

1 **Is environmental legislation conserving tropical stream faunas? A large-scale assessment of**
2 **local, riparian and catchment-scale influences on Amazonian stream fish**
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71 **Abstract**

72 1. Agricultural expansion and intensification are major threats to tropical biodiversity. In
73 addition to the direct removal of native vegetation, agricultural expansion often elicits other
74 human-induced disturbances, many of which are poorly addressed by existing environmental
75 legislation and conservation programs. This is particularly the case for tropical freshwater
76 systems, where there is considerable uncertainty about whether a legislative focus on
77 protecting riparian vegetation is sufficient to conserve stream fauna.

78 2. To assess the extent to which stream fish are being effectively conserved in agricultural
79 landscapes we examined the spatial distribution of assemblages in river basins to identify the
80 relative importance of human impacts at instream, riparian, and catchment scales in shaping
81 observed patterns. We used an extensive dataset on the ecological condition of 83 low-order
82 streams distributed in three river basins in the eastern Brazilian Amazon.

83 3. We collected and identified 24,420 individual fish from 134 species. Multiplicative diversity
84 partitioning revealed high levels of compositional dissimilarity (DS) among stream sites (DS =
85 0.74 to 0.83) and river basins (DS = 0.82), due mainly to turnover (77.8 to 81.8%) rather than
86 nestedness. The highly heterogeneous fish faunas in small Amazonian streams underscore the
87 vital importance of enacting measures to protect forests on private lands outside of public
88 protected areas.

89 4. Instream habitat features explained more variability in fish assemblages (15-19%) than
90 riparian (2-12%), catchment (4-13%) or natural covariates (4-11%). Although grouping species
91 into functional guilds allowed us to explain up to 31% of their abundance (i.e. for nektonic
92 herbivores), individual riparian- and catchment-scale predictor variables that are commonly a
93 focus of environmental legislation explained very little of the observed variation (partial R^2
94 values mostly < 5%).

95 5. *Policy implications.* Current rates of agricultural intensification and mechanisation in tropical
96 landscapes are unprecedented, yet the existing legislative frameworks focusing on protecting

97 riparian vegetation seem insufficient to conserve stream environments and their fish
98 assemblages. To safeguard the species-rich freshwater biota of small Amazonian streams,
99 conservation actions must shift towards managing whole basins and drainage networks, as
100 well as agricultural practices in already-cleared land.

101 **Keywords:** Amazon, Brazilian Forest Code, functional guilds, tropical landscapes, human-
102 modified landscapes, multiplicative diversity partitioning, physical habitat, small streams,
103 species turnover, watershed management

104 **Introduction**

105 Agricultural expansion and its associated forest disturbances are major threats to the
106 biodiversity of the humid tropics (Laurance, Sayer & Cassman 2014; Barlow *et al.* 2016).
107 Environmental legislation and conservation programs help countries to minimize these losses
108 and to meet their commitments to the Convention on Biological Diversity (CBD 2010).
109 However, the focus of legislative efforts has been largely based on maintaining terrestrial
110 forest extent, and has paid little heed to the critical features of hydrological systems such as
111 the size and distribution of river catchments (Castello & Macedo 2016). As such, it remains
112 unclear the extent to which existing environmental regulations safeguard the ecological
113 integrity of stream systems, which accumulate human impacts from many different terrestrial
114 activities, and whose biodiversity may be more imperilled than their terrestrial equivalents
115 (Strayer & Dudgeon 2010).

116 There are few places on Earth where the conservation of aquatic diversity is more
117 important than in the Amazon Basin, which has the world's most diverse freshwater fish fauna
118 (Reis, Kullander & Ferraris 2003; Castello & Macedo 2016). One of the most poorly studied
119 elements of this fauna is the fish diversity of small, wadable streams (Mojica, Castellanos &
120 Lobón-Cerviá 2009). Those streams are the most extensive and widespread freshwater
121 ecosystems in the basin (Beighley & Gummadi 2011), consisting of up to 90% of the total
122 channel length in some sub-basins (McClain & Elsenbeer 2001).

123 Brazil contains 60% of the Amazon Basin, and its environmental regulations seek to
124 conserve freshwater ecosystems in three ways: (1) establishing protected areas; (2) controlling
125 forest cover on private properties; and (3) regulating water resources that are considered to
126 be of high economic importance. Yet all of these approaches have important limitations.
127 Although protected areas represent 54% of the Brazilian Amazon, their distribution takes little
128 account of connectivity in and among watercourses, many of which extend across biomes and
129 jurisdictional boundaries (Castello *et al.* 2013). Effective protection of transboundary river

130 basins is particularly challenging because countries have different levels of international
131 cooperation, conservation priorities and conservation budgets (Dolezsai *et al.* 2015).
132 Environmental regulation on Brazilian private lands, which make up about half of the country's
133 native vegetation (Ferreira *et al.* 2012; Soares-Filho *et al.* 2014), is through the *Forest Code* (FC;
134 Law 12.651; Brasil 2012). Although the FC stipulates minimum-width riparian forests along
135 streams and limits deforestation outside riparian zones, it does not provide guidance for forest
136 protection at catchment or basin scales or for agricultural practices, both of which affect the
137 freshwater biota (Roth, Allan & Erickson 1996; Leitão *et al.* 2017). Last, the two Brazilian legal
138 instruments directly concerned with streams, the *Fisheries Code* (Law 11.959; Brasil 2009) and
139 the *Water Resources Regulation* (Law 9.433; Brasil 1997), focus on aquaculture and fishing
140 activities and water for human consumption, respectively. As such, they do not directly
141 address the biodiversity values of freshwater ecosystems (Castello *et al.* 2013). Moreover, all
142 three of these areas of legislation to conserve freshwater systems in the Brazilian Amazon
143 suffer from being poorly coordinated and weakly enforced (Castello & Macedo 2016).

144 Given the potential shortcomings in existing legislation to conserve stream biota, there
145 is an urgent need to assess the effectiveness of existing regulatory mechanisms for conserving
146 the fish assemblages in the Amazon Basin. Our current understanding of their effectiveness is
147 limited by three key knowledge gaps. First, there is a lack of data on the responses of
148 freshwater biota to human pressures across the biome. The vast majority of research on the
149 effects of habitat degradation in the Amazon is on terrestrial biota. For example, a review of 62
150 studies assessing faunal responses to land-use change in Amazonia (Peres *et al.* 2010) included
151 just one on fish (Dias, Magnusson & Zuanon 2010). Second, where fish responses to human
152 impacts have been studied in Amazonia, they have focused on large rivers, hydropower plants,
153 and commercially important species (Barthem, Ribeiro & Petreire 1991; Hurd *et al.* 2016,
154 Tregidgo *et al.* 2017). Very few studies have examined the consequences of human impacts on
155 the heterogeneous Amazonian fish assemblages in small streams. As such, little is known

156 about the responses of stream fauna to deforestation, agricultural intensification, and other
157 sources of forest degradation (Issues 2002; Dias, Magnusson & Zuanon 2010; Prudente *et al.*
158 2017; Leitão *et al.* 2017).

159 Third, we lack large-scale empirical studies evaluating the relative importance of
160 pressures affecting biotic change in streams at different spatial scales, and how amenable such
161 pressures are to changes in the management regime (Hughes, Wang & Seelbach 2006). There
162 is uncertainty regarding whether catchment disturbances (Roth, Allan & Erickson 1996; Allan,
163 Erickson & Fay 1997; Marzin, Verdonschot & Pont 2013) or local riparian disturbances (Wang
164 *et al.* 2003; Sály *et al.* 2011; Macedo *et al.* 2014) are the most critical drivers of changes in the
165 biotic condition of streams. Similarly, it is unknown to what extent management practices at
166 local, small scales are constrained by ecological processes at catchment scales (Palmer,
167 Menninger & Bernhardt 2010; Castello & Macedo 2016; Mantyka-Pringle *et al.* 2016). Answers
168 lie largely in the types and relative degrees of disturbance and natural variability at these two
169 scales and the biotic indicators of condition (Wang, Seelbach & Lyons 2006; Terra, Hughes &
170 Araújo 2016).

