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Anthropogenic impacts influence the functional traits of Chironomidae (Diptera) assemblages in a neotropical savanna river basin

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Abstract Increased demands for water affect its quality and availability and threaten biodiversity. In freshwaters, the Chironomidae (Diptera) represents ~ 50% of macroinvertebrate individuals and have great potential to improve ecological assessment tools. Incorporating trait-based approaches in those tools can further improve how we assess the effects of human disturbances on aquatic macroinvertebrate assemblages. Given that chironomid genera have different degrees of sensitivity to anthropogenic disturbances, we expected that composition, structure and functional characteristics of chironomid genera would be negatively affected by anthropogenic disturbances in a neotropical savanna river basin. We used nine traits in 32 categories related to Chironomidae functional roles. Out of 6147 individuals distributed in three subfamilies, we identified 52 chironomid genera collected from 30 randomly selected stream sites. The index of functional divergence was lower in places with greater anthropogenic disturbance of riparian vegetation. A RLQ matrix analysis revealed a significant relationship between genera abundance and environmental variables as well as with biological traits. We observed a positive relationship between Tanypodinae, which are mainly engulfer predators, with average embeddedness, % sand and catchment pasture. Three Chironomidae genera (*Stenochironomus, Endotribelos* and *Beardius*) were positively related to miner habit, herbivore feeding strategy and larger body size. We found that physical habitat structure and food resources were the

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most important factors structuring Chironomidae assemblages in the study sites and that chironomid genera were effective for assessing basin ecological status.

Keywords Traits · Macroinvertebrates · Monitoring · Ecological assessment · Cerrado · Bioindicators

Introduction

Rivers are important because they provide ecosystem services, such as water supply for domestic, industrial and agricultural use, power generation, navigation and recreation (Callisto et al. 2019b). In addition, they are home to a great diversity of species (Strayer and Dudgeon 2010). Increasing demands for water affects its quality and availability and threatens aquatic biodiversity (Gangloff et al. 2016; Reid et al. 2018). Important steps for preserving water quality and maintaining biodiversity are identifying human pressures and stressors, and understanding how they affect the conditions that favor maintaining biodiversity (Sánchez-Bayo and Wyckhuys 2019). In some regions, this becomes particularly important, such as in the neotropical savanna (Cerrado). Although this biome is home to important springs and hydrographic basins, housing high biodiversity and endemism and covering 2 million km^2, it is one of the most threatened biomes in South America (Strassburg et al. 2017; Latrubesse et al. 2019).

Those threats cause the simplification of aquatic habitats, such as the homogenization of the riverbed by siltation, which alters the structure of aquatic communities (Collen et al. 2014; Agra et al. 2021). Benthic macroinvertebrate assemblages respond to environmental changes resulting from anthropogenic activities, which is why they are commonly used in aquatic environmental assessment studies (Karr and Chu 1999; Ruaro et al. 2020). These organisms exhibit preferences regarding food acquisition and type of trophic resources, physical habitat preferences and water quality (Ferreira et al. 2015), and they respond to aquatic ecosystem disturbances through changes in their structure, composition and function. Those characteristics give biological indicators an advantage over traditional water quality assessments, which do not detect the effects of altered flow regimes and physical habitats (Karr 1981). Studies that use biological indicators and assessments of site and landscape variables have been more robust and have better responses (Roque et al. 2010; Herlihy et al. 2020).

In freshwaters, the Chironomidae represent ~ 50% of macroinvertebrate assemblage individuals (Serra et al. 2016) and have great value as bioindicators because they are widely distributed, taxonomically and functionally diverse, and responsive to environmental changes (Rosenberg 1992; Puntí et al. 2009; Nicacio and Juen 2015). Chironomids play fundamental roles in processing organic matter, scraping leaf detritus (Callisto et al. 2007), consuming fine particles of organic matter (Callisto and Graça 2013) and transferring energy and nutrients to the invertebrates, fish and birds that prey upon them (Serra et al. 2016). Despite their ecological importance and diversity in most freshwater ecosystems (Nicacio and Juen 2015), chironomid larvae are usually only identified to family or subfamily in ecological studies (Poff et al. 2006). However, if identified to genus, they have the potential to improve the signals provided in ecological assessments, because of the diversity of this family that includes both tolerant and sensitive genera (Morais et al. 2010; Serra et al. 2016). Although chironomids have great potential as bioindicators, their functional responses remain little explored in the neotropics (Gomes et al. 2018; Saulino and Trivinho-Strixino 2018a, b; Jovem-Azevêdo et al. 2019; Pereira et al. 2020). This is because their identification to genus is difficult and time-consuming, especially in tropical aquatic ecosystems (Rosenberg 1992; Roque et al. 2010).

