

REFORESTATION AND INSECTIVORY DYNAMICS: INSIGHTS FROM THE  
CHOCÓ RAINFOREST OF ECUADOR

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## **Abstract**

In the tropics, where deforestation rates are unparalleled, reforestation is a crucial strategy in combatting climate change, recovering biodiversity, and protecting ecosystem services. A nascent reforestation project in Ecuador's Chocó rainforest offers a unique system to study ecosystem recovery through the lens of insectivory. In this study, we used clay caterpillars to compare insectivory rate and insectivore assemblage between two reforestation treatments with different planting methods and four stages of successional forest. We found that the two reforestation treatments supported insectivory at an equal rate higher than that found in pasture. Caterpillars experienced increased predation pressure with succession. Between reforestation treatments, caterpillars were more likely to be predated by birds in sites with reduced herb cover; however, we found no compositional differences in the insectivore community between habitats of different successional stages. This study presents a novel application of the clay caterpillar method in order to assess reforestation's capacity to recover ecological processes that have been disrupted by forest fragmentation.

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## **Introduction**

### *Background*

Tropical forests are essential to life on Earth. They harbor unmatched levels of biodiversity (Turner 2004, Perfecto and Vandermeer 2008) and perform ecosystem services such as water and nutrient cycling, erosion control, oxygen release, food and medicinal plant provisioning, and climate regulation via carbon sequestration (Brandon 2014, Chazdon 2014). Despite their importance, these ecosystems face extensive deforestation: 8.6 million hectares of tropical forest were lost from 1990-2000 (Chazdon 2014). The loss of tropical forest is largely driven by agricultural expansion (Aide et al. 2013, Svampa 2019), and threatens ecosystems' biodiversity, function, and structure, along with the services they provide (Thébault and Loreau 2006, Correa-Cuadros et al. 2022). This is particularly true in Latin America, where primary commodity-based economies demand resource extraction at a grand scale and necessitate major land conversion, a pattern established in colonial times and expanding in the modern era (Svampa 2019). Given that most of the world's tropical forests are located in Latin America (Chazdon 2014), Neotropical deforestation is an issue of global concern.

To address the consequences of land conversion, reforestation is a major strategy (Chazdon 2017). Forest regeneration can be split into two technical categories: natural and assisted. In natural regeneration (also called "passive restoration"), the disturbing factor (e.g. a farm) is removed, and seeds are recruited from the existing seedbank or dispersed from nearby plant communities (Corbin and Holl 2012). In assisted regeneration (also called "active restoration"), plant establishment is human-mediated (e.g. planting trees) (Corbin and Holl 2012). These techniques have been implemented

around the world to varying degrees of success (Chazdon 2017). Despite alarming rates of deforestation in the Neotropics, parts of the region are actually gaining forest cover through active or passive restoration (Holl 2007, Chazdon 2017). In one Costa Rican province, for example, forest cover doubled between 1979 and 2005 as smallholders abandoned their farms, leaving them to regenerate naturally (Chazdon 2017). But while forest restoration is often successful in re-establishing tree cover in disturbed areas, it must be recognized that ecological processes do not all recover at an equal rate (Chazdon 2014, Jones et al. 2018). Thus, restoration's capacity to "restore" forest ecosystems in a given timeline is variable and depends on the ecological process being examined.

In this study, we investigate the recovery of one ecological process, insectivory (predation on insects), in habitats undergoing passive and active forest restoration. Insects play a substantial role in forest ecosystems, mediating many ecological processes, including decomposition, pollination, parasitism, and herbivory (Fáveri et al. 2008). Moreover, they are an important food source for a wide range of insect-eating animals, or insectivores (Seifert et al. 2016). Given their relevance to various ecosystem functions, restoring insects' interactions with other species, including plants and predators, is an important aspect of forest restoration. Here, we assess insectivory dynamics during forest regeneration using artificial caterpillars (larval moths and butterflies, Lepidoptera).

Previous studies investigating the effects of forest characteristics on Lepidoptera predation rates have yielded inconsistent results. While some suggest that tree density, fragmentation, and proximity to forest edges have no impact on insectivory rates (Koh & Menge, 2006; Leles et al., 2017), others indicate that tree density is positively correlated with predation by certain arthropod species (Yang et al., 2018). Since arthropods are the

dominant predators of Lepidoptera larvae across all habitats (Koh & Menge, 2006; Leles et al., 2017), Yang et al.'s (2018) finding suggests that insectivory rates in a deforestation context are partially regulated by arthropods' response to disturbance, which is likely to vary by species and region. In addition, diversity of the plant community may affect insect predation rates. It has been proposed that higher plant species richness should increase overall rates of predation due to the diversification of niches available to predators ('enemies hypothesis,' Root 1973), but research has demonstrated variable levels of support for this hypothesis (Leles et al. 2017, Yang et al. 2018). Given these incongruencies, the effects of forest characteristics on insectivory dynamics in regenerating forest are unclear. Our study seeks to address this knowledge gap.

Abandoned pastures, or "old fields," have long been a topic of interest for ecologists studying succession, as they provide a system to observe natural regeneration (Hobbs and Cramer 2007). At Fundación para la Conservación de los Andes Tropicales (FCAT), a reserve in the Chocó rainforest of northwest Ecuador, a reforestation project was recently established within a matrix of old fields, creating a unique system in which to study insectivory dynamics in the context of reforestation. The project uses an active restoration technique called "applied nucleation." Applied nucleation involves the planting of tree "islands", which are expected to accelerate regeneration by attracting animal seed dispersers to the area, thus helping to populate the inter-island zones with new plant growth (Figure 1; Holl et al. 2020). This method is an alternative to the commonly used "plantation" style of reforestation, in which seedlings are planted in a grid across the regenerating land. These reforestation techniques differ from passive restoration in many aspects including the speed that habitat is restored, which by

extension may influence how fast important ecological processes, like insectivory, are rescued.

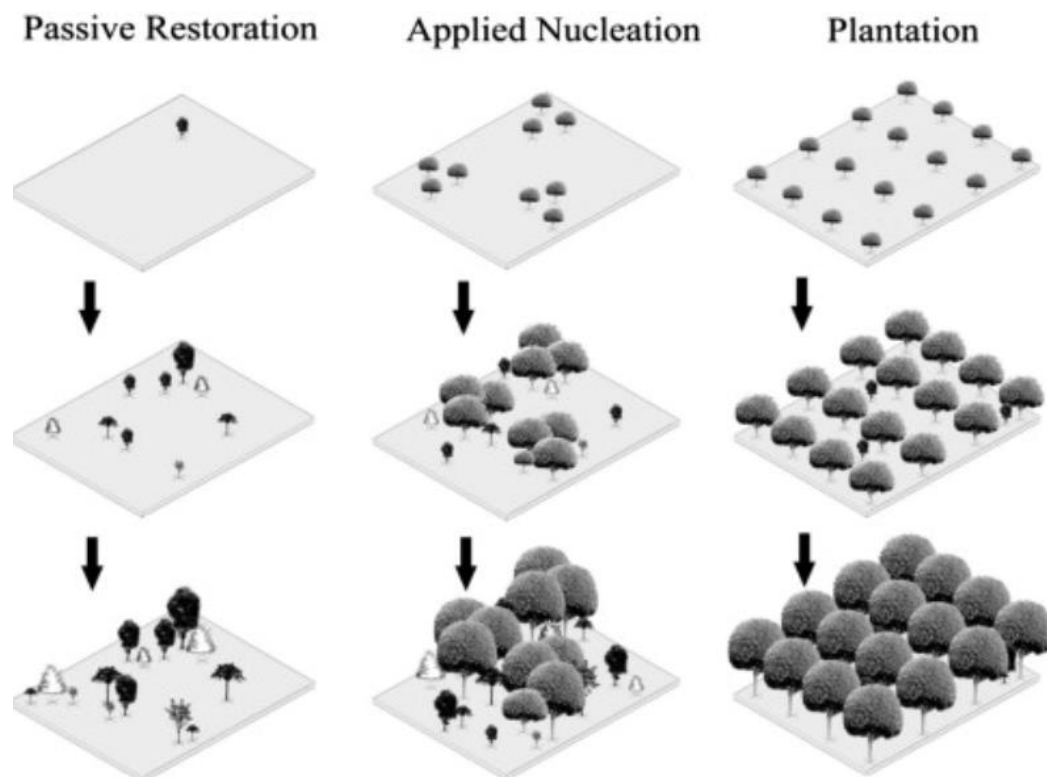


Figure 1: Comparison of three restoration techniques over time (Corbin and Holl 2012). Note the accelerated rate of succession in the active restoration methods, compared to passive restoration.

