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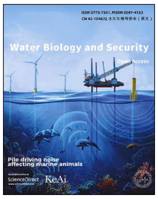

Research Article

Anthropogenic pressures reduce aquatic macroinvertebrate diversity through decreased physical habitat heterogeneity

Diego M.P. Castro[a,*], Gisele M. Santos[a], Diego R. Macedo[b], Tiago C.O. Tourinho[c], Felipe V. Manzano[c], Robert M. Hughes[d,e,1], Marcos Callisto[a]

[a] *Universidade Federal de Minas Gerais, Instituto de Ciências Biológicas, Departamento de Genética, Ecologia e Evolução, Av. Antônio Carlos 6627, Pampulha, CEP 31270-901, Belo Horizonte, MG, Brazil*
[b] *Universidade Federal de Minas Gerais, Instituto de Geociências, Geospatial Analysis and Landscape Management Research Group, Av. Antônio Carlos 6627, Pampulha, CEP 31270-901, Belo Horizonte, MG, Brazil*
[c] *Eletrobras, Av Graça Aranha, 26 – Centro, CEP 20030-000, Rio de Janeiro, RJ, Brazil*
[d] *Amnis Opes Institute, 2895 SE Glenn, Corvallis, OR, USA*
[e] *Department of Fisheries, Wildlife, & Conservation Sciences, Oregon State University, Nash 104, Corvallis, OR, USA*

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ABSTRACT

Habitat heterogeneity creates diverse habitats and resource distributions that affect community structure and dynamics. More environmentally heterogeneous areas are expected to support more diversified biological communities. However, environmental gradients induced by human activities can result in habitat homogenization, ultimately reducing biotic diversity and increasing biotic homogenization. Our objective was to evaluate whether increased anthropogenic disturbance reduces the diversity of aquatic macroinvertebrates by decreasing habitat heterogeneity. We sampled 40 randomly selected stream sites in the Atlantic Forest and Cerrado biomes surrounding one of the largest reservoirs in Brazil. We calculated 36 physical habitat metrics and used taxonomic and functional Hill numbers as response variables. Those Hill numbers indicated that increased anthropogenic disturbance reduced the diversity of aquatic invertebrates by decreasing habitat heterogeneity, for both rare and common taxa. The most-disturbed sites had a higher proportion of fine substrates and substrate embeddedness, but lower riparian vegetation cover. In contrast, the least-disturbed sites had higher proportions of shelters, riparian and channel canopy cover, benthic leaf litter, and pools. Our approach using taxonomic and functional Hill numbers proved effective in evaluating how environmental heterogeneity affects diversity along anthropogenic disturbance gradients. Consequently, this method can be employed by catchment and stream managers to enhance the effectiveness of stream ecosystem rehabilitation efforts.

1. Introduction

Human activities have increased pressure on ecosystems, their natural resources, and biodiversity, jeopardizing goods and services essential for maintaining life on the planet (Brauns et al., 2022; Díaz and Malhi, 2022). The conservation of biodiversity, goods, and ecosystem services is intrinsically linked to the maintenance and availability of water resources in river basins (Callisto et al., 2019; Kim et al., 2024). Although they occupy only 0.01 % of the planet's surface, aquatic ecosystems harbor 9.5 % of all animal species, especially in tropical regions (Barlow et al., 2018). However, aquatic ecosystems are constantly threatened by anthropogenic pressures, such as catchment land use intensification, hydrological modifications, water pollution, riparian vegetation removal, and physical habitat degradation (Dudgeon, 2019; Sundar et al., 2020).

These pressures are globally evident, as human activities continuously fragment natural landscapes, directly and indirectly altering the heterogeneity of the natural environment (Foley et al., 2005). The intensification of these activities generally results in environmental homogenization at various spatial extents, reducing biodiversity, and

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* Corresponding author.
E-mail address: dcastro@ufmg.br (D.M.P. Castro).

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[1] In memoriam – R. M. Hughes deeply discussed and participated in the first round review. After he passed away, we authors decided to celebrate his honor in memoriam.

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simplifying ecosystem structure and functioning (McKinney and Lockwood, 1999; Smart et al., 2006). On the other hand, high levels of habitat diversity are associated with increased diversity of fish and aquatic insects (Moi et al., 2024). However, in some cases, human activities can artificially generate more habitat heterogeneity and increase macroinvertebrate abundance and diversity in highly disturbed sites (e.g., Faria et al., 2023; Ligeiro et al., 2020).

Headwater streams are dynamic environments where spatial and temporal gradients in abiotic and biotic characteristics arise from variations in the diversity of physical habitats (such as substrate and flow types) and the quality of water (such as temperature, nutrient levels, turbidity, and contaminants). These gradients occur naturally and are modified by human activities (Muenchow et al., 2018; Tylianakis and Morris, 2017). Natural examples of gradients in streams and rivers arise from variations in altitude, latitude, or longitudinal position (upstream-downstream), which are associated with differences in vegetation cover, temperature, and other physical and chemical factors. These natural gradients can significantly influence the structure of aquatic communities (Agra et al., 2023; Herlihy et al., 2020; Vannote et al., 1980). On the other hand, human activities in drainage basins have caused substantial changes in water body quality, compromising the persistence and abundance of many aquatic organisms (Ahmed et al., 2022; Herlihy et al., 2020; Su et al., 2021). Moreover, the relative importance of these changes also varies regionally (Herlihy et al., 2020; Martins et al., 2021b; Silva et al., 2018).

Habitat heterogeneity, defined as the variety and complexity of different habitats within a specific area (Stein and Kreft, 2015), plays a crucial role in shaping the structure and dynamics of populations and communities (Agra et al., 2023; Moi et al., 2024; Yang et al., 2015). One of the key ecological hypotheses about habitat or environmental heterogeneity is the shape of the heterogeneity-diversity relationship (HDR), which has been extensively investigated in recent decades (Iacarella, 2022; Seiferling et al., 2014; van Galen et al., 2023). Rooted in the concept of niche differentiation (Chesson, 2000), it is theorized that a more heterogeneous environment can accommodate more species by greater partitioning of niche space, thus suggesting a positive relationship between heterogeneity and species diversity.

