Are tropical hummingbird pollination networks resistant to experimental extirpation of a common flowering plant?

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ABSTRACT

Plants and pollinators are experiencing parallel declines worldwide, despite theory predicting that pollination networks should withstand disturbance due to redundancy of pollinators, rarity of interactions between specialists, and flexible pollinator foraging behavior (rewiring). Experiments evaluating the stability of plant-pollinator networks may help resolve this incongruity but remain uncommon. In this study, we simulated the extirpation of a hummingbird-pollinated understory plant, *Heliconia tortuosa*, from tropical forest fragments using a replicated Before-After-Control-Impact experimental design while quantifying hummingbird space use (383 hummingbird captures and 72 radio-tagged individuals), floral visitation rates (6474 visits from >19,000 observation hours), and plant pollination success (529 flowers). To complement this experimental approach, we also examined these responses across a natural gradient in *H. tortuosa* density. We expected that declines of *H. tortuosa* would either result in (i) network collapse, in which hummingbirds vacate fragments and compromise the reproductive success of other flowering plants, or (ii) increased hummingbird reliance on alternative resources, leading to sustained fragment use. We also hypothesized that landscape and local context (i.e., connectivity to additional forest area and alternative resource abundance) could mediate hummingbird responses to *H. tortuosa* declines; for example, connectivity could facilitate hummingbird visitation to areas of reduced food availability. In our removal experiment, hummingbird persistence and plant pollination success were remarkably resistant to loss of *H. tortuosa*, a locally common plant species representing >40% of the available nectar resources on average. However, naturally low *H. tortuosa* densities were associated with reduced floral visitation rates and decreased pollination. Although landscape context (connectivity) led to higher hummingbird abundance, we found little evidence that connectivity or resource availability mediated hummingbird responses to declines in *H. tortuosa* availability. The exact

mechanisms enabling short-term hummingbird persistence after resource removal remain unclear, as we did not discover evidence of rewiring. Physiological adaptations (e.g., torpor and insectivory), may have contributed to hummingbird resilience, perhaps alongside high movement abilities that facilitated visitation to spatially dispersed floral resources. With the important caution that short-term experiments may not realistically emulate natural extinction processes, our study provides partial support for theoretical predictions that pollination networks may be relatively robust to plant species loss.

Key words: mutualistic networks, ecosystem function, foraging ecology, functional connectivity, fragmentation, Trochilidae

INTRODUCTION

Species loss can have cascading consequences for the broader community, including coextinctions of dependent species and declines in ecosystem function (Koh et al. 2004, Colwell et al. 2012). Linked extinctions may be especially likely for mutualistic interactions, such as those between plants and pollinators (Dunn et al. 2009, Aslan et al. 2013). For instance, plants that are not adequately pollinated produce fewer seeds, accruing extinction debt that becomes realized when the existing generation cannot be replaced by new recruits (Ashman et al. 2004, Vellend et al. 2006, Kuussaari et al. 2009). This repeated reproductive failure will eventually cause demographic collapse of plant populations (Anderson et al. 2011, Phillips et al. 2015), which could then hasten the decline of animals that consume pollen or nectar (Biesmeijer et al. 2006, Pauw and Hawkins 2011, Weiner et al. 2014, Scheper et al. 2014). Indeed, plants and pollinators are experiencing declines worldwide (Biesmeijer et al. 2006, Potts et al. 2010, Regan et al. 2015), which not only imperils the essential ecosystem services on which humans depend

but also threatens the maintenance of global biodiversity (Allen-Wardell et al. 1998, Klein et al. 2007, Ollerton et al. 2011).

Although the underlying reasons for plant and pollinator declines are not fully understood, one prominent hypothesis invokes the landscape-level processes of habitat loss and fragmentation (Kearns et al. 1998, Aguilar et al. 2006, Potts et al. 2010, Hadley and Betts 2012). Habitat destruction often creates small habitat patches unable to sustain large populations, which can increase rates of local extinction (Hanski 1998), reduce functional connectivity (sensu P. D. Taylor et al. 1993), and impede pollinator movement (Hadley and Betts 2012). Likely due to these combined mechanisms, land-use change and habitat isolation have been repeatedly linked to declines in pollinator diversity (Winfree et al. 2011, Montero-Castaño and Vilà 2012, Senapathi et al. 2015) and plant reproduction (Steffan-Dewenter and Tscharntke 1999, Taki et al. 2007, Farwig et al. 2009, Garibaldi et al. 2011).

However, parallel declines of pollinators and plants are at odds with theoretical predictions suggesting that species loss should generally not result in coextinction of interaction partners (Memmott et al. 2004, Bascompte et al. 2006). Tolerance to subsequent extinctions (i.e., robustness) primarily arises from the expectation that interaction networks exhibit strongly nested topologies, such that specialists interact with subsets of partners linked to generalists (Bascompte et al. 2003). Under this interaction pattern, connections between specialists are uncommon, so the functional role of a specialist can be absorbed by its more abundant generalist partners (Bascompte et al. 2003, Bascompte and Jordano 2007). In addition to the redundancy afforded by a nested structure, models suggest that flexibility in pollinator foraging behavior – i.e., rewiring to use alternative resources – may buffer networks against disturbance (Kaiser-Bunbury et al. 2010, Ramos-Jiliberto et al. 2012, Valdovinos et al. 2013, Timóteo et al. 2016).

Despite these overall predictions and mechanisms of robustness, pollination networks may be vulnerable to habitat loss and fragmentation under certain conditions. Although systematic loss of generalist pollinators may be less realistic than extinctions of rare, specialist pollinators (Aizen et al. 2012), simulation studies have revealed that removal of highly connected, generalist species thwarts a network's capacity to buffer perturbations, leading to rapid partner extinction (Memmott et al. 2004, Kaiser-Bunbury et al. 2010, Traveset et al. 2017). Thus, if external stressors such as habitat fragmentation threaten highly connected species ('topological keystones' sensu Jordán 2009), an extinction cascade could occur – particularly without rewiring by pollinators. However, uncertainty about the robustness of real-world plant-pollinator networks still exists because relatively few studies have quantified pollinator responses to deletions of other species (Appendix S1: Table S1).

In this study, we tested whether declines of a hummingbird-pollinated plant *(Heliconia tortuosa)* could have cascading consequences for communities of hummingbirds and flowering plants within a fragmented tropical forest landscape – or whether rewiring might forestall network collapse. In Neotropical forests, the genus *Heliconia* (Heliconiaceae) comprises understory herbs that have been suggested to function as "keystone mutualists" (Gilbert 1980). Large, brightly colored *Heliconia* inflorescences not only provide nectar to large-bodied hermit hummingbirds (Trochilidae: Phaethornithinae) throughout the year, but also support a variety of other hummingbird species during periods of resource scarcity (Stiles 1975, 1985). Because most hummingbird species visit multiple plant species (Rodríguez-Flores et al. 2019), loss of *H. tortuosa* and its associated hummingbird partners could jeopardize the reproduction of co-occurring plants, thus generating an extinction cascade (Gilbert 1980). Although *H. tortuosa* is locally common in the mid-elevation, premontane tropical forests of Costa Rica, Hadley *et al.*

(2014) found that *H. tortuosa* in smaller forest fragments produced fewer seeds, possibly due to short-distance pollen transfer associated with pollinator movement limitation. Although numerous hummingbird species visit *H. tortuosa* opportunistically, its primary pollinators are the green hermit (*Phaethornis guy*) and violet sabrewing (*Campylopterus hemileucurus*) (Stiles 1979, Wolf and Stiles 1989, Taylor and White 2007, Betts et al. 2015). These relatively large, forest-associated hummingbirds are high-reward trapliners (sensu Feinsinger and Colwell 1978) that visit spatially dispersed, nectar-rich floral resources in a routine sequence (Borgella et al. 2001). However, their movements become restricted in fragmented habitats (Hadley and Betts 2009, Volpe et al. 2016, Kormann et al. 2016).

To assess the potential consequences of *H. tortuosa* declines, we measured hummingbird space use, floral visitation rates, and plant pollination success before and after temporary experimental removal of *H. tortuosa* within forest fragments. Additionally, we assessed how these responses varied across a natural gradient in *Heliconia* density. We developed two alternative hypotheses about how hummingbirds would respond to declines of *H. tortuosa* (hereafter *Heliconia*) and potentially affect the pollination of co-occuring plant species (Table 1). Under the *robust network hypothesis* (**H1a**), hummingbirds do not emigrate from forest fragments following *Heliconia* decline, leading to uninterrupted visitation to remaining plants. Under this scenario, we hypothesized that hummingbirds compensate for *Heliconia* extinction by increasing their use of non-*Heliconia* floral resources (rewiring). Alternatively, hummingbirds may be highly dependent on *H. tortuosa* and inflexible in their foraging choices; therefore, local *Heliconia* declines should reduce nectar availability such that hummingbirds cannot afford the energetic costs to remain in (or travel to) focal forest fragments, leading to decreases in fragment use and pollination success (**H1b***: parallel extinction hypothesis*). Under both hypotheses, we

expected hummingbirds to change their behavior due to their exceptionally rapid metabolisms, high dependency on nectar, and limited fat stores (Suarez 1992, Powers et al. 2003). Additionally, we hypothesized that landscape and local context could mediate the influence of *Heliconia* declines on hummingbird behavior (Table 1). Connectivity to additional forest area (*connectivity hypothesis*, **H2**) may sustain hummingbird fragment use by facilitating travel to fragments (*rescue hypothesis*, **H2a**) or promote steeper declines in fragment use by facilitating hummingbird departure (*abandonment hypothesis*, **H2b**). Further, greater local availability of non-*Heliconia* floral resources may promote hummingbird persistence through increased opportunities for rewiring (*resource hypothesis*, **H3**). Lastly, we expected that changes in *Heliconia* density would affect green hermits and violet sabrewings more strongly than other hummingbird species (**H4**). Green hermits and violet sabrewings (hereafter '*Heliconia* specialists') inhabit the forest understory, have bills that are morphologically specialized to long, curved *H. tortuosa* flowers, and are thought to visit *H. tortuosa* more frequently than other hummingbirds (Borgella et al. 2001, Betts et al. 2015).

