

1 **Functional recovery of Amazonian bat assemblages following**
2 **secondary forest succession**

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18 **Abstract**

19 Regenerating forests occupy large areas in the tropics, mostly as a result of deforestation for
20 livestock and agriculture, followed by land abandonment. Despite the importance of
21 regenerating secondary forests for tropical biodiversity conservation, studies of temporal
22 effects of matrix regeneration on species responses in fragmented landscapes are scarce. Here,
23 we used an Amazonian whole-ecosystem fragmentation experiment to investigate how
24 changes in matrix quality over time through secondary forest regeneration affect bat
25 assemblages from a functional perspective. We found that forest regeneration in the matrix
26 positively affected functional α diversity, as well as species- and community-level functional
27 uniqueness, reflecting an increase of species that perform different ecological functions in
28 secondary forest over time. According to functional trait composition, animalivorous species
29 showed the clearest signs of recovery associated with matrix regeneration. Consequently,
30 between-period differences in functional β -diversity were highest in secondary forest
31 compared to fragments and continuous forest, determined mainly by trait gains. However, ~30
32 years of secondary forest regeneration were not sufficient for the functional recovery of bat
33 assemblages to levels observed in continuous forest. Restoring degraded habitats while
34 protecting primary forest will be an important strategy for safeguarding high functional
35 diversity of bats and their vital contributions to ecosystem functioning in fragmented tropical
36 landscapes.

37 **Keywords:** functional traits, forest succession, habitat fragmentation, long-term research,
38 matrix vegetation, temporal dynamics.

39 **1. Introduction**

40 Despite increased forest loss and fragmentation over the last decades (Haddad et al., 2015),
41 vast abandoned areas in the tropics are undergoing regeneration due to secondary forest
42 succession. As a result, secondary forests are now often the predominant forest type in many
43 tropical fragmented landscapes (Chazdon et al., 2009; Arroyo-Rodríguez et al., 2017). In the
44 Brazilian Amazon, approximately 21% of ~720,000 km² of deforested land is under forest
45 regrowth (Almeida et al., 2016). A secondary forest matrix in fragmented landscapes may
46 function as a buffer to the pervasive negative consequences of edge effects, expanding the
47 effective area of fragments, facilitating the movement of species between forest patches and
48 providing suitable habitat for numerous species, including many primary forest-interior
49 specialists (e.g. Antongiovanni and Metzger, 2005; Kupfer et al., 2006; Lindenmayer et al.,
50 2008; Franklin and Lindenmayer, 2009; Stouffer et al., 2011; Driscoll et al., 2013). On the
51 other hand, in systems with more pronounced patch-matrix contrast, environmental conditions
52 can act as a strong filter allowing only a narrow spectrum of species and functional traits to
53 persist (e.g. Meyer et al., 2008; Newbold et al., 2013; Lindenmayer et al., 2015; Wordley et
54 al., 2017). Promoting secondary forest regeneration and protection may effectively reduce
55 fragment-matrix contrast and maintain overall functional trait diversity of animal assemblages,
56 and hence ecosystem functioning (Kupfer et al., 2006; Farneda et al., 2015; Sayer et al., 2017).
57 Thus, the consequences of fragmentation for biodiversity can be better understood when the
58 vegetation dynamics in the matrix are considered in experimental design.

59 Functional diversity summarizes the variation in trait values of an organism that
60 potentially affects its performance, fitness and ecological function within a community (Violle
61 et al., 2007). Incorporating a temporal functional dimension into the study of fragmentation
62 effects can advance our understanding of ecosystem functioning and help devise more
63 effective conservation plans (Cadotte et al., 2011; Mouillot et al., 2013). However, the only

64 study exploring temporal changes in functional diversity of animal assemblages comes from
65 birds in a fragmented temperate landscape in Australia, reporting a decline over time in bird
66 functional diversity in the interiors of woodland patches embedded in a matrix dominated by
67 exotic pine plantations (Lindenmayer et al., 2015). For tropical ecosystems, the present study
68 is the first to quantify the temporal dynamics of fragmentation effects from a functional
69 perspective and to evaluate how functional trait responses are mediated by matrix regeneration
70 over time. Bats are a promising group for studying responses to landscape fragmentation
71 because they display a high diversity of ecological roles in tropical communities, acting as
72 important seed dispersers, key pollinators, prey and predators (Kunz et al., 2011). Moreover,
73 they are a group for which the effects of landscape change on the functional dimension of
74 biodiversity remain little explored (Cisneros et al., 2015; Meyer et al., 2016; Wordley et al.,
75 2017).

76 Surveys conducted between 1996-2002 by Sampaio (2000) and Bobrowiec and Gribel
77 (2010) documented rapid spatial turnover of phyllostomid bat species at the Biological
78 Dynamics of Forest Fragments Project (BDFFP) in the Central Brazilian Amazon. This
79 turnover was mainly explained by the disappearance of primary forest-interior species
80 following deforestation, and an increase of opportunistic shrub-frugivores in the secondary
81 forest matrix that are able to exploit the forest regrowth that surrounded forest fragments.
82 Using this extensive data set as a baseline, unique for this region of the Amazon, here we
83 evaluate how matrix regeneration over time affects bat assemblages from a functional
84 perspective in continuous primary forest, primary forest fragments, and secondary forest
85 matrix ~15 years after the studies by Sampaio (2000) and Bobrowiec and Gribel (2010).
86 Control sites in continuous forest sampled in both periods allow us to unveil whether an
87 observed temporal change in functional diversity of bat assemblages in fragments was in fact a

88 result of matrix regeneration or whether it is due to natural temporal variability of bat
89 populations.

