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Journal

Journal of Applied Ecology, 62(9)

ISSN

0021-8901

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Publication Date

2025-09-01

DOI

10.1111/1365-2664.70116

Peer reviewed

Lower-intensity restoration interventions drive greater seedling establishment for later-successional tree species

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Author contributions

R.A.Z., K.D.H., and F.H.J. conceived and designed the research; F.H.J. performed the experiments, analyzed the data, and wrote the manuscript; R.A.Z., K.D.H. edited manuscript drafts.

Acknowledgments

Funding was provided by NSF (DEB 14-56520 and DEB 20-16623). We thank Gerald Quirós, Juan Abel Rosales, Jeison Rosales, Vanessa Morales, An Bui, Christina Blebea, Richard Joyce, and Maina Handmaker for assistance in the field. We thank Rebecca Cole for use of facilities at Loma Linda Field Station and Román Gómez for assistance with seed collection. Leland Werden and Stacy Philpott provided helpful comments on drafts.

24 **Data availability statement**

25 Data available from the Dryad Digital Repository <https://doi.org/10.5061/dryad.cfxpvnvxp>
26 (Joyce et al. 2025).

Abstract

1. Recovery of tree community composition in restored tropical forests relies on successful recruitment of later-successional species. However, the long-term effects of different restoration interventions on establishment success of arriving seeds are poorly understood.
2. We evaluated the effects of three restoration treatments on the seed-to-seedling transition for later-successional tree species in a fragmented agricultural landscape in southern Costa Rica. Restoration plots (0.25 ha) were established in a block design nearly two decades prior and spanned a gradient of intervention intensity: natural regeneration (not planted), applied nucleation (planted tree clusters), and plantation (fully planted). We conducted seed addition experiments from 2021-2023 using eight species at seven replicate restoration sites and in four nearby remnant forests. We defined the seed-to-seedling transition as two stages: seedling emergence and post-emergence survival.
3. Seedling emergence was highest in natural regeneration (48%), intermediate in applied nucleation (37%), and lowest in plantation (25%) and remnant forests (27%), with high variability across species. First-year survival of emerged seedlings was greater in applied nucleation than in plantation and was lower in remnant forest than in all restoration treatments. On average seedlings grew slightly taller in natural regeneration compared to the other treatments.
4. Despite seed mass spanning four orders of magnitude among the eight species, this trait did not explain variation in seedling emergence or survival.
5. *Synthesis and applications:* Taken together, results suggest that after ~15 years, applied nucleation and natural regeneration plots may provide more favourable conditions for

24 establishment of later-successional trees than do plantation plots. When the seed-to-
25 seedling transition is not a major barrier for the recruitment of later-successional tree
26 species in restoration plots, practitioners should focus on increasing seed arrival for these
27 species. Seed addition could be a viable strategy to introduce later-successional species
28 that fail to colonize restoration sites, particularly in restoration sites with low intensity
29 interventions (i.e., natural regeneration or applied nucleation).

30
31 **Key words:** Applied nucleation, enrichment planting, establishment limitation, natural
32 regeneration, recruitment, seed addition, seedling emergence, seedling survival

Introduction

Restoring tropical forests is a priority for achieving global climate and biodiversity goals (Chazdon & Brancalion, 2019). Successfully restoring tropical forests at large scales will rely on both natural regeneration and active methods like tree-planting, depending on the context (Chazdon & Guariguata, 2016). Regardless of approach, recovery of later-successional tree species is critical for carbon (Brancalion et al., 2018; Osuri & Sankaran, 2016) and biodiversity (Martínez-Garza & Howe, 2003; Tabarelli et al., 2012) outcomes. Practitioners often lack the capacity to plant the full suite of later-successional species, particularly in hyper-diverse tropical forests (e.g., de Almeida et al., 2024), so regaining diverse tree assemblages must also rely on natural recruitment processes. Most studies focus on the first few years of recovery (e.g., Peña-Domene et al., 2013), however, so there is a paucity of information on the consequences of initial interventions on subsequent recruitment and secondary forest recovery.

The seed-to-seedling (STS) transition is a key demographic bottleneck for tropical tree establishment (Moles & Westoby, 2004; Poorter, 2007) and sets the stage for recruitment into sapling and tree strata. Differences in canopy cover among restoration treatments spanning the passive-active intervention continuum can shape ongoing recruitment of tree seedlings, not only by influencing seed rain patterns (Reid et al., 2015), but also by creating distinct microenvironmental conditions that affect STS transition success. Decreasing light availability is considered the dominant driver of successional change in tropical wet forests, with tree assemblages shifting toward more shade-tolerant, later-successional species as new recruits of pioneer species are filtered out (Finegan, 1996). However, non-pioneer species are also sensitive to light environment variation with seedling mortality of shade-tolerant species varying along gradients within low light conditions (Montgomery & Chazdon, 2002). At sites where multiple

restoration treatments have been tested side-by-side, recruits grew faster in naturally-regenerating plots with more open canopies compared to planted treatments (Caughlin et al., 2019; Kulikowski et al., 2023). Besides shaping light availability, differing vegetation structure among restoration treatments can also mediate many other abiotic (e.g., soil moisture and nutrients) and biotic factors (e.g., seed predation, herbivory, and disease) that influence STS transition success (Grubb, 1977). For example, greater canopy cover could result in more leaf litter, which can affect STS transitions (Muscarella et al., 2013) through multiple mechanisms (e.g., reducing desiccation or seed predation, but also excessively burying small seeds).

Variation in seedling establishment probabilities are often mediated by seed size (Khurana & Singh, 2001). Whereas pioneer species tend to have small seeds and high light requirements for germination and early seedling growth (Pearson et al., 2002), how these factors influence later-successional species in restoration contexts is less clear because later-successional species typically are more shade-tolerant and span a spectrum of seed sizes. The greater reserves of large seeds provide a survival advantage to seedlings (Baraloto et al., 2005; Moles & Westoby, 2004, 2006; Muscarella et al., 2013), although this advantage for seedling establishment may be dampened or reversed if large seeds are more heavily predated before emergence (Svenning & Wright, 2005).

STS transitions are difficult to quantify from observational data when naturally deposited seeds are scarce, so analyses are often constrained to common, early-successional species (e.g., Peña-Domene et al., 2017). Prior work during the initial decade of multi-treatment restoration experiments in Mexico (Caughlin et al., 2019) and in Costa Rica (Kulikowski et al., 2023) found differences in the survival and growth of naturally recruited seedlings among treatments, but these studies and others (e.g., Werden et al., 2020) have not assessed: (a) later-successional

species in all experimental restoration treatments, or (b) seedling establishment in restoration plots at older (intermediate) successional ages. Seed addition experiments quantify the STS transition for species of interest by standardizing per-species seed inputs among habitat types (Clark et al., 2007) and can elucidate the mechanisms for failed recruitment, providing insights to predict future compositional trajectories and enhance the success of future restoration interventions (Orrock et al., 2023).

We conducted a seed addition experiment within a long-term (16-18-year-old) multi-treatment forest restoration study in southern Costa Rica to examine the limitations on later-successional tree recruitment in natural regeneration, two active restoration treatments (applied nucleation and tree plantations), and nearby remnant forests. Applied nucleation is an intermediate-intensity intervention (Corbin & Holl, 2012), in which trees are planted in clusters ('nuclei') but most of the area is left unplanted, in contrast to natural regeneration (no planting) or more traditional tree plantations (fully-planted area). We asked: (1) How do seed-to-seedling transition probabilities and seedling growth of later-successional tree species vary across restoration treatments and compare to remnant forest? (2) Is variation in STS transitions explained by canopy cover? (3) Does seed size interact with restoration treatment to influence STS transition probability? We define the STS transition as encompassing two stages: seedling emergence and post-emergence survival through approximately one year after seed deposition.

For Questions 1 and 2, we expected that restoration treatments with more open canopies would favour greater post-emergence seedling survival. Likewise, we expected greater seedling growth in natural regeneration than applied nucleation and slowest growth in plantation and remnant forest, consistent with increasing shade from canopy cover (Holl et al., 2020). For Question 3, we anticipated a positive relationship between seed size and STS transition rates,

especially at the post-emergence survival stage, but how this relationship might vary among different restoration treatments was an open question.

Methods

Study region

We conducted this study from June 2021 to September 2023 in southern Costa Rica near Las Cruces Biological Station. The forests in this region are at the boundary between Tropical Premontane Wet and Rain Forest zones (Holdridge et al., 1971) and the study sites range from ~1100 to 1200 m in elevation. Soils are volcanic in origin, mildly acidic (pH ~5.5), low in P, and high in organic matter. Mean annual temperature is ~21°C, and annual precipitation is between 2700 and 5000 mm, with a dry season from December to March. The landscape was largely deforested between 1960 and 1980 and is now a fragmented mosaic of remnant forest, secondary forest, cattle pastures and agricultural fields.