Despite the relationship between species diversity and environmental heterogeneity being a well-documented pattern in ecology (Stein et al., 2014), most studies are seldom designed to uncover the underlying mechanisms (Ortega et al., 2018). Furthermore, there is limited evidence supporting the role of habitat heterogeneity in buffering stream ecosystems against anthropogenic disturbances (Moi et al., 2022). Habitat heterogeneity has been evaluated in freshwater ecosystems by measuring various environmental factors, including water flow types, channel morphology, bottom substrate composition, and the abundance of shelters provided by macrophytes and wood debris (Kaufmann et al., 2022; Tokeshi and Arakaki, 2012). Heterogeneous habitats provide a wider array of conditions (such as substrate and flow types) and greater variability of resources (such as coarse particulate organic matter and mosses), consequently enhancing biodiversity (Agra et al., 2021; Boyero and Bosch, 2004; Nessimian et al., 2008). Therefore, more environmentally heterogeneous sites are expected to support more diversified biological communities and greater functional redundancy within communities, as species coexistence is facilitated by the availability of varied resources (MacArthur and MacArthur, 1961; Valladares et al., 2015; Wellnitz and Poff, 2001). Stream ecosystems serve as good model systems for studying this relationship because of their high heterogeneity, which contributes to high biological diversity through the accumulation of species with different environmental requirements (Brown, 2003; Stein et al., 2014). However, environmental gradients created by human interventions can lead to habitat homogenization, which reduces diversity and promotes biotic homogenization (Castro et al., 2018).

Although the heterogeneity-diversity relationship has been extensively studied, the specific impacts of anthropogenic disturbances on this relationship remain underexplored in freshwater ecosystems (Moi et al., 2024). Therefore, gaining insight into how environmental gradients affect community structure and their connections to ecosystem services can deepen our understanding of these systems and inform better strategies for managing ecosystem conditions (Arthington et al., 2010).

Stream habitat heterogeneity is directly influenced by land use and land cover, with reduced habitat diversity being a direct consequence of anthropogenic impacts. However, this relationship may not be evident in highly altered sites, where habitat heterogeneity can even be artificially manipulated and improved through human activities, further highlighting the connection between land use practices and ecological structure. Based on that, we evaluated whether increased anthropogenic disturbance reduces the diversity of aquatic macroinvertebrates by decreasing habitat heterogeneity. We analyzed biological and environmental data from 40 stream sites in the Furnas hydrological unit, which surrounds one of the largest human-made reservoirs in Brazil. We hypothesized that the intensification of catchment and local anthropogenic activities would reduce stream habitat heterogeneity and, consequently, biodiversity. We predicted that higher levels of anthropogenic disturbance would increase the predominance of certain habitat characteristics that diminish habitat heterogeneity, consequently reducing aquatic macroinvertebrate diversity.

2. Methods

2.1. Study area

We sampled 40 stream sites from 1st to 3rd order (Strahler, 1964; 1:100,000 scale map) located in the Atlantic Forest and Neotropical Savanna (Cerrado) biomes in southeastern Brazil. Urbanization, industrialization, and agricultural expansion have led to economic growth and a historic loss and fragmentation of natural habitats in the Atlantic Forest and Cerrado biodiversity hotspots (Laurance, 2009; Rezende et al., 2018; Strassburg et al., 2017). Current estimates of the remaining vegetation cover of the Atlantic Forest in Brazil range from 11 to 16 % (Ribeiro et al., 2022). Approximately 40–55 % of the Cerrado biome has already been converted into agricultural areas (Colli et al., 2020; Sano et al., 2010).

Stream sites were distributed across the Furnas hydrological unit, delimited by the contributing drainage area within 35 km upstream of the Furnas Reservoir (Fig. 1). That distance was chosen due to its relevance to another objective of a sister study: the likely migration distance of small fish between the reservoir and spawning sites, along with project funding limitations. Sites were selected through a probability-based procedure that employed a spatially balanced design (Macedo et al., 2014; Olsen and Peck, 2008). This systematic approach ensures representation across various stream orders (limited to 3rd-to 5th-order reaches) and geomorphological factors such as slope, sinuosity, and channel width, which can influence physical habitat conditions and biological communities. Physical habitat measures and macroinvertebrate samples were collected at the same time in 2023 during the July to September dry season with one visit per site.

2.2. Sampling design and data collection

We marked six equidistant transects at each site to sample aquatic macroinvertebrates and environmental variables (more details in the Habitat Metrics section). We sampled macroinvertebrates using a D-frame kick net (30 cm mouth width, 500 μm mesh size). To establish a sampling area and obtain comparable sample sizes, each kick sample was taken in an area of 30 × 30 cm (0.09 m^2) where the sediment was disturbed for 30 s. Sampling followed a systematic zigzag sequence along the six transects at each site (Peck et al., 2006). We preserved each of the six samples separately in 80 % alcohol and took them to the laboratory for further processing and identification. All macroinvertebrate individuals in each

Fig. 1. Locations of sampling sites in the Furnas hydrological unit.

sample were identified to family using taxonomic keys (Pes et al., 2005; Mugnai et al., 2010; Hamada et al., 2014). We were limited to family-level identifications because species- or genus-levels of identification are not currently possible in Brazil due to insufficient taxonomic knowledge of most macroinvertebrate taxa (Buss and Vitorino, 2010; Godoy et al., 2019). Family-level taxonomy is sufficient for our objectives, with minimal information loss regarding bioassessment compared with genus- or species-level identifications (Carreira-Flores et al., 2024; Godoy et al., 2019; Jones, 2008; Melo, 2005; Piperac et al., 2024; Ruaro et al., 2024; Whittier and Van Sickle, 2010).

