

Submission date. 05/12/2024.

Associate editor's decision after peer review (21/02/2025).

Dear Dr. Sversut Dias,

Manuscript ID NI-2024-0130 entitled "Land use effects on the structure of trophic networks from Neotropical stream fish assemblages" which you submitted to the Neotropical Ichthyology, has been reviewed. The comments from reviewers are included at the bottom of this letter.

Both reviewers congratulate the efforts of producing this manuscript, but they also pointed out several important shortcomings. Observe that R1 suggested some improvements in the analysis and R2 commented on outdated information. Therefore, I must decline the manuscript for publication in the Neotropical Ichthyology at this time. However, a new manuscript may be submitted which takes into consideration these comments.

Please note that resubmitting your manuscript does not guarantee eventual acceptance, and that your resubmission will be subject to re-review by the reviewers before a decision is rendered. Provide a rebuttal letter with answers for all suggestions/comments made by R1 and R2. I will be glad in receiving a revised version.

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Because we are trying to facilitate timely publication of manuscripts submitted to the Neotropical Ichthyology, your resubmitted manuscript should be submitted by 21-Aug-2025. If you are unable to submit by this date please contact the Editorial Office for options.

I look forward to a resubmission.

Sincerely,

Dr. Lilian Casatti

Associate Editor, Neotropical Ichthyology

licasatti@gmail.com

Anonymous reviewer #1

Recommendation. Reject & Resubmit

Comments. The manuscript "Land use effects on the structure of trophic networks from Neotropical stream fish assemblages" shows an interesting topic to be explored in Neotropics. However, the methods employed by the authors are inadequate or aren't explained in detail.

Actually, the shortcomings of reusing interaction data from multiple sources are well-known in academia (see <https://doi.org/10.1371/journal.pbio.3002068>). To deal with this, several measures are indicated to have been performed previously, such as calculating the completeness (or sampling effort) of interaction networks to compare only networks with similar values. We can't compare a network that had 90% of their possible

interactions sampled with one with only 15% of sampled interactions.

Second, the authors used binary metrics to avoid sampling bias. In turn, binary metrics are known to be more biased by sampling bias than weighted data (see <https://doi.org/10.1111/1365-2656.12459>). So, if weighted data is available, it is more reliable to use than the binary one.

Third, instead of using many linear models to test the influence of land use types in network metrics, I suggest using path analysis (see <https://doi.org/10.1111/oik.06621>), also adding completeness as a variable in model.

In addition, I put some suggestions in .pdf file. I hope these suggestions will be useful to improve the manuscript.

Anonymous reviewer #2

Recommendation. Reject & Resubmit

Comments. NI-2024-0130 - Land use effects on the structure of trophic networks from Neotropical stream fish assemblages.

The manuscript presents a literature review of studies on fish feeding (at the community level) in Brazilian streams, from which data were extracted to calculate six (seven?) food web metrics and analyze their relationship to land use in those streams. It's a solid approach, and the authors have done a good job compiling and analyzing the data with comprehensive methods (that account for usually neglected aspects in this type of study, such as food web size and spatial autocorrelation). However, I've identified some key issues that need to be addressed to enhance the clarity of the manuscript and make it suitable for publication. For instance, the review is outdated, some chosen metrics seem to be redundant and/or erroneously considering only fish species (consumers) instead of all nodes, and the criteria for paper selection are unclear and appear to have unnecessarily excluded many studies. Thus, the manuscript needs a thorough revision that addresses both the major and minor comments to be accepted. Specific comments are provided below.

Major review:

- Title: should be "Brazilian streams" instead of Neotropical, as the review and data collection is restricted to Brazil. Although Brazil is by far the largest country in the Neotropical realm, it does not fully represent the entire region.
- Furthermore, whether considering the Neotropics or Brazil, differences among biomes or key stream features (such as stream width or altitude) are not accounted for, yet these factors may be more relevant than land use in defining stream food webs. How did authors deal with this challenge?
- I don't understand why connectance was not calculated, as it is a key metric that indicates how nodes are connected within the network, and is associated with food web complexity. Therefore, it is commonly used as a representative food web metric, allowing for comparison across studies. Why have authors not included this metric, once having data to calculate it?
- The review is significantly outdated. In Ecology, we come across many articles that publish data previously collected, some from years or even decades ago, and that's not a problem because sharing this data and insights is important, and sometimes revisiting the field is not feasible. However, there's no reason why a literature review could not be updated. Many papers on fish feeding and freshwater food webs may have been published in the past four years, and I believe they would enhance both the data available and the insights into the analyzed metrics and environmental factors. Since we are in 2025 and the manuscript was submitted in December 2024, I suggest the review be extended at least until mid-2024.
- Material and methods: The exact search terms used in the article search should be provided, as well as the exact number of articles retrieved. I imagine that just these three terms (fish* and feed* and stream*) could have retrieved hundreds, if not thousands, of

papers. How many studies are “many”? And which were the exclusion criteria to end up with only 49 articles? Actually, authors mention “49 trophic networks”. Does it equal 49 articles? The steps of this methodology should be elaborated further, and preferentially providing a Prisma diagram.