To our knowledge, this study not only constitutes one of most highly replicated, large-scale removal experiments conducted within the context of plant-pollinator interaction networks (Appendix S1: Table S1), but also represents the first test of how landscape and local context influence hummingbird responses to declines in a common floral resource. Importantly, understanding mechanisms of pollinator persistence in fragmented landscapes has implications for conservation planning, since fragmentation effects may be ameliorated by habitat restoration, preservation of riparian corridors, and/or implementation of living fences (Renjifo 2001, Kormann et al. 2016).

METHODS

Study area

This study was conducted in southern Costa Rica within a ~122 km^2 area surrounding the Las Cruces Biological Station (Coto Brus Canton, Puntarenas Province, 8°47´7´´ N, 82°57´32´´ W, Fig. 1: A-B). This study area comprises cattle pastures, coffee plantations, and fragments of tropical premontane wet forest created during a period of extensive land clearing concentrated between 1960 and 1980 (Zahawi et al. 2015). Remnant forest fragments (~30% of the study area) span a wide range of sizes (<1 to >1000 ha), with otherwise isolated fragments often connected by narrow riparian corridors and living fences (Hadley and Betts 2009, Zahawi et al. 2015). Mean annual temperature at the Las Cruces Biological Station is ~21 ºC, mean annual rainfall is 3500-4000 mm, and a dry season occurs from December to March (Borgella et al. 2001, Zahawi et al. 2015).

Study sites

We selected 14 focal fragments embedded within focal landscapes of 1000 m radius (‘sites’, Fig. 1: A, C). We chose the spatial extent of focal landscapes *a priori* based on the maximum estimated daily movement distance for large, forest-associated hummingbird species in this study system (Volpe et al. 2014).We identified potential sites using a stratified design with two categories: ‘connected to forest’ or ‘unconnected to forest’, based on visual examination of satellite imagery and previous work in this study area (Hadley et al. 2014, 2018, Kormann et al. 2016). This design was only for site establishment, as we later quantified connectivity as a continuous variable (see *‘Quantifying connectivity’* below). Sites were then included based on presence of flowering *Heliconia***,** landowner permission, vehicle accessibility, and navigable terrain. To ensure site accessibility, we conducted our study during the dry season

and beginning of the rainy season (February – May), because localized flooding can occur during periods of heavy rainfall. Elevation of sites ranged from ~900-1500 m (Appendix S2: Table S1).

Study design

Using the 14 focal-patch landscapes, we repeated the *Heliconia* removal experiment 16 times across three years (2016-2018). Each iteration of the experiment followed a BACI (Before-After-Control-Impact) design and used two sites paired based on elevation and connectivity (Appendix S2: Tables S2-S3). One of the paired sites received the treatment of *Heliconia* removal, and we designated the other site as a control (16 control replicates, 16 treatment replicates). To prevent hummingbirds from the treatment site relocating to the control site, paired sites were located at least 1000 m apart (mean ± SD: 2266 ± 1511 m, median: 3086 m). Most sites were used multiple times across the three-year study period, but only once per year (Appendix S2: Tables S2-S4). Sites used multiple times received the opposite treatment of the previous year, except for 3 of 16 instances (e.g., when covering *Heliconia* in that site again would have posed substantial logistical challenges). Initial treatment assignments were random. This design reduced the likelihood that any treatment differences were due to factors associated with individual sites (Appendix S2: Fig. S1).

Each experimental iteration lasted nine days and began with a period of pre-treatment data collection in each site (Fig. 2A). Starting around noon on the fourth day, we removed *Heliconia* from the treatment site and designated the following 36 hours as a behavioral adjustment period during which no data were collected. Although this adjustment period was necessarily relatively short due to the limited battery lifespan of hummingbird radio-transmitters (see *'Radio telemetry'*), we expected hummingbirds to respond rapidly due to their exceptionally high metabolisms and limited fat stores (Suarez 1992, Powers et al. 2003). To ensure that

Heliconia inflorescences were not overlooked during removal, field technicians positioned themselves ~2-3 meters apart and slowly walked through the forest while covering all *Heliconia* inflorescences (mature and immature) with dark-colored fabric bags (Fig. 2B). These bags prevented hummingbird access, obscured flowers from view, and allowed us to avoid long-term changes to floral resources associated with destructive sampling methods (e.g., cutting). The location of each *Heliconia* plant was recorded using a handheld GPS unit, and we retrieved the covering bags at the end of the experimental period. In each site, we also recorded the location and identity of all non-*Heliconia* floral resources available to hummingbirds (see *'Quantifying resource availability'*). The areas where we removed *Heliconia* and/or surveyed for floral resources are referred to as 'focal areas' (Fig. 1D), which we delineated using 100% minimum convex polygons. Focal areas also encompass locations where we captured hummingbirds, installed trail cameras, and set up plant arrays (Fig. 1D, see *'Response variables'*).

Response variables

Hummingbird captures

Within each focal area, we captured hummingbirds at the beginning and end of the experimental period (Fig. 2A) by deploying 5 - 20 mist nets (12-m and 6-m in length) near flowering plants visited by hummingbirds (e.g., the genera *Heliconia*, *Centropogon*, *Palicourea*, *Musa*). Nets were opened 30 minutes after sunrise and remained open for 2 - 5 hours per session. Capture effort (net-hours) and net locations were kept constant for both capture sessions, except in one instance (Appendix S2: Fig. S2). Following the North American Banding Council's hummingbird protocol (Russell and Russell 2001), we tagged hummingbirds with an aluminum tarsus band, measured body mass to the nearest 0.01 g using an electronic balance, and measured wing length to the nearest 0.5 mm using a wing rule. Tarsus bands allowed detection of

recaptured hummingbirds within and between capture sessions; individuals recaptured during the same capture session were only counted once. In 2017-2018, we also marked hummingbirds' heads with unique color combinations of nail polish following Hadley *et al.* (2014); this additional mark allowed us to identify individual hummingbirds seen on camera (see *'Camera observations'*). All birds were handled in accordance with Oregon State University Animal Care and Use Protocols (ACUP #4655, #5015).

Radio telemetry

We attached miniaturized radio transmitters (0.15-0.25g, Blackburn Transmitters, Nacogdoches, TX) to 72 hummingbirds according to the methods of Hadley & Betts (2009). We then followed hummingbirds on foot using portable receivers (Wildlife Materials Inc TRX 1000S) and handheld Yagi antennae, recorded locations continuously when the bird was found within receiver range (~250 m in the absence of topographical barriers). In total, we completed 447 hours of telemetry observation, averaging 3.1 ± 0.8 hours per session (typically ~8:00-11:00) and 5.3 ± 0.9 sessions per site (mean ± SD). From the recorded locations, we estimated the proportion of observation time that radio-tagged birds spent in the focal area (Appendix S2: Fig. S3-S4). Thirty-six hummingbirds spent time in the focal area during the 'pre' period and were included in analyses of the *Heliconia* removal experiment; for a comprehensive summary of radio-tracking outcomes, see Appendix S2 (Table S5).

Floral visitation rates

To understand hummingbird resource use within each site and to monitor for the presence of marked individuals, we positioned trail cameras (PlotWatcher Pro, Day 6 Outdoors) near inflorescences of plant species known or suspected to be visited by hummingbirds. Effort within each site was concentrated at two locations ('stations', Fig. 1D) located on roughly opposite ends

of the focal area (mean ± SD distance between stations: 60 ± 44 m). Stations were centered around a focal *Heliconia* plant expected to flower throughout the experimental period, not in an extremely steep area, and >10 m from the fragment edge. The two focal *Heliconia* plants remained uncovered for the entire experimental period, which allowed us to observe whether visitation rates changed after declines in surrounding *Heliconia* density. To partially standardize plant species composition between control and treatment replicates, we added a floral array comprising ~3-5 additional plant species at each station (see Appendix S2: Table S6 and *'Pollination success'*).