90 Our general hypothesis was that functional recovery of bat assemblages would occur in
91 fragments and matrix sites with secondary forest succession through the addition of different
92 species' functional traits. We anticipated that this increase in functional trait diversity in
93 secondary forest would in part come about by frugivorous bats responding positively to the
94 higher abundance of fruits of successional plant species, whereas gleaning animalivorous bats
95 would increase as the successional stage of secondary forest progresses due to increased
96 availability of roosts and food, such as arthropods and small vertebrates. We predicted that,
97 between periods, (1) functional and taxonomic α diversity and community-level functional
98 uniqueness (sensu Ricotta et al., 2016) would increase in fragments and matrix sites, (2)
99 species-level functional uniqueness would increase in fragments and matrix sites, (3)
100 functional trait composition in fragments and matrix sites would shift to mirror more closely
101 that of continuous forest, and (4) differences in functional β -diversity in fragments and matrix
102 sites are determined mainly by the replacement of functional traits and only to a limited extent
103 by the gain of traits.

104 **2. Material and methods**

105 ***2.1 Study area***

106 Fieldwork was conducted at the Biological Dynamics of Forest Fragments Project (BDFFP),
107 located ~80 km north of Manaus (2°25'S, 59°50'W), Central Amazon, Brazil (Fig. 1). The
108 area is characterized by a mosaic of unflooded (*terra firme*) Amazonian rainforest, primary
109 forest fragments (1, 10 and 100 ha), and secondary forest (~8,325 ha). Annual rainfall varies
110 from 1,900–3,500 mm, with a dry season between June and October (Laurance et al., 2017).
111 The topography is relatively flat (80–160 m elevation), intersected by small streams. The

112 primary forest canopy is ca. 23 m tall, with occasional emergent trees reaching 55 m (Gascon
113 and Bierregaard, 2001). In the early 1980s, 11 fragments were isolated from continuous forest
114 by distances of 80–650 m. Following abandonment of the cattle ranches, secondary regrowth
115 developed around the fragments, which were periodically re-isolated by clearance of a 100 m-
116 wide strip of vegetation around them (Rocha et al., 2017a). This 100 m distance from the
117 fragment edge represents the interface between younger and older secondary forest, which
118 together make up the inter-fragment matrix in our study landscape. The matrix is composed of
119 secondary forest dominated mainly by *Cecropia* spp. (areas that were cleared without fire) and
120 *Vismia* spp. (areas that were cleared and burned) (Mesquita et al., 2001). The most recent re-
121 isolation prior to this study occurred between 1999 and 2001 (Rocha et al., 2017a).

122 **2.2 Bat sampling**

123 The impacts of fragmentation on the bat fauna at the BDFFP were first assessed by Sampaio
124 (2000) who sampled six continuous forest sites and six forest fragments (three of 1 ha and
125 three of 10 ha) between January 1996 and June 1999. In addition, between October 2001 and
126 November 2002, Bobrowiec and Gribel (2010) sampled bats at seven sites in the secondary
127 forest matrix. That is, both studies were conducted ~15 years after fragment creation in the
128 early 1980s (Fig. 1). Using identical methods as those employed by Sampaio (2000), Sampaio
129 et al. (2003) and Bobrowiec and Gribel (2010), we resurveyed the same 19 sites between
130 August 2011 and June 2013, i.e. ~30 years after initial forest clearance.

131 In both periods, bats were sampled with ground-level mist nets (12 × 2.5 m) placed
132 along trails in the interior of continuous forest and fragments (for each habitat: period 1 = 18
133 to 24 nets, period 2 = 14 nets), and erected 100 m from one of the borders of each fragment in
134 the matrix (period 1 = 8 nets, period 2 = 7 nets). The nets were opened from dusk until six
135 hours later, and checked at intervals of ~25 minutes. Bias in capture rates due to net-shyness

136 (Marques et al., 2013) was avoided by spacing visits to the same site by three to four weeks,
137 and sampling was interrupted during heavy rains. Total sampling effort during both periods
138 was 28,959 mist-net hours (mnh), whereby 1 mnh equals one 12-m net open for 1 h
139 (continuous forest: period 1 = 8,757 mnh, period 2 = 4,009 mnh; forest fragments: period 1 =
140 9,429 mnh, period 2 = 3,963 mnh; secondary forest: period 1 = 860 mnh, period 2 = 1,941
141 mnh).

