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1 Edge effects and vertical stratification of aerial insectivorous
2 bats across the interface of primary-secondary Amazonian
3 rainforest
4

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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
40 composition. With over half of the world's tropical forests predicted to become forest edge by
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),
52 and edge foragers (six species). Modelling results revealed limited evidence of edge effects,
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.

60 **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
63 biodiversity loss (1). The Brazilian Amazon currently hosts over 10,000 plant species and is a
64 global hotspot for terrestrial vertebrate diversity (2,3). Declines in Amazonian deforestation
65 over the last two decades provided some with optimism for Brazil's commitment to
66 conservation. However, in 2020 the Brazilian Amazon experienced the highest deforestation
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
69 by the wake of the COVID-19 tragedy in Brazil (4–7). Such deforestation has massive
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).

84

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
87 edge influence, edge extent and edge magnitude, can be distinguished. The extent of edge
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

108 There have been over 405 reforestation projects across the Brazilian Amazon since 1950, and
109 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
115 (28,31,32). This helps to mitigate the impact of edge effects in primary forest and increase
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
127 predominantly on phyllostomids, the ecologically most diverse Neotropical bat family, as these
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

135 bat diversity and abundances between the canopy and understory in the Amazon (48,49).
136 However, due to sampling logistics, it is often difficult to incorporate canopy sampling into
137 mist-netting surveys. Alternatively, acoustic monitoring enables us to include aerial
138 insectivores in such studies and provides an effective method for cross-strata comparisons,
139 thereby providing a more comprehensive understanding of how the wider bat community may
140 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 da
156 Biodiversidade) permit (26877-3).

157

158 **Study sites**

159 Our study was conducted in the Central Brazilian Amazon, 80 km north of Manaus, at the
160 Biological Dynamics of Forest Fragments Project (BDFFP; 2024'26''S, 59043'40''W; Fig 1).
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
163 *terra firme* forest, with an average tree diversity of 280 species per hectare (50). In the early
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 ≥ 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
175 November and June (monthly rainfall > 250 mm) and the dry season occurs between July and
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

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

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

183 Brazil. Photographs show the plastic dividers used to ensure acoustic recordings from the
184 understory (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

207 with at least two distinguishable pulses to define a bat pass which was used as the surrogate
208 measure 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
213 classified using automated methods by first manually processing a subset of calls (all calls
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**

227 The 12 species we selected were assigned to guild depending on their ecological requirements
228 and family (61,62). Species/sonotypes that were considered forest specialists included
229 *Eptesicus brasiliensis*, *Furipterus horrens*, and *Myotis riparius*. *Pteronotus* spp. also typically
230 inhabit forest areas. However, in our study, we have defined them as ‘flexible forest’ species

231 as they are known to commonly exploit other habitats, such as forest edges, as well as hunting
232 in highly cluttered spaces (63,64). The final guild consisted of six ‘edge’ species/sonotypes
233 which typically forage along forest edges or in forest gaps. This included *Cormura brevirostris*,
234 *Centronycteris maximiliani/centralis*, *Peropteryx kappleri*, *P. macrotis*, *Saccopteryx bilineata*,
235 and *S. leptura*. By grouping species, we were able to assess guild-level responses to edge
236 effects. Continuous response functions, as described in Ewers and Didham (2006) (19), were
237 used to identify edge effects across the primary and secondary forest interface (Fig 2).

238

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

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

248

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

251

252 **(1) Null model**

$$253 \quad \eta_D = \bar{\eta} + \epsilon \quad (1)$$

254

255 With ϵ denoting the error term and $\bar{\eta}$ 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
$$\eta_D = \beta_0 + \beta_1 D + \epsilon \quad (2)$$

261

262 This model describes a simple linear gradient in mean bat activity for a particular guild across
263 the edge. β_0 and β_1 denote constants and D the distance from the habitat edge.

264

265 **(3) Power model**

266
$$\eta_D = \beta_0 e^{\beta_1 D} + \epsilon \quad (3)$$

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
$$\eta_D = \beta_0 + \frac{\beta_1 - \beta_0}{1 + e^{(\beta_2 - D)\beta_3}} + \epsilon \quad (4)$$

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**

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

279

280 This model describes a situation similar to the sigmoid model, but with a clear peak in the
281 response at the edge (i.e. groups with a preference for habitat edges). This is described through
282 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.
295 Only non-correlated variables were included in the models to avoid collinearity ($r_s < 0.5$).
296 Activity data was not log-transformed in the GLMMs (67). The final fixed covariates were
297 *Strata* (“understory” vs. “canopy,” categorical with two levels), *ForestType* (categorical with
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
302 top three models were determined based on their AICc values. We then undertook likelihood
303 ratio tests to determine which covariates from these models were statistically significant (S4

304 Table). The best-fit model included all covariates identified as statistically significant from the
305 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.