2.3. *Anthropogenic disturbance gradient*

To evaluate disturbance gradients at three spatial extents in each site, we used three indices developed by Ligeiro et al. (2013): the Local Disturbance Index (LDI), the Catchment Disturbance Index (CDI), and the Integrated Disturbance Index (IDI). The LDI is calculated from eleven observations that evaluate both the presence and proximity of anthropogenic disturbances within both the stream channel and its riparian zone. These include elements such as buildings, channel revetment, pavement, roads, pipes, trash and landfill, parks and lawns, row crops agriculture, pasture, logging, and mining (Kaufmann et al., 1999). The values are weighted according to the proximity of the observation from the stream channel. Thus, the LDI values quantify how much anthropogenic disturbances directly affect the site's riparian zone and stream channel. The CDI quantifies the potential impact of different land uses within the catchment area of each site. To compute the CDI, land use was first mapped using imagery acquired in July 2023 by the Sentinel-2 satellite's MSI sensor, which provides a spatial resolution of 10 m. The index is then calculated by summing the percentages of different land uses within each catchment, weighted by their estimated impact on aquatic ecosystems, using the formula (Eq. (1)):

$$CDI = (4 \times \%\text{urban}) + (2 \times \%\text{agriculture}) + \%\text{pasture} \qquad \text{(Eq. 1)}$$

The IDI represents a unified, quantitative measure of the overall level of human impact or disturbance affecting stream sites, integrating multiple sources of alteration into a single metric. It is the Euclidean distance between the site and the origin of the disturbance plane created by the LDI and CDI, using the formula (Eq. (2)):

$$IDI = \left[\left(\frac{LDI}{5}\right)^2 + \left(\frac{CDI}{300}\right)^2\right]^{1/2} \qquad \text{(Eq. 2)}$$

The scaling factors (5 and 300) correspond to the maximum observed values for LDI and CDI, respectively, thereby ensuring a balanced contribution of both indices to the final IDI value. Higher values of LDI, CDI, and IDI correspond to greater levels of local, regional and integrated disturbance, respectively (Ligeiro et al., 2013).

2.4. *Habitat metrics*

We used a slightly adapted version of the U.S. Environmental Protection Agency's National Rivers and Streams Assessment field protocol (Callisto et al., 2014; Peck et al., 2006). At each site, we established a 75-m reach, which was subdivided into five equidistant 15-m sections, defined by six cross-sectional transects. Along each transect, we measured channel dimensions—including wetted width, bankfull width, channel depth, and bank height—using a measuring tape and graduated rods. We visually classified substrate type and size at five equidistant points per transect, recorded substrate embeddedness, and assessed the presence of fine sediments. Thalweg depth was recorded at 1.5-m intervals along the main flow path between transects, and flow habitat types (riffles, pools, runs, glides, etc.) were identified visually at the same points. Stream slope was measured using a hand-held clinometer positioned between marked poles, and sinuosity was calculated from compass bearings and the ratio of channel to valley length. In-stream habitat features such as coarse woody debris, macrophytes, undercut banks, boulders, leaf packs, and artificial structures were recorded in a 5-m buffer upstream and downstream from each transect, and their percent cover visually estimated. Riparian vegetation was assessed in 10 × 10 m plots on both streambanks at each transect, where we visually estimated the percent cover of canopy, understory, and ground layer vegetation. Channel canopy cover was measured at the midpoint of each transect using a convex spherical densiometer facing upstream, downstream, and toward each bank. Water velocity was obtained by timing a floating object over a known distance, with multiple replicates near the channel margins and center. All field measurements were used to derive standardized physical habitat metrics following procedures outlined in Kaufmann et al. (1999) and Peck et al. (2006). This approach allowed for a standardized and spatially explicit characterization of environmental heterogeneity across sites.

We then calculated 36 key metrics of physical habitat structure that were important predictors and significantly related to the taxonomic and functional distribution patterns of macroinvertebrate assemblages in several previous Cerrado studies (e.g., Agra et al., 2021; Castro et al., 2017; Castro et al., 2018; Firmiano et al., 2021; Martins et al., 2021a; Silva et al., 2021a, 2021b). At each site, we characterized the physical habitat by channel hydromorphological variables (e.g., thalweg depth, bank angle, channel sinuosity), substrate variables (e.g., % of boulders, % of sand, % of total organic matter, % of large wood), flow variables (e.g. velocity, % of glides, % of pools), riparian cover variables (e.g., % woody cover, % exposed soil, % ground cover), and in-stream habitat variables (e.g., % aquatic macrophytes, % large wood). All site variables were computed following the methods outlined by Kaufmann et al. (1999), who detailed the concepts and analytical procedures for deriving metrics from data obtained through physical habitat field protocols. For each site, we calculated the average or percentage of the measured physical habitat variable across all six transects combined. Thus, we obtained a single value of each environmental metric per stream site.

2.5. *Biological metrics*

Taxonomic diversity corresponded to all taxa recorded at each stream site. Taxonomic diversity was evaluated using Hill numbers calculated in the vegan package (Oksanen, 2019). Hill numbers, also known as the "effective number of species," have been identified as an appropriate index to measure abundance-based taxa diversity (Chao et al., 2014). The Hill series is defined by the order q (D^q), which determines each index's weighting of rare to abundant taxa. D^0 corresponds to observed taxa richness, placing greater emphasis on rare taxa because they are insensitive to relative frequencies (i.e., evenness); D^1 is equivalent to Shannon's entropy exponent, which is weighted towards common taxa; and D^2 is equivalent to the inverse of Simpson's diversity, which is weighted towards highly abundant taxa (Tuomisto, 2010). Each point in the series thus offers complementary information on taxa richness and evenness.

We used a trait database fully described in Firmiano et al. (2021). This database contains biological traits and their respective trait categories describing taxa profiles in terms of morphology, life cycle, feeding behavior, and resilience or resistance to natural or anthropogenic disturbance. Six functional traits were considered: respiration, voltinism, feeding habits, locomotion, flexibility, and body shape. The affinity of each taxon for each trait category was determined using a fuzzy coding approach, ranging from 0 (no affinity) to 3 (maximum affinity). Affinity scores were standardized so that their sum for a given taxon and trait equaled 1 (Chevenet et al., 1994). This methodology allowed us to account for different types and levels of available information. The six selected traits were chosen because they were sufficiently documented in the literature, could be reliably compiled, and have been shown by several studies to be responsive to local stressors (e.g., Agra et al., 2021; Castro et al., 2017; Amaral et al., 2024; Castro et al., 2025).