Trait-based approaches have been successfully used to assess the effects of anthropogenic disturbances on aquatic macroinvertebrate assemblages (Dolédec and Statzner 2010; Kuzmanovic et al. 2017; Castro et al. 2018; Firmiano et al. 2021), including some focused on Chironomidae assemblages (Serra et al. 2016, 2017; Jovem-Azevêdo et al. 2019). Traits are generally defined as any measurable characteristics at the individual level that directly or indirectly affect general fitness or performance (Violle et al. 2007). Change in performance can affect population demographics, which in turn can affect the structure and dynamics of the community and the functioning of the ecosystem (Villéger et al. 2008). Different environmental factors act as filters by selecting species with a set of traits that determine the ability of individuals to coexist in a local community and allow them to persist under specific environmental conditions (Poff et al. 2006; Castro et al. 2018). Anthropogenic stressors are additional environmental filters that can alter the expected functional structure of assemblages observed under natural conditions (Floury et al. 2017). Anthropogenic disturbances can cause instability in habitat structure and select organisms that have specific functional characteristics and high abundance, such as resistant and generalist taxa (Poff 1997; Statzner and Bêche 2010; Li et al. 2019). Identifying assemblage traits filtered by specific environmental conditions enable mechanistic understanding of cause–effect relationships, indicating the stressors most likely responsible for biological impairment (Berger et al. 2018; Firmiano et al. 2021). One of the tools used is the functional diversity approach, which quantifies the value and range of characteristics of the organism that influence its performance, and therefore ecosystem functioning. Furthermore, considering that traits are stable across large spatial extents and natural environmental gradients, they offer a more reliable assessment of ecological condition than taxonomic composition, which varies naturally even within small spatial extents (Dolédec et al. 1996; Mouillot et al. 2013; Chen et al. 2019).

Despite the advantages of using this approach, knowledge gaps remain regarding how Chironomidae functional traits relate to specific environmental characteristics arising from anthropogenic stressors. Thus, we wanted to know which chironomid genera are effective for assessing ecological status in Cerrado streams and to understand how anthropogenic stressors affect traits and shape the taxonomic and functional structure of Chironomidae assemblages in neotropical streams. Given that chironomid genera have different degrees of sensitivity to anthropogenic disturbances, we hypothesized that the taxonomic and functional structure of Chironomidae genera would be negatively affected by the anthropogenic disturbances identified in the watershed. We expected to find trait combinations that are selected by specific stressors acting as environmental filters and to identify which stressors negatively affect the Chironomidae assemblages.

Methods

Study area

The Pandeiros River basin is in the northern region of the state of Minas Gerais, Brazil, in the Cerrado biome and has an area of 3960 km^2 (Fig. 1). Occurring in an area of "Special Biological Importance," it is a unique environment, having shrubby wetland, marginal lagoon and palm swamp complexes (Azevedo et al. 2009). The basin has a tropical savanna climate, with mean annual temperatures of 22 °C, annual precipitation close to 1000 mm and a water deficit between April and September (Alvares et al. 2013). Therefore, it is an international priority area for biome conservation (Drummond et al. 2005). Most of the basin area (85.7%) is part of the Rio Pandeiros State Environmental Protection Area (Instituto Estadual de Florestas 2019). It has less anthropogenic disturbance than other basins in the biome (Macedo et al. 2018; Callisto et al. 2019a,), making it fundamentally important for revitalizing the São Francisco River basin (Azevedo et al. 2009).