- How can authors be sure that five species represent a community? Some studies might focus only on the (five) most abundant species and yet it is not a study at the community level, but just a subset. On the other hand, some streams (e.g. oligotrophic or headwater streams) might have only 2 or 3 fish species and still be a whole community... Could you comment on that? And how was this number chosen?

- The number of nodes comprises consumers + resources, denotes food web size and is one of the dimensions of food web complexity. Do authors consider only consumers (fish) as nodes? This creates confusion, especially as it appears contradictory in different sections, leading to the conclusion that two metrics (links per species and linkage density) may be redundant. In any case, fish species richness is not typically considered a food web metric, even in bipartite networks.

- I don't understand the criterion of retrieving only papers that use an specific feeding index or other quantitative data, as there are other feeding indices available and as all the metrics analyzed (except H2) use only presence-absence data. It might have unnecessarily excluded important data (papers that use other indices or only binary data). Furthermore, a high correlation between modularity and H2 was observed; this could be another reason to exclude H2 and rely only on binary data, increasing the number of observations.

- The reference list is not standardized: some journal names are abbreviated while others are not, and some titles are in capital letters, just to cite some examples. A thorough revision is needed to ensure consistency, following the guidelines provided by NI for this section. This applies to the Supporting information references as well. And why are there some references in italic in this list?

Minor review:

Abstract: “We quantified six network metrics such as...” – why “such as”, as all 6 are cited here? Replace "such as" for colon (:) in the sentence. And it actually counts as 7, not 6. Nestedness and modularity are not the two sides of the same coin, hence not a single metric

- “We observed that the trophic networks are more... These findings highlight the relevance of preserving natural vegetation...” – what is the link between the two sentences? If almost no metric has changed, why should vegetation be preserved? The conclusion feels somewhat cursory, lacking the depth or analysis expected. The abstract needs to be both succinct and informative.

Introduction, Page 3:

L 7-10 – “Recently, theoretical studies have shown that the main determinants of network structure differentiation at different sites are variations in ecological and environmental factors (Arruda et al., 2020; Dugger et al., 2019; Emer et al., 2013). – Are these studies theoretical? They appear to be empirical studies. And these conclusions would not emerge from theoretical studies

L. 43-44 – Thus, the structure of the trophic network (Pimm et al., 1991) should vary according to the degree of land-use change (Winemiller, 1990) – the paper by Winemiller 1990 does not examine degrees of land use change, so this reference does not seem appropriate

Page 4:

L 9 – Neotropical stream fish assemblages – Brazilian (see Major comments)

L 10-21 – the introduction does not inform that these questions will be answered through a review...

- “that form Networks” – should be plural, right? Because each stream has its own network

M&M

Page 5:

L. 29-30 - from 1982 to 2021 – outdated (see major review comments)

L. 36 - freshwater fish assemblages in Brazil – stream only... stream fish assemblages

L. 36-41 - How many is “several”? The exact search terms should be provided, as well as the exact number of articles retrieved. The steps of this methodology should be elaborated further. Please see major comments for more details.

L. 40-42 - how can authors be sure that five species would represent a community? Please see major comments for more details.

L. 43-48 - “...we focused on the papers expressing the diet of species as the feeding index (IA_i) or numeric or volume percentage of the food item in the diet of each individual species... to make sure they were fully characterizing feeding habitats” – I don't understand this criterion. See major review comments for more details. And it should be ‘feeding habits’ instead of ‘habitats’, correct?

L. 48-49 - where were those theses obtained from? This should be mentioned. How can we ensure that all theses with such data were included?

L. 54-55 - “...that included different food items represented by different families of terrestrial and aquatic insects, algae, plant material, crustaceans, and mollusks.” – were food items classification kept as they appeared in the papers? For example, it is common to find papers where food items were analyzed to the lowest possible taxonomic resolution, whereas others merge items into coarser categories, and this will have implications to food web metrics. How did authors deal with this challenge? It is unclear which taxonomic resolution of resources (food items) was considered, and yet this is crucial for comparing food webs.

Page 6:

L. 11-22 - “We built bipartite networks...The number of fish species (i.e., nodes) is defined by the total number of consumers within each matrix (May, 1974; Tilman et al., 1996).” - The number of nodes should be the number of consumers + resources. Please see major comments. The number of links should be the number of non-empty cells in the resource-use matrix. Was this the case?

