**Title:** Land use effects on the structure of trophic networks from Neotropical fish.

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To the editorial board at *Hydrobiologia*

Dear Editors,

We present here a revised version of the manuscript HYDR-D-22-00442.R1 entitled “Land use effects on the structure of trophic networks from Neotropical fish”. We very much appreciate the time invested by the referees and the editors to provide a thorough review in the past, and we are excited about the overall positive reception during this first review.

This manuscript had been submitted to Hydrobiologia for a new volume on “Stream Ecology and Environmental Gradients”, but we have not completed the review processo due to major constraints. We appologize, however, for the time spent proceeding this review; the first author had a series of personal and professional problems and was unable to proceed with the review in the time scheduled. In this new version, we have gone deep in the review process, the analitical revision, and the writing of manuscript, which considerably improve our manuscript. Yet, we carefully considered all the suggestions made by the two reviewers in the first round, and find that the manuscript is much more consistent than the previous version. We hope this manuscript will be still of interest of Editors despite the long time since the first review, and for this reason we are sending you a new version as a new manuscript submission.

We look forward to hearing back from you.

Yours sincerely,

Murilo S. Dias and co-authors

**Editor's comments, Dr. Teixeria de Mello**

**Editor’s comment#1.** *We have received the reports from our advisors on your manuscript, "Land use effects on the structure of trophic networks from Neotropical fish", which you submitted to Hydrobiologia. Based on the advice received, I feel that your manuscript could be reconsidered for publication should you be prepared to incorporate major revisions, I recommend that you carefully consider reviewer 2 comments. When preparing your revised manuscript, you are asked to carefully consider the reviewer comments which are attached, and submit a list of responses to the comments.*

***Authors’ reply****: We appreciate the dedicated time and effort of the editor and reviewers in carefully reviewing our manuscript. We thank the reviewers and the editor for their valuable and constructive feedback. In response to reviewer #1's comments, we have added more details explaining why we expected a nested network. Additionally, we have addressed all of reviewer #2's concerns regarding the metrics considered in our analysis and have modified our discussion by removing speculation that was mentioned by the reviewer.We hope this manuscript is now in better shape than the previous version.*

**Reviewer 1’s comments**

**Reviewer 1’s comment#1.** *This is an interesting study about the anthropogenic effects on the trophic networks structure of Neotropical stream fish. Specifically, authors propose to evaluate the role of land use changes (pastures or cropland) on network structure (e.g., modularity, nestedness, links density).*

*I think the manuscript is well written and the conceptual framework is clearly developed. I have some minor comments, mainly about the analysis of the data, that I expect could improve the manuscript.*

***Authors’ reply****:* *We very much appreciate such positive feedback along with a summary highlighting the strongest points of our work. Please see below how addressing each of your comments improved the quality of our manuscript. Please note that line numbers refer to the manuscript version with inline tracked changes.*

**Reviewer 1’s comment#2.** *Line 43. "This nestedness pattern...." Why this? Which is the nestedness pattern that authors refer? Homogenization and reduction in network complexity*?

***Authors’ reply****: We appreciate the opportunity to improve our hypothesis and explain why we expected a modular network. Please see L112-119. “We hypothesize that increasing land use reduces biodiversity and modularity in fish web networks due to the loss of specialist species and faunal homogenization, resulting in nested networks”.*

**Reviewer 1’s comment#3.** *Link density and the average number of links per species is the same, not?*

***Authors’ reply****:* *We really appreciate this insightful comment. We now added some sentences to explain that these metrics are different. Please see L168-173. “On the other hand, we estimated the link density which is defined as the number of trophic links (L) divided by the total number of nodes (consumers and resources, S) in a food web (L/S). This metric is related to the number of trophic interactions in a food web, providing information on the complexity of the food web and the number of pathways along which energy can flow (Dunne, Williams, & Martinez, 2002). Lastly, we estimate the average number of links per species, which informs how connected species are within the food web (Dunne, Williams, & Martinez, 2002; Bersier, Dixon, & Sugihara, 1994).”*

**Reviewer 1’s comment#4.** *About table the table S3 that has the classes of anthropic impact on land use, the rows should sum 100%?*