There are multiple advantages to applied nucleation compared to the other techniques. First, it is both cheaper and more easily executed than the plantation technique because it requires fewer trees to be planted across smaller plots of land. Second, it is faster than passive restoration (Holl et al. 2020), which can be particularly slow in areas with degraded soil and few remnant trees (mature trees that are growing in an otherwise deforested area) (Corbin and Holl 2012). Third, applied nucleation mimics natural successional patterns, as it establishes “clumps” of plants from which new vegetation spreads outwards (Yarranton and Morrison 1974, Corbin and Holl 2012), while leaving room for diverse seedling establishment. Finally, by planting a mix of old-

growth species and trees of economic importance, people undertaking applied nucleation gain a degree of agency not offered by natural regeneration methods regarding which trees grow on their land, providing an economic incentive to reforest. Landowners can selectively harvest species of economic interest like balsa (*Ochroma pyramidalis*) while allowing other trees to mature and persist. With these considerations in mind, applied nucleation has recently gained popularity as a reforestation technique and is being implemented worldwide (Holl et al. 2020). The growing relevance of applied nucleation means that it is crucial we understand its effects on species interactions. FCAT established its reforestation plots in 2022 using an experimental design aimed to improve the efficacy of the applied nucleation technique by identifying the importance of island spacing, among other variables, for forest regeneration. In this study we consider applied nucleation parcels with either 30m or 10m spacing between islands. While previous studies have measured insectivory in naturally regenerating forest fragments (Koh and Menge 2006, Fáveri et al. 2008), ours is the first to investigate insectivory dynamics in an active restoration site.

We will evaluate how forest regeneration affects predation on caterpillars by collecting insectivory data from land parcels within FCAT's applied nucleation project, as well as from control sites in primary forest, secondary forest, and abandoned pastures that are naturally regenerating with and without remnant trees. In addition, in the context of the applied nucleation plots, we will provide a basis to examine the impacts of island spacing on insectivory. As the reforestation project is young, the results of this study will establish a baseline from an early-successional forest that will allow temporal

comparisons with future iterations of this study at later stages of succession. Specifically, we will address the following research questions:

### *Research Questions*

- 1) How does insectivory rate and predation by different predator groups vary among the following habitats: primary forest, secondary forest, pasture with and without remnant trees undergoing passive restoration, and applied nucleation parcels?
- 2) Within the applied nucleation parcels, how does the distance between tree islands affect insectivory rate and predation by different predator groups?

### *Predictions*

- 1) I predict that rate of caterpillar predation will increase with succession stage, with the primary forest and the high-impact, treeless pasture presenting the highest and the lowest rates of insectivory, respectively (Figure 2A). This follows the rationale that plant diversity and understory complexity positively affect predation on insects (Leles et al. 2017).
- 2) Among the applied nucleation parcels, I predict that parcels where tree islands are further apart (30m) will have higher insectivory rates than parcels where islands are closer to each other (10m), due to higher herb cover in the former group (Figure 2A). Herbs are a relevant distinguishing factor between the two treatment types, given the current small height of the planted trees, and the fact that islands are being managed to control grass, while the area between islands is left untrimmed. Compared to parcels where islands are separated by 10 meters, the 30m-spacing parcels have more untrimmed area and therefore higher herb cover

and, supposedly, higher herb diversity (Figure 4). Both herb cover and herb species richness have been found to increase predation on caterpillars (Leles et al. 2017).

- 3) In terms of predator type, I expect the relative importance of avian predation to increase with habitat openness (Figure 2B). As birds are visual predators and can move more freely in low-density vegetation structures (Yang et al. 2018), they may have greater success foraging in degraded habitats than arthropods, which are dispersal limited and often rely on a complex forest understory for movement, foraging and breeding (Leles et al. 2017). A negative correlation has been observed between rates of arthropod and bird predation (Yang et al. 2018). Thus, I predict the proportion of total predation attempts done by birds to be highest in the treeless pasture habitats and the applied nucleation parcels (regardless of spacing), where arthropods are limited. I predict that avian predation will be slightly less important, relative to arthropod predation, in the pasture with remnant trees, as trees create visual blocks for predators. For the same reason, I predict that the importance of avian predation will be lower in the secondary forest, and to be lowest in the primary forest (Figure 2B). Within the applied nucleation parcels, I predict that the rate of arthropod predation will be higher in 30m spacing parcels than in 10m spacing parcels, due to increased herb cover and presumed diversity in the former group, factors which increase predation by ants, the main arthropod insectivores (Leles et al. 2017) (Figure 2C). I predict that the

rate of avian predation will be consistent across spacing treatments due their equally treeless state (Figure 2D).

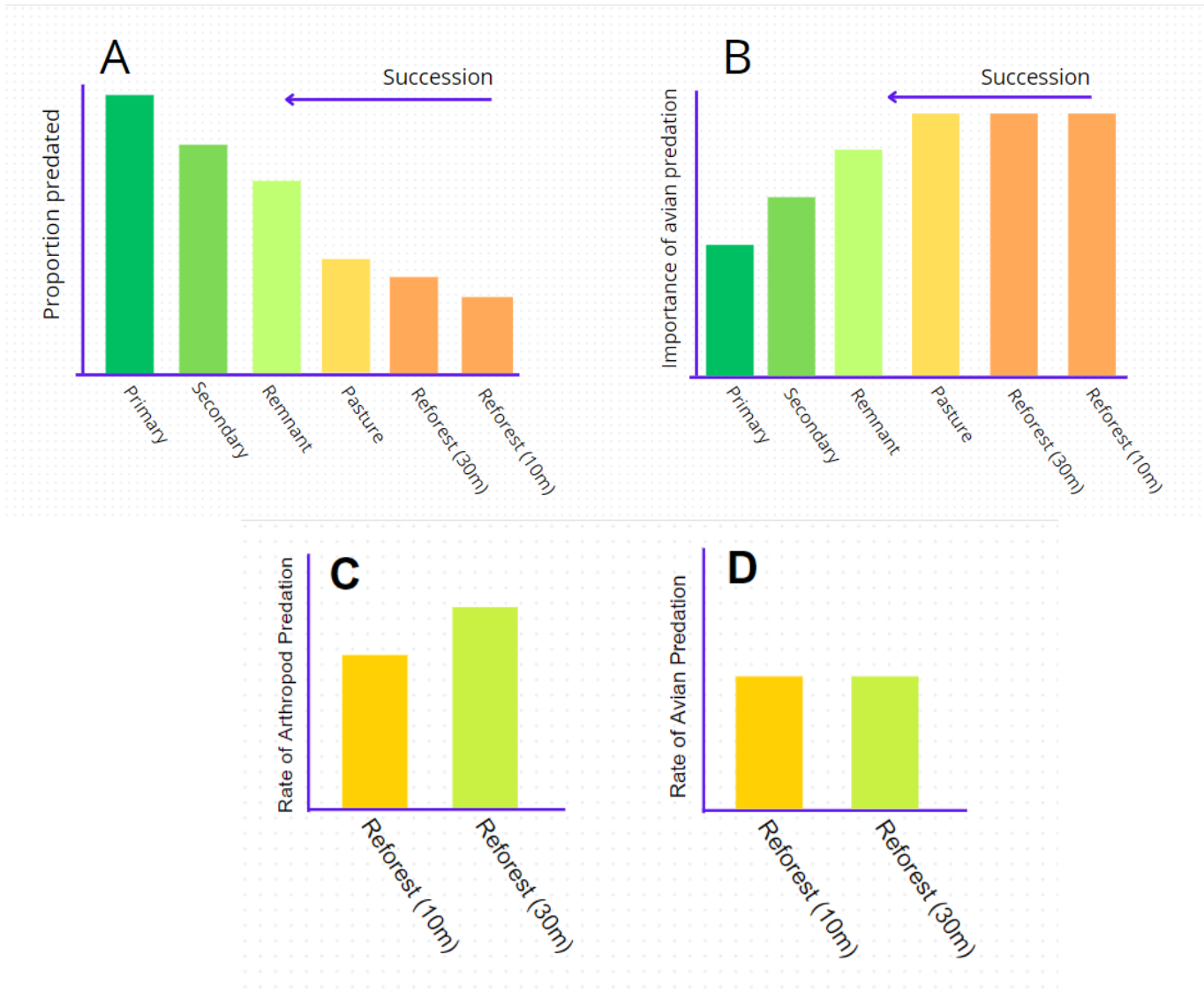


Figure 2: Predictive graphs. (A) Insectivory rates will increase with forest succession, due to increased tree diversity and habitat for predators. Insectivory will be lowest in the reforestation parcel with close island spacing, due to decreased herb cover. (B) The relative importance of avian predation will decrease with succession, due to increased tree cover and understory complexity which favors arthropod predators. (C) In applied nucleation sites, arthropods will predate caterpillars at a higher rate in parcels with 30m inter-island spacing than in parcels with 10m spacing, due to increased herb cover and diversity which facilitates arthropod (particularly ant) foraging. (D) Birds will predate caterpillars equally in applied nucleation parcels regardless of spacing, due to their similar lack of trees.

