Thesis

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Title

Effective Seed-Dispersers as Drivers of Natural Regeneration in Fragmented Landscapes.

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in front of the following jury:

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ABSTRACT

Natural forest regeneration is essential for carbon sequestration, biodiversity conservation, and sustaining local livelihoods. Frugivorous birds facilitate this process by dispersing seeds into open areas, yet their movements and effectiveness can be constrained in fragmented landscapes. The cascading effects of fragmentation on frugivore communities, their seed-dispersal–related functional traits, and the resulting consequences for seed-rain composition and regenerative potential in open areas remain poorly understood. In this study, I analysed bird-mediated seed rain across landscapes with 20%, 40%, and 60% forest cover and along distance gradients from forest edges. Genetic metabarcoding allowed identification of both the bird and plant species in each defecation event, enabling assessment of how species composition and functional traits shifted across forest covers and distance to the forest edge. Landscapes with 60% forest cover supported higher frugivore diversity and seed-rain richness, although only a subset of species acted as effective dispersers in open areas. Species richness in both birds and plants remained stable with distance, while community composition became increasingly dissimilar. Functional turnover in bird traits emerged only in the most fragmented landscape (20%), yet this shift did not translate into differences in seed-rain functional traits. These findings highlight the dominant role of generalist seed dispersers in open areas and emphasize the importance of tailoring restoration strategies to landscape context, particularly by enhancing connectivity in highly fragmented areas.

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1. INTRODUCTION

Forest regeneration in open areas is an essential tool for battling climate change and conserving biodiversity (Chazdon et al., 2016; Dent & Elsy, 2024). In the Neotropics, natural forest regeneration of secondary forests and abandoned farmland could account for approximately 31 petagrams of CO2 stored over 40 years, effectively offsetting carbon emissions produced by Latin America and the Caribbean for over two decades (Chazdon et al., 2016). A key driver of natural forest regeneration is animal-mediated seed dispersal. By consuming fruits and dispersing their seeds, frugivorous animals play a crucial role in shaping forest regeneration, structure, and community composition (Camargo et al., 2020; González-Varo et al., 2017; Wunderle, 1997). In particular, frugivorous birds, which make roughly one-third of all Neotropical terrestrial birds, engage in the highest known diversity of seed dispersal interactions with fleshy-fruited plants (Carlo et al., 2022; Daniel Kissling et al., 2009). However, the role of frugivore birds in natural forest regeneration for restoration projects is frequently overlooked, usually preferring active planting instead (Girardin et al., 2021; Seddon et al., 2020).

To effectively assess the role of frugivorous birds in forest regeneration, it is essential to examine their seed-dispersal behavior through their functional traits and their interactions with the surrounding landscape. Large-bodied species often have greater movement capacity (Neuschulz et al., 2013; Spiegel & Nathan, 2007) and may disperse seeds at longer distances than smaller species (Spiegel & Nathan, 2007). Large frugivores in particular tend to move over large areas because fruit resources are ephemeral and spatially unpredictable, requiring birds to track fruit availability across the landscape (Graham, 2001; Neuschulz et al., 2013). Similarly, the gape width (i.e., the width of the base of the beak where the mandible and the maxilla join) can also determine the fate of seeds. Birds with large gape widths can handle large and small fruits alike, while smaller gaped birds will have trouble handling large fruits. Hence, large gaped frugivores could potentially disperse a greater diversity of seeds (Carlo et al., 2022). Furthermore, dispersing large seeds which belong to long-lived, high-statured trees, will consequently contribute to sequestering more carbon in the regenerating forest (Bello et al., 2024; Galetti et al., 2017).

Beyond birds' traits, landscape configuration can also shape how frugivorous birds disperse seeds in heterogeneous landscapes. Factors such as proximity to old-growth forests, forest cover, and landscape connectivity can strongly influence the dispersal effectiveness in open areas (González-Varo et al., 2017; Wunderle, 1997). Forests regenerating near old-growth stands with intact frugivore communities tend to recover more rapidly and predictably, gradually converging with the community attributes of the surrounding older forests (Arroyo-Rodríguez et al., 2017). Furthermore, landscapes above 40% forest cover can sustain greater functional diversity among frugivorous birds, which in turn promotes the dispersal of a more even and diverse seed assemblage (Bello et al., 2024; Camargo et al., 2020). Landscape connectivity—facilitated by structures that act as steppingstones and perching sites, such as isolated trees, stumps, living fences, and hedgerows—can further enhance seed dispersal by enabling bird movement across open areas (Neuschulz et al., 2013; Wunderle, 1997).

Yet despite these facilitators, the benefits of connectivity are easily undermined when fragmentation increases, as bird populations are highly sensitive to habitat isolation (Carrara

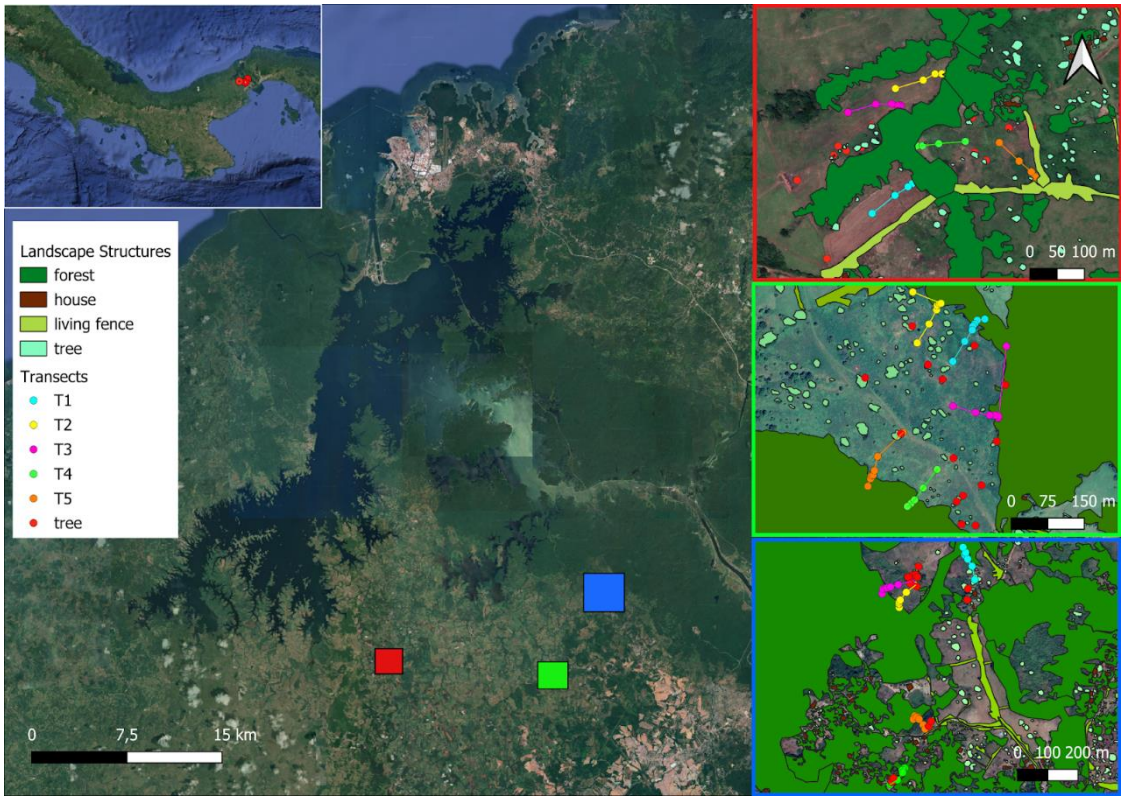
et al., 2015; Mariano-Neto & Santos, 2023; Mayhew et al., 2019). Increasing distance between forest remnants can severely disrupt seed dispersal, with density and richness of both birds and seeds declining exponentially as distance from forest fragments increases (Camargo et al., 2020). Additionally, few bird species forage in both open and forested areas, limiting potential movement and seed dispersal between these two types of systems (González-Varo et al., 2017). As a result, bird communities can experience ecological niche turnover across fragmentation gradients, where open habitat frugivores replace forest-dependent frugivores as fragmentation increases (González-Varo et al., 2017). This turnover can be accompanied by a change in birds' functional traits, which directly shape their capacity to disperse different plant species (Bello et al., 2024; Neuschulz et al., 2013). When certain functional traits are absent—for example, large-gaped birds—plant species with seeds that require those traits for ingestion and dispersal are effectively excluded from the seed rain. This, in turn, would lead to the regeneration of less diverse, more fragile forests with lower carbon storage capacity (Bello et al., 2024; Camargo et al., 2020).