***Authors’ reply****: Yes, you are right. Thank you for the opportunity to streamline our supplementary tables (please, be aware that this is now Table S2). Actualy, the rows could sum up 100%, but the landuse classes are not all shown at this table. Please note that all classes related to NATIVE landcover are not present here. Therefore,the sum of rows will not necessaraly be 100%. We have now made it clear at the figure caption “Classes of anthropic impact on land use in a 500-meter buffer on each local network. P (pasture); APC (annual and perennial culture); SPC (semi perennial culture); MAP (Mosaic of cropland and pasture); UI (urban infrastructure); ANVA (another non-vegetated area); PPF (percent planted forest); M (mining). Note that native land-uses are not provided here.”*

**Reviewer 1’s comment#5.** *I am not sure if Figure 2 support effect of land use on network metrics. There are a lot of dispersion in the data. Maybe a logistic regression between the probability of been nestedness (1) or modular (0) along the land use gradient could show the results more clearly. Complementary, a box-plot analysis showing the range of values of network structure along the gradient of land uses could be interesting.*

***Authors’ reply****:* *We appreciate the reviewer’s opinion, but our nestedness and modularity data are not probabilities; rather, they are Standardized Effect Sizes (SES) which could assume continuous positive or negative values. These values were estimates considering the null model and 999 permutations, resulting the values that varied among -2 to 2. Thus, we do not consider that the use of a logistic regression will improve the observed tends. However, we added a significant regression line where the effect is significant.*

**Reviewer 1’s comment#6.** *In addition, weighted matrices could be used to network structure estimations and then to the analysis.*

***Authors’ reply****:* *We appreciate the constructive suggestions aimed at improving the presentation of our work. However, the studies compiled during our systematic review diverge in their methods for quantifying consumed food items and sampling efforts. Such differences can introduce biases into the estimation of network metrics. Therefore, we minimized the potential effects of varying sampling effort across studies by focusing on the incidence, not the strength, of the cleaning interactions. That is, we converted all quantitative interactions into qualitative ones to focus only on the topologies of the network. See L136-140: “As these published studies may diverge in their way to quantify consumed food items and sampling efforts, we preferred using information on the presence/absence of interactions (i.e., binary matrices) for focusing mostly on the incidence, rather than strength of the interaction, and avoid biases regarding items quantification and sampling efforts. ”*

*The only exception is the H2 metric, for which relative abundance data was used; it is mentioned at the manuscript: “As it is based on frequencies of interactions, this was the only metric calculated using relative abundance consumed items (i.e., the strength of interactions).”*

**Reviewer 2’s comments****Reviewer 2’s comment#1.** *This study analyses how changes in land use affect the food web structure of different freshwater fish trophic networks among several sampling points in Brazil. To this aim, the authors gathered dietary information from 49 articles. From these studies, the authors estimated several network metrics related with the complexity and stability of the food webs. Additionally, the authors estimated the anthropogenic impact on riparian vegetation in each sampled site as the percentage of non-natural land-cover and evaluated its effect on food web structure and complexity. In general terms, I find that this study focuses on a relevant issue regarding the impact of anthropogenic activities on the stability and structure of ecological networks, and therefore has the potential to make a relevant contribution to the field of food webs and conservation. However, in its current form, the manuscript does not fulfil the requirements needed for publication. I detail below three main issues that the authors may want to consider. They range from writing to methodology. However, my major concern is that the discussion and conclusions are highly speculative and are based on results that lack statistical support.*

