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Natural regeneration triggers compositional and functional shifts in soil seed banks

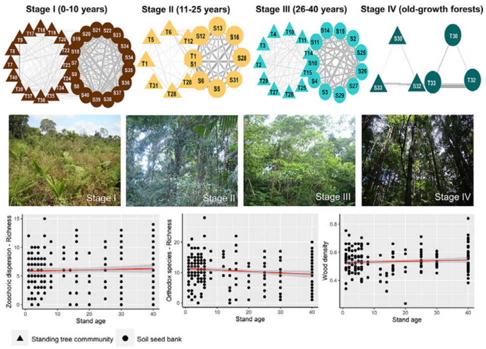
Priscila Sanjuan de Medeiros-Sarmento [a,b], Leandro Valle Ferreira [b], Markus Gastauer [a,*]

[a] Instituto Tecnológico Vale, Boaventura da Silva, 955, Umarizal, CEP 66055-200 Belém, Pará, Brazil
[b] Museu Paraense Emílio Goeldi, CBO, Avenida Perimetral, 1901, Terra Firme, CEP 66077-830 Belém, Pará, Brazil

HIGHLIGHTS

- Soil seed banks regenerating after shifting cultivation practices are dominated by herbs.
- Tree seed density and diversity increase with regeneration time and vegetation structure.
- Allochthonous seeds highlight the role of habitat connectivity in natural regeneration.
- Animal-dispersed, recalcitrant, slowly growing, large-fruited species increase with time.
- Long fallow periods guarantee the sustainability of shifting cultivation practices.

GRAPHICAL ABSTRACT



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ABSTRACT

Secondary forests emerging during traditional shifting cultivation practices are increasingly recognized for their fulfillment of ecosystem services and mitigation potential of climate change and biodiversity losses. The soil seed bank as a recruit reservoir is a limiting factor for natural forest regeneration of such secondary forests and is decisive for the formation and restitution of the post-disturbance community. The aim of this study was to compare the composition of the soil seed bank along a natural regeneration chronosequence from the Caxiuanã National Forest, eastern Amazon, including old-growth reference sites. We sampled standing vegetation, soil properties and soil seed banks and compared the density and species richness of different life forms among different regeneration stages. Using nonmetric, multiple scaling, we compared the composition of the soil seed bank among different regeneration stages and with standing vegetation composition. Furthermore, we outlined the influence of stand age, vegetation structure and soil properties on the density, richness and functional characterization of the soil seed bank using mixed effect models. The soil seed bank was dominated by herb seeds in all regeneration stages, and the density and richness of tree seeds increased with regeneration time and recovery of vegetation structure. Seed bank composition changed gradually with regeneration advance and differed from standing vegetation, containing a high amount of allochthonous seeds, especially in older stands. This observation highlights the importance of dispersal and habitat connectivity for the natural regeneration of these secondary forests. Shifts in soil seed bank composition towards slow-growing, animal-dispersed, non-pioneer species with larger, recalcitrant seeds in older regeneration stands indicate changes in vegetation composition along succession. Thus, our data indicate the importance of connectivity for forest regeneration and long fallow periods ($>$ 40 years) to increase the performance of ecosystem services, resilience and stability of secondary forests arising during shifting cultivation practices.

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* Corresponding author.
E-mail address: markus.gastauer@itv.org (M. Gastauer).

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1. Introduction

Shifting cultivation, or slash-and-burn agriculture, is a traditional land use system that guarantees the subsistence of many indigenous and non-indigenous people within the Amazon rainforest (Joslin et al., 2019; Ribeiro Filho et al., 2013; van Vliet et al., 2012). Clearcutting and burning of small areas of old-growth or regenerating forests give rise to short cultivation periods (mostly cassava), after which the areas were abandoned and regenerate naturally. Despite being considered a traditional form of land use, these practices and their intensification contribute to deforestation and the advance of secondary forests in the Amazon biome (Jakovac et al., 2017; Villa et al., 2018). Challenges faced to maintain biodiversity despite declining amounts of primary forests (Levis et al., 2020; Lovejoy and Nobre, 2019) increasingly recognize the importance of secondary forests for the performance of ecosystem services and as a mitigation strategy of climate change and biodiversity losses (Arroyo-Rodríguez et al., 2017; Barros et al., 2020; Chazdon, 2017; Elias et al., 2020), including that emerging after shifting cultivation (Villa et al., 2019, 2020).

The success of natural regeneration (Rezende and Vieira, 2019), an economic strategy for ecosystem restoration and the emergence of secondary forests (Brancalion et al., 2016; Shiferaw et al., 2018), depends on the physical characteristics of the surrounding landscape (Sprengelmeyer and Rebertus, 2015), the intensity and frequency of disturbances (Sanou et al., 2018) and the arrival of plant propagules for the colonization of the areas (Chazdon and Guariguata, 2016; Wandrag et al., 2015). As an important component of plant communities, the soil seed bank represents a reservoir of recruits that may emerge when environmental conditions are favorable (Faist et al., 2013; Vandvik et al., 2016). The soil seed bank is thus a limiting factor for natural forest regeneration, being decisive for the formation and restitution of the post-disturbance community (Sanou et al., 2018) and contributing to the conservation of genetic diversity and recovery of biodiversity (Nielsen et al., 2018; Taiwo et al., 2018).

In studies that assess the environmental factors shaping plant communities along regeneration gradients, most analyses are restricted to above-ground diversity patterns, resulting in a low understanding of how plant diversity below ground, e.g., seed bank diversity, can shape plant communities above ground (Plue et al., 2017). Community alterations following land use changes, such as shifting cultivation, cause not only the loss of aboveground biodiversity but furthermore change soil seed banks regarding their size, diversity and composition, significantly influencing the resilience and regeneration potential of natural vegetation (Bourgeois et al., 2017; Sanou et al., 2018; Sousa et al., 2017). The understanding of changes in the soil seed bank along secondary succession thus provides necessary information about the regeneration potential of the Amazon rainforest and the maintenance of biodiversity in this ecosystem, which is indispensable for land use planning as well as conservation and restoration policies.

In this context, functional approaches have improved the understanding of aboveground successional processes in tropical secondary forests (Meira-Neto et al., 2019; Muscarella et al., 2016). Gradual changes in the functional characterization of regenerating communities along successional trajectories, such as the substitution of acquisitive to more conserved species (Pinho et al., 2018), may follow shifts in the soil seed bank. Specifically, one might expect shifts towards more animal-dispersed, slower-growing species with larger fruits along regeneration advance. Thus, the functional characterization of the soil seed bank throughout the successional process and its relation with the aboveground community may provide useful insights into the ecological mechanisms driving successional dynamics.

The objective of this study was to compare the composition and functional changes of the soil seed bank along a natural regeneration chronosequence from the eastern Amazon, including old-growth reference sites, to subsidize the sustainable management of these areas. For that, we sampled standing vegetation, soil properties and soil seed banks. We outlined changes in density and species richness of the entire soil seed bank and different life forms among regeneration stages. Additionally, we analyzed the composition of the soil seed bank and compared it to that of standing vegetation using non-metric multiple scaling. We classify trees from soil seed banks regarding their storage capability, dispersal syndrome, wood density and fruit length. We focus on the tree species of the soil seed bank, as trees represent the main part of vegetation biomass in forest ecosystems and are thus the most important contributor to the performance of ecosystem services (Zeng et al., 2019). Finally, we modelled seed density, richness and functional characteristics of the soil seed bank as functions of different predictor variables to outline whether stand age, structural parameters such as canopy openness or standing tree density, standing tree diversity or soil properties are the best determinants of soil seed banks.

2. Methods

2.1. Study site

This study was carried out in *terra firme* forests, i.e., forest stands on well-drained, non-flooded oxisols and latisols (Stropp et al., 2011), from the Estação Científica Ferreira Penna (1°14′S; 48°17′W), Caxiuanã National Forest, situated between the interflows of the Xingu and Tocantins Rivers, Pará, Brazil (Fig. 1). The study site is situated in the ecoregion Xingu – Tocantins – Araguaia, an area considered priority for conservation and sustainable use (Castro et al., 2020).