How fragmentation shapes the turnover of frugivore communities and their functional traits—and how these changes cascade into seed-rain patterns and influence potential forest regeneration in open areas—remains poorly understood. Understanding these fragmentation effects on bird-mediated seed dispersal in open areas is essential for designing effective natural regeneration strategies and tailoring restoration practices to local landscape conditions. I conducted a metabarcoding analysis of seed rain deposited in open areas with the aim of identifying the birds and plants that are contributing to natural forest regeneration across a forest-fragmentation gradient. I ask 1) how does forest fragmentation shape the diversity, composition, and the related traits of effective seed-dispersing bird species in open areas? and 2) how potential turnovers in the bird seed-dispersing community, influenced by fragmentation, shape the composition and the related traits of the plants that can regenerate in open areas? First, 1) I expect that less fragmented areas will exhibit greater bird species and trait diversity contributing to the seed rain, whereas in highly fragmented landscapes, fewer bird species with lower trait diversity are likely to contribute to the seed rain in open areas. Likewise, 2) as bird functional diversity increases in less fragmented landscapes, I expect the seed rain deposited in open areas to reflect higher species and trait diversity. The findings of this research will provide insight into how fragmentation affects bird-mediated seed dispersal and its cascading impacts on seed rain and potential natural forest regeneration. These insights can help optimize resource allocation for forest regeneration efforts and inform more effective climate change mitigation policies.

2. MATERIALS AND METHODS

2.1. STUDY SITE

This project was developed in the buffer zone of the Barro Colorado Nature Monument (BCNM), Panama. The area is a mosaic of primary forest interspersed with secondary stands of varying ages and open areas dominated by pasture, isolated trees, and hedgerows. These regenerating forests are located on areas that were used for cattle pasture, swidden farming or fruit production between the 1880s and the establishment of BCNM in 1979 (Terborgh, 1983). The vegetation is classified as tropical moist forest and the climate is seasonal with a distinct dry season, typically from mid-December to early May. Annual rainfall is 1,900-3,600 mm (Turner et al., 2015). My study focused on three different landscapes of 360 000 m^2 with different levels of forest cover—20, 40, 60%— spanning a fragmentation gradient. 20% was called "Ananas", 40% was "Finca Isaac", and 60%, "Santa Clara", although I will refer to each landscape for its forest cover level in this study (see Map 1). 60% was divided into two plots of 360 000 m^2 due to the difficulty of finding large open areas in such a degree of forest cover.



Map 1: Study Site (BCNM buffer zone). Red, green, and blue squares mark the locations of the 20%, 40%, and 60% forest-cover landscapes, respectively. The panels on the right show each landscape in detail, including forest-cover and the positions of all seed traps along their corresponding transects.

2.2. DATA ACQUISITION

Seed collection

In each landscape, 50 seed traps were located in a total of five 105 meter transects that extend from forest edge to open areas, and below isolated trees. Seven traps were set along each transect at -5, 0, 5, 10, 20, 50, and 100 m, and 15 traps beneath isolated trees. Seed traps consisted of plastic trays (40 cm x 55 cm, 8 cm height) with small holes (1 mm) to allow water drainage and covered with wire mesh (1 cm) to prevent post-dispersal seed predation. Traps located along the transect had a pole perch to promote seed deposition (González-Castro et al., 2019). Traps were established at the end of the dry season, when most fruits are produced (March-April) and stand for two months. Traps were checked twice weekly to recover deposited seeds. Each seed was deposited, with minimum handling, into a 2.0-ml sterile tube. Tubes were labelled and stored in a freezer at -20°C until DNA extraction, following the recommendations of González-Varo et al., 2014.

Species identification

DNA metabarcoding analysis was used to identify the species of both the dispersed seeds and the associated bird disperser. Analysis was conducted in the Molecular Ecology lab at the Smithsonian Tropical Research Institute (STRI). DNA of animal origin can be extracted from the surface of defecated or regurgitated seeds, allowing for identification of frugivore species responsible for dispersal events (González-Varo et al., 2014). Bird identification was based on a 464-bp fragment of the mitochondrial COI region (cytochrome c oxidase subunit I). The QiaAmp Fast DNA Stool Mini Kit was used for incubating each seed directly in the extraction buffer (added to the 2.0-ml tube where the seed is stored). Primers COI-fsdF and COI-fsdR were used following PCR protocols described by González-Varo et al., 2014. In case of DNA degradation, e.g. due to rain or prolonged time from seed deposition and collection, primers for small DNA fragments, such as AvMiR1 and AWCintF2-AWCintR4 were used for avian DNA barcodes (Alcaide et al., 2009). For identification of plants, genetic material was extracted from seed samples using a CTAB extraction protocol (Doyle, 1991), then the ITS2 region was amplified (Chen et al., 2010). Bird and seed libraries were prepared using a 2-stage PCR approach and sequencing STRI Illumina MiSeq using 2x250 bp paired-end sequencing. R package DADA2 was used to assign taxonomy (Callahan et al., 2016). A custom reference database of > 2000 COI DNA barcodes was available for Panamanian birds, and an ITS2 database for Central American trees.

Species traits

Bird traits were obtained from the AVONET database, and a Principal Component Analysis (PCA) was done to select uncorrelated traits that explained the most variability in the data. A total of 5 uncorrelated bird traits were obtained to explain a particular ecological role in the seed dispersal behavior of birds. These were: Body Mass (BM), Beak Width (BM, the maximum lateral width of the bill, measured at the base of the upper mandible), Wing Length (WL, distance from wing "wrist" to the tip of longest primary wing), Kipp's Distance (KD, gap between the tip of longest primary wing and the first secondary wing), and Hand-Wind Index (HWI, kipp's distance / wing length) × 100). Depending on BM, birds are expected to exhibit different seed-dispersal behaviors. Larger birds may travel longer distances when food resources are less abundant, while smaller birds are more likely to move shorter distances (Carlo et al., 2022; Ramos et al., 2020). BW is used as a proxy for

gape size and feeding capacity (Carlo et al., 2022). HWI, KD, and WL are used as a proxy for the bird's flying capacity, meaning bird species with higher trait values will exhibit a larger wingspan that allows them to travel longer distances, hence potentially dispersing seeds further from the forest edge (Sheard et al., 2020). Traits were then linked to every seed rain sample through the birds' species name, meaning trait values were fixed per species. When the metabarcoding analysis did not reach species level identification, the mean trait value for all species belonging to that genus was assigned instead.

Plant traits were obtained from the TRY Plant Trait Database (Kattge et al., 2020) and selected based on their significance for determining their availability for ingestion, seed dispersal, and above-ground biomass in the case of successful establishment and development of the seed (Bello et al., 2015; Foster & Janson, 1985; Hodgson et al., 2017). Four plant functional traits were chosen based on these criteria while maximizing species coverage in the dataset: Seed Dry Mass (SDM, mg), Wood Density (WD, g cm^{-3}), Average Plant Height (APH, m), and Specific Leaf Area (SLA, mm^2 mg^{-1}). Similarly to bird traits, fixed plant trait values were used per species identification, and "all species in a genus" mean values were assigned where species level identification was not possible.

Bird community census

An additional bird visual census was conducted to assess the diversity of bird species moving across open areas in the three landscapes. Bird censuses were conducted in each landscape from a vantage point with clear visibility of both the surrounding area and the seed traps. Censuses were carried out from 6:00 to 9:00 a.m. over 24 days, totaling 72 hours of observation per landscape (Ramos et al., 2020). The purpose of these surveys was to serve as a reference level for knowing the baseline bird community that moved across open areas in each landscape. To identify the community that could potentially participate in seed dispersal, I filtered the species by diet, only maintaining those species that were classified as "Frugivores", "Invertivores", and "Omnivores". Invertivores were included, due to a large proportion of bird species identified in the seed rain metabarcoding being classified by AVONET as "Invertivores". Once I had both databases, I used these as proxies for the "bird community" that could potentially contribute to seed dispersal (visual census), and the "effective seed-dispersers" in each landscape (seed-rain data).