Survey design and physical habitat

The sample sites were selected through use of spatially balanced procedures following a random and systematic survey design, according to the method used by the USEPA (United States Environmental Protection Agency) in its National Rivers and Streams Survey (Olsen and Peck 2008). We sampled 30 sites at the beginning of the dry period (April to June 2016) in 3rd to 5th order wadeable streams (Strahler 1957). Site lengths were proportional to 40 × the mean width of each site, with a minimum length of 150 m. In each site, 11 transverse transects (perpendicular to the channel flow) were established defining 10 sections, where physical habitat measurements were taken (Peck et al. 2006; USEPA 2020).

The physical habitat metrics were calculated based on Kaufmann et al. (1999). From a list of potential anthropogenic stressors and pressures assessed by the physical habitat protocol and satellite images, plus results from other Cerrado studies, we selected 12 environmental indicators. The 10 local site variables were natural cover, leaf bank cover, canopy cover over the channel, riparian canopy cover, channel slope, % sand substrate, % substrate embeddedness, % fine

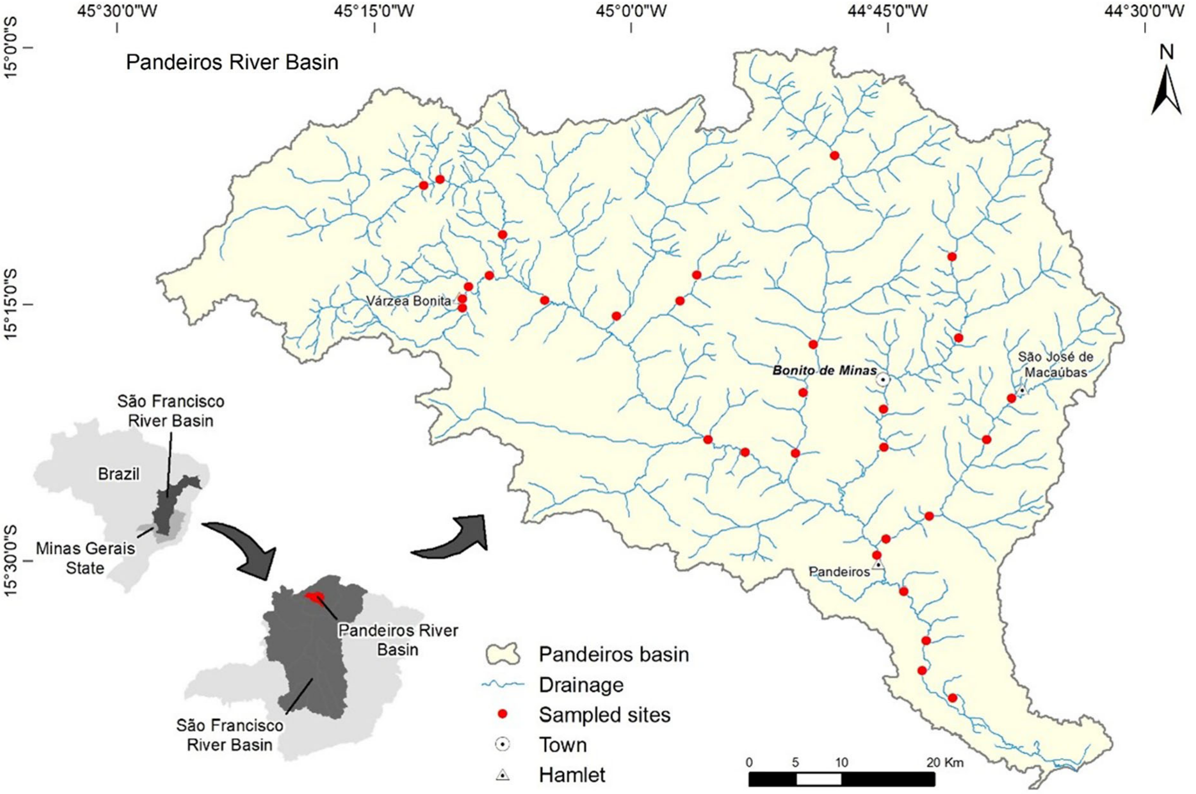

Fig. 1 Location of the Pandeiros River basin and the sample sites

substrates (< 16 mm), average site depth and W1_hall. W1_hall (Riparian Human Disturbance Index) is the proximity weighted total of anthropogenic pressures observed in the channel and riparian zone. We also determined two catchment disturbance indicators: % catchment agriculture and % catchment pasture.