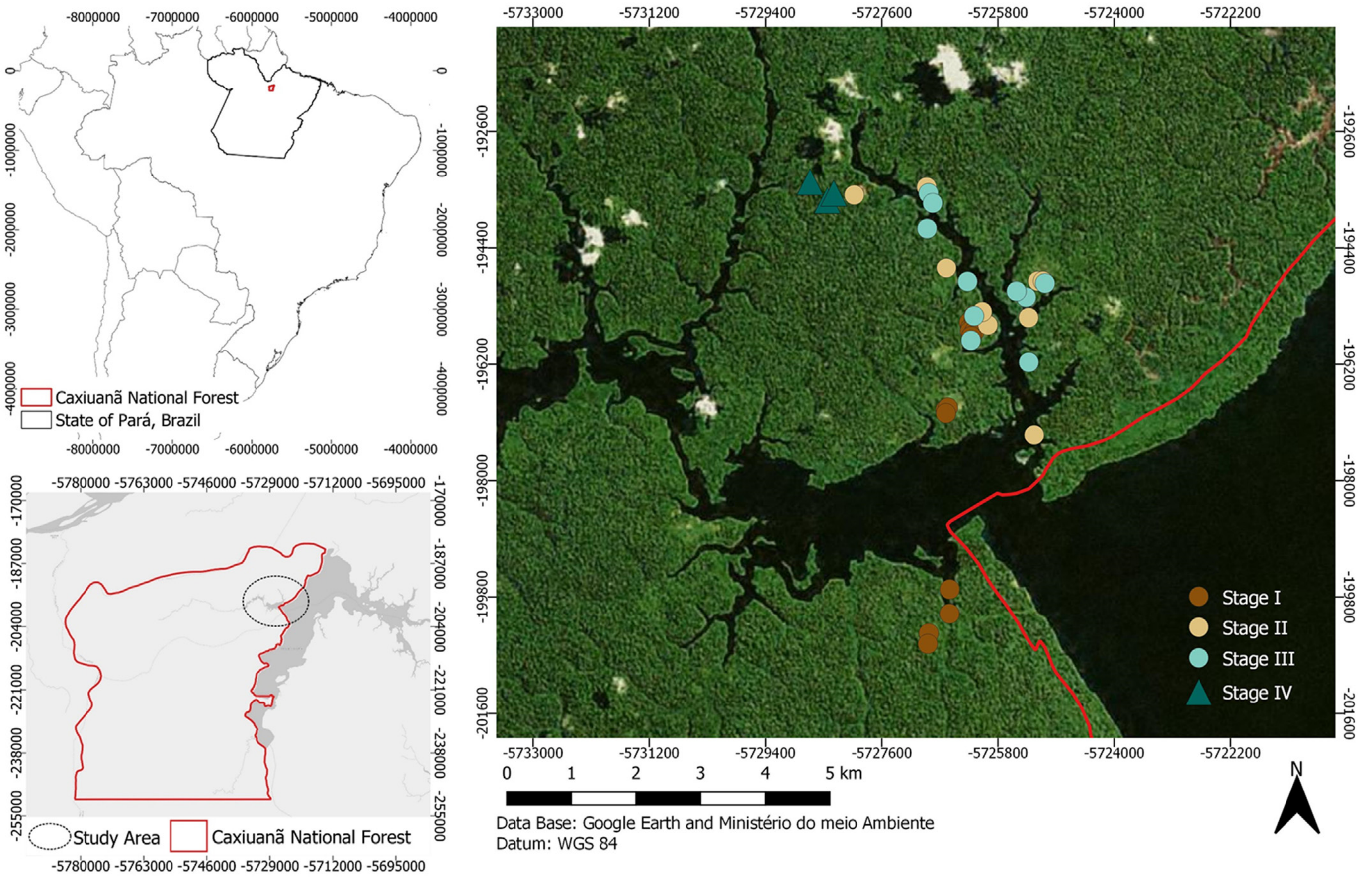
Old-growth *terra firme* forests represent the dominant vegetation formation in the Caxiuanã National Forest, but shifting cultivation practices during a long history of indigenous and riverine occupation created a mosaic of secondary forests embedded within an old-growth forest matrix (Lisboa et al., 2013). Additionally, minor anthropogenic impacts result from hunting, fishing, and extraction of timber and non-timber products. Shifting cultivation starts with the logging of vegetation, followed by cultivation for 2 to 3 years and a subsequent fallow period of eight or more years, before the cycle starts again. The main crop in shifting cultivation systems of the region is *cassava*, representing staff of life of the local population.

The climate is tropical warm and humid, Am in the Köppen classification (ICMBio, 2012). The mean air temperature oscillates at approximately 27 °C, with minimum and maximum values of 22 °C and 32 °C, respectively (ICMBio, 2012). The mean annual precipitation amounts to 2144 mm (Costa et al., 2018); precipitation is concentrated between January and June, while the season between July and December is considered the less rainy period.

2.2. Data collection

For sampling, we installed 40 permanent plots of 20 × 20 m in old growth (3 plots) and secondary forest patches (37 plots) varying in age from 1 to 40 years (Fig. 1). Information on regeneration age of different patches was achieved by means of interviews with people from local riverside communities. Due to a long occupation history of the region, we cannot eliminate the possibility that sampled patches suffered impacts from more than one shifting cultivation cycle, but we assured during our interviews that fallow time before the last logging event was 30 years or larger, and that forest patches presented a structure similar to old-growth forests.

Additionally, careful selection of patches with similar biotic and abiotic conditions guarantees that plots differ only regarding regeneration time, so spatially distinct areas constitute a temporal representation of the succession process. To avoid edge effects, each patch had a minimum size of 0.75 ha, and sampling was carried out in the patch's centers, at least 50 m from its edge. For statistical reasons we grouped forest patches in different regeneration stages. Stage I plots are regenerating for up to 10 years after abandonment of agricultural shifting cultivation, Stage II plots are regenerating for 11 to 25 years,



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Datum: WGS 84

Fig. 1. Geographic localization of the permanent plots and the study site in relation to the Caxiuanã National Forest and Brazil. The classification in regeneration stages refers to age after abandonment of shifting agricultural activities. Stage I is up to 10 years, Stage II is 11–25 years, Stage III is 26–40 years and Stage IV refers to old-growth forests without clearcutting for at least 80 years.

Stage III plots are regenerating for 26 to 40 years and Stage IV plots are old-growth forests not used for shifting cultivation practices for more than 80 years. Number of plots per stage differed, reflecting the availability of different regeneration stages within the region.

The soil seed bank was sampled six times in each plot between July 2013 and May 2014 at two month intervals to track seasonal variation along a single year. We used a 0.5 × 0.5 m template to mark 0.25 m^2 and collected the upper 8 cm of soil after removing the litter layer. During each field campaign, we collected three replicates homogeneously distributed within each plot. To avoid resembling the soil seed bank on the same place during the following campaigns, the exact localization of sampling points was marked using PVC tubes. After collection, soil samples were stored in unsealed plastic bags and kept in shady places to minimize seed degradation by overheating until the end of each field campaign. Samples were transported to a greenhouse in Belém as soon as possible and, upon arrival, immediately distributed in round plastic containers (45 cm in diameter, filling height approximately 15 cm). Soils were maintained at approximately 70% field capacity for germination for six months. Monthly emerging seedlings were counted, separated into morphospecies and identified to the species level. After the removal of emerging seedlings, remaining soils were thoroughly mixed to give all seeds the same chance to germinate. Nomenclature of species names follows the guidelines from the Missouri Botanical Garden (Missouri Botanical Garden, 2020), and systematic classification follows APG IV (The Angiosperm Phylogeny Group, 2016).