Design of long-term restoration experiment

We used seven restoration sites set up in 2004-2006 (Figure 1), a subset of the 15 sites described in Holl et al. (2020). All sites were previously used for ≥ 18 years for grazing or coffee production, and most are steeply sloped (15-35°). Minimum distance between sites is 700 m. Each restoration site had three 50 × 50 m (0.25 ha) plots: natural regeneration, applied nucleation, and plantation. Natural regeneration plots were not planted, whereas applied nucleation and plantation plots were planted with seedlings of four tree species: *Erythrina poeppigiana* (Walp.) Skeels, *Inga edulis* Mart., *Terminalia amazonia* (J.F. Gmel.) Exell, and *Vochysia guatemalensis* Donn. Sm. *E. poeppigiana* and *I. edulis* are naturalized, fast-growing N-

fixers commonly used in agricultural intercropping, whereas *T. amazonia* and *V. guatemalensis* are native timber species. In applied nucleation plots trees were planted in six nuclei or patches, two each of 4×4 , 8×8 , and 12×12 m, whereas plantation plots were planted uniformly throughout; the number of trees planted in applied nucleation plots was 27% of the total in plantation plots. Four sites also have paired remnant forest (2 to >300 ha) located 5-50 m from restoration plots, which serve as a reference system for comparing the conditions and ecological processes of restoration plots. On average, percent canopy cover was lower and cover of exotic pasture grasses was much greater in natural regeneration than planted treatments (Kulikowski et al., 2023).

Seed addition experiment

We selected eight tree species (Table 1; Figure S1) for seed addition experiments, based on: (a) their later-successional status, categorized by expert opinion as occurring exclusively in late-successional forest or both early- and late-successional forest (Schubert et al., 2025); (b) a continuum of seed sizes; and (c) regional availability of seeds (>700) for the experiment. Study species were all animal-dispersed and ranged from 2.4 to 24.8 mm seed width.

We collected seeds from at least three mother trees per species, encountered in conditions that differed by species: (1) mature fruits/nuts from the ground (*Pseudolmedia mollis*, *Quercus benthamii*, *Trophis mexicana*, and some *Erythroxylum macrophyllum* and *Otoba novogranatensis*), (2) seeds with pulp already removed by birds from the ground (*Ocotea puberula* and some *O. novogranatensis*), and (3) ripe fruit directly from trees (*Lacistema aggregatum*, *Palicourea padifolia*, some *E. macrophyllum*). We manually removed any fruit pulp from seeds and briefly submerged all seeds in water to check for insect damage. Seeds that

148 floated were assumed to be non-viable and not used. We measured fresh mass and width for ≥ 50
149 seeds per species (Table 1; Figure S1). We thoroughly mixed conspecific seeds to avoid bias in
150 seed source or quality among sites or habitat types (hereafter ‘treatments’). We conducted
151 concurrent germination trials to confirm that no species completely failed to germinate in a
152 nursery setting.

153 Within each restoration plot and remnant forest, we established four sets (hereafter ‘stations’)
154 of three 1-m² quadrats separated by ≥ 15 m. In natural regeneration, plantation, and reference
155 forest plots, one station was located in each quarter of the plot (Figure 2a). In applied nucleation
156 plots, stations were systematically distributed with one station at each of four positions relative
157 to the initial planting design (Figure 2b): (1) within a large nucleus; (2) at the edge of a medium
158 nucleus; (3) between two nuclei, and (4) far from any nucleus. Nuclei have spread considerably
159 beyond the planted area due to crown expansion and natural recruitment; however, these
160 positions spanned a representative range of microsite conditions. Two quadrats per station were
161 used for seed additions to accommodate all eight species and one non-sown control quadrat was
162 surveyed to assess background abundance of naturally recruited seedlings of the study species
163 (Figure 2c). Each control quadrat was located 2 m from a seed addition quadrat and on the uphill
164 side if on a slope. We placed quadrats to avoid large tree trunks but did not remove existing
165 vegetation or leaf litter.

166 At each station, we sequentially added seeds of three species (*P. mollis*, *O.*
167 *novogranatensis*, and *O. puberula*) to one quadrat (26 total seeds m⁻²) and five species (*Q.*
168 *benthamii*, *E. macrophyllum*, *L. aggregatum*, *T. mexicana*, and *P. padifolia*) to the second
169 quadrat (47 total seeds m⁻²) as seeds of each species became available between July 2021 and
170 August 2022. The number of seeds added per quadrat ranged from 7 to 11 seeds per species

(Table 1) because of varying seed availability. Realized densities of total added individuals were lower than the cumulative densities because of (a) temporal staggering of additions by species and (b) seed mortality. We placed seeds on top of the litter or soil to simulate how seeds would naturally be deposited from primary dispersal, slightly pressing down to minimize rolling on steeper slopes. We secured a roll of fine mesh ~5 cm high on the downhill side of each seed addition quadrat to catch seeds washed downslope by runoff. Seeds were placed in rows with 10-20 cm minimum spacing and assigned an identifier based on grid position. For the five smallest-seeded species, locations were marked with popsicle sticks to facilitate monitoring.

We monitored seedling emergence, survival, and height (to nearest 0.5 cm) for each species at least four times. Census intervals were timed to: (a) to capture seedling emergence approximately 1-2 months post seed addition and every 2-4 months thereafter, and (b) measure the first-year survival percentage of emerged seedlings 12-15 months after seed addition. Because some seedlings may have emerged and died between censuses or prior to the first census, we may have underestimated emergence and overestimated seedling survival. This uncertainty was mitigated by our observations of obviously dead seeds (indicating failure to emerge) or dead seedlings (indicating emergence but subsequent mortality). For the four larger-seeded species for which ungerminated seeds could be reliably re-found, missing seeds were interpreted as pre-emergence mortality. Assuming that missing seeds were predated was reasonable because a companion study found that almost all seeds removed by vertebrates were eventually predated (Joyce et al., 2024). Variable durations of the “first year” period are typical in tropical tree seed addition experiments involving multiple species with differing phenologies (e.g. Svenning & Wright, 2005). One of the seven sites where we added seeds in 2021 was then cleared by the landowner, so in 2022 we added seeds of the remaining six species to an alternate

site. The other six sites were used for the duration of the study. Accordingly, *P. mollis* and *Q. benthamii*, were tested at six sites rather than seven.

We did not tag seedlings but were able to distinguish between natural recruits and seedlings that recruited from added seeds based on the location of seedlings relative to the grid and the size of seedlings compared to the experimental cohort. We sampled the non-sown control quadrat at each station at a single timepoint (July 2023) for seedlings similarly sized to our added individuals.

Microsite characterization

We quantified canopy cover (%) above each quadrat as a proxy for light environment by averaging measurements taken with a convex spherical densiometer at 1 m above the centre of each quadrat in four orthogonal directions in July 2023. We also measured litter depth at five points in each addition quadrat in an X pattern in June 2022.

Data analysis

We used the combination of species $\times$ station as the unit of statistical analysis. We divided the STS transition into two stages: (1) seedling emergence, i.e., the proportion of added seeds that germinated and had a measurable shoot, and (2) early seedling survival, i.e., the proportion of emerged seedlings that survived to ~ 1 year (12-15 months after seed addition). Because a global model with all predictor variables and their interactions failed to converge, we fit multiple models with structures that matched our specific research questions.

To assess the overall and species-specific effects of treatment (habitat type) on binomial proportions of seedling emergence ($n = 776$ species $\times$ station combinations) and seedling

survival ($n = 598$), we fit generalized linear mixed models (GLMMs) with treatment, species, and their interaction as fixed predictors using package *lme4* v. 1.1-35.1 (Bates et al., 2015). We included site ($n = 7$) as a random intercept in both models because stations were grouped within sites; models specifying the full nested design of stations within treatment plots within sites failed to converge.

To assess whether seedling emergence and survival were predicted by percent canopy cover (as a proxy for light availability) while excluding treatment, we fit binomial GLMMs with canopy cover as the fixed predictor and site and species as random intercepts.

To assess the effect of seed mass on emergence and survival, we fit binomial GLMMs with only seed mass (species-level mean), treatment, and their interaction as fixed predictors and site as a random intercept.

We used height of seedlings that survived to one year as a final metric of seedling performance; we did not use relative growth rates because the assumption of exponential growth was not met (see growth curves in Figure S8). To assess treatment effects on height for all species, we fit a linear mixed model (LMM) with quadrat-level median height of surviving seedlings after one year as the response variable, treatment as the fixed-effect predictor, and random intercepts for species and site. We performed this analysis at the quadrat-level rather than for individual seedlings to avoid the issue of non-independence for seedlings in close proximity.

We used R version 4.3.2 (R Core Team, 2022) for all analyses. For mixed-effects models we report conditional and marginal pseudo- R^2 values (R^2_C and R^2_M), which summarize goodness of fit for all predictors (fixed and random) and for only fixed predictors, respectively, using package *performance* v 0.11 (Lüdtke et al., 2021). We performed post-hoc comparisons to

evaluate differences among treatments (overall and within species) using packages *emmeans* v 1.8.9 (Lenth et al., 2023) and *multcomp* v.1.4-25 (Hothorn et al., 2008).

Results

Overview

Of 7084 seeds added across all species, 2691 (38.0%) emerged as seedlings. Of these, 1550 (57.6%) survived to one year, resulting in seedling establishment of 21.9% of added seeds. For all 32 species $\times$ treatment combinations (8 species $\times$ 4 treatments), at least one seedling emerged in $\geq 50\%$ of sites (Figure S2a) and in $>30\%$ of stations (median 82%, Figure S2b). Accordingly, the sample size for assessing seedling survival (i.e., the number of stations with seedling emergence) was at least 10 (median = 19 stations) for 28/32 species treatment combinations. Overall seedling establishment percentages were 32.8% in natural regeneration, 30.0% in applied nucleation, 18.4% in plantations, and 11.2% in remnant forest. The maximum height attained after one year ranged from 12 cm for *T. mexicana* to 47 cm for *Q. benthamii*. Maximum height attained (after two years) was 64 cm for *Q. benthamii* and 53 cm for *P. mollis*, both in a natural regeneration plot.

