1]	Edge effects and vertical stratification of aerial insectivorous						
2	bats across the interface of primary-secondary Amazonian							
3		rainforest						
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37 Abstract

38 Edge effects, abiotic and biotic changes associated with habitat boundaries, are key drivers of 39 community change in fragmented landscapes. Their influence is heavily modulated by matrix composition. With over half of the world's tropical forests predicted to become forest edge by 40 41 the end of the century, it is paramount that conservationists gain a better understanding of how 42 tropical biota is impacted by edge gradients. Bats comprise a large fraction of tropical 43 mammalian fauna and are demonstrably sensitive to habitat modification. Yet, knowledge 44 about how bat assemblages are affected by edge effects remains scarce. Capitalizing on a 45 whole-ecosystem manipulation in the Central Amazon, the aims of this study were to i) assess 46 the consequences of edge effects for twelve aerial insectivorous bat species across the interface 47 of primary and secondary forest, and ii) investigate if the activity levels of these species differed 48 between the understory and canopy and if they were modulated by distance from the edge. 49 Acoustic surveys were conducted along four 2-km transects, each traversing equal parts of 50 primary and ca. 30-year-old secondary forest. Five models were used to assess the changes in 51 the relative activity of forest specialists (three species), flexible forest foragers (three species), and edge foragers (six species). Modelling results revealed limited evidence of edge effects, 52 53 except for forest specialists in the understory. No significant differences in activity were found 54 between the secondary or primary forest but almost all species exhibited pronounced vertical 55 stratification. Previously defined bat guilds appear to hold here as our study highlights that 56 forest bats are more edge-sensitive than edge foraging bats. The absence of pronounced edge 57 effects and the comparable activity levels between primary and old secondary forests indicates 58 that old secondary forest can help ameliorate the consequences of fragmentation on tropical 59 aerial insectivorous bats.



Keywords (5): Chiroptera, secondary forest, acoustic sampling, vertical stratification

61 Introduction

62 Deforestation and fragmentation of tropical forests continue to be major contributors to global biodiversity loss (1). The Brazilian Amazon currently hosts over 10,000 plant species and is a 63 64 global hotspot for terrestrial vertebrate diversity (2,3). Declines in Amazonian deforestation over the last two decades provided some with optimism for Brazil's commitment to 65 conservation. However, in 2020 the Brazilian Amazon experienced the highest deforestation 66 67 rates for the last decade (4). This was largely driven by the dismantling of environmental 68 regulations and enforcement capacity, compounded by political and economic uncertainty left by the wake of the COVID-19 tragedy in Brazil (4-7). Such deforestation has massive 69 70 implications for global biodiversity, as well as global carbon emissions (5,8,9).

71

72 Deforestation creates a patchwork of isolated forest fragments across modified landscapes. The 73 interface between these artificially created fragments and the matrix (e.g., pasture or 74 agricultural land) is subjected to edge effects (10). Edge effects, the changes in abiotic 75 conditions and biotic interactions at the boundary between two contrasting habitats, are strong 76 determinants of ecological processes in humanized landscapes (1,11). As edge conditions 77 exceed the variability typically associated with habitat interiors, environmental deterioration 78 often decreases the habitat suitability for the assemblages it previously supported (8,11–15). 79 Approximately 70% of remaining global forests are within 1 km of the edge (1) and 85% of 80 1,673 vertebrate populations are already affected by edge effects (11), with edge area globally 81 increasing from 27% to 37% over the last decade (16). By 2100, half of tropical forest is 82 predicted to become forest edge (16). In the Brazilian Amazon, at least 35,000-50,000 km of 83 new edge is created annually (17).

85 Despite being one of the most well-studied ecological phenomena of the last century (18), edge 86 effects are still not well understood due to their diversity and complexity. Two components of edge influence, edge extent and edge magnitude, can be distinguished. The extent of edge 87 88 effects is defined as the distance over which changes in natural conditions that are associated 89 with habitat boundaries penetrate habitat interiors, whereas magnitude is the relative strength 90 of an edge effect (19). Both metrics are highly taxon and context specific, and the range of 91 edge-effect extent is widely debated. Most edge effects have been documented to occur 92 between 100-300 m from the edge (e.g., changes to canopy height and understory bird 93 densities; 15,19,20). However, other studies estimate they may extend 1–10 km into forest 94 interiors (e.g., shifts in carnivore abundance; 21-25). Matrix composition is known to 95 significantly affect both the extent and magnitude of edge effects, with low-contrast matrices 96 (e.g., secondary forest in advanced regeneration) increasing connectivity between remnant 97 forest patches and reducing the gradient of microclimatic change (12,26–28). Therefore, forest 98 regeneration can lead to 'edge sealing' or 'edge softening' (26), as the disturbed, secondary 99 forest can provide habitat for primary forest (forest relatively undisturbed by human activities) 100 specialists. Many tropical studies fail to consider source-sink dynamics between populations 101 in primary forest and the matrix (29). This is the process whereby species can persist in the 102 secondary forest (a "sink" habitat) so long as there is continual immigration from primary forest 103 (a "source" habitat). Without such proximity to the source habitat, populations in the sink 104 habitat would begin to decline (29). As such, studies comparing species responses across a 105 habitat boundary should consider the habitats on either side as interactive and not as 106 independent units.