To control contamination of containers by seeds dispersed to the greenhouse during the germination experiment, we maintained identical plastic containers containing sterilized sand during the entire germination period. No emerging seedlings were detected in these control containers during the germination period, indicating that all emerging seedlings belong to the original soil seed bank from Caxiuanã National Park.

Emerging seedling species were classified according to their life form (herbs, lianas and trees as proposed by (Jardim Botânico de Rio de Janeiro, 2020)), dispersal syndrome (zoochoric and non-zoochoric species) and resistance of seeds for drying and freezing (orthodox and recalcitrant seeds). For each plot, we computed the community weighted mean value of the functional traits wood density and fruit size. For all identified seedling species, we gathered wood density using the 'getWoodDensity' function from the BIOMASS package (Réjou-Méchain et al., 2017) in R Environment (R Development Core Team, 2018), setting the region to Tropical South America. This function grasps mean wood density for species from the Global Wood Density Database (Chave et al., 2009; Zanne et al., 2009). For species not available in this database, 'getWoodDensity' builds means from all species in the database belonging to the same genus. Fruit size was detected by measuring fruits from three herbarium exsiccatae per species from the Museum Paraense Goeldi Herbarium or, when not available there, from online sources. For each exsiccatae used for measurement, we checked the occurrence in *terra firme* forests as close as possible to our study site.

During the first field campaign, all trees within plots with diameters at breast height (dbh) larger than 10 cm were identified to the species level to obtain the species richness and tree density of standing vegetation. To detect canopy openness, we used a commercially available digital camera that was adjusted on a tripod to take vertical photos at five homogeneously distributed points within each plot. By means of a magnetic compass, the upper margem of the photo was oriented to the north. All photos were taken during dust or down or during cloudy days to avoid reflections of solar radiation by vegetation and subsequent misinterpretation of pixels. Photos were analyzed using ImageJ software. For that, the original photos were transformed into black and white images, where black pixels represent the dossel and the proportion of white pixels was considered the canopy openness.

2.3. Soils samples and analysis

Soil texture and fertility for each plot were assessed using a composite sample per plot. For that, 12 homogeneously distributed sampling points were defined within each plot. Litter layers were removed, and a soil cylinder was collected with an auger (0–20 cm depth). The cylinders from all sampling points within a plot were mixed, and 500 g was weighed, air dried and analyzed for organic matter, fertility (N, P, K, Ca, Mg), pH and texture (coarse sand, find sand, silt and clay) in the soil lab of Embrapa Amazônia Oriental.

2.4. Statistical analysis

All statistical analyses were carried out in R software (R Development Core Team, 2018). As far as not stated otherwise, we pooled seed amounts and species richness of the soil seed bank from different campaigns for each plot. To check for sample sufficiency, we built species-accumulation curves for each regeneration stage for the entire soil seed bank and the tree species from the soil seed bank separately. For that, we used the 'iNEXT' function from the homonymous package to rarefy and extrapolate the 95% confidence intervals of the species-sampling curve from the species abundance distribution (Hsieh et al., 2016). As all curves' inclinations decrease with sampling effort (Fig. S1), we conclude that sampling was sufficient within our study.

To compare old-growth forests and further regenerations stages regarding species richness, tree density and canopy openness of standing vegetation and seed density and richness of the entire soil seed bank and different life forms therein, we used one-way ANOVAs followed by post hoc Tukey tests after checking the data distribution for normality and homoscedasticity.

Differences in soil properties between regeneration stages were outlined using PERMANOVA analysis. To illustrate differences in soil properties between regeneration stages and to reduce the number of soil variables for modelling, we computed the principal components (PCs) using 'princomp' function from R Environment. This principal component analysis transforms soil data to a new, orthogonal coordinate system such that the greatest variance comes to lie on the first coordinate (called PC1), the second greatest variance on the second coordinate, and so on. Individual PCs are statistically independent (orthogonal) of each other and summarize more variance than single variables, which predestines them for modelling purposes. We used the parallel analysis to decide on the number of PCs in subsequent analysis: If the PC's eigenvalue based on observed data is larger than the average corresponding eigenvalues from a set of random data matrices, PC is maintained (Hayton et al., 2004). For that, functions and scripts provided in the 'factorAnalysis' package were used (Kabacoff, 2020). For visualization, soil variables were fitted on this ordination using the 'envfit' function (vegan package).

Community composition was assessed by nonmetric multiple scaling (NMDS) using the 'metaMDS' function from the 'vegan' package (Oksanen et al., 2017). Significance levels for differences among regeneration stages and between seed bank and standing vegetation were outlined using the R statistic from the analysis of similarity ('anosim' function). Additionally, we computed the dissimilarity matrix based on the Jaccard-type overlap among plots and between the soil seed bank and standing vegetation using the 'pair_dis' function and built the qgraph network diagram with the 'pair_dis_plot', both functions from the 'hilldiv' package (Alberdi and Gilbert, 2019).

To check whether species richness or density of entire soil seed bank species richness or that belonging to distinct life forms, zoochoric dispersal strategy and orthodox seeds in regenerating sites are influenced by stand age, canopy openness, standing tree density, standing tree species richness or soil attributes (significant soil PCs revealed by the parallel analysis), we used a model-selection procedure. Using the same model-selection procedure, we further outlined whether abundance-weighted mean values of wood density and fruit length of seed bank species change as a function of the mentioned predictor variables. As no accurate age estimations are available for old-growth plots, we withdraw Stage IV plots from modelling.

For modelling, data were fitted by general linear mixed effect models using the 'glmer' function from the 'lme4' package (Bates et al., 2014), considering the effects of plots as fixed effects, as different field campaigns were considered separately. Count data, i.e., species richness and seed densities, were modelled using the Poisson family, and Gaussian error distributions were assumed for abundance-weighted mean trait values.

For each response variable, we built a global model containing all five predictor variables. Then, we selected the most parsimonious models containing three or less uncorrelated ($r < 0.6$), scaled predictor variables by the 'dredge' command from the MuMIn package (Bartón, 2015) using the Akaike Information Criterion (AIC) (Symonds and Moussalli, 2011). Models with more than three predictor variables were ignored to avoid overfitting; to compute the maximum correlation coefficient between predictor variables, we used the dredge_mc function (https://github.com/rojaff/dredge_mc). All models with ΔAIC less than 2 were considered equally parsimonious (Burnham and Anderson, 2002). When more than one model was selected, we calculated model-averaged parameters and unconditional standard errors using the 'model.avg' function from the 'MuMIn' package, and likelihood ratio tests evaluated the significance of each parameter estimate in this conditional averages model.

3. Results

The standing vegetation in all plots was composed of 1.073 trees belonging to 192 species from 130 genera and 53 families. Tree density and tree species richness were lower in Stage I plots than in more advanced regeneration stages or old growth forests, and canopy openness showed the opposite tendency, being higher in Stage I than in further stages (Fig. S2).

From a total of 13 principal components, the first three PCs each explained more than 10% of the variance in soil data; together, they capture more than 75% of the overall soil variance (Fig. S3a). pH, Ca, fine and coarse sand contents were positively related with PC1, and N, K, organic matter, clay content and Al^{3+} availability reduced with PC1 (Fig. S4). K increased significantly with PC2 and P with PC3 (Fig. S3b). Soil properties differed between regeneration stages according to a PERMANOVA (F-value = 4.6314, $p = 0.003$). In the PCA biplot, old-growth forests were placed near the transition between the second and third quadrants, indicating average soil texture and Al availability (PC1) but lower K availability than average (PC2). Plots from further rehabilitation stages showed higher variation regarding soil texture and higher average fertility, especially in Stage I plots (Fig. S3b).