Catchment anthropogenic pressures

We used digital land-use and land-cover maps for measuring catchment pressures. The quantification of types of land use and cover was carried out using supervised classification of digital images, whereby classes are assigned to the pixels of the satellite images, creating homogeneous patterns to which different classes of land use and cover are associated (Santos et al. 2017). We used 2016 imagery from the Landsat-8 satellite, sensor OLI, orbit scene 219/71 and 219/70 made available by Instituto Nacional de Pesquisas Espaciais (INPE, http://www.dgi.inpe.br). The anthropogenic land-use classes included urban areas, row crop agriculture and pasture and were calculated as the percent of each class in the total site catchment, as described in Macedo et al. (2014).

Integrated anthropogenic pressures

For quality assessments across a gradient of environmental conditions, it is necessary to establish reference conditions for comparison and standardization (Stoddard et al. 2008). We used the concept of "least disturbed" or minimally disturbed (Stoddard et al. 2006; Martins et al. 2018), because there were no pristine sites in the basin (Hughes et al. 1986). To identify these sites, we used IDI (*Integrated Disturbance Index*) scores that were calculated from local anthropogenic pressures (LDI—*Local Disturbance Index*) and catchment pressures (CDI—*Catchment Disturbance Index*). The CDI was based on the % of human land uses in the site's total catchment, weighted by the potential degradation that each land-use class has on aquatic ecosystems (CDI = 4x% urban + 2x% agricultural + % pasture) (Ligeiro et al. 2013). The LDI (W1_hall) summarizes the amount of anthropogenic disturbances observed in the channel and the riparian zone for 11 types of disturbances. The disturbances were walls/dikes/revetments, buildings, pavement, roads/railroads, pipes, landfills/trash, parks/lawns, row crops, pasture/range/hay fields, logging operations and mining activities. Each disturbance was assessed on both sides of the channel and at each of the 11 transects (Ligeiro et al. 2013).

Chironomidae sampling

We collected chironomids at each of the 11 transects (Peck et al. 2006) per site by use of a D-frame kick-net (500 µm mesh, 0.9 m^2 area). Each sample was placed in a plastic bag and fixed with 50 ml of formaldehyde. The samples were taken to the UFMG (Universidade Federal de Minas Gerais) Benthos Ecology laboratory, where they were washed on a 500 µm mesh screen. The washed material was placed in transparent trays on a light box, and each chironomid individual was separated and later identified to genus (Trivinho-Strixino 2011; Hamada et al. 2019). Each individual was photographed in a stereomicroscope (Leica M80) equipped with a digital camera (Leica IC 80 HD). The length of each photographed specimen was measured using Motic Image Plus 2.0 software. All specimens were deposited in the Reference Collection of Benthic Macroinvertebrates at the UFMG Institute of Biological Sciences.

Chironomidae traits

Traits that are associated with species morphology, behavior and life history strategies were used for analyzing the functional structure of the Chironomidae assemblages (Armitage et al. 1995; Trivinho-Strixino 2011). The trait categories were based on studies carried out in the neotropics (Butakka et al. 2016; Saulino et al. 2017; Jovem-Azevêdo et al. 2019; Pereira et al. 2020), and when not available, the search was expanded to studies carried out in other locations (USEPA 2012; Serra et al. 2017) (Table 1).

Nine traits in 32 categories related to the functional role of genera were used. Body size was obtained by direct measurement of all individuals, from the cephalic capsule to the last segment of the body, excluding the cephalic and terminal appendices. Then, individuals were grouped into three body size classes (Table 1). This procedure was done to have a better understanding of which body size classes, if any, are mainly affected by a particular environmental variable. Regarding feeding or trophic habits, the larvae were divided into five classes according to their food preferences and eating habits. The trophic food groups are associated with the organic matter available to the species, allowing us to infer the trophic dynamics in ecosystems. The feeding strategy categories were analyzed based on the size and type of organic particles ingested and reflect the adaptation of genera to capture available food, which varies with the taxonomic composition of the assemblages (Tomanova et al. 2006). The trait table is given in Supplementary Material 1.

