.g. bore holes) by any invertebrate, (4) *rodent predation*—when the seed presented typical teeth marks caused by rodents, (5) *fungi infested*—with signs of fungal pathogen attack, (6) *termite infested*—with presence of termites, (7) *beetle infested*—with presence of beetle, (8) *rotten*—seed with signs of decomposition, (9) *removed*—seed no longer present.

#### 2.5. Data analysis

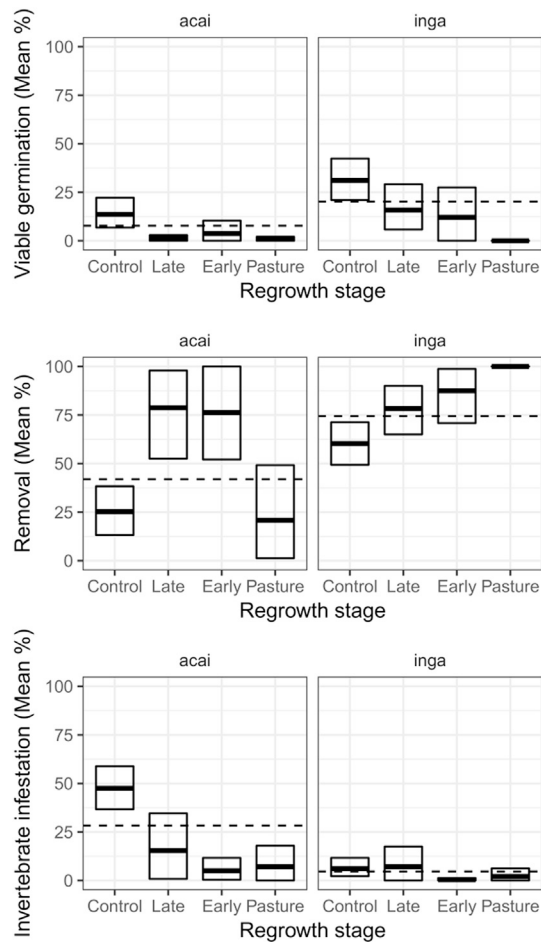
We used generalized linear mixed-effects models (GLMMs) with a binomial error distribution to explain patterns in seed germination. We reclassified the seed conditions to generate four presence/absence seed fate responses: germination (seed germinated at any time), viable germination (germinated and intact/seedling at the end of the experiment), removal and invertebrate infestation (combination of classes 3, 6 and 7). To explain patterns in seed fate we included the forest regrowth stage (control as the reference level, late-regrowth, early-regrowth and pasture), seed species, plot type (enclosure vs open), and litter treatment as categorical fixed effects. Two-way interactions between seed species and the regrowth stage, plot type and litter treatments were also included. To model the split-plot design, the four combinations of plot and litter treatments were nested within the 30 sites (15 control and 15 regrowth) as random effects. We performed all statistical analyses in R 3.4.0 (R Development Core Team., 2017) and we used package lme4 (Bates et al., 2015) to run the GLMMs. Spatial dependence in model residuals was examined using semi-variograms.

### 3. Results

The experiment started with 2880 seeds available, of which 632 (21.9%) were still healthy (intact but ungerminated (209, 7.3%) or intact germinated (423, 14.7%)) at the end of the experiment. More than half of all seeds were removed (1676, 58.2%), with increased (test for equality of proportions,  $p < 0.001$ ) removal of ingá (1072, 74.4%) compared with açaí (604, 41.9%). The 572 (19.9%) remaining seeds were likely to be unviable in the long term due to insect infestation (452), vertebrate predation (12) or fungal/mold infection (108).

A total of 997 seeds germinated (34.6% of all seeds, 36.9% for açaí and 32.3% for ingá) during the sample period (Fig. 2). However, by the end of the experiment only 403 of the germinated seeds were still viable. Over time, 143 of the germinated seeds were removed, additionally 430 were likely to be unviable due to insect infestation (345), vertebrate predation (4) or fungal/mold infection (81). Overall germination increased significantly with regrowth stage (abandoned pasture to late second-regrowth forest) and also increased when mid to large bodied animals were excluded (Table 1). Germination also differed between seed species, but a significant interaction showed that the difference between species depended on the regrowth stage, with germination differing between species in Late and Early secondary-regrowth areas but both species showing close to zero germination in abandoned pasture areas. A similar pattern was observed for viable germination at the end of 60 days (Fig. 2), however a significant species regrowth stage interaction was only found in Late secondary-regrowth areas, with increased ingá germination compared with close to zero germination of açaí (Fig. 2, Table 1).