L. 30-31 - “...algorithm modified for two-mode networks (Dormann, Strauss, 2014)” – what do authors mean by two-mode networks?

Page 7:

L. 10-13 - “Lastly, we also estimated the mean number of links per species, which informs how connected species are within the food web (Bersier et al., 1999; Dunne et al., 2002).” - This information would rather be given by connectance, which considers also the potential links. See also major comments

Page 9:

L. 27 - outlier ‘remotion’? – should it be ‘removal’? Please check it throughout the text

Page 10:

L. 3 - 20 - Please provide mean values found also for the other metrics evaluated (number of nodes, number of links/ species, linkage density...). On the contrary, the percentage of each guild does not seem relevant to the manuscript and should be replaced by those missing food web metrics

Page 15:

L.21 - replace consume for consumption. Please check it throughout the text

L. 16 - 28 - The addition of generalist species or an expansion of the trophic niche width of existing species will increase connectance within the food web, thereby enhancing complexity and, presumably, stability. Generalism expands the number of pathways for resource use, ultimately contributing to food web stability. This point should be addressed in the discussion and supported by recent literature.

Page 16:

L. 26-28 - What do authors mean by “large-scale variation”?

L. 30–31 – nestedness supposedly increases stability

Kortsch et al. (2019) focuses on gradients in the Arctic Sea; marine environments present different challenges and connectivity dynamics, making comparisons with the findings of this paper less relevant

Table S1 – R2 values seem too low to be significant with only 49 samples. Could you comment on that?

Author's Rebuttal Letter (20/08/2025).

Dear Dr. Lilian Casatti,

Thank you for the opportunity to revise and resubmit our manuscript NI-2024-0130 entitled “High pasture land cover reduces modularity of trophic networks from Brazilian stream fish assemblages”. We very much appreciate the time invested by the referees and the editors to provide a thorough review, and we are excited about the overall positive reception during this second review.

All comments have been carefully considered and we modified the manuscript accordingly. We also gave special attention to the grammar and the flow of sentences. The entire document has been revised and we think it considerably improved in this new version.

We submit two versions of the manuscript—one clean version (as the Main Body) and one with track changes (as the Supporting Document). In our responses, we detail all changes to each comment provided by the reviewers (in blue ink and in italics). Should this formatting be lost, we also precede each response with an ‘Authors’ response’. Please note that the line numbers in the response to reviewers document correspond to the document without track changes, which make it easier to identify changes made during the revision process.

Once more, we appreciate such an insightful review and excellent editorial services provided by Neotropical Ichthyology. We look forward to hearing back from you.

Yours sincerely,

Murilo S. Dias and co-authors

Reviewer 1's comments

Major review

Reviewer 1's comment#1. Line 19. Humans have led to an intensification in land use. Humans did not lead to an intensification, but a land use change.

Authors' reply: Thank you for this observation. We agree with the reviewer that “land-use change” is more accurate than “intensification in land use.” The sentence was revised accordingly.

Revised sentence: “Humans have driven extensive land-use changes, posing serious threats to ecosystems”.

Reviewer 1's comment#2. Line 20: Despite advances, it is unknown at large... It is not unknown. There are many articles showing the impacts on this scale. Maybe this topic is poorly known, but not unknown

Authors' reply: We appreciate the reviewer's comment and agree that several studies have already documented the impacts of land-use change on aquatic ecosystems at broad scales. Our intention was to emphasize that, despite these advances, the effects on trophic network structure remain poorly understood rather than entirely unknown. We have revised the sentence to clarify this point.

Revised sentence: “Despite recent advances, the impact of human activities on ecological interactions and multiple food web metrics in freshwater ecosystems remains poorly understood at large spatial scales.”

Reviewer 1's comment#3. Line 26. How did you analyze the influence of land use on network metrics? Please include in the abstract.

Authors' reply: We thank the reviewer for this helpful suggestion. We have revised the

abstract to clarify how land-use influence was analyzed. Specifically, we added a sentence describing the use of MapBiomass data within 500 m buffers around sampling sites to quantify land-use effects on trophic structure.

Reviewer 1's comment#4. Lines 91–92. You mention both mutualistic and antagonistic networks. I think it is better to cite only antagonistic networks, which relate to your theme.

Authors' reply: We appreciate the reviewer's suggestion and agree that focusing only on antagonistic networks is more appropriate for the scope of our study. Accordingly, we removed the reference to mutualistic networks from the text and kept only the discussion related to antagonistic networks.

Reviewer 1's comment#5. Line 100. Fresh water. Be consistent with the term fresh water/freshwater. Revise the term throughout the text.

Authors' reply: We thank the reviewer for this observation. We revised the entire manuscript to ensure consistency, using the term "freshwater" throughout the text.