Data analyses

We first computed the functional distance between each pair of species. The dimensionality of the functional space was defined from a principal coordinate analysis (PCoA) based on a Gower distance matrix. We kept the first four PCoA axes after testing the quality of the functional space (Maire et al. 2015). Then, we calculated four functional diversity indices using the relative abundance of taxa in each trait category. (1) Functional richness (FRic) represents the range of functional strategies within an assemblage. FRic is the minimum convex hull occupied by an assemblage in the functional space defined by species traits (Villéger et al. 2008). (2) Functional divergence (FDiv) represents how abundance is spread along a functional characteristic axis within the range occupied by the assemblage. Many groups having greater than average abundances indicate greater functional divergence (Villéger et al. 2008). (3) Functional evenness (FEve) describes the evenness of abundance distribution in a functional trait space (Villéger et al. 2008) and thus shows whether all species are equally distant in the trait space or whether the assemblage is composed of a group of functionally similar species. (4) Functional dispersion (FDis) represents the dispersion of species in the space of characteristics from the centroid of all species weighted by their relative abundances (Laliberte and Legendre 2010). This functional diversity index provides a measure of trait heterogeneity across assemblages structurally independent from species richness. After testing the

Table 1 Description of traits used, codes and references

Trait	Category	Codes	References
Tube construction	Tube absent	TUBNON	(Serra et al. 2016)
	Tube without shape, unorganized	TUBUNO	
	Tube rigid/case-like	TUBRIG	
Hemoglobin	Hemoglobin present	HBPRES	(Serra et al. 2016; Saulino et al. 2017; Jovem-Azevêdo et al. 2019)
	Hemoglobin absent	HBNONE	
Substrate relation	Free living	FREELV	(USEPA, 2012; Serra et al. 2016)
	Burrower	BURROW	
	Miner	MINER	
	Fixed	FIXED	
Body size	< 2.5 mm	SIZE1	(Serra et al. 2016)
	> 2.5–5 mm	SIZE2	
	> 5–10 mm	SIZE3	
Feeding/trophic habits	Fine sediment eater	DEFEE	(USEPA, 2012; Jovem-Azevêdo et al. 2019; Saulino et al. 2017; Serra et al. 2016)
	Shredder	SHR	
	Scraper grazer	SCR	
	Filterer	FFEEDT	
	Predator	PRED	
Feeding strategy	Filters	FI	(Butakka et al. 2016; Saulino et al. 2017; Jovem-Azevêdo et al. 2019)
	Gatherer	GA	
	Herbivore	HE	
	Engulfer	EN	
Habit	Sprawler	SP	(USEPA, 2012; Jovem-Azevêdo et al. 2019; Saulino et al. 2017)
	Silk tube	ST	
	Climber	CL	
	Miner	MI	
Pseudopods	Elongated	EL	(Trivinho-Strixino 2011; Jovem-Azevêdo et al. 2019)
	Short	SH	
	Absent	AB	
Lauterborn organs	Present	PR	(Trivinho-Strixino 2011; Jovem-Azevêdo et al. 2019)
	Absent	AB	

normality of residuals and homoscedasticity, we used linear regression to assess the degree to which the functional diversity indices were affected by the IDI values.

To assess associations between trait categories and local environmental variables, we applied RLQ and fourth-corner methods. RLQ produces three tables: environmental (*R*), taxa abundance (*L*) and trait (*Q*) tables. RLQ aims to identify the main co-structures between traits and environmental characteristics weighted by taxa abundances (Dolédec et al. 1996), and provides classification scores to summarize the joint structure between the three tables. The fourth-corner method primarily tests relationships between individual characteristics and the environment (that is, one characteristic and one environmental variable at a time) (Dray et al. 2014). We standardized local environmental metrics (mean = 0 and standard deviation = 1) before running all analyses. Overall significance was assessed via a global Monte Carlo test using 9999 random permutations of the table rows of R (sites, model 2) and of the rows of Q (species, model

4). A combination of RLQ and fourth-corner analyses was used to evaluate the significance of associations between traits and combinations of environmental variables identified by RLQ. Significance was tested using a permutation procedure with model 6, which is a combination of models 2 (permutation of sites) and 4 (permutation of species). We used 9999 permutations and the false discovery rate adjustment (FDR) method to correct P-values for multiple-test comparisons (Dray et al. 2014). All analyzes were performed in R (R Core Development Team 2016) with vegan (Oksanen et al. 2017), FD (Laliberté et al. 2014) and ade4 (Chessel et al. 2004) packages.