171 We address these knowledge gaps using a large-scale assessment of the fish fauna
172 among 83 stream sites in the human-modified landscapes of the eastern Brazilian Amazon.
173 First, we examine the importance of forest reserves on private lands for conserving fish
174 diversity by assessing patterns of species turnover among stream sites within three river basins
175 and among those basins. Second, we examine the effectiveness of the FC for protecting
176 Amazonian stream biota by investigating how fish assemblages are affected by human
177 disturbances assessed at three spatial scales: (1) the riparian scale, reflecting the explicit focus
178 of the FC in conserving aquatic systems; (2) the catchment scale, accounting for the
179 requirement of private landholders to conserve 50-80% of their forest cover outside the
180 riparian zone, although the FC does not explicitly regulate at the catchment scale; (3) the
181 instream habitat scale, characterizing conditions that are strongly affected by riparian and

182 catchment disturbances, and that have a direct impact on fish assemblages, but for which
183 there is virtually no legislative protection (Fig. 1). We use our findings to discuss the challenges
184 involved in understanding the links between human disturbances and fish assemblages in
185 tropical streams, the effectiveness of the FC in protecting stream biota, and the implications
186 for large-scale conservation planning in human-modified tropical forest landscapes more
187 generally.

188 **Materials and methods**

189 SAMPLING DESIGN

190 We studied two regions in the eastern Brazilian Amazon state of Pará. Santarém (STM) covers
191 1 million ha at the confluence of the Amazonas and Tapajós Rivers; Paragominas (PGM) covers
192 1.9 million ha in the far eastern Amazon basin. Both regions are characterized by a patchwork
193 of pasture (3.9% in STM and 21.1% in PGM, data from 2010), annual crops (2.0% and 3.5%)
194 including mechanised agriculture, secondary forest (10.4% and 17.6%), and retain around two-
195 thirds of their native primary forest, albeit in varying stages of degradation from
196 fragmentation, logging, and fires (Gardner *et al.* 2013; Almeida *et al.* 2016). Wadable stream
197 sites (1st to 3rd Strahler order on a digital 1:100,000 scale map) were chosen to encompass a
198 gradient in the extent of riparian and catchment forest cover, resulting in 33, 26, and 24 sites
199 in the Curuá-Una (STM), Capim (PGM), and Gurupi (PGM) River Basins, respectively (Fig.1).

200 We sampled fish during the Amazonian dry season June-August 2010 (STM) and 2011
201 (PGM). Each 150 m long site was subdivided into 10 continuous sections by 11 cross-sectional
202 transects (isolated by block nets) (Fig. 1). Three people sampled fish for 120 min (12 min per
203 section) with seines (6.0 x 1.5 m, 5 mm stretched mesh size) and semi-circular hand nets (0.8
204 m in diameter, 2 mm stretched mesh size) (Appendix S4). Specimens were euthanized in
205 Eugenol and then fixed in 10% formalin. In the laboratory, all sampled fishes were transferred
206 to 70% ethanol and identified to species. Voucher specimens from all species are deposited at
207 the Fish Collection of the Instituto Nacional de Pesquisas da Amazônia (INPA) and the Museu
208 Paraense Emílio Goeldi (MPEG), Brazil.

209 Physical habitat data were collected along the thalweg and from 11 transects every 15
210 m (Fig. 1; see Appendix S1 in Supporting Information; Hughes & Peck 2008). Between the
211 transects we quantified large wood volume in the channel and measured thalweg depth and
212 substrate size at 10 equidistant points. At each of the 11 transects, we measured bankfull
213 width and depth, and at five equidistant points along each transect, we measured water depth

214 and assigned a surficial bed particle diameter class. Cover for fish was assessed at each
215 transect along 10 m long plots inside the stream channel using semi-quantitative estimates of
216 the areal cover of leaf packs, roots, overhanging vegetation, wood, undercut banks, boulders,
217 filamentous algae, and aquatic macrophytes. Forest canopy cover above the channel was
218 measured with a convex densiometer at the centre of each transect (facing upstream,
219 downstream, left and right margins) and the mean values were used as a proxy for channel
220 shading. We measured conductivity and temperature with a portable digital meter placed
221 below the water surface in the centre of the site. From these measurements we calculated 11
222 metrics (Table 1; Kaufmann *et al.* 1999) representing complementary attributes of instream
223 conditions likely affected by land-use changes (Leal *et al.* 2016) and influencing stream fish
224 assemblages (Leitão *et al.* 2017).

225

226 RIPARIAN- AND CATCHMENT-SCALE MEASURES

227 We mapped the drainage network using the hydrological model ArcSWAT (Di Luzio, Srinivasan
228 & Arnold 2004), allowing us to calculate hydrological distance between each site and the main
229 river downstream (4th order reaches). We determined catchment boundaries, mean elevation,
230 and slope through use of digital elevation models (SRTM images, 90 m resolution).

231 We assessed site pressures at three spatial scales (Fig. 1): (1) whole catchment
232 upstream from a site (catchment); (2) 100 m buffer along the entire drainage network
233 upstream from the site (riparian network); and (3) 100 m riparian buffer along the site (local
234 riparian). Riparian buffer widths and the basis for their definition vary greatly among ecological
235 studies and environmental regulations worldwide (e.g. Lee *et al.* 2004). The FC establishes a
236 minimum buffer width of riparian vegetation to be protected (or restored in case of illegal
237 deforestation) alongside watercourses inside private properties. However, this width is based
238 on several criteria (e.g. size of the property, stream width, when deforestation occurred, etc.)
239 and there is no set width that could be applied across the landscape in the absence of data on

240 land tenure and deforestation history. Therefore, we selected 100 m buffers to provide
241 estimates of land-use within the riparian zone considering the resolution of the land use maps
242 and the digital elevation models (30 to 90 m), and what is considered in other studies (e.g. Van
243 Sickle *et al.* 2004), without linking these to the requirements specified by Brazilian laws.

244 We calculated forest cover proportion for 2010 using classified Landsat images with 30
245 m of resolution (Gardner et al 2013). Forest cover included primary forest (whether
246 undisturbed or disturbed from fire or logging), and secondary forest older than 10 years, which
247 was considered sufficiently developed to provide important hydrological services (e.g. soil
248 stabilization, sediment and nutrient filtration). The history of mechanised agriculture was
249 calculated from annual MODIS data from 2001 to 2010 (Gardner et al 2013).

250 We noted the human activities in the local riparian zone (e.g. pipes, buildings, trash
251 etc.; Hughes & Peck 2008) and calculated an index of proximity of human impact (W1_HALL;
252 Kaufmann et al. 1999). We used Rapideye images (2010 for STM and 2011 for PGM, 5 m
253 resolution) to estimate riverscape fragmentation from upstream and downstream road
254 crossings within a 5 km circular buffer from the stream site. All landscape analyses were
255 conducted in ArcGIS 9.3© (Environmental Systems Research Institute, Redlands, CA, USA).

