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Disentangling the pathways of land use impacts on the functional structure of fish assemblages in Amazon streams

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Abstract

Agricultural land use is a primary driver of environmental impacts on streams. However, the causal processes that shape these impacts operate through multiple pathways and at several spatial scales. This complexity undermines the development of more effective management approaches, and illustrates the need for more in-depth studies to assess the mechanisms that determine changes in stream biodiversity. Here we present results of the most comprehensive multi-scale assessment of the biological condition of streams in the Amazon to date, examining functional responses of fish assemblages to land use. We sampled fish assemblages from two large human-modified regions, and characterized stream conditions by physical habitat attributes and key landscape-change variables, including density of road crossings (i.e. riverscape fragmentation), deforestation, and agricultural intensification. Fish species were functionally characterized using ecomorphological traits describing feeding, locomotion, and habitat preferences, and these traits were used to derive indices that quantitatively describe the functional structure of the assemblages. Using structural equation modeling, we disentangled multiple drivers operating at different spatial scales, identifying causal pathways that significantly affect stream condition and the structure of the fish assemblages. Deforestation at catchment and riparian network scales altered the channel morphology and the stream bottom structure, changing the functional identity of assemblages. Local deforestation reduced the functional evenness of assemblages (i.e. increased dominance of

Supplementary material (Appendix ECOG-02845 at <www.ecography.org/appendix/ecog-02845>). Appendix 1–5.

specific trait combinations) mediated by expansion of aquatic vegetation cover. Riverscape fragmentation reduced functional richness, evenness and divergence, suggesting a trend toward functional homogenization and a reduced range of ecological niches within assemblages following the loss of regional connectivity. These results underscore the often-unrecognized importance of different land use changes, each of which can have marked effects on stream biodiversity. We draw on the relationships observed herein to suggest priorities for the improved management of stream systems in the multiple-use landscapes that predominate in human-modified tropical forests.

Introduction

Tropical ecosystems are facing high levels of human-induced disturbances, with conversion and degradation of habitats being a primary cause of biodiversity loss (Limburg et al. 2011, Newbold et al. 2015). Global demand for agricultural commodities, mainly through pasture and cropland expansion, removes hundreds of thousands of hectares of tropical forest on a yearly basis (Hansen et al. 2013). In the case of the Brazilian Amazon more than 20% of the original forest cover has already been cleared (INPE 2013). Amazonian riverine ecosystems, which host a large part of the Earth's freshwater biodiversity, are of particular concern given typically low levels of compliance with environmental legislation protecting riparian zones (Nunes et al. 2014), and recent modifications of the Brazilian Forest Code that relaxed restoration requirements in these areas (Soares-Filho et al. 2014).

Changes in land use across catchment and riparian zones are important considerations in ongoing efforts to protect headwater streams and conserve their biodiversity (Allan 2004). Deforestation may lead to several alterations in stream channel structure and shifts in energy sources (Allan et al. 1997, Paula et al. 2011, Leal et al. 2016). Besides forest clearing, road crossings and dams adversely affect streams (i.e. riverscape fragmentation), acting either on the habitat conditions or directly on the organisms' dispersal possibilities (Perkin and Gido 2012, Johnson et al. 2013). Although those changes are known to significantly alter stream biological communities in temperate regions, their effects on tropical freshwaters have received little attention. This represents a critical gap in terms of biodiversity conservation of Amazon streams. Given the remarkable environmental stability of these systems under natural conditions (Espírito-Santo et al. 2009), it is possible that Amazonian biota have lower levels of tolerance and resilience to human-induced disturbances than temperate streams (Peres et al. 2010), where organisms have evolved in highly unstable and harsher environmental conditions (Walser and Bart 1999).

The consequences of land use on the structure of stream fish assemblages have been traditionally investigated from a taxonomic perspective with often contrasting results. For instance, fish species richness was reported to increase (Lorion and Kennedy 2009) or be unaffected by deforestation (Bojsen and Barriga 2002). This purely taxonomic approach is thus limited in helping identify general conclusions that can inform effective management strategies. By contrast, the functional structure (FS) of biological communities, often assessed through the identity and diversity of species functional traits, has the potential to reveal more consistent and monotonic relationships with the level of disturbance and to provide early warning signals of impacts ahead of actual species loss (Flynn et al. 2009,

Villéger et al. 2010, Mouillot et al. 2013). Furthermore, ecological processes that underpin ecosystem functioning are closely related to the diversity of functional traits beyond the mere number of taxa (Hooper et al. 2005, Mora et al. 2014). Therefore, studying changes in FS may facilitate disentangling the effects of disturbances on species assemblages as well as forecasting potential changes in key ecological processes (Mouillot et al. 2011, Naeem et al. 2012, Leitão et al. 2016). Despite these promising perspectives, assessments of land use effects on the FS of stream fish assemblages are still highly overlooked, especially in tropical species-rich regions of the world (but see Teresa and Casatti 2017).

Based on a uniquely comprehensive multi-scale assessment, we investigated how riverscape fragmentation and deforestation, mediated by instream habitat changes, affected the functional structure of stream fish assemblages in human-modified regions of the Amazon. The multifaceted and complex nature of these relationships undermines the development of more effective management approaches for stream systems. It also illustrates the need for more in-depth studies to assess the relative importance of alternative mechanisms in determining changes in aquatic biodiversity. To address this complexity we employed an analytic framework that enables joint consideration of predictors at different spatial scales to identify plausible causal pathways of land use on fish assemblage structure.

Methods

Study area

This study is part of the Sustainable Amazon Network (Rede Amazônia Sustentável), a multidisciplinary research initiative focusing on the assessment of the social and ecological dimensions of land use sustainability in the eastern Brazilian Amazon (Gardner et al. 2013). We sampled 94 headwater stream sites (150-m long reaches in 1st to 3rd order streams) from two regions: Santarém (STM; 7 July to 13 August, 2010), located near the confluence of the Amazonas and Tapajós Rivers; and Paragominas (PGM; 20 June to 8 August, 2011), in the lower Amazon Basin. Samples were distributed along a gradient of previously known anthropogenic impacts based primarily on the amount of remnant forest cover in the catchment of each site (Gardner et al. 2013; Supplementary material Appendix 1 Fig. A1). The landscapes in the two regions constitute mosaics of well-established mechanized agriculture, extensive and intensive cattle pastures, silviculture, and small landowner colonies, as well as regenerating secondary forests and undisturbed primary forests, the latter mostly found within officially protected areas.

Although both study regions are part of the same overall hydrographic basin (the Amazon Basin) and show similarities in some environmental attributes (Supplementary material Appendix 2 Table A1), STM and PGM are more than 1400 km apart and exhibit important differences in patterns of both current and past land use. Once a center of pre-Columbian civilization, STM was founded in 1661 and has been densely settled by small-scale farmers for more than a century. By contrast, PGM had a very low population density prior to its colonization by cattle ranchers in the 1960s, and a boom in the timber industry during the 1980s and 1990s (Gardner et al. 2013). Paragominas has also experienced a rapid recent expansion of silviculture (mostly *Eucalyptus* spp. and *Schizolobium amazonicum*). Historical differences in land use changes, and hence the environmental legacies related to

them, are recognized as critical factors that affect relationships among landscape, instream habitat and stream biodiversity, confounding interpretation of effects or hiding patterns and processes resulting from those differences (Allan et al. 1997, Uriarte et al. 2011, Leal et al. 2016). As such we focused most our analytical procedures separately for each region, treating them as independent case studies. More than reducing historical and spatial bias, doing so also provides a valuable and unusual opportunity to better understand the extent to which our inferences regarding biodiversity responses to land use can be generalized across multiple regions.