2.3. DATA ANALYSIS

I analyzed the effects of forest fragmentation on the seed-dispersal behavior of frugivorous birds by studying the relationship among 2 landscape explanatory variables and 3 bird-plant response variables. As landscape explanatory variables, I used: (1) the percentage of forest cover, which reflects the effect of landscape fragmentation, and (2) the distance to the nearest forest edge, which reflects the local effect of isolation from the forest. As response variables I used: (1) the number of samples deposited in open areas, (2) species richness of birds and seeds, and (3) the functional traits of birds and seeds. For the landscape variables, I calculated the percentage of forest cover in each landscape after having manually classified and delimited forest polygons over satellite imagery from Google Earth in QGIS. For the minimum distance to the forest edge, I computed the minimum distance of every seed trap to the closest forest polygon edge using the *Nearest Neighbour Distance* tool in QGIS. I ultimately did not use the designated transect distances as the measure of 'distance to the nearest forest edge,' because in several cases seed traps that were positioned progressively further from their original forest edge were actually approaching other forest patches. As a

result, the assigned distances did not accurately represent the true nearest-edge distance. Seed traps that were classified in the transect at distances "- 5m" (5 meters within the forest), "0m" (forest edge), and "5m" (5 meters away from the forest edge) remained with their transect distance value as in these cases the transect distance was thought to be more accurate than the one computed in QGIS.

Sample abundance

Sample abundance—the number of defecation events belonging to one seed-dispersal interaction between bird and plant species—was compared among forest cover landscapes for a descriptive assessment. To evaluate how sample abundance varied with distance from the nearest forest edge across forest-cover landscapes, I fitted a generalized linear mixed-effects model (GLMM) with a negative binomial distribution and a log link using the glmer.nb function in the lme4 package in R. The logarithm of the mean sample abundance was modeled as a function of the log-transformed minimum distance to the forest edge and its interaction with forest cover. To account for sampling design locations, Transect was included as a random intercept.

$$Samples_{i,j,k} \sim NegBin(S_{i,j,k}, \theta)$$

$$log(S_{i,j,k}) = (\mu + a_j) + (\beta + \gamma_j)\, x_{i,j} + b_k$$

$$b_k \sim N(0, \sigma^2)$$

Where: $Samples_{i,j,k}$ is the random variable representing the number of samples at seed trap i, landscape j, and transect k. $S_{i,j,k}$ is the expected number of samples, θ is the dispersion parameter of the negative binomial distribution, μ is the intercept of the regression line in the reference landscape (20%), a_j is the added effect on the intercept due to landscape j, β is the slope of the regression line in the reference landscape, γ_j is the additive effect of landscape j on the slope of the regression line, $x_{i,j}$ is the logarithm of the distance with an added constant ($log(distance_{ij}+10)$). b_k captures transect-specific deviations (random effect), σ^2 is the variance of the random effect.

The log-transformation of distance with a constant added was implemented to accommodate for zero and negative values. The interaction term allowed the distance–sample abundance relationship to vary among landscapes. For sample abundance and species richness models, I tested for model performance with AIC, having compared whether a GLM, a GLM with Transect as random intercept, or a GAM, would better fit the data (see Appendix 2). I also tested for the significance of adding the random effect with ANOVA (Appendix 3).

Species richness

Species richness of both birds and plants was compared among forest covers for a descriptive assessment. In the case of birds, Beta-diversity analyses were conducted to compare community composition among landscapes for both the overall bird community and the effective seed-dispersers. Additionally, within each landscape, beta-diversity was assessed to compare the overall bird community with the effective seed-disperser community.

Secondly, to evaluate how effective dispersers and plant species richness responded to forest edge distance across landscapes, I fitted a generalized linear mixed model (GLMM) with a negative binomial distribution and log link, using the glmer.nb() function from the lme4 package in R. Richness was modeled as a function of the log-transformed minimum

distance to the forest edge and its interaction with forest cover. To account for sampling design locations, Transect was included as a random intercept:

$$Richness_{i,j,k} \sim NegBin(R_{i,j,k}, \theta)$$

$$log(R_{i,j,k}) = (\mu + a_j) + (\beta + \gamma_j)\, x_{i,j} + b_k$$

$$b_k \sim N(0, \sigma^2)$$

Where: $Richness_{i,j,k}$ is the random variable representing the number of species at seed trap i, landscape j, and transect k. $R_{i,j,k}$ is the expected number of species, θ is the dispersion parameter of the negative binomial distribution, μ is the intercept of the regression line in the reference landscape (20%), a_j is the added effect on the intercept due to landscape j, β is the slope of the regression line in the reference landscape, γ_j is the additive effect of landscape j on the slope of the regression line, $x_{i,j}$ is the logarithm of the distance with an added constant ($log(distance_{ij}+10)$). b_k captures transect-specific deviations (random effect), σ^2 is the variance of the random effect.

I explored the turnover of bird and plant communities as distance to the forest increases in each landscape using the Jaccard dissimilarity index. This index assumes that communities can be compared meaningfully based on presence–absence data, giving equal weight to all species regardless of abundance. To do so, I first converted species occurrence data into presence–absence matrices by collapsing observations to unique combinations of forest cover, distance, and species. Each species was coded as present (1) or absent (0). I then grouped matrices by forest-cover landscapes and categorized distances into reference sites (–5 m and 0 m) versus all other distances, with the aim of comparing how species composition in open areas differs from the one in the forest edges (reference sites). For each landscape, I calculated pairwise Jaccard dissimilarities between reference sites and other distances, and paired these with the corresponding geographic distances. Finally, I tested for distance-dissimilarity relationships to assess how community dissimilarity changed with distance and whether this relationship differed among vegetation cover types. I fitted a linear model of the form:

$$Dissimilarity_{i,j} \sim Normal(D_{i,j}, \sigma^2)$$

$$D_{i,j} = (\mu + a_j) + (\beta + \gamma_j)\, x_{i,j}$$

Where: $Dissimilarity_j$ is the random variable representing the Jaccard dissimilarity between two seed traps (replica i) in landscape j. $D_{i,j}$ is the expected dissimilarity index, σ^2 is the variance of the normal distribution, μ is the intercept of the regression line in the reference landscape (20%), a_j is the added effect on the intercept due to landscape j, β is the slope of the regression line in the reference landscape, γ_j is the additive effect of landscape j on the slope of the regression line, $x_{i,j}$ is the logarithm of the distance with an added constant ($log(distance_{ij}+10)$).

Species Traits

To evaluate how bird and plant traits responded across forest-cover gradients and distances from the nearest forest edge, I used abundance-weighted trait values. The frequency with which each bird and plant species appeared in the seed traps is ecologically meaningful, as it reflects the relative abundance of dispersers and seeds entering the system and thus their potential influence on seed dispersal and community assembly. Because seed rain density directly shapes regeneration dynamics, weighting plant traits by their observed abundances offers a more realistic representation of the functional traits most likely to drive forest recovery across landscapes.

For trait-distance relationships, I modeled plant traits and bird traits as a function of log-transformed distance, forest cover landscape, and their interaction using a GLM with a Gamma distribution and a log link. SDM was log-transformed due to several extreme outliers. This specification accounts for the positive, continuous nature of the response variable and allows the effect of distance on traits to vary among cover types.

$$Trait_{i,j} \sim Gamma(T_{i,j}, \phi)$$

$$T_{i,j} = (\mu + a_j) + (\beta + \gamma_j)\, x_{i,j}$$

Where: $Trait_{i,j}$ is the random variable representing the traits value in seed trap i in landscape j. $T_{i,j}$ is the expected trait value, ϕ is the gamma dispersion parameter, μ is the intercept of the regression line in the reference landscape (20%), a_j is the added effect on the intercept due to landscape j, β is the slope of the regression line in the reference landscape, γ_j is the additive effect of landscape j on the slope of the regression line, $x_{i,j}$ is the minimum distance to the forest edge.

3. RESULTS

The number of samples collected in each forest cover type was 524 in 20%, 457 in 40%, and 666 in 60%. The model for sample abundance against distance to forest edge showed no significant effect of distance or forest-cover landscape on sample abundance (all $p > 0.05$), except for a negative interaction observed for 40% forest cover ($\gamma_2 = -0.091$, $p = 0.025$, see Appendix 4).

Objective 1

From the bird visual census, forest cover was found to influence the overall bird community richness, with higher forest-cover landscapes supporting more species. This pattern remained even when I filtered species based on diet, retaining only potential frugivores—those categorized in AVONET as frugivores, invertivores, or omnivores—. Using this subset, I observed an increase in species richness as forest cover increased with 75 species at both 20% and 40% forest cover, and 94 species at 60% forest cover (Fig. 1.A).