Reviewer 1's comment#2. "Line 121 and the entire text. Revise the term landuse/land-use/land use"

Authors' reply: We thank the reviewer for this helpful observation. We have revised the entire manuscript to ensure consistent use of the term. Following standard English scientific conventions, land use is employed as a noun (e.g., "effects of land use"), and land-use is used as a compound adjective (e.g., "land-use change," "land-use data"). All instances have been corrected accordingly.

Methods:

Reviewer 1's comment#2. Network metrics.

"Did you test the correlation among network metrics? Some metrics can be related, and using them can be useless, such as connectance, link density, and mean links per consumer."

Authors' reply: Thank you for this insightful comment. Yes, we tested the correlations among all network metrics to verify potential collinearity issues. The results are presented in Table S5 (Supporting Information), where some expected relationships are shown (e.g., between connectance, link density, and mean number of links per consumer). Nevertheless, we retained all metrics because they describe distinct ecological properties of the trophic networks.

We also checked redundant link to multicollinearity among predictors previous our multiple regression analyses. We calculated the Variance Inflation Factor (VIF) for all predictors, which remained low ($VIF < 2$). Redundancy among predictors was not an issue in our analyses, and each metric contributed unique ecological information to our interpretations.

Reviewer 1's comment#4. Line 240. "Why only a 500m buffer and not more? I do not think that 500m buffers can capture local and regional scale changes, because it also depends on the river width".

Authors' reply: Identifying the size and the format of the buffer is far from trivial and many distances are considered (see Bregão et al., 2021). A recent local scale analyses suggest 100 m circular buffer is fine to characterize and explain fish diversity patterns (Pessoa et al., 2025), but large buffer sizes (especially 500m radius) also provide significant and similar results (see Table 1, Pessoa et al. 2025).

We choose the 500 m circular buffer as a compromise between local and regional spatial scales based on previous studies evaluating land-use impacts on stream fish communities in Brazil (e.g., Bregão et al., 2021; Pessoa et al., 2025). Importantly, our database included only stream ecosystems (i.e., small to medium-sized headwater systems), where this buffer size is commonly applied and adequate to capture both riparian and catchment-scale influences. The 500 m radius also ensures comparability among studies with different stream widths and drainage areas. In our dataset, the original studies did not consistently report river width or reach length, preventing us from applying site-specific buffer

sizes. Therefore, the standardized 500 m buffer provided a consistent and reproducible spatial unit across all networks while still capturing the main land-use gradients around each study. We have added an explanation to the text L (235–246).

Pessoa et al. 2025 – <https://link.springer.com/article/10.1007/s10750-024-05699-9>

Brejão et al. 2021 – <https://revistas.ufrrj.br/index.php/oa/article/view/475>

Reviewer 1's comment#5. Stream covariates. Can these covariates change according to the month? In North and Northeastern, some rivers can increase and shrink their size depending on the season. So, this measure can change.

Authors' reply: Thank you for your comment. Some of our chosen stream covariates are invariable to annual climatic variation (stream order, elevation, upstream area, distance from river source), whereas river width can drastically change, specially during the dry season. Those variations are more drastic in the dry regions of North East Brazil, but we had no studies from this region (see Fig 1). Anyway, we did not use in situ habitat covariates such as discharge or stream width that could indeed change a lot annually because site information was not available across studies; we did include large scale predictors from WorldClim that represent mean annual and monthly variability in climatic conditions over studies locations. Therefore, the annual variability in stream flow, width or discharge are already represented and controlled for in our models by using large scale climatic conditions.

Linear models.

Reviewer 1's comment#6. Lines 287–288. “Why did you only use the proportional area of anthropogenic land-use, cropland, and pasture, and not the natural?”

Authors' reply: We focused on the proportional area of anthropogenic land-use (cropland and pasture) because our objective was to assess the degree of human impact on stream trophic networks. Natural land cover is implicitly represented as the complementary proportion (i.e., 100% minus the anthropogenic percentage) and therefore provides redundant information in the models. Using only anthropogenic land-use variables avoids perfect collinearity between predictors and allows us to interpret their effects directly along a gradient of habitat disturbance. We have now clarified this rationale in the Materials and Methods section (lines 247–268) with the following text.

Reviewer 1's comment#7. Figure 2. “What represents the Land use gradient (x axis)?”

Authors' reply: The land-use gradient on the x-axis represents the proportion of anthropogenic land cover (i.e., the sum of cropland, pasture, and other human-modified areas) within a 500 m buffer around each sampling site. This variable was extracted from the MapBiomias land-use dataset and ranges from 0 (completely natural cover) to 1 (fully modified landscape).

We have clarified this information in the caption of Figure 2 and in the Materials and Methods section (lines 373–374). The revised caption now reads.