Landscape assessment

We analyzed landscape features at three different spatial scales (Supplementary material Appendix 1 Fig. A2): the whole catchment upstream from the site (‘catchment’); a 100-m wide buffer along the entire drainage network upstream from the site (‘riparian network’); and a 100-m wide buffer around the sampled site only (‘local’). Catchment boundaries and area were obtained from digital elevation models for STM (Shuttle Radar Topography Mission images with 90 m resolution; NASA) and for PGM (TopoData with 30 m resolution; INPE, Brazil). The drainage network was extracted using the hydrological model ArcSWAT (Soil and Water Assessment Tool extension for ArcGis). The percentage of deforestation at each of the three spatial scales was obtained using land use maps (Landsat TM and ETM+ images, 30 m resolution, year 2010), allowing them to be fully comparable. We defined deforestation as the sum of cleared areas in 2010, deforested primary forest areas in the past, old-regeneration-deforestation (i.e. deforestation of secondary forest areas in baseline year – 1990 STM, 1988 PGM) and young regeneration areas (i.e. deforestation < 10 yr). Natural non-forested areas are negligible in both regions. The percentage of mechanized agriculture at the catchment scale was calculated considering annual Moderate Resolution Imaging Spectroradiometer (MODIS) data from 2001 to 2010. We did not consider urbanization as a land cover change because 1) we aimed to investigate the effects of agricultural land use on biodiversity, thus, directing our sampling to rural zones, and 2) urban settlements are restricted to a small portion of each studied region (Gardner et al. 2013, Leal et al. 2016).

Riverscape fragmentation was estimated by two measures: density of upstream and downstream road crossings in the drainage network, both calculated within a 5 km buffer from the sampling site and scaled by the catchment area. The road crossings were mapped by aerial interpretation using georeferenced color Rapideye images (2010 for STM and 2011 for PGM, 5 m resolution), identifying transversal linear structures along the drainage network (Jensen 2000). A subset of half of these identified crossings were validated using Google Earth images. The vast majority of roads across both regions are unpaved and river crossings are generally ad hoc structures made by landowners that have little if any technical support from engineers. Such conditions, combined with the high number of crossings (e.g. > 4 000 estimated for PGM), prevented a thorough mapping of the crossing types (e.g. bridge, culvert, fording). Besides small ponds caused by some of the road crossings, other types of infrastructure (e.g. dams and weirs) causing fragmentation were negligible in our studied systems. Distances between each sample site and the main river downstream (4th

order reaches) were calculated using Landsat images. All landscape analyses were carried out using ArcGis 9.3 (Environmental Systems Research Inst., Redlands, CA, USA).

Instream physical habitat structure

We adapted the field methods of Peck et al. (2006) to characterize instream physical habitat structure. Each 150-m long site was subdivided into 10 contiguous sections by 11 cross-sectional transects (Supplementary material Appendix 1 Fig. A3). Before measurements, the site extremities were blocked with nets (5 mm mesh) to prevent fish from escaping. Section characterization included the quantification of large wood volume in the channel and 15 longitudinal equidistant measurements of thalweg depth. At each of the 11 transects we estimated the proportion of different substrate types and channel depth along five equidistant points, and measured bankfull width and depth. Cover for fish was assessed at each transect along 10-m long plots inside the stream channel using semi-quantitative estimates of the areal cover of leaf packs, standing cover (i.e. roots, overhanging vegetation, undercut banks, and boulders), submerged grassy vegetation, filamentous algae and aquatic macrophytes (mainly submerged rooted groups). Forest canopy cover above the channel was measured with a convex densiometer at the center of each transect (facing upstream, downstream, left and right margins) and the mean values were used as a proxy for channel shading. We measured temperature with a digital thermometer placed below the water surface in the center of the site. From these field measurements we calculated 10 final physical habitat metrics (based on Kaufmann et al. 1999, 2009, Hughes and Peck 2008, Kaufmann and Faustini 2012): water-column depth, bankfull width/depth ratio, $\log_{10}$ relative bed stability, bottom complexity (i.e. relative residual thalweg depth), wood volume, coarse litter cover, standing cover, aquatic vegetation (i.e. macrophyte + grass + algae) cover, channel shading, and water temperature. Those environmental metrics were chosen because they represent complementary attributes of the local instream conditions that are expected to be affected by land use changes and to affect the structure of fish assemblages.

Fish sampling

Following the physical habitat assessment, three people sampled fish in the entire area of the site for 120 min in an upstream direction. During this procedure, each 15-m section was isolated with block nets, allowing for effective sampling of fish abundances within well-delimited boundaries. Fishes were collected during daylight hours using different equipment to encompass different microhabitats and groups; i.e. hand nets to capture species associated with litter banks, roots and aquatic vegetation; and seines to capture species associated with the sandy bottom and open waters (Supplementary material Appendix 1 Fig. A3). Specimens were euthanized in Eugenol, fixed in 10% formalin, and returned to the lab for identification and preservation in ethanol. Voucher specimens are deposited in the fish collections of the Inst. Nacional de Pesquisas da Amazônia (INPA) and the Museu Paraense Emílio Goeldi (MPEG), Brazil.

Functional structure of fish assemblages

To evaluate the functional structure of fish assemblages we first conducted an ecomorphological analysis. Using a set of 18 morphological traits, we characterized each species with respect to three key functions: food acquisition, locomotion, and habitat

preference (Supplementary material Appendix 3). We then computed the functional distance between each pair of species in each regional pool (STM and PGM). Some functional traits were not coded as continuous variables, so we used the Gower distance, which allows considering different types of traits while standardizing them (Villéger et al. 2008). We then ran a principal coordinate analysis (PC) on the distance matrix to build a multidimensional functional space for each region and estimate the different functional facets of assemblage structure. The number of dimensions (i.e. PC axes) was chosen based on the quality of the functional space, i.e. the extent to which it accurately represented the initial functional distances between species pairs, quantified by the mean squared-deviation index (mSD; Maire et al. 2015). We kept the first four PC axes, as this was the minimum number of axes that provided a high-quality functional space (i.e. mSD < 0.01) for each regional species pool, while minimizing the number of assemblages we had to exclude (i.e. those with fewer species than PC axes) to attain computation requirements (Villéger et al. 2008). This choice led us to remove only five sites, all from STM.