Trail cameras took one photograph per second during daylight hours (5:30-17:30) and automatically combined these photographs into a time-lapse video. We reviewed videos for hummingbird sightings using the motion detection program MotionMeerkat (Weinstein 2015) or by manually watching the sped-up video. When hummingbirds were detected on camera, we identified the species, recorded any color marks, and noted whether they visited any flowers. From video, we also recorded the number of open flowers available per day. Dates with no available flowers were excluded from analysis, yielding 20,735 total hours of video (Appendix S2: Table S7).

Pollination success

To investigate whether *Heliconia* declines influenced pollination success, we examined styles for the presence of pollen tubes, which indicate pollen grain germination and subsequent movement toward the flower ovule(s) (Kearns and Inouye 1993). We focused our pollen tube investigation on the two focal *Heliconia* plants per replicate, as well as several species from the focal arrays (Appendix S2: Table S6). Throughout the experimental period, we collected flowers from these focal plants every 1-2 days, which corresponds to the maximum floral longevity for

most of the selected species (*Heliconia tortuosa*, *Hamelia patens*: Stratton 1989, *Stachytarpheta frantzii*: Thomas-Granger 2003). Styles were fixed in formalin-acetic acid-alcohol for at least 24 hours, stained with aniline blue, and examined for pollen tubes using epifluorescence microscopy (Kearns and Inouye 1993, Betts et al. 2015). Due to low pollen tube presence and/or challenges with pollen tube visualization, we were only able to analyze two plant species: *H. tortuosa* (N = 327 styles) and *H. patens* (N = 202 styles). Additional details about style collection and lab procedures are available in Appendix S2.

Additional response variables (Heliconia removal experiment only)

To supplement the primary response variables described above, we examined several additional metrics for the *Heliconia* removal experiment: (1) recapture probability (i.e., proportion of hummingbirds recaptured in the 'post' period), (2) floral visitation rates from individual hummingbirds marked with nail polish, and (3) body mass of hummingbirds captured during both experimental periods (pre and post). The first two response variables assessed the possibility that hummingbirds caught during the initial capture sessions were rapidly replaced by new individuals, potentially obscuring treatment effects. Body mass measurements allowed us to explore whether birds that remained in (or returned to) treatment focal areas paid an energetic cost. To compare mass changes across hummingbirds of different structural sizes, we calculated a relative body mass measure: observed mass divided by the mass predicted by species-specific allometric equations (i.e., Ln(body mass) ~ Ln(wing length), Appendix S2: Fig. S5).

Quantifying connectivity

Within each focal landscape, we digitized forested habitat using high resolution (1 m) Airbus/CNES satellite imagery in Google Earth Pro. When digitizing, we combined closed-canopy forest and regenerating forest because functional connectivity experiments suggest that

both forest types facilitate hummingbird movement (Jackson 2017). We then delineated connected areas of forest ('patches') assuming a hummingbird gap-crossing ability of 50 m (Volpe et al. 2016, Kormann et al. 2016). Lastly, we quantified functional connectivity as the amount of reachable forest within each focal landscape, following a modified version of Hanski's (1994) incidence function model for metapopulation connectivity. Substituting forested pixels for patches used in the original model, we assigned exponentially less importance to pixels farther away from the focal area (Fig. 1E, Appendix S2: Fig. S6). These weights were calculated as:

$$W_j = \exp(-\alpha\, d_{ij}), \qquad \textbf{(Eq. 1)}$$

where α is the decay rate parameter that scales the effect of distance and *d* is the shortest cumulative distance between the focal area centroid *i* and each forested pixel *j*, staying within the patch boundary. The decay rate parameter is inversely related to the average movement distance of the focal species (Moilanen and Nieminen 2002) and was calculated using the mean home range length of green hermit hummingbirds (282 m: Volpe et al. 2016). Then, to calculate functional connectivity, we then summed the weighted pixel areas as follows:

$$S_i = \Sigma\, W_j\, A_j, \qquad \textbf{(Eq. 2)}$$

where W*j* is the weight of each forested pixel and *A* is the area of each forested pixel. Geospatial processing was conducted using Google Earth Engine (Gorelick et al. 2017).

Quantifying resource availability

From the floral resource surveys (see *'Study design'*), we estimated the combined energetic value of *Heliconia* and non-*Heliconia* flowers. Briefly, we converted resource counts (i.e., numbers of flowers and inflorescences) into calories, the most biologically relevant unit for hummingbird energetics (e.g., Wolf et al. 1972, Stiles 1975). To calculate calories, we measured

nectar volume and concentration from >1400 flowers (*N* = 38 species) growing in the focal landscape and/or the Wilson Botanical Garden at the Las Cruces Biological Station, supplementing these measurements with literature data when necessary. We then converted average nectar volume and concentration per flower to calories per plant. Full methods and nectar data used in calorie calculations are available in Appendix S2 (Tables S8-S14).

Because tropical hummingbirds generally interact with subsets of the available floral resources (e.g., Stiles 1985, Maglianesi et al. 2014, Vizentin-Bugoni et al. 2014), we generated estimates of caloric availability specific to each hummingbird group examined (e.g., all species versus *Heliconia* specialists). We developed these calorie estimates before conducting any statistical analysis by summarizing visitation rates to 35 flowering plant species (15,119 video hours; Appendix S2: Table S7). The most frequently visited plant species for each hummingbird group was given a weight of one; other species were down-weighted accordingly (Fig. 3, Tables S15-S16). We divided these calorie estimates by focal area size, then natural log-transformed the resulting densities. Floral corolla length was measured from flowers collected in the field, supplemented with literature values when necessary (Appendix S2: Table S17).

Statistical analysis

We used linear mixed models (LMMs) and generalized linear mixed models (GLMMs) to analyze how experimental *Heliconia* extinction and natural variation in *Heliconia* density influenced our primary response variables: (i) captured hummingbird abundance, (ii) proportion of time spent in focal area, (iii) visitation rates to flowering plants (*Heliconia* and non-*Heliconia*), and (iv) plant pollination success. Analyses of the *Heliconia* removal experiment also included several additional, individual-level response variables (see '*Additional response variables'*). To determine whether declines in *Heliconia* affected *Heliconia* specialists more

strongly than other hummingbird species (**H4**), we analyzed all hummingbird responses with (i) the full dataset containing all species, and (ii) a dataset including green hermits and violet sabrewings.

We modeled each response variable using appropriate statistical distributions and controlled for variable effort when applicable (Appendix S2: Tables S18-S20). All statistical analyses were conducted in R version 4.1.1 (R Core Team 2021) using 'glmmTMB' (Brooks et al. 2017). For LMMs, we checked for normality, homoscedasticity, and linearity using the 'performance' package (Lüdecke et al. 2021). For GLMMs, we checked for overdispersion, zero-inflation, and linearity by visually inspecting residual plots from 'DHARMa' (Hartig 2020). Estimated marginal means were calculated using 'emmeans' (Lenth 2020) and visualized using the 'ggemmeans' function within 'ggeffects' (Lüdecke 2018). Results were considered statistically significant if $P < 0.05$.

Heliconia removal experiment

In a BACI experimental design, an overall treatment effect is indicated by a statistically significant interaction between the variables representing treatment and experimental period (Underwood 1994, McDonald et al. 2000). We adopted this standard analytical approach rather than calculating change scores (e.g., pre-to-post differences) because change scores are nearly always negatively correlated with baseline measurements; when baseline measurements also covary with predictor variables of interest, misleading (i.e., spuriously negative) relationships can emerge (Allison 1990). Therefore, we tested the *parallel extinction hypothesis* (**H1a**) and *robust network hypothesis* (**H1b**) using a two-way interaction between *Heliconia* removal and period (pre *versus* post) for all response variables measured during both experimental periods

(recaptures were measured in the post period only). All models included a random intercept for 'Site'. Except when modeling recapture probability, we also included a nested random effect for 'Replicate ID' (Site-Year), which paired pre-to-post observations within each replicate. Additional nested random effects, namely for plant species and individual birds, were included where applicable (Appendix S2: Tables S18-S19). Because we were interested in pre-to-post changes in our response variables, we only included covariates that varied between pre and post periods. For example, when analyzing hummingbird visitation rates, we accounted for the attractiveness of a given focal plant by including a covariate for the number of open flowers per day, averaged across the experimental period. Since the magnitude of our experimental *Heliconia* extinction varied widely (see '*Results'*), we ran parallel models using a categorical treatment variable (Control/Treatment) and a quantitative treatment variable (Calories removed/hectare). Because results were qualitatively similar, only results from the categorical approach are presented in the main text.

Natural variation in Heliconia density

To complement the *Heliconia* removal experiment, we also examined hummingbird responses over a natural gradient in *Heliconia* availability using data from the 'pre' period of the experimental sites. This 'space-for-time' approach elucidates how hummingbird space use and foraging behavior respond to natural variation present in the study area – presumably emerging over longer time scales than those of our removal experiment. We also used this approach to test the connectivity hypothesis and resource hypothesis by modeling the interaction term between *Heliconia* density and either connectivity (**H2**) or non-*Heliconia* resource availability (**H3**). These hypotheses were not tested using the experimental framework due to limited sample size

(and thus statistical power) for estimating a three-way interaction between treatment, experimental period, and resources/connectivity. Because we did not collect resource availability data in the control sites from 2016, these replicates (N = 6) were excluded.