Reviewer 1's comment#8. Lines 387–391. “This sentence is hard to follow. Maybe break it in two?”

Authors' reply: Thank you for this helpful suggestion. We agree that the original sentence was too long and difficult to read. We have now rewritten it as two clearer sentences to improve readability (lines 400–432).

Reviewer 1's comment#9. Line 403. “The lack of broad land use effects on network metrics was thus contrary to our initial hypotheses and unexpected. It can also be a bias in relation to the buffer size.”

Authors' reply: Thank you for this important comment. We included a sentence at discussion to account with that possibility (line 445–448).

Reviewer 1's comment#10. Line.25

I agree with Reviewer 2, who suggested that the review should be up to mid 2024's and thus include more recent studies as well.

Authors' reply: Thank you for your thoughtful comment. We agree that updating systematic reviews is important to reflect the progress of knowledge in the field.

In our study, we chose to focus on the period from 1982 to 2021 in order to build a robust dataset based on consistent, long-term studies that have been widely used in both national and international literature. Many of these older datasets are essential for identifying long-term trends, assessing structural changes in food webs, and representing different biomes and historical land-use contexts in Brazil, and are therefore fundamental to our objectives. Nevertheless, recognizing the relevance of more recent data, we conducted an additional literature search covering the period from 2022 to 2024, including peer-reviewed articles, theses, and dissertations (e.g. <https://doi.org/10.1590/S2179-975X0322>; <http://repositorio.ufc.br/handle/riufc/76951>; <https://hdl.handle.net/11449/257097>). From this search, we found only a few studies that met the inclusion criteria (at least five species, quantitative diet data, and stream ecosystems). We have also quickly screened some published papers that met our criteria and found no more than five.

Though the low number, we opt to not include them in our study as the computing time for processing everything is really high. The compiling time, the GIS processing of land use extraction, the topology metrics calculation, and the adaptation of all routines is really time consuming and unfeasible for us at this stage. The first author (Dra Nuven) was a PhD candidate at the time of compiling, but she has had a new baby born and is involved in intensive teaching loads while searching for a permanent job position. Therefore, it is impossible for us to go further in such updating exercise, and we believe that incorporating these few additional references would not significantly change the patterns observed in the 49 food webs already included, which span a wide ecological and geographic range across nearly four decades. Moreover, these newer studies do not provide substantial complementary spatial coverage, as they are from regions and contexts already represented in our dataset. Yet, we acknowledge and highlight in the manuscript that the database can and should be updated in the future as more studies make their raw diet data available. We also emphasize in the discussion that encouraging data sharing is essential for future syntheses.

Once again, we appreciate your valuable suggestion, which reinforces the importance of dynamic and periodically updated reviews — a practice we also support.

See L494–498: “Future studies could build upon this work in several key areas. While our literature search was thorough, it is possible that some relevant trophic matrices were missed, and our dataset represents a fixed snapshot in time. We acknowledge this limitation and have made all trophic matrices fully available to facilitate future updates and refinements (see Methods for data and code repository).”

Reviewer 2's comments

Major review

Reviewer 2's comment#1. The manuscript to hand is a reviewed version of a former submission. In this version, the authors corrected many changes suggested by previous reviewers and included new analyses. Overall, the manuscript addresses the impacts of different land uses on fish trophic networks in Brazilian streams. The authors gathered literature data (over 300 studies) on fish diet as an effort to detect general patterns in consumer–resource trophic networks in various regions. They expected nestedness to increase and modularity to decrease in more impacted areas, though no effect of land use on trophic network metrics was observed. However, when land uses were analyzed separately, the pasture percent cover negatively influenced modularity as expected. This is an interesting result likely related to riparian forest removal in pasture landscapes.

In general, the introduction section, the analyses used in the manuscript, and the results (along with tables and figures) are well done. However, the discussion and conclusions need to be improved. The discussion section could be restructured and better connected to the aims and results. As written, it is a bit wordy and, at the same time, seems to lack important subjects, such as (i) the importance of the riparian forest for fish trophic networks and how it is usually lacking in pasture streams; (ii) could increased nestedness

be related to trophic specialization because it is quite common to find many rare food items in fish diets (i.e. high frequency of occurrence in low amounts)? (iii) What could be the role of detritus and organic matter in the trophic networks observed? Perhaps it could be an especially important food source in highly impacted areas and/or for generalist species. It is important to recognize the limitations of the study, but they are emphasized in all but three paragraphs – way more than needed. Instead of focusing on what they didn't or couldn't do, authors should remember that their contribution is mostly to detect macro patterns of network metrics and land use (although they can discuss findings on a smaller scale, as mentioned above, based on available literature). It would also be important to discuss, on a macro scale, why the main hypothesis was not corroborated. Perhaps the number of trophic species and trophic links in Brazilian (i.e., tropical) stream food webs is quite high even in impacted areas, and a complex network in terms of links and species can be more stable against perturbations. These are some suggestions that could be approached in the discussion.

