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Restoration fosters later-successional seed rain, yet large seeds remain scarce after two decades

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Abstract

Seed dispersal patterns play a crucial role in tropical forest restoration, and especially for later-successional species, which are vital for carbon storage and the structural integrity of old-growth forests. While early-successional species typically colonize quickly, the dispersal and establishment of later-successional, large-seeded species represents a consistent challenge to restoration efforts. We examined the seed deposition rate, species richness, and composition of seed rain from mid- and late-successional (i.e., later-successional) species across three restoration treatments (natural regeneration - no planting, applied nucleation - planting in patches, plantation - planting throughout) as well as in remnant reference forests in southern Costa Rica, nearly two decades after restoration began. Small-seeded (<5 mm) zoochorous tree species dominated seed assemblages, particularly *Ficus* spp., a keystone genus for frugivores. Reference forests exhibited the highest deposition rate, with values 8 to 11 times greater for small-seeded, mid-successional species and 6 to 11 times greater for small-seeded late-successional species compared to restoration plots. Large-seeded (≥5 mm) species were significantly underrepresented across all restoration treatments, likely due to limited seed sources within and surrounding restoration plots. Compositional differences between restoration treatments and reference forests were minimal, suggesting convergence of the more abundant species over time. Mid-successional species, such as *Palicourea padifolia* and *Miconia* spp., showed very high seed deposition rates in restoration treatments (up to 36 seeds m^{-2} yr^{-1} in applied nucleation plots), possibly resulting from naturally recruited reproductive individuals

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generating successional feedbacks. Results emphasize the need for complementary restoration strategies, such as early planting of large-seeded species and enhancing ecological interactions through landscape connectivity to promote dispersal and facilitate the establishment of later-successional species.

Keywords: dispersal limitation, diversity, ecological restoration, frugivores, long-term, seed arrival, seed dispersers.

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INTRODUCTION

A key element for forest recovery in both naturally regenerating and actively restored forests is arrival of seeds (Wunderle 1997, Wijdeven and Kuzee 2000, Rozendaal et al. 2019). Many studies have focused on early stages of tropical forest recovery, where seed deposition is largely dominated by small-seeded, early-successional species that quickly colonize open spaces (Zimmerman et al. 2000, Cole et al. 2010, de la Peña-Domene et al. 2017). Over time, these species proliferate and contribute to the local species pool in important ways (Reid et al. 2015, Huanca Nuñez et al. 2021). However, gaps remain in our understanding of the arrival and establishment dynamics of mid- to late-successional (hereafter "later-successional"), large-seeded species, which are essential for mature forest development, carbon storage, biodiversity, and ecosystem resilience (Bello et al. 2015, Chazdon and Guariguata 2016, Werden et al. 2020). This later-successional species are usually seed and dispersal limited.

Later-successional tropical species are typically dependent on animal-mediated dispersal, which is sparse or entirely absent in many restoration sites (Martínez-Garza et al. 2009, Bello et al. 2024). Larger seeds are commonly dispersed long distances by large-bodied fauna via endozoochory (Wheelwright 1985), although some small-bodied fauna are also able to manipulate and transport fruits as well (McConkey et al., 2024; Ong et al., 2022). Moreover, there is a well-known tradeoff between seed size and number of seeds a species produces (Moles and Westoby 2006), namely large-seeded species produce fewer seeds. Together these characteristics limit arrival of larger seeds. However, large-seeded species are usually long-lived hardwood trees crucial for carbon storage (Bello et al. 2015, 2024). The structural complexity of long-lived tropical trees, especially their height, branches, and canopies, creates diverse microhabitats that support a wide range of organisms. Moreover, they provide shelter and

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essential food sources, including fruit, seeds, leaves, and nectar, for numerous species (Ali and Wang 2021). Despite their ecological importance, few studies have assessed the deposition patterns of later-successional species in restored tropical forests beyond the first decade of recovery (de la Peña-Domene et al. 2016, Werden et al. 2021, Pohlman et al. 2021, Rivas-Alonso et al. 2021).

We previously tracked long-term seed rain dynamics across a gradient of restoration interventions in southern Costa Rica, documenting both initial seed rain patterns and changes in species composition over time at multiple sites across the landscape (Cole et al. 2010, Reid et al. 2015, Werden et al. 2021, Schubert et al. 2025). The current study builds upon this previous research by examining the patterns of mid- to late-successional species seed deposition in three restoration treatments (planting seedlings throughout the plot, applied nucleation planting, and natural regeneration) nearly two decades after restoration efforts began. Here we address two primary questions: (1) How does the seed deposition rate (SDR) differ across restoration treatments and in remnant reference forest for mid- and late-successional species by seed size? and (2) How does later-successional (mid- and late-successional combined) species richness and composition vary among treatments? We expected SDR and species richness to be highest in reference forests, followed by planted treatments and, lastly, natural regeneration, with these differences being most pronounced for large-seeded species, which typically have lower SDRs. Additionally, we explored local fruiting dynamics to understand which species were producing fruit within sites versus those depending exclusively on dispersal from the surrounding landscape.

MATERIALS AND METHODS

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Study sites

Study sites are located between the Las Cruces Biological Station (LCBS; 8° 47′ 7″ N, 82° 57′ 32″ W) and the town of Agua Buena (8° 44’ 52’’N, 82° 56’ 39’’W) in southern Costa Rica (Figure S1). The region, once predominantly covered by pre-montane tropical forest, has undergone significant land-use changes since the 1950’s, primarily due to coffee production and subsequent conversion to pasture and crops. This has resulted in a highly fragmented landscape, with forest elements such as remnant patches and riparian forests now comprising approximately 35% of the study area (Zahawi et al. 2015). Most remaining forest is in small patches, and larger more-intact forested areas are protected, so regional conservation efforts are focused largely on ecosystem restoration.

We established a well-replicated study between 2004 and 2006 to test hypotheses about long-term tropical forest recovery under different restoration interventions; here we report on data from seven, ~1-ha experimental sites. Sites range from 1100–1200 m in elevation, receive an annual rainfall of 3–4 m, and are mostly steeply sloped (15-30%). Vegetation at the onset of the experiment was dominated by non-native grasses primarily, and ruderal herbs and ferns (Holl et al. 2011). At each site, we established three 50 × 50 m treatments: (1) No planting/natural regeneration; (2) Applied nucleation planting – planting with six tree nuclei of three sizes (4×4, 8×8, and 12×12 m); and (3) Plantation – planting the entire plot. In both applied nucleation and plantation treatments, four tree species (one animal-dispersed: *Inga edulis* [11.0 mm]; and three wind-dispersed: *Erythrina poeppigiana* [6.4 mm], *Terminalia amazonia* [6.3 mm], and *Vochysia guatemalensis* [38.5 mm]) were planted in a grid and separated by ~2.8 m; 313 trees were planted in plantation, 86 in applied nucleation and none in natural regeneration plots (for additional experimental design details see Holl et al., 2020). Additionally, we selected four

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relatively well-preserved and nearby remnant forest sites (ranging from 2 to 320 ha) to serve as references. Sites were separated by ≥700 m with treatment plots separated by ≥5 m within sites. Forest cover within 500-m buffers at our sites ranged from 11–88%.

After nearly two decades, natural regeneration plots exhibit high variability in recovery, ranging from sites still dominated by tall non-native grasses to others with substantial woody recruitment, primarily of early-successional species (Holl et al. 2020, Schubert et al. 2025). These plots generally show patchy canopy development concentrated in the 2–5 m stratum, with canopy cover exceeding 60% but interspersed with dense grass cover, although some plots support more continuous tree and shrub cover (Holl et al. 2020, Kulikowski et al. 2023). In applied nucleation plots, tree clusters have coalesced into a heterogeneous canopy that extends well beyond the original planted nuclei, with canopy cover exceeding 90% and a broad vertical structure spanning 2–25 m (Zahawi et al., unpublished data). In contrast, plantation plots are closed-canopy environments with consistently high cover (>90%), a relatively homogeneous tall canopy (20–25 m) currently dominated by *Vochysia guatemalensis* following the mortality of most *Erythrina poeppigiana* and *Inga edulis* individuals (Kulikowski et al. 2023, Zahawi et al., unpublished data). Plantation understories are fairly open with the greatest relative abundance of late-successional large-seeded recruits compared to the other restoration treatments (Figure S2).