Natural recruits were absent from control quadrats for 15 of 32 study species $\times$ treatment combinations; in 8 of the 17 remaining combinations for which we did observe natural recruits, seedlings occurred in $<10\%$ of control quadrats (Figure S3). Natural recruits were most frequent ($>25\%$ of stations) for *O. puberula* and *P. mollis* in remnant forests and for *P. padifolia* in restoration treatments. Canopy cover (%) was significantly lower and more variable in natural regeneration (Figure S4, $p < 0.0001$) compared to all other treatments, among which canopy

cover did not differ. Litter depth averaged ~2-3 cm and did not differ significantly across treatments (Figure S5, $p = 0.55$).

Seedling emergence

Seedling emergence was highest in natural regeneration (48%, [42%, 55%]) (estimated marginal mean, [95% CI]), intermediate in applied nucleation (37%, [31%, 43%]), and lowest in plantation (25%, [20%, 30%]) and remnant forest (27%, [21%, 33%]) (Figure 3a, $R^2_C = 0.23$ $R^2_M = 0.03$).

These differences were driven by a few species (e.g., *P. padifolia*, *Q. benthamii*); others showed weaker or no difference across treatments (e.g., *L. aggregatum*) (Figure 4a). Canopy cover had a small but significant negative relationship with seedling emergence (Table S1, $p < 0.001$), while seed mass was negatively related to seedling emergence only in plantation and remnant forest plots (Figure S6; Table S2, $p < 0.01$).

Seedling survival

Overall, first-year survival of emerged seedlings was >60% across the three restoration treatments and was greater in applied nucleation than in plantation plots, with intermediate survival in natural regeneration (Figure 3b, $R^2_C = 0.14$ $R^2_M = 0.05$). Survival was significantly lower ($30\% \pm 4\%$) in remnant forests (Figure 3b, $p < 0.001$). Patterns were more variable at the species level with no significant treatment effect for *P. padifolia* or *O. puberula* (Figure 4b).

Seedling survival was not associated with canopy cover (Table S3, $p = 0.12$). Evidence for a positive relationship between seed mass and seedling survival was weak (Figure S7; Table S4), with a significant effect only in applied nucleation ($p = 0.017$).

Seedling height

Treatment effects on seedling height after one year were small, with slightly taller average height in natural regeneration, compared to all other treatments (Figure 5, Figure S8, $p < 0.001$). The growth of some species slowed after the second or third census following emergence (Figure S8), as might be expected for shade-tolerant seedlings under low light conditions.

Discussion

Overall, the probability of seed-to-seedling transition success, as measured by combined emergence and early post-emergence survival, was greatest in natural regeneration and applied nucleation, intermediate in plantation, and lowest in remnant forest. This pattern is consistent with our hypothesis relating treatments to canopy cover, but surprisingly, we found that canopy cover was weakly correlated with seedling emergence and had no effect on seedling survival (Tables S2, S4), suggesting that canopy cover alone does not explain treatment effects and that other aspects of vegetation structure may contribute to treatment differences. Seed mass only significantly predicted seedling outcomes in a subset of treatments, and was negatively related to seedling emergence, opposite of our expectations, highlighting the potential importance of other traits for explaining performance of later-successional tree species.

Potential mechanisms underlying treatment differences

The differences in STS transition rates among treatments could have resulted from multiple mechanisms acting either on seeds, seedlings, or both. Differences in vertical canopy structure may be driving lower seedling survival in plantations and remnant forests by limiting light filtration despite those treatments having similar canopy cover to applied nucleation. Moreover,

shading by dense cover of exotic pasture grasses could offset the effect of lower canopy cover on light availability, as well as temperature and humidity, for small seedlings in natural regeneration (Hooper et al., 2002). Lower seedling survival in plantations and remnant forest, where there are more large trees, may also result from physical damage from falling woody debris, which is known to be important in mature forest (Alvarez-Clare & Kitajima, 2009) but has only recently been considered in restoration contexts (Quirós Cedeño et al., 2025). In this rainy region with steep terrain, seedlings in sparse, shaded understories may also be more susceptible to erosion. Although litter depth can influence seedling establishment (Muscarella et al., 2013), in our study it did not vary significantly among treatments.

Top-down effects of consumers likely played a role. At the seed stage, a companion study found that mammal and bird seed predators consumed a substantial portion of seeds for three of the four largest-seeded study species (Joyce et al., 2024) in both plantations and remnant forest. That study did not evaluate seed predation in natural regeneration and applied nucleation, but it seems likely that in these treatments greater grass and herbaceous cover may limit the presence of key vertebrate seed predators (Boron et al., 2019) or their foraging efficiency. Prior research found greater effects of insect herbivory on seedling mortality in remnant forests compared to plantations (Kulikowski et al., 2022), which could further contribute to the lowest overall establishment we observed in remnant forests.

Conspecific negative density-dependence (CNDD) is pervasive in tropical forests (Harms et al., 2000), and could be at play in remnant forests, where more conspecific individuals and high humidity could facilitate fungal pathogen transmission (Swinfield et al., 2012). However, our restoration treatments overwhelmingly lack reproductive individuals of later-successional tree species and therefore have low seed densities, so CNDD is unlikely to have driven

differences in establishment among restoration treatments. Moreover, a prior study found no evidence of CNDD effects on natural recruit survival in restoration plots (Kulikowski et al., 2023).

Comparisons to prior results in this system illustrate that the mechanisms influencing relative seedling establishment across different restoration treatments shift over time. For example, seedling survival for five large-seeded later-successional tree species directly seeded (buried) was greater in 3-year old plantation plots than in natural regeneration plots (Cole et al., 2011), likely due to enhanced initial N availability from litter inputs from planted N-fixing trees (Celentano et al., 2011), which have since suffered high mortality in the past decade. At 7-12 years after plot establishment, an analysis based on seed rain and seedling recruitment data suggested that STS transition rates were similar across restoration treatments and lower in remnant forest, albeit only for small-seeded species (Werden et al., 2020); but, they had limited sample sizes to detect differences for later-successional species.

Seed mass as a weak predictor of seedling establishment

Whereas STS transition probabilities varied greatly across species, consistent with nearly all past studies (e.g., Harms et al., 2000; Muscarella et al., 2013; Peña-Domene et al., 2017), STS transition probabilities were not related to species-level seed mass in all treatments and stages. This contrasts with the patterns documented in global meta-analyses and local studies (Baraloto et al., 2005) but has been observed in other regional studies (Augspurger, 1984; Norden, Chave, et al., 2009; Svenning & Wright, 2005) and has multiple potential explanations. First, we selected non-pioneer species given our focus on recruitment limitation of later-successional species. As a result, we may have selected for traits linked to shade-tolerant life history strategies

that promote seedling establishment and compensate for small seed size. Second, we did not include extremely small-seeded species (e.g., *Ficus* spp.), and used a high proportion of larger-seeded species. Third, larger-seeded species may have been disproportionately killed by vertebrate seed predators, as reported by Svenning and Wright (2005), diminishing any emergence advantage; indeed, we found a negative relationship between seed mass and emergence in plantations and remnant forest, where predation of large-seeded species is high (Joyce et al., 2024).

Another seed trait that likely affected seedling emergence patterns is time to germination. The three species that emerged the fastest, *E. macrophyllum*, *L. aggregatum*, and *O. puberula*, also had the greatest observed emergence (Figure S8), consistent with reduced probability of seed mortality (Norden, Daws, et al., 2009). Moreover, cotyledon function can be a key trait affecting seedling establishment (de Souza & Engel, 2024), with storage cotyledons predicting higher establishment percentages. Despite their high levels of emergence, *E. macrophyllum* and *L. aggregatum* had among the lowest seedling survival rates, possibly because they have photosynthetic cotyledons and thus cannot rely on seed reserves.

Implications for longer-term successional trajectories and management

Our eight later-successional study species experienced 18-33% establishment across the three restoration treatments. Seed addition resulted in the establishment of at least one seedling of each species in the vast majority of quadrats (Figure S2), whereas control quadrats lacked naturally recruiting seedlings for most species × treatment combinations (Figure S3). This suggests that seed arrival is more limiting to recruitment than are microsite conditions across our restoration treatments, consistent with past studies in our and other sites showing that recruitment of shade-

tolerant tree species is strongly limited by seed sources in both intact forests (Norden, Chave, et al., 2009; Svenning & Wright, 2005) and in ≥ 10 -year-old restored forests (Sangsupan et al., 2018; Werden et al., 2020; Zahawi et al., 2021).

In this study we only monitored for the first 1-2 years after seed addition, but evidence from this system and elsewhere indicates that recruits in natural regeneration could also be favored at later life stages. For example, recruits in natural regeneration may grow faster at the sapling stage (Kulikowski et al., 2023) and reproduce sooner due to less shading (Caughlin et al., 2019). It is unclear, however, whether seedlings surviving to one year in natural regeneration would continue to persist long enough to benefit at later stages. Because tropical tree seedlings show size-dependent mortality (Johnson et al., 2017) and median seedling height was < 20 cm for most species, survivorship would likely have continued to drop over several years, especially for individuals shaded by pasture grasses. Accordingly, a longer timespan might enhance the advantage of seedlings in applied nucleation, but a longer experiment would be required to assess this possibility, as later-successional recruits have been too rare in natural regeneration for demographic analyses (Kulikowski et al., 2023).