Finally, the manuscript also needs a thorough language review by the authors. There are some grammatical, punctuation, and style mistakes throughout the text, and formal scientific language needs improvement. I have listed some of them, but it was unfeasible to list them all. Overall, the manuscript concerns an interesting subject and was based on a large dataset, though some improvements should be addressed before it is published. Please check for the minor concerns, corrections, comments, and suggestions in the Word files.

Authors' reply: We thank the reviewer for the constructive and detailed comments, which greatly helped us to improve the clarity, focus, and scientific contribution of our manuscript. We summarize below the main changes implemented according to these suggestions.

1. General structure of the Discussion

As suggested, we thoroughly revised the Discussion to make it more concise, better structured, and directly connected to our aims and results. The section now begins with the main findings, followed by contextual comparisons with other studies, and ends with a synthesis emphasizing ecological mechanisms and implications.

2. Importance of riparian forests

We incorporated a specific discussion on the role of riparian vegetation in maintaining food-web modularity and trophic diversity (see revised Discussion). We now emphasize that riparian forest loss in pasture-dominated areas reduces allochthonous inputs, habitat heterogeneity, and thus the compartmentalization of trophic networks.

3. Relationship between nestedness and trophic specialization

We added a paragraph linking the observed nested structures to patterns of trophic specialization in stream fish communities. Following the reviewer's suggestion, we now discuss how high nestedness can emerge when many species feed on rare or low-frequency resources, a common feature of fish diets in tropical streams.

4. Role of detritus and organic matter

We expanded the discussion about detritus and fine organic matter as key resources in degraded systems. We now argue that increased sedimentation and primary production in open, pasture-dominated streams enhance detritus pathways, sustaining generalist consumers and buffering network collapse.

5. Macroecological explanation for the lack of strong land-use effects

We addressed the reviewer's suggestion to explain, at a broader scale, why the main hypothesis was not corroborated. We now discuss that Brazilian stream food webs—characterized by high richness and redundancy in trophic links, may be inherently resilient to environmental disturbance, which could explain the weak relationship between land use and network metrics.

6. Reduction of excessive emphasis on limitations

We revised the text to remove redundant statements about limitations and to focus

instead on the study's contributions. We acknowledge, however, that most inclusions of biases/gaps have been suggested by other reviewers/editors, so this current version represents the best compromise of our draft to comport all previous suggestions. This is also important in our view to guide new studies and considerably improve their methods compared to our first assessment. Key limitations and further suggestions are also succinctly presented in one final paragraph, highlighting opportunities for future research rather than constraints.

7. Language and style revision

We performed a thorough language revision to correct grammatical and stylistic issues, improving fluency and consistency throughout the text.

Reviewer 2's comment#2. L. XX-XX. "Were quantitative trophic networks constructed? If not, why exclude qualitative diet data?"

Authors' reply: Thank you for this important question. As depicted in the first paragraph of Material and Methods, we selected studies based on the presence of any quantitative food item data; more specifically on studies having diet of species as the feeding index (IAi) and/or numeric and/or volume percentage of the each food item in the diet of each individual species. This was necessary to make sure they were fully characterizing species feeding habits as a primarily study goal in a highly confident protocol and not only reporting food items from occasional sampled individuals. However, as these published studies may diverge in sampling efforts and their way to quantify consumed food items, we preferred using here information on the presence/absence of interactions (i.e., binary matrices) to focus mostly on the incidence of links, rather than on the strength of interactions, and avoid biases regarding items quantification. This was also necessary to maintain consistency and comparability across all networks.

We have clarified this point in the Materials and Methods section (lines 132–158).

Reviewer 2's comment#3. L. XX-XX. Is this information accurate? Or is it the sum of all fish species from each trophic network?

Authors' reply: We confirm that the reported numbers (583 fish species, 185 food items, and 24,214 trophic interactions) refer to the consolidated totals of unique species and items across all networks. Each species and food item was counted only once.

Reviewer 2's comment#4. L. XX-XX. Rewrite more clearly. In fact, the whole discussion section needs improvement of language style and grammar. Authors should carefully check the whole text. I've listed several corrections throughout the text but it's unfeasible to list them all.

Authors' reply: We carefully revised the Discussion section to improve clarity, grammar, and overall style throughout the text, as suggested by the reviewer. We believe this a much stronger version with higher readability.

Associate editor's decision after peer review (25/09/2025).