We included several additional covariates not included in the analyses of the *Heliconia* removal experiment. First, we used estimates of non-*Heliconia* resource availability tailored to each hummingbird group being examined. Because resource availability was moderately correlated with connectivity (Pearson's r = 0.57, P = 0.002, Appendix S2: Fig. S7), we included both covariates in all models to statistically control for their independent effects. Analyses of radio telemetry data (i.e., proportion of time spent in focal area) included a covariate for the focal area size. When analyzing visitation rates of *Heliconia* specialists to non-*Heliconia* resources, we also included plant corolla length as a covariate, since these relatively long-billed hummingbirds (mean bill lengths of 40 and 33 mm: Borgella et al. 2001) are expected to select plant species with long corolla tubes (e.g., Maglianesi et al. 2014, Vizentin-Bugoni et al. 2014, Dalsgaard et al. 2021). We included random effects for 'Site' in all models; additional random effects were included where applicable (Appendix S2: Table S20).

RESULTS

Across all study sites and years, *Heliconia* density ranged from 3 to 1131 inflorescences per ha (mean ± SD: 186 ± 228, median: 145, Appendix S3: Table S1). The magnitude of our experiment therefore varied widely; we removed between 8 and 526 *Heliconia* inflorescences per treatment replicate (mean ± SD: 172 ± 138, median: 138, Appendix S3: Table S2). These inflorescences represented, on average, 96% (± 5.5%) of the *Heliconia* inflorescences encountered during removal (two focal *Heliconia* plants/site remained uncovered, and sometimes plant inaccessibility limited our covering capacity). Sizes of focal treatment areas ranged from

0.27 to 7.49 ha (mean ± SD: 1.7 ± 1.9, median: 1.25, Appendix S3: Table S2), depending on the size of the forest fragment and the navigability of the terrain. We estimate that, on average, the covered *Heliconia* plants supplied 42% (± 31%, range: 1 – 90%) of the calories available for the entire hummingbird community (*N* = 19 species total, Appendix S3: Table S3), and at least 66% (± 32%, range: 2 – 98%) of calories for *Heliconia* specialists (Fig. 2C, Appendix S3: Table S4). Focal *Heliconia* plants were visited by hummingbirds 6.4 ± 5.7 times per day (range: 0-25, median: 5), with green hermits visiting most frequently (75% of 1,055 visits, Appendix S3: Table S5).

Overall responses to changes in Heliconia density (H1)

Hummingbird behavior and pollination success were remarkably resistant to experimentally induced *Heliconia* extinction. Despite the magnitude of *Heliconia* removal and confirmed hummingbird visitation to *Heliconia*, we found no overall effect of extirpation on hummingbird space use, floral visitation rates, or plant pollination success. However, when examining these response variables across a natural gradient in *Heliconia* availability (i.e., the space-for-time approach), we found that floral visitation rates and pollination success declined as *Heliconia* became scarce. We provide detailed evidence for these findings below.

Hummingbird captures & radio telemetry

We found little evidence that *Heliconia* removal reduced hummingbird abundance or decreased time spent in the focal area. Although we caught 23% fewer hummingbirds in the ‘post’ capture session than the ‘pre’ capture session in treatment replicates (95% CI: -41% to 1%), this pre-to-post decline fell within the natural variability of the control replicates (all species: z = -1.11, P = 0.27; *Heliconia* specialists: z = -0.5, P = 0.62; Fig. 4: A-B; Appendix S3: Table S6). Similarly, although radio-tagged hummingbirds spent 25-30% less time in focal areas

during the ‘post’ period, we found no evidence that pre-to-post changes in space use were linked to our experimental manipulation (95% CI, treatment: -56% to 11%; 95% CI, control: -56% to 28%). Results were similar when examining all species together (Fig. 4C) and *Heliconia* specialists separately (Fig. 4D). We also found no evidence that natural variation in *Heliconia* density influenced hummingbird space use, quantified either with mist net captures or radio telemetry (Appendix S3: Tables S6-S7).

When examining individual-level hummingbird responses, we also did not observe an effect of *Heliconia* removal. On average, the estimated probability of recapturing a hummingbird from the ‘pre’ capture session was 18% in control replicates (95% CI: 11% to 29%), compared to 14% in treatment replicates (95% CI: 9% to 23%). This difference was not statistically significant, and recaptures of *Heliconia* specialists showed qualitatively similar results (Appendix S3: Fig. S1: A-B, Table S8). We also found no evidence that pre-to-post changes in relative body mass differed between control and treatment replicates (Appendix S3: Table S9).

Floral visitation rates & pollination success

We continuously monitored hummingbird visits to the two focal *Heliconia* inflorescences that remained uncovered throughout the experimental period, as well as numerous other flowering plants (mean ± SD: 9.3 ± 2 species/replicate; range: 6-14). Under the *robust network hypothesis*, we expected hummingbirds to rewire and increasingly use alternative (non-*Heliconia*) resources. However, pre-to-post changes in hummingbird visitation rate were similar between control and treatment replicates, both when examining the focal *Heliconia* plants (z = 0.83, P = 0.41, Fig. 5: A-B, Appendix S3: Table S10) and non-*Heliconia* plant species (z = -0.69, P = 0.49, Fig. 6: A-B, Appendix S3: Table S11). Responses by *Heliconia* specialists mirrored the responses observed for all species, although these species visited non-*Heliconia* species

relatively infrequently (<1 visit/day, on average: Fig. 6B). Results were similar when examining pre-to-post changes in visitation rates for individual color-marked hummingbirds observed on camera (Appendix S3: Fig. S1: C-F, Tables S12-S13).

In contrast to the experimental results, we found that natural variation in *Heliconia* availability strongly influenced visitation to focal *Heliconia* plants (Fig. 5: D-E). For every standard deviation decrease in the caloric density of *Heliconia*, we found that overall *Heliconia* visitation rates decreased by 49% (95% CI: 15% to 93%, $z = 3.03$, $P = 0.0024$, Appendix S3: Table S10). Visitation to non-*Heliconia* plants also declined when natural *Heliconia* availability was low, but only for *Heliconia* specialists ($z = 2.02$, $P = 0.044$, Fig. 6E, Appendix S3: Table S11).

We found no evidence that experimental *Heliconia* removal influenced pollination success for the focal *Heliconia* plants ($z = 0.23$, $P = 0.82$, Fig. 5C, Appendix S3: Table S14) or another plant species from the floral arrays (*Hamelia patens:* $z = 0.19$, $P = 0.85$, Appendix S3: Table S14). However, focal *Heliconia* plants were pollinated less when surrounding *Heliconia* availability was naturally low; for every standard deviation decrease in the caloric density of *Heliconia*, the odds of pollination declined by 2.4 times (95% CI: 1.1 times to 5.4 times, $z = 2.2$, $P = 0.028$, Fig. 5F).

Effects of connectivity (H2) and resource availability (H3)

Hummingbird captures & radio telemetry

Connectivity to forested habitat influenced hummingbird abundance, and alternative (non-*Heliconia*) resource availability was associated with the amount of time radio-tagged birds spent in focal areas. Specifically, hummingbird captures increased by 6% for every one-unit increase in functional connectivity (95% CI: +1% to +12%, $z = 2.31$, $P = 0.021$, Appendix S3: Table S6), and the odds of being in the focal area increased by 2.3 times (95% CI: 1.3 to 4.1

times) for every standard deviation increase in caloric density (all species only: $z = 2.78$, $P = 0.0055$, Appendix S3: Table S7). However, neither connectivity or resource availability appeared to mediate hummingbird responses to *Heliconia* declines, either with experimental removal or across the natural density gradient ($P > 0.05$ for all interactions of interest, Appendix S3: Tables S6-S7).

Floral visitation rates & pollination success

Connectivity showed no statistically significant main effects on hummingbird visitation rates, either to focal *Heliconia* plants or non-*Heliconia* species. However, after *Heliconia* removal, visitation rates to focal *Heliconia* plants by *Heliconia* specialists declined more sharply when connectivity was low ($z = -1.76$, $P = 0.08$, Appendix S3: Fig. S2, Table S10). This potential interactive effect did not emerge when examining *Heliconia* pollination success ($z = -0.55$, $P = 0.58$). Although we expected that rewiring might be more likely to occur when alternative resources were abundant, we found no evidence for this hypothesis in the visitation rates to non-*Heliconia* plant species (Appendix S3: Table S11).

DISCUSSION

In this study, we experimentally induced the local extinction of a locally abundant tropical plant, *Heliconia tortuosa*, to assess the robustness of plant-hummingbird interaction networks in a fragmented tropical landscape. Additionally, across a natural gradient in *Heliconia* density, we assessed whether low *Heliconia* abundance would lead to parallel declines in hummingbirds and their pollination services – and whether landscape context (connectivity) and local context (alternative resource availability) could buffer against potential declines. Based on suite of complementary response variables we found that, even when very high proportions of total available calories were experimentally removed (~42-66% on average), hummingbird responses fell within the natural variability of control replicates. As might be expected with such

small changes in hummingbird behavior, we did not observe any cascading effects of experimental manipulation on pollination success of flowering plants, which supports the *robust network hypothesis* (**Hlb**). On the other hand, when examining hummingbird responses and pollination success across a natural gradient in *Heliconia* density, we found evidence for the *parallel extinction hypothesis* (**H1a**). In sites where *Heliconia* density was low, hummingbirds visited individual *Heliconia* plants less frequently, and these plants experienced reduced pollination success. Other flowering plants also exhibited lower visitation rates from *Heliconia*-associated hummingbirds, suggesting that high *Heliconia* densities may attract these hummingbirds (and/or incentivize repeat visitation) and therefore facilitate pollination for other species.