In total, 16,073 seedlings belonging to 47 families, 106 genera and 158 species emerged from the collected soil seed banks (Table S1). Of these seedling species, 51.9% were trees, 33.5% herbs and 14.5% liana. With a total of 67% seedlings, herbs were the most abundant life form, followed by trees (30.15%) and liana (2.85%). From the overall soil seed bank, 99 seedling species were zoochorous (62.7% of overall seedlings), and 125 species were orthodox (79%). Among tree seedlings only, 72 species (87.8% of all trees) were zoochorous, and 70 (85.4%) were orthodox. Overall, seedling richness and density were higher in regenerating plots than in old-growth forests (Fig. 2). The number and density of seedlings belonging to herb species were higher in Stage I plots than in further stages. The number of tree species seedlings in regeneration stages did not differ from the scores achieved in old-growth forests, but the richness of this life form was higher in Stages II and III (Fig. 2). Further life forms showed no differences (data not shown).

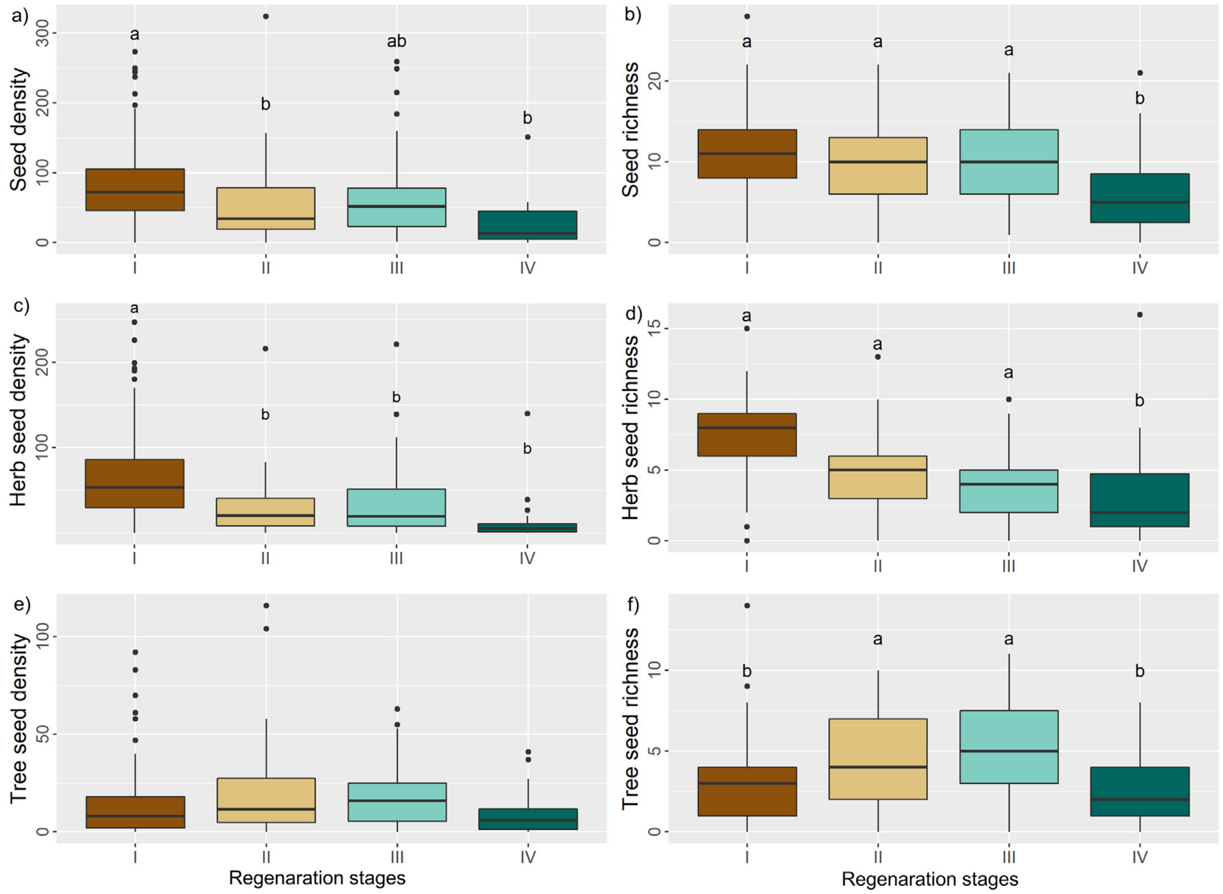

Fig. 2. Seedling richness and density for the overall seed bank and distinct life forms from four different regeneration stages from the Caxiuanã National Forests, Eastern Amazon, Brazil. For color codes and definition of regeneration stages, see legend of Fig. 1. Different letters indicate significant differences between regeneration stages ($p \leq 0.05$).

Regarding seed bank composition, we found significant differences among regeneration stages (all seeds: $R = 0.191$, $p = 0.001$, trees only: $R = 0.110$, $p = 0.019$, Fig. 3). In both NMDS ordination biplots, there is a strict separation between plots from Stage I (first and second quadrants) and further stages (third and fourth quadrants). For tree species only, plots from Stages II and III are placed nearer to old-growth forests (Stage IV) than Stage I plots, indicating that the seed bank becomes closer to that of regenerating forests with regeneration time. Additionally, variability in seed bank composition decreased with regeneration time, as plots belonging to Stage I are widely dispersed along axes 1 and 2, Stage II showed dispersion along axis 2 only (explains less variance in the overall dataset), and Stage III showed little variance regarding both axes (Fig. 3).

Additionally, we found significant differences among the composition of the seed bank and the standing tree communities ($R = 0.136$, $p = 0.001$, Fig. 3). Standing vegetation in Stage I plots showed higher similarity with seed bank species than in more advanced stages.

In regenerating sites, stand age reduced the richness of herbaceous seeds from soil seed bank, but increased the number and the richness of all and orthodox tree species (Fig. 4). Richness of standing vegetation reduced density and richness of the entire soil seed bank, herbs and orthodox trees. Tree density increased the number of zoochoric tree species in the soil seed bank. Soil PC1 contributed positively to the richness of entire seed bank and the number of orthodox tree species, while PC2 increased richness and density of the entire seed bank, herbs, all, zoochoric and orthodox trees and PC3 reduced the density of the entire and herbaceous seed bank (Fig. 4). Abundance-weighted wood density and fruit length of tree seed bank species were positively related to stand age; additionally, PC2 and standing tree richness increased wood density and fruit length, respectively.

4. Discussion

In terms of abundance, soil seed bank in all regeneration stages, including old-growth forests, was dominated by herbs, confirming previous studies (Martins and Engel, 2007), although their abundance and richness decreased with regeneration advance (Fragoso et al., 2018). This is because herbaceous species tend to produce more and smaller sized seeds compared to trees, which are more easily dispersed by abiotic agents or generalist dispersers. Furthermore, herbaceous species tend to have more persistent seeds (Bossuyt and Honnay, 2008). Nevertheless, the abundance and richness of herbaceous species in the soil seed bank decreased with standing tree diversity and/or regeneration time, indicating lower seed rain, larger dispersal limitations and/or larger seed predation of herbaceous seeds in older regeneration stages. In contrast, seeds of tree species become more abundant and more diverse with regeneration time, indicating that tree seed arrival and/or persistence in the soil seed bank depends on the recovery of the forest structure.