256

257 LINKING ENVIRONMENTAL PREDICTORS WITH BRAZILIAN LEGISLATION

258 Our direct (riparian and catchment) and indirect (instream habitat) measures of human
259 disturbance reflect different aspects of Brazilian legislation regulating the protection of
260 watercourses (Fig.1, Table S1). The forest-cover variables and the index of proximity of human
261 impact represent the FC regulation on the protection of riparian vegetation and Legal Reserves
262 elsewhere in the properties. Roads alter both the streams they cross (Macedo *et al.* 2013; Leal
263 *et al.* 2016; Leitão *et al.* 2017) and the riparian forests adjacent to the crossing; however, the
264 FC regulates only the forests. The extent and type of agricultural mechanisation is not
265 governed by the FC or any other regulation in the country. Measures of instream habitat are

266 very difficult to regulate because they reflect both natural characteristics of the landscape and
267 the outcomes of human disturbances. However, dissolved oxygen is used for water body
268 classification by Law No 9.433 (Brasil 1997).

269

270 DATA ANALYSES

271 *Diversity partitioning*

272 We used multiplicative diversity partitioning to analyse the spatial distribution of fish diversity
273 considering the following decompositions: $\gamma_{\text{region}} = \alpha_{\text{river-basin}} \times \beta_{\text{river-basin}}$ (for PGM) and $\gamma_{\text{river-basin}} =$
274 $\alpha_{\text{stream-site}} \times \beta_{\text{stream-site}}$ (for the Curuá-Una, Capim, and Gurupi Basins). We compared the
275 magnitude of variation in $\beta_{\text{river-basin}}$ and $\beta_{\text{stream-site}}$ using the relative compositional dissimilarity
276 (DS) following Arroyo-Rodríguez *et al.* (2013). DS varies from 0 (identical assemblages) to 1
277 (completely different assemblages). Next we decomposed the components of $\beta_{\text{stream-site}}$ to
278 investigate whether variation in species composition across sites in each river basin was a
279 result of turnover (species replacement) or nestedness (species loss or gain) by using Sørensen
280 (β_{SOR}) and Simpson (β_{SIM}) indices (Baselga 2010).

281

282 *Assemblage–environment modelling*

283 We conducted variance-partitioning analysis (Borcard, Legendre & Drapeau 1992) for each
284 river basin separately, which allowed us to estimate the amount of variation in taxonomic
285 composition in assemblages explained by the four sets of environmental predictors. We
286 performed variance partitioning for functional guilds by combining fish trophic and habitat-use
287 characteristics for all river basins together (Appendix S2). Species biological traits can help to
288 uncover responses to human disturbances (Mouillot *et al.* 2013), especially in systems
289 dominated by rare species. Several species were singletons (e.g. 12 species in Capim) or
290 occurred at very few sites (e.g. 50% of the Curuá-Una species occurred in three or fewer sites)
291 (Appendix S3), which hindered development of robust species-specific models.

292 We used R_a^2 values from adjusted redundancy analysis, which account for the number of
293 predictor variables in each group and the number of observations in the response variables to
294 produce unbiased estimates (Peres-Neto *et al.* 2006). Explained variance was split into 16
295 fractions using partial ordination methods: four individual components explained
296 independently by each group of predictor variables, 11 fractions for the explained variance
297 shared by two or more groups, and a residual fraction of the unexplained variance (Borcard,
298 Legendre & Drapeau 1992).

299

300 *Relative effects of policy-relevant environmental predictors*

301 To examine the influence of variables that are frequently targeted by environmental
302 legislation, we used random forest models (RF; from Breiman 2001) to evaluate changes in
303 functional guild abundance for the combined river basins. We considered riparian and
304 catchment predictors and natural covariates in the models to investigate the effect of those
305 governed by the FC (CAT_FOR, LOC_FOR, NET_FOR, W1_HALL) and possibly governable
306 (DNS_RDS, CAT_MAG) (See Table 1 for variable codes). RF incorporates interactions among
307 predictors and non-linear response-predictor relationships. We calculated a pseudo- r^2 value as
308 $1 - \text{MSE}/\text{Var}(y)$, where MSE is the mean squared error of the out-of-bag predictions (Ellis, Smith
309 & Roland Pitcher 2012). This value estimates the reliable proportion of variation predicted by
310 the ensemble model. All models were fitted with 10,000 trees, with one third of variables
311 randomly sampled as candidates at each split (one variable selected if total variables < 3).

312 Next, we used RF to model the partial responses of functional guilds to the six predictor
313 variables listed above. Those partial responses show the relative odds of detecting each guild
314 along a predictor gradient while holding all other predictors constant (Barlow *et al.* 2016). Last,
315 we used latent trajectory analysis (LTA) to group guild partial responses into homogeneous
316 classes, which summarize the main types of response to the predictors and the extent of
317 species turnover. We considered LTA models with up to five classes and selected the model

318 with the lowest Bayesian Information Criterion score. We show the LOWESS smoothed
319 response of each guild class along the associated predictor variable with bandwidth set to the
320 default value of 0.75.

321 All analyses were performed in R (R Core Team 2013) and are outlined in Appendix S1.
322 Diversity partitioning (`beta.multi` function) and variance partitioning (`varpart` function) were
323 performed using the `vegan` library (Oksanen *et al.* 2013). Random forest models and the
324 relative importance (RI) of individual predictor variables were calculated using the conditional
325 permutation method in the `randomForest` function of the `extendedForest` library (Smith, Ellis
326 & Pitcher 2011). Latent trajectory analysis used the `lcmm` library (Proust-Lima *et al.* 2016).

327 **Results**

328 DIVERSITY PARTITIONING TO ASSESS LANDSCAPE PATTERNS OF STREAM FISH DIVERSITY

329 We collected 24,420 individual fish from 134 species, with 60 species (5,846 specimens) in
330 Curuá-Una, 83 in Capim (7,421 specimens) and 83 in Gurupi (11,153 specimens) (Table S2). The
331 relative compositional dissimilarity for the PGM basins was $DS = 0.46$. Among stream sites, DS
332 $= 0.82$ for PGM, 0.74 for Gurupi, 0.78 for Capim, and 0.83 for Curuá-Una, indicating that river
333 basins and stream sites within river basins are distinct from each other (Fig. 2A), showing the
334 high level of environmental heterogeneity in Amazonian streams. The contribution of turnover
335 to the $\beta_{\text{stream-site}}$ component was much higher than nestedness in all river basins: 81.8% (Curuá-
336 Una), 78.6% (Capim) and 77.8% (Gurupi) (Fig. 2B). All values were significantly different from
337 those expected by chance obtained from 1000 permutations ($P < 0.001$).

338

339 ASSEMBLAGE-ENVIRONMENT RELATIONSHIPS TO ASSESS THE EFFECTIVENESS OF CURRENT
340 LEGISLATION TO PROTECT STREAM FISH DIVERSITY

341 Despite the diverse set of environmental predictor variables included in our analysis, together
342 they explained only $0.9\text{--}19.5\%$ of the variation in taxonomic and 19.8% in functional guild
343 assemblage composition (Fig. 3). Instream habitat was the most important predictor in the
344 Curuá-Una Basin (22.3%) and for all stream sites (8.8%) (Fig. 3). In the Capim and Gurupi
345 Basins, the effect of instream habitat was through its interactions with other predictor
346 variables. Riparian and catchment predictors explained smaller proportions of assemblage
347 variation for both species and guilds abundance, and mostly through interactions with other
348 predictor variables. Natural characteristics of stream sites were mainly important in the Capim
349 River basin (3.8%).

350 Assessing the effects of each group of predictor variables independently showed a
351 similar pattern of responses (Fig. 4). Instream habitat had the greatest contribution in
352 explaining the observed variability in fish assemblages from the Curuá-Una (19.2%), Capim

353 (19.2%) and Gurupi (7.3%) Basins, and in the functional guild composition for all stream sites
354 combined (15.7%). The contribution of riparian pressures differed greatly, accounting for
355 16.5% in the Capim Basin, 5.8% in the Gurupi Basin, and 6.8% in all river basins together, but
356 effectively none of the variability in the Curuá-Una Basin. Overall, catchment disturbance was
357 associated with smaller proportions of the variability in assemblage composition than riparian
358 pressures, except for the Curuá-Una Basin. Natural characteristics were only important in the
359 Capim Basin (13.2%); however, they accounted for variability in the other assemblages through
360 interactions with other predictor variables.