Seed sampling

We placed 16 square seed traps (0.5 m^2, mesh size 1 mm) in each of the three restoration treatment plots at seven sites and in each of the four reference forest plots (Figure S1). Traps were distributed around four seedling monitoring areas previously established within each plot

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(see Zahawi et al., 2013) with a median (±SD) seed trap distance of 23.4 ± 10.9 m (range 5-51 m). We obtained precise coordinates of each trap using the average waypoint tool with a Garmin GPS. We collected all seeds ≥1 mm twice monthly for two years (Mar 2021-Feb 2023). All seeds were dried and identified to the lowest possible taxonomic level using a reference seed collection established by this project and housed at the Las Cruces Biological Station. We identified 97% of collected seeds to at least the family level and distinguished at least 228 taxonomic entities (164 to species, 20 to genus, 4 to family, and 40 remained as morphospecies).

We classified all identified species into successional categories (early, mid, or late), based on expert opinion, herbarium specimens at Las Cruces, and literature resources (e.g., Souza and Válio 2001; San-José et al. 2021) using the following criteria. Early-successional species are found mainly in open areas, gaps, secondary forests and edges; they are generally soft wooded, fast growing, shade intolerant, and short-lived species. Late-successional species are those that typically establish once a canopy is well developed and are mainly found in the interior of old-growth forests; they are generally hard wooded, slow growing, shade tolerant, and long-lived species. Mid-successional species are those occurring both in early- and late-successional forest habitat and are fast-growing, long-lived species. We separated and counted only seeds of mid- and late-successional species (combined as 'later-successional' for some analyses, referred to as 'both' in Werden et al., 2021). We focused on quantifying the arrival of seeds of later-successional species only, given that the dispersal dynamics of early-successional species (65 species in this study) have been thoroughly assessed in previous studies in our field sites (Cole et al. 2010, Reid et al. 2015, Werden et al. 2021). Moreover, our results, as well as extensive past research at other sites (e.g., Norden et al. 2011, de la Peña-Domene et al. 2013), indicate that these species colonize most disturbed habitats early in succession and do not limit

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recovery. Although Asteraceae, Piperaceae, and Solanaceae have some >1 mm later-successional species, these families produce copious early-successional species that are difficult to distinguish from the few later-successional species and hence were not identified to species level nor assessed in analyses.

We used previously reported information on dispersal mode (animal-, wind-, gravity-, or ballistic-dispersed), growth form (canopy tree, understory tree, liana, shrub, vine, epiphyte, or forb) and seed width (mm) (Werden et al. 2021). We combined explosive- and gravity-dispersed seeds (0.001% of observed seeds) with those dispersed by wind (collectively referred to hereafter as "abiotic" dispersal). Forbs were excluded from analyses as our traps were too tall to adequately sample this growth form. Seed width was categorized into small- (<5 mm) and large-seeded (≥5 mm). Less than 1% of zoochorous tree seeds (22 species) were >10 mm, hence we included these in the ≥5 mm category for analyses. We combined mid- and late-successional, large-seeded species for analyses, given the small number of mid-successional, large-seeded species.

Finally, we recorded data on which trees were flowering or fruiting in restoration plots from January 2021 to April 2023. Observations were made during seed collection fieldwork and during bird surveys (4 times per year). Following San-José et al. (2019), we assumed a seed was local if the species was present at least once in the records of fruiting trees within the plot; otherwise a seed was considered to be dispersed.

Statistical analyses

All analyses were performed using R version 4.4.2 (R Development Core Team 2024). Seed deposition rate (SDR) was considered at the seed-trap level, and the four planted tree species

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were excluded from this analysis. Thus, we focus solely on seeds arriving from species external to a given plot, or that established in a plot naturally over time. To quantify SDR (seeds m^{-2} yr^{-1}), we divided the total number of collected seeds in each trap by the seed trap area (0.52 m^2) * 2 yr Due to large variance in seed abundance across species and samples, seed deposition rate was log-transformed. Spatial distances (m) were calculated for each pair of trap locations using the *geosphere* package. Due to the spatial autocorrelation of the data (Mantel test: r = 0.18; p = 0.0001), we compared SDR at the trap level among treatments using spatial Generalized Linear Mixed Models (GLMM) with a Gaussian distribution error in R package *spaMM*. We tested for the interaction between treatment and successional stage by seed size (i.e., mid <5 mm, late <5 mm, and later ≥5 mm) as fixed factors, using the GPS coordinates for each trap location as a random factor [logSDR ~ Treatment * Successional stage by seed size + (1|Longitude + Latitude)]. Differences among treatments for each successional stage by seed size category were analyzed with a one-way ANOVA. We used likelihood- ratio F tests to detect significant model terms (*anova* function *spaMM* package), and when significant differences were detected, a Tukey's HSD post hoc tests to determine differences in SDR between treatments (*glht* function *multcomp* package; Hothorn et al. 2008).

To evaluate species richness by successional stage and seed size (i.e., mid <5 mm, late <5 mm, and later ≥5 mm), we calculated species accumulation curves and sampling coverage for each treatment and reference forest using the *iNext* package (Hsieh et al. 2016). Sampling coverage for all treatments was high (>98% for small seeds and >89% for large seeds), ensuring robust estimates of species diversity. We used incidence data from a species-by-trap matrix to determine gamma (all plots together) and alpha (plot average) diversity estimates (Hill numbers

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q = 0, 1, and 2; Jost, 2006), and calculated rarefied, observed and extrapolated diversity. The confidence intervals were based on the bootstrap method developed by Chao et al. (2014).

To assess compositional differences of seed assemblages, we used non-metric multidimensional scaling (NMDS) of the ln transformed (ln + 1) species abundance matrices of each treatment or reference plot, with composition distances calculated from the Bray-Curtis dissimilarity index (95% confidence intervals) using the *vegan* package (Chao et al. 2005, Oksanen et al. 2022). Then, we compared groups using pairwise permutational analysis of variance (PERMANOVA) between centroids, using the Benjamani-Hochberg method to adjust p-values for multiple comparisons (*adonis2* function, *vegan* package). We also conducted these analyses using the Chao similarity index (Chao et al. 2014) and obtained similar results; we report the results of the Bray-Curtis analysis since they showed a better fit with the log-transformed data.

Finally, to explore whether seeds may have come from within the restoration plots, we merged the fruiting trees database with the seed database, using the respective site and treatment, and seeds were categorized based on the presence of fruiting trees and/or seeds as "Fruiting tree only", "Seeds only", or "Fruiting tree + seeds". The visualizations were produced using *ggplot2* with facet grids to plot a matrix of treatments and species category combinations.

RESULTS

We collected and identified 142,501 later-successional seeds belonging to 104 species and 41 families (Table S1). Of these, 84.4% belonged to 34 mid-successional species, and 15.6% were classified as 70 late-successional species. Zoochorous tree species comprised 98.3% of seeds and 70.2% of species (140,148 seeds, 73 tree species, Table S2). Although the vast majority of later-

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successional seeds captured were small (<5 mm) and zoochorous (95% of collected tree seeds), the number of large seeded species (45) substantially exceeded those with small seeds (28). Moreover, most large-seeded species were late- (37 species) rather than mid-successional (8 species).