Our finding that that experimental removal of *Heliconia* did not elicit parallel extinctions in pollinators aligns with theoretical predictions (Memmott et al. 2004, Kaiser-Bunbury et al. 2010) and several other experiments that removed plant species from mutualistic networks (Ferrero et al. 2013, Goldstein and Zych 2016, Costa et al. 2018). However, if hummingbirds remained in forest fragments despite *Heliconia* declines, we assumed that they would rewire to use alternative floral resources; hummingbirds as a taxonomic group are highly opportunistic foragers (Arizmendi and Ornelas 1990, Maruyama et al. 2013) and the most frequent *Heliconia* visitor, the green hermit, has a relatively wide niche breadth (Borgella et al. 2001, Betts et al. 2015). Unexpectedly, we found no evidence that hummingbirds increasingly visited non-*Heliconia* resources when *Heliconia* became less available, suggesting that the mechanisms underlying network robustness in this study may not necessarily align with the rewiring observed in previous empirical tests (e.g., Timóteo et al. 2016, Kaiser-Bunbury et al. 2017, Costa et al. 2018). This apparent lack of foraging flexibility may emerge from various constraints, such as

morphological trait-matching, search costs, and/or competition from other hummingbirds (see also Weinstein and Graham 2017). For example, high levels of exploitative competition for non-defendable resources may have discouraged hummingbirds from modifying their trapline and risking overlap with another bird's foraging route (Young 1971, Gill 1988, Ohashi and Thomson 2009). Lastly, is possible that fine-scale rewiring did occur but was not detected using our analytical approach, which aggregated visitation rates to the level of hummingbird species; further analysis examining the diversity of pollen grains carried by individual hummingbirds may shed light on the possibility of rewiring at finer scales.

In the absence of rewiring, the resilience in hummingbird behavior, abundance, and pollination services following experimental *Heliconia* removal is surprising. Brief pauses in nectar intake can disrupt a hummingbird's daily energy balance, given their high metabolic rate and limited energy reserves (Calder and Booser 1973, Tooze and Gass 1985, Brice 1992). Moreover, previous nectar manipulations with hummingbirds – albeit within defended territories – led to rapid changes in territory size, foraging patterns, and/or defense behavior, generally within a day (e.g., Hixon et al. 1983, Gass and Sutherland 1985, Eberhard and Ewald 1994, Temeles et al. 2004). One explanation for our result is that hummingbirds permanently emigrated from fragments after *Heliconia* removal but, because of high intruder pressure, were quickly replaced by new individuals (Stiles and Wolf 1970). However, we monitored individual birds using several different approaches (radio telemetry, recaptures, visitation rates) and found no evidence that *Heliconia* removal caused emigration and subsequent replacement. An alternative possibility is that hummingbirds relied on physiological adaptations, such as a torpor and/or insectivory, to maintain body condition despite large declines in nectar availability – resulting in the stable body mass measurements we observed in recaptured hummingbirds. Torpor is an

energy-saving adaptation characterized by declines in body temperature and metabolic rate (Hainsworth et al. 1977, Spence and Tingley 2021), and even tropical hummingbirds inhabiting low-to-mid elevations appear to possess this capability, including the green hermit (Krüger et al. 1982, Schuchmann and Prinzinger 1988, Bech et al. 1997, Shankar et al. 2020). Additionally, hummingbirds are highly insectivorous and, although they cannot completely replace nectar with insects (Brice 1992, Stiles 1995), they may supplement their diet with arthropods when nectar becomes scarce (Young 1971, Kuban and Neill 1980, Montgomerie and Redsell 1980, Hazlehurst and Karubian 2018). Finding torpid hummingbirds at night is extremely challenging (Carpenter and Hixon 1988), but future research could explore changes in arthropod consumption using techniques such as DNA metabarcoding (Moran et al. 2019).

Another explanation for hummingbird persistence following experimental *Heliconia* removal involves their high movement ability. *H. tortuosa* is a patchily distributed species that necessitates long-distance foraging movements, as a single clump does not make territoriality energetically profitable (Stiles 1975). Densities can also vary substantially across short distances; for example, Stiles (1979) notes that some Costa Rican *Heliconia* species can be common in one watershed, yet nearly absent in an adjacent watershed < 1 km away (see also Bruna and Kress 2002). The most frequent *Heliconia* visitor, the green hermit, is therefore capable of traveling long distances (>500m) while foraging within home ranges of >150 ha, although home range sizes of ~2 ha are more typical (Volpe et al. 2014, 2016). Thus, returning to our focal areas (typically ~1.5 ha) to visit the few remaining *Heliconia* resources could be perceived as a relatively low-cost option for long-distance trapliners, at least in the short term. Notably, although highly mobile pollinators might buffer against network collapse (Volpe et al. 2016), they may be vulnerable to habitat loss and fragmentation once thresholds are crossed, leading to

parallel pollinator declines observed in nature (Andrén 1994, Hadley and Betts 2012). This strategy is also unlikely to be profitable over longer time scales than measured by our experiment, which only removed *Heliconia* for five days.

The relatively short length of our removal experiment might also explain why – when examining hummingbird responses across a natural gradient in *Heliconia* availability – lower *Heliconia* densities were indeed associated with reduced floral visitation rates. Although a short adjustment period (<1 day) is standard in similar species removal experiments (e.g., Hixon et al. 1983, Brosi and Briggs 2013, Hazlehurst and Karubian 2018), extending the experimental timeline may have more realistically simulated a future extinction and provided more time for behavioral or demographic adjustment. If hummingbirds were not permanent residents of our focal areas – as suggested by our relatively low recapture rates (~15%) and proportion of time spent in the focal area (~20%) – then transient individuals might have not immediately noticed changes in *Heliconia* density and changed their foraging patterns accordingly. Likewise, hummingbirds might not have persisted had we removed *Heliconia* over larger areas, because traveling to isolated plants would become even more energetically costly (Heinrich and Raven 1972). Unfortunately, covering (and then uncovering) *Heliconia* plants in steep terrain with dense understories limited the area we could manipulate.

We also acknowledge that our ability to detect experimental effects may have been limited by low statistical power, owing to high individual-level variation and limited sample size. Low statistical power may have also weakened our tests of the connectivity hypothesis and resource hypothesis, as those datasets only included a subset of the replicates included in the experimental analyses (resource availability data were not available for all sites). Still, we emphasize that the spatial scale of our experiment and amount of replication surpasses most

other empirical studies simulating species extinctions within plant-pollinator networks (Appendix S1: Table S1). Nevertheless, future removal experiments with highly mobile pollinators should consider increasing the amount of replication, and, if possible, manipulate larger areas.

In the space-for-time component of our study, we found that visitation rates to individual plants decreased with natural declines in *Heliconia* density, suggesting that *Heliconia* and its pollinators might facilitate pollination of co-flowering plants in this study system. Indeed, facilitative effects arising from the overall attractiveness of the floral neighborhood are expected to operate most strongly in systems where plants are spatially dispersed and pollinator density is low (Schemske 1981, Moeller 2004, Ghazoul 2005), as is the case in the tropical forest understory. If *Heliconia* population densities decrease due to habitat fragmentation (Hadley et al. 2014), plants visited frequently by *Heliconia*-associated hummingbirds might therefore experience a higher risk of reproductive failure (e.g., *Pitcairnia imbricata*: Fig. 3). Whether visitation rates map onto pollination success for these species remains an open question, since pollinator sharing may lead to heterospecific pollen transfer and pollen loss (Morales and Traveset 2008, Ashman and Arceo-Gómez 2013) – tipping the balance toward interspecific competition rather than facilitation (Rathcke 1983, Ghazoul 2005, Mitchell et al. 2009). However, we did discover a positive relationship between visitation rate and pollination success for *Heliconia*. This relationship has been predicted for other *Heliconia* species but yielded mixed results (Bruna et al. 2004, Stein et al. 2013), in accordance with accumulating evidence that facilitation effects can be highly context-specific (Bergamo et al. 2020). Nevertheless, this finding suggests that any reproductive declines of *Heliconia* in this study system might accelerate once extinction debt is realized and conspecific density begins to decline.