107

There have been over 405 reforestation projects across the Brazilian Amazon since 1950, and
vast areas of abandoned pastureland are now under natural forest regeneration (9). Between

110 1986 and 2018, over 260,000 km² of secondary forest has regenerated in the Brazilian Amazon 111 which equates to almost 60% of the area of old-growth forest which was lost between 1988 112 and 2019 (4,30). Secondary forests are increasingly recognized for improving species' 113 persistence in tropical human-modified landscapes (31). As secondary forests mature, they 114 reduce the gradient of structural differences between the matrix and the primary forest (28,31,32). This helps to mitigate the impact of edge effects in primary forest and increase 115 116 habitat suitability across the landscape (32,33). Whilst secondary forests are no substitute for 117 old-growth forests, they typically support around 57% of the diversity of primary forests (31), 118 even after only 14–19 years of regeneration (34). For indicator species, such as dung beetles 119 and birds, there is evidence to suggest secondary forests can support the equivalent diversity 120 of primary forests within 15 to 30 years, respectively (35). As secondary regrowth continues 121 to mature, it has been shown to support more forest specialist species, including bats (32,35-122 38).

123

124 The Amazon supports over 200 bat species that perform important ecological roles in tropical 125 forests, such as pollination, seed dispersal and insect suppression (39,40). Few studies to date 126 have investigated how tropical bats respond to edge effects and existing studies have focused predominantly on phyllostomids, the ecologically most diverse Neotropical bat family, as these 127 128 species can be reliably sampled using mist nets (e.g., 40,22,12). These studies suggest bats may 129 be affected by edge effects up to 3 km from the habitat boundary (22), with most studies 130 indicating species richness declines at the forest edge, whereas the abundance of several 131 dominant generalist species increases (13,42). Aerial insectivores, which represent a large 132 fraction of Amazonian bat diversity (43), have so far been overlooked. There have also been 133 limited studies investigating how fragmentation and edge effects may affect bats differently 134 between forest strata (but see 43–45). It is widely accepted that there are differences in both bat diversity and abundances between the canopy and understory in the Amazon (48,49).
However, due to sampling logistics, it is often difficult to incorporate canopy sampling into
mist-netting surveys. Alternatively, acoustic monitoring enables us to include aerial
insectivores in such studies and provides an effective method for cross-strata comparisons,
thereby providing a more comprehensive understanding of how the wider bat community may
be impacted by edge effects.

141

142 Working within an experimentally fragmented landscape with low fragment-matrix contrast, 143 the overarching goal of this study was to assess edge influence, both in terms of extent and 144 magnitude, on Amazonian aerial insectivorous bats. Specifically, we evaluated how bat activity 145 varied along a habitat gradient of increasing distance from the habitat boundary in both 146 secondary and primary forest. We assessed how this response in activity varied along this 147 gradient between the understory and canopy. These comparisons were conducted for common 148 species/sonotypes and three functional guilds. We hypothesized that forest specialist activity 149 would exhibit a negative edge effect response in both habitats, whereas we expected to see a 150 positive or null response for flexible forest foragers and edge foragers. Furthermore, we 151 anticipated that responses to edge effects differ between the understory and canopy, with a 152 greater extent and magnitude being observed in the canopy.

153

154 Materials and methods

155 This research was conducted under ICMBio (Instituto Chico Mendes de Conservação da156 Biodiversidade) permit (26877-3).

158 Study sites

159 Our study was conducted in the Central Brazilian Amazon, 80 km north of Manaus, at the Biological Dynamics of Forest Fragments Project (BDFFP; 2024'26''S, 59043'40''W; Fig 1). 160 161 The BDFFP is the world's most comprehensive, long-term experimental study into the effects 162 of habitat fragmentation across a broad range of taxa (12). The primary forest is classified as *terra firme* forest, with an average tree diversity of 280 species per hectare (50). In the early 163 164 1980s, a series of primary forest fragments (1, 10, and 100 ha) were experimentally isolated 165 within cattle ranches, separated 80-650 m from continuous forest. However, forest 166 regeneration quickly occurred after the ranches were abandoned 5-10 years later due to 167 economic unviability (51,52). Regrowth forest was dominated by Vismia spp., in areas that 168 were cleared and burned, or *Cecropia* spp., in areas that were cleared without fire (53). The 169 understory is dominated by palms (52) and is characterized by an average canopy height of 23 170 m (52). The secondary forest at the time of the study was classified as 'old secondary forest' 171 using the age classes proposed by Powell et al. (2015; 27–31 years old with a mean canopy 172 height \geq 19 m) (38). A small strip has been periodically cleared to ensure fragment isolation, 173 most recently between late 2013 and early 2014 (54). Average annual rainfall ranges between 174 2.3-2.5 m, with large interannual variation (1.9-3.5 m). The wet season occurs between November and June (monthly rainfall > 250 mm) and the dry season occurs between July and 175 176 October (monthly rainfall < 100 mm). The average temperature is between 26-30°C and the 177 study area is characterized by low-lying topography (80-160 m elevation (32,55)).

178

Figure 1. Location of the primary-secondary forest transects at the Biological Dynamics of Forest Fragments Project, Central Amazon, Brazil.

(a) Transect location within the BDFFP is presented in the inserts, where primary forest isdenoted in white and secondary forest is presented in green. (b) Location of the BDFFP in

Brazil. Photographs show the plastic dividers used to ensure acoustic recordings from theunderstory (c) and canopy samples (d) were independent.