***Authors’ reply****: We really appreciated this carefull reading and all suggestions.*

**Reviewer 2’s comment#2.** *Speculative discussion supported on results with lack of statistical support*

*The authors detected a negative and statistically significant relationship between network modularity and link density with land use gradient. These results are used to suggest that there is a reduction in the umber of specialist species and an increase in the number of generalist consumers. However, the authors tried to reinforce this hypothesis based on the relationships observed for nestedness and trophic specialization, which are not statistically significant and showed low effect size. It seems to me that the results are not robust enough to support the idea stated by the authors. In order to address this issue, the authors may consider: (i) analyzing if a nested pattern may also be detected among rows or columns since the metric NODF allows to perform these estimations; (ii) estimating the degree of turnover of specialist and generalist species using a beta diversity metric among sites. Finally, the reduction in modularity along the gradient of land use could also emerge because of changes in the structure of body size of both predators and prey. If, for example, the distribution of body size of prey changes from being aggregated to continuous, the diet of consumers is expected to change from modular to nested. I suggest the authors include to the discussion how land use could potentially affect the structure of food webs considering not only the diversity of prey but also their size.*

***Authors’ reply****:* *Thank you very much for the careful review highlighting the strongest points of our study and identifying the areas that needed improvement. It is important to note that all metrics have been double checked and entirely recalculated by MSD and JPQ; there was some inconsistencies related to the use of abundance vs incidence data between metrics (all but H2 are calculated with incidence; see above) and that justified our new computation. All metrics and scripts are now open in a GitHub repository for checking. We unfortunaly did not follow most of your suggestions regarding other metrics not used here:*

*(i) NODF metric over rows and colums: as we were more interested in metrics related to the full trophic matrices, we believe calculating NODF values over rows or colums would not add to our understanding. Yet, NODF is the only metric (to our knowlegde) capable of descriminating between rows and colums, and that would produce inconsistences among metrics; therefore, we opt to maintain only NODF for the full matrices juste like the others;*

*(ii) beta diversity metrics of specialists and generalists among sites: it would be interesting to perform such comparision, but again that would prevent us from focusing iniatialy in metrics related to the full matrices. Yet, we would need to classify fish species in specialists of generalists, but we do not have such information; we could estimate such information for each node with each of our gathered matrices, but that could produce the same species being classified diferently among matrices. Instead, se opt to continue with our full matrix framework and avoid here increasing complexity by performing exploratory analyses;*

*(iii) analyses with body size: we do agree that evaluating body size would be interesting, but none of our matrices had information on it for fish or preys; It is important to note that we are dealing with information gathered from available manuscripts or thesis; therefore, body size on preys and predators had not been available from our literature review; any use of such information here would be highly biased, and threfore it has been avoided.*

**Reviewer 2’s comment#3.** *Not accurate estimation of the anthropogenic impact on each sampling site and lack of discussion of some methodological decisions and their potential limitations If I understood well, the authors estimate the anthropogenic impact only considering the loss of natural land-cover across sites. I find this criterion not properly correct, since there is an implicit assumption that the categories of land use identified by the authors have the same impact on the communities. For example, assuming two sites with 10% of non-natural cover, the impact on the food web structure should be higher if it is an urban area compared to a crop land. I think that including the relative weight of each anthropogenic impact to the estimation of the land use gradient may improve the results of the study. On the other hand, the authors decided to estimate the land use gradient considering a buffer area of 500m even though they tested different buffer lengths. This decision needs to be better justified and authors should discuss the potential consequences of it.*

***Authors’ reply****: We understand the reviewer’s concern regarding the anthopogenic metric, but we advocated the MapBiomas iniciative (MapBiomas, 2020) is the most complete information regarding land-cover, land classificatation, and is highly consistent over the entire the entire Brazilian country. With this dataset, we can descriminate the percentage of cover in each landscape according to the defined classe os landuse. We agree with the reviewer that it would be useful to include weights to each vegetation type, but those values would be largelly didictated by our own impressions, and hence questionable. If we would set 0.25 weight to urban and 0.10 to crop pixels, these choices would hardly be justifiy by the literature and would give us questionable support. We opt to avoid such imprecise and questionable strategy and keep only percentage cover as our main metrics.*

*Moreover, the choice buffer radius is highly variable and is an open avenue of research. We now illustrate such variaty and better explain our motivation on the manuscript (see L201-206: “The form and extent of buffers to characterize local scale impacts is largely heterogeneous over studies, ranging from circular to riparian buffers of 50 - 500 m (Brejão et al. 2021; Pessoa et al. 2024). We opt for 500m circular buffers as it captures at the same time local and regional scale changes, and is large enough to deal with major land-use changes over distinct Brazilian biomes.”).*