Based on the position of fish species in the multidimensional functional spaces and their relative abundance in the sampled assemblages we computed five complementary indices to describe the functional structure of fish assemblages: functional richness (FRic), functional evenness (FEve), functional divergence (FDiv), functional originality (FOri), and community-weighted mean of a trait (CWM). FRic is the convex-hull volume of the functional space filled by all species within the local assemblage, indicating the range of trait combinations (Villéger et al. 2008). We standardized FRic values for each assemblage by expressing them as a proportion of the volume filled by its regional pool of species. FEve measures the regularity of distribution of abundance in the functional space, and is constrained between 0 and 1, increasing when species and their abundances are more evenly distributed in the functional space (Villéger et al. 2008). FDiv quantifies how the species abundances diverge from the center of the volume filled by the assemblage in the functional space, and ranges between 0 and 1, approaching unity when dominant species are very distant from the assemblage center (Villéger et al. 2008). FOri reflects the degree of uniqueness (i.e. the opposite of redundancy) of species traits in the assemblage, and is expressed as the mean distance between each species and its nearest neighbor in the functional space (Mouillot et al. 2013). The raw values of FOri were standardized between 0 and 1 by dividing them by the maximum nearest-neighbor distance observed over all species present in each region. CWM indicates the functional identity of an assemblage (Lavorel et al. 2008), being expressed as the abundance-weighted average value for each PC axis. We computed the functional indices by using the cluster, ape, and geometry packages in R (R Development Core Team).

Structural equation modeling (SEM)

To evaluate potential causal pathways of land use on the functional structure of fish assemblages we performed structural equation modeling (SEM). SEM is a statistical framework that deals simultaneously with multiple processes to explain the functioning of a whole system (Shipley 2000). It is based on theoretically justified models that are parameterized by finding a solution minimizing the difference between the model predictions and observed data (Grace 2008). Our structural hypothesis was based on

personal knowledge and on previous studies, with the final set of variables including: four land cover and two fragmentation predictors; two natural landscape predictors; 10 instream habitat variables; and two taxonomic and five functional structure indicators for the fish assemblages (Fig. 1).

We hypothesized that land use acts mostly indirectly on the structure of fish assemblages, mediated by changes in instream habitat conditions (Fig. 1). Stream bankfull channels are expected to widen (increase in width/depth ratio), whereas bottom complexity, bed stability, and water-column depth are expected to decrease with increasing deforestation at all spatial scales and upstream fragmentation. We hypothesized these pathways because those landscape disturbances tend to increase flood frequency and magnitude, reduce tree roots along the stream channel, and increase the runoff of fine sediments into the channel by erosion of exposed soil (Allan et al. 1997, Allan 2004, Allan and Castillo 2007, Hughes and Peck 2008). This latter process (i.e. sedimentation) is most active in low current velocities, a condition that characterizes our streams (Leal et al. 2016). Decreased amounts of roots and increased flood frequency destabilize bed and stream banks, which leads to decreased bed stability and widening of the stream, thereby the stream cross-section becomes shallower (Kaufmann et al. 2009, Kaufmann and Faustini 2012). We also expected that deforestation at all spatial scales and upstream fragmentation should decrease the amount of wood and coarse litter delivery to the stream channel (Paula et al. 2011). Deforestation at the local scale is expected to decrease standing cover and shading over the channel which, in turn, should increase water temperature and aquatic vegetation cover (Bojsen and Barriga 2002, Casatti et al. 2012). Water temperatures should also increase with increasing deforestation at catchment and riparian network because of increased soil warming (Leal et al. 2016). Aquatic vegetation cover should also increase with increasing levels of mechanized agriculture in the catchment that increase nutrient inputs to the streams.

Downstream road crossings were used as an indication of riverscape fragmentation directly influencing local assemblages (Fig. 1) by potentially impairing dispersal of organisms from downstream of the sample site. We did not consider a direct effect of upstream fragmentation on fish dispersal because headwaters are not expected to act as fish species sources at the microbasin scale (Matthews 1998). Catchment area and distance to larger rivers were used as natural landscape predictors of the structure of fish assemblages (Fig. 1), representing, respectively, the natural size and the isolation of each site (i.e. considering the potential importance of fish colonization from larger rivers; Hitt and Angermeier 2008).

Given the expected correlation of some functional indices with the taxonomic structure of assemblages (Villéger et al. 2008), we included species richness (affecting FRic) and the evenness of abundance distribution among species (Pielou index J; affecting FEve) in the model. This ultimately would provide a causal framework linking environmental gradients with the functional structure of assemblages directly and indirectly, via taxonomic structure (Fig. 1).

Linearity among variables was assessed by inspection of dispersion plots, and transformations ($\ln(x + 1)$ or arc-sine($\sqrt{x}$)) were used when necessary. We tested individual-variable and multivariate normality using, respectively, Shapiro–Wilk's and Mardia's test.

Even after transforming several variables, normality was not attained for some of them. Therefore, we used 'Bollen–Stine' bootstrap (1000 draws) to evaluate the overall fit of the models. This is a modification of the chi-square statistic that is considered robust to non-normal data distributions (Bollen and Stine 1992), and measures the correspondence between the model and the observed data structure. Standardized path coefficients that were not statistically significant were retained in the model (i.e. we did not re-specify the structural model a posteriori). SEM procedures were carried out using the lavaan package in R (R Development Core Team).

Multi-regional analyses

Despite strong arguments for analyzing biodiversity responses separately in STM and PGM, a thorough understanding of the environmental consequences of land use change dynamics can be assisted by conducting analyses at multiple scales (Brondizio and Moran 2012). As a complementary approach, we followed the same analytical procedures presented above for STM and PGM samples combined, helping to assess the potential for emergent patterns of functional response in stream fish assemblages to overall land use changes in the eastern Amazon. For a detailed description of methods and results see Supplementary material Appendix 4.

Data deposition

Data available from the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.j7d32> (Leitão et al. 2017).

Results

Landscape and habitat characteristics

Our sampling captured a broad gradient of land use, particularly for the proportion of deforestation at the different spatial scales, which ranged from 0 to ca 100% in both regions (Supplementary material Appendix 2 Table A1). We also captured a high variability in habitat characteristics among streams, although the ranges for each metric substantially differ between regions. For example, the bankfull width/depth ratio ranged from 0.8 (deep and narrow) to ca 86.0 (very shallow and wide) in STM and 2.7 to 38.6 in PGM sites. Coarse litter covered from 0 to 95.2% of the stream bottom in STM and 0 to 64.8% in PGM sites, and the proportion of aquatic vegetation cover ranged from 0 to 52.3% in STM and 0 to 76.4% in PGM sites.

Ichthyofauna

We caught a total of 25 132 fish specimens (STM = 6634; PGM = 18 498) and a total of 141 species (STM = 67; PGM = 112), representing 27 families (STM = 22; PGM = 26), and seven orders (Supplementary material Appendix 5). The species composition was very different between STM and PGM, with only 27% of them occurring in both regions (Supplementary material Appendix 1 Fig. A4). The stream sites supported an average of 11 (6 to 20) and 23 (6 to 44) species in STM and PGM regions, respectively. The mean taxonomic evenness across sites was relatively high in both regions (J = 0.66), ranging from 0.20 to 0.86 in STM and 0.29 to 0.93 in PGM.

Land use effects on fish functional structure

Santarém sites—Land use and stream fragmentation affected multiple habitat conditions. Increased riverscape fragmentation by upstream road crossings reduced water-column depth, bed stability and bottom complexity of the STM sites (Fig. 2). Increased mechanized agriculture increased the bankfull width/depth ratio. Increased local deforestation reduced bottom complexity, wood volume, coarse litter cover, and channel shading, thereby increasing water temperature and aquatic vegetation cover (Fig. 2). Unlike PGM (see below), deforestation at the catchment and riparian network scales had no significant effect on any habitat metric assessed in STM.