185

186 Acoustic sampling

187 Two static detectors (SM2Bat+, Wildlife Acoustics) with omnidirectional microphones (SMX-188 US Ultrasonic Microphone) were placed in the understory and canopy of 164 sample points. 189 These sample points were spaced 50 m apart along four 2-km transects. Transects were located 190 across two spatially independent sites to reduce site bias (Fig 1). Each transect extended 191 through 1 km of secondary forest and then continued 1 km into the neighboring primary forest. 192 Surveys were conducted in the dry season of 2013 and the wet season of 2014 to minimize 193 seasonal bias, equating to eight transect visits in total. As bats are known to favour established 194 flyways for commuting (56), each transect was established specifically for this study.

195

196 As Amazonian bats are known to exhibit vertical stratification (45,57), we recorded bats 197 separately using two detectors simultaneously, with one detector in the understory and one in 198 the canopy. For this study, the understory was defined as extending from the ground to a height 199 of approximately 10 m and the canopy was defined as approximately 30 m from ground level. 200 To ensure the understory and canopy samples could be considered independent, plastic dividers 201 were attached to the detectors to create discrete directional microphones (Fig 1c; 56). 202 Additionally, sample points were manually rotated so that actively recording detectors were 203 always 250 m apart. Each active detector was programmed to record for 12 hours (18:00-06:00) 204 for three consecutive nights, amounting to 11,808 recording hours. Detectors recorded at 384 205 kHz sampling rate in full spectrum with 16-bit resolution. The high pass filter was set at 12 206 kHz (fs/32), with a trigger level of 18SNR. Recordings were split into five-second fragments

with at least two distinguishable pulses to define a bat pass which was used as the surrogatemeasure of bat activity (59).

209

210 Call classification to species/sonotype

211 We used a combination of manual and automatic methods to classify calls to species or 212 sonotype (a group of species with similar calls). We tested which species could be reliably classified using automated methods by first manually processing a subset of calls (all calls 213 214 recorded in the understory) and then comparing the results against those generated using the 215 classifier for Amazonian bats developed by López-Baucells et al. (2019) (60). To improve the 216 performance of the classifier, we included additional reference calls into the classifier training 217 dataset (S1 Table) following the methodology of López-Baucells et al. (2019) (60). We 218 compared the difference between manual identification (45,554 bat passes) and automatic 219 identification (41,702 bat passes) of the understory data using non-paired Wilcoxon Signed-220 Rank tests to confirm the reliability of the automatic classifications. Overall, the automatic 221 classifier generated comparable results to calls identified manually (W = 123,260, p = 0.87). 222 However, to increase consistency and robustness for the edge-effect analysis, we only included 223 the calls for those 12 species where there was no difference between manual identification and 224 automatic identification. See supporting information for full classifier performance results.

225

226 Statistical analysis

The 12 species we selected were assigned to guild depending on their ecological requirements and family (61,62). Species/sonotypes that were considered forest specialists included *Eptesicus brasiliensis, Furipterus horrens*, and *Myotis riparius. Pteronotus* spp. also typically inhabit forest areas. However, in our study, we have defined them as 'flexible forest' species as they are known to commonly exploit other habitats, such as forest edges, as well as hunting
in highly cluttered spaces (63,64). The final guild consisted of six 'edge' species/sonotypes
which typically forage along forest edges or in forest gaps. This included *Cormura brevirostris*, *Centronycteris maximiliani/centralis, Peropteryx kappleri, P. macrotis, Saccopteryx bilineata*,
and *S. leptura*. By grouping species, we were able to assess guild-level responses to edge
effects. Continuous response functions, as described in Ewers and Didham (2006) (19), were
used to identify edge effects across the primary and secondary forest interface (Fig 2).

Figure 2. Visual representation of the five models proposed by Ewers & Didham (2006) to delineate species' theoretical responses to edge effects.

(a) For the null, linear, and power models it is not possible to calculate extent or magnitude as
there is either no response present or the response exceeds the sampling area; (b) in the sigmoid
model, species exhibit a negative response to edge effects and asymptotes are reached in each
habitat; and (c) in the unimodal model, species demonstrate a preference for edge habitat. Note,
we have illustrated here a hypothetical preference for secondary forest using the linear and
sigmoid model and a preference for primary forest using the power model. However, each of
these models can be used to demonstrate a preference for either habitat.

The five models can be used to describe mean bat activity per guild, η, at a certain distance (D)
from the edge, and these models are as follows:

(1) Null model

$$\eta_D = \bar{\eta} + \epsilon \tag{1}$$

255	With ϵ denoting the error term and $\overline{\eta}$ mean bat activity across all distances from the edge.	This
256	model describes a scenario in which no discernible edge effect can be detected using the	data
257	(i.e., generalist activity).	
258		
259	(2) Linear model	
260	$\eta_D = \beta_0 + \beta_1 \mathbf{D} + \epsilon$	(2)
261		
262	This model describes a simple linear gradient in mean bat activity for a particular guild ac	ross
263	the edge. β_0 and β_1 denote constants and D the distance from the habitat edge.	
264		
265	(3) Power model	
266	$\eta_D = \beta_0 e^{\beta_1 D} + \epsilon$	(3)
267		
268	This model describes a scenario in which there is an asymptote on one side of the edge.	
269		
270	(4) Sigmoid model	
271	$\eta_D = \beta_0 + \frac{\beta_1 - \beta_0}{1 + e^{(\beta_2 - D)\beta_3}} + \epsilon$	(4)
272		
273	This model describes a scenario in which there is an asymptote on each side of the edge,	
274	with β_2 and β_3 as constants. This represents groups in which there is a discrete change in	
275	activity from one habitat to the next.	
276		
277	(5) Unimodal model	
270	$\beta_1 - \beta_0$	