361

362 FUNCTIONAL GUILD RESPONSES TO POLICY-RELEVANT MEASURES OF HUMAN IMPACT

363 Random forest models explained up to 31% (for the nektonic herbivore guild) of the observed
364 variation in guild abundance (Table S3). Four of the 31 guilds had no variation explained and
365 another ten could not be modelled because they occurred at too few sites or were
366 represented by too few individuals. Single riparian- and catchment-scale predictor variables
367 explained very little of the observed variation (partial R^2 values mostly < 5%) in most functional
368 guilds (Fig. 5). This result reflects the low level of assemblage turnover relative to most of our
369 measures of human disturbance, which was shown by the latent trajectory analysis on guild
370 partial responses (Fig. 6, Table S4). Guilds responses mainly were to forest-cover variables.
371 Most guilds responded negatively to network forest cover (Fig. 6B) and some showed a
372 positive increase at ca 70%. Few guilds responded to local forest cover, and those mainly
373 decreased in more forested streams (Fig. 6A). Catchment forest accounted for sharp increases
374 of guilds at ca 60%. However, most responses also related to guilds decreasing in abundance
375 along the gradient of human impact (Fig. 6D). We did not find consistent changes in guild
376 abundance in response to road density, the proportion of mechanised agriculture in
377 catchments, or the index of proximity of human impact (Fig. 6 C, E, F).

378 **Discussion**

379 Our large-scale assessment of Amazonian stream fishes provides four sets of insights relevant
380 to the research and management of aquatic diversity in human-dominated landscapes. First,
381 we observed very high levels of species turnover, even within the same river basin, highlighting
382 the importance of conservation measures beyond protected areas. Second, we found that
383 changes in fish abundance were more strongly associated with instream habitat pressures than
384 with the variables more frequently addressed by Brazilian environmental legislation, such as
385 those related to riparian and landscape-scale measurements of forest cover. Third, despite our
386 extensive sampling of environmental features generally thought to affect fish assemblages, our
387 understanding of the relative importance of different impacts was diluted by the amount of
388 unexplained variance, region-specific relationships, and the complex interdependent
389 associations amongst predictor variables. Such challenges are to be expected in biodiversity-
390 rich regions with a diverse mosaic of land uses and natural characteristics, and poses particular
391 difficulties for assessments of the most disturbance-sensitive fish species. Last, our results
392 underscore a number of priorities for future research on human impacts on tropical stream
393 fish assemblages, including the assessment of a wide range of impacts at multiple scales, the
394 importance of pre-disturbance information, and the relevance of different species traits in
395 determining species' tolerance to disturbance impacts. We examine these four issues in more
396 detail below.

397

398 CONSERVATION OF STREAM FAUNA BEYOND PROTECTED AREAS

399 While high levels of species turnover are typical of many tropical landscapes (Solar *et al.* 2015),
400 ours is the first study to report such a finding for stream systems in Amazonian agricultural-
401 forest landscapes. This very high level of species turnover in fish assemblages among streams
402 and river basins (Fig. 2) lends strong support for legislation, such as the Brazilian FC, which
403 targets the maintenance and rehabilitation of forest cover in private properties throughout

404 agricultural landscapes. However, our results demonstrate that planning needs to consider the
405 scale of entire landscapes and river basins, and cannot be focused on individual private
406 properties or on municipalities, where most environmental legislation (including the FC) is
407 enacted (Viana *et al.* 2016). Therefore, our results have two important implications for the
408 spatial implementation of FC legislation to conserve aquatic biodiversity.

409 First, our results provide guidance on forest restoration. The FC offers two alternative
410 mechanisms for land owners to address previous illegal deforestation (the so-called legal
411 reserve deficit; Soares-Filho *et al.* 2014; Nunes *et al.* 2016) and come into compliance with the
412 law – land owners can either undertake on-farm rehabilitation or invest in compensation by
413 renting or purchasing forest in other regions. However, the FC does not specify which action
414 should occur, and any compensation only needs to occur within the same biome, that is, in the
415 entire Brazilian Amazon (Nunes *et al.* 2016). By demonstrating the high turnover in species
416 composition, our results provide strong empirical support for the recommendations of Nunes
417 *et al.* (2016) to encourage compliance efforts to take place locally, either by focusing on
418 rehabilitation in landscapes that are heavily deforested or by undertaking off-farm
419 compensation within the same river basin.

420 Second, our results show that the FC focus on land use in the riparian zone to protect
421 streams should not undermine the necessity to maintain and restore forest cover elsewhere in
422 the catchment. In some cases, catchment-scale pressures were of comparable importance to
423 riparian-scale pressures in shaping fish assemblages (Figs. 3, 4), which supports other studies
424 that show how management practices in the riparian zone are insufficient for restoring
425 biodiversity unless incorporated with improved catchment and channel network management
426 (Fausch *et al.* 2002; Mantyka-Pringle *et al.* 2016). Within the Brazilian Amazon, this is
427 particularly important in areas that have been designated as ‘consolidated zones’ for
428 agriculture as part of ecological-economic zoning plans, where properties that have cleared
429 more than 50% of their forest cover only have to restore (or compensate) back to 50%.

430 However, our results show that even 50% forest cover in catchments risks altering the
431 abundance and composition of fish functional guilds (Fig. 6). More work is needed to identify
432 thresholds in the abundance of species of the highest conservation concern (e.g. de Oliveira-
433 Junior *et al.* 2015; Leitão *et al.* 2016).

434

435 THE IMPORTANCE OF LOCAL STREAM CONDITION

436 Our findings show that fish assemblages are influenced by changes in local stream condition,
437 which includes a suite of factors that are not currently addressed by any environmental
438 legislation. This is important because it implies that disregarding changes in local stream
439 condition can lead to an underestimation of the effects of human disturbances at the
440 catchment and riparian scales, given that many such impacts are only observable through
441 changes in instream habitat condition (Leal *et al.* 2016). The question remains as to whether
442 management can address such impacts.

443 First, it is important to examine to what extent these changes in instream condition
444 are an outcome of indirect interactions with broader-scale human pressures, such as forest
445 cover, that are already being addressed by existing legislation. For example, while our results
446 were statistically independent of our catchment and riparian scale variables, linkages between
447 landscape change and instream condition can be complex and diverse (Leal *et al.* 2016), and it
448 is unlikely that they were fully represented by our explanatory variables. It is highly probable
449 that the human alterations at riparian and catchment scales play indirect roles in influencing
450 fish assemblages by, say, regulating channel morphology, bed substrate composition, wood
451 and leaf litter inputs, shade, and water quality (Kaufmann & Hughes 2006; Leal *et al.* 2016;
452 Leitão *et al.* 2017). These linkages between human disturbances and instream habitat
453 conditions are further complicated by interactions with factors such as the degree of basin
454 disturbance (Wang, Seelbach & Lyons 2006; Sály *et al.* 2011), type of disturbance (USEPA
455 2016), biotic group (Marzin *et al.* 2012), and the intrinsic geomorphological characteristics of

456 the systems (Kaufmann & Hughes 2006); all of these factors may have contributed to low
457 levels of explained variation in our models. Given these complexities, there is a genuine risk
458 that monitoring and assessment programs that focus only on instream habitat or riparian
459 zones are likely to underestimate the effects of cumulative human disturbances on streams
460 (e.g. Schinegger *et al.* 2012; USEPA 2016).