This study suggests that almost twenty years after restoration interventions, less-intensive restoration treatments benefit STS transition probabilities of later-successional tree species. Since deposition rates of large-seeded, animal dispersed tree species were previously found to be similar between applied nucleation and plantation (Reid et al., 2015), choosing applied nucleation as an initial intervention might help balance seed and establishment limitation in the long term. We suggest that practitioners (a) consider less-intensive initial restoration interventions such as applied nucleation and natural regeneration when appropriate (Shono et al., 2007) that may initially have slower accumulation of biomass but facilitate the establishment of

later-successional species over time, and (b) consider direct seeding for enrichment of species that do not colonize naturally (Palma & Laurance, 2015) and are not heavily shaded.

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573

Tables

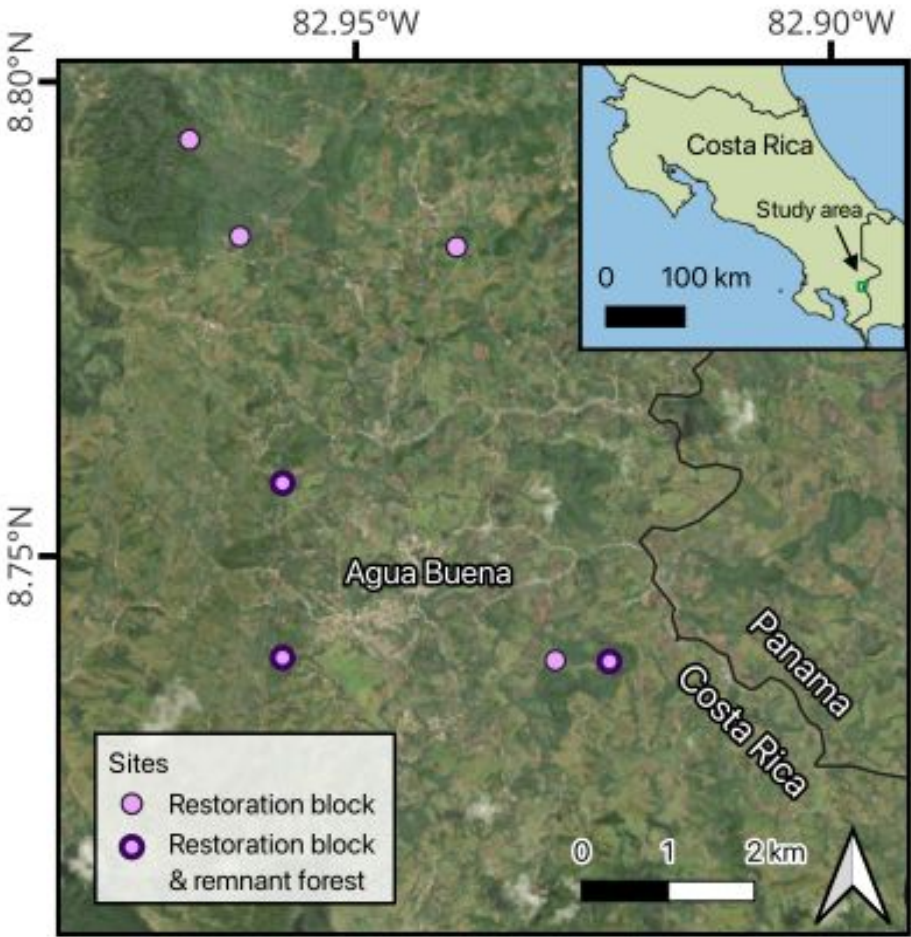
Table 1. Later-successional tree species used in seed addition experiments, listed by increasing seed mass. Values reported for width and fresh mass are mean $\pm$ 1 sd. Sample size (n) is the number of seeds added per station. Species names follow Tropicos nomenclature (Missouri Botanical Garden, 2024).

Species	Authority	Code	Family	Width (mm)	Fresh mass (g)	n
<i>Palicourea padifolia</i> †	(Willd. ex Roem. & Schult.) C.M. Taylor & Lorence	PAL_PAD	Rubiaceae	2.4 $\pm$ 0.5	0.02 $\pm$ 0.01	10
<i>Trophis mexicana</i>	(Liebm.) Bureau	TRO_MEX	Moraceae	3.9 $\pm$ 0.3	0.04 $\pm$ 0.01	10
<i>Lacistema aggregatum</i>	(P.J. Bergius) Rusby	LAC_AGG	Lacistemataceae	4.3 $\pm$ 0.4	0.07 $\pm$ 0.01	10
<i>Erythroxylum macrophyllum</i>	Cav.	ERY_MAC	Erythroxylaceae	4.7 $\pm$ 0.3	0.1 $\pm$ 0.1	10
<i>Ocotea puberula</i> †	Nees	OCO_PUB	Lauraceae	10.1 $\pm$ 1.0	1.1 $\pm$ 0.2	7
<i>Otoba novogranatensis</i> †	Moldenke	OTO_NOV	Myristicaceae	20.4 $\pm$ 2.2	5.1 $\pm$ 1.4	8
<i>Pseudolmedia mollis</i>	Standl.	PSE_MOL	Moraceae	20.7 $\pm$ 1.9	5.7 $\pm$ 1.4	11
<i>Quercus benthamii</i>	A. DC.		Fagaceae	24.8 $\pm$ 2.8	10.1 $\pm$ 2.6	7

579 Notes: *P. mollis* and *Q. benthamii* were added in 2021 and the remaining six species were added in 2022.

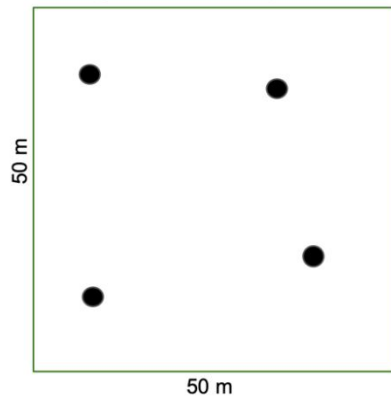
580 † Occurs in both early-successional and late-successional contexts.

582 **Figures**

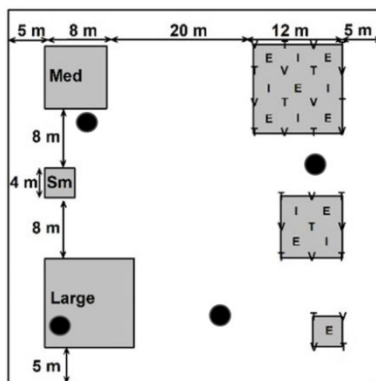


583
584 Figure 1. Map of seven study sites in southern Costa Rica.

(a) Plot-level design for natural regeneration and plantation



(b) Plot-level design for applied nucleation

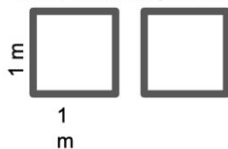


(c) Station-level design

Natural recruit
quadrat
(control)



Seed addition quadrats



586 Figure 2. Schematic of seed addition experimental design at the plot (a and b) and station (c)
587 levels. There were 100 stations total: 28 stations per restoration treatment (7 restoration sites $\times$ 4
588 stations/plot) and 16 remnant forest stations (4 sites $\times$ 4 stations).

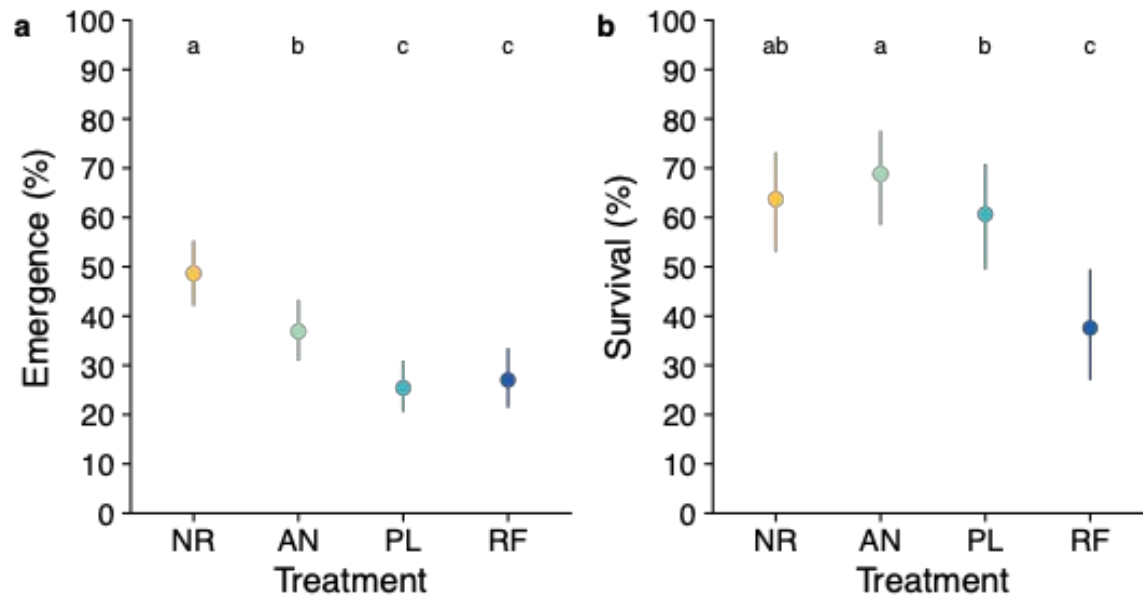


Figure 3. Effect of treatment on overall seedling emergence (a) and survival (b). Points represent estimated marginal means with 95% confidence intervals. Treatments are NR, natural regeneration; AN, applied nucleation; PL, plantation; RF, remnant forest. Means with the same letter do not differ significantly ($p < 0.05$).