278
$$\eta_{\rm D} = \beta_0 + \frac{\beta_1 - \beta_0}{1 + e^{(\beta_2 - D + \beta_4 D^2)\beta_3}} + \epsilon$$
(5)

279

This model describes a situation similar to the sigmoid model, but with a clear peak in the response at the edge (i.e. groups with a preference for habitat edges). This is described through the inclusion of the constant β_4 .

283

284 The canopy and understory data for each guild were analysed separately as we expected that 285 the model of best fit would depend on forest stratum. Average activity was log-transformed to 286 ensure normality assumptions were met. Non-linear models were fit using the "nlsLM" 287 function from the R package "minpack.lm" (65). Once each model was fitted, we compared 288 them using the second-order Akaike Information Criterion (AICc) to determine the model of 289 best fit whilst correcting for small sample sizes (66). An advantage of using these models is 290 the ability to calculate the magnitude and extent of the edge effects for equations 4 and 5, if 291 they were the best-fit models. Full model parameters are available in S2 Table.

292

293 We then applied generalized linear mixed effect models (GLMMs) with negative binomial 294 distributions to determine if bat activity varied with distance from the edge or between strata. Only non-correlated variables were included in the models to avoid collinearity ($r_s < 0.5$). 295 Activity data was not log-transformed in the GLMMs (67). The final fixed covariates were 296 Strata ("understory" vs. "canopy," categorical with two levels), ForestType (categorical with 297 298 three levels) and Distance (continuous). We included Transect as a random intercept, to 299 incorporate the dependency among observations of the same transects, as well as Season, to 300 account for any seasonal variation in activity. All covariates were centred and standardized 301 before analysis (68). We fit the models using the package "glmmADMB" (69)(S3 Table). The top three models were determined based on their AICc values. We then undertook likelihood 302 303 ratio tests to determine which covariates from these models were statistically significant (S4

Table). The best-fit model included all covariates identified as statistically significant from the
likelihood ratio tests. This analysis was repeated for each guild and species/sonotype.

306

307 **Results**

308 In total 252,912 bat passes were automatically identified to 12 aerial insectivorous species or 309 sonotypes. This included species from four families: two Vespertilionidae species/sonotypes, 310 six Emballonuridae species/sonotypes, three Mormoopidae species and one species of 311 *Furipteridae* (Table 1). Three species/sonotypes were not included in the edge effect analysis. 312 This includes *Emballonuridae* spp. (n = 8,205) and *Pteronotus personatus* (n = 459), which 313 had insufficient bat passes manually identified in the understory to test for agreement between 314 the manual and automatic identification methods, and *Molossidae* spp. (n = 9,236) as we found 315 the automatic classification for this sonotype was significantly different from manual 316 identification, suggesting incorrect classifications (S1 Table). Finally, three bat passes were 317 manually identified as *Thyroptera tricolor* in the understory but this species is not specified in 318 the automatic classifier and therefore was excluded.

	Understory Canopy				T-4-1		
	Secondary	Edge	Primary	Secondary	Edge	Primary	Total
Forest specialists			-				
Eptesicus brasiliensis	99	2	29	589	21	945	1,685
Furipterus horrens	25	0	10	27	1	13	76
Myotis riparius	489	16	148	2,629	105	1,390	4,777
Flexible forest foragers							
Pteronotus gymnonotus	164	5	72	336	17	143	737
Pteronotus alitonus	5,573	278	4,444	5,579	159	4,020	20,053
Pteronotus cf. rubiginosus	5,773	136	2,660	1,699	37	959	11,264
Edge foragers							
Cormura brevirostris	188	4	259	1,781	50	3,317	5,599
Centronycteris maximiliani/centralis	10,838	7	4,370	51,742	4,352	50,651	121,960
Peropteryx kappleri	82	1	22	3,196	151	3,717	7,169
Peropteryx macrotis	337	3	195	6,923	1,174	5,238	13,870
Saccopteryx bilineata	604	4	2,416	6,089	836	30,319	40,268
Saccopteryx leptura	271	0	651	7,231	957	16,344	25,454
Excluded from analysis							
Emballonuridae spp.	177	10	182	2,629	982	4,225	8,205
Molossidae spp.	438	44	733	5,195	159	2,667	9,236
Pteronotus personatus	46	0	17	222	5	169	459
Rhynchonycteris naso	0	0	0	8	0	2	10
Total	25,104	510	16,208	95,875	9,006	124,119	270,822

Table 1. Total number of bat passes per species/sonotype in both the understory and canopy of secondary forest, forest edge, and primary forest.

320 These values represent bat passes as determined by the automatic classifier. Data for *Thyroptera tricolor* not given as this

321 species was only identified manually and is not included in the classifier.