Seed deposition rate

Reference forest sites showed the highest seed deposition rate (SDR) in all cases, whereas differences among restoration treatments varied slightly more by successional stage (treatment × successional stage $F = 3.3$; $p = 0.02$) than seed size (treatment × seed size $F = 2.4$; $p = 0.06$, Figure 1). SDR (mean ± SE) of small-seeded, mid-successional species in reference forest (1,340.0 ± 389.0) was between 8 and 11 times higher than applied nucleation (169.0 ± 66.6) and natural regeneration (118.0 ± 45.5), respectively, and 78 times higher than in plantation (17.2 ± 4.3); applied nucleation had a significantly higher SDR than natural regeneration while plantation had the lowest SDR for this seed size and successional category ($F = 14.4$, $p < 0.001$; Figure 1a). For small-seeded, late successional species, SDR in reference forest (252.0 ± 101.0) was 6× that of applied nucleation (40.0 ± 21.9) and 10× that of natural regeneration (26.8 ± 6.3) and plantation (18.7 ± 4.4), though none of these differences were significant given high within-treatment variation ($F = 1.2$, $p = 0.32$; Figure 1b). Finally, the SDR of later-successional large-seeded species was at least an order of magnitude higher in reference forest (82.9 ± 21.2) than any of the restoration treatments ($F = 14.5$, $p < 0.001$): it was significantly higher in applied nucleation (7.4 ± 2.2) than plantation (4.1 ± 1.2) and natural regeneration (3.6 ± 0.8; Figure 1c).

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Canopy tree species had >10× times greater SDR for reference forests (1613 ± 408.0; F = 32.1, p < 0.001; Figure 2a), followed by applied nucleation (144 ± 63.2), while SDR in both natural regeneration (89.5 ± 44.8) and plantation (21.8 ± 4.2) were significantly lower. Understory trees showed a different trend, dominated by *Palicourea padifolia* a species that fruits abundantly in the restoration plots, with applied nucleation (54.1 ± 13.3) and natural regeneration (52.6 ± 14.6) yielding slightly higher mean SDR than plantation (9.8 ± 3.0) and reference forest (6.1 ± 1.0), though all were statistically equivalent (F = 0.9, p = 0.44; Figure 2b). Other growth forms (lianas, vines, and shrubs) also varied significantly across treatments (F = 5.3, p = 0.003), but they were recorded in very low numbers across treatments (Figure 2c).

Later-successional tree species richness

Species richness (Hill number order q = 0) of later-successional zoochorous seed assemblages arriving at plots differed by seed size and restoration treatment (Figure 3, Table S3). Rarefied and extrapolated species accumulation curves for mid- and late-successional, small-seeded species (<5 mm) indicated statistically equivalent richness (overlapping 95% CIs) across all treatments, including reference forests (Figure 3a&b, Table S3). For later-successional large-seeded species (≥5 mm), reference forests had the highest observed species richness (34 species) though the number of traps was less than for restoration treatments. However, applied nucleation estimated gamma richness was the highest (41.5 ± 17.9) followed by reference forest (35.5 ± 6.0), while plantations and natural regeneration exhibited similar lower richness (21.6 ± 5.6 and 27.1 ± 9.8, respectively; Figure 3c). When assessing alpha diversity (i.e., plot average estimated species richness ± SD), reference forests showed the highest (18.8 ± 4.7), followed by applied nucleation (15.1 ± 6.4), natural regeneration (12.2 ± 9.2), and plantation (11.9 ± 10.1). Diversity

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261 at higher orders (q=1 and 2) showed similar trends, with reference forests consistently displaying
262 the greatest diversity of both small- and large-seeded species (Table S3).
263
264 *Later-successional tree species seed composition*
265 Non-metric multidimensional scaling (NMDS) analysis revealed similar patterns of species
266 composition both for small- and large-seeded species across restoration treatments (Figure 4);
267 namely, while there were subtle differences among centroids for restoration vs. reference forest
268 sites, pairwise PERMANOVA comparisons showed no significant differences (Table S4). Stress
269 values were high for small-seeded species (0.23) and moderate for large-seeded species (0.19),
270 indicating the ordination might not adequately capture the structure of the data in two
271 dimensions.
272 Approximately 40% of seeds recorded in restoration treatments were from species that
273 had within-plot fruiting individuals, so could have been, though were not necessarily, locally
274 dispersed (Figure S3). Of the remaining 60% of seeds that definitely were dispersed from
275 external sources, the number of zoochorous tree species in the largest size category (≥10 mm)
276 was higher in applied nucleation (12), followed by natural regeneration and plantation (9 and 8
277 species, respectively). Half of the largest seeds (≥10 mm) were from the late-successional
278 canopy palm *Euterpe precatoria* (410 seeds in reference forests and 75 seeds in restoration
279 plots), which was observed fruiting in reference forests and one natural regeneration plot. Most
280 of these palm seeds (80%) were dispersed into the restoration plots. The SDR of externally-
281 sourced dispersed species was greater in applied nucleation than in plantation plots and highly
282 variable in natural regeneration plots (Figure S4).

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If all *Ficus* spp. seeds were combined (here representing canopy trees with ~1-mm sized seeds embedded in a fig syconium or fruit), they comprised 83.7% of seeds in reference forest and 40.9% in applied nucleation, 29.2% in plantation, and 12.5% in natural regeneration plots. *Palicourea padifolia*, an abundant mid-successional understory tree observed fruiting within the plots, was common in natural regeneration (26.2% of all seeds), applied nucleation (18.2%) and plantation plots (13.5%) (Table 1). Finally, *Vochysia guatemalensis*, a wind-dispersed tree and one of the four planted species in our plots (hence not included in the analyses and figures), was not surprisingly the most numerous abiotic seed-dispersed species in plantation plots (15.2% of seeds) but not in applied nucleation (1.6%). Remaining planted species, including *Erythrina poeppigiana* and *Inga edulis* were not recorded in any of the restoration plots, whereas *Terminalia amazonia* seeds comprised fewer than 1% of recorded seeds in both types of restoration plots.

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DISCUSSION

Our results show that seed rain of later-successional species in restoration plots remains much lower than reference forest nearly two decades after recovery efforts began. Reference forests exhibited the highest seed deposition rates (SDR) and species richness across all seed size categories, emphasizing the critical role that mature remnant forests serve as both seed sources and habitat for seed-dispersing fauna. Whereas restoration interventions have successfully promoted the arrival of small-seeded, mid-successional species, significant gaps persist in the abundance and diversity of large-seeded species that are critical to long-term forest recovery and ecosystem function. Natural regeneration still lags behind planted treatments in terms of the SDR and diversity of later-successional species, although some naturally regenerated nuclei are now

present, which should facilitate seed dispersal going forward. Composition of dominant species was similar across restoration treatments and reference forests, indicating that seeds of common later-successional species do arrive in restored areas, but in low numbers. Unsurprisingly, most later-successional species came from sources outside the plots, while some mid-successional species established in the plots are likely contributing heavily to overall seed rain patterns found.

Our seed rain results, combined with complementary studies of direct seeding and a complete census of seedling recruitment in the sites (Joyce et al. 2025, Schubert et al. 2025), suggest that limited arrival of large-seeded, later-successional species remains the main barrier to recovery in restoration treatments after nearly 20 years. A concurrent direct seeding experiment in our sites showed greater establishment limitation in remnant forest and plantation plots than applied nucleation and natural regeneration plots (Joyce et al. 2025), which supports our assertion that dispersal limitation affects patterns of seedling recruitment (Schubert et al. 2025, in press) more strongly than establishment limitation. Prior seed rain studies conducted at our sites (Reid et al. 2015, Werden et al. 2021) and other tropical restoration studies (Palma et al. 2021, Pohlman et al. 2021) concur that large-seeded (≥5 cm) species are not being dispersed in high numbers, and unlike reference forests, restoration plots do not yet host fruiting trees that could provide seeds of these species.

The majority of the seeds in our restoration plots arrived by dispersal from the surrounding matrix (>60%); in contrast, in Mexican old-growth tropical forest fragments, most seeds are from local sources and dispersed seeds (i.e., over ~30 m) are less abundant (<30%; San-José et al. 2020). As such, distance and connectivity to seed sources in remnant forests and isolated trees or fencerows in the agricultural landscape has proven to be a significant factor determining the presence of large-seeded species within restoration plots in this (Zahawi et al.