## Methods

### *Study Area*

We collected data from June 12 to July 12, 2023 at Fundación para la Conservación de los Andes Tropicales (FCAT) (79°68'W, 0°40'N), a nearly-1500 acre private rainforest reserve within the federally operated Mache Chindul Ecological Reserve in Northwest Ecuador, located in the Chocó biogeographic region (Figure 3) (Durães et al. 2013, FCAT n.d.). The Chocó is characterized by high species richness and endemism and is recognized globally as a biodiversity hotspot (Lamperty et al. 2021). The study region has a mean annual temperature of 23-26 °C and mean annual rainfall of 2-3m, with a wet season (January-June) and a dry season (July-December) (Durães et al. 2013), although in 2023 it experienced heavy rains into July due to effects of El Niño. The area surrounding FCAT has experienced increased deforestation since the 1970's, when internal immigration to the region grew, and small-scale agriculture and cattle ranching ensued (Durães et al. 2013). Due to these land use changes, the landscape around FCAT comprises a patchwork of successional stages, including recently abandoned pasture, secondary forest that has regenerated naturally after being abandoned 15-20 years ago, and old growth rainforest (Lamperty et al. 2021). We sampled insectivory data in each of these natural regeneration habitat types.

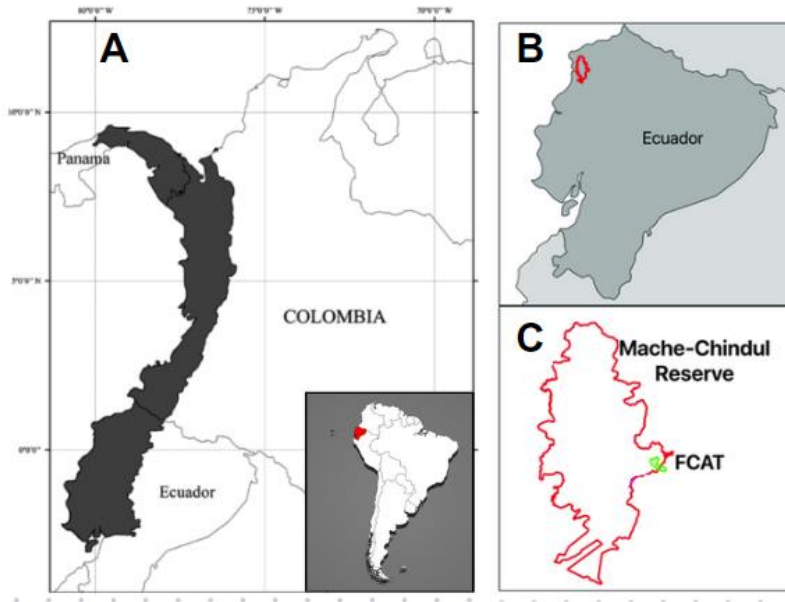


Figure 3: Map of study area. (A) Map of the Chocó biogeographic region with Ecuador highlighted in red (Correa-Cuadros et al. 2022, Freepik n.d.). (B) Map of Ecuador with the Mache-Chindul Ecological Reserve outlined in red. (C) Map of the Mache-Chindul Ecological Reserve with the FCAT reserve outlined in green (B,C, Lueder et al. 2022).

### *Natural regeneration sites*

We collected data in four parcels of land (125m×125m) undergoing natural regeneration (Table 1, Figure 5). We sampled two parcels of land containing recently abandoned pasture. The first had been used for cattle grazing before FCAT purchased the land and removed the cattle in December 2021. At the time of this study, the site contained few trees and was dominated by grasses, which had grown to over a meter high. Due to its complete deforestation, we consider this parcel to be the most highly impacted by anthropogenic activity out of the sites we study. We refer to it as “highly impacted pasture” or simply “pasture.”

The second pasture parcel also bore cattle before 2021 but did not undergo a complete clear cut in its conversion to grazing land. Rather, the previous landowner left several trees (“remnant trees”) which remain today, along with grasses, forbs, and fallen

logs. In disturbed tropical forests, remnant trees drive succession by acting as “recruitment foci,” providing seeds and habitat for animal seed dispersers and thus promoting seedling recruitment (Corbin and Holl 2012). Because remnant trees give this site a successional head start, we consider it to be less disturbed than the first pasture. We refer to this parcel as “remnant tree pasture” or simply “remnant.”

We sampled one parcel in secondary forest, which has been regenerating naturally from a fragmented state for 15-20 years. In the manner of mid-successional tropical rainforests, this site is characterized by the presence of recently established old growth tree species, which are slow-growing and shade tolerant, sheltering a crowded understory dominated by vines, woody lianas, and large-leaved herbaceous plants such as camacho (*Xanthosoma sagittifolium*) (Chazdon 2014). Light intensity is much lower than in pasture, and grasses are absent.

Lastly, we sampled one parcel containing primary forest, which has no recent history of land conversion. This forest is characterized by low light conditions, a relatively open understory, a predominance of old growth tree species and the presence of tree fall gaps (Clark 1996, Chazdon 2014).

#### *Reforestation sites*

We collected data in eight parcels (125m×125m) undergoing reforestation by applied nucleation (Table 1, Figure 5). Previously containing pasture, FCAT established these reforestation parcels in 2022 using different planting variables, including tree diversity, frugivore network connectivity, and inter-island spacing. We focused on the inter-island spacing variable. We sampled from four parcels with 10m spacing between tree islands and four parcels with 30m spacing. The islands (15m×15m) were mowed

regularly to prevent competition with the seedlings, while the space between islands was unkempt, the grass left to grow tall. Due to this maintenance, parcels with 10m spacing were dominated by open space with close-cut vegetation, punctuated by narrow aisles of grass between the tree islands. In contrast, parcels with 30m spacing, having fewer mowed islands, were dominated by the grasses of the inter-island zones (Figure 4).

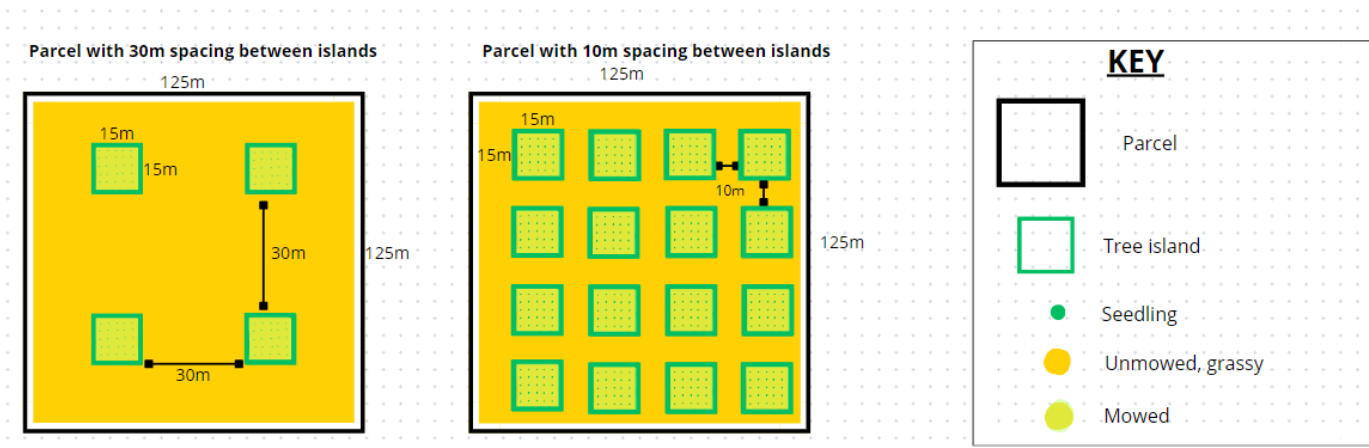


Figure 4: In the applied nucleation parcels, tree islands were maintained by regular mowing, whereas the inter-island spaces were left unmowed. Parcels with 10m inter-island spacing have more islands, and thus more mowed area, than parcels with 30m spacing.