The null model provided the model of best fit for forest specialists in the canopy which indicates that there was no edge effect on mean activity in this stratum (Table 2). The linear and power models provided the best fit for forest specialists in the understory. There was little variation between the linear and power model fit (Fig 3). Despite considerable variation in the data, both models showed that activity increased from the interior in primary forest towards the edge and into the secondary forest, whereby activity peaked in secondary forest farthest from the forest edge, therefore indicating a preference for secondary forest (Fig 3).

Guild	Habitat	Model	AICc	
Forest specialists				
	Canopy			
		Null	116.357	*
		Linear	118.245	
		Power	118.258	
		Sigmoid	121.775	
		Unimodal	125.423	
	Understory			
		Null	91.427	
		Linear	89.507	*
		Power	89.722	*
		Sigmoid	90.100	
		Unimodal	98.100	
Flexible forest foragers				
	Canopy			
		Null	114.132	*
		Linear	116.003	
		Power	115.974	
		Sigmoid	116.072	
		Unimodal	119.413	
	Understory			
		Null	127.412	*
		Linear	129.054	
		Power	129.066	
		Sigmoid	131.469	
		Unimodal	137.224	
Edge foragers				
	Canopy			
		Null	148.845	*
		Linear	149.609	

Table 2. Comparison of model fit using Ewers and Didham's (2006) edge effect models.

	Power	149.654	
	Sigmoid	155.166	
	Unimodal	157.421	
Understory			
	Null	162.503	*
	Linear	164.652	
	Power	164.655	
	Sigmoid	169.718	
	Unimodal	171.593	

329 Results are provided for each of the three guilds in both the understory and

330 canopy. **Bold*** - model/(s) of best fit

331

Figure 3. Edge effect model fit for forest specialists in the understory.

Log forest specialist activity (bat passes) per 50 m sample point averaged across all transects with corresponding lines of best fit. Activity increases from the edge in the secondary forest and decreases from the edge in the primary forest. Dark blue dash – power model, light blue – linear model. Standard error provided for the linear model. Model parameters listed in S2 Table.

338

For both flexible forest foragers and edge foragers, we found the null model provided the best fit for both the canopy and understory. This indicates there is no evidence that edge effects were affecting either of these guilds (Table 2). In contrast to our hypothesis, the unimodal models provided the poorest fit for edge foragers. No calculations were possible for edge extent or magnitude as no guild demonstrated a relevant edge effect response (e.g., sigmoid or unimodal), and it is not recommended to infer magnitude or extent from the power model (19).

346

347 Distance from the edge did not explain edge forager activity or forest specialist activity based
348 on GLMMs (Table 3, S3-S4 Tables). However, compared to the habitat boundary, flexible

forest forager activity was significantly higher with increasing distance from the edge (Table 3). There was no difference in response between primary and secondary forest and the forest edge for any guild (Table 3). We also observed no significant differences in bat activity between the primary and secondary forest or edge for any species/sonotype (Table 3). Only one species demonstrated a significant response to distance from the edge, *Peropteryx macrotis*, which had greater activity closer to the edge.

355

356 Table 3. Summary of the best-fit Generalized Linear Mixed Effect Models for each bat

		Estimate	SE	Z	<i>p</i> -value	
Forest specialist	s					
	Intercept	3.595	0.446	8.05	8.1 ^{e-16}	***
	Understory	-1.609	0.144	-11.17	< 2 ^{e-16}	***
	Primary forest	0.530	0.426	1.24	0.210	
	Secondary forest	-0.036	0.427	-0.08	0.930	
Flexible forest for	oragers					
	Intercept	4.287	0.464	9.24	< 2 ^{e-16}	***
	Understory	0.449	0.140	3.26	0.001	**
	Primary forest	0.170	0.416	0.41	0.683	
	Secondary forest	-0.193	0.426	-0.45	0.650	
	Distance	1.233 ^{e-03}	2.66^{e-04}	1.97	0.048	*
Edge foragers						
	Intercept	6.504	0.607	10.72	< 2 ^{e-16}	***
	Understory	-2.177	0.181	-12.06	< 2 ^{e-16}	***
	Primary forest	0.552	0.525	1.05	0.294	
	Secondary forest	0.896	0.538	1.66	0.096	
Eptesicus						
brasiliensis						
	Intercept	2.797	0.102	27.43	< 2 ^{e-16}	***
	Understory	-1.350	0.202	-6.67	2.6 ^{e-11}	***
Furipterus						
horrens						
	Intercept	1.183	0.208	5.69	1.2^{e-08}	
Myotis riparius	-					
_	Intercept	3.393	0.473	7.18	7 ^{e-13}	***
	-					