Results

We identified 6147 individuals in 52 chironomid genera distributed in 3 subfamilies. The body size of the organisms ranged from 1.5 to 7.2 mm ($\bar{x}$= 3.3, SD = 1.02). Functional richness (FRic: $F_{1,28} = 0.13$, $P = 0.72$), evenness (FEve: $F_{1,28} = 0.21$, $P = 0.64$) and dispersion (FDis: $F_{1,28} = 0.41$, $P = 0.52$) did not significantly differ among the anthropogenic disturbance categories. Functional divergence index scores were significantly lower in sites with greater anthropogenic disturbances (FDiv: $F_{1,28} = 5.54$, $P = 0.02$) (Fig. 2).

The global RLQ test revealed a significant relationship between abundance of genera and environmental variables (model 2, $P = 0.01$), as well as abundance of genera and biological traits (model 4, $P = 0.01$). The cross-variance between traits and environmental variables can be summarized by the first two RLQ axes (60.6% and 23.7% for axis 1 and 2, respectively). These axes were responsible for 86% of the variability of the environmental variables and 83% of the variance of the traits table (Fig. 3).

Regarding the first RLQ axis, we observed a positive relationship between *Tanypus, Nilotanypus, Larsia, Labrundinia, Djalmabatista, Denopelopia, Ablabesmyia, Monopelopia, Procladius* and *Fittkauimyia* with average substrate embeddedness, % sand substrate and % catchment pasture. Most of these taxa are predators (PRED) with an engulfer (EN) feeding strategy, and the greater the amount of stream sand and catchment pasture, the greater the abundance of those chironomid genera.

The positive relationship of RLQ axis 1 with *Stenochironomus, Endotribelos* and *Beardius*, which have a miner habit (MI), herbivorous feeding strategy (HE) and larger body size, were related to riparian and channel vegetation cover (Fig. 3). The presence of *Polypedilum* was related to % catchment agriculture, % site fine sediments and W1_hall.

We also assessed the relationships between individual traits and the two RLQ environmental axes and individual environmental variables and the two RLQ trait axes by combining both RLQ and fourth-corner analysis. The first environmental axis (AxcR1, combination of environmental variables) was positively correlated with a herbivorous feeding strategy (Fig. 4a). The first RLQ trait axis (AxcQ1, combination of traits) was positively related to riparian and channel canopy cover (Fig. 4b).

Discussion

We found strong relationships between environmental variables (local and catchment) and Chironomidae assemblage traits (predators, herbivores, miners, engulfers and body size). The most important trait for structuring Chironomidae assemblages was herbivorous feeding strategy, positively related to riparian and channel canopy cover. Functional divergence (FDiv) increased with increased disturbance (IDI), but functional richness (FRic), uniformity (FEve) and dispersion (FDis) showed no relationship with IDI scores. These results, taken together, suggest a high divergence of chironomid functional traits between sites with low versus high anthropogenic disturbance.

We also found that the structure of habitat and food resources were important factors structuring Chironomidae assemblages, as did Specziár et al. (2018). The abundance of Tanypodinae was positively associated with higher percentages of sand, embeddedness by fine sediments and amount of catchment pasture. Tanypodinae species are widely distributed, occupying a wide variety of habitats (Cortelezzi et al. 2020), and they are known to be more tolerant of intermediate flow conditions (Puntí et al. 2009). These organisms are predators, one of the most important components of assemblages, because they strongly influence food chain structure (Saulino and Trivinho-Strixino 2018a) and are often related to human disturbances (Feio et al. 2015). Consequently, they lead to changes in