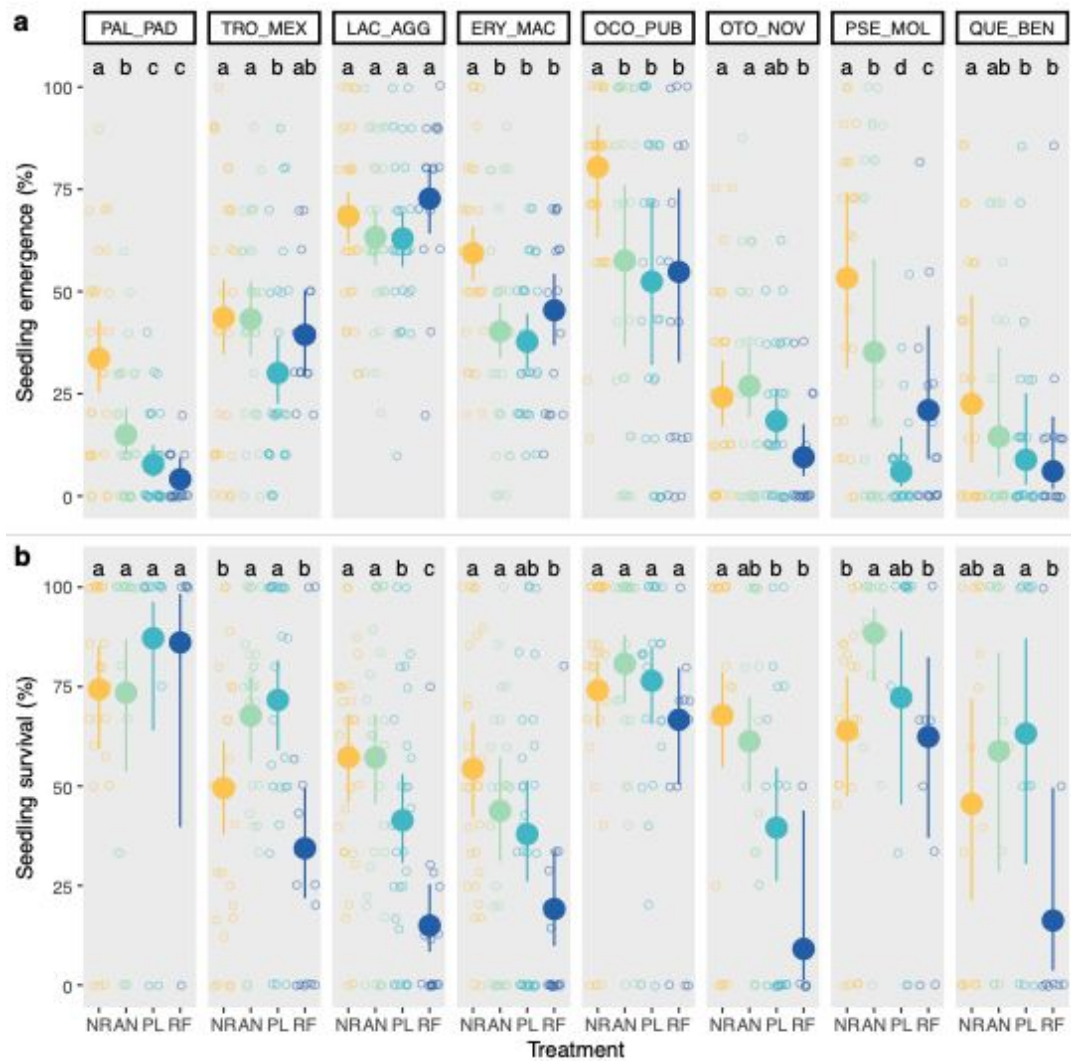
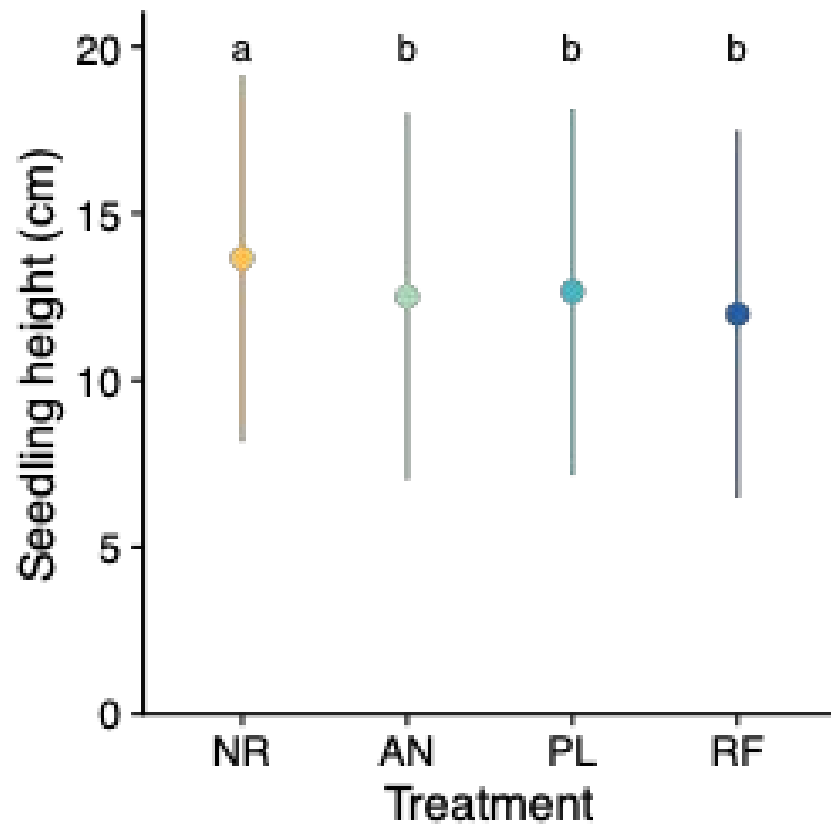


Figure 4. Seedling emergence (a) and survival (b) by species and treatment. Large points with vertical lines represent estimated marginal means with 95% confidence intervals. Individual stations are represented by small open points, randomly offset to reduce overplotting. For restoration treatments, $n = 24$ or 28 (6 or 7 sites $\times$ 4 stations); for remnant forests, $n = 16$ (4 sites $\times$ 4 stations). Sample sizes for survival vary due to differences in emergence percentages. Species are ordered from left to right by increasing seed mass. Means with the same letter do not differ significantly.



603

604 Figure 5. Estimated marginal means of seedling height one year after seed addition, based on

605 model with site and species as random effects.

Spanish-language abstract

Resumen

1. La recuperación de la composición de comunidades arbóreas en los bosques tropicales restaurados depende del reclutamiento exitoso de especies de sucesión tardía. Sin embargo, se desconocen los efectos a largo plazo de las diferentes intervenciones de restauración sobre la probabilidad de que semillas depositadas se establecen exitosamente.
2. Evaluamos los efectos de tres tratamientos de restauración sobre la transición de semilla a plántula para especies arbóreas de sucesión tardía en un paisaje agrícola fragmentado del sur de Costa Rica. Las parcelas de restauración (0,25 ha) se establecieron en un diseño de bloques casi dos décadas antes y abarcaron un gradiente de intensidad de intervención: regeneración natural (sin plantación), nucleación aplicada (grupos de árboles plantados) y plantación (plantación completa). Realizamos experimentos de adición de semillas entre 2021 y 2023 utilizando ocho especies en siete réplicas de sitios de restauración y en cuatro bosques remanentes cercanos. Definimos la transición de semilla a plántula como dos etapas: emergencia de la plántula y supervivencia de la plántula hasta un año.
3. La emergencia de plántulas fue mayor en la regeneración natural (48%), intermedia en la nucleación aplicada (37%) y menor en plantaciones (25%) y bosques remanentes (27%), con alta variabilidad entre especies. La supervivencia de las plántulas emergidas durante el primer año fue mayor en parcelas de nucleación aplicada que en plantación y menor en el bosque remanente que en todos los tratamientos de restauración. En promedio, las

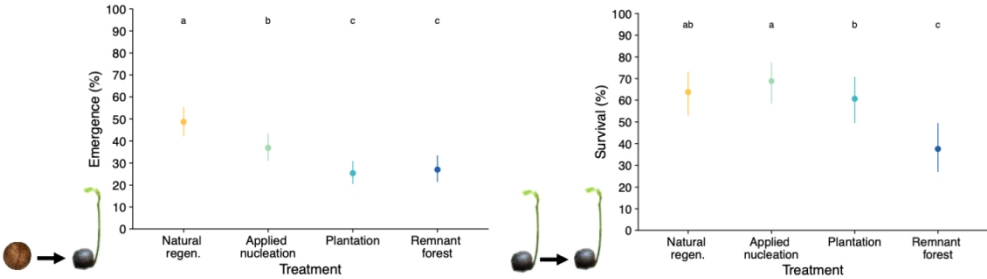
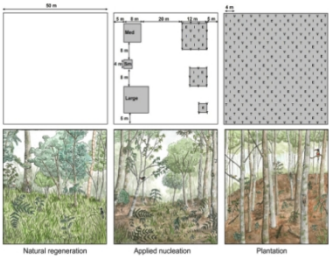
plántulas crecieron más altas en la regeneración natural en comparación con los otros tratamientos, pero por un pequeño margen.

4. A pesar de que la masa de semillas abarcó cuatro órdenes de magnitud entre las ocho especies, este rasgo generalmente no predijo la variación en la emergencia o supervivencia de las plántulas.