The overall richness of tree species in the soil seed bank was lower than the established tree species richness in all regeneration stages, including old-growth forests. This indicates that soil seed banks do not mirror above-ground communities because not all established tree species deposit viable seeds in the soil seed bank. Some established tree species may not have achieved maturity yet; furthermore, not all trees may fruit and produce viable seeds within each year. Third, some

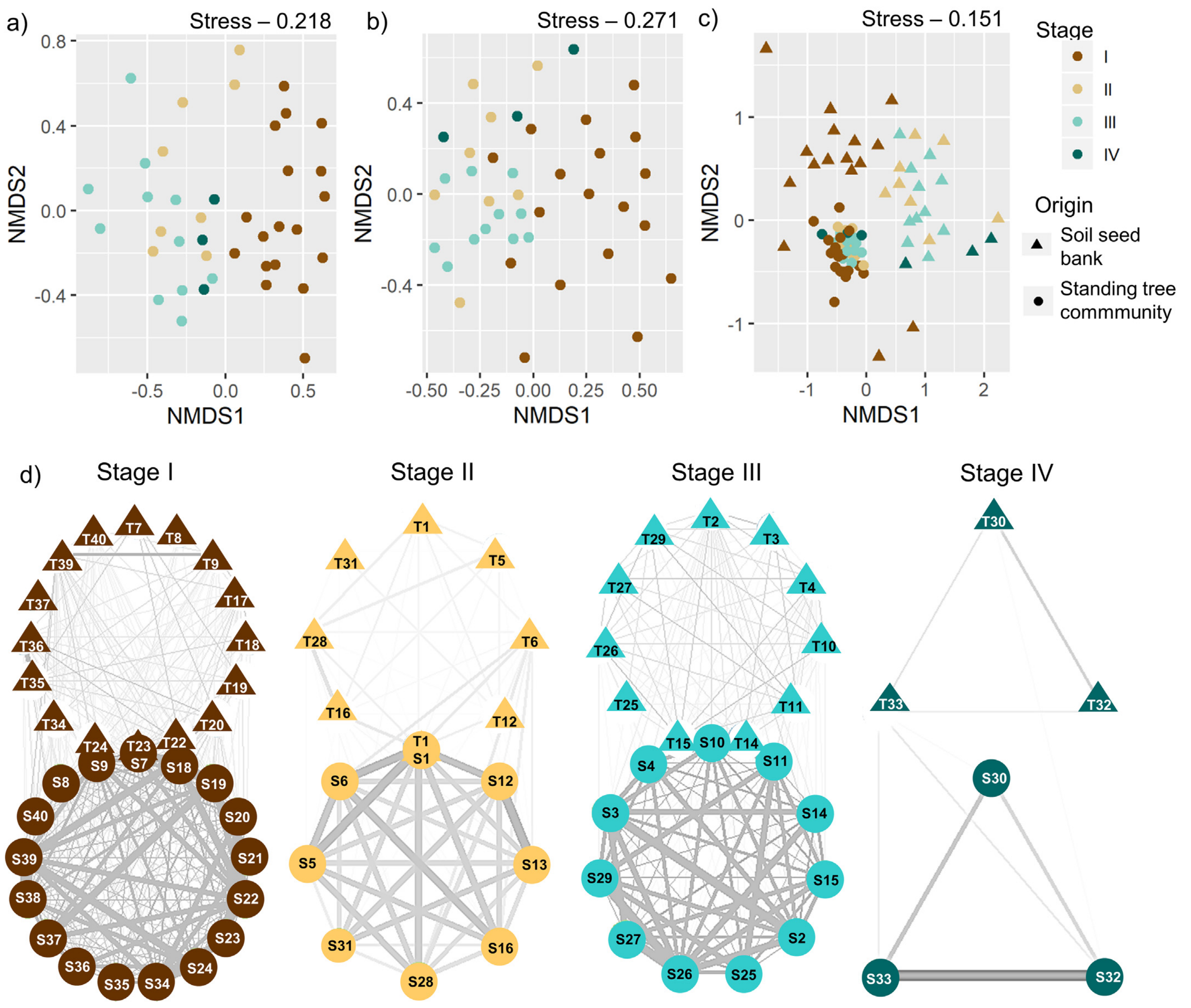

Fig. 3. NMDS biplots of the overall seed bank (a) and tree seedling seed bank (b) in comparison with the standing forest community (c) and qgraph network diagram (d); the thicker the lines connecting two samples (among and between soil seed bank samples [circles] and plot standing vegetation [triangles]), the higher is the Jaccard similarity among them. For color codes, please see Fig. 1.

established species may produce short-lived seeds that stay short periods only in the soil seed bank. Specifically, large seeds without hard shells persist for only periods only in the soil seed bank (Shiferaw et al., 2018), and two-month sampling intervals may be enough for transient seeds to die or germinate and to escape sampling within this study. Fourth, findings are consistent with the Connell-Janzen hypothesis, which postulates that seedling emergence and establishment increases with distance to the mother plants to reduce pathogen and predation pressure (Comita et al., 2014; Mangan et al., 2010). As soils containing microbial communities have been transferred, it is possible that specific pathogens and predators were conveyed unintentionally together with the seeds in the seed germination experiment.

The lower richness of the soil seed bank compared to standing vegetation and differences in the composition of standing vegetation and soil seed bank in all regeneration stages, including old-growth forests, demonstrate that some tree species recruit without maintaining persistent propagules in the soil seed bank. This highlights the importance of allochthonous seed arrival to maintain natural regeneration dynamics (Gomes et al., 2019; Shiferaw et al., 2018). As 50 to 90% of tree species from tropical forests are dispersed by animals (Howe and Smallwood, 1982), the landscape context, the fauna conservation status and the connectivity of restoring areas with net seed sources such as old-growth or primary forests (Gastauer et al., 2015; Gastauer and Meira-Neto, 2013; Sousa et al., 2017; Zimmerman et al., 2000) are thus decisive for the success of natural regeneration (Arroyo-Rodríguez et al., 2017; Reid et al., 2015). Based on that, we conclude that the evaluation of naturally regenerating forests by age only (e.g., Barros et al., 2020) may thus be oversimplified.

Regarding tree species only, the variation in soil seed bank composition among plots and regeneration stages was smaller than that in standing vegetation, indicating that soil seed banks are spatially more homogeneous than the established vegetation. Additionally, the degree of seed bank similarity to standing vegetation decreased with regeneration time, indicating that tree recruitment from the soil seed bank reduces with regeneration advance, and species less abundant or not persistent in the soil seed bank establish in later regeneration stages only, increasing the differences between the soil seed bank and standing vegetation. Spatially homogeneous seed persistence in the soil seed