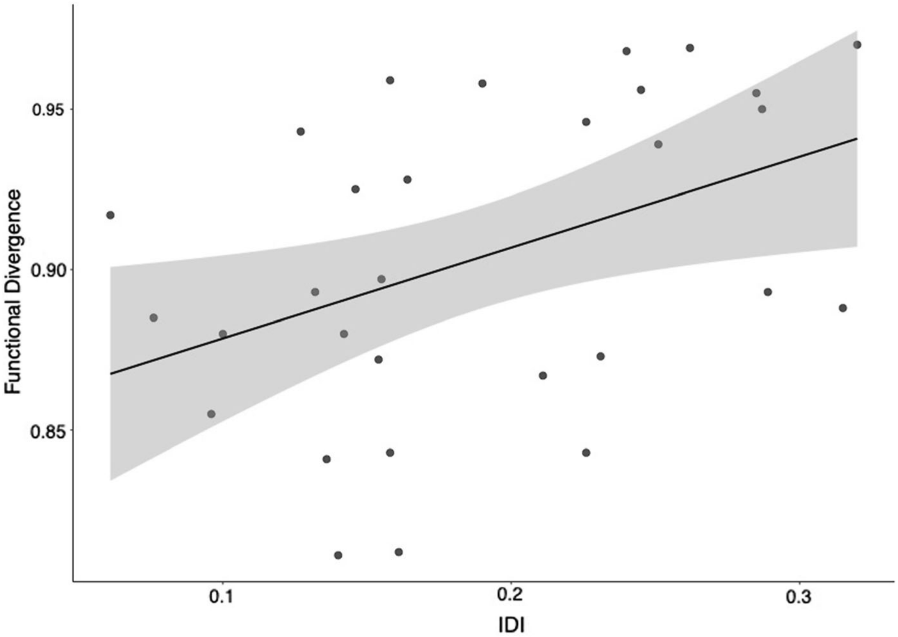

Fig. 2 Linear regression between the Functional Divergence Index (FDiv) and the Integrated Disturbance Index (IDI)

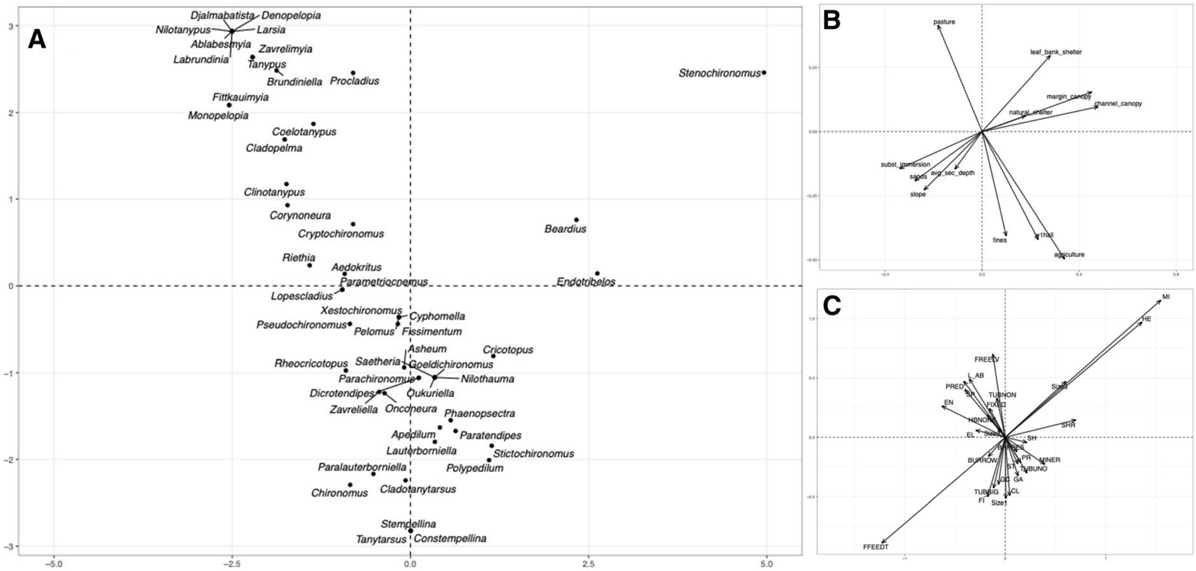

Fig. 3 Axis 1 and axis 2 of the RLQ analysis of 30 stream sites in the Pandeiros basin. **a** Chironomidae scores; **b** environmental variable scores; **c** trait scores. The values for “d” indicate grid sizes for scale comparison between the four figures. Each type of circle represents a significant relationship between the variables analyzed