The interaction between landscape and site characteristics resulted in significant indirect land use effects on the functional structure of fish assemblages (Fig. 2, Supplementary material Appendix 2 Table A2). Increased local deforestation negatively affected functional richness (FRic), via species richness. This effect was mediated by reduced bottom complexity and coarse litter cover in deforested streams. The total effect of increased local deforestation on functional evenness (FEve) was also negative, because FEve decreased both with increased aquatic vegetation cover and reduced bottom complexity (Fig. 2a). Also mediated by reduced bottom complexity, FEve was slightly reduced by increased upstream fragmentation. Downstream fragmentation influenced several assemblage structure indicators, negatively affecting FRic (directly and via species richness), FEve, and functional divergence (FDiv). Via species richness, FRic increased with catchment area and decreased with distance to larger rivers (Fig. 2a). Taxonomic evenness did not predict FEve, and functional originality (FOri) was not affected by land use in STM. Although affected by land use, bankfull width/depth ratio and temperature did not affect any of the fish assemblage metrics that we evaluated.

Land use had significant effects on the functional identity of fish assemblages (Fig. 2b, Supplementary material Appendix 2 Table A2). Mediated by reduced bottom complexity and bed stability, local deforestation and upstream fragmentation negatively affected traits related to the occupation of the stream bottom or highly structured microhabitats (i.e. high CWM1). On the other hand, traits related to the occupation of mid/upper layers of the water column (i.e. low CWM1) were negatively associated with upstream fragmentation via reductions in channel depth (Fig. 2b, Supplementary material Appendix 1 Fig. A5). Mediated by reduced wood volume, local deforestation had a negative effect on species with good maneuverability in structured microhabitats (i.e. high CWM2). Increased downstream fragmentation decreased CWM2 and CWM4, but increased CWM3, indicating a negative effect on species with lower propulsion and acceleration efficiency as well as on large and elongated-body carnivorous fishes (Fig. 2b).

Paragominas sites—PGM sites showed both similar and different responses to land use as STM sites. Increased catchment deforestation strongly increased water temperature, and decreased bed stability and wood volume in PGM (Fig. 3). On the other hand, increased riparian network deforestation increased wood volume. Increased local deforestation increased the bankfull width/depth ratio, and decreased wood volume and channel shading, thereby increasing water temperature and aquatic vegetation cover (Fig. 3). Increased

upstream fragmentation increased the bankfull width/depth ratio. Unlike STM, mechanized agriculture had no significant effect on any PGM habitat metric assessed. Although they influenced assemblage structure, water-column depth, bottom complexity, coarse litter, and standing cover were not significantly affected by land use in PGM (Fig. 3).

Despite being related, increased riparian network deforestation reduced FRic, but catchment and local deforestation increased FRic (Fig. 3a, Supplementary material Appendix 2 Table A3). The negative relation between wood volume and species richness mediated those effects. Mediated by increased aquatic vegetation cover, increased local deforestation reduced FEve. FOri was only slightly affected by local deforestation, because the negative impact mediated by aquatic vegetation cover was offset by the positive impact mediated by reduced wood volume. Given its negative relation with increased wood volume, FOri was positively affected by catchment deforestation but negatively affected by riparian network deforestation (Fig. 3a). FDiv increased with distance to large rivers and decreased with catchment area.

Land use also had significant effects on the functional identity of fish assemblages (Fig. 3b, Supplementary material Appendix 2 Table A3). Riparian network deforestation negatively affected traits related to the occupation of hard substrates or the stream bottom (i.e. high CWM1), mediated by the negative relation with wood volume (Fig. 3b, Supplementary material Appendix 1 Fig. A5). CWM2, negatively weighted by species with well-developed fins, was positively affected by downstream fragmentation and negatively affected by upstream fragmentation and local riparian deforestation, both mediated by the negative relationship with bankfull width/depth ratio (Fig. 3b). CWM3, positively weighted by species with viliform, comb- or spoon-shaped teeth, was positively affected by riparian network deforestation and negatively affected by catchment deforestation (Fig. 3b). The positive relation of wood volume with CWM3 mediated these effects. Local deforestation had a small total effect on CWM3, with aquatic vegetation cover offsetting the influence of wood volume. CWM4, negatively weighted by large elongated-body species, was only significantly affected by catchment area (Fig. 3b).

Discussion

The multifaceted nature of land use effects on habitat and fish assemblage structure in streams is widely recognized (Wang et al. 2001, Allan 2004, Leal et al. 2016), but the complexity of these relationships has often impeded efforts to draw conclusive outcomes. This challenge is even more acute for small tropical streams, where basic knowledge of their natural dynamics and species ecology is often lacking (Carvalho et al. 2009). By analyzing complementary spatial scales and contrasted intensities of land use, we disentangled key pathways through which deforestation and riverscape fragmentation affected the structure and functional properties of fish assemblages in these ecosystems. We identified distinct, sometimes contrasting responses to land use between different components of assemblage structure, illustrating the need to consider multiple biotic indicators when assessing biodiversity in changing landscapes (Villéger et al. 2010, Gardner et al. 2013). Moreover, our results suggest that the combined effects of different forms of disturbance (e.g. local deforestation + river network fragmentation) can exacerbate long-term impacts on stream

ichthyofauna. Such impacts may be further worrisome if considering the yet poorly understood interactions of land use and the climate changes that are projected for Amazonian freshwater systems (Castello and Macedo 2016).

Land use, instream habitat structure and the functional structure of fish assemblages

Although many potential causal pathways identified by our modelling work differed between the studied regions, land use consistently altered the channel morphology and the physical structure of stream bottoms. The bankfull width/depth ratio increased with upstream fragmentation and local deforestation in PGM, whereas this habitat change was driven by increased mechanized agriculture in STM. Relative bed stability decreased with catchment deforestation in PGM, whereas that metric, water-column depth, and bottom complexity decreased with upstream fragmentation in STM. Regardless of the predominant pathway, the process behind those structural alterations of streams is likely the same: the destabilization of the banks, and the erosion of exposed soil with subsequent runoff of high amounts of fine sediments into the channel. This process was also identified by the complementary model combining STM and PGM samples.

Sedimentation alters fish assemblage structure, and is one of the main threats facing some functional groups (e.g. habitat specialists) in both temperate and tropical streams (Walser and Bart 1999, Casatti et al. 2006, Bryce et al. 2010). We had not expected such severe consequences for lowland Amazon streams with bottoms naturally dominated by sand and fines. However, our findings indicate that the enhanced load of fine sediment into Amazonian streams leads to significant changes in the functional structure of their fish assemblages, evidencing strong trait-filtering mechanisms across land use gradients. For instance, species with traits associated with the use of the benthic compartment and structurally complex microhabitats were most affected by reductions in bottom complexity and bed stability. On the other hand, species having morphological traits related to the occupation of mid and upper layers of the water column were negatively affected by reductions of water-column depth (see CWM1 in Fig. 2b).