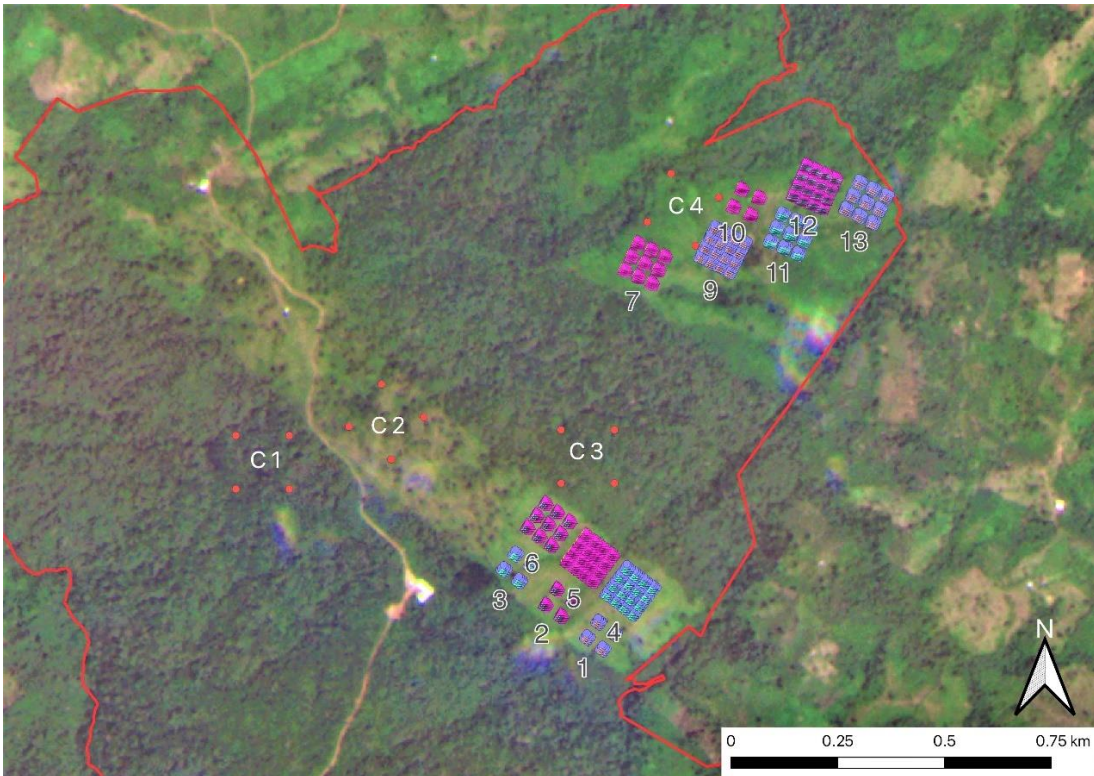


Figure 5: Map of sampling parcels. (C1) Secondary forest. (C2) Remnant tree pasture. (C3) Primary forest. (C4) Pasture. (1,2,3,10) Reforestation parcels with 30m interisland spacing. (4,5,9,12) Reforestation parcels with 30m interisland spacing. (6,7,11,13) Reforestation parcels with intermediate spacing (1,2,3,10) were not sampled in this study (Dr. Leighton Reid, personal correspondence).

### *Assessing insectivory using clay caterpillars*

Given the challenges of observing natural predation events, previous studies have found it useful to gauge predation using artificial insect prey objects made of clay. Caterpillars (larval moths and butterflies, order Lepidoptera) are a logical choice to represent insectivory using this method due to their ubiquity and simple morphology; moreover, their important role as major herbivores (Fáveri et al. 2008), important food sources for insectivores, and future pollinators in forest ecosystems makes them of ecological interest. When an insectivore attempts to predate a decoy caterpillar, it indents the clay, and the shape of the mark can be associated with the predator's general taxonomic identity (Figure 6). While species-level predator identification is usually not

possible using clay caterpillars, the coarse visual identification they provide (e.g. “bird” or “mammal”) makes them a valuable and widely accepted research method. Ecologists have used caterpillars made of dough or clay in a diverse array of ecosystems over the past several decades to study correlates of predation rate ranging from phenotypic rarity (Allen and Anderson 1984), aposematism and camouflage (Cuthill et al. 2016, Barnett et al. 2017), group size (Reader and Hochuli 2003, Kuussaari and Singer 2017), and habitat use (Loiselle and Farji-Brener 2002, Low et al. 2016, Roslin et al. 2017). This study falls into the final category as we investigate the effects of different successional habitats on insectivory.

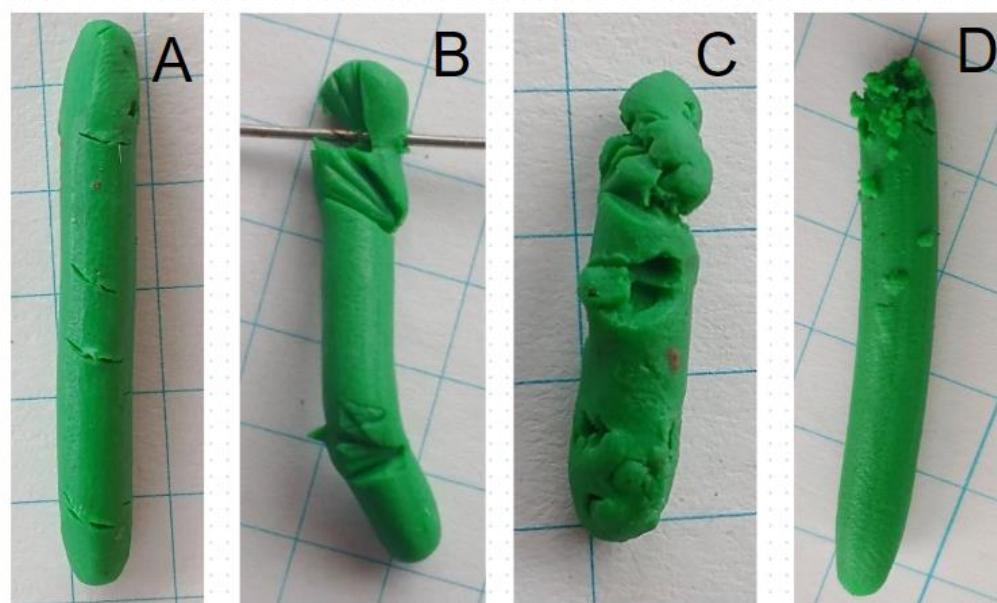


Figure 6: examples of attack marks on clay caterpillars by different predators: (A) Arthropod, (B) Bird, (C) Mammal, (D) Ants.

We made the caterpillar models (30mm × 5 mm) out of green plastilina, a non-toxic, non-hardening clay, using a craft syringe to ensure uniformity. In each of the 125m×125m reforestation parcels, we exposed caterpillar models in four tree islands (each 15m×15m). In the natural regeneration parcels, four islands were randomly established for sampling;

the natural regeneration parcels and islands had the same dimensions as those in the reforestation sites. We deployed 20 caterpillar models in each island (n= 80 caterpillars per parcel). To avoid disturbing newly established seedlings in the reforestation parcels, we placed the models within 10-meter transects extending from either end of the islands; we maintained this transect sampling procedure in the natural regeneration parcels. The transects did not overlap (Figure 7).