5. *Síntesis y aplicaciones:* En conjunto, los resultados sugieren que, después de ~15 años, las parcelas de nucleación aplicada y regeneración natural pueden proporcionar condiciones más favorables para el establecimiento de árboles de sucesión tardía que las parcelas de plantación. Cuando la transición de semilla a plántula no constituye un obstáculo importante para el reclutamiento de especies arbóreas de sucesión tardía en las parcelas de restauración, los profesionales deberían centrarse en aumentar la llegada de semillas de estas especies. La adición de semillas podría ser una estrategia viable para introducir especies de sucesión tardía que no colonizan los sitios de restauración, especialmente en sitios de restauración con intervenciones de baja intensidad.

Recruitment of later-successional tree species is critical for ongoing recovery of restored tropical forests

Seed addition experiments in 16-18 year-old restoration plots revealed that the probability of seedling establishment was lower in plantations compared to less-intensively planted treatments due to both lower emergence and survival of seedlings.



337x189mm (113 x 113 DPI)

Supporting Information for:

Lower-intensity restoration interventions drive greater seedling establishment for later-successional tree species

Figure S1. Fresh seed mass distributions by species.

Figure S1. Percentage of (a) sites and (b) stations where seedlings emerged for each species × treatment combination

Figure S2. Percentage of stations where natural recruits were observed for focal species × treatment combinations

Figure S3. Canopy cover by treatment

Figure S4. Litter depth by treatment

Figure S5. Seedling emergence by mean seed mass

Figure S6. Seedling survival by mean seed mass

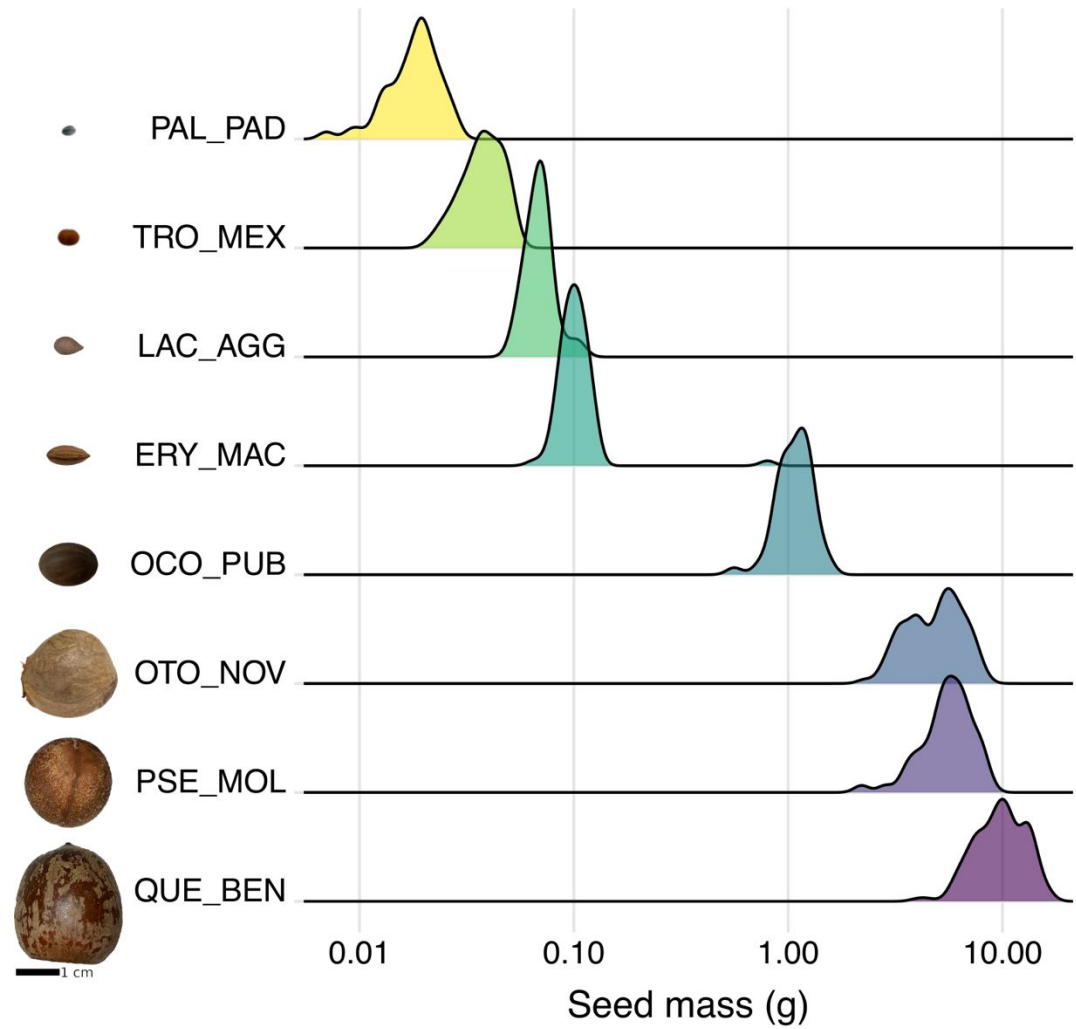
Figure S7. Growth curves for seedlings surviving to one year.

Table S1. Summary of GLMM estimating effects of canopy cover (%) on seedling emergence.

Table S2. Summary of GLMM estimating effects of seed mass on seedling emergence.

Table S3. Summary of GLMM estimating effects of canopy cover (%) on seedling survival.

Table S4. Summary of GLMM estimating effects of seed mass on seedling survival.



19

20 Figure S1. Fresh seed mass distributions by species ($n \geq 50$ seeds per species).

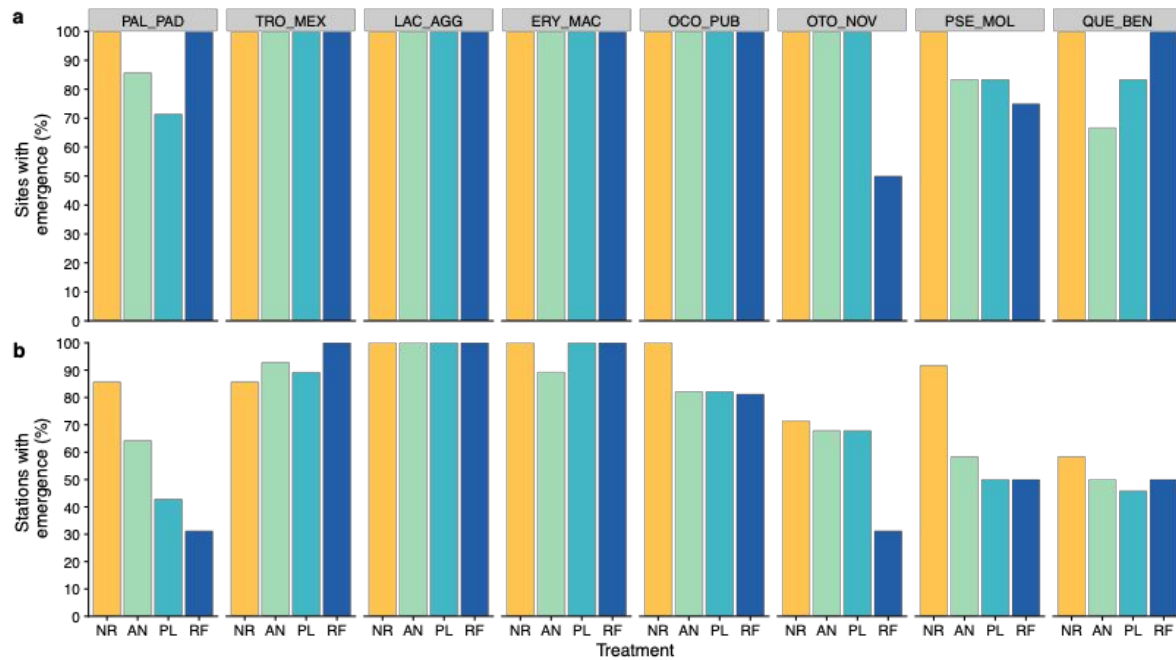


Figure S2. Percentage of (a) sites and (b) stations where seed additions resulted in emerged seedlings for each species $\times$ treatment combination. Total number of sites for each combination was four (RF for all species), six (restoration sites with PSE_MOL and QUE_BEN), or seven (restoration plots with remaining six species), with corresponding numbers of stations 16, 24, 28. Treatments abbreviations are NR, natural regeneration; AN, applied nucleation; PL, plantation; RF, remnant forest.