However, from the seed rain data, I found that the number of effective seed-dispersing bird species did not differ substantially between landscapes. I observed 48 species in 20%, 44 species in 40%, and 49 species in 60% (Fig. 1.A). Landscapes showed to have around ~ 60-65% of shared species, 6-10% of species shared amongst two landscapes, and 15-20 % of species exclusively found on each landscape (see Appendix 1). The community of effective seed-dispersing birds was also not shared in totality by the community surveyed in the visual census data. Some species appeared exclusively in the seed rain data: 17 species in 20%, 15 in 40%, and 13 in 60%.

Effective disperser richness showed no significant relationship with distance from the forest edge, nor with forest cover, with no landscape exhibiting a significant interaction between distance and richness (all $p > 0.15$, see Appendix 5). Including Transect as a random effect significantly improved model fit relative to the fixed-effects model (likelihood ratio test: $\chi^2 = 9.56$, df = 1, p = 0.002), with lower AIC, indicating that accounting for transect-level variation better explains species richness.

Jaccard dissimilarity of bird communities did show a positive relationship with distance (Fig.1.C). First of all, forest cover types did not differ significantly from 20% in their baseline dissimilarity (40%: $a_2 = 0.147$, $p = 0.38$; 60%: $a_3 = -0.056$, $p = 0.75$). In the 20% landscape, there was a significant positive effect of distance on community dissimilarity ($\beta = 0.086$, $p = 0.0048$), indicating an increment in compositional turnover with increasing distance from the forest edge. Interaction terms between distance and cover type were not significant (40%: $\gamma_2 = -0.034$, $p = 0.34$; 60%: $\gamma_3 = 0.0004$, $p = 0.99$), indicating an overall increase in dissimilarity with distance among all three landscapes. The model explained a moderate proportion of the variance (adjusted $R^2 = 0.24$; $F_{5,82} = 6.42$, $p < 0.001$). Due to all forest-cover classes exhibiting similar positive slopes, I simplified the model by fitting a single overall prediction line across landscapes to improve model parsimony and predictive performance. Jaccard dissimilarity increased significantly with distance across all forest covers ($\beta = 0.070$, $p < 0.001$), and this simplified model showed improved fit based on AIC.

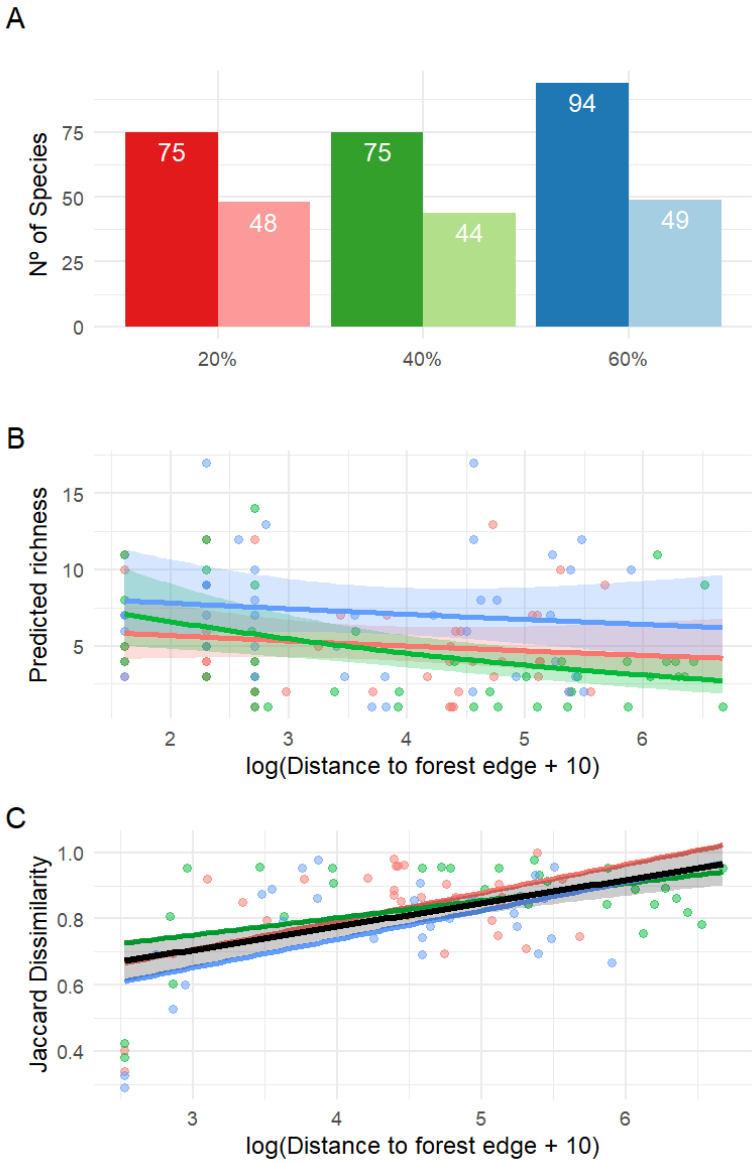


Figure 1: Relationship between bird species richness and landscape variables: A: Bird Species Richness per Forest Cover. *The number of bird species found in each forest cover landscape from two different sources.* ***Left columns:*** *bird visual census, and* ***Right columns:*** *seed rain metabarcoding.* ***B: Bird species richness per minimum distance to forest edge:*** *The number of bird species as a function of log minimum distance to forest edge per forest cover landscape.* ***C: Distance-dissimilarity relationship in Bird Species:*** *Jaccard dissimilarity index as a function of log minimum distance to forest edge per forest cover landscape. Forest cover landscapes are represented 20% in red, 40% in green, and 60% in blue. Black line represents the overall predictive trend. CIs indicate uncertainty on the regression curve.*

For bird traits, the effects of distance differed significantly among landscapes. Both 40% ($a_2 = 0.369$, $p < 0.05$) and 60% ($a_3 = 0.288$, $p < 0.05$) had larger mean BM at baseline level compared to 20%. BM increased significantly with greater distance from the forest edge in 20% ($\beta = 0.003$, $p < 0.001$), while 40% and 60% landscapes showed a negative trend in comparison with 20% (40%: $\gamma_2 = -0.003$, $p < 0.001$), 60%: $\gamma_3 = -0.003$, $p < 0.001$). A similar pattern can be seen in the other four traits, where 20% shows a positive significant

relationship with distance, while the trend in the other two landscapes is significantly different from the one in 20% (all $p < 0.05$, see Appendix 6).