142 We restricted our analyses to phyllostomids and the mormoopid *Pteronotus parnellii*,
143 which can be sampled adequately with mist nets (Kalko et al., 1996). Same-site recaptures
144 were excluded. Canopy net data obtained in the interiors of the continuous forest sites and
145 fragments in both periods were used merely for assigning species to a particular forest stratum
146 ('vertical stratification' trait, see below), and were not included in the analyses. We analyzed 1
147 and 10 ha fragment interiors jointly because they did not differ significantly in species
148 richness (see Rocha et al., 2017b). We also considered secondary forest as a single habitat due
149 to small sample size for *Cecropia*- (three sites) and *Vismia*-dominated regrowth (four), and
150 because none of the most common bat species differed significantly in abundance between
151 these habitats (see Bobrowiec and Gribel, 2010). A total of 6,109 bats of 46 species were used
152 in the analysis. Bat sampling was conducted under ICMBio permit (26877-2) and followed the
153 ethical guidelines approved by the Animal Care and Use Committee of the American Society
154 of Mammalogists (Sikes, 2016).

155 ***2.3 Species functional traits***

156 We used five functional traits that reflect diet and foraging behaviour, and which describe
157 important functional components of Neotropical bat assemblages (Meyer et al., 2008; Cisneros
158 et al., 2015): (1) body mass, (2) trophic level, (3) dietary specialization, (4) vertical
159 stratification, and (5) wing morphology (see Table A1 in Supplementary data for trait values

160 of individual species). These traits are related to species responses to local habitat
161 fragmentation in human-modified tropical landscapes (Meyer et al., 2008; Farneda et al.,
162 2015) and are considered to be linked to the species' capacity to provide ecosystem services
163 (Luck et al., 2012). See Farneda et al. (2015) for a description of these traits, and Meyer et al.
164 (2008) for a detailed explanation of the rationale behind selecting these particular traits.
165 Logarithmic transformations were performed on body mass to normalize values. Continuous
166 traits (body mass and wing morphology) were standardized to a mean of zero and a standard
167 deviation of one prior to analysis to facilitate comparison of their relative effects on the same
168 scale.

169 **2.4 Data analysis**

170 We followed the methodological framework recently introduced by Ricotta et al. (2016) to
171 quantify functional and taxonomic α diversity (i.e. diversity intrinsic of each community) and
172 community-level functional uniqueness of bat assemblages. This approach takes into account
173 relative species abundances, thus allowing to adequately deal with differences in sampling
174 effort between study periods. Relative species abundance was based on capture rate in each
175 assemblage (bats per mist-net-hour). We calculated Rao's quadratic diversity index Q for
176 functional α diversity, which takes the differences (trait-based variance) between species pairs
177 into account, as well as the Simpson index D for taxonomic α diversity, which considers all
178 species maximally dissimilar (Botta-Dukát, 2005). Rao's index Q is a functional
179 generalization of the Simpson index D of species diversity, since both are based on the same
180 species pairwise distances and relative species abundances (see Botta-Dukát, 2005 for details).
181 Furthermore, we calculated community-level functional uniqueness U (Q/D) (Ricotta et al.,
182 2016). For all index values (Q , D and U) pairwise comparisons between periods were
183 performed for each habitat (continuous forest, fragments, secondary forest) using paired

184 permutational tests with 9999 randomizations.

185 To analyze how important (unique) a species is in supporting a certain function and
186 whether this varies over time, we calculated species-level functional uniqueness (\bar{K}_i) for both
187 study periods. \bar{K}_i is the mean distance of a given species from all other species in the
188 assemblage in terms of their functional traits (see Ricotta et al., 2016). As for α diversity, \bar{K}_i
189 was computed using relative species abundances. The calculations of Q , D , U and \bar{K}_i were
190 performed with the ‘uniqueness’ function provided by Ricotta et al. (2016).

191 To assess how habitat-level changes affect bat functional traits, we calculated
192 community-weighted mean trait values (CWM; Lavorel et al., 2008). This method allows
193 assessing shifts in mean trait values weighted by relative species abundances (Lavorel et al.,
194 2008), equalizing differences in capture effort between studies. We calculated CWM traits
195 using the ‘functcomp’ function of the R package FD (Laliberté and Legendre, 2010). For each
196 trait, pairwise comparisons between periods were performed separately for each habitat
197 (continuous forest, fragments, secondary forest) with paired permutation tests, using 9999
198 randomizations.

199 To elucidate the ecological processes underlying community structuring, we
200 partitioned the total functional β -diversity (i.e. dissimilarity between α [local] and γ [regional]
201 diversities) into their respective replacement (turnover) and richness difference components
202 (Cardoso et al., 2013). This framework can be represented as: $\beta_{\text{total}} = \beta_{\text{repl}} + \beta_{\text{rich}}$, where β_{total}
203 represents the total community functional variation, β_{repl} denotes the fraction resulting from
204 turnover of species functional traits, and β_{rich} is determined by trait loss or gain (Cardoso et al.,
205 2013; Cardoso et al., 2015). Functional β -diversity components were calculated based on a
206 functional tree (sum of branch lengths of a functional dendrogram using the unweighted pair
207 group method with arithmetic mean, Petchey and Gaston, 2007), and on presence-absence data
208 using a sites \times species matrix with Jaccard dissimilarity index. The β -diversity components

209 were computed using rarefaction (1,000 runs) to 602 individuals (corresponding to the total
210 number of individuals sampled in continuous forest during the second study period) for each
211 habitat to account for differences in capture effort between periods. The analyses were
212 conducted using the ‘beta’ function of the R package BAT (Cardoso et al., 2015). Temporal
213 changes in functional β -diversity components were quantified as the difference between mean
214 values between both study periods, and significance was inferred by examining the overlap of
215 95% confidence levels (Kiflawi and Spencer, 2004).