357 guild and species/sonotype

	Understory	-1.397	0.162	-8.64	< 2 ^{e-16}	***
	Primary forest	0.469	0.454	1.03	0.300	
	Secondary forest	-0.182	0.457	-0.40	0.690	
Pteronotus						
gymnonotus						
	Intercept	1.670	0.327	5.11	3.2^{e-07}	***
	Understory	-0.321	0.127	-2.53	0.011	*
	Primary forest	0.390	0.331	1.18	0.239	
	Secondary forest	-0.034	0.336	-0.10	0.919	
Pteronotus						
alitonus						
	Intercept	4.340	0.303	14.30	< 2 ^{e-16}	***
Pteronotus cf.						
rubiginosus						
	Intercept	3.456	0.327	10.56	< 2 ^{e-16}	***
	Understory	0.821	0.182	4.51	6.5 ^{e-06}	***
Cormura						
brevirostris						
	Intercept	3.641	0.294	12.37	< 2 ^{e-16}	***
	Understory	-1.548	0.164	-9.41	< 2 ^{e-16}	***
Centronycteris						
maximiliani/						
centralis						
	Intercept	6.558	0.408	16.08	< 2 ^{e-16}	***
	Understory	-1.743	0.214	-8.15	3.7^{e-16}	***
Peropteryx						
kappleri						
	Intercept	4.112	0.150	27.40	< 2 ^{e-16}	***
	Understory	-2.632	0.257	-10.20	< 2 ^{e-16}	***
Peropteryx						
macrotis						
	Intercept	4.967	0.334	14.85	$< 2^{e-16}$	***
	Understory	-2.107	0.194	-10.88	< 2 ^{e-16}	***
	Distance	-0.001	3.25^{e-03}	-4.19	2.8^{e-05}	***
Saccopteryx						
bilineata					4 -	
	Intercept	5.154	0.618	8.34	< 2 ^{e-16}	***
	Understory	-1.858	0.204	-9.11	< 2 ^{e-16}	***
	Primary forest	-0.452	0.586	0.77	0.440	
	Secondary forest	0.928	0.598	1.55	0.120	

Saccopteryx leptura

1	Intercept	5.884	0.647	9.10	< 2 ^{e-16}	***
	Understory	-2.577	0.177	-14.59	< 2 ^{e-16}	***
	Primary forest	-1.034	0.648	-1.59	0.110	
	Secondary forest	-0.294	0.648	-0.45	0.650	

358 See S3 Table, S4 Table for complete models

359

We found that stratum was an important predictor for the activity of each guild (Table 3, S3– S4 Tables). Activity was highest in the canopy for edge foragers and forest specialists, but highest in the understory for flexible forest foragers. We observed that ten of the twelve species were significantly more active in the canopy than the understory (Table 3; Fig 4). Only one species, *Pteronotus* cf. *rubiginosus*, showed a significant preference for the understory.

366 Figure 4. Vertical stratification of twelve Amazonian bat species

367 Comparison of total bat activity (bat passes) per species/sonotype recorded in the understory
368 and canopy at the Biological Dynamics of Forest Fragments Project. Significance values * <
369 0.05, *** < 0.001

370

371 **Discussion**

An expanding body of literature supports the conservation benefits to bats, and multiple other taxonomic groups, associated with the regeneration of secondary forests in fragmented tropical landscapes (32,36,37,70). By providing evidence of edge sealing, our study supports this by showing that old secondary forest adjacent to primary forest can support comparable activity to primary forest for 12 aerial insectivorous bat species/sonotypes. However, we still found evidence of the impact of edge effects for both forest specialists and flexible forest foragers at the guild level, although results between different statistical approaches were conflicting. 379

380 Guild and species specific responses

381 We did not find evidence of edge effects for both flexible forest foragers and edge foragers, as 382 well as forest specialists in the canopy, using Ewers and Didhams' (2006) models (19). As 383 suggested by Powell et al. (2015) (38), the old secondary forest at the BDFFP might have 384 reached the point of recovery where edge effects can no longer be detected for most 385 species/sonotypes. This would be consistent with findings for other taxonomic groups (e.g., 386 dung beetles; 33,36), suggesting that old secondary forest provides valuable habitat for 387 common aerial insectivores. In contrast, forest specialist activity demonstrated a response to 388 edge effects using Ewers and Didhams' (2006) models, suggesting the secondary forest was 389 not yet sufficiently mature to prevent edge effects penetrating the primary forest. However, 390 different statistical approaches demonstrated conflicting results. Using the GLMM approach, 391 we did find evidence that flexible forest forager activity increased with increasing distance 392 from the edge, whereas no response was detected for forest specialists. Therefore, it is possible 393 these models do not capture the full breadth of response. As such, we advise multiple 394 approaches are used when assessing edge effects.

395

396 Whilst old secondary forests at the BDFFP may support several common aerial insectivorous 397 bat species, López-Baucells (2019) (63) demonstrated that a complete assemblage-level 398 recovery was not observed after 15 years of forest regrowth. However, recovery rates can vary 399 between bat species and guilds. Even after ~30 years, phyllostomid assemblages in secondary 400 forest may not fully resemble the assemblages within primary forest (32,70). Trophic level, 401 dispersal ability, and habitat specialization all affect a species' sensitivity to edge effects 402 (47,71). Species which are highly dependent on primary forest interiors are more likely to be 403 edge sensitive, to be affected over a larger extent, as well as at greater magnitudes (8,24). Forest 404 specialist bats typically have low wing loading which gives them the maneuverability to 405 navigate dense forest clutter (72). Other traits related to their echolocation call design, also 406 facilitate navigating and locating prey in clutter and are poorly suited for more open spaces 407 (72,73). Compounded, these traits limit their dispersal ability. Fast-flying, more mobile species 408 are less affected by fragmentation as they are more capable of exploiting landscape mosaics 409 (13,63,74). Whilst we did not observe a significant difference in activity between secondary 410 and primary forest based on the GLMMs, there was evidence forest specialists were to some 411 degree influenced by edge effects in the understory using the Ewers and Didhams' (2006) 412 models. However, contrary to our expectations, they exhibited higher activity in the secondary 413 forest. This response may be driven by increased prey availability (75) however we were not 414 able to test this. Increased food availability can lead to an increased abundance of generalist 415 phyllostomids up to 3 km from the forest edge (22). As the linear model provided the model of 416 best fit for forest specialists in the understory, our results indicate these species may also be 417 impacted by edge effects beyond 2 km.