Dear Dr. Sversut Dias,

Manuscript ID NI-2025-0149 entitled "High pasture land cover reduces modularity of trophic networks in Brazilian stream fish assemblages" which you submitted to the Neotropical Ichthyology, has been reviewed. The comments of the reviewers are included at the bottom of this letter. R2 also attached two files, with comments and suggestions. Please, when sending the revised version, provide a rebuttal letter with the answers to each of their comments.

The reviewer(s) have recommended publication, but also suggest some revisions to your manuscript. Therefore, I invite you to respond to the reviewer(s)' comments and revise your manuscript.

To revise your manuscript, log into <https://mc04.manuscriptcentral.com/ni-scielo> and enter your Author Center, where you will find your manuscript title listed under "Manuscripts with Decisions." Under "Actions," click on "Create a Revision." Your manuscript number has been appended to denote a revision.

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Because we are trying to facilitate timely publication of manuscripts submitted to the Neotropical Ichthyology, your revised manuscript should be submitted before 24-Nov-2025. If it is not possible for you to submit your revision by this date, we may have to consider your paper as a new submission.

Once again, thank you for submitting your manuscript to the Neotropical Ichthyology and I look forward to receiving your revision.

Sincerely,

Dr. Lilian Casatti

Associate Editor, Neotropical Ichthyology

licasatti@gmail.com

Anonymous reviewer #1

Recommendation. Major Revision

Comments. The authors did a good job improving the study, but some issues still need to be clarified.

My major and minor appointments are cited below.

Abstract.

Line 19. Humans have led to an intensification in land use. Humans did not lead to an intensification, but a land use change.

Line 20: Despite advances, it is unknown at large... It is not unknown. There are many articles showing the impacts on this scale. Maybe this topic is poorly known, but not unknown.

Line 26. How did you analyze the influence of land use on network metrics? Please include in the abstract.

Resumo. Line 36. This sentence is different in the Abstract. Verify the entire Resumo to match the Abstract

Introduction.

Lines 62–64. Communities can be represented by a graph...

Line 64. The study of communities through networks

Lines 91–92. You mention both mutualistic and antagonistic networks. I think it is better to cite only antagonistic networks, which relate to your theme.

Line 100. Fresh water. Be consistent with the term fresh water/freshwater. Revise the term throughout the text.

Lines 100–101. See <https://doi.org/10.1002/oik.11253>

Line 121 and the entire text. Revise the term landuse/land-use/land use
Methods

Network metrics.

Did you test the correlation among network metrics? Some metrics can be related, and using them can be useless, such as connectance, link density, and mean links per consumer.

Anthropogenic impact in streams – on streams?

Line 240. Why only a 500m buffer and not more? I do not think that 500m buffers can capture local and regional scale changes, because it also depends on the river width
Stream covariates. Can these covariates change according to the month? In North and Northeastern, some rivers can increase and shrink their size depending on the season. So, this measure can change.

Linear models.

Lines 287–288. Why did you only use the proportional area of anthropogenic land-use, cropland, and pasture, and not the natural?

Figure 2. What represents the Land use gradient (x axis)?

Discussion.

Line 380. Fish trophic networks IN BRAZIL

Lines 387–391. This sentence is hard to follow. Maybe break it in two?

Lines 392–420. This paragraph is too extensive. Consider breaking it in line 403.

Line 403. The lack of broad land use effects on network metrics was thus contrary to our initial hypotheses and unexpected. It can also be a bias in relation to the buffer size.

Line 455 and on. Isn't it better to create a subsection entitled "Gap and biases" here?

Anonymous reviewer #2

Recommendation. Minor Revision

Comments. The manuscript to hand is a reviewed version of a former submission. In this version, the authors corrected many changes suggested by previous reviewers and included new analyses. Overall, the manuscript addresses the impacts of different land uses on fish trophic networks in Brazilian streams. The authors gathered literature data (over 300 studies) on fish diet as an effort to detect general patterns in consumer–resource trophic networks in various regions. They expected nestedness to increase and modularity to decrease in more impacted areas, though no effect of land use on trophic network metrics was observed. However, when land uses were analyzed separately, the pasture percent cover negatively influenced modularity as expected. This is an interesting result likely related to riparian forest removal in pasture landscapes.