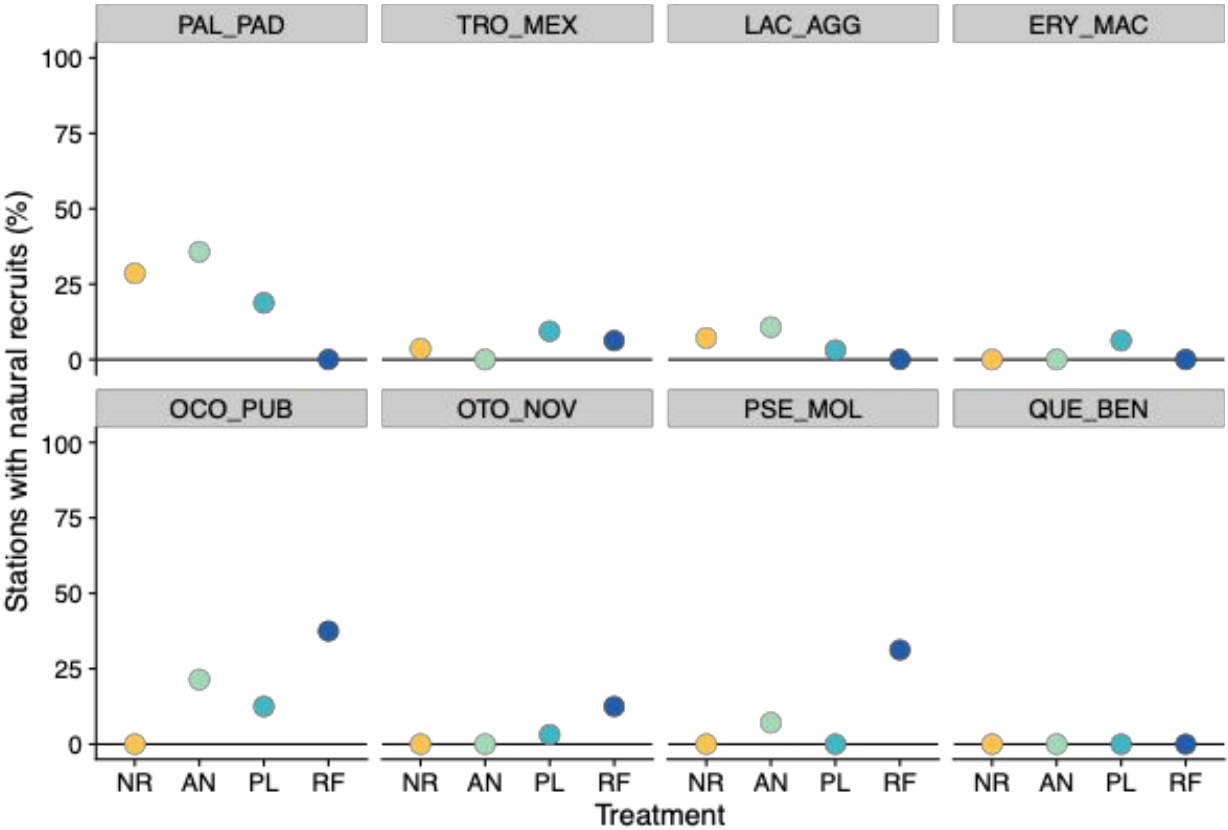
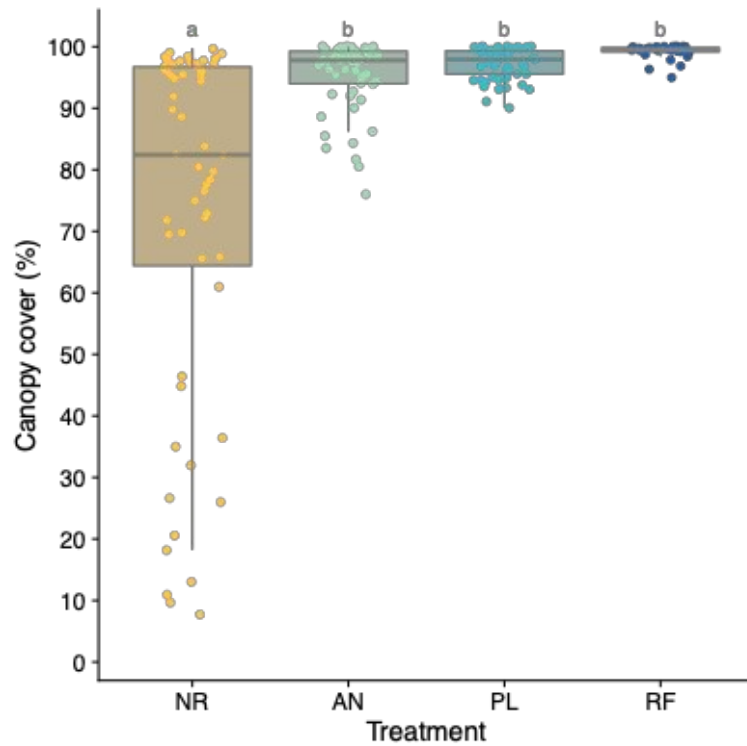
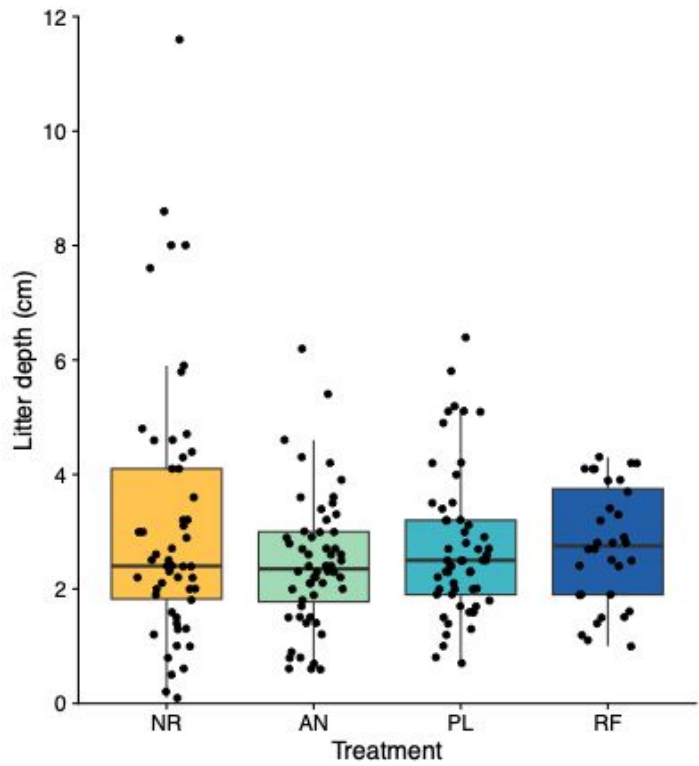


Figure S3. Percentage of stations in which natural recruits were observed for each focal species × treatment combination (n = 28 quadrats in restoration plots, n = 16 in remnant forest. Natural recruits were sampled in one non-seeded 1-m² quadrat per station.



33

34 Figure S4. Canopy cover by treatment. Points represents the canopy cover (mean of four
35 densiometer measurements 1 m above ground) above each seed addition quadrat.



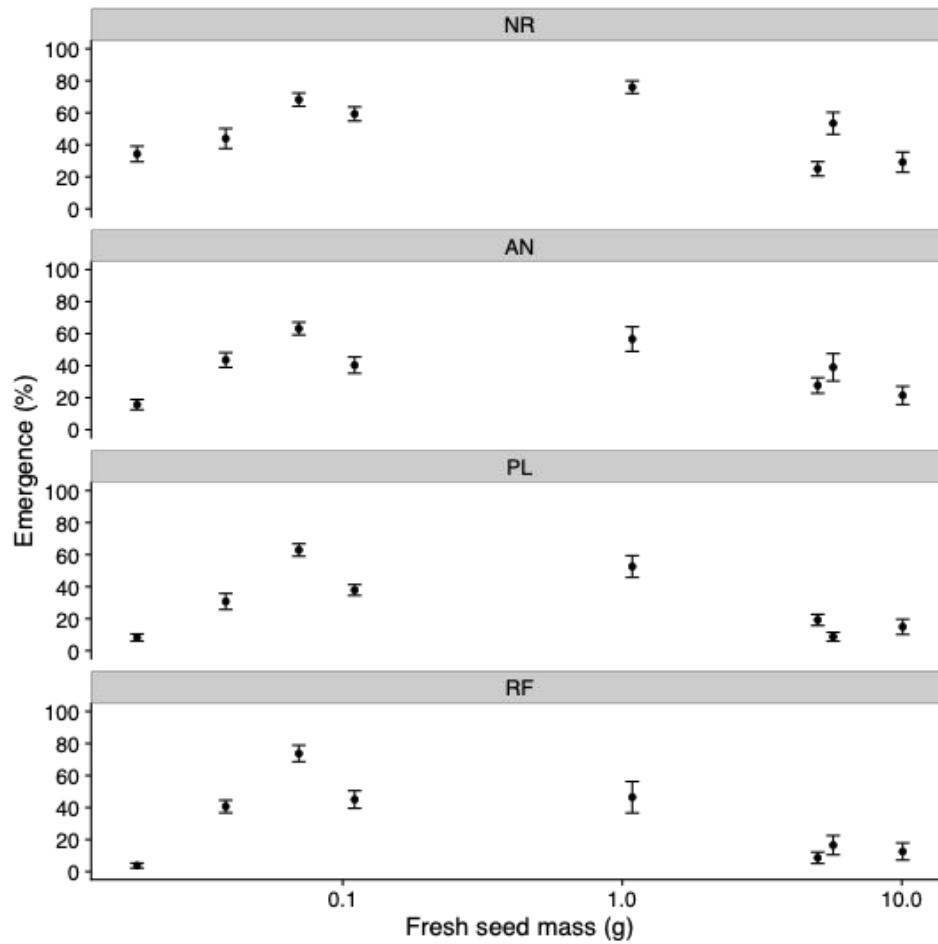
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Figure S5. Litter depth by treatment. Points represent quadrat-level means (5 measurements per quadrat). Litter depth did not vary significantly by treatment (binomial linear mixed model, $p = 0.55$).

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41 Figure S6. Seedling emergence (mean $\pm$ SE of stations by treatment) by species-level mean seed

42 mass. Note log10 x-axis scale.