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2021) and other tropical forests (Vespa et al. 2014, de la Peña-Domene et al. 2016, Piotto et al. 2019).

Planted treatments attract more dispersing fauna (Peña-Domene et al. 2014, Mendes et al 2021, Joyce et al. 2024) and hence receive more later-successional seeds than natural regeneration; however, the canopy composition and cover might be playing an important role in seed dispersal (Kwit et al. 2004, Bonilla and Pringle 2015). For example, natural regeneration plots now have some early- and mid-successional fruiting trees (Schubert et al. 2025) that produce fruits (Holl, unpublished data) and attract frugivorous birds (Schubert, unpublished data), but SDR and species richness in this treatment was the lowest compared to the other restoration treatments, probably due to the low canopy cover. On the other hand, plantations generally showed low SDR, possibly because at the time of the study the canopy cover was dominated mostly by *V. guatemalensis* trees (a wind-dispersed species), which might not attract as much frugivorous fauna as the fruiting trees of the other two restoration treatments (i.e., through contagious seed dispersal; (Kwit et al. 2004, Camargo et al. 2020)). However, Schubert et al. (2025) found greater seedling and sapling abundance of large-seeded (later successional) species in plantation than in applied nucleation and natural regeneration plots, possibly because of earlier arrival of these species due to more rapid canopy development which enhanced frugivorous bird abundance and foraging (Fink et al. 2009, Reid et al. 2014).

Small-seeded species were the most abundant in the seed rain some due to high external dispersal and others due to local dispersal (i.e., successional feedbacks *sensu* Huanca Nuñez et al. 2021). *Ficus* spp., a keystone tropical species, were the most abundant in both restoration and reference plots. They produce a copious amount of fruit which are available all year, and are

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amongst the most important plants for tropical forest frugivores worldwide (Shanahan et al. 2001). Interestingly, despite the high SDR of *Ficus* spp., all of which were dispersed into restoration plots, these species have not recruited abundantly (Schubert et al. 2025), suggesting that while these species do arrive at restoration plots, their establishment is hindered. This may be explained by at least two reasons. First, in many cases either the whole syconium or parts of it are dispersed, but the seeds do not germinate while still embedded in the syconium pulp. The pulp typically needs to be removed to enable germination and to reduce the risk of pathogens (Heer et al. 2010). In this regard, bats—important seed dispersers—often carry the fruits to other trees where they consume most of the pulp and discard parts of the syconium. This process neither enhances nor hinders seed germination (Saldaña-Vázquez et al. 2019). Second, the strongest filtering effect observed in facilitating *Ficus* seedling emergence is canopy openness; in contrast, drier and cooler soils are less favorable for seedling emergence, particularly for hemiepiphytic species (Castillo-Díaz et al. 2021). Thus, even when seeds are present, successful recruitment is strongly constrained by microsite conditions and growth form, through filtering mechanisms that operate during the transition from seedling emergence to establishment.

Some of the most common species falling in the plots were of species that were observed fruiting in the plots, particularly *Palicourea padifolia* and *Miconia doneana* in applied nucleation, and *Vochysia guatemalensis* in plantation plots. These species' higher seed deposition rates likely reflect their rapid maturation and local dispersal within plots, creating a successional feedback loop (Huanca Nuñez et al. 2021). Nonetheless, species composition of seed rain was similar among all restoration treatments and reference plots, particularly for small seeded species (Figure 3), highlighting that treatments are slowly converging over time, and suggesting that

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large-seeded, later-successional species will establish eventually in restored sites, but only in sufficient numbers once initially established trees are old enough to produce seeds, which will likely take decades. Finally, the estimated gamma diversity of later-successional large-seeded species was surprisingly higher in applied nucleation plots than in the reference forest. However, this result may be biased by the greater number of seed traps deployed in applied nucleation plots (112) compared to the reference forest (64), which could inflate richness estimates and contribute to the larger associated error when extrapolating. In contrast, alpha diversity patterns revealed that, on average, reference forests still harbored higher species richness than any restoration treatment. This underscores that restoration plots—regardless of planting strategy—still lag behind remnant forests in supporting diverse assemblages of later-successional large-seeded species.

After nearly two decades of recovery, the seed rain of large-seeded, later-successional species remains limited across restoration treatments, consistent with prior studies showing that rare and large-seeded zoochorous species may not colonize for decades or at all (Martínez-Garza and Howe 2003, Pohlman et al. 2021, Elsy et al. 2024). Although there is no shortcut to obtain an old-growth forest, planting trees partially or throughout an area as a restoration strategy can help overcome dispersal barriers and increases the SDR and diversity of species arriving compared to natural regeneration. As such, future restoration strategies must prioritize the early planting of large-seeded, later successional species, which are slow growing and take decades to bear fruit and act as seed sources (Zahawi et al. 2021) and can establish in these sites if direct seeded (Joyce et al. 2025). These results suggest that enrichment planting after a canopy is established may be a viable option for actively reintroducing these species, particularly in canopy

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gaps with higher light levels (Martínez-Garza and Howe 2003, Bertacchi et al. 2016). Restoration efforts must consider these temporal dynamics to ensure that seed arrival leads to sustained recovery of biodiversity and ecological interactions.

Acknowledgements

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Figures and Tables

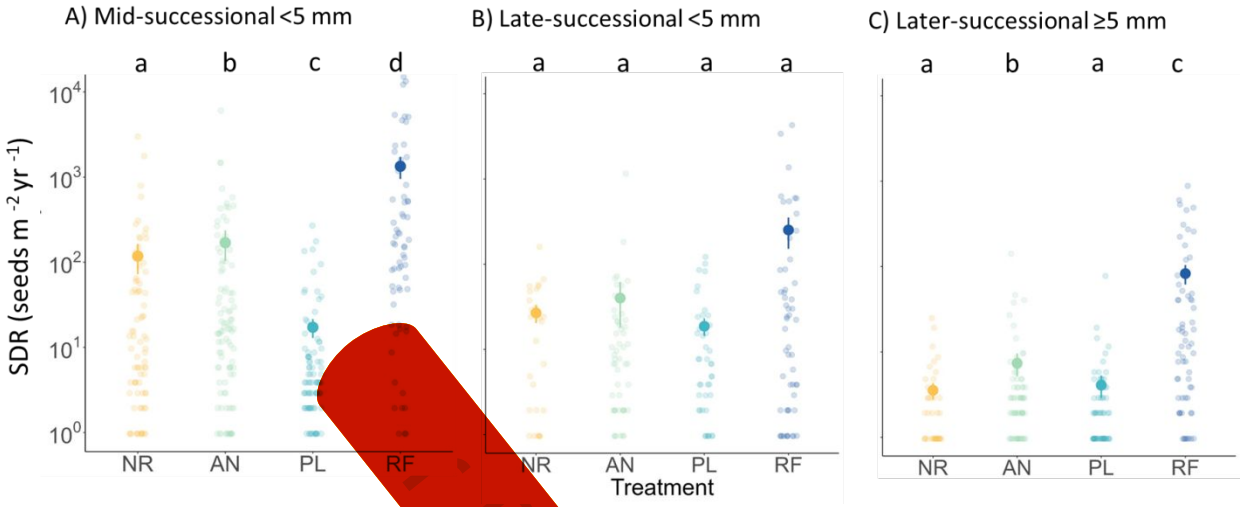

Figure 1. Seed deposition rate (SDR, seeds m^{-2} yr^{-1}) for A) mid- (<5 mm), B) late- (<5 mm), and C) later- (≥5 mm) successional tree species in restoration treatments and reference forest sites. Large point indicates the mean, vertical lines the standard error, and small dots the sampling units. Letters indicate significant differences ($p < 0.05$, one-way ANOVA test). NR = Natural Regeneration, AN = Applied Nucleation, PL = Plantation, RF = Reference Forest.