Functional diversity (FD) was assessed using functional diversity Hill numbers as described by Chao et al. (2019). This set of indices facilitates the calculation of FD based on the pairwise distances between taxa and their respective assemblage weights. The indices represent the effective number of taxa that are functionally equally distinct (or virtual functional groups) and can be directly compared to taxonomic Hill numbers, including taxa richness (Magneville et al., 2022).

Integrating functional diversity with taxonomic diversity highlights the role of habitat heterogeneity in shaping biotic communities. Functional diversity indices capture trait-based variations that directly influence ecosystem processes, allowing for a deeper understanding of how diverse habitats support a wider range of ecological functions (Cadotte et al., 2011). By linking habitat heterogeneity to functional traits, this approach underscores the importance of maintaining structural and environmental complexity to preserve both biodiversity and ecosystem resilience in the face of anthropogenic disturbances (Agra et al., 2021).

2.6. *Data analyses*

Habitat heterogeneity was initially described by 36 environmental metrics. Each metric was categorized based on its potential to increase or decrease habitat heterogeneity (Table S1). To avoid multicollinearity among them, final analytical metrics were selected in three screening steps. (i) Metrics with values of 0 in >80 % of sites were excluded, as were (ii) those that had coefficients of variation (CV) < 0.2. (iii) We then eliminated all but one of any strongly correlated metrics ($r > 0.70$), retaining the metric that we assumed was most biologically meaningful. This decision was based on previous studies conducted in similar tropical stream systems, expert knowledge of the metrics, and relevance to macroinvertebrate ecology. After this procedure, 21 metrics were retained. A Variance Inflation Factor (VIF) analysis was performed on the environmental predictors, and all variables exhibited VIF values < 10, indicating low multicollinearity (Montgomery et al., 2021). We then performed a principal component analysis (PCA) on the retained scaled metrics to determine which were responsible for generating the most variation among the sites. Permutation-based PCA (Camargo, 2022) was implemented to evaluate the overall significance of each principal component axis. The analysis revealed that the first four principal component axes were significant and thus were selected. Lastly, we used the function *dimdesc* in the FactoMineR package (Lê et al., 2008) to point out the most characteristic variables according to each dimension obtained by the PCA. The PCA axes were used as proxies for habitat heterogeneity because they synthesize the variability in environmental metrics.

To assess the gradient of environmental disturbance across sites, we used PERMANOVA and PERMDISP analyses. Initially, we delineated the boundaries of least- and most-disturbed sites using quantiles derived from the Integrated Disturbance Index (IDI) distribution (Castro et al., 2018). Sites below the 25th quantile (IDI <0.375) were classified as least-disturbed (reference), whereas those above the 75th quantile (IDI >0.575) were categorized as most-disturbed (impaired). Sites falling within the interquartile range were designated as intermediate. Klemm et al. (2003) also used the 25th and 75th percentiles for scoring macroinvertebrate MMI metrics. To ensure comparability across different metric scales, all environmental metrics were standardized by using the range method to rescale values between 0 and 1. A PERMANOVA analysis based on Hellinger distance was then used to assess differences in environmental metrics among the three impact categories. Subsequently, we assessed environmental heterogeneity within each impact category using PERMDISP analysis based on centroid distance, which calculates the average distance of sites to their respective centroids as a measure of environmental heterogeneity. The significance of PERMDISP was determined through analysis of variance (ANOVA).

To test whether the selected environmental metrics influenced the taxonomic and functional diversities, we used Generalized Linear Models (GLM's) with a Gamma distribution, where the response variables were the taxonomic and functional Hill diversity indices, and the explanatory variables were the four PCA axes selected. The significance of the constructed models was tested using an Analysis of Deviance (*F*-test for Gamma distribution). Model residuals and overdispersion were checked using the DHARMa package (Hartig, 2022). All analyses were performed using the vegan package (Oksanen, 2019) in R version 4.3.2 (R Core Team, 2023).

3. Results

We collected a total of 24,577 organisms distributed in 20 orders and 75 families. The Chironomidae (Diptera) was the most abundant family, followed by Simuliidae (Diptera), Baetidae (Ephemeroptera), and Hydropsychidae (Trichoptera). Across the gradient of sites examined in this study, Integrated Disturbance Index (IDI) values varied from 0.01 (observed in protected areas) to 0.79 (urban sites). Consequently, the sampled sites encompassed a broad spectrum of anthropogenic disturbances. Among the 40 sampling sites, based on IDI, 10 were classified as least-disturbed (reference), 20 as intermediate, and 10 as most-disturbed (degraded).

3.1. Environmental characterization

The PERMANOVA on the distance matrix on environmental variables showed a significant difference among disturbance classes (pseudo