Removal was the single largest cause of seed loss. Increased removal was recorded in Late and Early second-regrowth compared with control sites (Table 1, Fig. 2). There was a small but significant reduction in the removal of both seed species with the exclusion of mid to large bodied animals (45–39% and 75 to 73%, açaí and ingá respectively, Table 1). In contrast



**Fig. 2.** Seed fate along an Amazon forest regrowth gradient. Comparison of percentage viable germination, removal and the number of infested açai and ingá seeds. Experiments were conducted in 30 sites (15 control and 15 regrowth). Boxplots show means and 95% confidence limits estimated via nonparametric bootstrap. Dashed horizontal lines are the mean for all sites.

removal tended to increase with the clearing of litter (Table 1). There was also a significant difference in removal between species, with increased ingá removal compared with açai (Table 1), particularly in control and pasture sites (Fig. 2).

Control areas had greater invertebrate infestation compared with the other regrowth stages (Table 1). There was also increased invertebrate infestation in plots with litter cover and a significant difference between species with açai infested more by invertebrates (Table 1, Fig. 2). Excluding mid to large bodied vertebrates had no significant effect on invertebrate infestation, but a significant interaction showed that excluding mid to large bodied animals did modulate differences in infestation between species. Similarly to germination, the differences in invertebrate infestation of species depended on regrowth stage, with infestation differing between species in Late and Early secondary-regrowth areas but both species infested similarly in abandoned pasture areas. None of the unexplained variation (model residuals) was related to the geographic distance among plots (Supplemental Material S3).

#### 4. Discussion

We establish that seed removal is the most important driver of patterns in short term germination success in two important NTFP species across different forest regrowth stages. The experimental approach adopted also enabled us to determine that direct seeding is unlikely to be viable in pasture areas for these species. Here we discuss how these findings contribute to the application of direct seeding for cost effective restoration in Amazon small holder properties.

Both species tested appear to have limited use for direct seeding during the initial restoration stages of pasture land, even though they are early successional species. After 60 days we found effectively zero viable germination in pasture areas. Although we also found that on average less than 20% of seeds had germinated and remained viable after 60 days in late and early regrowth forest areas, this rate is within that expected for early stage pioneers (Camargo et al., 2002). Previous studies show that late second-regrowth forest are generally likely to have more optimal biotic and abiotic conditions for the



**Table 1**

Results from generalized linear mixed models (GLMMs). GLMMs of four responses evaluated against additive and interaction effects of experimental treatments.

Fixed effect	Germination		Viable germination		Removal		Invertebrate infestation	
	OR	CI	OR	CI	OR	CI	OR	CI
(Intercept)	1.16	0.41–3.22	0.04***	0.01–0.12	0.38	0.11–1.28	0.47	0.18–1.24
Regrowth stage								
Late second-regrowth	<b>0.02***</b>	0.00–0.12	<b>0.06*</b>	0.01–0.68	<b>36.79**</b>	3.43–394.76	<b>0.05***</b>	0.01–0.31
Early second-regrowth	<b>0.00***</b>	0.00–0.02	<b>0.03**</b>	0.00–0.39	<b>118.02***</b>	9.53–1462.20	<b>0.01***</b>	0.00–0.09
Abandoned Pasture	<b>0.00***</b>	0.00–0.01	<b>0.04*</b>	0.00–0.65	0.28	0.02–3.21	<b>0.02***</b>	0.00–0.12
Plot (Exclosure vs open)	<b>6.02***</b>	3.35–10.80	<b>2.31*</b>	1.12–4.76	<b>0.59*</b>	0.37–0.94	1.04	0.53–2.04
Litter (Litter vs clear)	1.11	0.63–1.96	0.78	0.38–1.59	0.65†	0.41–1.03	<b>2.60**</b>	1.32–5.10
Species (Ingá vs açai)	0.74	0.49–1.13	<b>4.60***</b>	2.65–7.99	<b>7.45***</b>	4.77–11.66	<b>0.12***</b>	0.07–0.21
Species:Exclosure	<b>0.20**</b>	0.12–0.32	0.70	0.38–1.29	1.25	0.78–2.00	<b>0.06***</b>	0.02–0.15
Species:Regrowth Late	<b>16.32***</b>	8.18–32.59	<b>4.87*</b>	1.28–18.54	<b>0.10***</b>	0.05–0.18	<b>4.91***</b>	3.75–21.19
Species:Regrowth Early	<b>15.83***</b>	5.34–46.90	2.09	0.63–7.00	<b>0.43*</b>	0.21–0.89	<b>1.16***</b>	0.12–11.21
Species:Regrowth Pasture	4.20	0.75–23.54	Inf	Inf	Inf	Inf	2.70	0.75–9.69
Species:Litter	0.90	0.55–1.46	1.59	0.86–2.92	1.30	0.81–2.08	<b>0.48*</b>	0.23–0.97
Discrimination <sup>a</sup>	0.565		0.411		0.578		0.511	
Model deviance	1735.8		1270.4		1825.1		1227.6	