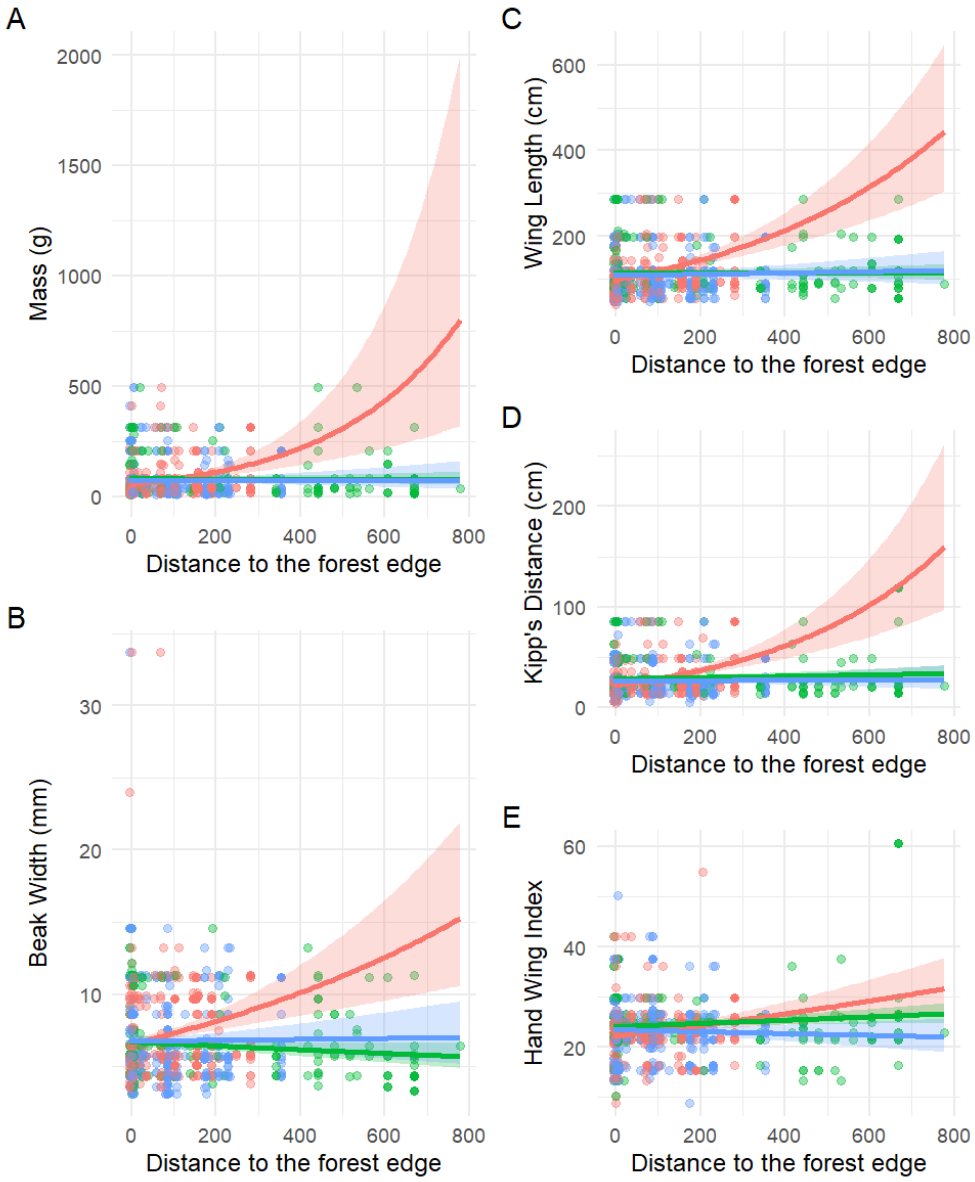


Figure 2. Bird traits relationship with log minimum Distance to Forest Edge: *(A) Body Mass, (B) Beak Width, (C) Wing Length, (D) Kipp's Distance, (E) Hand-Wing Index. Forest cover landscapes are represented 20 % in red, 40 % in green, and 60% in blue. CIs indicate uncertainty in the regression curve.*

Objective 2

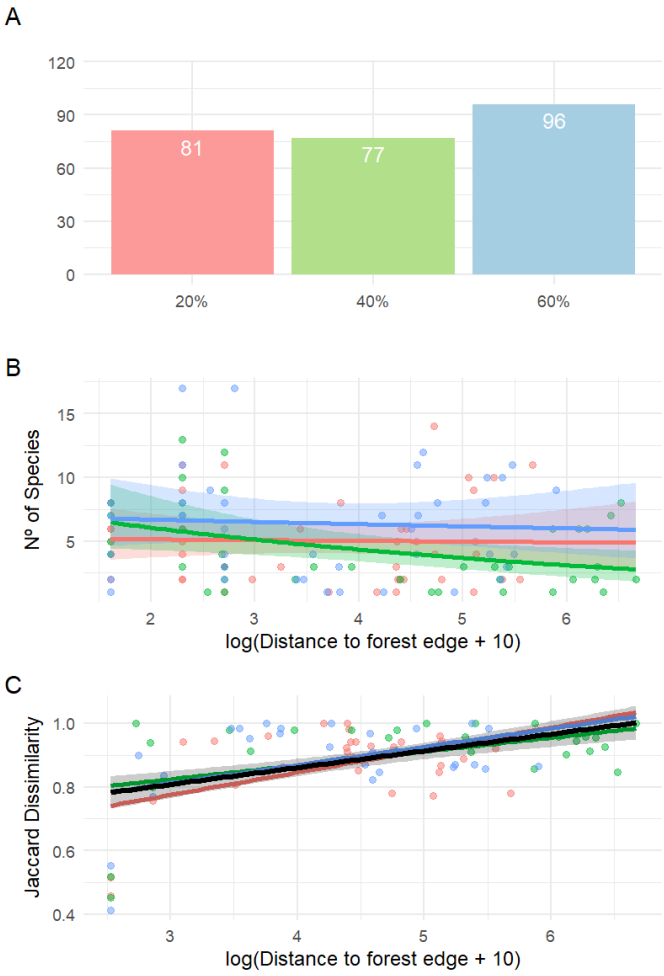


Figure 3: Relationship between plant species richness and landscape variables: A: Plant Species Richness per Forest Cover*. The number of plant species found in each forest-cover landscape.* ***B: Plant species richness per minimum distance to forest edge:*** *The number of plant species as a function of log distance to nearest forest edge per forest cover landscape****. C: Distance decay of dissimilarity in Plant Species:*** *Jaccard dissimilarity index as a function of log distance to nearest forest edge per forest cover landscape. Forest cover landscapes are always represented 20% in red, 40% in green, and 60% in blue. Black line represents the overall predictive trend. CIs indicate uncertainty on the regression curve.*

Plant species richness identified in the birds' seed rain varied between forest cover types. At 20% forest cover, I found 81 species, 77 species at 40%, and 96 species at 60% (Fig. 3.A).

When looking at plant species richness-distance relationships, the model showed no evidence that distance to the forest edge influenced plant species richness at 20% forest cover (β =0.007, p = 0.93, Fig. 3.B). No landscape exhibited a significant relation between distance and richness (all p > 0.100, see Appendix 7). Overall, plant richness remained effectively constant across distances and did not vary meaningfully among landscapes.

However, dissimilarity in plant community composition increased significantly with distance from the forest edge across all landscapes (Fig. 3.C). In 20%, there was a significant

positive effect of distance on community dissimilarity (β =0.07, p = 0.006). No interaction effect of forest cover distance–dissimilarity relationships was found (both interaction terms p > 0.36). Thus, plant communities became more dissimilar with increasing distance overall, maintaining a similar pattern across all cover landscapes.

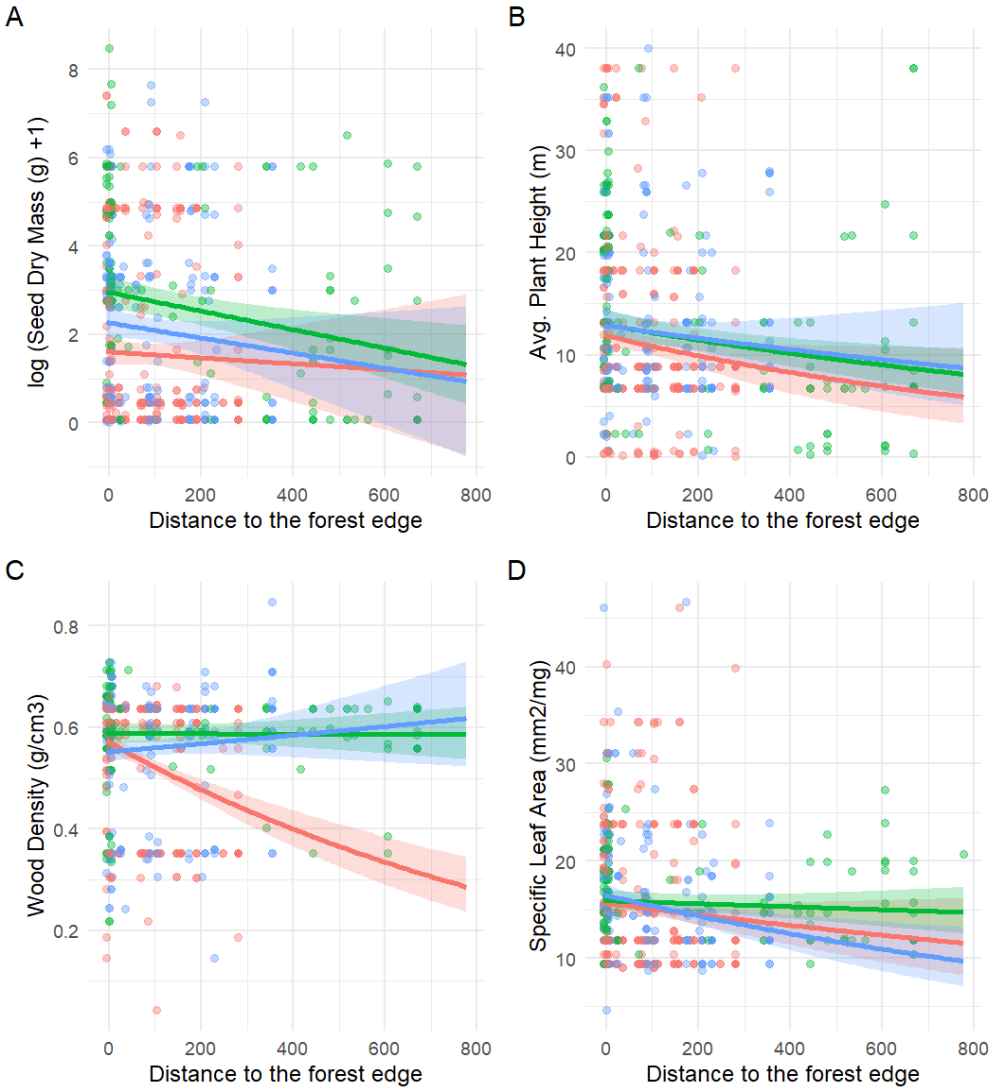


Figure 4. Plant traits relationship with log minimum Distance to Forest Edge: *(A) SDM, (B) APH, (C) WD, (D) SLA. Forest cover landscapes are represented 20% in red, 40% in green, and 60% in blue. CIs indicate uncertainty of the regression curve.*