CONCLUSIONS

Although pollinators seemed able to persist during our experiments that removed floral resources for a short period of time (several days), this persistence may not be sustainable or reflect the outcome of a natural extinction process. In fact, we observed declines in hummingbird visitation when *Heliconia* availability was naturally low, with cascading consequences for pollination success. Thus, longer-term experiments will be key for predicting consequences of species loss and understanding mechanisms of network robustness – at least for pollinator species that forage at large spatial scales. Our study also suggests that behavioral flexibility to use alternative floral resources might be limited, even in taxonomic groups thought to be highly opportunistic foragers. Thus, even rewiring rules with strong biological underpinnings (e.g., Vizentin-Bugoni et al. 2020) may not necessarily predict real-world responses to species loss, perhaps due to additional constraints such as competition. To further understand the mechanisms underlying network robustness or collapse, we encourage future experiments that consider how temporal scale, species traits (e.g., movement ability), competition, and landscape context interact to determine the outcome of species extinctions. These experiments could be especially valuable at geographic range limits, where climate change will likely lead to species replacements (HilleRisLambers et al. 2013), and in the tropics where landscape change and species loss are most rapid (Hansen et al. 2013).

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TABLES

Table 1. Hypotheses testing whether declines of a locally common, tropical flowering plant (*Heliconia tortuosa*) affect hummingbird space use, hummingbird floral visitation rates, and plant pollination success. Predicted effects are indicated in the column below each hypothesis. To address Question 1, we (i) experimentally removed *H. tortuosa* using a BACI (Before-After-Control-Impact) experimental design and (ii) studied a natural gradient in *H. tortuosa* density. Under the experimental framework, predicted effects can be conceptualized as pre-to-post changes in treatment replicates, compared to pre-to-post changes in control replicates. To address Question 2, we examined changes in hummingbird and plant responses across a natural gradient in *H. tortuosa* density only.

			Question 1: How do hummingbirds respond to declines in *Heliconia*, and are there cascading effects on the remaining plants?		Question 2: What factors mediate hummingbird responses to *Heliconia* declines?		
	Response variable	Methodological approach	Robust network hypothesis (H-1a)	Parallel extinction hypothesis (H-1b)	Connectivity hypothesis: Rescue (H-2a) *In highly connected forest fragments…*	Connectivity hypothesis: Abandonment (H-2b) *In highly connected forest fragments…*	Resource hypothesis (H-3) *In forest fragments with high resource availability…*
Space use	Abundance	Mist net captures	No change	Decrease			
	Time spent in focal area	Radio telemetry					
Floral visitation rates	Visitation to focal *Heliconia* plants	Remote camera observations	No change or increase	Decrease	Smaller decrease	Larger decrease	Smaller decrease
	Visitation to non-*Heliconia* plants	Remote camera observations	Increase (rewiring)	Decrease			
Pollination services	Pollination success	Pollen tubes	No change or increase	Decrease			

FIGURES

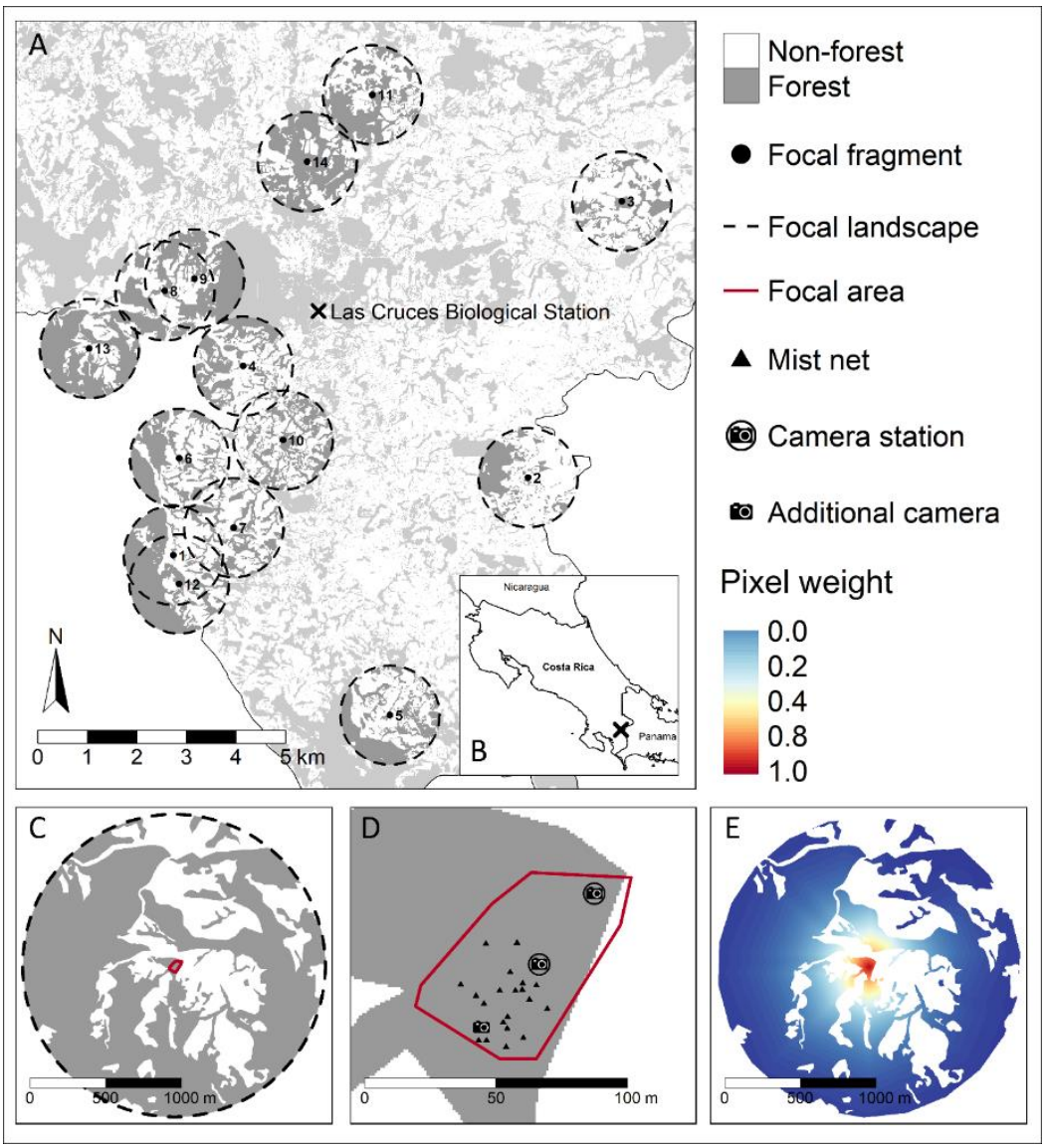

A
Non-forest
Forest
Focal fragment
Focal landscape
Focal area
Mist net
Camera station
Additional camera
Pixel weight
0.0
0.2
0.4
0.8
1.0
Las Cruces Biological Station
N
0 1 2 3 4 5 km
Nicaragua
Costa Rica
Panama
B
C
0 500 1000 m
D
0 50 100 m
E
0 500 1000 m

Figure 1. This study was conducted in the fragmented landscape surrounding the Las Cruces Biological Station in southern Costa Rica **(A-B).** Forested areas are shown in grey. The dark grey forest layer was hand-digitized for this study; the light grey forest layer, available within the geographic boundary of Coto Brus Canton, was obtained from Mendenhall & Wrona ***(2018)***. Non-forested areas (white) primarily comprise pasture. **(A)** Within the study system (rectangular extent), we utilized fourteen focal landscapes of 1000 m radius ('sites', dashed circles). Sites are numbered by increasing connectivity to other areas of forest (1 = least connected, 14 = most connected). Within a year (2016-2018), each site received either the *Heliconia* removal treatment or was assigned to be a control. Treatments were reversed in alternate years for a total sample size of 16 control replicates and 16 treatment replicates. **(C)** An example site, with red line representing a focal area [i.e., where we removed *Heliconia* (for treatment replicates) and/or surveyed for floral resources (for treatment and control replicates)]. **(D)** An example focal area, showing where we captured hummingbirds using mist nets (triangles) and monitored plant-hummingbird interactions using trail cameras (camera icons). **(E)** An illustration of how we calculated functional connectivity. We first identified any forested pixels (1 x 1 m) that were connected to the focal area, assuming a hummingbird gap-crossing ability of 50 m; in the example shown, all pixels were connected to the focal area (center of map). We then down-weighted pixel areas based on their distance to the focal area centroid (higher weights = closer to focal area). Finally, we summed all weighted pixel areas within the 1000 m focal landscape (see Appendix S2 for details).