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

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

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.

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

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

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	

	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

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

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

338

339 For both flexible forest foragers and edge foragers, we found the null model provided the best
340 fit for both the canopy and understory. This indicates there is no evidence that edge effects
341 were affecting either of these guilds (Table 2). In contrast to our hypothesis, the unimodal
342 models provided the poorest fit for edge foragers. No calculations were possible for edge
343 extent or magnitude as no guild demonstrated a relevant edge effect response (e.g., sigmoid
344 or unimodal), and it is not recommended to infer magnitude or extent from the power model
345 (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

349 forest forager activity was significantly higher with increasing distance from the edge (Table
 350 3). There was no difference in response between primary and secondary forest and the forest
 351 edge for any guild (Table 3). We also observed no significant differences in bat activity
 352 between the primary and secondary forest or edge for any species/sonotype (Table 3). Only
 353 one species demonstrated a significant response to distance from the edge, *Peropteryx*
 354 *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**
 357 **guild and species/sonotype**

	Estimate	SE	z	p-value	
Forest specialists					
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 foragers					
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	
<i>Distance</i>	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	
<i>Eptesicus brasiliensis</i>					
Intercept	2.797	0.102	27.43	< 2 ^{e-16}	***
Understory	-1.350	0.202	-6.67	2.6 ^{e-11}	***
<i>Furipterus horrens</i>					
Intercept	1.183	0.208	5.69	1.2 ^{e-08}	
<i>Myotis riparius</i>					
Intercept	3.393	0.473	7.18	7 ^{e-13}	***

	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	
<i>Pteronotus gymnonotus</i>						
	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	
<i>Pteronotus alitonus</i>						
	Intercept	4.340	0.303	14.30	$< 2^{e-16}$	***
<i>Pteronotus cf. rubiginosus</i>						
	Intercept	3.456	0.327	10.56	$< 2^{e-16}$	***
	Understory	0.821	0.182	4.51	6.5^{e-06}	***
<i>Cormura brevirostris</i>						
	Intercept	3.641	0.294	12.37	$< 2^{e-16}$	***
	Understory	-1.548	0.164	-9.41	$< 2^{e-16}$	***
<i>Centronycteris maximiliani/centralis</i>						
	Intercept	6.558	0.408	16.08	$< 2^{e-16}$	***
	Understory	-1.743	0.214	-8.15	3.7^{e-16}	***
<i>Peropteryx kappleri</i>						
	Intercept	4.112	0.150	27.40	$< 2^{e-16}$	***
	Understory	-2.632	0.257	-10.20	$< 2^{e-16}$	***
<i>Peropteryx macrotis</i>						
	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}	***
<i>Saccopteryx bilineata</i>						
	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

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

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

365

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**

372 An expanding body of literature supports the conservation benefits to bats, and multiple other
373 taxonomic groups, associated with the regeneration of secondary forests in fragmented tropical
374 landscapes (32,36,37,70). By providing evidence of edge sealing, our study supports this by
375 showing that old secondary forest adjacent to primary forest can support comparable activity
376 to primary forest for 12 aerial insectivorous bat species/sonotypes. However, we still found
377 evidence of the impact of edge effects for both forest specialists and flexible forest foragers at
378 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
452 species' spatial distribution. Fluctuations in prey availability and moon illumination influence
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
467 forest when locating prey (45). If edge effects are increasing understory clutter or altering prey
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

478 them at their current magnitude. More specialist species are less adaptable and therefore are
479 more 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.

501

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.

523

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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545

546 **Authors Contributions**

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

551

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784

785 **Supporting Information**

786 **S1 Table – The training data for the classifier and the comparison between manual** 787 **classification and automatic classification.**

788 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
791 files for statistical comparison. Training data represents the total number of individual pulses
792 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.**

796 Each model below represents the best-fit model(s) per guild and stratum as determined using
797 the second-order Akaike Information Criterion (AICc). These include the raw estimates on the
798 logarithmic scale as well as the back-transformed estimates (true bat passes). Mean number of
799 bat passes in stratum (η or β_0). Change in bat passes with distance from the edge (β_1).
800 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).**