SDM differed among landscapes at baseline level (Fig. 4.A): 40% and 60% had significantly higher seed mass than 20% at baseline level (40%: a_2 = 1.347, p < 0.001; 60%: a_3 = 0.659, p = 0.005). At 20% there was no relationship with distance to the forest edge (β = <–0.001, p = 0.613), and this trend was not significantly different in the other two landscapes (40%: γ_2 = –0.001, p = 0.331; 60%: γ_3 = –0.001, p = 0.562). APH (Fig. 4.B) did not differ among landscapes at baseline level (40%: a_2 = 0.084, p = 0.274; 60%: a_3 = 0.080, p = 0.316). There was however a significant decline with increasing distance from the forest edge in 20% (β = <0.001, p = 0.035), while the additive terms in the other two landscapes were not significant (40%: γ_2 = <–0.001, p = 0.525; 60%: γ_3 = <0.001, p = 0.497). This indicates that the decreasing distance–height relationship was similar across all landscapes. For WD (Fig.

4.C), no differences at baseline level were found among landscapes (40%: a_2 = 0.029, p = 0.225, 60%: a_3 = –0.034, p = 0.175). WD declined significantly with increasing distance from the forest edge in 20% (β = <–0.001, p < 0.001), while the two other landscapes exhibited significantly shallower distance-related declines compared with the one in 20% (40%: γ_2 = <0.001, p < 0.001; 60%: γ_3 = 0.001, p < 0.001). This indicates that the distance-related decrease in WD was strongest in site 20% while practically flat in the other two landscapes. SLA (Fig. 4.D) did not show variations at baseline level between 20% and the other two landscapes (40%: a_2 = 0.017, p = 0.702; 60%: a_3 = 0.050, p = 0.251). In 20%, no significant relationship with distance from the forest edge was found (β = <–0.001, p = 0.107), and additive terms were non-significant (40%: γ_2 = <0.001, p = 0.282; 60%: γ_3 = <–0.001, p = 0.380), indicating no effect of distance nor forest cover on SLA (see Appendix 8).

4. DISCUSSION

Natural forest regeneration is a key ecological process that contributes to climate mitigation by sequestering carbon in recovering biomass, while simultaneously safeguarding biodiversity and enhancing local livelihoods (Chazdon et al., 2016; Chazdon & Uriarte, 2016; Koch & Kaplan, 2022). Such regeneration typically occurs in open areas where prior land degradation or deforestation has taken place as a result of human activities or natural disturbances (Chazdon & Uriarte, 2016). These disturbed areas represent critical opportunities for natural regeneration, particularly when regeneration is facilitated by bird-mediated seed dispersal. In tropical ecosystems—where the vast majority of canopy trees rely on animals to disperse their seeds—birds play a pivotal role in enabling and accelerating natural forest recovery. However, this study shows that although frugivore communities were richer in higher forest cover landscapes, effective seed-dispersers remained similar across all forest covers. Distance from forest edges implied bird and plant species turnover, although only bird functional turnover happened in highly fragmented landscapes. This functional turnover was not clearly translated into seed-rain composition, and in consequence, contribution to natural forest regeneration by seed dispersing birds was maintained across all levels of fragmentation.

Higher forest cover is typically associated with increased habitat connectivity and the presence of older or more structurally complex forests. These conditions provide a wider range of ecological niches, allowing a richer assemblage of bird species to persist (Martensen et al., 2012). However, not all the species present in the landscape were effective seed-dispersers in open areas. The number of bird species that were effectively dispersing seeds in the landscape was not only lower in comparison to the overall community across all three landscapes, but this reduction in species richness was not proportionate to forest cover. All three forest cover landscapes had a similar effective seed-disperser species richness (~ 44 - 49 species), and the species composition was majorly shared by them all. Landscapes showed to have around two thirds of shared species, meaning only a small proportion was exclusive to each landscape. These species were mostly generalist passerines belonging to genus such as *Turdus*, *Tyrannus,* or *Vireo* (see Appendix 1).

The community of effective seed-dispersing birds was also not shared in totality by the community surveyed in the visual census data. Some species appeared exclusively in the seed rain data: 17 species in 20%, 15 in 40%, and 13 in 60%. This means that, contrary to my expectations, I can not use the community found in the visual census as the overall community, as there are species that clearly were missed in this census that did actively contribute to seed dispersal. But most importantly, there was a large proportion of frugivorous species present in the visual census that were not recorded in the seed rain metabarcoding. These results highlight a key insight: even in landscapes with higher forest cover and greater frugivore diversity, only a subset of species is actually dispersing seeds into open areas. This effect might be caused by forest-specialist birds consistently restricting their foraging to forested patches (Kennedy et al., 2017). When forest remnants are heavily degraded or lack sufficient resources, these specialists may be forced to move more frequently between patches or enlarge their territories to meet their energetic needs (Graham, 2001; Kennedy et al., 2017). This dynamic helps explain my results: although higher forest-cover landscapes support richer bird communities overall, the subset of frugivores that actually disperse seeds into open areas represents only a small fraction of the total community. The missing fraction may consist of forest specialists, who, despite being observed flying across the landscape, likely rely exclusively on forest patches for

foraging, thereby avoiding open areas and contributing little to seed dispersal beyond the forest edge (Kennedy et al., 2017). This result is particularly relevant for studies that solely use the presence, or movement of frugivore birds across the landscape to determine the potential seed dispersal happening in open areas.

In each landscape, the number of effective seed-dispersers remained constant across distances from the nearest forest patch. Contrary to my expectations, species richness was not significantly higher at the forest edge compared to open areas, although there was a non-significant tendency for richness to decline with increasing distance (Fig. 1.B). However, when examining changes in community turnover across distance, all three forest-cover landscapes showed higher community dissimilarity with increasing distance (Fig. 1C). In other words, although mean species richness remained relatively stable, the identity of species present shifted markedly with increasing distance from the forest edge. This pattern was consistent across forest-cover landscapes, indicating that forest cover did not influence the rate of community turnover as birds moved from forest edges into open areas. Because each forest-cover landscape hosts a similar species pool, their respective distance gradients reflect generalist-frugivore community responses to open area environments.

My results now further indicate that the subset of species that does disperse seeds forms two distinct assemblages: one operating near the forest edge and another active in open areas. Such differentiation aligns with well-documented behavioral shifts in bird communities in response to fragmentation, where species do not respond uniformly. Bird assemblages in open areas can consist of both highly mobile guilds capable of broad-scale movements and less mobile species restricted to short-range movements (Neuschulz et al., 2013). Additionally, forest specialists rarely venture into open habitats and generally avoid using resources outside forest patches (Kennedy et al., 2017), however, brief foraging flights near the forest edge remain possible if forest patches are poor in resources (Graham, 2001; Imbeau et al., 2003; Schlinkert et al., 2016). This behavior from forest specialists may also explain this pattern, where the taxonomical turnover is driven by forest specialists dispersing seeds close to the forest edge.