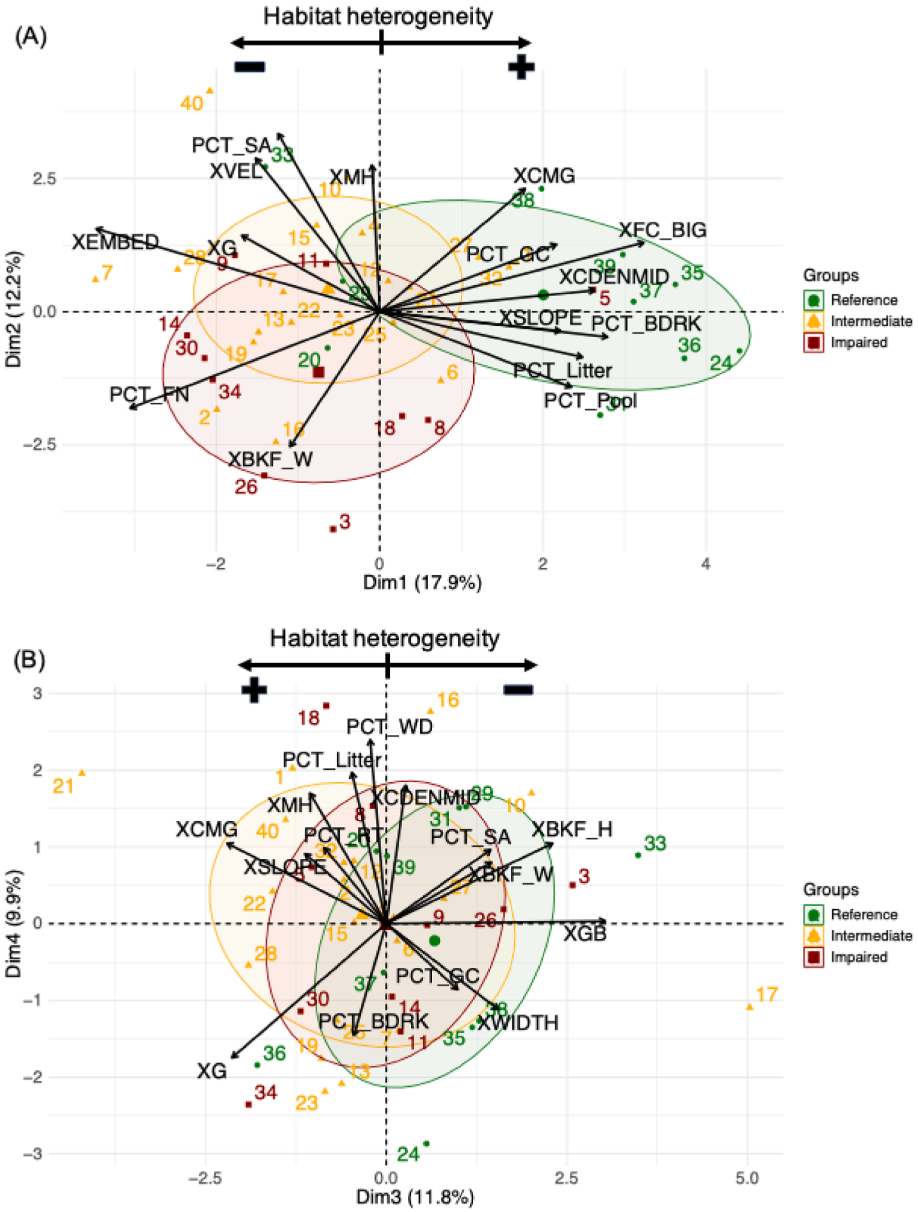

Fig. 2. (**A**) Representation of physical habitat variables on PCA axes 1 and 2. (**B**) Representation of physical habitat variables on PCA axes 3 and 4. Confidence interval ellipses = 70 %. Each point represents a site categorized as Reference, Intermediate, or Impaired. The arrows represent the direction and strength of correlation for each physical habitat variable with the PCA axes. The percentage values on the axes indicate the proportion of variance explained by each principal component.

– $F_{(2,37)} = 2.74$, $p < 0.0001$). However, the PERMDISP ($F_{(2,37)} = 0.55$, $p = 0.58$) test did not show significant differences in dispersion among those three disturbance classes. That indicates that differences among disturbance classes partly result from changes in the composition of physical habitat metrics amongst those classes, as well as indistinct class boundaries.

The first four PCA components explained 17.9 %, 12.2 %, 11.8 %, and 9.9 % of the total variation in the environmental metrics data, so these four components combined explained 51.7 % of the variation. Considering the first PCA axis, the least-disturbed sites (reference) had a positive relationship with increasing levels of large shelters (XFC_BIG), percentage of bedrock (PCT_BDRK), average mid-channel canopy cover (XCDENMID), percentage of coarse leaf cover over the channel bottom (PCT_Litter), and percentage of pools (PCT_Pool) (Fig. 2A). The first PCA axis also indicated a negative relationship with increasing substrate embeddedness (XEMBED), percentage of fines (PCT_FN), and percentage of riparian ground-layer vegetation cover (XG). The second PCA axis was positively related to the percentage of substrate sand (PCT_SA), water velocity (XVEL), herbaceous mid-layer canopy cover (XMH), and total riparian vegetation cover (XCMG). PC-2 was negatively related to bankfull width (XBKF_W), percentage of substrate fines (PCT_FN), and percentage of wood (PCT_WD) (Fig. 2A). The third PCA axis was positively related to the amount of exposed riparian soil (XGB), bankfull height (XBKF_H), wetted width (XWIDTH), and percentage of substrate sand (PCT_SA). In contrast, riparian ground-layer vegetation cover (XG) and total riparian cover (XCMG) were negatively related to the third axis. Finally, the fourth PCA axis was positively correlated with the percentage of wood (PCT_WD), percentage of coarse leaf cover over the channel bottom (PCT_Litter), midchannel canopy cover (XCDENMID), and herbaceous riparian mid-layer canopy cover (XMH). PC-4 was negatively correlated with ground cover (XG) and percentage of channel bedrock (PCT_BDRK) (Fig. 2B).

Hence, the PCA components represented a gradient of habitat heterogeneity, with increasing scores indicating more diverse habitats in the first and fourth PCs and decreasing scores reflecting more habitat complexity in the third PC. For the second PC we did not observe a gradient. The most-disturbed sites had higher proportions of fine substrates, substrate embeddedness, and lower riparian vegetation cover. In contrast, the least-disturbed sites had higher proportions of large shelters (i.e., large wood, boulders, overhanging banks), higher proportions of riparian and channel canopy cover, abundant leaf litter on the channel bottom, and more pools.

3.2. *Taxonomic and functional diversities*

The first PCA axis showed a positive correlation with taxonomic diversity Hill indices of orders $q = 0$ ($R^2 = 0.36$, $p < 0.001$) and $q = 1$ ($R^2 = 0.11$, $p = 0.034$) where lower values were observed in the most-disturbed sites and higher values were observed in the least-disturbed sites (Fig. 3). Hill diversity indices of order $q = 0$ placed greater emphasis on rare taxa, whereas indices of order $q = 1$ placed greater emphasis on common taxa. The other three PCA axes showed no correlation with the taxonomic diversity indices.