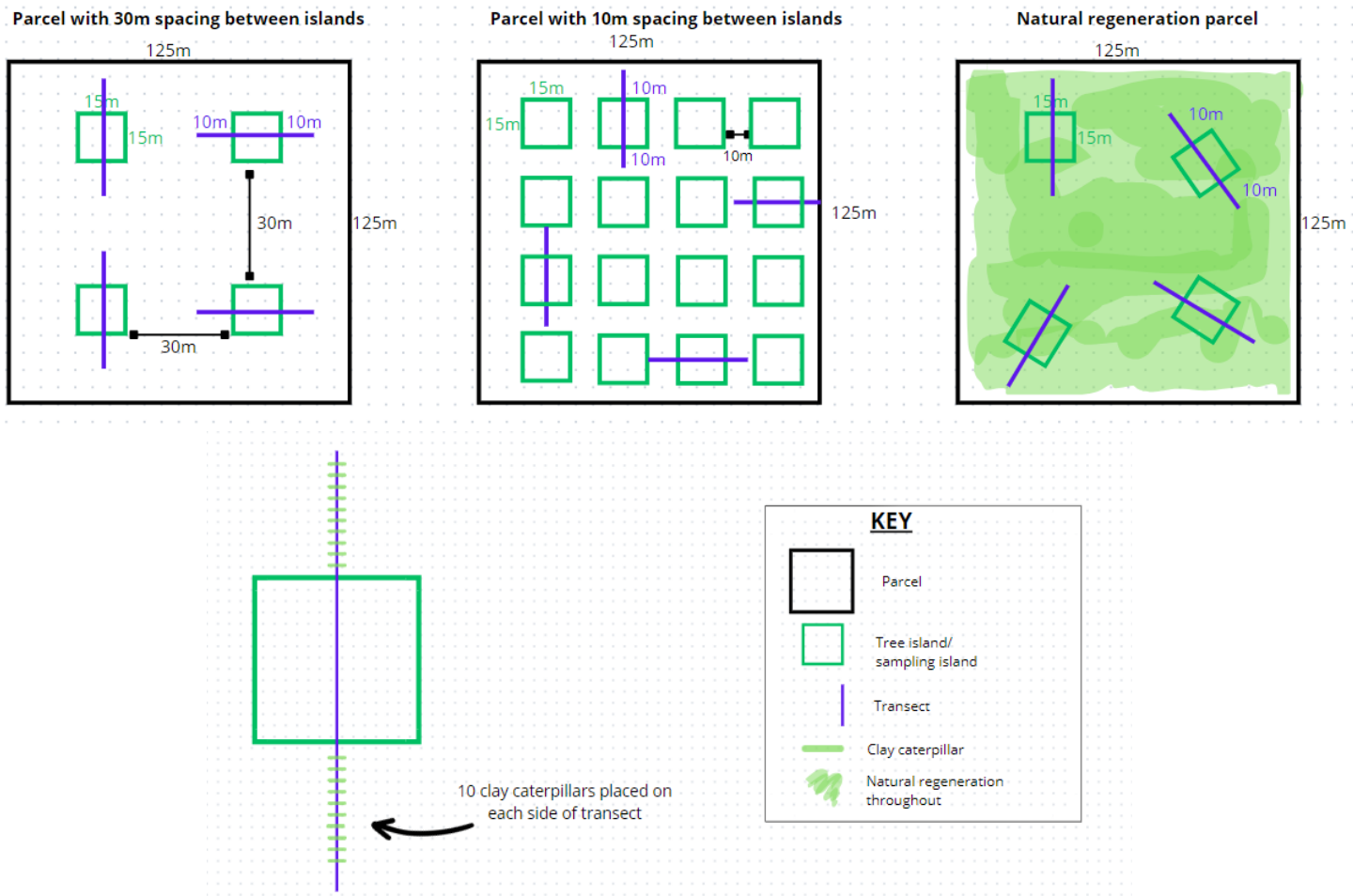


Figure 7: We placed caterpillar decoys in transects extending from either side of tree/sampling islands. Each parcel, both reforestation and natural regeneration, contained four sampling islands.

Following Koh and Menge (2006), we attached the decoys with sewing pins to the upper side of leaves, approximately 1m above ground level, with >1m spacing between models. We avoided pinning decoys to leaves with significant herbivory damage, as this can alert predators to the presence of herbivores (Sam et al. 2014). We also avoided plants with known ant symbionts, such as trees in the genus *Cecropia*. Beyond these criteria we used any available plant substrate. While pinning the caterpillar models to leaves we ensured that they were free of all markings which could later be mistaken for signs of predation.

We left the caterpillar decoys in the field for 48 hours before recollecting them. If we observed signs of a predation attempt, we profiled them using the predator marking identification guides from Tvardikova and Novotny (2012), Low et al. (2014, 2016), and Roslin et al. (2017). Caterpillars bearing predation markings of unclear origin were included in the analyses as having been attacked by “unknown predator”. Caterpillars that went missing in the field were excluded from the analyses, as we could not determine their predation status (2.5% of sample caterpillars). We sampled each parcel twice, with two weeks in between sampling (Table 1).

Table 1: Sampling effort. We sampled insectivory rates in eight parcels of land (125m×125m) undergoing reforestation by applied nucleation: four with 10m spacing between tree islands, four with 30m spacing. We also sampled in four parcels of land (125m×125m) undergoing natural regeneration: one in primary forest, one in secondary forest, one in a pasture with mature remnant trees, and one in a highly impacted pasture without remnant trees. Each parcel contained eight 10m sampling transects in which we exposed 10 clay caterpillars. We sampled each transect twice with a 2-week interval.

Number of parcels	Type	Description	Number of transects sampled	Number of clay caterpillars exposed
4	Reforestation	10m spacing	64	640
4	Reforestation	30m spacing	64	640
1	Natural regeneration	Primary forest	16	160
1	Natural regeneration	Secondary forest	16	160

1	Natural regeneration	Remnant tree pasture	16	160
1	Natural regeneration	Highly impacted Pasture	16	160

### *Statistical analysis*

We used the transect as the unit of analysis. To compare predation rates in the reforestation parcels with different spacing treatments, we first used a t-test to compare parcels with 10m and 30m inter-island spacing.

To compare predation rates relative to succession stage, we used a  $\beta$  regression model to perform pairwise comparisons between each type of parcel. Regardless of inter-island spacing, we grouped applied nucleation parcels together as “Reforestation” after the t-test suggested equivalent predation rates in the two spacing treatments ( $t = -0.065$ ,  $df = 60.894$ ,  $p = 0.948$ ). We increased all observed predation rates by 0.00000001 in order include transects where no predation occurred in the model.

To compare rates of avian and arthropod predation in the reforestation parcels with different spacing treatments, we performed two t-tests: first, we compared the rate of predation by birds in parcels with 10m vs 30m inter-island spacing; second, we compared the rate of predation by arthropods in parcels with 10m vs 30m inter-island spacing.

We used a  $\beta$  regression model to compare the relative importance of avian predation in different habitats, quantified as the number of predation attempts by birds out of the total number of predation attempts. Caterpillar models that exhibited signs of predation by multiple predators were counted as multiple attacks.

For the  $\beta$  analyses, we performed Tukey’s Honest Significant Difference tests to adjust for multiple comparisons. The p-values we report are Tukey-adjusted. For all tests, we used an  $\alpha$  level of 0.05 to determine statistical significance.

## Results

### *Predation Rates*

We exposed a total of 1920 artificial caterpillars for two days each, out of which we recovered >97% (n=1873; the rest disappeared and were excluded from the analyses). We observed a 27% overall predation rate (n=521). In the applied nucleation reforestation parcels, we found no difference in predation rate between parcels with 10m and 30m inter-island spacing ( $t = -0.065$ ,  $df = 60.894$ ,  $p = 0.948$ ), so we grouped the reforestation parcels together in the subsequent predation rate analysis.

Predation rate was highest in the primary forest (39%) and lowest in the highly impacted pasture (14%). Predation rate was significantly lower in the pasture compared to the primary forest, and marginally lower than in the reforestation sites (27%). Predation in secondary forest (26%) and in pasture with remnant trees (25%) were intermediate between primary forest and highly impacted pasture (Figure 8).

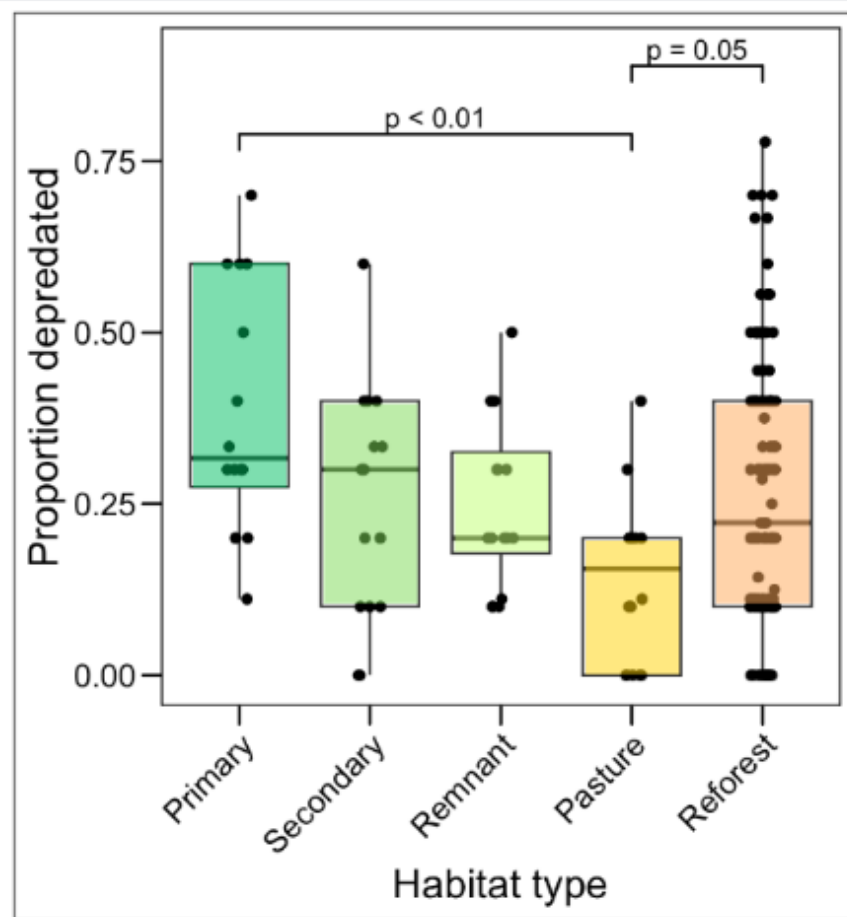


Figure 8: Rates of predation on clay caterpillars in primary forest, secondary forest, pasture with remnant trees, highly impacted pasture, and reforestation parcels (includes parcels with both 10m and 30m spacing). Points represent sampling transects.