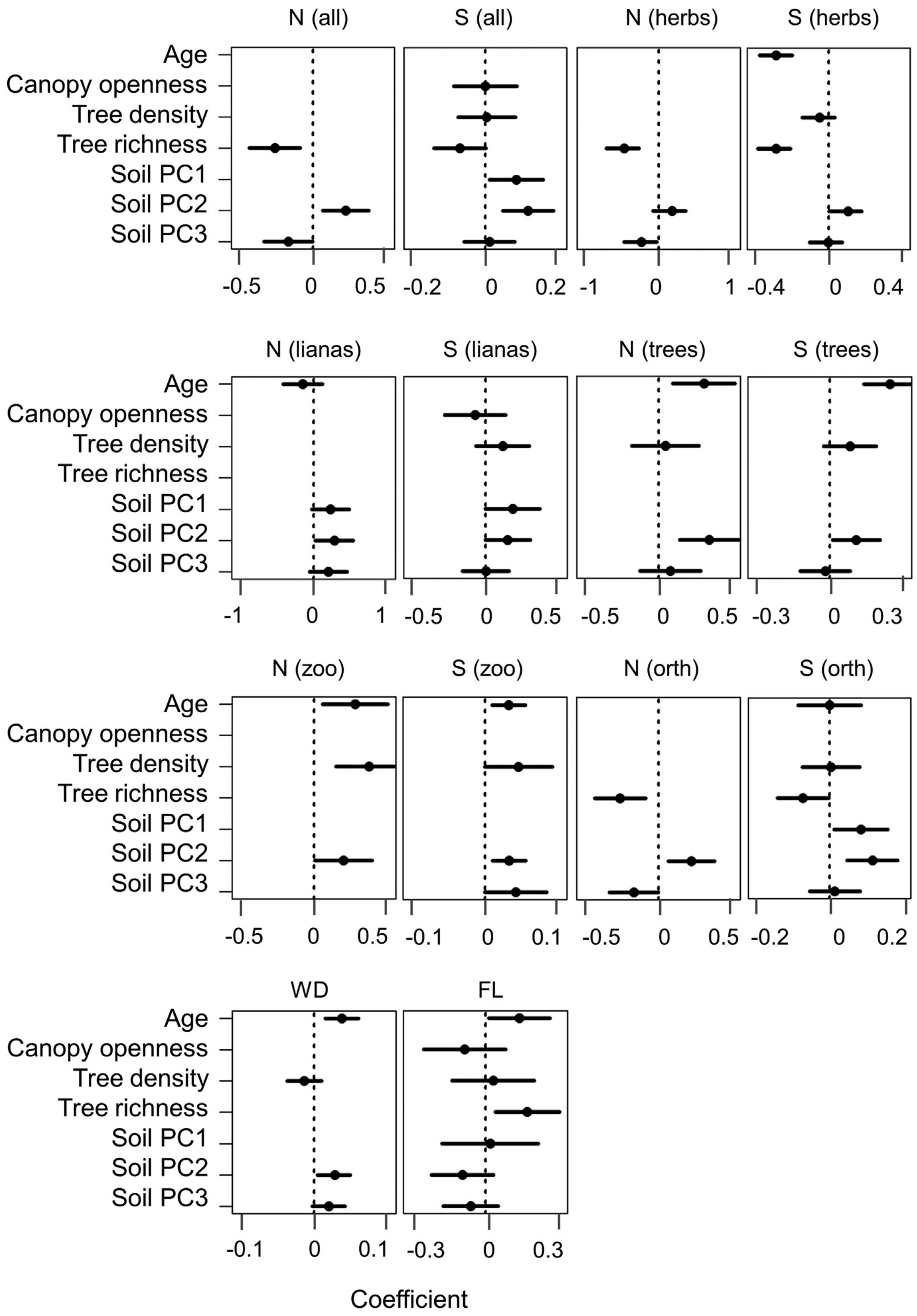

Fig. 4. Model-averaged coefficients (points) and standard errors (lines) for predicting soil seed bank characteristics from stand age, canopy openness, tree density, standing tree richness and soil PCs in a regeneration chronosequence from the Caxianã National Forests, Eastern Amazon, Brazil; model selection results are shown in Table S2 from ESM. Error bars not touching or crossing the zero-line are significant with $p \leq 0.05$. S is the number of seed species, N is the seed density, all is the entire soil seed bank, herbs are herbaceous species, lianas are liana species, trees are tree species, zoo are zoochorous tree species, orth are tree species with orthodox seeds, WD and FL are abundance-weighted mean wood density and fruit lengths of trees from the soil seed bank.

bank highlights the importance of dispersal of non-persistent seeds for the regeneration of spatially heterogeneous, diverse forest communities, especially in older regenerating stands.

Stand age, standing tree density and standing tree richness influence soil seed bank characteristics, reducing entire seed bank density and species richness and that of herbaceous species, while tree species gain in dominance, highlighting the importance of regeneration advance for the recovery of the tree component of the soil seed bank. Our models showed that most seed bank properties are furthermore influenced by soil properties. First, influence of PC1 indicates that finer textured soils with higher contents of soil organic matter and nitrogen favor emergence, survival and/or arrival of overall seed bank richness and the number of orthodox seeds. Once this is plausible, as finer textured soils maintain water availability and may facilitate seed imbibition and seedling emergence. Furthermore, nitrate is successfully used to accelerate germination in some species (Yan and Chen, 2020). On contrast, underlying mechanisms how these factors influence seed survival or arrival are unknown. Increasing with pH, Ca and Mg availability, PC2 influenced positively the richness and the density of the entire seedbank, different life forms, zoochoric and orthodox seeds; the influence of pH on seed emergence, seed arrival and survival in the soil seed bank requires further investigation.

Although differences in soil seed bank composition among regeneration stages are smaller than in standing vegetation, our data show changes in the functional characterization of the soil seed bank along the observed regeneration gradient. The importance of zoochorous species and the community-weighted wood density of species from the soil seed bank increased with stand age, while desiccation-tolerant orthodox seeds declined with standing tree vegetation richness and mean fruit length increased with stand age and standing diversity. As larger fruits require larger dispersers such as birds or mammals, the addition of larger-fruited, animal dispersed species to the soil seed bank with regeneration advance highlights the gradative regeneration of animal dispersal with regeneration time within the analyzed forest fragments. This indicates that larger, effective seed-dispersing animals are attracted by denser, more mature forest stands, where they are compensated by shelter or foraging opportunities (fruits, leaves). This finding is consistent with observations of fauna return after stand establishment in regenerating forests (Acevedo-Charry and Aide, 2019; Edwards et al., 2017; Omeja et al., 2016).

Furthermore, the amount of desiccation-tolerant, longer living orthodox seeds declines with standing tree vegetation richness, indicating their substitution by recalcitrant species along the regeneration gradient as the pattern of overall tree seed richness and abundance does not follow this tendency. Finally, wood density is inversely related to growth speed, so that the increase in the soil seed bank's wood density indicates the incorporation of slower growing, non-pioneer species in the soil seed bank. Species with higher wood density are able to sequester higher amounts of carbon, so that their increase in soil seed banks plus subsequent establishment indicates that regenerating forests may exponentiate their performance to mitigate climate change (Cavanaugh et al., 2014).

Our results thus show that the sustainability of shifting cultivation depends on the connectivity of the logged patches to old-growth forests and long fallow periods of four decades or more. This is because initial regeneration stands represent sinks for secondary and climax species that are able to colonize secondary forests after recovery of forest structure only, benefiting biodiversity and ecosystem services after long periods of abandonment. In regions without an old-growth forest matrix or elevated population density reducing fallow periods, other forms of subsistence should be preferred, as shifting cultivation can further degrade the forests of a region.

5. Conclusion

By analyzing a regenerating chronosequence after shifting cultivation using multivariate analysis, we showed that the recovery of important soil seed bank characteristics is possible in short time periods only, including biological (life form) spectra, seed density and species richness. Nevertheless, small differences in soil seed bank composition resulting from the addition of slow-growing, animal-dispersed, non-pioneer tree species with large fruits and recalcitrant seeds were detected along the chronosequence and affect the functional characterization of the soil seed bank. These differences contribute to tree recruitment and the establishment of species-rich, resilient forest communities with regeneration advance. Simultaneously, the amounts of allochthonous seeds within soil seed banks and the recruitment from allochthonous seeds increased with the advance of regeneration, increasing thus the importance of dispersal for regeneration and forest maturing. Unclear remains, why some regenerating tree species do not persist in the soil seed bank and by which mechanisms soil properties affect seed dispersal, survival and/or persistence in the soil seed bank and should be addressed by follow-up research, as this information may be useful for active forest restoration approaches. In conclusion, our data highlight the importance of connectivity for forest regeneration to increase the performance, resilience and stability of secondary forests. As long as practiced traditionally in large forested areas, with low population density and long fallow periods, shifting cultivation can thus be considered sustainable.

CRediT authorship contribution statement

PSMS and LVF designed the study and carried out field work, PSMS and MG carried out statistical analysis and wrote the paper with important contributions from LVF.

Declaration of competing interest

Authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2020.141934.

References

Acevedo-Charry, O., Aide, T.M., 2019. Recovery of amphibian, reptile, bird and mammal diversity during secondary forest succession in the tropics. Oikos 128, 1065–1078.

Alberdi, A., Gilbert, M.T.P., 2019. hilldiv: an R package for the integral analysis of diversity based on Hill numbers. Biorxiv https://doi.org/10.1101/545665.

Arroyo-Rodríguez, V., Melo, F.P.L., Martínez-Ramos, M., Bongers, F., Chazdon, R.L., Meave, J.A., Norden, N., Santos, B.A., Leal, I.R., Tabarelli, M., 2017. Multiple successional pathways in human-modified tropical landscapes: new insights from forest succession, forest fragmentation and landscape ecology research. Biol. Rev. Camb. Philos. Soc. 92, 326–340.