Regarding the functional diversity Hill indices, the first PCA axis exhibited a positive correlation with the indices of orders $q = 0$ ($R^2 = 0.18$, $p = 0.0065$), $q = 1$ ($R^2 = 0.15$, $p = 0.014$), and $q = 2$ ($R^2 = 0.14$, $p = 0.015$). Additionally, PC-3 displayed a negative correlation with the indices of orders $q = 0$ ($R^2 = 0.15$, $p = 0.012$) and $q = 1$ ($R^2 = 0.25$, $p = 0.0009$) (Fig. 4).

4. Discussion

Our findings demonstrate that anthropogenic disturbances measured at local and regional spatial extents affect the relationships between

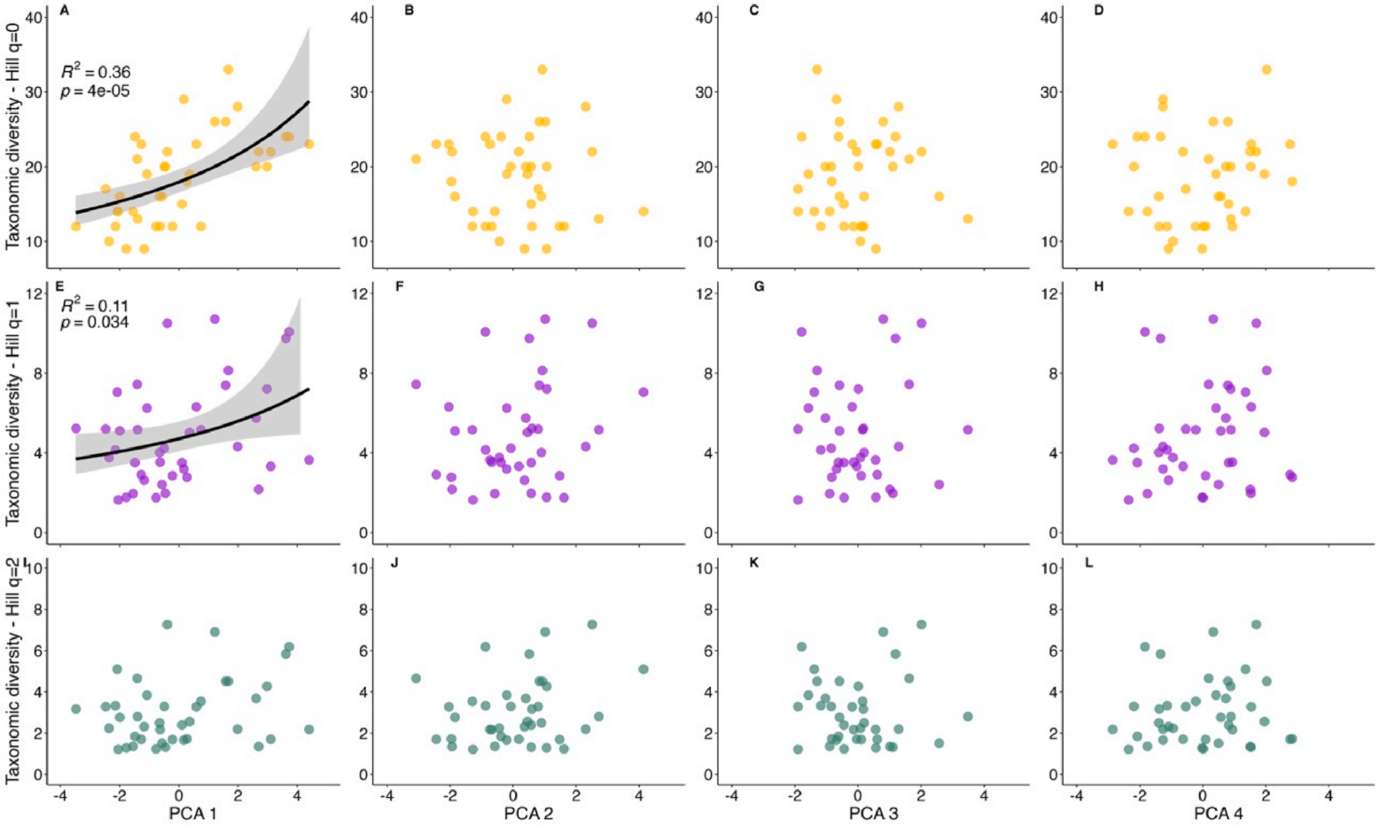

Fig. 3. Relationships between environmental metrics (PCA axis) and Taxonomic Diversity Hill Indices. The dark line indicates predicted values; dots indicate observed values, and the gray band indicates 95 % confidence intervals. Regression lines are shown only for relationships that were statistically significant ($p < 0.05$).