461 A second argument against legislating for instream condition relates to evidence from
462 other systems. Although management practices in temperate and tropical nations are often
463 restricted to reach or riparian scales (Bernhardt & Palmer 2011; Giling, Mac Nally & Thompson
464 2015), there is growing recognition of the importance of implementing catchment- or basin-
465 scale management (Abell, Allan & Lehner 2007). Moreover, there is a lack of evidence
466 supporting the effectiveness of reach-scale interventions (e.g. channel re-configuration or the
467 addition of boulders and logs) or point-source pollution treatment for restoring aquatic
468 biodiversity in Europe and the United States (Palmer, Menninger & Bernhardt 2010; Hughes *et*
469 *al.* 2014). Most aspects of instream habitat are difficult and costly to manage directly, and it
470 would be nearly impossible to monitor effectively across very large spatial scales such as the
471 Amazon basin (Castello *et al.* 2013).

472 Although there are many challenges to developing management strategies that focus on
473 changes in instream condition in complex tropical landscapes, our results do nevertheless
474 highlight the importance of these changes for stream-fish assemblages. Perhaps a more
475 effective approach would be to develop a better understanding of the linkages between
476 landscape-scale changes and instream condition, through assessing key indicators (e.g. volume
477 of wood, water temperature, discharge, measures of sedimentation) as part of a wider
478 approach to monitor and improve the effectiveness of riparian and catchment-scale
479 interventions. Such monitoring programs have been established in developed countries, and
480 incorporate multiple biotic and abiotic indicators, catchment and riparian conditions, and
481 relative risk assessments for linking instream conditions with multiple pressures. The results of

482 such assessments have been effective in providing the scientific evidence for mitigating or
483 preventing further reductions in instream biotic condition in a cost-effective manner (Hughes
484 & Peck 2008; Davies *et al.* 2010; USEPA 2016).

485 Developing these assessments in the Amazon would be challenging, particularly given
486 the current changes in environmental laws in Brazil (e.g. Ferreira *et al.* 2014; Fearnside 2016;
487 Azevedo-Santos *et al.* 2017). One option would be to use demonstration studies at ecoregion
488 (McCormick *et al.* 2001) or basin (Jiménez-Valencia *et al.* 2014) scales to develop these
489 schemes – effective protocols could then be rolled out to other regions.

490

491 THE CHALLENGE OF UNEXPLAINED VARIANCE AND REGION-SPECIFIC RELATIONSHIPS

492 Among river basins, fish assemblages often showed different responses to the partial effects of
493 the predictors (Fig. 4), further illustrating the heterogeneity of Amazonian streams. For
494 example, we found no substantial effects of riparian-scale pressures on Curuá-Una fish
495 assemblages (Fig. 4A), but these were as important as instream habitat variables in structuring
496 Capim fish assemblages (Fig. 4B). Although road crossings and the extent of mechanised
497 agriculture were unrelated to the composition of fish functional guilds (Fig. 6) and had limited
498 effects on fish assemblages (Fig. 5), both are known to affect instream habitat and fish
499 functional structure of Amazonian streams in agricultural landscapes (Macedo *et al.* 2013; Leal
500 *et al.* 2016; Leitão *et al.* 2017) – and have impacts on stream condition that are both
501 cumulative and potentially multiplicative. Without clear empirical evidence, it is even harder to
502 translate these findings into guidance for decision makers, and current legislation may miss
503 some of the key impacts by focusing on a limited number of management variables (e.g. the FC
504 focuses only on forest cover).

505 Despite including detailed trophic and habitat-use information that is considered to be
506 ecologically relevant to Amazonian stream fish assemblages, we found few clear associations
507 between fish and gradients of human pressures or specific impacts. Up to 22.5% of the

508 variation in insectivorous fish was explained by riparian and catchment pressures (Table S3),
509 yet partial effects from single predictor variables were mostly small (partial R^2 values < 5%).
510 However, the best explained guild, nektonic herbivores, increased with decreased forest cover
511 at all three spatial scales (Table S3, Fig. S1). Deforestation increases insolation and aquatic
512 vegetation, which favours herbivores. However, the lack of expected guild associations with
513 forest cover, road crossings, mechanised agriculture, and the index of proximity of human
514 impact highlights the complex nature of linking multiple human disturbances to aspects of
515 aquatic condition. This seems to be a nearly ubiquitous problem because researchers
516 developing multimetric indices of fish assemblage condition in Europe, the USA, and Brazil
517 have had to reject the majority of candidate metrics because of low range, insensitivity to
518 disturbance, or poor reproducibility (Pont *et al.* 2006; Esselman *et al.* 2013; de Carvalho *et al.*
519 2017b).

520

521 IMPLICATIONS FOR UNDERSTANDING FISH DISTRIBUTIONS IN TROPICAL STREAMS

522 Results from this study provide the basis for four recommendations for future applied research
523 on fish-environment relationships. First, the importance of regional context suggests we need
524 more multi-scale studies in other river basins to understand the factors that underpin this
525 context specificity. This would allow us to scale up these results to the rest of the Amazon and
526 to other tropical systems, and would assist with regional conservation planning. Future work
527 should also address the specific design parameters of existing environmental legislation and
528 current management and conservation strategies from other Amazonian countries to identify
529 and help address potential inadequacies.

530 Second, we recommend that studies account for the full range of potential human
531 disturbances. Both of our study regions have relatively high levels of catchment forest cover
532 (60-69%) and a recent history of intensified agricultural land use (i.e. mechanised agriculture
533 was established in the early 2000s), so that we did not sample the most heavily disturbed

534 catchments affected by mining, oil and gas drilling, or urbanization. Allan (2004) noted that
535 temperate streams may show little change in biota until reaching 30-50% of agriculture
536 extension in the catchment, although Fitzpatrick *et al.* (2001) reported thresholds at 10-20%
537 agriculture in the riparian zone. We did not account for degradation of the riparian forest (e.g.
538 fire or cattle), which can affect functioning in agricultural landscapes (Ferraz *et al.* 2014).
539 Similarly, the recent spread of mechanised agriculture in Amazonia means it is important to
540 investigate the effects of pesticides and fertilizers that result in high levels of contamination in
541 surface and groundwater supplies, soil, and biota (Schiesari & Grillitsch 2011).

542 Third, we encourage more monitoring to investigate how time lags and shifting
543 baselines in undisturbed forests influence stream condition responses to human disturbances.
544 Our study was a temporal snapshot, which has two shortcomings. First, we have no
545 information on pre-disturbance conditions, which is important because there is evidence that
546 space-for-time approaches may lack the statistical power to detect changes identified by
547 before-and-after studies (Larsen *et al.* 2004; França *et al.* 2016). Second, lag effects mean the
548 full effects of disturbance may only become evident over longer times (Harding *et al.* 1998;
549 Hylander & Ehrlén 2013).

550 Last, further studies are needed to relate fish ecophysiology (e.g. tolerance to pollutants
551 and hypoxia), life history traits (e.g. reproduction strategy, dispersal ability), and finer-tuned
552 information on energetic sources (e.g. isotopic analysis revealing the real interdependence
553 between terrestrial and aquatic food webs) to predict their tolerance to human impacts (Leitão
554 *et al.* 2017; de Carvalho *et al.* 2017a). Such information is scarce for the majority of Amazonian
555 stream fish species, and would be of great value for improving our understanding of fish
556 responses to human disturbances and the FC effectiveness.