418

419 It is important to note that only four transects in two locations were sampled in this study. 420 Therefore, there may be location-specific factors which have influenced the patterns we 421 observed and the results may not necessarily generalize across the Amazon. Old-growth, 422 continuous forest acts as a source for many species across the BDFFP landscape. Elsewhere in 423 the Amazon, many remaining forest fragments are isolated within a matrix of pasture. As the 424 wider, landscape-scale effects of fragmentation are known to strongly influence edge effects 425 and disrupt source-sink dynamics (11,76), it is likely the magnitude of edge effects in these 426 fragments will be exacerbated. Similarly, whether secondary forest neighbours primary forest 427 is an important determinant of bat abundance and diversity (28). Many resources may not be 428 available in secondary forest until it matures, e.g., mature/dead trees for roosting. However,

429 more mobile species such as flexible forest foragers and edge foragers may move between 430 habitats to exploit the resources available in each (29). This could explain why we observe high 431 activity in the secondary forest and would contradict previous findings that suggest that the 432 intermediate disturbance hypothesis does not apply to neotropical bats (28). Finally, we stress 433 that our study should only be used to draw conclusions about the responses of common species 434 and not to infer how more specialist species are impacted. Nevertheless, our results align with 435 previous studies highlighting that forest species are more edge-sensitive than generalist species 436 (8,24). As such, primary forest is of irreplaceable value, not only for edge-sensitive 437 phyllostomid bats but also for aerial insectivorous bats (63,77).

438

439 Vertical stratification

440 Our results support previous findings that tropical bat activity differs between strata, with most 441 species showing a strong preference for the canopy (46,49,57,78). However, we found different 442 stratum preferences than those previously reported. *Myotis riparius* has previously been shown 443 to prefer the understory in Costa Rica (46) and in French Guiana, where C. maximiliani also 444 demonstrated a preference for the same stratum (78). Both were significantly more active in 445 the canopy in our study. C. maximiliani is known to vary its activity in the understory and 446 canopy across the night, with peak canopy activity in the middle of the night (78). However, 447 this does not account for the differences demonstrated in our study as recordings were collected 448 across the whole night. Both species are relatively small, slow fliers with short call durations 449 (< 6ms) (48,79) which suggests they are well suited to foraging in understory vegetation. 450 Similarly, there has previously been a lack of vertical stratification reported for *Saccopteryx* 451 bilineata and S. leptura (48,78). Forest structure is not the only consideration affecting a species' spatial distribution. Fluctuations in prey availability and moon illumination influence 452 453 how bats utilize different strata (46,78,80). Gomes et al. (2020) demonstrated how species

454 modulate their stratification preferences across the night to forage opportunistically (78).
455 However, the scale of the differences we observed in our study (e.g., a seven-fold increase in
456 *C. maximiliani* activity between the understory and canopy) suggests a strong affiliation with
457 the canopy. Unlike understory specialists, species that forage in the canopy are considered less
458 vulnerable to the effects of fragmentation, including edge effects (47). Almost all of the species
459 assessed in this study showed a preference for the canopy. Therefore, our study should not be
460 used to infer how interior, understory specialists will be affected by edge effects.

461

462 Whilst we did not detect many direct changes in bat activity in response to edges, the deviation 463 we observed from typical stratum use may reflect the potential for more subtle effects on bat 464 populations. Habitat disturbance, including edge effects, can affect a species' behaviour, 465 physiology, and other fitness parameters (8,11,81,82). At least two Amazonian phyllostomids 466 change their habitat preferences to utilize more strata in forest fragments than in continuous forest when locating prey (45). If edge effects are increasing understory clutter or altering prey 467 468 distributions, this may have knock-on effects on where bats can forage. This may partially 469 explain why we observe lower understory activity than expected for forest specialists. 470 However, more research is needed to test this hypothesis. Habitat deterioration can also reduce 471 the richness of prey in insectivorous bat diets in disturbed habitats and the long-term impacts 472 of this are not yet fully understood (81,82). Similarly, Estrada-Villegas et al. (2010) showed 473 fragmentation increased the activity of aerial insectivorous forest bats and altered their 474 assemblage composition (74). This is also reflected in the responses of other taxonomic groups, 475 including birds, plants, and invertebrates (1). Therefore, we cannot rule out the presence of 476 edge effects by measuring activity alone. Nevertheless, our study does demonstrate that if edge 477 effects are present, common bat species have been able to adapt their behaviour to cope with them at their current magnitude. More specialist species are less adaptable and therefore aremore vulnerable to potential edge effects.