We also found some congruent patterns in biotic responses to stream disturbance across both study regions. For instance, local deforestation increased aquatic vegetation cover via decreased channel shading, thereby reducing functional evenness. Therefore, local deforestation increased the dominance of a few trait combinations (see Fig. 4 for examples of contrasting patterns of occupation of the functional space), indicating that the most abundant species in aquatic-vegetation dominated streams are functionally similar. Although this habitat change decreased FEve, it had no effect on taxonomic or functional richness. This suggests that fish assemblages in streams subjected to some level of deforestation may not show lower species richness but their functional trait combinations become more unevenly distributed. Another pathway reducing FEve was through changes in bottom complexity and wood volume, exacerbating the total negative effect of local deforestation on this functional component.

Decreasing functional evenness, especially when functional richness remains constant, may have critical negative consequences for ecosystem functioning if some key traits are greatly underrepresented or aggregate assemblage properties are important to ecological processes.

For example, niches unoccupied by native taxa favor invasion by non-natives (Hillebrand et al. 2008), which are often more tolerant and tend to be more successful in colonizing streams after habitat alterations (Baltz and Moyle 1993, Hughes et al. 2005, Lomnicky et al. 2007). Compared with many rivers worldwide, successful invasions of non-native fish within the Amazon Basin are less common (Leprieur et al. 2008). However, the consistent decrease in FEve of fish assemblages along the deforestation gradients assessed in this study could increase their susceptibility to invasive species. This constitutes an advanced warning that cascading effects on native assemblages across Amazonian streams may result if non-native introductions occur along with anthropogenic disturbance.

Beyond effects on FEve, the increases in aquatic vegetation cover resulting from local deforestation in streams decreased the functional originality of assemblages, which is a measure of the level of species uniqueness (Mouillot et al. 2013; Fig. 4). The decrease of both these indices indicates that deforestation-induced expansion in aquatic vegetation cover increases the proportion of functionally redundant species, corroborating previous findings that environmental degradation led to replacement of species having unique traits by functionally redundant ones (Villéger et al. 2010, Casatti et al. 2015). This can be further linked with recent findings showing that the most unique and distinct combinations of traits are disproportionately supported by rare species (Leitão et al. 2016), which often have greater sensitivity to human-induced disturbances.

Opposing effects of land use occurred for the functional identity of the assemblages (e.g. see CWM3 in Fig. 3). On the one hand, local deforestation negatively affected wood-eating species (those with spoon-shaped teeth), mediated by reductions in wood volume. On the other hand, this landscape alteration positively affected periphyton-grazing fishes (those with comb-shaped teeth), mediated by increases in aquatic vegetation cover. Notably, both trophic groups are represented by species of the same family (Loricariidae), which has been reported as being favored by deforestation (Bojsen and Barriga 2002). These contrasting effects on the same family and by the same landscape predictor illustrate common limitations faced by most investigations of land use on taxonomic aspects of fish assemblages. In this context, we suggest that further studies should search for the finest possible trait-based information, incorporating it in a functional perspective capable of differentiating properties within taxonomic groups (i.e. not all loricariids are periphyton-grazers). This is particularly critical for species-rich tropical ecosystems, where high levels of niche diversification are likely (Winemiller 1991). These findings also indicate the need to explore the mechanistic and simultaneous causal pathways through which disturbances affect stream ecosystems (Riseng et al. 2011), going further than just examining direct landscape–assemblage relationships.

A non-expected result was the positive influence of deforestation at catchment and local scales on assemblage functional richness, particularly for PGM, mediated by a negative relationship between wood volume and species richness. Whilst this result appears counter-intuitive it is possible that the relatively undisturbed nature of both study regions, both of which retain approximately two-thirds of their original forest cover, can lead to elevated number of species in streams with intermediate levels of disturbance (i.e. the intermediate disturbance hypothesis, sensu Connell 1978).

Loss of connectivity affects the functional structure of assemblages

Land use change not only alters local instream habitat structure, but also impedes movement of organisms throughout river networks (Urban et al. 2006, Perkin and Gido 2012). One of the most striking findings of our study was the strong influence of downstream fragmentation on several components of fish assemblage structure, particularly in STM. The density of road crossings downstream from sample sites was negatively correlated with FEve and FDiv, suggesting a trend toward functional homogenization of local assemblages. Furthermore, this disturbance had a direct negative impact on FRic, which, in addition to the indirect effect via taxonomic richness, indicates that losing regional connectivity potentially reduces the range of ecological strategies in local assemblages. These combined responses of complementary functional facets to riverscape fragmentation might have critical consequences for Amazonian streams, such as disrupting refined interactions among species, eradicating specialized forms of resource use, and undermining the integrity of important ecological processes.

These results are likely linked to reduced dispersal of species from larger rivers or of fish groups unable to maintain local populations in small streams, as indicated by the predominant morphological traits across sites. For instance, large elongated-body carnivorous species were strongly negatively correlated with downstream fragmentation. Because of the oligotrophic conditions of Amazonian streams, these top predators are probably wanderers that alternate the search for food resources across different streams and microbasins, resulting in greater dependence on spatial connectivity. Given that predation is an important mechanism for the structure and function of stream ecosystems (Jackson et al. 2001), the loss of these functional entities may result in severe impacts to local communities due to changes in top-down dynamics within their food webs. Other fish groups vulnerable to local extinctions in small streams following riverscape fragmentation are those characterized by body morphologies indicative of weaker swimming ability (Fig. 3), which potentially have poor dispersal capability (Olden et al. 2008).

The strongest effects of fragmentation on functional structure were found in STM streams, even though the mean density of road crossings was greater in PGM (Supplementary material Appendix 2 Table A1). We believe this reflects the differing permeability of the road crossings in the two regions. Appropriately constructed bridges often do not necessarily represent effective obstacles for stream fishes, whereas passages with undersized and elevated culvert outlets, frequently observed in STM, prevent species from dispersing upstream (Nislow et al. 2011, Evans et al. 2015). Further research using in situ assessments of road crossing characteristics and permeability to fish dispersal would likely yield deeper insights to support management efforts to reduce stream fragmentation.

Study limitations

This study offers important insights concerning functional responses of stream fish assemblages to landscape alterations in the Amazon. Nevertheless, we also recognize that it is a starting point, and its limitations suggest fruitful opportunities for future investigations. First, although we have used a relatively high number of traits to characterize the species, they were restricted to functions related to food acquisition, locomotion and habitat

preferences. Including traits describing fish ecophysiology and life history are clearly desirable to better interpret potential critical disturbance processes (e.g. increases in water temperature or the loss of reproductive sites resulting from deforestation and siltation). An additional step including traits directly related to fish roles (e.g. nutrient recycling, Vanni et al. 2002) would facilitate modeling the impact of disturbance on ecosystem functioning. Second, as pointed out by Riseng et al. (2011), structural equation modeling is a simplification of a much more complex reality (including unmeasured variables), meaning that it can only support or contradict causal hypotheses, but cannot prove causation (as is only possible in controlled experiments). However, considering the logistical impossibility of making direct large-scale experimentation in species-rich regions, we considered SEM a powerful analytical tool for addressing the relationships between land use and biodiversity changes.