Importantly, these behavioral responses are closely tied to species functional traits, which shape their capacity and willingness to cross open areas (Neuschulz et al., 2013; Ramos et al., 2020). When paying attention to how bird functional traits changed with distance to the nearest forest edge, differences among forest cover landscapes appeared. All five traits (BM, BW, WL, HWI, and KD) showed a positive relationship with distance exclusively at the lowest forest cover landscape (20%). Despite community species composition gradually becoming more different with distance across all forest covers, it was only at the lowest forest cover where the community experienced functional turnover. Meaning in this landscape, bird communities not only became more dissimilar taxonomically but also larger in size the further they moved away from the forest edge. It is important to note that this tendency in the 20% landscape began with lower mean trait values at the forest edge, and showed higher values at larger distances, when compared with the other two landscapes (Fig 2). In contrast, in the 40% and 60% landscapes, birds of all sizes ventured into open areas without exhibiting significant changes in mean trait values across distance. In 20%, however, there was a clear tendency for smaller birds to disperse seeds closer to the forest edge, whereas larger birds were more frequently associated with open areas.

This trend is likely driven by the distance between patches and lack of connectivity in highly fragmented landscapes. At lower forest covers, large-sized frugivores, that is, heavier, with wider gapes and longer wingspans, are forced to venture into open areas (Graham, 2001; Neuschulz et al., 2013; Ramos et al., 2020). These species would otherwise use the

continuous forest patch to move across the landscape (Ramos et al., 2020; Zhu et al., 2025). Due to the perceived lack of safety in exposed perching sites: many large bird species can face heightened risks from both poaching and natural predators and therefore preferentially perch within dense vegetation, where structural cover provides greater protection (Thornton et al., 2012). However, in the absence of continuous forest, highly mobile forest specialists can cross open areas looking for distant forest patches with higher availability of resources (Graham, 2001; Neuschulz et al., 2013). In this process, they may use landscape structures as steppingstones where they occasionally disperse seeds in open areas (González-Varo et al., 2014; Mueller et al., 2014; Wunderle, 1997). In contrast, 40 and 60% forest covers, thanks to a better connected landscape, allow forest specialists to navigate through a continuous forest patch. The remaining seed-dispersers in open areas would then consist of habitat generalists adapted to disturbed environments that share similar functional traits (De Coster et al., 2015; Imbeau et al., 2003; Zhu et al., 2025). Therefore, the dispersing bird community near the forest edge and in open areas is functionally the same one. These results are opposite to my first hypothesis, which is that I would find higher variability of frugivore functional traits in less fragmented landscapes (Martin & Fahrig, 2018; Price, 2006).

Through the changes in bird community composition across distances, the diversity and functional traits of plants that can regenerate in open areas can be filtered as well. I found a solid higher species richness of seeds at 60% forest cover (Fig. 3.A), most probably due to a higher plant species richness in the highest forest cover landscape. However, seed species richness, similarly to bird's one, did not vary significantly with distance across all forest cover landscapes (Fig. 3.B). Although the turnover of bird functional traits in 20% could serve as an indicator that larger birds disperse a greater diversity of seeds in open areas (Carlo et al., 2022), this effect is not seen in my results. Hence, it is safe to assume that as bird species' richness remains stable across distance, the regenerating plant species richness they are dispersing does as well. Although seed richness does not change, species composition does. Seed species composition—much like bird species composition—became increasingly dissimilar with distance from the forest across all forest-cover landscapes (Fig 3.C). This indicates that the assemblage of seed species found near the forest edge differs markedly from the assemblage deposited in open areas. Given that these two ecotones are used by distinct bird communities, it is likely that differences in their diets lead to the dispersal of different sets of plant species (Carlo et al., 2022), ultimately producing the observed divergence in plants that can regenerate in open areas.

It is expected that with the functional turnover of the bird community across distance in the 20% forest cover landscape, the cohort of seeds being dispersed would see a change in their functional traits too. As the bird community becomes dominated by larger-bodied species further from the forest, so does their ability to consume and disperse larger seeds. However, this pattern is not reflected in my results: SDM— a trait associated with seed size— did not show any significant relationships with distance. Despite this, there is a weak tendency in the two higher forest cover landscapes for SDM to diminish with distance, where larger seed sizes are not reaching open areas. In these cases, larger seeds are likely missing from the seed rain in open areas due to the absence of large-bodied frugivores (Bello et al., 2024). However, SDM is consistently lower across the distance gradient in the 20% landscape compared to the other two, making it difficult to attribute any observed patterns in seed-rain traits to the influence of large-bodied frugivores. APH declined across all forest covers, contrary to my expectations. A positive response was not seen in the 20% landscape, which would confirm the hypothesis that larger birds are dispersing seeds from potentially larger trees, as seed size and plant height are positively correlated (Bello et al., 2015). Interestingly, WD displayed the opposite pattern to SDM, with a decreasing trend

detected only in the 20% landscape. Given that WD is positively related with seed size (Bello et al., 2015), WD should show an increase with distance as bird gapes become wider as well, but I did not observe this coherent pattern. Additionally, SLA did not show any significant trends whatsoever. These inconsistent patterns in plant traits are likely driven by the abundance-weighted nature of the data, in which the high density of small seeds in open areas outweighs the occasional presence of larger seeds. Birds with wider gape widths can consume both small and large seeds, and do not necessarily prefer larger fruits; thus, when larger fruits are scarce, their dispersal activity may be dominated by small-seeded species.

My results should be considered suggestive rather than definitive and generalized with caution. Several limitations in the study design may have influenced the patterns observed. First, although I used landscape-level forest cover and distance to the nearest forest edge as proxies for fragmentation, bird movement is often more strongly shaped by other landscape factors. The proximity of each seed trap to neighboring forest remnants, stepping stones, and resource-rich patches likely plays a more decisive role than broad forest-cover categories (Graham & Blake, 2001). Future work should incorporate these spatially explicit metrics of connectivity and patch quality.

Secondly, sample abundance was uneven across forest-cover landscapes and distances to the nearest forest edge. Not all seed traps yielded comparable numbers of samples, introducing potential bias in the representation of species and trait relationships. In particular, the 20% forest-cover landscape did not exhibit a decline in sample abundance with increasing distance, whereas both the 40% and 60% landscapes did. This imbalance may partly explain why patterns observed in the 20% landscape differ from those in more forested landscapes.

Lastly, the metabarcoding dataset contained taxonomic uncertainties. While bird reference libraries are nearly complete, tropical plant DNA databases remain incomplete, leading to many unresolved or ambiguous reads. In several cases, sequences matched multiple species with equal probability, requiring random assignment—an inherent limitation of DNA metabarcoding due to reference-library gaps, amplification biases, and degraded plant DNA in avian gut contents (Ando et al., 2020). Additionally, incomplete coverage in TRY Plant Trait Database meant several species and genuses lacked trait values, reducing sample size for trait-based analyses and potentially biasing community-level estimates.

5. CONCLUSION

The results of this study show that forest fragmentation strongly influences bird-mediated seed dispersal in open areas. Not all frugivore species act as effective dispersers outside forest patches, and—contrary to expectations—forest cover did not affect the richness of effective seed-dispersers nor their taxonomic turnover with distance from the forest edge. However, functional turnover in bird traits emerged exclusively in the most fragmented landscape (20% forest cover), where larger-bodied species ventured further into open areas. Yet, this shift did not produce corresponding differences in seed-rain composition or functional traits. Overall, effective seed-disperser communities remained similar across open areas, dominated by open-area generalists that disproportionately sustain seed dispersal. These species play a crucial role in delivering a diverse seed cohort to open areas and thereby supporting natural forest regeneration. Nonetheless, highly fragmented landscapes are more prone to shifts in disperser identity and function, underscoring the need to tailor regeneration actions to local conditions. In such settings, active restoration interventions may improve bird movement of generalist species across the matrix (Wunderle, 1997). These measures may help homogenize seed-rain functional composition across space, promoting the arrival of higher-carbon-storage seed types in open areas. Enhanced connectivity may also attract richer frugivore assemblages from nearby mature forests (Arroyo-Rodríguez et al., 2017), further strengthening regeneration processes.