Bold font shows significant variables in each model.

Signif. Codes (probability of Z score): \*\*\*\* 0.001 \*\*\* 0.01 \*\* 0.05 \* † 0.1 "Inf": denotes parameters that could not be reliably estimated.

Acronyms: OR: Odds ratio, CI: confidence interval of odds ratio.

<sup>a</sup> Tjur's Coefficient of Discrimination (D).

germination of native species (Guariguata and Ostertag, 2001; Camargo et al., 2002; Mesquita et al., 2015; Holl et al., 2017). In contrast abandoned pasture is likely to provide the least suitable habitat for the germination of native species due to more extreme microclimatic factors such as direct light incidence, elevated temperatures and poor quality soils (Aide et al., 2000; Camargo et al., 2002).

Losses were largely attributable to removal and invertebrate infestation for both açai and ingá. A previous study showed that exclosure plots can have less removal and higher proportion of germination in large seeds when compared to unfenced (open) plots (Asquith et al., 1997) and that on islands these differences were largely attributable to differences in the terrestrial mammal community (Asquith et al., 1997). Indeed studies from the Peruvian rainforest showed that mid to large bodied mammals had little influence on short term germination, but excluding large animals had a much stronger impact on seedling establishment, generating up to six fold differences in seedling community recruitment (Beck et al., 2013). In the case of our small holder properties, which are all connected to continuous forest with elevated diversity of small and large vertebrate dispersers, exclosure had a much weaker effect on removal and viable germination than forest regrowth stage and seed species. Although both species are consumed by mid to large bodied vertebrates, they are also consumed by small mammals and birds. The weak effect of exclosure on the germination success of both species is therefore to be expected. The small percentage point differences in removal between plots (exclosure vs open) mean that we attribute the majority of removal to these other important seed predators.

Both seed species are early succession pioneers, therefore the most likely sites for their direct seeding as part of regeneration and/or restoration actions would be pasture and/or early second-regrowth sites. We found that viable germination was <10% from direct seeding in early regrowth and pasture sites. Indeed germination in pasture areas was effectively zero for both species. The low germination was due to elevated ( $\approx 75\%$ ) removal of both species in early second regrowth areas. Whereas in pasture the removal was 25% and 100% (açai and ingá, respectively). Both species require relatively humid conditions to thrive. As such they seem unlikely candidates for accelerating initial regeneration and restoration of pasture sites without additional interventions such as irrigation.

Germination success was most strongly affected by forest regrowth stage and the NTFP seed species in our lowland Amazon sites. We found that the additive and interaction effects between regrowth stage and seed species explained approximately 40% of the variation in viable germination of the pioneer seeds. Additionally, these same two factors also explained more than 50% of the variation in seed removal and invertebrate infestation. These findings support those of previous studies from tropical regions that show the importance of land-cover type and seed species for the success of direct sowing (Camargo et al., 2002; Shoo and Catterall, 2013; Palma and Laurance, 2015; Shoo et al., 2016; Holl et al., 2017). The rural population density across the Brazilian Amazon has generally remained stable or has increased (IBGE, 2010), see supplemental material in Norris and Michalski (2013)). Additionally, with the stricter controls on deforestation in large farms, the relative contribution of small-scale (<100 ha) rural farmers for the remaining deforestation in Brazil has also increased in comparison to that of large (>2500 ha) landowners (Godar et al., 2014). As in much of the tropics, agroforestry is playing an increasingly important role as a replacement to slash and burn practices across rural Amazonia (Lasco et al., 2014; Reed et al., 2016). Direct seeding of these important NTFP species remains a viable option for regeneration, restoration and agroforestry of small-holder properties.