Conservation implications of inter-regional variability in biodiversity responses

Whilst some biotic responses to land use changes were similar between regions, the general pattern of structural models in STM and PGM was notably distinct. An important implication of this result is that even from a functional perspective, which is often assumed to provide great potential for extracting general insights (Mouillot et al. 2013), inferences on land use and biodiversity relationships derived from one region cannot necessarily be applied to another. This finding tightly corroborates a recent study assessing functional responses of fish assemblages to environmental degradation across three Neotropical regions (Teresa and Casatti 2017). The authors concluded that, although having higher predictive performance than taxonomic indices, most functional indicators were context-dependent; and caution is needed when generalizing them across distinct regions. Idiosyncrasies were also found in parallel assessments in STM and PGM for other taxonomic groups (e.g. birds; Moura et al. 2016), indicating that endogenous regional characteristics such as topography and geology, as well as the differing deforestation histories and disturbance time lags may play significant roles. Environmental legacies related to historical differences in land use changes have been widely evoked to explain current differences among regions, including for stream ecosystems (Allan et al. 1997, Uriarte et al. 2011, Leal et al. 2016).

Although limiting our ability to draw more generalized conclusions, those results reinforce the importance of the regional-scale approach for assessing and guiding the development of conservation strategies (Riseng et al. 2011). Such approach has been widely used for mapping ecoregions (e.g. based on differences in land use, potential natural vegetation and soils) which were found useful for classifying patterns of fish assemblages and indicating priorities for management of surface waters (Hughes et al. 1987, Van Sickle and Hughes 2000, Pinto et al. 2009). In this context,Gardner et al. (2013) emphasized that a ‘meso-scale’ level (i.e. spanning hundreds of kilometers and coincident with the scale of individual municipalities in Brazil, such as Santarém and Paragominas) is a particularly relevant spatial scale. This meso-scale captures important variability in environmental and land use gradients that drives widespread ecological changes that cannot be discerned by finer-scale studies at a small number of intensively sampled sites. At the same time, a meso-scale approach does not obscure important inter-regional processes with starkly different land use histories – differences that are lost with macro-scale analyses that encompass, for example,

the entire Amazon Basin (which drains a land area of ca 7 million km^2 and encompasses enormous variability in natural aspects as well as in the prevalent human activities). A particularly interesting finding illustrating the importance of regional differences in ecological responses to disturbance was the absence of any influence of downstream fragmentation on the ichthyofauna when analyzing STM and PGM together (Supplementary material Appendix 4). Yet this disturbance was identified as one of the most important drivers of biodiversity changes in STM when assessed separately. Masking this result would also mask the urgent need for management interventions to address stream fragmentation, an issue that has hitherto received comparatively little attention by decision makers.

Conclusions

The rapid and intense pace of agricultural development in the tropics is resulting in highly degraded landscapes in many regions, and the ecological integrity of even relatively well-preserved biomes such as the Amazon is severely threatened. This study illustrates how land use, through several disturbance processes and across multiple spatial scales, has markedly altered the functional composition of fish assemblages in the most speciose freshwater system on Earth. Management strategies are urgently needed for the effective conservation of stream biota in these human-modified landscapes. One important consideration in efforts to improve the long-term effectiveness of stream conservation strategies that emerges from our research is the need to consider the dendritic structure of river networks and their linkages at multiple landscape scales, whilst also recognizing that aquatic systems commonly demand additional and tailored management strategies as distinct from terrestrial systems (Castello and Macedo 2016). Moreover, extending such an approach to other tropical regions where deforestation and land use change is ongoing, but where taxonomic and functional structures of assemblages are quite different (e.g. southeastern Asia and Africa, Toussaint et al. 2016), would certainly add important insights for the conservation of freshwater biodiversity at a global scale. Overall, our findings clearly corroborate the current view in ecology and conservation biology that biodiversity should be assessed in a multifaceted framework that explicitly takes into account the functional elements of biotic assemblages and underscores the need to consider the conservation of aquatic systems in their own right.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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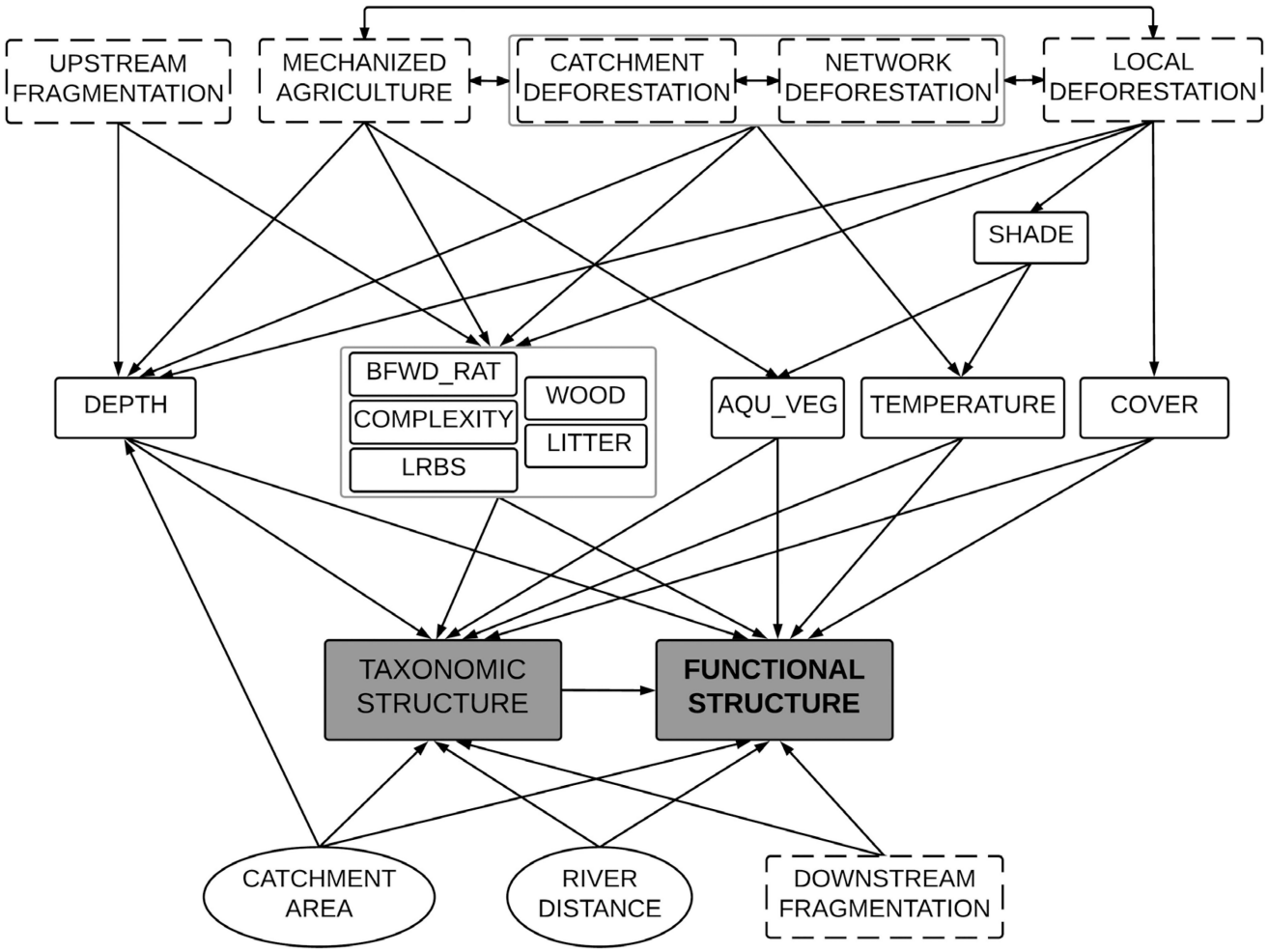

Figure 1.