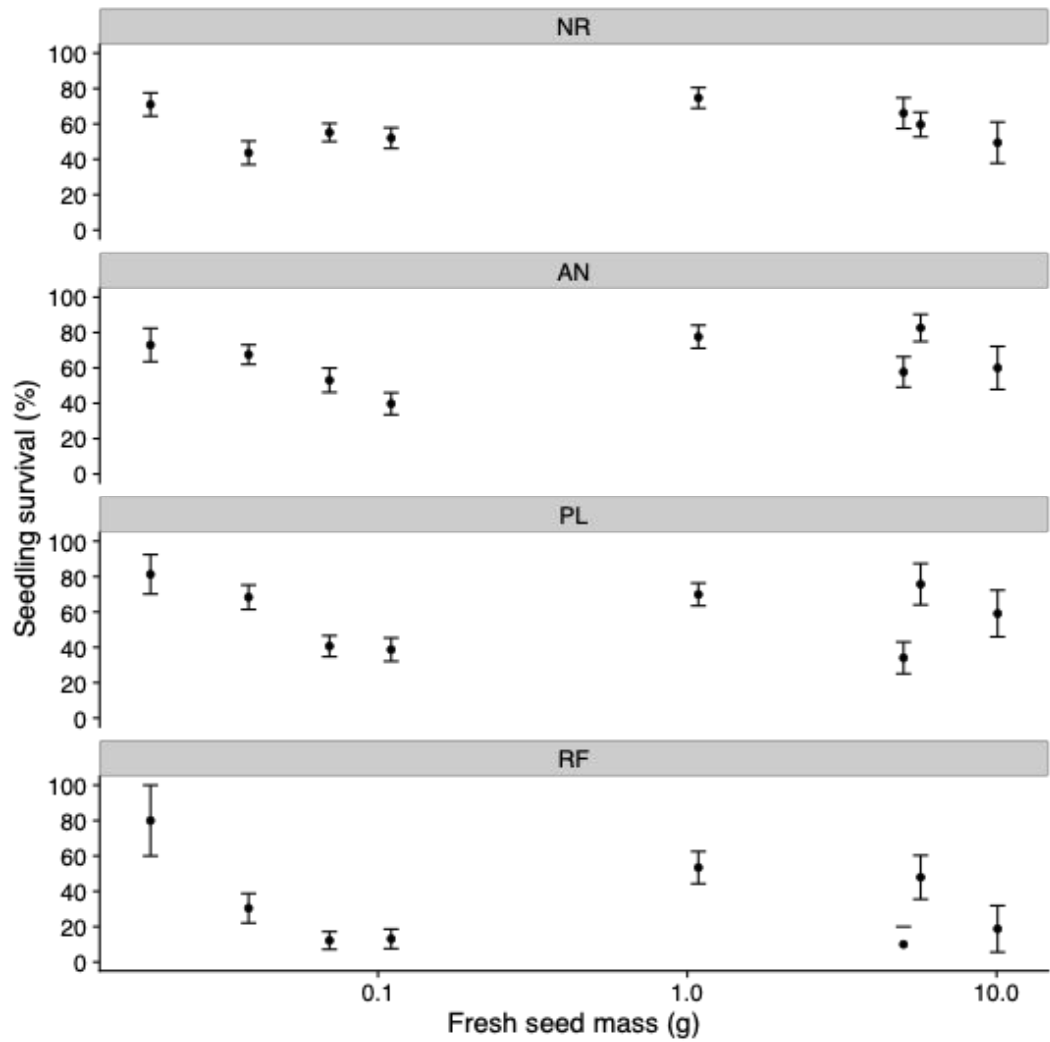
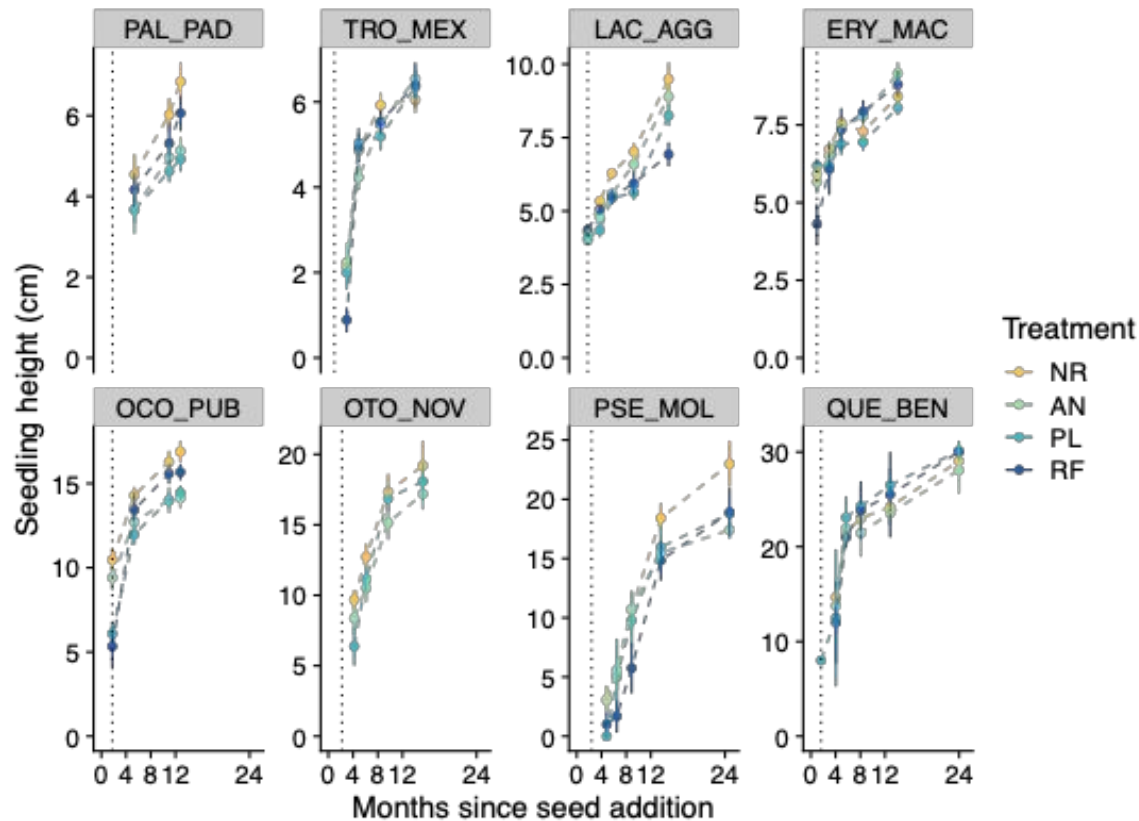


Figure S7. First-year seedling survival (mean $\pm$ SE of stations by treatment) by species-level mean seed mass. Note log10 x-axis scale.

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48 Figure S8. Growth curves for six seedling species surviving to one year and for two species (*P.*
 49 *mollis* (PSE_MOL) and *Q. benthamii* (QUE_BEN)) that were monitored for two years. Points
 50 represent the mean of median heights in quadrats with seedlings that survived. Error bars
 51 represent standard error of the mean. Sample size (number of stations with seedlings) varies by
 52 species $\times$ treatment combination due to variation in emergence and survival: range is 1-28
 53 stations; median sample size is 13 stations. Dotted vertical lines represent the timing of the first
 54 census for each species, which preceded emergence for four species. Note different y-axis scales.

Table S1. Summary of GLMM estimating effects of canopy cover (%) on seedling emergence.

Predictor	log(OR) ¹	95% CI ¹	p-value
(Intercept)	0.91	0.22, 1.6	0.010
Canopy cover (%)	-0.02	-0.02, -0.01	< 0.001

¹OR = Odds Ratio, CI = Confidence Interval

Table S2. Summary of GLMM estimating effects of seed mass on seedling emergence. The reference level for treatment is natural regeneration (NR).

Predictor	log(OR) ¹	95% CI ¹	p-value
(Intercept)	0.24	-0.44, 0.92	0.5
Treatment			
NR	—	—	
AN	-0.54	-0.71, -0.38	<0.001
PL	-0.85	-1.0, -0.67	<0.001
RF	-0.52	-0.73, -0.32	<0.001
seed mass	-0.11	-0.25, 0.03	0.13
Treatment * seed mass			
AN * seed mass	0.02	-0.02, 0.07	0.2
PL * seed mass	-0.07	-0.12, -0.02	0.007
RF * seed mass	-0.11	-0.17, -0.05	<0.001

¹OR = Odds Ratio, CI = Confidence Interval

Table S3. Summary of GLMM estimating effects of canopy cover (%) on seedling survival.

Predictor	log(OR) ¹	95% CI ¹	p-value
(Intercept)	0.80	0.18, 1.4	0.012
Canopy cover (%)	0.00	-0.01, 0.00	0.12

¹OR = Odds Ratio, CI = Confidence Interval

Table S4. Summary of GLMM estimating effects of seed mass (species-level mean wet mass) on seedling survival. The reference level for treatment is natural regeneration (NR).

Predictor	log(OR) ¹	95% CI ¹	p-value
(Intercept)	0.44	0.16, 0.72	0.002
Treatment			
NR	—	—	
AN	0.00	-0.23, 0.24	>0.9
PL	-0.29	-0.53, -0.05	0.019
RF	-1.2	-1.6, -0.94	<0.001
seed mass	0.02	-0.02, 0.07	0.3
Treatment * seed mass			
AN * seed mass	0.09	0.02, 0.17	0.017
PL * seed mass	0.03	-0.05, 0.12	0.4
RF * seed mass	0.09	-0.01, 0.19	0.073

¹OR = Odds Ratio, CI = Confidence Interval