216 The functional pairwise dissimilarity matrices in all analyses were calculated using
217 Gower’s distance because our trait matrix (see Table A1) included a mix of continuous and
218 categorical traits. All analyses were conducted using R software (R Development Core Team,
219 2017).

220 **3. Results**

221 For both study periods, continuous forest was functionally more diverse than fragments and
222 secondary forest. However, temporal changes were most marked in secondary forest; forest
223 regeneration in the matrix was accompanied by a significant increase in functional α diversity
224 (Fig. 2). In contrast, in forest fragments and continuous forest, functional α diversity did not
225 significantly change over time (Fig. 2). Taxonomic α diversity significantly differed between
226 sampling periods for both forest fragments and secondary forest, yet exhibited opposite trends;
227 it increased in secondary forest but decreased in fragments (Fig. 2). In secondary forest,
228 species- and community-level functional uniqueness U increased over time (Figs. 2 & 3),
229 reflecting an elevated representation of species that perform different ecological functions (e.g.
230 *Chrotopterus auritus*, *Lophostoma schulzi*, *Trinycteris nicefori*, *Uroderma bilobatum*) (Fig. 3).

231 The species-level functional uniqueness in secondary forest over time increased mainly for
232 *Artibeus cinereus*, *Mesophylla macconnelli*, *Rhinophylla pumilio*, *Sturnira lilium* and *S. tildae*
233 (Fig. 3).

234 The most pronounced differences in functional trait composition between study periods
235 also occurred in secondary forest (Fig. 4; Table A2). Paired permutation tests comparing
236 CWM trait values revealed significant changes over time for trophic level, dietary
237 specialization and body mass. While animalivores increased with secondary forest
238 regeneration, phytophagous species, mostly frugivores with intermediate levels of diet
239 specialization, decreased (Fig. 4). Body mass decreased significantly over time in forest
240 fragments (Fig. 4). Statistical support for changes in vertical stratification and wing
241 morphology traits was limited (see Table A2).

242 Differences in functional β_{total} between periods were lowest in continuous forest and
243 higher in forest fragments and in secondary forest (Fig. 5). Differences in functional β_{total}
244 among bat assemblages in fragments were driven by both replacement of species traits (β_{repl})
245 and loss or gain of traits (β_{rich}). In contrast, in secondary forest the most important contribution
246 to functional β_{total} was by β_{rich} , which was significantly higher than for fragments and
247 continuous forest (Fig. 5).

248 **4. Discussion**

249 Fragmentation studies have often considered the matrix as a static component of the landscape
250 (Driscoll et al., 2013), and little is known about the temporal dynamics of fragmented tropical
251 ecosystems and how several aspects of functional diversity respond to habitat fragmentation
252 over time (but see Lindenmayer et al., 2015). Our study provides novel insights into bat
253 functional responses to habitat fragmentation and matrix regeneration. Our findings indicate
254 that forest regeneration in the matrix was associated with a significant recovery in bat

255 functional and taxonomic α diversity. We also found strong evidence for an increase in
256 species- and community-level functional uniqueness, and shifts in functional trait composition
257 of bat assemblages in response to secondary forest maturation. Moreover, matrix regeneration
258 between study periods also appears to be a major determinant of functional β_{total} in secondary
259 forest, which was mainly driven by trait gains (β_{rich}).

260 Our results show that an increase in secondary forest quality over time is accompanied
261 by an overall increase in bat species richness (higher taxonomic α diversity) and functionally
262 more diverse assemblages (higher functional α diversity and community-level functional
263 uniqueness) in matrix sites. The effects of secondary forest maturation over time are often
264 associated with pronounced changes in animal species composition and abundance, with some
265 species decreasing and others becoming hyper-abundant (e.g. Antongiovanni and Metzger,
266 2005; Stouffer et al., 2006; Lindenmayer et al., 2008). Changes in bat species composition and
267 abundance may reflect significant changes in functional diversity (Cisneros et al., 2015;
268 Wordley et al., 2017) since different bat ensembles respond differently to habitat complexity.
269 Bat ensembles adjust their foraging activities according to the availability of food, roosts, and
270 vegetation structure (e.g. Marciente et al., 2015; Meyer et al., 2016; Voss et al., 2016;
271 Gonçalves et al., 2017).

272 Matrix regeneration increased the connectivity between forest fragments and
273 continuous forest sites in our study area (Rocha et al., 2017b). However, the majority of bat
274 species in small fragments (≤ 10 ha) depend on their ability to use the matrix (Farneda et al.,
275 2015), and although some species have recolonized the fragments (e.g. *Carollia castanea*,
276 *Glossophaga soricina*, *Lamproncycteris brachyotis*, *Lophostoma carrikeri*, *L. schulzi*), others
277 seem to have disappeared (e.g. *Chiroderma trinitatum*, *Micronycteris hirsuta*, *Phyllostomus*
278 *hastatus*, *Platyrrhinus helleri*, *Sturnira lilium*). These species replacements and a more

279 dissimilar distribution of relative abundance between species probably explain the significant
280 declines in taxonomic α diversity in fragments over time.