Although germination success from direct seeding was reduced compared with control areas, both species are widespread and their seeds are easily acquired. As such direct seeding remains a viable option for these pioneers in both late and early regrowth sites. The exploitation of NTFPs in the ANF is incipient with an association of 21 local smallholders informally (i.e. lacking commercial production chains) harvesting açai fruits and producing soap from native tree oilseed and resin extracts (e.g. *Carapa guianensis* and *Copaifera multijuga* (Norris et al., 2016). With low human density and low income, cost is of primary importance for the success of any restoration action. However, further long term studies are required to evaluate the establishment of seedlings and trees of these important NTFP species within small holder regeneration, restoration and agroforestry actions.

## 5. Conclusions

Our study showed that short term germination success in late and early regrowth forest is limited by elevated seed removal rates. Although germination success was reduced in late and early regrowth sites compared with control sites, we suggest that direct seeding remains a viable alternative for the establishment of these relatively common and widely available NTFP species. However, long term monitoring is required to confirm the sowing density and cost effectiveness of directly sown seeds required to provide meaningful establishment of these important NTFP species.

## Acknowledgements

The Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) and the Amapá National Forest staff (Érico Emed Kauano and Sueli Gomes Pontes dos Santos) and the Federal University of Amapá (UNIFAP) provided logistical support. We thank the Brazilian Ministério do Meio Ambiente (“MMA”) for authorizing data collection (SISBIO permits 40355–1 and 47859–2). We also thank the local landowners who gave permission for data collection at their properties. We are deeply indebted to Cremilson and Cleinaldo Alves Marques and family for their dedication, commitment and assistance during the fieldwork. We are grateful to Víctor Rodríguez-Chuma for help during data collection. We thank the following Brazilian agency for financially supporting data collection: Conselho Nacional de Desenvolvimento Científico e Tecnológico (“CNPq” project #446926/2014–0). Authors also thank CAPES (scholarship to AAS, # 12002011003PO). We are also grateful to the editor and two anonymous reviewers for providing comments that improved earlier version of the text.

## Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.gecco.2017.e00371>.