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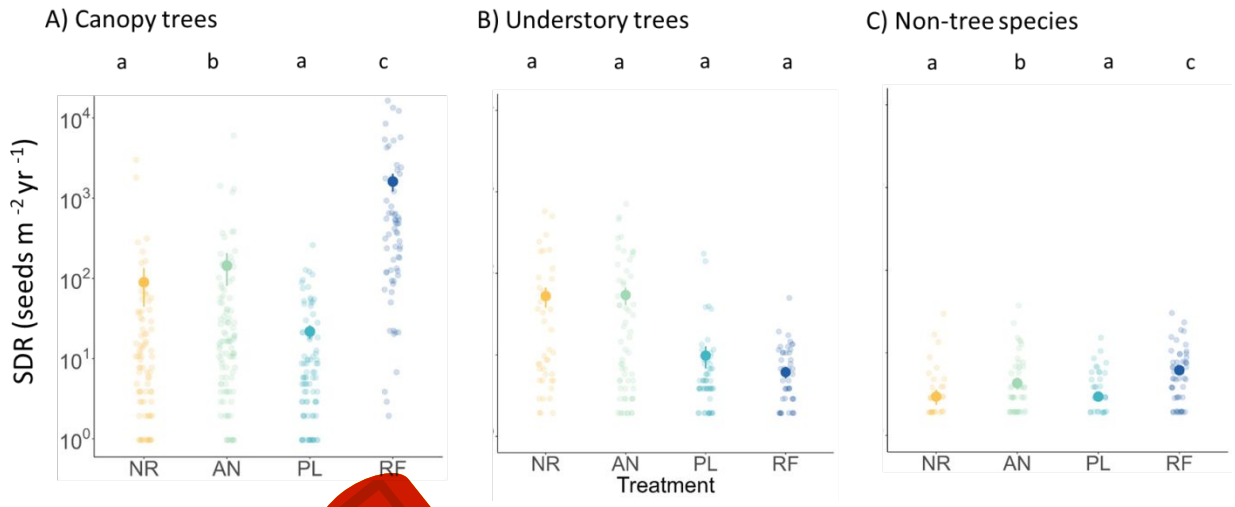

Figure 2. Comparison of the seed deposition rate (m^{-2} yr^{-1}) of seeds from different growth forms of later-successional species collected at restoration treatments and reference sites. Non-tree species comprise lianas, shrubs, epiphytes and vines. Large point indicates the mean, vertical lines the Standard Error and small dots the sampling units. Letters indicate significant differences ($p < 0.05$, one-way ANOVA test). NR = Natural Regeneration, AN = Applied Nucleation, PL = Plantation, RF = Reference Forest

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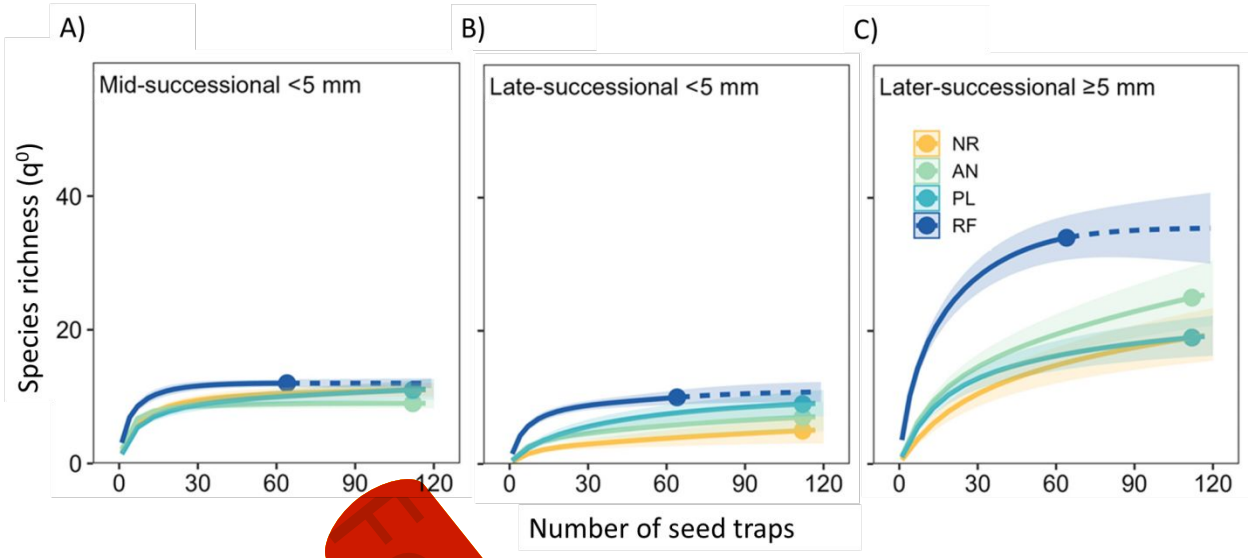

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565 **Figure 3.** Rarefied later-successional species accumulation curves (solid line: rarefaction; dotted
566 line: extrapolation, dot: observed richness) for gamma species richness (Hill number order q = 0)
567 of zoochorous seed rain for A) mid-successional small-seeded tree species, B) late-successional
568 small-seeded species, and C) later-successional large-seeded tree species arriving to traps located
569 in experimental plots (NR =natural regeneration; AN =applied nucleation; PL = plantation, RF =
570 reference). Shaded areas denote bootstrapped 95% confidence intervals.

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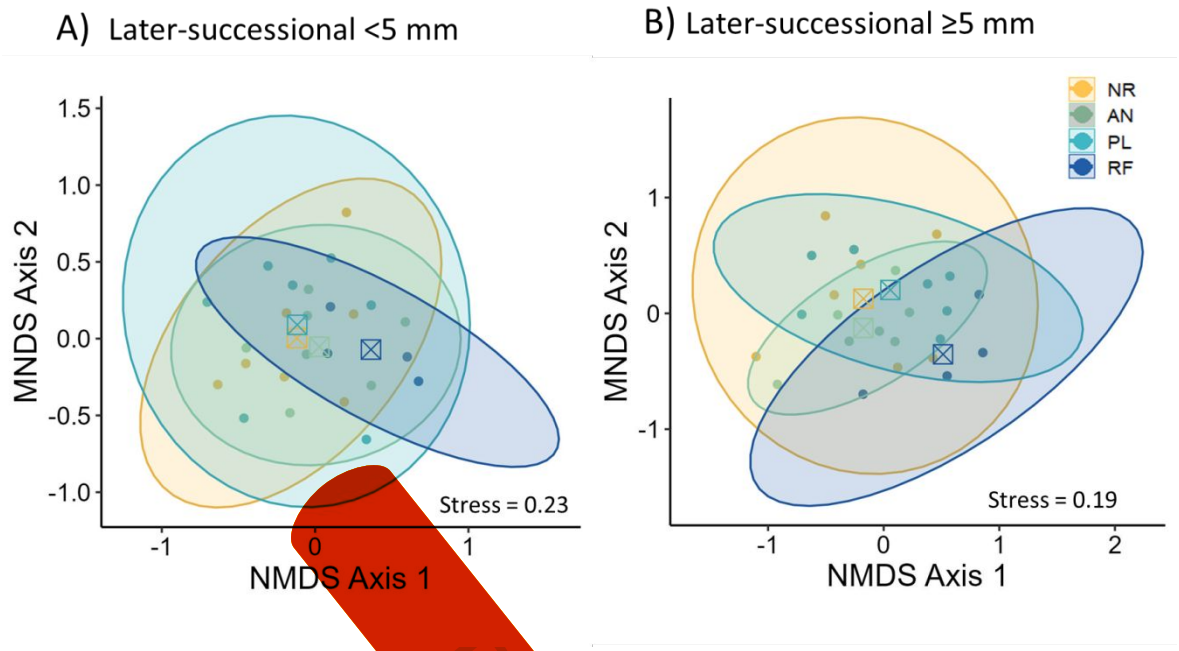

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573 **Figure 4**. Non-metric multidimensional scaling (NMDS) fits based on Bray-Curtis dissimilarity
574 of later-successional small- (A) and large-seeded (B), animal-dispersed trees community
575 composition in restoration treatments and reference forests. Each point corresponds to a plot and
576 colors denote restoration treatments: Natural Regeneration (NR, yellow), Applied Nucleation
577 (AN, green), Plantation (PL, light blue), and Reference Forest (RF, blue). Ellipses represent 95%
578 confidence intervals around the centroid for each treatment group.