Barros, T.C., Elias, F., Romano, L.L., Ferreira, J., 2020. Natural recovery of plant species diversity in secondary forests in Eastern Amazonia: contributions to passive forest restoration. Rev. Bras. Bot. 43, 165–175.

Bartón, K., 2015. MuMIn R package version 1.13.4.

Bates, D., Meachler, M., Bolker, B., Walker, S., 2014. Lme4: linear mixed-effects models using Eigen and S4. R package version 1, 1–7.

Bossuyt, B., Honnay, O., 2008. Can the seed bank be used for ecological restoration? An overview of seed bank characteristics in European communities. J. Veg. Sci. 19, 875–884.

Bourgeois, B., Boutin, C., Vanasse, A., Poulin, M., 2017. Divergence between riparian seed banks and standing vegetation increases along successional trajectories. J. Veg. Sci. 28, 787–797.

Brancalion, P.H.S., Schweizer, D., Gaudare, U., Mangueira, J.R., Lamonato, F., Farah, F.T., Nave, A.G., Rodrigues, R.R., 2016. Balancing economic costs and ecological outcomes of passive and active restoration in agricultural landscapes: the case of Brazil. Biotropica 48, 856–867.

Burnham, K.P., Anderson, D.R., 2002. Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach (2nd ed).

Castro, R.B., Pereira, J.L.G., Saturnino, R., Monteiro, P.S.D., Albernaz, A.L.K.M., 2020. Identification of priority areas for landscape connectivity maintenance in the Xingu Area of Endemism in Brazilian Amazonia. Acta Amazon. 50, 68–79.

Cavanaugh, K.C., Gosnell, J.S., Davis, S.L., Ahumada, J., Boundja, P., Clark, D.B., Mugerwa, B., Jansen, P.A., O'Brien, T.G., Rovero, F., Sheil, D., Vasquez, R., Andelman, S., 2014. Carbon storage in tropical forests correlates with taxonomic diversity and functional dominance on a global scale: biodiversity and aboveground carbon storage. Glob. Ecol. Biogeogr. 23, 563–573.

Chave, J., Coomes, D., Jansen, S., Lewis, S.L., Swenson, N.G., Zanne, A.E., 2009. Towards a worldwide wood economics spectrum. Ecol. Lett. 12, 351–366.

Chazdon, R.L., 2017. Landscape restoration, natural regeneration, and the forests of the Future1. Ann. Mo. Bot. Gard. 102, 251–258.

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.

Comita, L.S., Queenborough, S.A., Murphy, S.J., Eck, J.L., Xu, K., Krishnadas, M., Beckman, N., Zhu, Y., Gómez-Aparicio, L., 2014. Testing predictions of the Janzen-Connell hypothesis: a meta-analysis of experimental evidence for distance- and density-dependent seed and seedling survival. J. Ecol. 102, 845–856.

Costa, A.C.L. da, Rodrigues, H.J.B., Silva Junior, J. de A., Nunes, L.R.C., Moraes, B.C., Costa, A. da C.e., da Cunha, A.C., Meir, P.W., Malhi, Y.S., 2018. Variabilidade Horária, Diária e Sazonal da Frequência e Intensidade de Precipitação em uma Floresta Tropical Chuvosa na Amazônia Brasileira. RBGF 11, 1290–1302.

Edwards, D.P., Massam, M.R., Haugaasen, T., Gilroy, J.J., 2017. Tropical secondary forest regeneration conserves high levels of avian phylogenetic diversity. Biol. Conserv. 209, 432–439.

Elias, F., Ferreira, J., Lennox, G.D., Berenguer, E., Ferreira, S., Schwartz, G., Melo, L. de O., Reis Júnior, D.N., Nascimento, R.O., Ferreira, F.N., Espirito-Santo, F., Smith, C.C., Barlow, J., 2020. Assessing the growth and climate sensitivity of secondary forests in highly deforested Amazonian landscapes. Ecology 101, e02954.

Faist, A.M., Ferrenberg, S., Collinge, S.K., 2013. Banking on the past: seed banks as a reservoir for rare and native species in restored vernal pools. AoB Plants 5. https://doi.org/10.1093/aobpla/plt043.

Fragoso, R. de O., Carpanezzi, A.A., Zuffellato-Ribas, K.C., Koehler, H.S., 2018. Seed bank from abandoned pastures in the coastal region of Paraná. Floresta Ambient 25. https://doi.org/10.1590/2179-8087.029515.

Gastauer, M., Meira-Neto, J.A.A., 2013. Interactions, environmental sorting and chance: phylostructure of a tropical forest assembly. Folia Geobot 1–17.

Gastauer, M., Guerra Sobral, M.E., Meira-Neto, J.A.A., 2015. Preservation of primary forest characteristics despite fragmentation and isolation in a forest remnant from Viçosa, MG. Brazil. Revista Arvore 39. https://doi.org/10.1590/0100-67622015000600001.

Gomes, F.M., de Oliveira, C.C., da Rocha Miranda, R., da Costa, R.C., Loiola, M.I.B., 2019. Relationships between soil seed bank composition and standing vegetation along chronosequences in a tropical dry forest in north-eastern Brazil. J. Trop. Ecol. 35, 173–184.

Hayton, J.C., Allen, D.G., Scarpello, V., 2004. Factor retention decisions in exploratory factor analysis: a tutorial on parallel analysis. Organ. Res. Methods 7, 191–205.

Howe, H.F., Smallwood, J., 1982. Ecology of seed dispersal. Annu. Rev. Ecol. Syst. 13, 201–228.

Hsieh, T.C., Ma, K.H., Chao, A., 2016. iNEXT: an R package for rarefaction and extrapolation of species diversity (Hill numbers). Methods Ecol. Evol. 7, 1451–1456.

Plano de manejo da floresta nacional de caxiuanã. In: ICMBio (Ed.), Instituto Chico Mendes de Conservação da Biodiversidade, Brasilia/dF.

Jakovac, C.C., Dutrieux, L.P., Siti, L., Peña-Claros, M., Bongers, F., 2017. Spatial and temporal dynamics of shifting cultivation in the middle-Amazonas river: expansion and intensification. PLoS One 12, e0181092.

Jardim Botânico de Rio de Janeiro, 2020. Flora do Brasil 2020. [WWW Document]. URL. http://www.floradobrasil.jbrj.gov.br/reflora/listaBrasil/ConsultaPublicaUC/ConsultaPublicaUC.do (accessed 5.8.20).

Joslin, A.H., Vasconcelos, S.S., de Assis Oliviera, F., Kato, O.R., Morris, L., Markewitz, D., 2019. Forests | free full-text | a slash-and-mulch improved-fallow agroforestry system: growth and nutrient budgets over two rotations. For. Trees Livelihoods 10, 1125.

Kabacoff, R., 2020. Using the Factor Analysis Package [WWW Document]. URL. https://rkabacoff.github.io/factorAnalysis/articles/factorAnalysis.html accessed 8.10.20.

Levis, C., Flores, B.M., Mazzochini, G.G., Manhães, A.P., Campos-Silva, J.V., Borges de Amorim, P., Peroni, N., Hirota, M., Clement, C.R., 2020. Help restore Brazil's governance of globally important ecosystem services. Nat Ecol Evol 4, 172–173.

Lisboa, P.L.B., Cardoso, A.L. de R., Bezerra, M. das G.F., 2013. Caxiuana: História natural e ecologia de uma Floresta Nacional da Amazônia. Museu Paraense Emílio Goeldi.

Lovejoy, T.E., Nobre, C., 2019. Amazon tipping point: last chance for action. Sci. Adv. 5, eaba2949.

Mangan, S.A., Schnitzer, S.A., Herre, E.A., Mack, K.M.L., Valencia, M.C., Sanchez, E.I., Bever, J.D., 2010. Negative plant-soil feedback predicts tree-species relative abundance in a tropical forest. Nature 466, 752–755.

Martins, A.M., Engel, V.L., 2007. Soil seed banks in tropical forest fragments with different disturbance histories in southeastern Brazil. Ecol. Eng. 31, 165–174.