480

481 **Considerations for study design**

482 One limitation of the statistical approach employed here is that the models by Ewers and 483 Didham (2006) assume a unidirectional response to edge effects in each habitat (19,24). This 484 does not necessarily account for the interaction between habitats at the border. As previously 485 discussed, individuals may leave the primary forest to exploit resources in the secondary forest 486 within a certain distance from the edge (29,33,83). This may create an inflated decrease of 487 activity in the immediate area adjacent to the edge in the primary forest (Fig 5). Habitat 488 complementation, the use of different habitats across a landscape, is the key process thought to 489 underpin the distribution of mobile species in heterogeneous landscapes, including bats (83). 490 Further studies should consider incorporating a model (e.g., a spline regression model) which 491 could test for bidirectional responses to edge effects, e.g., where activity increases in the first 492 200 m from the edge but then decreases for 400 m before stabilizing to natural activity levels 493 (Fig 5; hypothetical values). Whilst it would not be possible to calculate magnitude and extent 494 from this type of model, it would help to test for source-sink dynamics (see 29).

495

496 Figure 5. Schematic of bidirectional response to edge effects

497 A theoretical example of how activity/abundance may exhibit a bidirectional response to edge 498 effects. The complementation zone would be the area between the first asymptotes from the 499 edge in each habitat. The full extent of edge effects is observed at the second asymptotes from 500 the edge in each habitat whereby activity stabilises.

502 For management purposes, future research could also examine the impact that different land 503 clearing approaches have on later regeneration. This study was predominantly restricted to 504 Vismia-dominated regrowth, therefore further studies could also investigate whether 505 comparable patterns are observed with a matrix dominated by Cecropia regrowth. This would 506 enable land-owners to clear the forest in a manner (with or without the use of fire) that would 507 minimize its effects on bat communities (see 47). Additionally, we recommend future studies 508 extend the transect length and repeat across more replicates. This will help identify the extent 509 of edge effects for forest specialists, as well as eliminate the risk that extent is not being 510 detected for other guilds due to sampling design. Increased replication may also facilitate 511 species-specific analyses using Ewers and Didham's (2006) models which were not possible 512 in this study due to small sample sizes.

513

514 Only one species classified as an edge forager demonstrated a preference for the forest edge in 515 our study. This may be because our "edge" did not represent a hard edge between forest and 516 non-forest. Therefore, our findings support Jantzen & Fenton (2013) (84) which suggests this 517 type of labelling oversimplifies the relationship between species and edge effects and does not 518 capture variation in species responses due to different types of edge. As matrix contrast plays 519 a pivotal role in determining the impact of edge effects (12,26–28), future research would also 520 benefit from comparing the responses we observed in a low-contrast matrix to those detected 521 in high-contrast matrix landscapes, e.g., in soy plantations, without first classifying species into 522 guilds.

524 Conclusions

525 Investigating how we can buffer the impacts of edge effects will be increasingly important to 526 protect species in human-modified tropical landscapes. Our results demonstrate that 527 maintaining secondary forest in an advanced regeneration state (> 30 years) adjacent to primary 528 forest can help support common aerial insectivorous bats at the landscape level. However, it 529 also highlights that edge effect responses can be guild and species-specific and that their 530 increased specialization means forest specialists are more susceptible to edge effects, even in 531 a mosaic of primary and ca. 30-year-old secondary forests. Consequently, primary forest 532 remains irreplaceable for supporting the whole bat assemblage. We advocate that future studies 533 also consider how vertical stratification and source-sink dynamics may affect species responses 534 to edge effects. Whilst secondary forest in isolation may not be able to support the same bat 535 diversity and abundance as primary forest, we argue it can reduce extinction pressure from 536 edge effects at the landscape level and mitigate habitat degradation in the remaining primary 537 forest. Therefore, the long-term protection of secondary forests would greatly benefit the 538 conservation of neotropical bats in human-modified landscapes.

539

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546 Authors Contributions

AL-B, CFJM, RR and P.E.D.B designed research; NY, JC, MM and AL-B performed the bioacoustic and data analyses; NY led the writing of the manuscript; AL-B and MM collected data at the BDFFP; All authors contributed critically to the drafts, gave final approval for

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- 551

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785 Supporting Information

S1 Table – The training data for the classifier and the comparison between manual classification and automatic classification.

The Wilcoxon test was used to compare the difference between the number of bat passes

789 (≥ 2 pulses) automatically identified by the classifier to at least 60% confidence (Auto ID)

790 compared to manual identification (Manual ID) in the understory. "-" represents insufficient

files for statistical comparison. Training data represents the total number of individual pulses

available to train the classifier, see López-Baucells et al. (2019) for full methodology.

793

794 S2 Table – Model parameter estimates after fitting Ewers and Didham's (2006) edge

795 effect models.

Each model below represents the best-fit model(s) per guild and stratum as determined using the second-order Akaike Information Criterion (AICc). These include the raw estimates on the logarithmic scale as well as the back-transformed estimates (true bat passes). Mean number of bat passes in stratum (η or β 0). Change in bat passes with distance from the edge (β 1). Confidence intervals (CI) for the transformed scale were calculated using the delta method.

801

802 S3 Table – Generalized linear mixed-effect model equations.

803 Generalized linear mixed-effect model (GLMMs) equations used to model bat activity (n) as a 804 function of the distance from the forest edge (*Distance*), forest type (*ForestType*) and stratum 805 (*Strata*) for each of the three bat guilds and per species. The models are ordered based on their

806 AICc. **Bold** – top three models per guild.

- 807
- 808 S4 Table S4 Table Results of likelihood ratio tests comparing the top generalized
- 809 linear mixed-effect models for each guild and species/sonotype (see Table S3).