In general, the introduction section, the analyses used in the manuscript, and the results (along with tables and figures) are well done. However, the discussion and conclusions need to be improved. The discussion section could be restructured and better connected to the aims and results. As written, it is a bit wordy and, at the same time, seems to lack important subjects, such as (i) the importance of the riparian forest for fish trophic networks and how it is usually lacking in pasture streams; (ii) could increased nestedness be related to trophic specialization because it is quite common to find many rare food items in fish diets (i.e. high frequency of occurrence in low amounts)? (iii) What could be the role of detritus and organic matter in the trophic networks observed? Perhaps it could be an especially important food source in highly impacted areas and/or for generalist species. It is important to recognize the limitations of the study, but they are emphasized in all but three paragraphs – way more than needed. Instead of focusing on what they didn't or couldn't do, authors should remember that their contribution is mostly to detect macro patterns of network metrics and land use (although they can discuss findings on a smaller scale, as mentioned above, based on available literature). It would also be important to discuss, on a macro scale, why the main hypothesis was not

corroborated. Perhaps the number of trophic species and trophic links in Brazilian (i.e., tropical) stream food webs is quite high even in impacted areas, and a complex network in terms of links and species can be more stable against perturbations. These are some suggestions that could be approached in the discussion.

Finally, the manuscript also needs a thorough language review by the authors. There are some grammatical, punctuation, and style mistakes throughout the text, and formal scientific language needs improvement. I have listed some of them, but it was unfeasible to list them all. Overall, the manuscript concerns an interesting subject and was based on a large dataset, though some improvements should be addressed before it is published. Please check for the minor concerns, corrections, comments, and suggestions in the Word files.

Author's Rebuttal Letter (19/11/2025).

No rebuttal letter was sent by the authors when submitting the revised version of the manuscript.

Associate editor's decision after peer review (15/12/2025).

Dear Dr. Sversut Dias:

It is a pleasure to accept your manuscript entitled "High pasture land cover reduces modularity of trophic networks from Brazilian stream fish assemblages" in its current form for publication in the *Neotropical Ichthyology*.

Congratulations for the acceptance of your article, and be aware on the following topics:

1. Publication Fee.

NI will charge a publication fee if none of the co-authors is an active SBI member. This measure is essential to strengthen SBI and thus ensure the continuity of our journal, scientific society, and biannual meetings. SBI is not limited to Brazilians but is open to anyone interested in freshwater and marine Neotropical fishes. More details on SBI are available at <https://www.sbi.bio.br>. Please email tesouraria.sbi@gmail.com to confirm whether any of your co-authors is a current SBI member and to activate your SBI membership if needed. Otherwise, if you will cover the publication fee of R\$1.000, please inform us at the same email. For authors outside Brazil, the fee will be converted to US dollars based on the official exchange rate on the date of payment. The only exceptions to this fee are invited articles.

2. Science Communication and Social Media.

NI actively promotes published articles to both academic colleagues and the general public, including science journalists. To support this, we create social media posts and require images and/or videos of fish related to your work. If your article does not include such images, please send a photo of a representative fish species, preferably alive in its natural habitat. If you do not have your own photo, you may provide a link to an online image, along with the source, author, and, if applicable, authorization for its use. We also publish video summaries of articles in Portuguese on our Instagram (@neoichth). We ask you to designate one author to record a short video using a mobile phone, following the attached instructions. The video should include visual materials (photos, graphics) and a script for subtitles to enhance accessibility. Please email the completed material to neoichth@nupelia.uem.br within 30 days. For now, please provide the name and email address of the author responsible for recording the video.

Additionally, if your article is taxonomic in nature and has been submitted to Zoobank, it is your responsibility to update the manuscript's status on Zoobank once it has been published.

All of the above information and materials are mandatory for the publication of your article, including the scientific dissemination component, which is crucial in the current climate of science denial and misinformation. If you have any questions, please feel free

to contact us at neoichth@nupelia.uem.br.

Please respond to this e-mail within five working days to let us know you are aware of all the important points mentioned above.

Thank you for your fine contribution. On behalf of the Editors of the Neotropical Ichthyology, we look forward to your continued contributions to the Journal.

Sincerely,

Dr. Lilian Casatti

Associate Editor, Neotropical Ichthyology

licasatti@gmail.com

Anonymous reviewer #1

Recommendation. Accept

Comments. The authors did a good job improving the manuscript

Anonymous reviewer #2

Recommendation. Accept

Comments. The manuscript in hand is a version revised by the authors, who carefully considered the annotations and corrections that had been suggested. They also provided thorough answers to all comments, justifying their choices in the review process. The authors addressed confounding sentences and improved the overall manuscript structure, which improved manuscript quality and understanding. More importantly, the discussion section was greatly improved. Thus, I congratulate them for their effort and consider the manuscript ready for publication.

Neotropical Ichthyology

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HOW TO CITE THIS ARTICLE

- Nuven DMAS, Quimbayo JP, Rodrigues AA, Gonçalves Júnior JF, Dias MS. High pasture land cover reduces modularity of trophic networks from Brazilian stream fish assemblages. Neotrop Ichthyol. 2026; 24(2):e250149. <https://doi.org/10.1590/1982-0224-2025-0149>



Official Journal of the
Sociedade Brasileira de Ictiologia