281 The importance of individual species in supporting a determined function (species-
282 level functional uniqueness) varied over time in our study area. At the BDFFP, an increase in
283 bat ecological functions is strongly associated with matrix vegetation moving towards the
284 structural and compositional complexity of continuous primary forest, with larger trees and
285 greater vertical vegetation density. This increase led to a reduction in functional redundancy
286 (i.e. when several species perform similar functions, Rosenfeld, 2002) of bat assemblages in
287 secondary forest over time. Examination of the composition of the bat assemblages (Fig. 3)
288 shows that besides animalivorous bats (e.g. *C. auritus*, *Glyphonycteris daviesi*, *Lophostoma*
289 *brasiliense*, *L. schulzi*), some frugivores with a highly specialized diet (e.g. *Ametrida centurio*,
290 *Carollia castanea*, *Vampyriscus bidens*) have benefited from secondary forest regeneration – a
291 result supported by our analysis of α diversity. Secondary forests in an advanced successional
292 stage offer more favorable conditions for recolonization of animalivorous species, which are
293 usually more abundant in old-growth forests and the first ones to be adversely affected by
294 fragmentation and habitat loss (e.g. Meyer et al., 2008; Muylaert et al., 2016; Gonçalves et al.,
295 2017). Species at higher trophic levels (such as secondary consumers) are more extinction-
296 prone than those at lower trophic levels due to more unstable population dynamics (Colinvaux,
297 1980; Henle et al., 2004).

298 A higher extinction risk is also commonly attributed to large-bodied species as they
299 tend to have smaller populations and lower reproductive rates (Colinvaux, 1980; Henle et al.,
300 2004). However, the reasons for a significant decrease in body mass observed in forest
301 fragments over time remain unclear. It is possible that this finding is associated with
302 differences in species richness and abundance in forest fragments between periods, as the
303 largest bat species (*Phyllostomus hastatus*) was not recorded in fragments during the second

304 study period. Changes in functional trait composition were also observed for frugivorous bats
305 with intermediate levels of diet specialization, which decreased over time in secondary forest.
306 This finding is probably linked to opportunistic shrub-frugivores of open-forest environments
307 (e.g. *Sturnira lilium* and *Sturnira tildae*), which consume fruits of early-successional plants
308 (mainly *Solanum* spp., Mello et al., 2008) and that typically occurred in higher abundance in
309 the matrix during the first study period (Bobrowiec and Gribel, 2010). Abundant production of
310 fruits of early-successional plant species in the matrix during the first study period probably
311 facilitated inter-patch movements and habitat use by some species of frugivorous bats, which
312 are generally more tolerant to disturbed areas than animalivorous phyllostomids (e.g. Cisneros
313 et al., 2015; Farneda et al., 2015; Muylaert et al., 2016; Gonçalves et al., 2017). The capacity
314 of bat species to persist in fragments and use patches in fragmented landscapes is mediated by
315 the quality and permeability of the matrix (e.g. successional stage, spatial extent) and
316 landscape characteristics (e.g. forest cover, fragment connectivity) via functional traits (Meyer
317 et al., 2008; Farneda et al., 2015).

318 Our findings regarding functional β -diversity suggesting stable coexistence of
319 functionally dissimilar species (limiting similarity hypothesis, MacArthur and Levins, 1967)
320 were more marked in forest fragments and continuous forest than in secondary forest. In more
321 stable environments, such as fragments and particularly continuous forest, colonization and
322 extinction are stochastic events, and functional replacement (β_{repl}) predominantly contributes
323 to functional β_{total} (Aguirre et al., 2016; Si et al., 2016). In this case, a high functional β_{repl} may
324 occur if species performing different ecological functions are not shared in the same habitat
325 over time (Cardoso et al., 2013). On the other hand, a high functional β_{total} may also result
326 from marked functional trait loss or gain (β_{rich}) via selective environmental filtering (Aguirre
327 et al., 2016; Si et al., 2016). We found that matrix regeneration translated into an increase in
328 functional traits β_{rich} over time, as supported by our results of species-level functional

329 uniqueness. This finding suggests that ~30 years of matrix regeneration were not sufficient to
330 reduce the strong effect of trait-mediated environmental filters, which still selectively benefit
331 the phytophagous species in the secondary forest matrix at the BDFFP (Farneda et al., 2015).

332 *4.1 Conservation implications*

333 In our study area, the successional process in the matrix is still ongoing, and a further increase
334 in functional diversity in fragments and secondary forest sites can be expected. Approximately
335 30 years of secondary forest regeneration in the matrix were not sufficient for functional
336 diversity to effectively recover to levels similar to those observed in continuous forest. Bat
337 assemblages in continuous forest are functionally more diverse and stable compared to those
338 in fragments and secondary forest. This emphasizes the importance of old-growth
339 megareserves in the Amazon (Laurance, 2005) for safeguarding the full set of key ecological
340 functions provided by bats and other animal groups (Laurance et al., 2017). However, our
341 findings also highlight that restoring and protecting secondary forest might be a valuable
342 strategy to prevent new anthropogenic land use changes and to sustain bat community
343 functioning in fragmented landscapes.