### *The Predator Community*

In all habitat types, arthropods were the main predators, followed by birds. The relative importance of bird predation was highest in the highly-impacted pasture (38%) and lowest in the secondary forest (14%), but we found no statistically significant differences in the relative proportion of avian predation among the different sites (Figure 9).

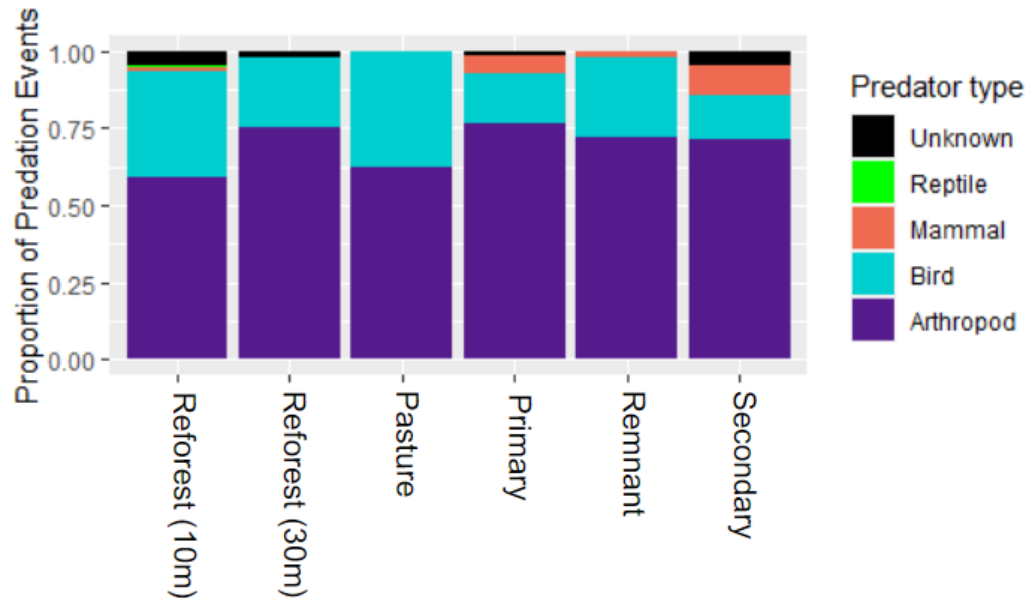


Figure 9: Predator communities. Arthropods were the dominant predator across habitats. There was no difference in the relative proportion of avian predation across habitats.

In the applied nucleation sites, the rate of predation by birds was significantly higher in parcels with 10m inter-island spacing than in parcels with 30m spacing ( $t = 2.164$ ,  $df = 66.001$ ,  $p = 0.034$ ) (Figure 10A). There was no difference in the rate of predation by arthropods between the two spacing treatments ( $t = -0.565$ ,  $df = 93.048$ ,  $p = 0.573$ ) (Figure 10B).

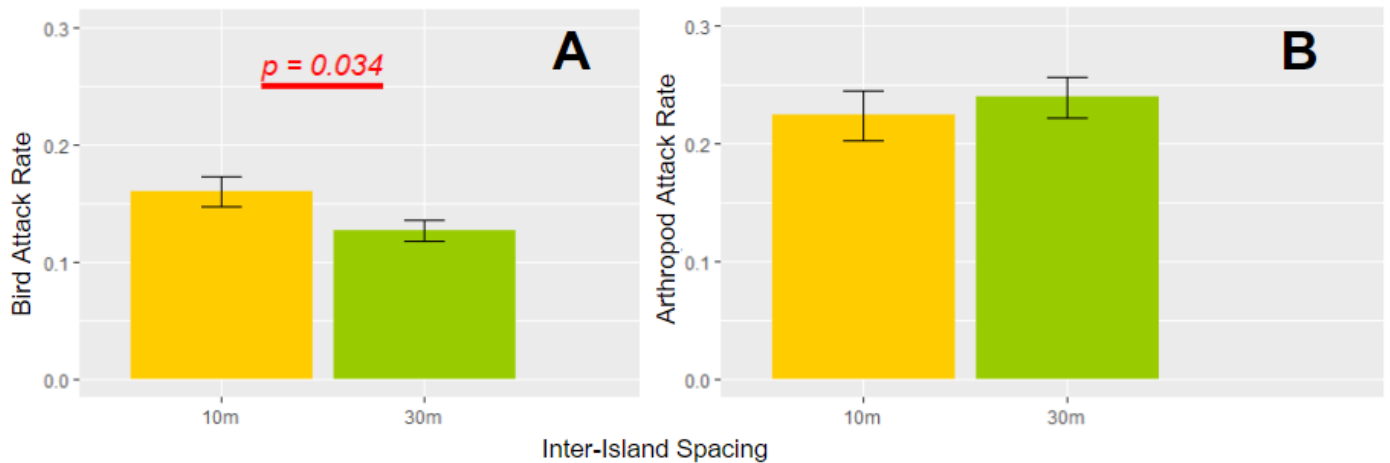


Figure 10: Average predation pressures exerted by different predators in the applied nucleation (AN) parcels ( $\pm$ SE). (A) Birds predated caterpillars at a significantly higher rate in parcels with 10m spacing than in parcels with 30m spacing. (B) Arthropods attacked caterpillars at an equivalent rate in the two spacing treatments.

## Discussion

In this study, we examined insect-predator relationships across multiple stages of forest succession, including early-stage sites undergoing restoration by applied nucleation. Our results support the prediction that rates of insectivory increase with tree cover and diversity, as rates of caterpillar predation were highest in the primary forest and lowest in the degraded pasture, with intermediate predation levels in secondary forest and reforestation sites. We did not find significant evidence of a difference in predator community composition between habitat types, although our results show trends consistent with our prediction that the relative importance of bird predation would decrease with tree cover. Contrary to our predictions that arthropod predation rate would be higher in applied nucleation parcels with 30m inter-island spacing than in parcels with 10m spacing, and that avian predation rates would be the same between the two spacing

treatments, we found no difference in arthropod predation rates across the active restoration sites, and predation by birds was higher in the 10m spacing parcels than in the 30m spacing parcels.

### *Insectivory along the succession gradient*

The observed insectivory rates fall within the wide range reported by other artificial caterpillar predation experiments conducted in the Neotropics. While Seifert et al. (2016) recorded a 52% attack rate on clay caterpillars in primary forest in French Guinea, Fáveri et al. (2008) observed predation rates as low as 4.7% in forest fragments of the Brazilian Amazon. Our findings reaffirm this variability of insectivory, ostensibly due to habitat structure differences, between pasture and primary forest, indicating a gradient of habitat suitability for insectivores. This gradient may be shaped by structural complexity and/or plant diversity. The “enemies hypothesis” proposes that top-down control on herbivores is mediated by increased plant species richness, as complex floral environments provide more niches for predators to exploit, and thus foster more diverse predator assemblages (Root 1973, Yang et al. 2018). Although we did not directly measure plant diversity, the “intermediate disturbance hypothesis” provides that moderate levels of disturbance maintain species diversity in an ecosystem (Connell 1978), suggesting that after being disturbed, mid- and late-stage successional forests have high rates of species richness and turnover in species composition, but that areas experiencing chronic disturbance, such as long-term clear-cutting and cattle grazing, are less biodiverse. Indeed, our field observations noted floristic homogeneity of herbs in the highly degraded, grass-dominated pasture, where predation rates were lowest (14%),

possibly due to the loss of plant richness or the simplification of habitat structure. The high predation rates in primary and secondary forests could point to high plant species diversity mediated by their unique disturbance regimes, including tree fall in the primary forest and a history of logging in the secondary forest (Chazdon 2014). The observed gradient of insectivory rates roughly mirrors the stages of succession in our study sites, providing support for the Enemies Hypothesis in this system. Future studies could explore this further by measuring herb and tree diversity in the reforestation sites to investigate how plant diversity mediates insectivory under different regeneration treatments.