557 **Authors' contributions**

558 CGL wrote the first draft of the manuscript; JB, TG, JF coordinated the project;

559 CGL, JB, TG, RMH, RPL, PRK, SFBF, JZ, JF, PSP designed the experiment; CGL, RMH, RPL, SFBF,

560 JZ, FRP, EPD, CPR collected the data; CGL, RMN, JRT, GDL analysed the data; CGL, JB, TG, PSP

561 led the manuscript writing with substantial contributions from all authors. All authors gave

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582 **Data accessibility**

583 All relevant data used in this manuscript is publicly available at Dryad Digital Repository :

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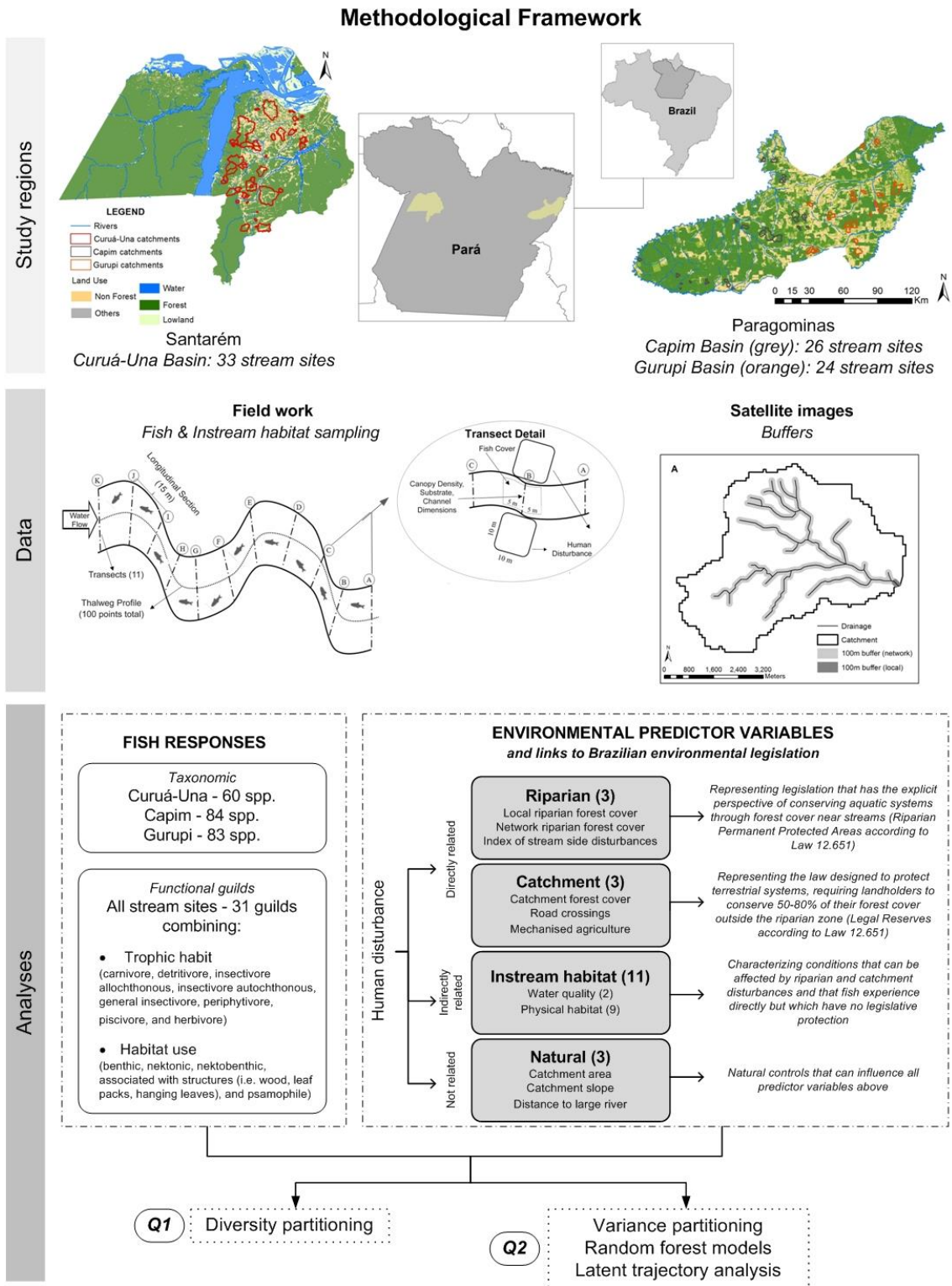
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820

821 Table 1. Environmental variables used to predict fish assemblage composition in Amazonian
 822 stream sites.

Environmental predictor variables		Definition
Group	Code	
Riparian	NET_FOR	% riparian network forest
	LOC_FOR	% local riparian forest
	W1_HALL	Proximity weighted tally of riparian/stream side disturbances (Kaufmann <i>et al.</i> 1999)
Catchment	CAT_FOR	% catchment forest
	CAT_MAG	% mechanised agriculture
	DEN_RCS	Number of road crossings within a 5 km circular buffer upstream and downstream the stream site divided by catchment area
<i>Water quality</i>		
	TEMP	Water temperature – °C
	COND	Electrical conductivity – µS/cm
<i>Substrate</i>		
	FINE	Streambed surficial fines < 0.6 mm diameter – % areal cover
<i>Cover and wood</i>		
Instream habitat	AMCV	In-channel algae and macrophytes – % areal cover
	NTCV	In-channel natural cover (wood, live trees and roots, leaf packs, overhanging vegetation, undercut banks, boulders) – % areal cover
	WOOD	Wood volume – m ³ /m ² wetted channel area
<i>Channel morphology</i>		
	DPTH	Standard deviation of thalweg depth – cm
	BKWD	Ratio: Bankfull width to bankfull thalweg depth – dimensionless
	RP100	Mean residual depth at thalweg – (m ² /m)/cm
<i>Other</i>		
	LRBS	Log ₁₀ of relative bed stability estimated at bankfull flow conditions (Kaufmann <i>et al.</i> 2008; Kaufmann, Larsen & Faustini 2009)
	SHAD	Canopy density (shading) measured at mid-channel – %
Natural	CAT_ARE	Catchment area – ha
	CAT_SLO	Catchment slope
	DST_RIV	Distance to large river (≥ 4th Strahler order) - m