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579 **Table 1.** Seed deposition rate (SDR = seeds m^{-2} yr^{-1}) for the top five most abundant later-successional zoochorous species in each

580 restoration treatment and in reference forest. All species were small seeded (<5 mm) and successional stage is indicated by superscript

581 M (mid-successional) or L (late-successional).

	Natural Regeneration		Applied Nucleation		Plantation		Reference Forest	
Rank	**Species**	**SDR**	**Species**	**SDR**	**Species**	**SDR**	**Species**	**SDR**
1	*Miconia donaeana*^{M}	28.1	*Ficus colubrinae*^{M}	61.5	*Palicourea padifolia*^{M}	5.5	*Ficus costaricana*^{M}	355.4
2	*Palicourea padifolia*^{M}	27.8	*Palicourea padifolia*^{M}	36.1	*Ficus hartwegii*^{L}	5.2	*Ficus americana*^{M}	345.6
3	*Miconia trinervia*^{M}	23.5	*Miconia donaeana*^{M}	29.1	*Ficus americana*^{M}	4.0	*Ficus hartwegii*^{L}	79.7
4	*Ficus hartwegii*^{L}	6.5	*Miconia trinervia*^{M}	12.4	*Miconia donaeana*^{M}	2.6	*Conostegia icosandra*^{L}	42.4
5	*Ficus americana*^{M}	5.6	*Conostegia icosandra*^{L}	11.1	*Ficus tonduzii*^{L}	1.3	*Ficus cervantesiana*^{M}	37.0

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Table S1. Species recorded in seed traps from February 2021 to February 2023 at seven sites in southern Costa Rica. Growth form: CT = Canopy tree, CP = Canopy palm, ET = Emergent tree, UT = Understory tree, UP = Understory palm. Dispersal mode: ZOO = animal-dispersed, WIND = wind-dispersed, GRAV = gravity-dispersed, EXPL = explosive dispersal. Seed size category is classified as 1: seed width <5 mm, 2: 5-10 mm, 3: 10-15 mm, 4: >15 mm.

Species name	Family	Growth form	Dispersal mode	Successional stage	Seed size category
Mendoncia retusa Turrill	Acanthaceae	Vine	ZOO	Mid	2
Viburnum costaricanum (Oerst.) Hemsl.	Adoxaceae	UT	ZOO	Mid	1
Desmopsis oerstedii Saff.	Annonaceae	UT	ZOO	Late	2
Guatteria dolichopoda Donn. Sm.	Annonaceae	CT	ZOO	Late	1
Forsteronia myriantha Donn. Sm.	Apocynaceae	Liana	WIND	Mid	3
Prestonia portobellensis (Beurl.) Woodson	Apocynaceae	Liana	WIND	Mid	3
Monstera deliciosa Liebm.	Araceae	Epiphyte	ZOO	Late	1
Monstera spp.	Araceae	Liana	GRAV	Late	2
Dendropanax sessiliflorus (Standl. & A.C. Sm.) A.C. Sm.	Araliaceae	CT	ZOO	Mid	1
Caryota maxima Blume ex Mart.	Arecaceae	CP	ZOO	Late	3
Caryota sp.	Arecaceae	CP	ZOO	Mid	2
Chamaedorea pinnatifrons (Jacq.) Oerst.	Arecaceae	UP	ZOO	Late	2
Chamaedorea tepejilote Liebm.	Arecaceae	UP	ZOO	Late	2
Euterpe precatoria Mart.	Arecaceae	CP	ZOO	Late	3
Geonoma sp.	Arecaceae	UP	ZOO	Late	1
Hyospathe elegans Mart.	Arecaceae	UP	ZOO	Late	1
Oenocarpus mapora H. Karst.	Arecaceae	CP	ZOO	Late	3
Amphilophium pannosum (DC.) Bureau & K. Schum.	Bignoniaceae	Liana	WIND	Late	1
Martinella obovata (Kunth) Bureau & K. Schum.	Bignoniaceae	Liana	WIND	Late	4
Cordia eriostigma Pittier	Boraginaceae	CT	ZOO	Late	2
Calophyllum brasiliense Cambess.	Calophyllaceae	CT	ZOO	Late	3
Jacaratia dolichaula (Donn. Sm.) Woodson	Caricaceae	UT	ZOO	Mid	2
Chrysochlamys glauca (Oerst., Planch. & Triana) Hemsl.	Clusiaceae	UT	ZOO	Mid	1
Clusia sp.	Clusiaceae	CT	ZOO	Mid	1

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Terminalia amazonia (J.F. Gmel.) Exell	Combretaceae	CT	WIND	Late	4
Maripa nicaraguensis Hemsl.	Convolvulaceae	Liana	ZOO	Late	3
Cayaponia granatensis Cogn.	Cucurbitaceae	Vine	ZOO	Late	3
Sechium tacaco (Pittier) C. Jeffrey	Cucurbitaceae	Vine	ZOO	Mid	4
Canavalia oxyphylla Standl. & L.O. Williams	Fabaceae	Liana	EXPL	Late	3
Erythrina lanceolata Standl.	Fabaceae	UT	GRAV	Late	2
Inga densiflora Benth.	Fabaceae	CT	ZOO	Late	3
Inga punctata Willd.	Fabaceae	CT	ZOO	Mid	2
Inga skutchii Standl.	Fabaceae	UT	ZOO	Late	2
Mucuna spp.	Fabaceae	Liana	GRAV	Late	4
Rhynchosia quercetorum Standl.	Fabaceae	Liana	GRAV	Mid	1
Quercus benthamii A. DC.	Fagaceae	CT	ZOO	Late	4
Lacistema aggregatum (P.J. Bergius) Rusby	Lacistemataceae	CT	ZOO	Late	1
Aegiphila valerii Standl.	Lamiaceae	CT	ZOO	Late	1
Beilschmiedia tovarensis (Klotzsch & H. Karst. ex Meisn.) Sach. Nishida	Lauraceae	CT	ZOO	Late	3
Cinnamomum triplinerve (Ruiz & Pav.) Kosterm.	Lauraceae	CT	ZOO	Late	2
Ocotea oblonga (Meisn.) Mez	Lauraceae	CT	ZOO	Late	3
Ocotea puberula Nees	Lauraceae	CT	ZOO	Mid	2
Ocotea sp.	Lauraceae	CT	ZOO	Late	3
Ocotea stenoneura Mez & Pittier	Lauraceae	CT	ZOO	Late	3
Persea americana Mill.	Lauraceae	CT	ZOO	Late	1
Peristethium leptostachyum (Kunth) Tiegh.	Loranthaceae	Epiphyte	ZOO	Mid	2
Struthanthus sp.	Loranthaceae	Epiphyte	ZOO	Mid	2
Mascagnia divaricata (Kunth) Nied.	Malpighiaceae	Liana	WIND	Late	4
Tetrapterys discolor (G. Mey.) DC.	Malpighiaceae	Liana	WIND	Late	2
Conostegia icosandra (Sw. ex Wikstr.) Urb.	Melastomataceae	CT	ZOO	Late	1
Miconia appendiculata Triana	Melastomataceae	UT	ZOO	Late	1
Miconia dolichopoda Naudin	Melastomataceae	CT	ZOO	Mid	1
Miconia doniana Naudin	Melastomataceae	CT	ZOO	Mid	1
Miconia punctata (Desr.) D. Don ex DC.	Melastomataceae	UT	ZOO	Mid	1
Miconia trinervia (Sw.) D. Don ex Loudon	Melastomataceae	CT	ZOO	Mid	1
Cedrela odorata L.	Meliaceae	CT	WIND	Late	4