*Predator types: relative proportions, rates of predation, and habitat structure*

Our experiment revealed a somewhat low proportion of predation by arthropods (76% of total attacks) when compared to similar studies, which have recorded proportions as high as 94% (Koh and Menge 2006) or 98.5% (Seifert et al. 2016). Even so, Tvardikova and Novotny (2012), and Low et al. (2016) found arthropod predation proportions lower than ours (46% and 57% respectively), indicating that the predator community we observed fits within the spectrum of community compositions described by previous research. In previous studies, as in ours, birds were responsible for all or most of the non-arthropod predation events, with predation by mammals or reptiles being rare (Tvardikova and Novotny 2012, Seifert et al. 2016, Low et al. 2016, Leles et al. 2017). We observed 521 predation events in total, out of which eleven were done by mammals, and one by a reptile. Although we did not separate ants from other arthropods in our assessment of predation markings, we observed that many clay caterpillars were

seemingly attacked by ants (Figure 6), which follows previous studies' findings that ants are caterpillars' foremost enemies (Loiselle and Farji-Brener 2002, Fáveri et al. 2008, Tvardikova and Novotny 2012, Seifert et al. 2016). Given their predatory prominence, future studies should consider identifying signs of predation by ants to assess their role as insectivores in different types of regenerating forest.

Following that the avian/arthropod composition of insectivore communities changes with habitat location and structure, including factors such as elevation and latitude (Roslin et al. 2017), tree density and diversity (Yang et al. 2018), and canopy height (Loiselle and Farji-Brener 2002), we may attribute the high proportion of avian predation in our study to structural features, particularly the prevalence of pasture-like environments in the reforestation parcels. Pastures could present a dispersal challenge to cursorial arthropods, who rely on understory structure to navigate their environment while foraging (Yang et al. 2018). Birds, on the other hand, are less dispersal-limited and have been found to contribute more to total insectivory in low-density, species-poor habitats (like pasture) than in primary forest. Yang et al. (2018) attributes this partially to increased prey visibility. In our study, the pattern of avian predator importance matches the successional gradient, with birds committing the highest proportion of caterpillar attacks in highly degraded pasture (38%) and the lowest in primary and secondary forests (16% and 14% respectively). Although not statistically significant, this pattern is compelling as it reinforces the habitat gradient characterized by predation rates.

Given the dominance of arthropod predators across habitat types, assessing the predator community via relative proportions could mask the variability in predation performed by non-arthropods. Therefore, considering predator assemblages in terms of

predation rate for each predator may yield better ecological insight into habitat-specific predation pressures. When we assessed predation rates for birds and arthropods separately, we found that birds predated caterpillars at a higher rate in applied nucleation parcels with 10m inter-island spacing (16%) than in parcels with 30m spacing (12%). This could be attributed to structural differences between the two habitats: parcels with 10m spacing have lower herb cover and form a more open habitat than parcels with 30m spacing (Figure 4). Birds' ability to detect clay caterpillars is likely similar between spacing treatments, since the decoys were pinned among tall grasses in both 30m and 10m spacing parcels. It is possible that a reduced abundance of arthropods due to lack of understory connectivity created food scarcity for birds foraging in the 10m spacing parcels, thus increasing the predation pressure on clay caterpillars. Future studies could address this by assessing arthropod diversity and abundance in the assisted regeneration parcels.

#### *Land management and rescuing insectivory*

At the time this study was conducted, the trees in FCAT's reforestation parcels had been planted three years prior on cleared pastureland. Despite their recent establishment, these parcels already displayed higher rates of insectivory than pasture habitats undergoing passive restoration. This finding indicates that applied nucleation can produce changes in certain ecological processes in a short period, even before substantial tree cover is established, and suggests the potential for a relatively rapid rescue of insect-predator interactions following disturbance. Early-stage reforestation sites and pasture with remnant trees had similar and intermediate rates of insectivory between pasture and

primary forest. For landowners in the region clearing land for grazing and farming, it should be known that the decision to leave some trees standing rather than deforest completely has a palpable effect on species interactions, evidencing a less extreme modification of the rainforest ecosystem. Maintaining ecosystem functions through remnant trees also protects ecosystem services which benefit residents, such as pollination and pest control for nearby crops (Brandon 2014).

### *Study limitations*

The results of our study should be interpreted with the understanding that clay caterpillars are not real prey objects. They are disproportionately attractive to visual predators because they lack chemical signals, which may exclude them from predation by many arthropods, potentially producing artificially lower natural predation rates (Koh and Menge 2006, Seifert et al. 2016). On the other hand, the decoys also lack the defense and evasion mechanisms employed by lepidoptera larvae, including spines, leaf rolling, and the ability to move (Tvardikova and Novotny 2012); in this sense, they are more vulnerable to predation than real caterpillars, which could potentially increase natural predation rates. Artificial predation studies can partially address decoys' shortcomings by making them more realistic prey objects, through adding coloration (Barnett et al. 2017, Hiltbold and Shriver 2018), rolling them into leaves (Tvardikova and Novotny 2012), or scenting them to mimic real caterpillars, all of which are possibilities for future studies in this system. With these considerations in mind, clay caterpillars are most useful in comparing the relative predation pressure between habitats rather than estimating actual predation rates.

Because we deployed our caterpillar decoys on leaves 1m above the ground, our findings reflect insectivory dynamics in the understory. This methodology was probably inconsequential in the highly impacted pasture and applied nucleation sites, where the vegetation did not reach heights much past 1m. However, in the primary forest, secondary forest, and pasture with remnant trees, our decision to sample at a low height probably excluded predators foraging in higher strata, such as canopy-dwelling birds. Loiselle and Farji-Brener (2002) found that insectivory rates were 25% higher in forest canopy than understory. This suggests that the predation rates we report may be lower than those experienced by real insect prey. Future studies could consider insectivory in multiple forest strata to better assess changes in insect-predator interactions as canopy cover is re-established.

Another limitation in our study is the constrained sampling of the natural regeneration parcels. Because we sampled from eight transects within a single parcel for each non-reforestation habitat, our results may be affected by site characteristics other than forest successional stage, for example: proximity to rivers, roads, or primary forest, and composition of local plant communities. Previous studies have shown that plant substrate type has a significant effect on clay caterpillar predation, as some predators forage preferentially on certain plant species (Yang et al. 2018). In the reforestation and pasture sites, we nearly always pinned caterpillars to grasses or camacho (*Xanthosoma sagittifolium*), a common forb. In the forest, the available substrates were much more varied. Our results may be affected by our inability to standardize our usage of plant substrates, given the fundamentally different plant communities present in forested and

pasture habitats. If possible, future studies should consider a broader range of sampling sites to account for these local differences.

Lastly, our results are limited by statistical incongruencies. While the  $\beta$ -models accounted for non-independence between sampling transects, which were nested within islands and parcels, the t-tests did not make this adjustment. The t-tests allowed us to biologically interpret rates of predation by different predators, but their failure to account for the nested data structure is problematic. Future studies should use  $\beta$ -models to assess the predator community via rates of predation, rather than relative proportions, which may mask the effects of minority predator groups like birds.

### *Conclusion*

We conducted this study at the “start” of a forest, exploring insectivory dynamics in the early stages of FCAT’s reforestation project. Future research may observe differential predation patterns between applied nucleation treatments, making deeper insights into the technique’s effects on insect-predator interactions, considering variables of tree diversity and frugivore network connectivity, in addition to inter-island spacing. With time, insectivory will become a clearer lens through which to observe human-mediated regeneration and its capacity to rescue ecological processes in disturbed forests.