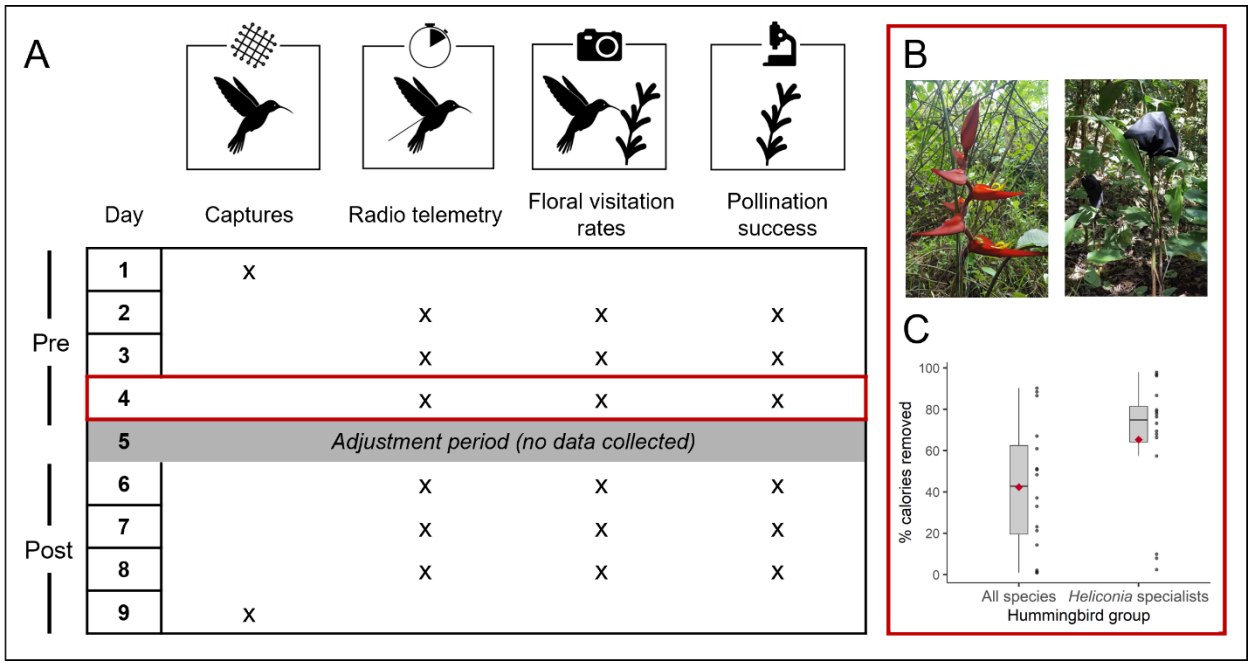


Figure 2. (A) During the *Heliconia* removal experiment, we measured four main response variables: captured hummingbird abundance, proportion of time radio-tagged hummingbirds spent in the focal area, floral visitation rates, and plant pollination success. Each response variable was measured during both experimental periods (pre and post); the sampling schedule for each is denoted with an 'X'. Each experimental replicate used two sites (control and treatment) sampled using this schedule, offset by one day. Starting around noon on the fourth day, we covered *Heliconia* inflorescences in the treatment site. **(B)** *Heliconia* inflorescences were covered with dark-colored bags that prevented hummingbirds from accessing any nectar present. **(C)** Our manipulation removed, on average, 42% (± 31% SD) of the calories available for the entire hummingbird community and 66% (± 32% SD) of the available calories for *Heliconia* specialists (green hermits and violet sabrewings). Mean values are denoted by red diamonds, and the darker black line represents the median. Lower and upper lines within boxplots represent the first and third quartiles. Whiskers extend from the first and third quartiles to the lowest and highest values within 1.5 times the interquartile range. Individual data points

appear next to each boxplot. *Response variable icons in (A) were created using images from the Noun Project (see 'Acknowledgments'). Photos in (B) were provided by Marion Donald.*

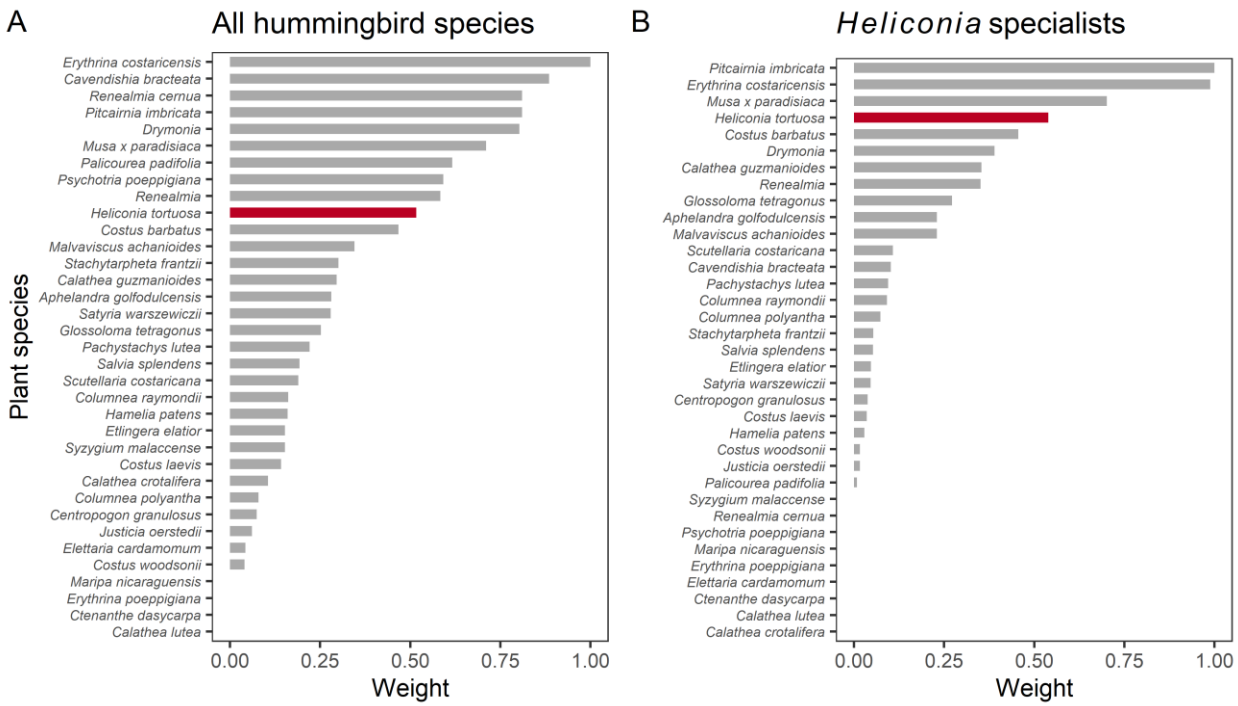


Figure 3. Relative visitation rates (weights) used to tailor our resource availability estimates to different groups of hummingbird species. Within each hummingbird group, the most frequently visited plant species was assigned a weight of one. Visitation rates are summarized from the 15,119 hours of 'normal' (i.e., unmanipulated) camera observations collected in the landscape surrounding the Las Cruces Biological Station across 14 study sites, three field seasons (2016-2018), and 35 plant species. **(A)** Weights summarized across all hummingbird species detected on camera (*N* = 17 species). **(B)** Weights for green hermits (*P. guy*) and violet sabrewings (*C. hemileucurus*), two hummingbird species with morphologically specialized bill shapes that mirror the curvature of *Heliconia* flowers. These two species are also the most frequent *Heliconia* visitors (Appendix S3: Table S5).

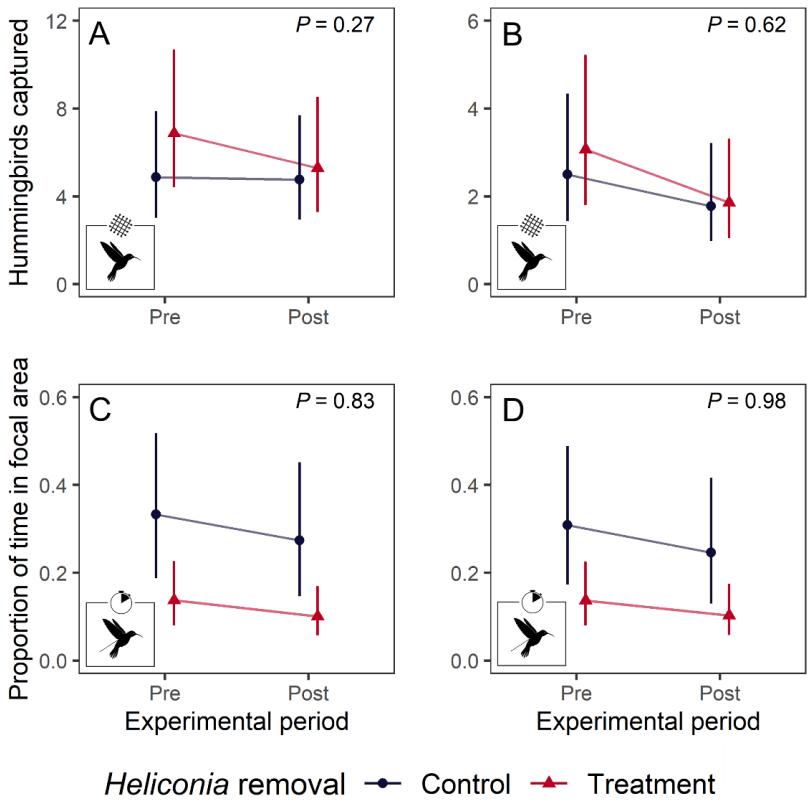


Figure 4. Hummingbird space use before and after experimental local extinction of an understory plant, *Heliconia tortuosa*, within forest fragments in southern Costa Rica. Despite removing at least 42% (± 31 SD) of calories available to hummingbirds within focal areas of 1.7 ha (± 1.9 SD), we did not observe an effect of *Heliconia* removal on the number of hummingbirds captured (A-B) or the proportion of time that radio-tagged hummingbirds spent in the focal areas (C-D). Results are shown for all species analyzed (left) and green hermits and violet sabrewings (right). Estimated marginal means from GLMMs are presented alongside 95% confidence intervals. *P*-values refer to the interaction testing the effect of *Heliconia* removal, relative to control replicates; conceptually, a treatment effect is indicated by non-parallel lines. The estimated number of captured hummingbirds is calculated for 45.8 net-hours, the mean capture effort across all replicates.