## References

- Aide, T.M., Zimmerman, J.K., Pascarella, J.B., Rivera, L., Marcano-Vega, H., 2000. Forest regeneration in a chronosequence of tropical abandoned pastures: implications for restoration ecology. *Restor. Ecol.* 8, 328–338.
- ANA, 2017. Sistema de Monitoramento Hidrológico (Hydrological Monitoring System). Agência Nacional de Águas [nl] National Water Agency. Available at: <http://www.hidroweb.ana.gov.br>.
- Asquith, N.M., Wright, S.J., Clauss, M.J., 1997. Does mammal community composition control recruitment in Neotropical forests? Evidence from Panama. *Ecology* 78, 941–946.
- Bagchi, R., Gallery, R.E., Gripenberg, S., Gurr, S.J., Narayan, L., Addis, C.E., Freckleton, R.P., Lewis, O.T., 2014. Pathogens and insect herbivores drive rainforest plant diversity and composition. *Nature* 506.
- Bates, D., Maechler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* 67, 1–48.
- Beck, H., Snodgrass, J.W., Thebpanya, P., 2013. Long-term enclosure of large terrestrial vertebrates: implications of defaunation for seedling demographics in the Amazon rainforest. *Biol. Conserv.* 163, 115–121.
- Bilia, D., Barbedo, C., Cicero, S., Marcos Filho, J., 2003. Ingá: uma espécie importante para recomposição vegetal em florestas ripárias, com sementes interessantes para a ciência. *Inf. Abrates* 13, 26–30.
- Brondízio, E.S., 2008. The Amazonian Caboclo and the Açai Palm: Forest Farmers in the Global Market. New York Botanical Garden Press, New York.
- Brondízio, E.S., Safar, C.A., Siqueira, A.D., 2002. The urban market of açai fruit (*Euterpe oleracea* Mart.) and rural land use change: ethnographic insights into the role of price and land tenure constraining agricultural choices in the Amazon estuary. *Urban Ecosyst.* 6, 67–97.
- Camargo, J.L.C., Ferraz, I.D.K., Imakawa, A.M., 2002. Rehabilitation of degraded areas of central Amazonia using direct sowing of forest tree seeds. *Restor. Ecol.* 10, 636–644.
- Chazdon, R.L., Guariguata, M.R., 2016. Natural regeneration as a tool for large-scale forest restoration in the tropics: prospects and challenges. *Biotropica* 48, 716–730.
- Facelli, J.M., Pickett, S.T., 1991. Plant litter: its dynamics and effects on plant community structure. *Botanical Rev.* 57, 1–32.
- Ganade, G., Brown, V.K., 2002. Succession in old pastures of central Amazonia: role of soil fertility and plant litter. *Ecology* 83, 743–754.
- Godar, J., Gardner, T.A., Tizado, E.J., Pacheco, P., 2014. Actor-specific contributions to the deforestation slowdown in the Brazilian Amazon. *Proc. Natl. Acad. Sci.* 111, 15591–15596.
- Gond, V., Freycon, V., Molino, J.-F., Brunaux, O., Ingrassia, F., Joubert, P., Pekel, J.-F., Prévost, M.-F., Thierron, V., Trombe, P.-J., Sabatier, D., 2011. Broad-scale spatial pattern of forest landscape types in the Guiana Shield. *Int. J. Appl. Earth Observation Geoinformation* 13, 357–367.
- Griffiths, H.M., Bardgett, R.D., Louzada, J., Barlow, J., 2016. The value of trophic interactions for ecosystem function: dung beetle communities influence seed burial and seedling recruitment in tropical forests. *Proc. R. Soc. B* 283, 20161634.
- Guariguata, M.R., Ostertag, R., 2001. Neotropical secondary forest succession: changes in structural and functional characteristics. *For. Ecol. Manag.* 148, 185–206.
- He, J., Zhou, Z., Weyerhaeuser, H., Xu, J., 2009. Participatory technology development for incorporating non-timber forest products into forest restoration in Yunnan, Southwest China. *For. Ecol. Manag.* 257, 2010–2016.
- Holl, K.D., 2017. Restoring tropical forests from the bottom up. *Science* 355, 455–456.