## Literature Cited

- Aide, T. M., M. L. Clark, H. R. Grau, D. López-Carr, M. A. Levy, D. Redo, M. Bonilla-Moheno, G. Riner, M. J. Andrade-Núñez, and M. Muñiz. 2013. Deforestation and reforestation of Latin America and the Caribbean (2001-2010). *Biotropica* 45:262–271.
- Allen, J. A., and K. P. Anderson. 1984. Selection by passerine birds is anti-apostatic at high prey density. *Biological Journal of the Linnean Society* 23:237–246.
- Barnett, J. B., I. C. Cuthill, and N. E. Scott-Samuel. 2017. Distance-dependent pattern blending can camouflage salient aposematic signals. *Proceedings of the Royal Society B: Biological Sciences* 284:20170128.
- Brandon, K. 2014. Ecosystem services from tropical forests: Review of Current Science. CGD Working Paper 380. Center for Global Development Climate and Forest Paper Series. [DOI:10.2139/ssrn.2622749]
- Chazdon, R. L. 2014. *Second Growth: The promise of tropical forest regeneration in an age of deforestation*. University of Chicago Press.
- Chazdon, R. L. 2017. Landscape restoration, natural regeneration, and the forests of the future. *Annals of the Missouri Botanical Garden* 102:251–257.
- Clark, D. B. 1996. Abolishing virginity. *Journal of Tropical Ecology* 12:735–739.
- Connell, J. H. 1978. Diversity in tropical rain forests and coral reefs. *Science*, 199: 1302–1310.
- Corbin, J. D., and K. D. Holl. 2012. Applied nucleation as a forest restoration strategy. *Forest Ecology and Management* 265:37–46.
- Correa-Cuadros, J. P., A. Gómez-Cifuentes, and J. A. Noriega. 2022. Comparative effect of forest cutting and mammal hunting on dung beetle assemblages in Chocó

- Biogeographic forests in Colombia. *International Journal of Tropical Insect Science* 42:3045–3055.
- Cuthill, I. C., N. S. Sanghera, O. Penacchio, P. G. Lovell, G. D. Ruxton, and J. M. Harris. 2016. Optimizing countershading camouflage. *Proceedings of the National Academy of Sciences* 113:13093–13097.
- Durães, R., L. Carrasco, T. B. Smith, and J. Karubian. 2013. Effects of forest disturbance and habitat loss on avian communities in a Neotropical biodiversity hotspot. *Biological Conservation* 166:203–211.
- Fáveri, S. B., H. L. Vasconcelos, and R. Dirzo. 2008. Effects of Amazonian forest fragmentation on the interaction between plants, insect herbivores, and their natural enemies. *Journal of Tropical Ecology* 24:57–64.
- Freepik. (n.d.). Ecuador country nation map highlighted in red on South America continent map vector.
- Hiltbold, I., and W. G. Shriver. 2018. Birds bug on indirect plant defenses to locate insect prey. *Journal of Chemical Ecology* 44:576–579.
- Hobbs, R. J., and V. A. Cramer. 2007. Why old fields? Socioeconomic and ecological causes and consequences of land abandonment. Pages 1-14 *in* Old fields: Dynamics and restoration of abandoned farmland. Island Press, Washington, D.C.
- Holl, K. D. 2007. Old field vegetation succession in the neotropics. Pages 93–118 *in* Old fields: Dynamics and restoration of abandoned farmland (edited by R.J. Hobbs and V.A. Cramer). Island Press, Washington, D.C.
- Holl, K. D., J. L. Reid, R. J. Cole, F. Oviedo-Brenes, J. A. Rosales, and R. A. Zahawi. 2020. Applied nucleation facilitates tropical forest recovery: Lessons learned from

- a 15-year study. *Journal of Applied Ecology* 57:2316–2328.
- Jones, H. P., P. C. Jones, E. B. Barbier, R. C. Blackburn, J. M. Rey Benayas, K. D. Holl, M. McCrackin, P. Meli, D. Montoya, and D. Moreno Mateos. 2018. Restoration and repair of Earth's damaged ecosystems. *The Royal Society Proceedings B* 285:20172577. [DOI: 10.1098/rspb.2017.2577]
- Koh, L. P., and D. N. L. Menge. 2006. Rapid assessment of lepidoptera predation rates in Neotropical forest fragments. *Biotropica* 38:132–134.
- Kuussaari, M., and M. C. Singer. 2017. Group size, and egg and larval survival in the social butterfly *Melitaea cinxia*. *Annales Zoologici Fennici* 54:213–223.
- Lamperty, T., J. Karubian, and A. E. Dunham. 2021. Ecological drivers of intraspecific variation in seed dispersal services of a common neotropical palm. *Biotropica* 53:1226–1237.
- Leles, B., X. Xiao, B. O. Pasion, A. Nakamura, and K. W. Tomlinson. 2017. Does plant diversity increase top–down control of herbivorous insects in tropical forest? *Oikos* 126:1142–1149.
- Loiselle, B. A., and A. G. Farji-Brener. 2002. What's up? An experimental comparison of predation levels between canopy and understory in a tropical wet forest. *Biotropica* 34:327.
- Low, P. A., C. McArthur, K. Fisher, and D. F. Hochuli. 2014a. Elevated volatile concentrations in high-nutrient plants: Do insect herbivores pay a high price for good food? *Ecological Entomology* 39:480–491.
- Low, P. A., K. Sam, C. McArthur, M. R. C. Posa, and D. F. Hochuli. 2014b. Determining predator identity from attack marks left in model caterpillars: Guidelines for best

- practice. *Entomologia Experimentalis et Applicata* 152:120–126.
- Low, P. A., C. McArthur, and D. F. Hochuli. 2016. It is safe to go out on a limb: Within-tree leaf age and location do not alter predation risk for insect herbivores. *Austral Entomology* 55:284–290.
- Lueder, S., K. Narasimhan, J. Olivo, D. Cabrera, J. G. Jurado, L. Greenstein, and J. Karubian. 2022. Functional traits, species diversity and species composition of a Neotropical palm community vary in relation to forest age. *Frontiers in Ecology and Evolution* 10:678125.
- Perfecto, I., and J. Vandermeer. 2008. Biodiversity conservation in tropical agroecosystems. *Annals of the New York Academy of Sciences* 1134:173–200.
- Reader, T., and D. F. Hochuli. 2003. Understanding gregariousness in a larval Lepidopteran: the roles of host plant, predation, and microclimate. *Ecological Entomology* 28:729–737.
- FCAT n.d., *Reserve*, accessed 20 April 2024, <<https://fcatecuador.org/reserve/>>.
- Root, R. B. 1973. Organization of a plant-arthropod association in simple and diverse habitats: The fauna of collards (*Brassica oleracea*). *Ecological Monographs* 43:95–124.
- Roslin, T., B. Hardwick, V. Novotny, W. K. Petry, N. R. Andrew, A. Asmus, I. C. Barrio, Y. Basset, A. L. Boesing, T. C. Bonebrake, E. K. Cameron, W. Dáttilo, D. A. Donoso, P. Drozd, C. L. Gray, D. S. Hik, S. J. Hill, T. Hopkins, S. Huang, B. Koane, B. Laird-Hopkins, L. Laukkanen, O. T. Lewis, S. Milne, I. Mwesige, A. Nakamura, C. S. Nell, E. Nichols, A. Prokurat, K. Sam, N. M. Schmidt, A. Slade, V. Slade, A. Suchanková, T. Teder, S. Van Nouhuys, V. Vandvik, A. Weissflog, V.

- Zhukovich, and E. M. Slade. 2017. Higher predation risk for insect prey at low latitudes and elevations. *Science* 356:742–744.
- Sam, K., B. Koane, and V. Novotny. 2014. Herbivore damage increases avian and ant predation of caterpillars on trees along a complete elevational forest gradient in Papua New Guinea. *Ecography* 38.
- Seifert, C. L., C. H. Schulze, T. C. T. Dreschke, H. Frötscher, and K. Fiedler. 2016. Day vs. night predation on artificial caterpillars in primary rainforest habitats – an experimental approach. *Entomologia Experimentalis et Applicata* 158:54–59.
- Svampa, M. 2019. Neo-extractivism in Latin America: Socio-environmental conflicts, the territorial turn, and new political narratives. First edition. Cambridge University Press.
- Thébault, E., and M. Loreau. 2006. The relationship between biodiversity and ecosystem functioning in food webs. *Ecological Research* 21:17–25.
- Turner, J. R. G. 2004. Explaining the global biodiversity gradient: Energy, area, history and natural selection. *Basic and Applied Ecology* 5:435–448.
- Tvardikova, K., and V. Novotny. 2012. Predation on exposed and leaf-rolling artificial caterpillars in tropical forests of Papua New Guinea. *Journal of Tropical Ecology* 28:331–341.
- Yang, B., B. Li, Y. He, L. Zhang, H. Bruelheide, and A. Schuldt. 2018. Tree diversity has contrasting effects on predation rates by birds and arthropods on three broadleaved, subtropical tree species. *Ecological Research* 33:205–212.
- Yarranton, G. A., and R. G. Morrison. 1974. Spatial dynamics of a primary succession: Nucleation. *Journal of Ecology* 62:417–428.