Hypothesized model tested using structural equation modeling, indicating the expected pathways (single-headed arrows) for the effects of land cover and riverscape fragmentation (dashed-line rectangles) on the structure of stream fish assemblages in the Amazon. Most effects are expected to be indirect, mediated by changes in the habitat conditions within streams (solid-line rectangles), such as: water-column depth (DEPTH); bankfull width/depth ratio (BFWD_RAT); bottom complexity (COMPLEXITY); relative bed stability (LRBS); wood volume (WOOD); coarse litter cover (LITTER); channel shading (SHADE); aquatic vegetation cover (AQU_VEG); water temperature (TEMPERATURE); and standing cover (COVER). Natural landscape factors were also considered (ovals). Taxonomic structure comprises species richness and evenness. Functional structure comprises functional richness, evenness, divergence, originality, and identity. Double-headed arrows indicate expected correlations. For the sake of graphical simplicity, variables acting similarly on the model are grouped (surrounded by gray-line rectangles).

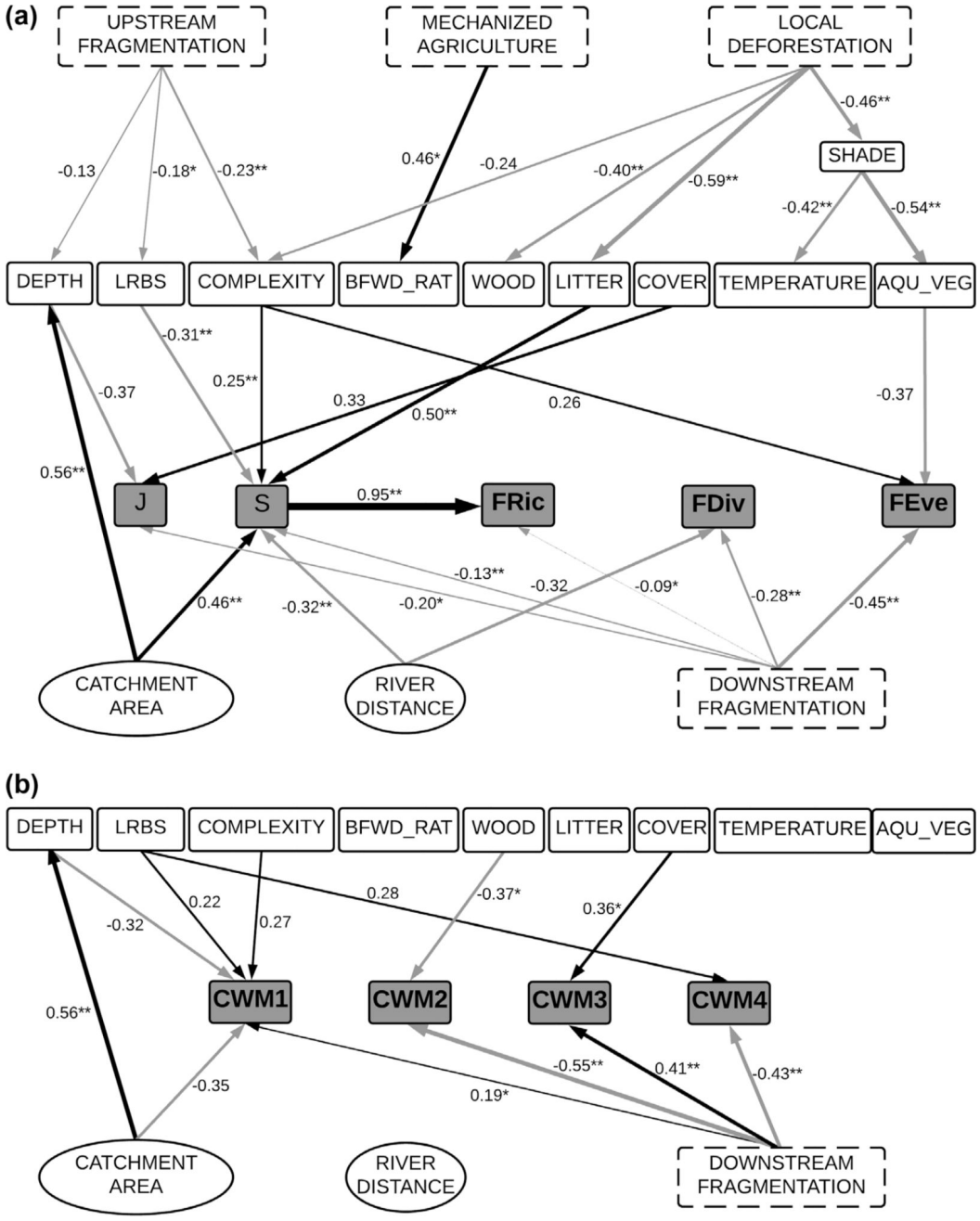

Figure 2.

Structural equation model diagrams showing the effects of land cover and riverscape fragmentation (dashed-line rectangles), instream habitat characteristics (solid-line rectangles; see code meaning in Fig. 1), and natural landscape factors (ovals) on the structure of stream fish assemblages (n = 40) in the Santarém region, Amazon. For the sake of graphical simplicity, biodiversity metrics are divided in two diagrams: (a) species richness (S) and evenness (J), functional richness (FRic), functional divergence (FDiv), and functional evenness (FEve); (b) functional identity (CWM1-4). Unidirectional arrows indicate positive (black) and negative (gray) significant direct effects ($p < 0.10$; *$p < 0.05$;

**p < 0.01), with thickness proportional to their power (standardized path coefficients along arrows). Model fit: $\chi^2 = 473.1$, df = 180, p = 0.55. See overall model explanation (R^2) for each variable in Supplementary material Appendix 2 Table A4.