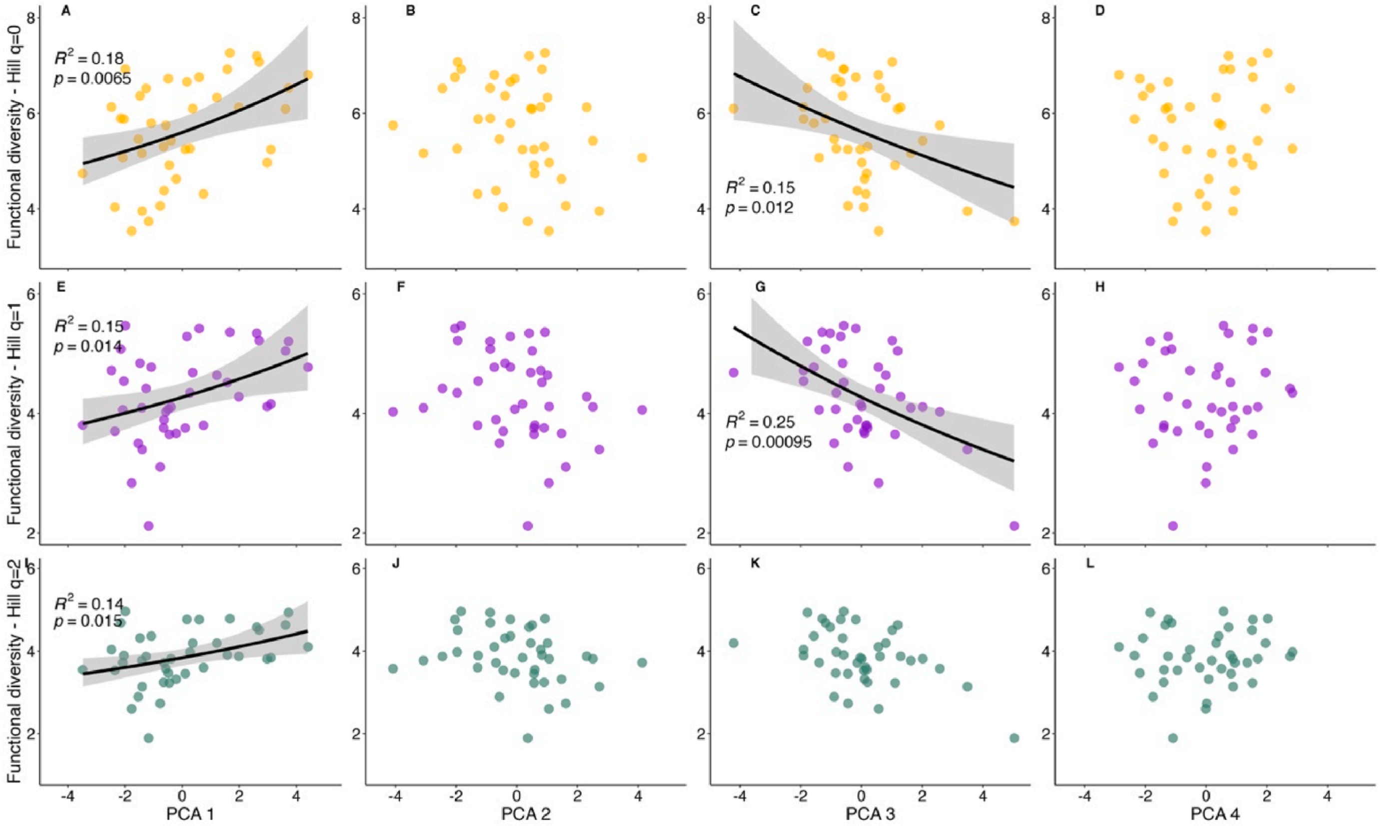

Fig. 4. Relationships between environmental metrics (PCA axis) and Functional Diversity Hill Indices. The dark line indicates predicted values; dots indicate observed values, and the gray band indicates 95 % confidence intervals. Regression lines are shown only for relationships that were statistically significant ($p < 0.05$).

habitat heterogeneity and taxonomic and functional diversities. Our hypothesis was corroborated, as we observed that increased anthropogenic disturbance, as measured by IDI, CDI, and LDI, reduced the taxonomic and functional diversities of aquatic macroinvertebrates by decreasing habitat heterogeneity, especially for rare and common taxa and traits.

The IDI, CDI, and LDI directly determined the physical habitat metrics and revealed a distinct separation between areas with higher and lower heterogeneity. Reference environments were more strongly associated with metrics indicating greater habitat heterogeneity (e.g., proportion of large shelters, percentage of litter, and pools), whereas the impaired environments were associated with metrics indicating habitat homogenization (e.g., higher proportions of fine substrate, substrate embeddedness, and more riparian ground cover than canopy and mid-layer cover). These findings show that anthropogenic impacts influence not only the composition but also the heterogeneity of stream environments, significantly affecting the diversity and structure of aquatic macroinvertebrate assemblages.

Human activities alter the features of local fluvial habitats, including the presence and composition of riparian vegetation and the characteristics and granulometry of streambed substrates (Bylak and Kukuła, 2022; Kaufmann et al., 2022; Pusey and Arthington, 2003). These alterations directly influence the composition of local macroinvertebrate and fish assemblages (Kaufmann et al., 2022; Reynoldson et al., 2001; Moi et al., 2024). In the current investigation, the substantial variations in habitat characteristics among the three disturbance classes significantly impaired the taxonomic and functional diversity observed.

Geological factors and soil composition naturally influence the quantity and types of sediments found in streams and exhibit a negative correlation with the richness and abundance of macroinvertebrates. This relationship arises because an increase in fine sediment content diminishes the available area for shelter and breeding, negatively affecting macroinvertebrate assemblages (Bryce et al., 2010; Downes et al., 2006; Jones et al., 2012). Degraded sites exhibited higher proportions of fine sediments and substrate embeddedness, which resulted in less substrate heterogeneity. Diverse substrates foster the development of varied benthic communities and offer refuge opportunities, whereas uniform substrates expose organisms to greater risks of physical disturbances, such as high current velocity and shear stress (Beisel et al., 2000; Milesi et al., 2016). Furthermore, the increased predominance of fine substrate is correlated with erosion processes, which are frequently intensified by the removal of riparian and catchment vegetation (Wood and Armitage, 1997).

Flow diversity (i.e., the presence of pools, glides, and rapids in a site) is closely linked to substrate diversity (Boyero, 2003), and the interplay between these factors contributes to the formation of distinct habitat patches within riverine landscapes. These habitat patches are interpreted uniquely by different aquatic insect taxa (Boyero and Bosch, 2004; Wiens, 2002).