**Reviewer 2’s comment#4.** *Awkward writing structure. Overall, I find that the structure of the introduction is messy, alternating between sections more related with network theory and the structure of ecological networks, and sections that describe the impacts of land use on ecological communities. I suggest the authors reorganize the introduction to make it more concise and straightforward to the point. Moreover, I detect several writing mistakes and style inconsistencies that could have been avoided with a more comprehensive review of the manuscript before its submission.*

***Authors’ reply****: We appreciate the constructive critique and endeavoured to reorganize this section. We hope the Introduction now reads better than the previous version.*

**Reviewer 2’s comment#5.** *L13-15: The hypothesis should be presented in a more comprehensive way, explicitly including the mechanisms by which land use intensity is expected to affect food web structure.*

***Authors’ reply****: We appreciate the opportunity to improve our hypothesis and explain why we expected a modular network. Please see L112-119. “We hypothesize that increasing land use reduces biodiversity and modularity in fish web networks due to the loss of specialist species and faunal homogenization, resulting in nested networks. Conversely, high land use values determine less specialized assemblages, more simplified (less number of links) and more nested trophic networks.”*

**Reviewer 2’s comment#6.** *L19-20: Define clearly what network complexity means (how was it estimated). Additionally, results are presented only for modularity and link density but those related with nestedness, trophic specialization and number of links should also be described.*

***Authors’ reply****: We follow the review suggestion and carefully reviewed the description of each metric. We also added the results found for all network metrics. Please see L32-42. “We quantified six network metrics based on the species richness and distribution of interactions (nestedness and modularity), trophic specialization, number of links per species (links density), and number of trophic links according to the number of nodes (number of links). We observed that the trophic networks are more nested than modular, thus supporting our hypothesis. However, we only observed a negative effect of land use on modularity, and the number of links, whereas other metrics such as nestedness, trophic specialization, number of species, and link density are not influenced by land-use.”.*

**Reviewer 2’s comment#7.** *L28-38 I find that devoting an entire paragraph to introduce the concept of network, its representation, etc. is unnecessary in the context of this study. I suggest reducing these explanations to the minimum and devote more attention to the central point of the study.*

***Authors’ reply****:* *We understand the reviewer’s concern, but we have decided to maintain this paragraph in the introduction section because it defines the metrics that were used. This can promote an easy comprehension of our study for the reader. However, we tried to reduce this paragraph as suggested.*

**Reviewer 2’s comment#8** *L40-44: The connection between nestedness and human effects on network structure in not clear. Please, clarify. L44-46: This point should be clarified. L51-52: Please, clarify.*

***Authors’ reply****: Thank you for the opportunity to streamline our arguments. We clarified these sentences. Please see L59-74. “Recent research has been focused on the human actions on the dynamic and structure of networks (Wang et al., 2021; Effert‐Fanta et al., 2023; Neves et al., 2024). For instance, habitat degradation promotes homogenization and reduction in network complexity, resulting in network more nested (i.e., species interactions are hierarchically organized, with a few species interacting with many others) than modular (i.e., species interactions are compartmentalized with some species interacting only within specific subsets) (Bascompte et al., 2003; Olesen et al., 2007; Pires & Guimarães, 2013; Dormann & Strauss, 2014). In trophic interactions, other network descriptors, such as trophic specialization (a metric that indicates the degree of species consumption on exclusive food items), number of links per species (links density), and number of trophic links according to the number of nodes (number of links given consumers and resources) also can be affected by human actions (Felipe-Lucia et al., 2020). For instance, human actions can reduce aquatic habitat heterogeneity and promote habitat fragmentation, disrupting the balance of species interactions within ecosystems (Staudacher et al., 2017). Thus, examining how the properties of networks are determined by anthropogenic stressors have the potential to provide insights into how the trophic networks are structured (Pellissier et al., 2017) and their consequences on ecosystem functioning. ”*

**Reviewer 2’s comment#9.** *L69: What does "more complex networks" mean? This point should be*

*clarified. L83: Change "reduce" to "reduces" L84: Change "increase" to "increases" and "change" to "changes"*