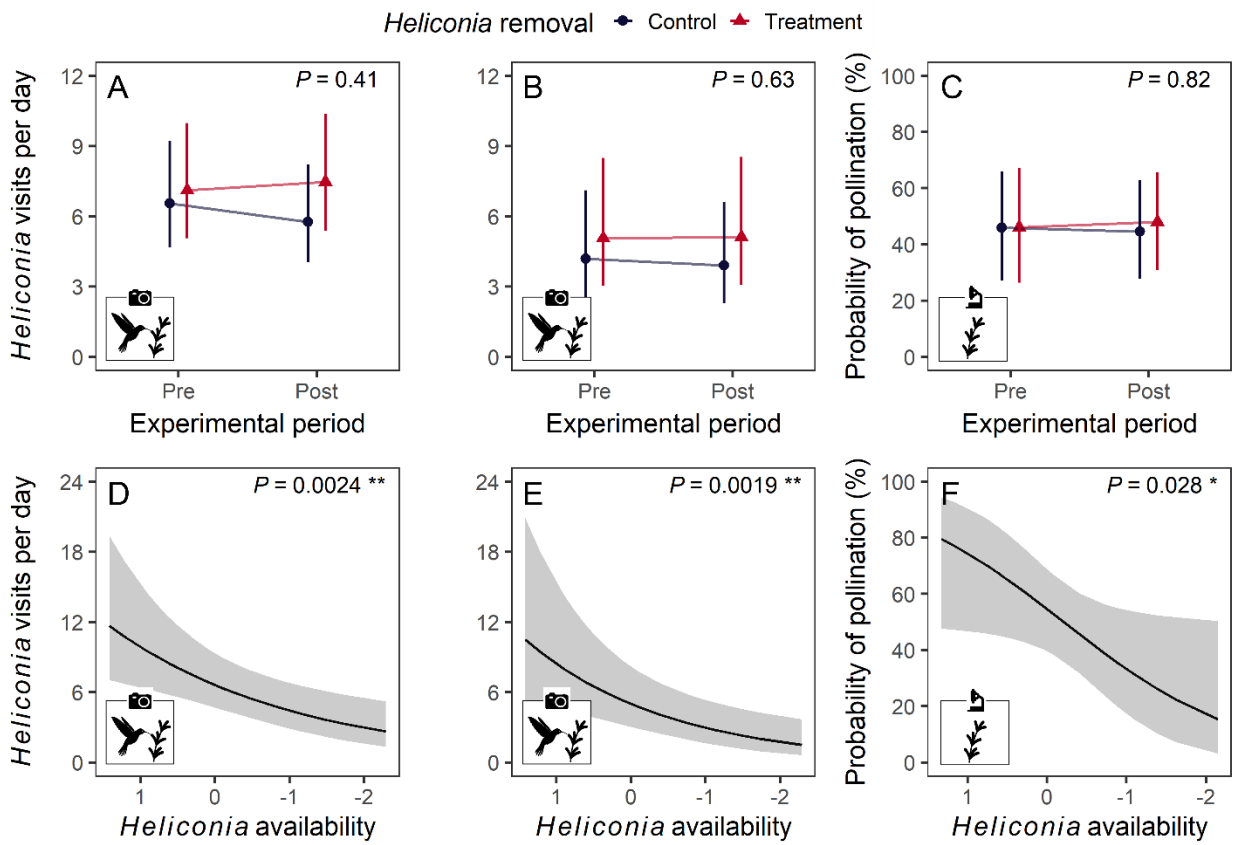
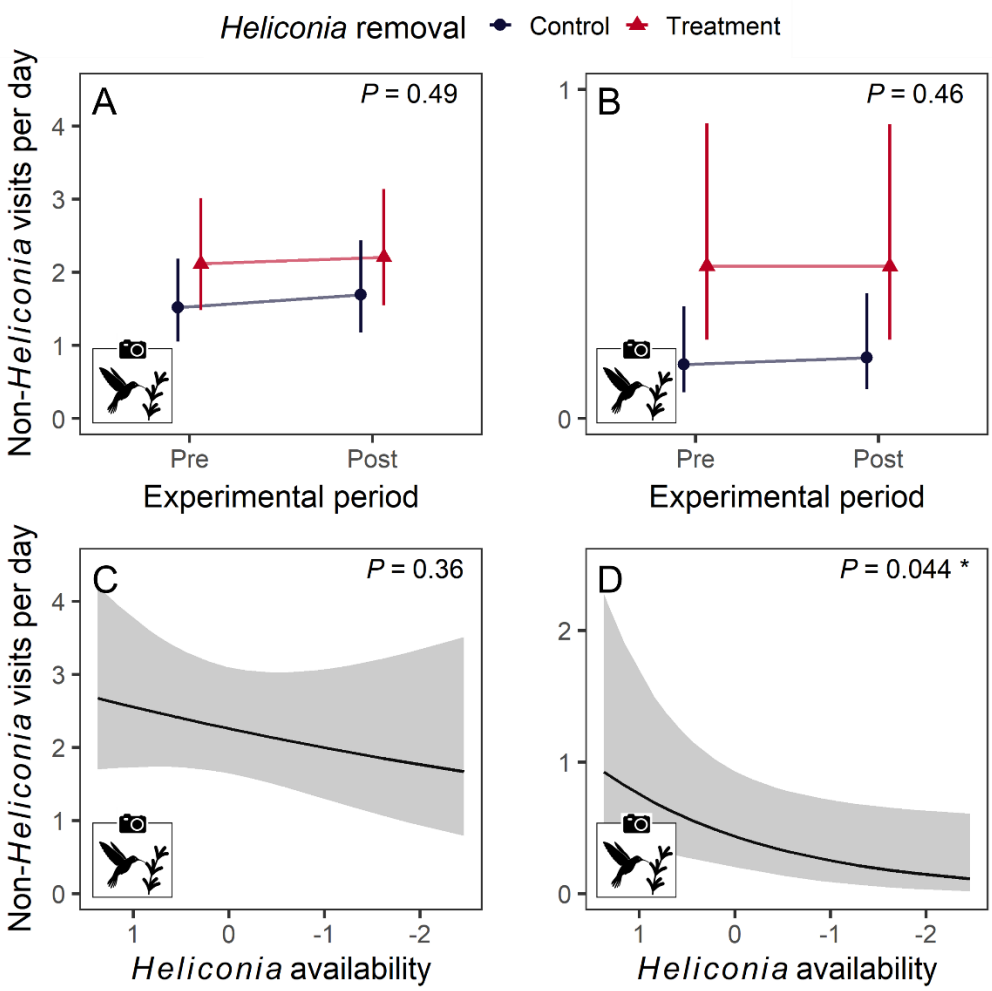


Figure 5. Hummingbird visitation rates to focal *Heliconia* plants and *Heliconia* pollination success before and after experimental *Heliconia* removal (A-C) and across a natural gradient in *Heliconia* density (D-F). For hummingbird visitation rates, results are shown for the entire hummingbird community (A, D) and green hermits and violet sabrewings separately (B, E). Estimated marginal means from GLMMs are presented alongside 95% confidence intervals. (A-C) *P*-values refer to the interaction testing the effect of *Heliconia* removal, relative to control replicates; conceptually, a treatment effect is indicated by non-parallel lines. (D-F) Values of *Heliconia* availability (*x*-axis) are standard deviations (1 SD = 1.35), with zero representing the mean log-transformed caloric density (mean = 8). Note that the *x*-axis is reversed, such that declining visitation is associated with declining *Heliconia* availability. *P*-values refer to the coefficient for *Heliconia* availability.


Heliconia removal
Control
Treatment
A
P = 0.49
B
P = 0.46
C
P = 0.36
D
P = 0.044 *
Non-*Heliconia* visits per day
Pre
Post
Experimental period
Heliconia availability

1076

Figure 6. Hummingbird visitation rates to non-*Heliconia* plant species before and after experimental *Heliconia* removal (A-B) and across a natural gradient in *Heliconia* density (C-D). Results are shown for the entire hummingbird community (A, C) and green hermits and violet sabrewings separately (B, D). Estimated marginal means from GLMMs are presented alongside 95% confidence intervals. (A-B) *P*-values refer to the interaction testing the effect of *Heliconia* removal, relative to control replicates; conceptually, a treatment effect is indicated by non-parallel lines. (C-D) Values of *Heliconia* availability (*x*-axis) are standard deviations (1 SD = 1.35), with zero representing the mean log-transformed caloric density (mean = 8). Note that the *x*-axis is reversed, such that declining visitation is associated with declining *Heliconia* availability. *P*-values refer to the coefficient for *Heliconia* availability.