More rare and common taxa were associated with sites with larger shelters, higher percentages of bedrock, higher riparian and channel canopy cover, and higher proportions of coarse leaf cover over the channel bottom. The percentage of coarse leaf cover over the channel bottom indicates the amount of litter available in the streambed for use by macroinvertebrates as shelter, substrate, and food (Ligeiro et al., 2020). Wooded riparian zones play a crucial role in maintaining aquatic ecosystem ecological conditions and trophic dynamics (Castro et al., 2016; Gregory et al., 1991; Kaufmann et al., 2022). Alterations such as replacement, reduction, or removal of riparian vegetation cover often result in impaired physical habitat, hydromorphological features, and water quality (Gregory et al., 1991; Pusey and Arthington, 2003; Ferreira et al., 2012), which, in turn, can lead to more homogeneous macroinvertebrate assemblages (Castro et al., 2018).

We observed reductions in functional Hill indices in streams with less canopy cover or without riparian vegetation, indicated by bare ground cover (XGB). Reduced canopy cover and riparian vegetation lead to declines in key stream functions, like organic matter processing (LeRoy et al., 2023; Vannote et al., 1980). Furthermore, we observed an inverse relationship between functional Hill indices, stream size, and aquatic biodiversity. Stream size plays a pivotal role in shaping aquatic biodiversity. Generally, a positive association between stream size and aquatic biodiversity is observed (McGarvey and Terra, 2016; Vannote et al., 1980). However, considerable variation exists in the mechanisms underlying this relationship (Vander Vorste et al., 2017).

Increased functional richness implies that taxa fill more niches. Higher functional diversity reflects greater variation in species trait values, which leads to a wider array of ecological functions and potentially enhances the resilience of these functions to human impacts or environmental stressors (Mouillot et al., 2013). In our study, functional richness, in terms of rare, common, and abundant species, was increased in the least-disturbed sites, suggesting that many niches were occupied and the species were well distributed. In contrast, the most-disturbed sites showed reduced functional richness. Also, sites with lower functional richness, especially fewer rare species, may indicate functional homogenization, reducing ecosystem resilience and resistance to environmental changes (Leitão et al., 2016; Olden et al., 2004). Environmental variables act as filters on these traits, limiting the occurrence of sensitive taxa and facilitating the occurrence of tolerant taxa. Our approach using taxonomic and functional Hill numbers proved to be effective in evaluating how environmental heterogeneity affects diversity along gradients of anthropogenic disturbances.

Although we found significant relationships between environmental heterogeneity and aquatic macroinvertebrate diversity, our models showed relatively low explanatory power (R^2 0.11 to 0.36). Different mechanisms may explain the diversity-heterogeneity relationship in the field (Stein and Kreft, 2015). Various ecological factors, including natural variability, spatially extensive influences, competitive interactions, unmeasured variables, and stochastic events, contribute to shaping the structure of biological assemblages (McCabe and Gotelli, 2000; Perez Rocha et al., 2018), in addition to environmental heterogeneity. Consequently, our models' relatively lower explanatory powers could be attributed to the influence of these multifaceted ecological factors alongside habitat heterogeneity.

Furthermore, seasonality can be an important factor in structuring physical habitats and, consequently, aquatic communities. While seasonal changes—particularly during the wet season—can indeed alter both physical habitat structure and biological communities, evaluating temporal variation was beyond the scope of this study. We restricted our sampling to the dry season in order to minimize variability due to seasonal flow regimes and ensure consistency across sites (Hughes and Peck, 2008). According to Moya et al. (2011), physical habitat characterization should preferably be conducted when natural variability is minimal, when sites are accessible (i.e., not flooded, to avoid risks to field teams), and when human disturbances are more easily detected. During the dry season, more stable flows result in lower natural variability, greater homogeneity in species distribution, easier site access, and lower risk to field teams (Fierro et al., 2021). Additionally, despite the marked climatic seasonality in tropical regions, long-term studies have not shown significant seasonal differences in macroinvertebrate communities (Feio et al., 2015).

Understanding how human pressures alter the functional composition of biological assemblages can enhance our ability to predict patterns and processes in freshwater ecosystems. Moreover, this knowledge can assist in developing tools that supplement traditional assessments for informing management actions and conservation initiatives (Jonsson et al., 2017; Pallottini et al., 2017). In a context where freshwater ecosystems face substantial threats from anthropogenic disturbances (Collen et al., 2014), our findings offer insights into the mechanisms by which tropical stream macroinvertebrate assemblages respond to varying gradients of habitat heterogeneity across three differing levels of anthropogenic disturbance. However, there is a paucity of studies employing Hill functional diversity indices to investigate anthropogenic impacts on aquatic ecosystems despite the potential of this approach for biomonitoring and stream conservation. Incorporating functional diversity and habitat heterogeneity measures holds promise for advancing biomonitoring and bioassessment efforts and stream conservation. By examining how human activities influence biological assemblage functional and taxonomic composition via habitat heterogeneity, researchers and conservationists can develop more comprehensive frameworks for understanding and managing freshwater ecosystems.

CRediT authorship contribution statement

Diego M.P. Castro: Writing – review & editing, Writing – original draft, Formal analysis, Data curation, Conceptualization. **Gisele M. Santos:** Writing – review & editing, Data curation, Conceptualization. **Diego R. Macedo:** Writing – review & editing, Formal analysis, Data curation, Conceptualization. **Tiago C.O. Tourinho:** Writing – review & editing, Validation, Conceptualization. **Felipe V. Manzano:** Writing – review & editing, Validation, Conceptualization. **Robert M. Hughes:** Writing – review & editing, Validation, Conceptualization. **Marcos Callisto:** Writing – review & editing, Validation, Supervision, Funding acquisition, Conceptualization.

Ethical approval

This study did not require ethical approval as it did not involve human participants or sensitive data. Field surveys were conducted based on collection permit number (IEF-10327-6 and SISBIO-10.635-7).

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests. The authors declare that Tiago Tourinho and Felipe Manzano are affiliated with Eletrobras, the research presented in this paper was conducted impartially and independently, with the company having no influence over the study's design, data collection, analysis, or writing of the manuscript. All opinions, findings, and conclusions are solely those of the authors and do not necessarily reflect the views of their company. Marcos Callisto is an editorial board member for *Water Biology and Security* and was not involved in the editorial review or the decision to publish this article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.watbs.2025.100496.

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