Meira-Neto, J.A.A., Nunes Cândido, H.M., Miazaki, Â., Pontara, V., Bueno, M.L., Solar, R., Gastauer, M., 2019. Drivers of the growth–survival trade-off in a tropical forest. J. Veg. Sci. 30, 1184–1194.

Missouri Botanical Garden, 2020. Tropicos [WWW Document]. URL. https://www.tropicos.org/ (accessed 3.21.20).

Muscarella, R., Uriarte, M., Aide, T.M., Erickson, D.L., Forero-Montaña, J., Kress, W.J., Swenson, N.G., Zimmerman, J.K., 2016. Functional convergence and phylogenetic divergence during secondary succession of subtropical wet forests in Puerto Rico. J. Veg. Sci. 27, 283–294.

Nielsen, D.L., Campbell, C., Rees, G.N., Durant, R., Littler, R., Petrie, R., 2018. Seed bank dynamics in wetland complexes associated with a lowland river. Aquat. Sci. 80, 23.

Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Szoecs, E., Wagner, H., 2017. No Title [WWW Document]. vegan: Community Ecology Package. URL. https://cran.r-project.org/package=vegan.

Omeja, P.A., Lawes, M.J., Corriveau, A., Valenta, K., Sarkar, D., Paim, F.P., Chapman, C.A., 2016. Recovery of tree and mammal communities during large-scale forest regeneration in Kibale National Park, Uganda. Biotropica 48, 770–779.

Pinho, B.X., de Melo, F.P.L., Arroyo-Rodríguez, V., Pierce, S., Lohbeck, M., Tabarelli, M., 2018. Soil-mediated filtering organizes tree assemblages in regenerating tropical forests. J. Ecol. 106, 137–147.

Plue, J., De Frenne, P., Acharya, K., Brunet, J., Chabrerie, O., Decocq, G., Diekmann, M., Graae, B.J., Heinken, T., Hermy, M., Kolb, A., Lemke, I., Liira, J., Naaf, T., Verheyen, K., Wulf, M., Cousins, S.A.O., 2017. Where does the community start, and where does it end? Including the seed bank to reassess forest herb layer responses to the environment. J. Veg. Sci. 28, 424–435.

R Development Core Team, 2018. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing.

Reid, J.L., Holl, K.D., Zahawi, R.A., 2015. Seed Dispersal Limitations Shift Over Time in Tropical Forest Restoration. https://doi.org/10.1890/14-1399.1.

Réjou-Méchain, M., Tanguy, A., Piponiot, C., Chave, J., Hérault, B., 2017. biomass: an r package for estimating above-ground biomass and its uncertainty in tropical forests. Methods Ecol. Evol. 8, 1163–1167.

Rezende, G.M., Vieira, D.L.M., 2019. Forest restoration in southern Amazonia: soil preparation triggers natural regeneration. For. Ecol. Manag. 433, 93–104.

Ribeiro Filho, A.A., Adams, C., Murrieta, R.S.S., 2013. The impacts of shifting cultivation on tropical forest soil: a review Impactos da agricultura itinerante sobre o solo em florestas tropicais: uma revisão. Bol. Mus. Para. Emílio Goeldi. Cienc. Hum. Belém 8, 693–727.

Sanou, L., Zida, D., Savadogo, P., Thiombiano, A., 2018. Comparison of aboveground vegetation and soil seed bank composition at sites of different grazing intensity around a savanna-woodland watering point in West Africa. J. Plant Res. 131, 773–788.

Shiferaw, W., Demissew, S., Bekele, T., 2018. Ecology of soil seed banks: implications for conservation and restoration of natural vegetation: a review. Int. J. Biodivers. Conserv. 10, 380–393.

Sousa, T.R., Costa, F.R.C., Bentos, T.V., Leal Filho, N., Mesquita, R.C.G., Ribeiro, I.O., 2017. The effect of forest fragmentation on the soil seed bank of Central Amazonia. For. Ecol. Manag. 393, 105–112.

Sprengelmeyer, E.E., Rebertus, A.J., 2015. Seed bank dynamics in relation to disturbance and landscape for an ant-dispersed species. Plant Ecol. 216, 371–381.

Stropp, J., Sleen, P.V. der, Assunção, P.A., Silva, A.L. da, Steege, H.T., 2011. Tree communities of white-sand and terra-firme forests of the upper Rio Negro. Acta Amazon. 41, 521–544.

Symonds, M.R.E., Moussalli, A., 2011. A brief guide to model selection, multimodel inference and model averaging in behavioural ecology using Akaike's information criterion. Behav. Ecol. Sociobiol. 65, 13–21.

Taiwo, D.M., Oyelowo, O.J., Ogedengbe, T.C., Woghiren, A.I., 2018. The role of soil seed bank in forest regeneration. Asian Journal of Research in Agriculture and Forestry 1, 1–10.

The Angiosperm Phylogeny Group, 2016. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG IV. Bot. J. Linn. Soc. 181, 1–20.

van Vliet, N., Mertz, O., Heinimann, A., Langanke, T., Pascual, U., Schmook, B., Adams, C., Schmidt-Vogt, D., Messerli, P., Leisz, S., Castella, J.-C., Jørgensen, L., Birch-Thomsen, T., Hett, C., Bech-Bruun, T., Ickowitz, A., Vu, K.C., Yasuyuki, K., Fox, J., Padoch, C., Dressler, W., Ziegler, A.D., 2012. Trends, drivers and impacts of changes in swidden cultivation in tropical forest-agriculture frontiers: a global assessment. Glob. Environ. Change 22, 418–429.

Vandvik, V., Klanderud, K., Meineri, E., Måren, I.E., Töpper, J., 2016. Seed banks are biodiversity reservoirs: species-area relationships above versus below ground. Oikos 125, 218–228.

Villa, P.M., Martins, S.V., de Oliveira Neto, S.N., Rodrigues, A.C., Martorano, L.G., Monsanto, L.D., Cancio, N.M., Gastauer, M., 2018. Intensification of shifting cultivation reduces forest resilience in the northern Amazon. For. Ecol. Manag. 430, 312–320.

Villa, P.M., Martins, S.V., Rodrigues, A.C., Safar, N.V.H., Bonilla, M.A.C., Ali, A., 2019. Testing species abundance distribution models in tropical forest successions: implications for fine-scale passive restoration. Ecol. Eng. 135, 28–35.

Villa, P.M., Ali, A., Venâncio Martins, S., Oliveira Neto, S.N., Rodrigues, A.C., Teshome, M., Alvim Carvalho, F., Heringer, G., Gastauer, M., 2020. Stand structural attributes and functional trait composition overrule the effects of functional divergence on aboveground biomass during Amazon forest succession. For. Ecol. Manag. 477, 118481.

Wandrag, E.M., Dunham, A.E., Miller, R.H., Rogers, H.S., 2015. Vertebrate seed dispersers maintain the composition of tropical forest seedbanks. AoB Plants 7. https://doi.org/10.1093/aobpla/plv130.

Yan, A., Chen, Z., 2020. The control of seed dormancy and germination by temperature, light and nitrate. Bot. Rev. 86, 39–75.

Zanne, A.E., Lopez-Gonzalez, G., Coomes, D.A., Ilic, J., Jansen, S., Lewis, S.L., Miller, R.B., Swenson, N.G., Wiemann, M.C., Chave, J., 2009. Global Wood Density Database.

Zeng, Y., Gou, M., Ouyang, S., Chen, L., Fang, X., Zhao, L., Li, J., Peng, C., Xiang, W., 2019. The impact of secondary forest restoration on multiple ecosystem services and their trade-offs. Ecol. Indic. 104, 248–258.

Zimmerman, J.K., Pascarella, J.B., Aide, T.M., 2000. Barriers to forest regeneration in an abandoned pasture in Puerto Rico. Restor. Ecol. 8, 350–360.