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7. LIST OF ABBREVIATIONS

STRI : Smithsonian Tropical Research Institute

BCNM : Barro Colorado Nature Monument

BM : Body Mass

BW : Beak Width

WL : Wing Length

HWI : Hand-Wing Index

KD : Kipp's Distance

SDM : Seed Dry Mass

APH : Average Plant Height

WD : Wood Density

SLA : Specific Leaf Area

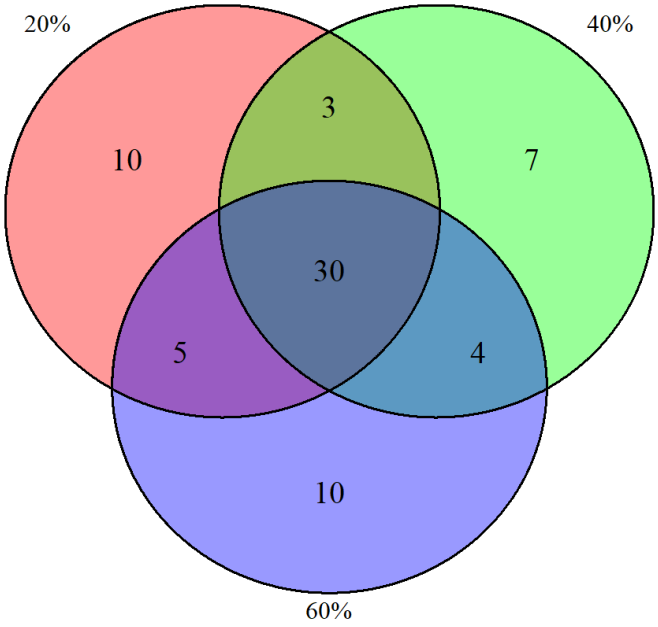
CI : Confidence Interval

8. APPENDICES

TABLE OF APPENDICES

APPENDIX 1

Venn Diagram of effective seed disperser communities across forest cover landscapes.



Species common to all three landscapes: *Baryphthengus martii, Brotogeris jugularis, Cyanerpes cyaneus, Dacnis cayana, Elaenia flavogaster, Euphonia luteicapilla, Leptotila verreauxi, Manacus vitellinus, Melanerpes pucherani, Melanerpes rubricapillus, Milvago chimachima, Mimus gilvus, Myiodynastes maculatus, Myiozetetes cayanensis, Myiozetetes similis, Ortalis cinereiceps, Psarocolius decumanus, Ramphocelus dimidiatus, Saltator striatipectus, Tangara inornata, Tangara larvata, Thraupis episcopus, Thraupis palmarum, Trogon massena, Turdus grayi, Tyrannus forficatus, Tyrannus melancholicus, Vireo altiloquus, Vireo flavoviridis, Vireo olivaceus.*

APPENDIX 2

Richness x Distance Model Comparisons

Model	Samples		Birds		Plants	
Term	**df**	**AIC**	**df**	**AIC**	**df**	**AIC**
GLM	7.00	924.87	7.00	670.86	7.00	649.42
GAM	11.06	915.07	10.24	669.21	7.50	649.81
GLM with Transect as random intercept	8.00	915.00	8.00	663.30	8.00	650.38

APPENDIX 3

ANOVAs for testing Transect as random factor

Response variable	Base model	Random-effect model	ΔAIC	χ^2	df	p-value
Samples	samples ~ SITE × log(dist + 10)	+ (1 | TRANSECT_TREE)	−9.87	11.87	1	<0.001***
Bird richness	richness ~ SITE × log(dist + 10)	+ (1 | TRANSECT_TREE)	−7.56	9.56	1	0.002**
Plant richness	richness ~ SITE × log(dist + 10)	+ (1 | TRANSECT_TREE)	+0.97	1.04	1	0.309

APPENDIX 4

Sample abundance x Distance model output

Model terms definitions: Intercept = μ, distance = β, 40% = a_2 , 60% = a_3 , distance*40% = γ_2 , distance*60% = γ_3

Term	Estimate	Std.error	z-value	p-value
Intercept	2.680	0.313	8.554	<0.001***
distance	-0.064	0.077	-0.825	0.409
40%	0.680	0.379	1.795	0.073
60%	0.687	0.414	1.658	0.097
distance *40%	-0.214	0.095	-2.245	0.024
distance *60%	-0.091	0.110	-0.833	0.405

*** p(H0)<0.05; ** p(H0)<0.01; *** p(H0)<0.001**

APPENDIX 5

Bird Richness x Distance model output

Term	Estimate	Std.error	z-value	p-value
Intercept	1.869	0.268	6.954	<0.001***
distance	-0.065	0.068	-0.950	0.342
40%	0.397	0.340	1.168	0.243
60%	0.286	0.356	0.803	0.422
distance *40%	-0.124	0.087	-1.429	0.153
distance *60%	-0.015	0.094	0.166	0.868

*** p(H0)<0.05; ** p(H0)<0.01; *** p(H0)<0.001**

APPENDIX 6

Bird traits x Distance model output

Trait	Term	Estimate	Std.Error	t-value	p-value
BM	**Intercept**	3.502	0.168	20.836	<0.001***
	distance	0.212	0.043	4.970	<0.001***
	40%	0.853	0.228	3.739	<0.001***
	60%	1.044	0.229	4.551	<0.001***
	distance * 40%	-0.209	0.057	-3.649	<0.001***
	distance * 60%	-0.281	0.058	-4.819	<0.001***
BW	**Intercept**	1.722	0.066	25.920	<0.001***
	distance	0.065	0.016	3.882	<0.001***
	40%	0.220	0.090	2.444	<0.05*
	60%	0.302	0.091	3.332	<0.001***
	distance *40%	-0.085	0.023	-3.760	<0.001***
	distance *60%	0.095	0.023	-4.128	<0.001***
WL	**Intercept**	4.315	0.070	61.675	<0.001***
	distance	0.113	0.018	6.405	<0.001***
	40%	0.424	0.094	4.472	<0.001***
	60%	0.446	0.095	4.671	<0.001***
	distance *40%	-0.113	0.024	-4.749	<0.001***
	distance *60%	-0.131	0.024	-5.402	<0.001***

Trait	Term	Estimate	Std.Error	t-value	p-value
HWI	**Intercept**	3.034	0.033	91.520	<0.05*
	distance	0.026	0.008	3.161	<0.05*
	40%	0.119	0.045	2.653	<0.05*
	60%	0.123	0.045	2.724	<0.05*
	distance *40%	-0.013	0.011	-1.196	0.232
	distance *60%	-0.029	0.011	2.529	<0.05*
KD	**Intercept**	2.741	0.092	29.716	<0.001***
	distance	0.150	0.023	6.423	<0.001***
	40%	0.561	0.125	4.485	<0.001***
	60%	0.613	0.125	4.872	<0.001***
	distance *40%	0.128	0.031	-4.092	<0.001***
	distance *60%	-0.171	0.032	-5.355	<0.001***

*** p(H0)<0.05; ** p(H0)<0.01; *** p(H0)<0.001**

Appendix 7

Plant richness x Distance model output

Term	Estimate	Std.error	z-value	p-value
Intercept	1.662	0.301	5.511	<0.001
distance	-0.011	0.075	-0.148	0.882
40%	0.473	0.384	1.231	0.218
60%	0.295	0.402	0.735	0.463
distance*40%	-0.155	0.096	-1.611	0.107
distance*60%	-0.016	0.104	-0.153	0.878

* p(H0)<0.05; ** p(H0)<0.01; *** p(H0)<0.001

APPENDIX 8

Plant traits x Distance model output

Trait	Term	Estimate	Std.Error	t-value	p-value
SDM	**Intercept**	1.606	0.159	10.100	<0.001***
	distance	<-0.001	0.001	-0.506	0.613
	40%	1.347	0.246	5.478	<0.001***
	60%	0.659	0.236	2.797	0.005**
	distance * 40%	-0.001	0.001	-0.972	0.331
	distance * 60%	-0.001	0.001	-0.579	0.563
APH	**Intercept**	2.474	0.051	47.956	<0.001***
	distance	<-0.001	<0.001	-2.103	0.036*
	40%	0.084	0.077	1.095	0.274
	60%	0.080	0.080	1.003	0.316
	distance*40%	<0.001	<0.001	0.635	0.525
	distance*60%	<0.001	<0.001	0.679	0.497
WD	**Intercept**	-0.560	0.016	-33.873	<0.001***
	distance	<-0.001	<0.001	-6.579	<0.001***
	40%	0.029	0.024	1.215	0.225
	60%	-0.034	0.025	-1.358	0.175
	distance*40%	<0.001	<0.001	5.910	<0.001***

Trait	Term	Estimate	Std.Error	t-value	p-value
	distance*60%	0.001	<0.001	5.645	<0.001***
SLA	**Intercept**	2.751	0.029	95.815	<0.001***
	distance	<-0.001	<0.001	-1.615	0.107
	40%	0.017	0.044	0.383	0.702
	60%	0.050	0.044	1.148	0.251
	distance*40%	<0.001	<0.001	1.077	0.282
	distance*60%	<-0.001	<0.001	-0.878	0.380

*** p(H0)<0.05; ** p(H0)<0.01; *** p(H0)<0.001**