344 Maintaining a high-quality matrix in fragmented landscapes, as represented here by
345 advanced-stage secondary forest, is pivotal to preserving their key ecological functions.
346 Furthermore, this minimizes local extinction risk for fragmentation-sensitive species and
347 increases species-level functional uniqueness with the possible addition of species that
348 perform different ecological functions. The restoration and, crucially, long-term protection of
349 tropical secondary forests independently of their age is fundamental for safeguarding
350 functionally diverse bat assemblages in the future. We thus recommend increasing habitat
351 availability at the landscape scale, investing in the natural regeneration or active restoration of
352 degraded matrix habitats to increase bat functional diversity and accelerate functional recovery

353 in fragmented tropical landscapes. Furthermore, tropical conservation strategies need to
354 consider the inclusion of secondary forest within legally protected areas, particularly in
355 fragmented landscapes where little primary forest remains.

356 **Conflict of interest**

357 The authors declare that they have no conflict of interest.

358 **Funding**

359 Funding was provided by a project grant (PTDC/BIA-BIC/111184/2009) to C.F.J.M. and PhD
360 fellowships to R.R. (SFRH/BD/80488/2011) and A.L.-B. (PD/BD/52597/2014) from
361 Fundação para a Ciência e a Tecnologia (FCT). F.Z.F. and P.E.D.B. are supported by a
362 fellowship from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES),
363 and C.E.V.G. by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

364 **Acknowledgements**

365 We thank the Instituto Nacional de Pesquisas da Amazônia (INPA) and BDFFP, especially
366 José Luís Camargo, Ary Ferreira, and Rosely Hipólito, for logistic support. The following
367 people helped with fieldwork: Gilberto Fernandez, Diogo Ferreira, Milou Groenenberg,
368 Rodrigo Marciente, Madalena Boto, Inês Silva, Kevina Vulinec, Julia Treitler, Joana
369 Carvalho, Solange Farias, Leonardo Oliveira, Ileana Mayes, Ubirajara Capaverde Jr, Alaércio
370 Reis, Luiz Queiroz, Josimar Menezes, Osmaildo Silva, and José Tenaçol. We are grateful to
371 Pedro Cardoso for helpful insights concerning the BAT package. We further thank Zulmira
372 Coimbra, Eduardo Arcoverde de Mattos, Vinicius Farjalla and two anonymous reviewers for
373 comments on earlier drafts. This is publication number *** in the BDFFP technical series.

374 **Appendix A. Supplementary data**

375 Supplementary data to this article can be found online.

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549 **Fig. 1.** Map of the Biological Dynamics of Forest Fragments Project (BDFFP) in the Central
550 Amazon. Dark green: continuous primary forest and primary forest fragments; light green:
551 secondary forest matrix. Sampling sites are represented by green squares (continuous forest),
552 blue circles (forest fragments) and red triangles (secondary forest matrix). The general location
553 of the study area in South America is shown in the inset map.

554 **Fig. 2.** Functional α diversity (Rao's index Q), taxonomic α diversity (Simpson index D), and
555 community-level functional uniqueness U of the bat assemblages sampled in continuous
556 primary forest (CF), primary forest fragments (FF) and secondary forest (SF) during 1996-
557 2002 (circles) and 2011-2013 (triangles) at the BDFFP, Brazilian Amazon. Values represent
558 means \pm 95% confidence intervals. $*P < 0.05$.

559 **Fig. 3.** Species-level functional uniqueness for the first (circles) and second (triangles) study
560 periods. Bat species were sampled in continuous primary forest, primary forest fragments and
561 secondary forest at the BDFFP, Brazilian Amazon.

562 **Fig. 4.** Community-weighted mean (CWM) trait values for three statistically significant
563 functional traits: body mass, dietary specialization (category: intermediate), and trophic level
564 (animalivorous and phytophagous). The analyses were based on relative abundance of bat
565 species sampled in continuous primary forest (CF), primary forest fragments (FF) and
566 secondary forest (SF) in 1996-2002 (circles) and 2011-2013 (triangles) at the BDFFP, Central
567 Amazon. Values represent means \pm 95% confidence intervals. $*P < 0.05$. Results for all traits
568 are provided in Supplementary data, Table A2.

569 **Fig. 5.** Changes in the components of bat functional β -diversity between study periods (1999-
570 2002 and 2011-2013) in continuous primary forest, primary forest fragments and secondary
571 forest at the BDFFP, Brazilian Amazon. Values represent means (horizontal lines) \pm 95%
572 confidence levels (color bars) and maximum and minimum (vertical lines). Points represent
573 outliers in secondary forest β_{repl} . Grey: β_{total} , functional beta diversity total; yellow: β_{repl} , beta
574 diversity due to replacement of species functional traits; blue: β_{rich} , beta diversity due to loss or
575 gain of traits.



Fig. 1.