***Authors’ reply****: We removed these sentences to avoid future confusion.*

**Reviewer 2’s comment#10.** *L85-91: It seems to me that this is the main idea that leads the study, connecting the expected impacts of land use on the structure of food webs. Therefore, it should be presented before in the introduction in a more direct way.*

***Authors’ reply****: We followed the reviewer’s suggestion and changed the order of paragraphs to present these ideas earlier in the introduction.*

**Reviewer 2’s comment#11.** *L92: Anthropogenic effects of what? Please, clarify.*

***Authors’ reply****: We clarified this sentence to specify that we evaluated the effects of land use on the structure of trophic networks.*

**Reviewer 2’s comment#12.** *L93-95: I find these questions well presented.*

***Authors’ reply****:* *We appreciate the reviewer’s opinion and positive feedback.*

**Reviewer 2’s comment#13.** *L93: Change "are" to "is"*

***Authors’ reply****: Done*

**Reviewer 2’s comment#14.** *L97: Change to "more complex ... and specialized ... networks"*

***Authors’ reply****: We removed the term 'more complex' from the entire manuscript to avoid potential misinterpretations, as this term can vary depending on the network metric. For instance, a nested network is not necessarily more complex than a modular network.*

**Reviewer 2’s comment#15.** *L92-101: In order to present the expected effects of land use on network structure, the authors stated two different hypotheses. This seems unnecessary since the set of predictions are complementary (i.e. along a gradient in resource diversity and supply, networks are expected to be more modular and less nested). Please, reduce the two hypotheses to a single one.*

***Authors’ reply****: We followed the reviewer’s suggestion and rephrased our hypothesis. Please see L30-38. “We hypothesize that increasing land use reduces biodiversity and modularity in fish networks due to the loss of specialist species and faunal homogenization, resulting in nested networks. We quantified six network metrics based on the species richness and distribution of interactions (nestedness and modularity), trophic specialization, number of links per species (links density), and number of trophic links according to the number of nodes (number of links). We observed that the trophic networks are more nested than modular, thus supporting our hypothesis. However, we only observed a negative effect of land use on modularity, and the number of links, whereas other metrics such as nestedness, trophic specialization, number of species, and link density are not influenced by land-use. ”.*

**Reviewer 2’s comment#16.** *L107: Please, specify the other terms of interest included in the search*

***Authors’ reply****: We here is described the words used to find all the manuscripts we found, so we cannot change the strings of words. That would mask the procedure we used and prevent fully documenting and methods paths. So we prefered to maintain it as follows. Please see L122-140. “We conducted a systematic literature review of articles published from 1982 to 2021 from electronic databases and search engines, including Scopus, Web of Science and Google Scholar. Our focus was on studies examining the diet of freshwater fish assemblages in Brazil. Our search focuses on several combinations of keywords: (fish\*) AND (stream\*) AND (feed\*). The literature survey returned many studies, but we only considered articles with more than five species that represent the local community because we were interested in describing the local community network structure. Additionally, we focused on the papers expressing the diet of species as the feeding index (IAi) or numeric or volume percentage of the food item in the diet of each individual species (Bonato et al., 2012; Peressin et al., 2018; Santos et al., 2021; Souza et al., 2022; Caldatto et al., 2023), to make sure they were fully characterizing feeding habitats. We also considered thesis and gray literature reporting fish trophic networks with the same condition described above (N=18). In total, we compiled 49 trophic networks across Brazil (Table S1). We extracted information from the dietary tables of the papers (row food items and column species) that included different food items represented by different families of terrestrial and aquatic insects, algae, plant material, crustaceans, and mollusks. As these published studies may diverge in their way to quantify consumed food items and sampling efforts, we preferred using information on the presence/absence of interactions (i.e., binary matrices) for focusing mostly on the incidence, rather than strength of the interaction, and avoid biases regarding items quantification and sampling efforts. ”*

**Reviewer 2’s comment#17.** *L125-127: Consider rephrasing to something like "...while the links between them represent trophic interactions" L132: Change to "The number of species (nodes), is defined..."*