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Guarea cf. *bullata*	Meliaceae	CT	ZOO	Late	2
Guarea chiricana Standl.	Meliaceae	UT	ZOO	Late	2
Guarea sp.	Meliaceae	CT	ZOO	Late	2
Ruagea glabra Triana & Planch.	Meliaceae	CT	ZOO	Late	2
Trichilia havanensis Jacq.	Meliaceae	CT	ZOO	Late	2
Abuta panamensis (Standl.) Krukoff & Barneby	Menispermaceae	Liana	ZOO	Late	4
Anomospermum reticulatum (Mart.) Eichler	Menispermaceae	Liana	ZOO	Late	4
Hyperbaena eladioana Q. Jiménez	Menispermaceae	Shrub	ZOO	Late	3
Mollinedia costaricensis Donn. Sm.	Monimiaceae	UT	ZOO	Late	3
Mollinedia viridiflora Tul.	Monimiaceae	UT	ZOO	Late	2
Ficus americana Aubl.	Moraceae	CT	ZOO	Mid	1
Ficus caldasiana Dugand	Moraceae	ET	ZOO	Late	1
Ficus cervantesiana Standl. & L.O. Williams	Moraceae	CT	ZOO	Mid	1
Ficus colubrinae Standl.	Moraceae	CT	ZOO	Mid	1
Ficus costaricana (Liebm.) Miq.	Moraceae	CT	ZOO	Mid	1
Ficus hartwegii (Miq.) Miq.	Moraceae	ET	ZOO	Late	1
Ficus sp.	Moraceae	CT	ZOO	Mid	1
Ficus tonduzii Standl.	Moraceae	CT	ZOO	Late	1
Pseudolmedia glabrata (Liebm.) C.C. Berg	Moraceae	CT	ZOO	Late	2
Pseudolmedia mollis Standl.	Moraceae	CT	ZOO	Late	3
Sorocea trophoides W.C. Burger	Moraceae	UT	ZOO	Late	1
Trophis mexicana (Liebm.) Bureau	Moraceae	UT	ZOO	Late	1
Trophis racemosa (L.) Urb.	Moraceae	CT	ZOO	Late	2
Otoba novogranatensis Moldenke	Myristicaceae	CT	ZOO	Mid	4
Virola chrysocarpa D. Santam. & Aguilar	Myristicaceae	CT	ZOO	Late	4
Eugenia pittieri Standl.	Myrtaceae	UT	ZOO	Late	2
Syzygium jambos (L.) Alston	Myrtaceae	UT	ZOO	Mid	4
Passiflora ambigua Hemsl.	Passifloraceae	Liana	ZOO	Late	2
Passiflora edulis Sims	Passifloraceae	Vine	ZOO	Mid	1
Ardisia standleyana P.H. Allen	Primulaceae	Shrub	ZOO	Late	1
Drypetes brownii Standl.	Putranjivaceae	CT	ZOO	Late	3
Palicourea padifolia (Willd. ex Roem. & Schult.) C.M. Taylor & Lorence	Rubiaceae	UT	ZOO	Mid	1

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Psychotria limonensis K. Krause	Rubiaceae	UT	ZOO	Late	2
Psychotria microbotrys Ruiz ex Standl.	Rubiaceae	Shrub	ZOO	Mid	1
Zanthoxylum acuminatum (Sw.) Sw.	Rutaceae	CT	GRAV	Late	1
Meliosma allenii Standl. & L.O. Williams	Sabiaceae	UT	ZOO	Late	4
Casearia sp.	Salicaceae	CT	ZOO	Late	2
Allophylus psilospermus Radlk.	Sapindaceae	CT	ZOO	Mid	2
Cupania americana L.	Sapindaceae	CT	ZOO	Late	3
Paullinia alata G. Don	Sapindaceae	Liana	ZOO	Late	2
Paullinia bracteosa Radlk.	Sapindaceae	Liana	GRAV	Late	4
Paullinia capreolata (Aubl.) Radlk.	Sapindaceae	Liana	GRAV	Late	2
Serjania mexicana (L.) Willd.	Sapindaceae	Liana	WIND	Mid	4
Serjania racemosa Schumach.	Sapindaceae	Liana	WIND	Late	4
Siparuna gesnerioides (Kunth) A. DC.	Siparunaceae	Shrub	ZOO	Mid	1
Smilax domingensis Willd.	Smilacaceae	Liana	ZOO	Mid	1
Gordonia fruticosa (Schrad.) H. Keng	Theaceae	CT	WIND	Mid	4
Vochysia guatemalensis Donn. Sm.	Vochysiaceae	CT	WIND	Late	4

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Table S2. Total number of later-successional species and number of collected seeds (in parentheses) by dispersal mode, growth form and seed size in the seed rain of restoration and reference plots in southern Costa Rica. Canopy trees include emergent trees and canopy palms. Understory trees include understory palms. All early successional species and all Asteraceae, Piperaceae, and Solanaceae were excluded from census.

Dispersal mode	Growth form	<5 mm	≥5 mm	All sizes
Zoochorous		**33 (134,158)**	**51 (6,587)**	**84 (140,745)**
	Canopy tree	18 (125,083)	28 (6,176)	46 (131,259)
	Understory tree	9 (8,583)	12 (306)	21 (8,889)
	Liana	1 (27)	5 (14)	6 (41)
	Vine	1 (43)	3 (64)	4 (107)
	Shrub	3 (239)	1 (1)	4 (240)
	Epiphyte	1 (183)	2 (26)	3 (209)
Non-zoochorous		**4 (74)**	**16 (1,683)**	**20 (1,757)**
	Canopy tree	1 (3)	4 (1,307)	5 (1,310)
	Understory tree	1 (1)	0	1 (1)
	Liana	2 (69)	12 (376)	14 (445)
Total		**37 (134,232)**	**67 (8,270)**	**104 (142,502)**

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Table S3. Observed and estimated diversity of small (<5 mm) mid- and late-successional and large (≥5 mm) later-successional zoochorous tree species seeds recorded over a 2-yr period in three restoration treatments (7 sites each) and reference forests (4 sites).

			Mid-successional <5 mm seeds		Late-successional <5 mm seeds		Later-successional ≥5 mm seeds	
Treatment	**# Traps**	**Diversity order**	**Observed**	**Estimated ± S.E.**	**Observed**	**Estimated ± S.E.**	**Observed**	**Estimated ± S.E.**
Natural Regeneration	112	q^0	11	11.0 ± 0.7	5.0	7.0 ± 2.5	19	27.1 ± 9.8
		q^1	7.6	7.8 ± 0.4	2.9	3.3 ± 0.6	13.7	16.9 ± 2.1
		q^2	6.2	6.3 ± 0.4	2.4	2.6 ± 0.3	10.9	12.9 ± 1.7
Applied Nucleation	112	q^0	9	9.0 ± 0.2	7.0	9.0 ± 2.1	25	41.5 ± 17.9
		q^1	7.5	7.6 ± 0.2	4.0	4.3 ± 0.5	14.8	17.3 ± 1.9
		q^2	6.7	6.9 ± 0.3	3.2	3.4 ± 0.4	10.8	11.7 ± 1.2
Plantation	112	q^0	11	12.0 ± 1.3	9.0	10.0 ± 2.2	19	21.6 ± 5.6
		q^1	7.2	7.5 ± 0.5	5.4	5.9 ± 0.7	13.5	14.9 ± 1.2
		q^2	6	6.1 ± 0.5	3.7	4.0 ± 0.8	10.9	11.9 ± 1.3
Reference Forest	64	q^0	12	12.0 ± 0.4	10.0	11.0 ± 1.1	34	35.5 ± 6.0
		q^1	8.7	8.9 ± 0.4	7.1	7.4 ± 0.5	19.9	21.5 ± 1.3
		q^2	7.1	7.3 ± 0.4	5.9	6.2 ± 0.5	13.3	13.8 ± 1.1

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Table S4. PERMANOVA pairwise comparisons among community data matrices using NMDS with Chao dissimilarity measures from four treatments: natural regeneration (NR), applied nucleation (AN), plantation (PL), and reference forest (RF). Adjusted P-values were calculated using the Benjamani-Hochberg method for multiple tests.