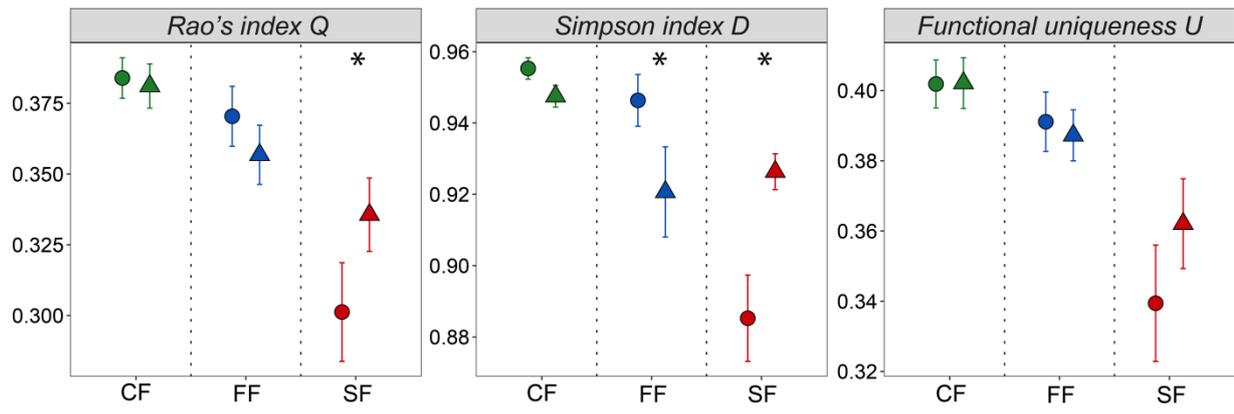


Fig. 2.

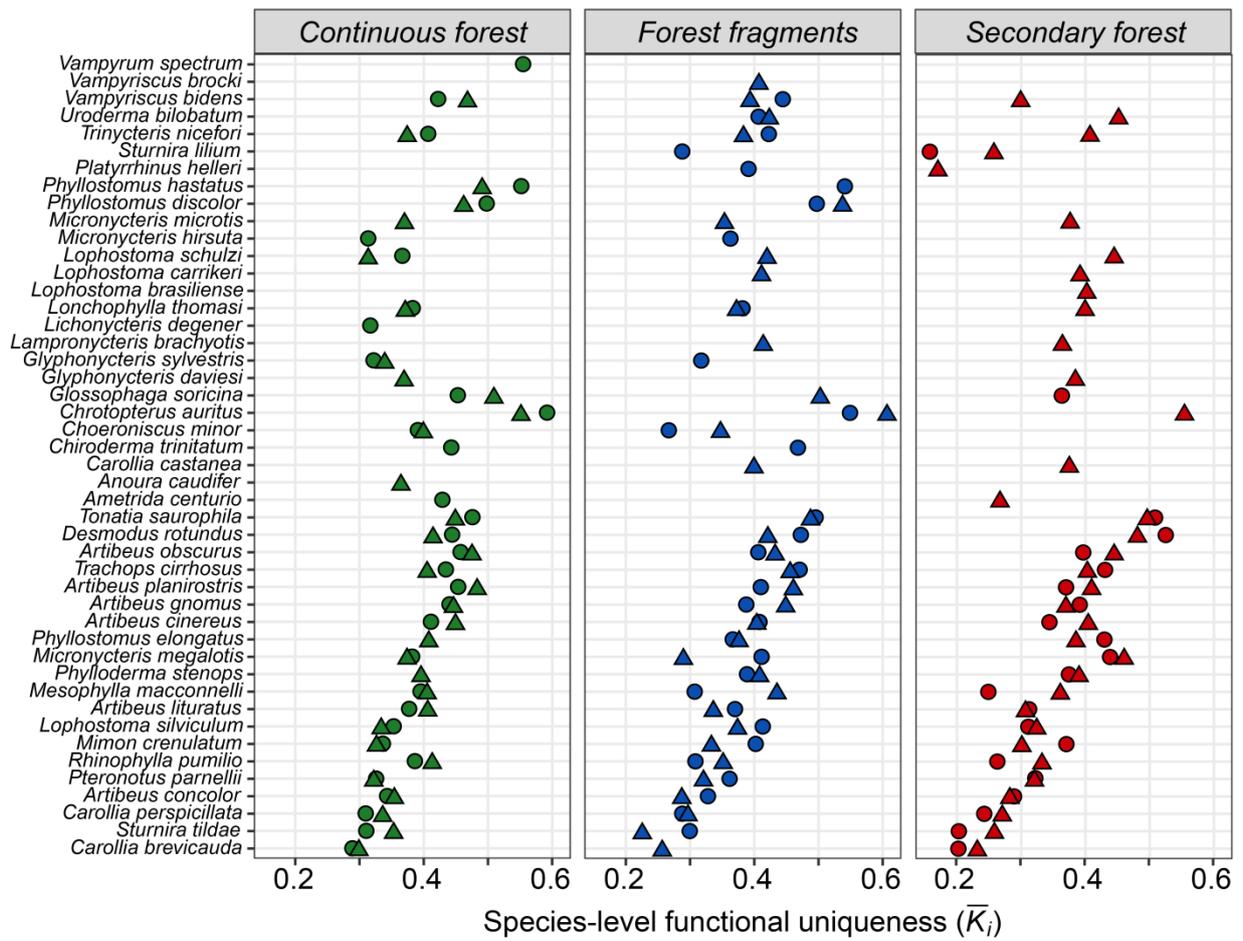


Fig. 3.