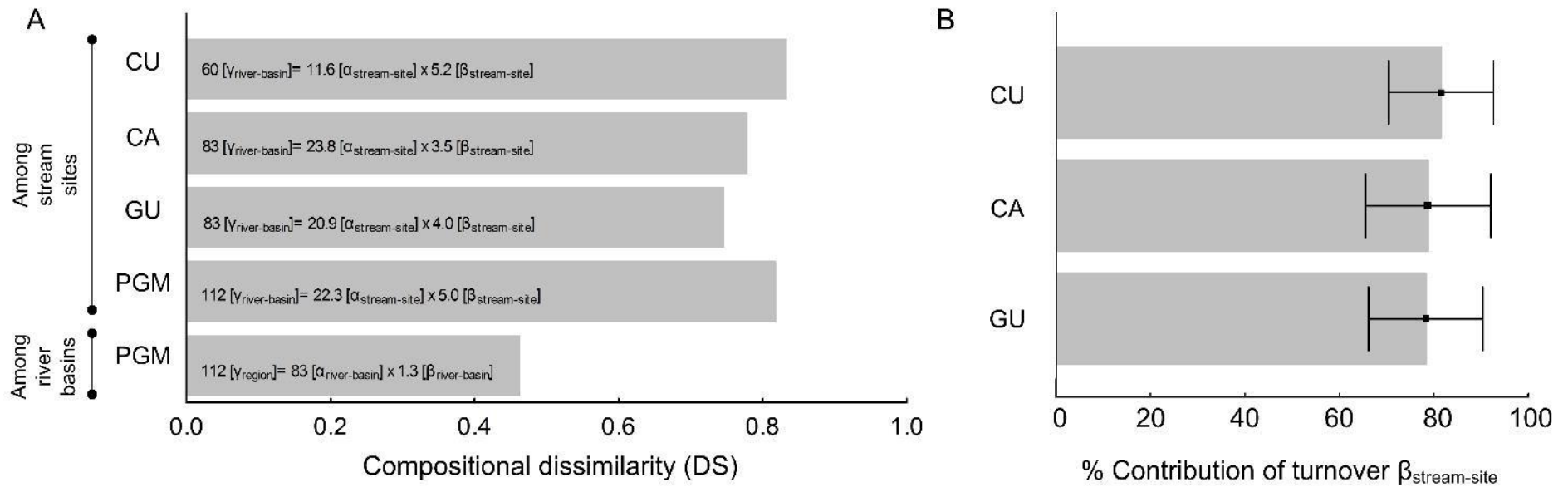
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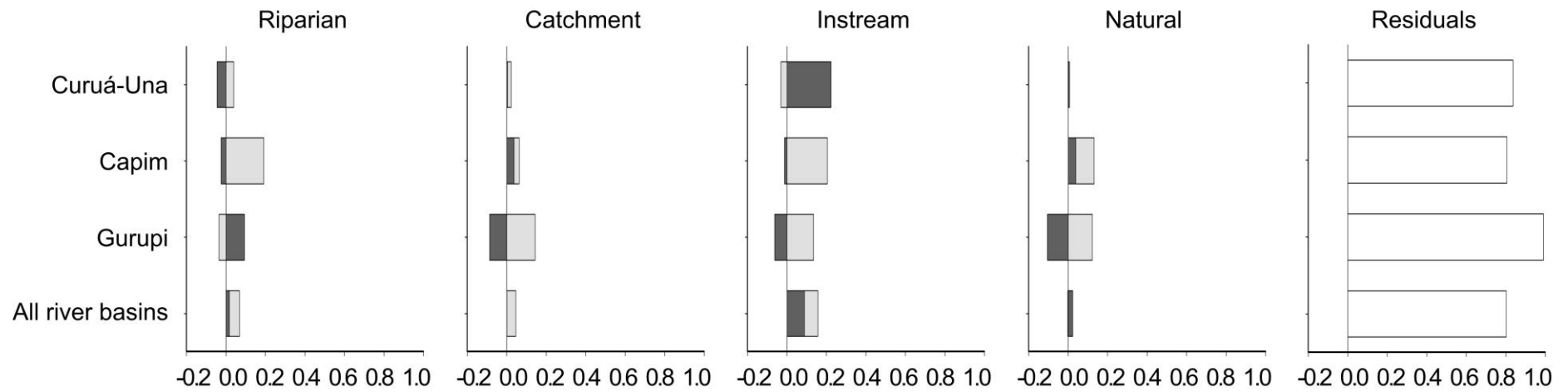
825 Figure 1. Methodological framework to investigate fish species responses to human

826 disturbances in Amazonian landscapes.



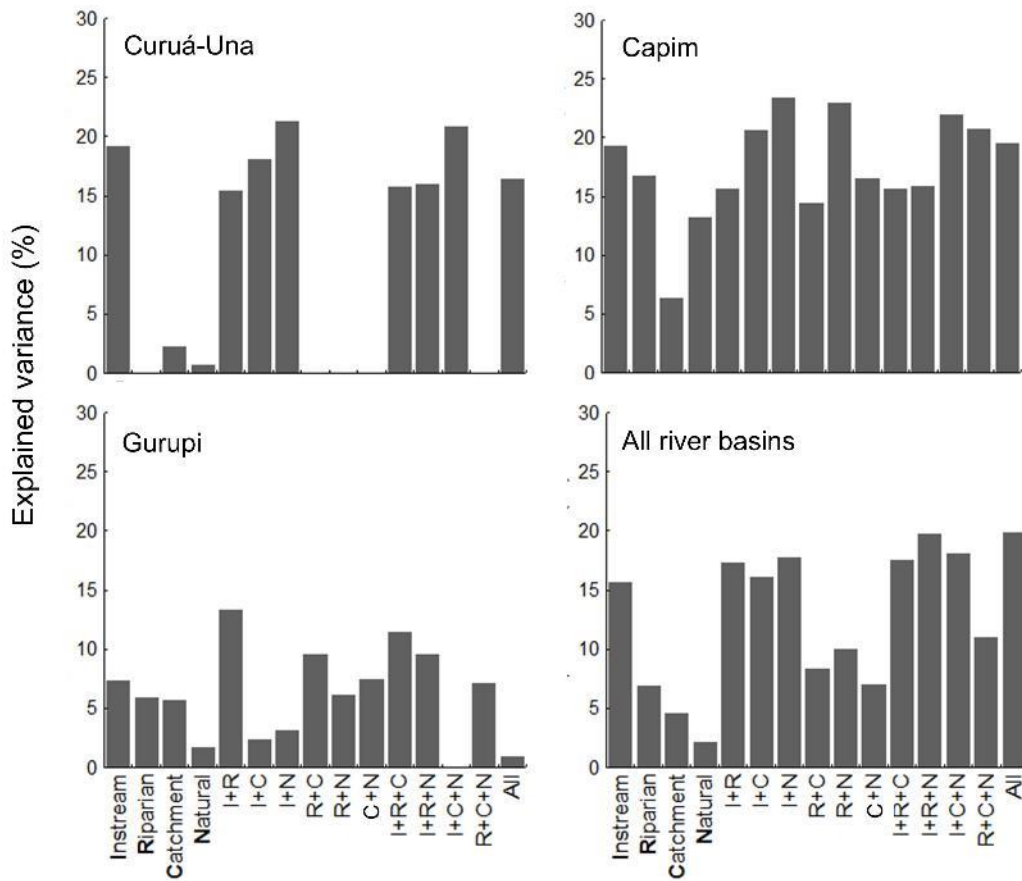
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828 Figure 2. Multiplicative diversity partitioning for Amazonian stream sites and river basins: Curuá-Una (CU), Capim (CA) and Gurupi (GU). (A) Relative
 829 compositional dissimilarity among stream sites and river basins; DS varies from 0 (identical assemblages) to 1 (completely different assemblages). (B)
 830 Percentage contribution of turnover to $\beta_{\text{stream-site}}$ with standard deviation bars.



831

832 Figure 3. Partitioning of the variation in occupancy of stream fish assemblages in Curuá-Una, Capim and Gurupi River Basins (species abundance), and
 833 river basins together (functional guilds abundance) showing the variance explained by each group of predictor variables (dark grey) when partitioning out
 834 the effects of the other groups through redundancy analysis (partitions [a], [b], [c] and [d] according to variance partition analysis) and the fractions shared
 835 between the groups (light grey). Unexplained variance is represented in white. Negative values of R_a^2 indicate that the predictor variables explain less
 836 variation than random normal variables, and should be interpreted as zeros (Legendre 2008).