Small-seeded species (<5mm)				
Pairs	F	R^2	p	p-adjusted
NR-AN	0.523	0.042	0.840	1.000
NR-PL	0.367	0.030	0.927	1.000
NR-RF	2.586	0.223	0.010	0.060
AN-PL	0.444	0.036	0.877	1.000
AN-RF	2.361	0.208	0.013	0.078
PL-RF	2.573	0.222	0.017	0.102

Large-seeded species (≥5mm)				
Pairs	F	R^2	p	p-adjusted
NR-AN	1.164	0.088	0.301	1.000
NR-PL	0.421	0.034	0.933	1.000
NR-RF	1.987	0.181	0.041	0.246
AN-PL	1.236	0.093	0.273	1.000
AN-RF	2.236	0.199	0.028	0.168
PL-RF	1.823	0.168	0.078	0.468

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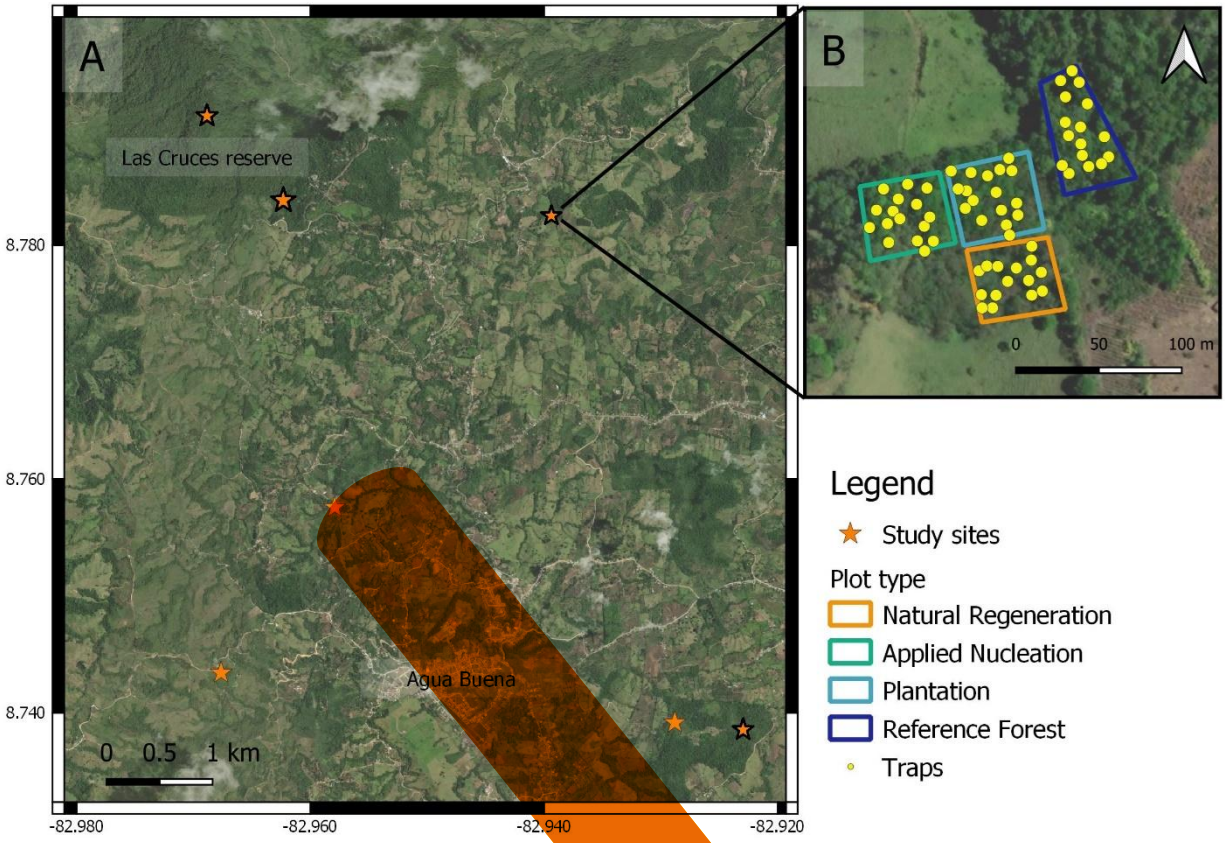

Figure S1. Study sites in southern Costa Rica (A) and example of trap locations at each of three restoration plots and one reference forest plot at one site (B). Each site comprised three restoration treatments (natural regeneration, applied nucleation, and plantation), some sites (bolded stars) included a reference forest, and each treatment or reference forest plot had 16 seed traps spread throughout the plot.

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Figure S2. Representative photographs of the three restoration treatments at the San Gabriel site (February 2025), approximately two decades after restoration began. The natural regeneration plot (top) is among the most canopy-closed examples of this treatment, yet still exhibits relatively open structure compared to planted treatments. The applied nucleation plot (middle) shows formerly discrete tree islands of varying sizes that have largely coalesced, while remaining canopy gaps allow light to reach the forest floor. The plantation plot (bottom) exhibits the darkest understory conditions, with rows of planted trees still clearly distinguishable, reflecting persistent structural legacies of the original planting design.

Figure S2. Number of plots per restoration treatment per zoochorous species where only a fruiting tree was detected (red), only a seed was detected (blue) and both fruiting trees and seeds were detected (green). Species names represent a code built with the first three letters of the genus and first three letters of the specific epithet.

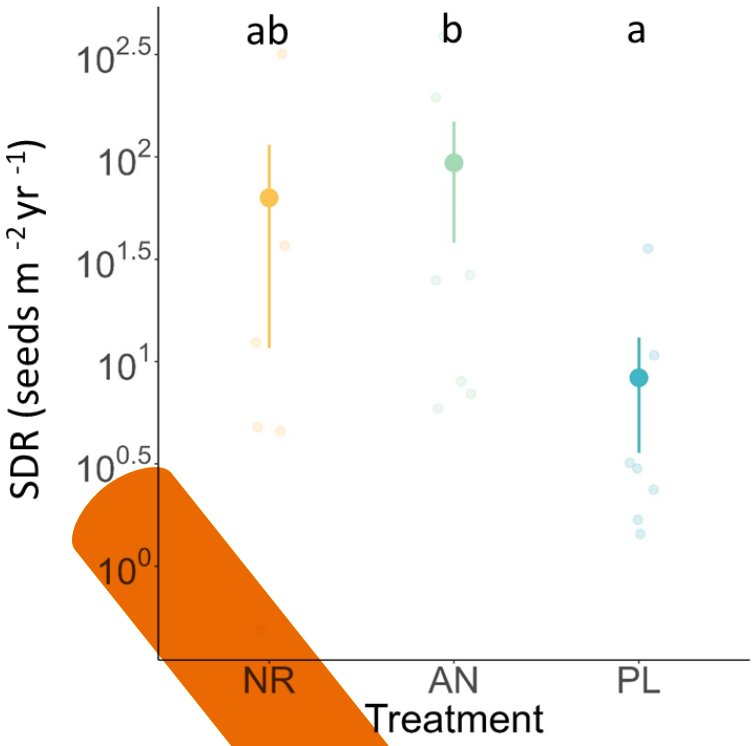

Figure S4. Comparison of the seed deposition rate (seeds $m^{-2}yr^{-1}$) of tree seeds from exclusively externally dispersed mid-successional small-seeded species into restoration treatments. Large point indicates the mean, vertical lines the Standard Error and small dots the sampling units (plots in this case). Different letters indicate significant differences ($p < 0.05$, one-way ANOVA). NR = Natural Regeneration, AN = Applied Nucleation, PL = Plantation.

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