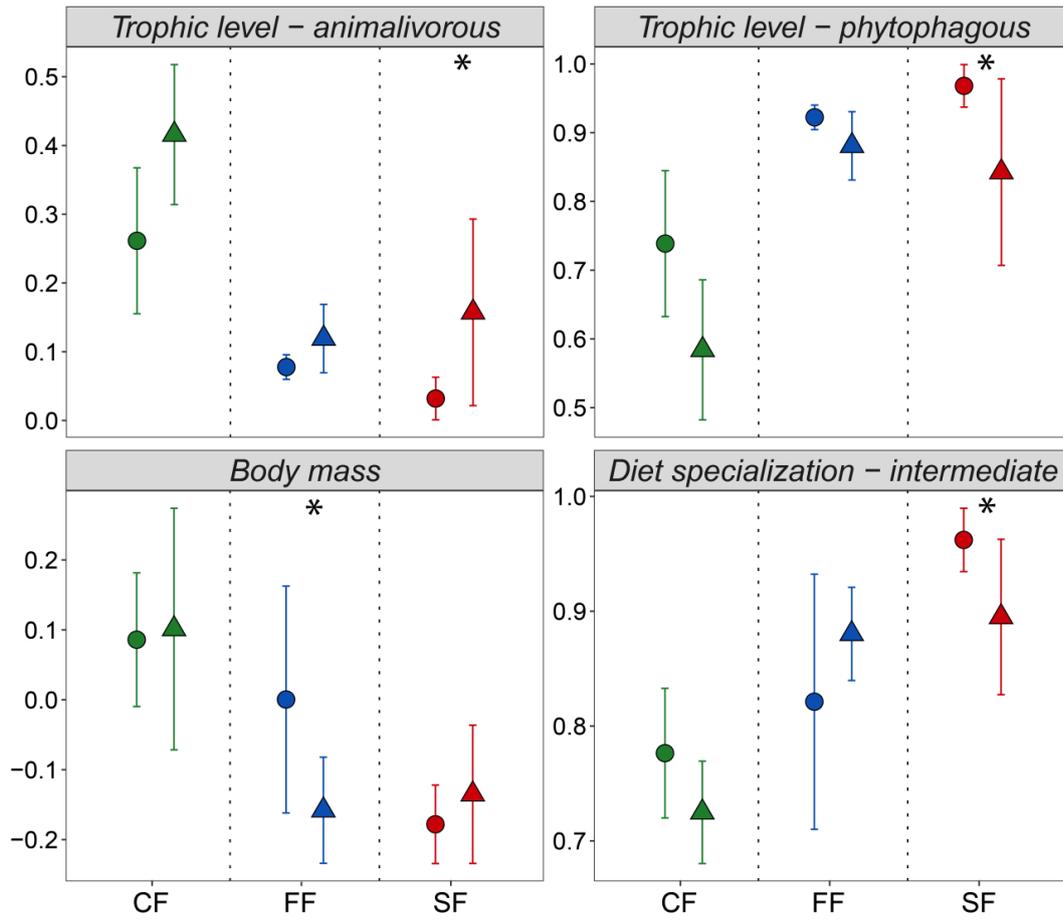


Fig. 4.

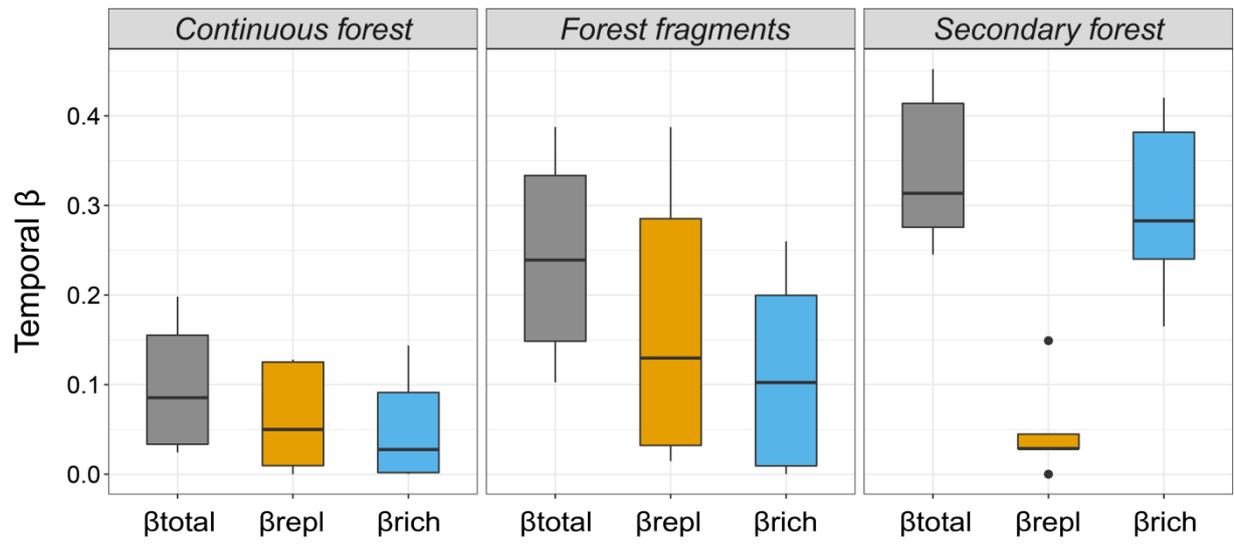


Fig. 5.