- Holl, K.D., Reid, J.L., Chaves-Fallas, J.M., Oviedo-Brenes, F., Zahawi, R.A., 2017. Local tropical forest restoration strategies affect tree recruitment more strongly than does landscape forest cover. *J. Appl. Ecol.* 54, 1091–1099.
- IBGE, 2010. Censo Demográfico 2010. Instituto Brasileiro de Geografia e Estatística, Rio de Janeiro.
- ICMBIO, 2014. FLONA Do Amapá. <http://www.icmbio.gov.br/portal/>. (Accessed 14 May 2015).
- Kottek, M., Grieser, J., Beck, C., Rudolf, B., Rubel, F., 2006. World map of the Koppen-Geiger climate classification updated. *Meteorol. Z.* 15, 259–263.
- Lasco, R.D., Delfino, R.J.P., Catacutan, D.C., Simelton, E.S., Wilson, D.M., 2014. Climate risk adaptation by smallholder farmers: the roles of trees and agroforestry. *Curr. Opin. Environ. Sustain.* 6, 83–88.
- Martínez-Garza, C., Howe, H.F., 2003. Restoring tropical diversity: beating the time tax on species loss. *J. Appl. Ecol.* 40, 423–429.
- Meli, P., Herrera, F.F., Melo, F., Pinto, S., Aguirre, N., Musálem, K., Minaverry, C., Ramírez, W., Brancalion, P.H.S., 2017. Four approaches to guide ecological restoration in Latin America. *Restor. Ecol.* 25, 156–163.
- Mesquita, R.D.C.G., Massoca, P.E.D.S., Jakovac, C.C., Bentos, T.V., Williamson, G.B., 2015. Amazon rain forest succession: stochasticity or land-use legacy? *BioScience* 65, 849–861.
- Moegenburg, S.M., Levey, D.J., 2003. Do frugivores respond to fruit harvest? An experimental study of short-term responses. *Ecology* 84, 2600–2612.
- Nepstad, D.C., Uhl, C., Pereira, C.A., da Silva, J.M.C., 1996. A Comparative Study of Tree Establishment in Abandoned Pasture and Mature Forest of Eastern Amazonia. *Oikos*, pp. 25–39.
- Norris, D., Michalski, F., 2013. Socio-economic and spatial determinants of anthropogenic predation on Yellow-spotted River Turtle, *Podocnemis unifilis* (Testudines: pelomedusidae), nests in the Brazilian Amazon: implications for sustainable conservation and management. *Zool. (Curitiba)* 30, 482–490.
- Norris, D., Rodriguez Chuma, V.J.U., Arevalo-Sandi, A.R., Landazuri Paredes, O.S., Peres, C.A., 2016. Too rare for non-timber resource harvest? Meso-scale composition and distribution of arborescent palms in an Amazonian sustainable-use forest. *For. Ecol. Manag.* 377, 182–191.
- Paine, C.E., Beck, H., Terborgh, J., 2016. How mammalian predation contributes to tropical tree community structure. *Ecology* 97, 3326–3336.
- Palma, A.C., Laurance, S.G.W., 2015. A review of the use of direct seeding and seedling plantings in restoration: what do we know and where should we go? *Appl. Veg. Sci.* 18, 561–568.
- Parolin, P., Wittmann, F., Ferreira, L.V., 2013. Fruit and seed dispersal in Amazonian floodplain trees—a review. *Ecotropica* 19, 15–32.
- Pennington, T.D., 1997. The Genus *Inga*: Botany. Royal Botanic Gardens.
- Piironen, T., Valtonen, A., Roininen, H., 2017. Vertebrate herbivores are the main cause of seedling mortality in a logged African rainforest—implications for forest restoration. *Restor. Ecol.* 25, 442–452.
- Poffenberger, M., Singh, S., 1992. 'Forest management partnerships: regenerating India's forests'. *Unasylva* 43, 46.
- Pompeu, D.R., Silva, E.M., Rogez, H., 2009. Optimisation of the solvent extraction of phenolic antioxidants from fruits of *Euterpe oleracea* using Response Surface Methodology. *Bioresour. Technol.* 100, 6076–6082.
- Pritchard, H., Haye, A., Wright, W., Steadman, K., 1995. A comparative study of seed viability in *Inga* species: desiccation tolerance in relation to the physical characteristics and chemical composition of the embryo. *Seed Sci. Technol.* 23, 85–100.
- Queiroz, J. A. L. d., Mochiutti, S., 2012. Guia prática de manejo de açaiçais para produção de frutos, 2 edition. Embrapa Amapá, Macapá, Brazil.
- R Development Core Team, 2017. R: a Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.
- Reed, J., Van Vianen, J., Deakin, E.L., Barlow, J., Sunderland, T., 2016. Integrated landscape approaches to managing social and environmental issues in the tropics: learning from the past to guide the future. *Glob. Change Biol.* 2540–2554.
- Richards, M., 1993. The potential of non-timber forest products in sustainable natural forest management in Amazonia. *Commonw. For. Rev.* 21–27.
- Sabbe, S., Verbeke, W., Deliza, R., Matta, V., Van Damme, P., 2009. Effect of a health claim and personal characteristics on consumer acceptance of fruit juices with different concentrations of açai (*Euterpe oleracea* Mart.). *Appetite* 53, 84–92.
- Shoo, L.P., Catterall, C.P., 2013. Stimulating natural regeneration of tropical forest on degraded land: approaches, outcomes, and information gaps. *Restor. Ecol.* 21, 670–677.
- Shoo, L.P., Freebody, K., Kanowski, J., Catterall, C.P., 2016. Slow recovery of tropical old-field rainforest regrowth and the value and limitations of active restoration. *Conserv. Biol.* 30, 121–132.
- Smith, N., 2015. *Euterpe Oleracea*. Pages 239–258 *Palms and People in the Amazon*. Springer, Switzerland.
- Stege, H. t., Persaud, C.A., 1991. The phenology of Guyanese timber species: a compilation of a century of observations. *Plant Ecol.* 95, 177–198.
- Stoner, K.E., Riba-Hernández, P., Vulinac, K., Lambert, J.E., 2007. The role of mammals in creating and modifying seedshadows in tropical forests and some possible consequences of their elimination. *Biotropica* 39, 316–327.
- Xiong, S., Nilsson, C., 1999. The effects of plant litter on vegetation: a meta-analysis. *J. Ecol.* 87, 984–994.