***Authors’ reply****: We followed the reviewer’s suggestion and rephrased this sentence. Please see lines: L146-147. “The number of species (i.e., nodes) is defined by the total number of consumers and resources (May 1973; Tilman 1996).”*

**Reviewer 2’s comment#18.** *L174-177: If I understood well, the method used to standardize the metrics associated to network structure retains both the total number of nodes and links when the observed matrix is randomized. If this is the case, I wonder how a standard value for number of links can be obtain, since the algorithm does not change this parameter. This point requires more clarification.*

***Authors’ reply****: Thank you for the opportunity to clarify that we estimated the Standardized Effect Size (SES) of each network metric (e.g., NODF, Q, Link density, and Number of links) to control the difference associated with given the number of species and trophic links. Please see lines: L175-192. “When calculating network descriptors, it is important to control for a possible sampling bias related to network dimensions (i.e., number of species and trophic links), which could prevent comparing descriptors among networks. Therefore, we compared three main (NODF, Q, and H2’) observed descriptors from individual networks to those calculated under null models with the same randomized matrix (Kortsch et al., 2019;**Quimbayo et al., 2018; Dáttilo & Vasconcelos, 2019). We randomized the observed trophic networks over 499 matrices for each network descriptor, using a null model that fixes both marginal totals and connectivity (‘swap.web’ null model), i.e., maintaining constant the number of interactions (and therefore connectivity), as implemented in the “bipartite” package in R (Dormann et al., 2009). Then, we quantified the Standardized Effect Size (SES) of each observed network metric (i.e., NODF, Q, and H2’) as the difference between observed and null estimated values of network metrics using the following equation: (observed - μ) / σ, where ‘observed’ is the value of the focal network metric, μ is the mean value of focal metric over all null matrices, and σ is its standard deviation of all null matrices. Negative and positive SES values indicate observed values that are lower and higher, respectively, than the expectation, given the number of species and trophic links. Empirical values of trophic network descriptors were considered to deviate strongly from the randomized food webs if these were outside the 0.05 to 0.95 quantile range of the null distribution.”*

**Reviewer 2’s comment#19.** *L178: Change "strongly" to "significantly".*

***Authors’ reply****: Done*

**Reviewer 2’s comment#20.** *L231: Change the comma for a period when reporting the number of total trophic interactions*

***Authors’ reply****: Done*

**Reviewer 2’s comment#21.** *L234: Remove "and".*

***Authors’ reply****: Done*

**Reviewer 2’s comment#22.** *L238: Change "then" to "than".*

***Authors’ reply****: Done*

**Reviewer 2’s comment#23.** *L241-250: The description of the relationship between each variable and land use should match the order of the plots presented in Figure 2. In addition, and following the coherence of figure 2, the plot corresponding to Number of species should not show the regression line since the trend is not significant.*

***Authors’ reply****: Done*

**Reviewer 2’s comment#24.** *L560-564: The order of the plots does not match the caption of the figure*

***Authors’ reply****: Done*

**Reviewer 2’s comment#25.** *Table S1: What does "Network size" refer to? Clarification is needed.*

***Authors’ reply****: We avoid such information as it does not improve understanding.*

**Reviewer 2’s comment#26.** *Table S2: This table is confusing. Are the values p-values? If yes, I suggest the authors change them for the Z-score values. Table S5: Should the sum of each row not sum 1 since they are proportions?*

***Authors’ reply****: There are no p-values at Table S2, these are proportion of land cover in buffers at the region of each study. We also made it clear that not all landcover classes are shown, hence their row sums should not sum up 100%. We thank the referee for noting, and hope these sentences solve much of confusion.*

**Reviewer 2’s comment#27.** *Table S6: The authors define "number of links" as the total number of links observed in a local food web (L136-137 in the ms). Why does this variable have decimals? Should it not be a variable with positive integer values?*

***Authors’ reply****: Thank your for noting. We have double chacked and it is better explained that the number of links actually refers to the mean number of link per species, which is a measure of how much individual fish species is connected with other items in the network. Therefore, those metrics are indeed decimals.*