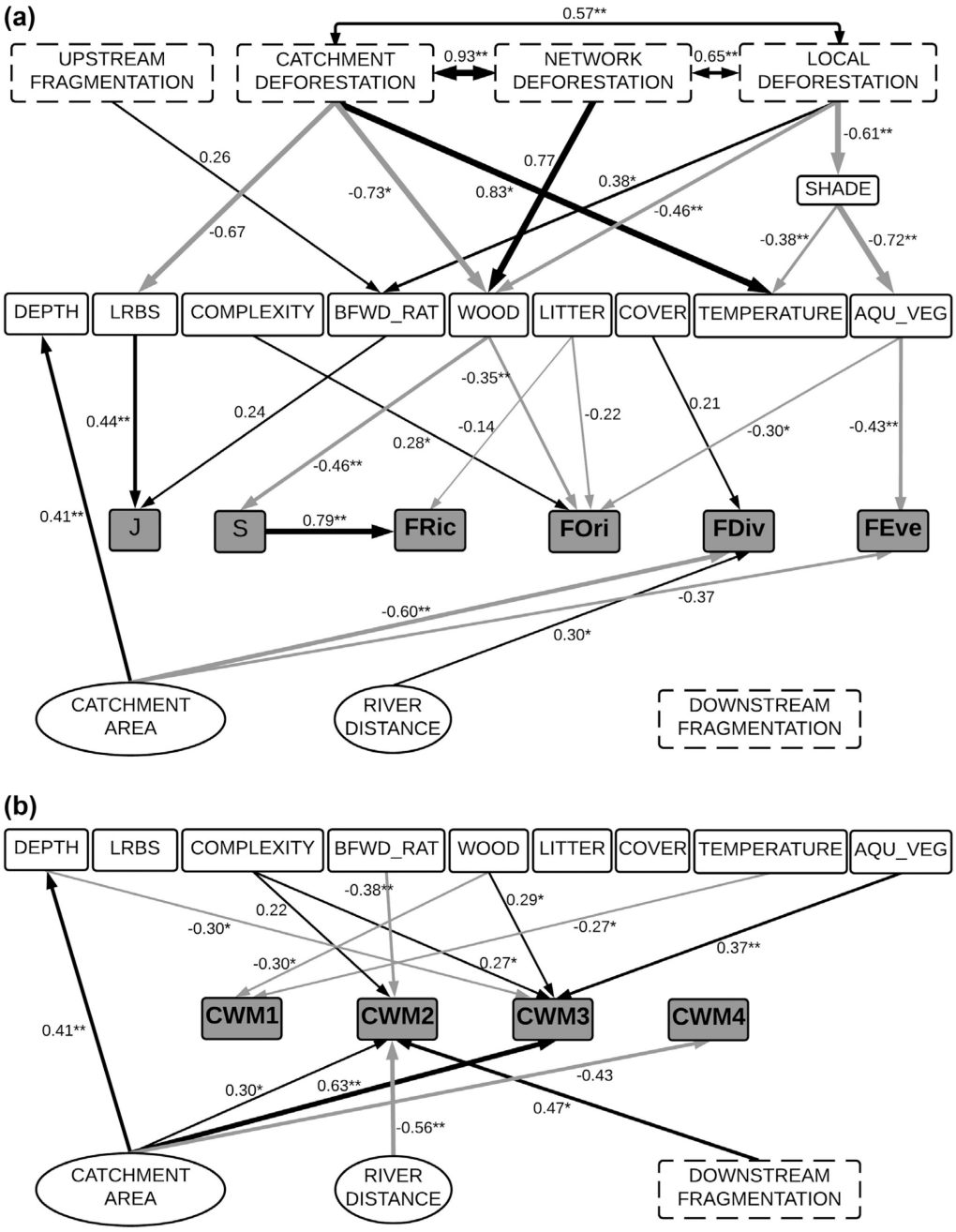

Figure 3.

Structural equation model diagrams showing the effects of land cover and riverscape fragmentation (dashed-line rectangles), instream habitat characteristics (solid-line rectangles; see code meaning in Fig. 1), and natural landscape factors (ovals) on the structure of stream fish assemblages (n = 49) in the Paragominas region, Amazon. For the sake of graphical simplicity, biodiversity metrics are divided in two diagrams: (a) species richness (S) and evenness (J), functional richness (FRic), functional divergence (FDiv), functional evenness (FEve), and functional originality (FOri); (b) functional identity (CWM1-4). Unidirectional arrows indicate positive (black) and negative (gray) significant

direct effects ($p < 0.10$; *$p < 0.05$; **$p < 0.01$), with thickness proportional to their power (standardized path coefficients along arrows). Bidirectional arrows indicate significant correlations. Model fit: $\chi^2 = 412.5$, df = 180, $p = 0.62$. See overall model explanation (R^2) for each variable in Supplementary material Appendix 2 Table A4.

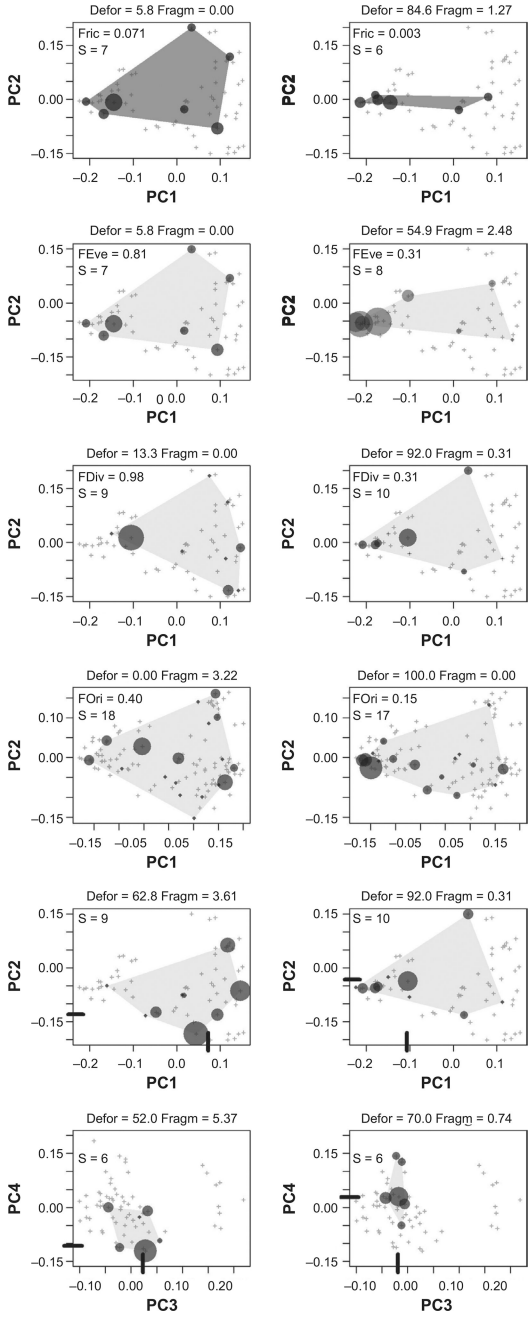

Figure 4.

Contrasting patterns of occupation of the functional space by different stream fish assemblages from the Amazon. Each plot represents two axes of a principal coordinate analysis (PC), where species are plotted according to their respective trait values. Gray crosses indicate all species from the regional pool (67 for Santarem or 112 for Paragominas), whereas dark-gray dots indicate the species present within a given local assemblage (delimited by the convex gray polygon); dot sizes are proportional to species abundances in that assemblage. The top four pairs of plots illustrate cases of high (left) and low (right) values for each index of functional diversity: functional richness (FRic), evenness (FEve),

divergence (FDiv), and originality (FOri). The two pairs of plots on the bottom of the figure illustrate assemblages with contrasting values of functional identity (CWM indicated by black bars along each PC axis). For each case considered, the percentage of local deforestation (Defor) and the level of downstream fragmentation (Fragm) are indicated above the plot, as those are the most important land use predictors affecting the functional structure of the assemblages. S: number of species in the local assemblage.