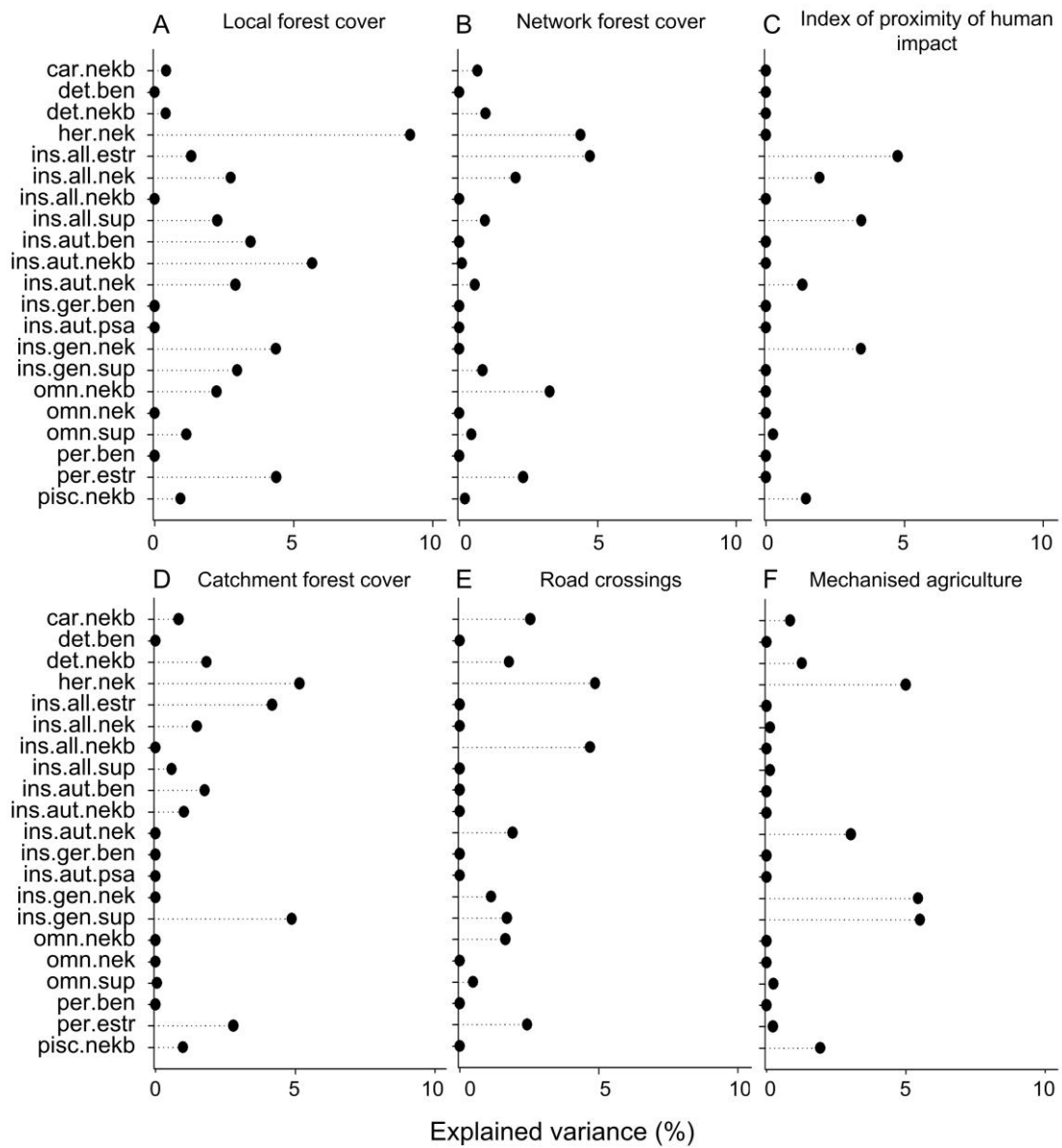


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838 Figure 4. Individual and joint effects of instream habitat (I), riparian (R), catchment (C), and

839 natural (N) predictor variable groups on taxonomic (Curuá-Una, Capim, and Gurupi River

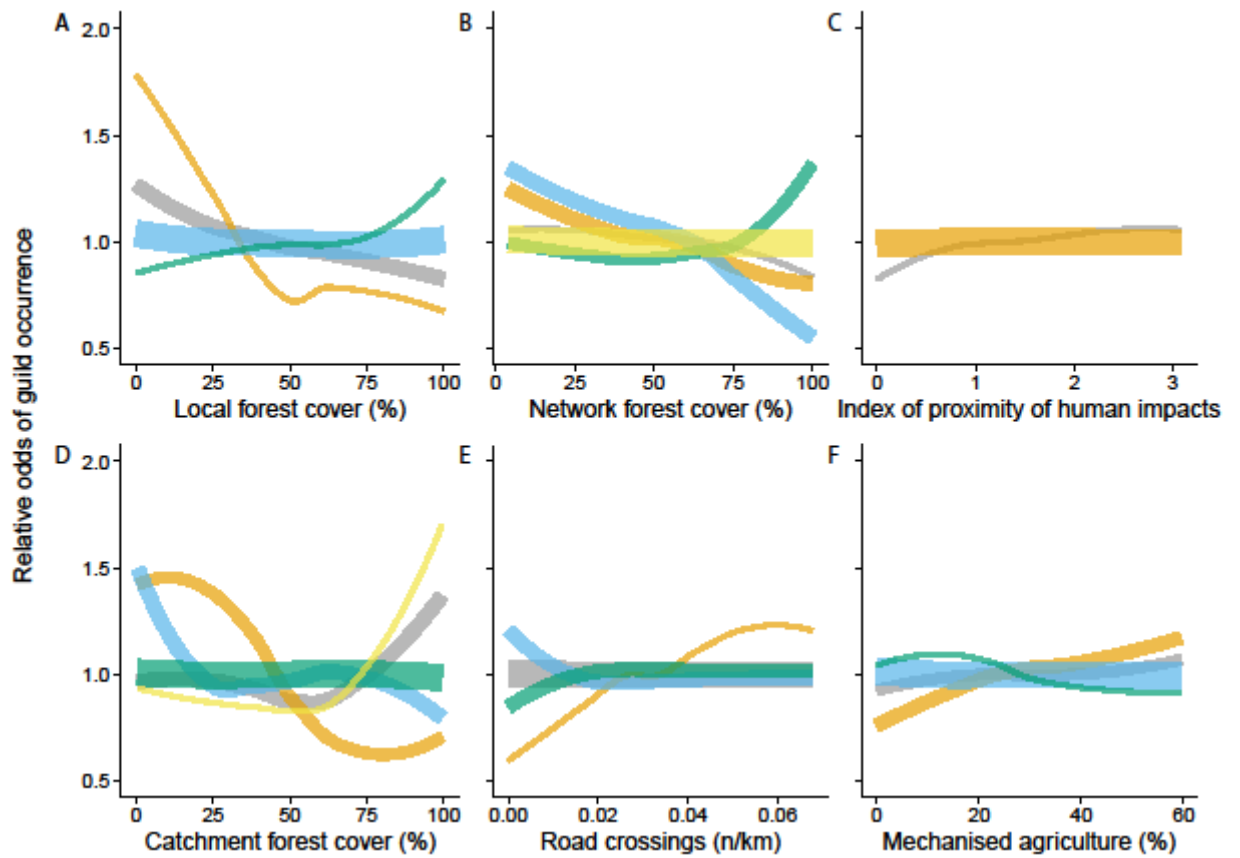
840 Basins) and functional guild (all river basins together) composition.



841

842 Figure 5. Partial effects from random forest models showing the percentage of functional guild

843 variation explained by the environmental predictors.



844

845 Figure 6: The relative odds of detecting fish functional guilds along gradients of governable
 846 management predictor variables. Different coloured lines show classes of guilds with similar
 847 responses to human disturbance (see Table S4 for constituent species). Line thickness
 848 represents the relative number of guilds in each LTA-defined class.

849 **Supporting Information**

850 Additional Supporting Information may be found in the online version of this article:

851

852 **Appendix S1.** Characterization of site instream habitats.

853 **Appendix S2.** Supplementary methods.

854 **Appendix S3.** Rank of relative species abundance and occurrence in stream sites.

855 **Appendix S4.** Species-based accumulation curves and estimation of species richness.

856 **Figure S1.** Partial responses of herbivore nektonic guild to riparian and catchment pressures.

857 **Table S1.** Links between the environmental predictor variables and Brazilian legal instruments.

858 **Table S2.** List of fish species.

859 **Table S3.** Performance of random forest models for functional guilds.

860 **Table S4.** Guild class membership for each governable predictor variable in latent trajectory

861 analysis.