ecological processes, because they are directly linked to energy transfer processes within ecosystems (Sih et al. 2010). Genera of this subfamily have been associated with high nitrogen concentrations, low dissolved oxygen concentrations (Cortelezzi et al. 2020), drought (Jovem-Azevêdo et al. 2019) and pesticides (Kuzmanovic et al. 2017). This indicates that they are tolerant to ecological changes as observed in this study. Some characteristics of these organisms may explain this tolerance. They can change their

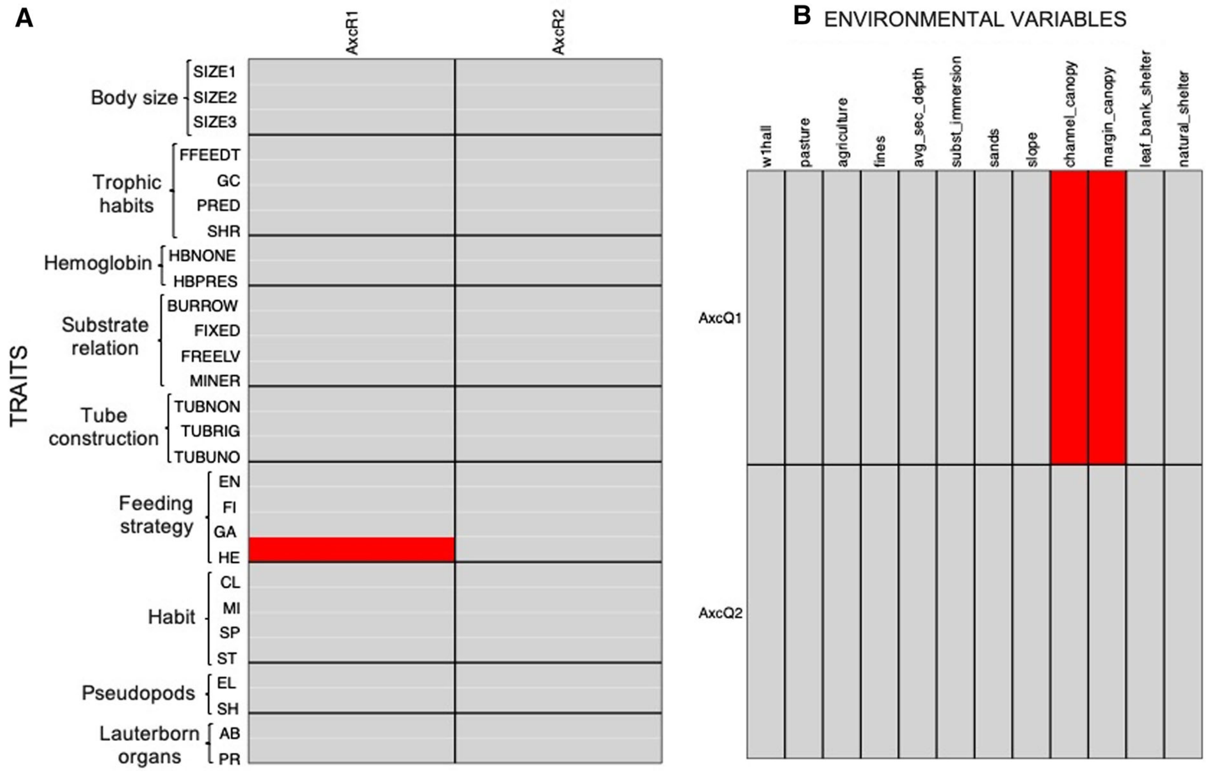

Fig. 4 Significant relationships (P-adjusted < 0.05) between (**a**) the RLQ environmental axes and individual traits and (**b**) between the RLQ trait axes and environmental variables. Red indicates positive correlations between factors. Nonsignificant relationships are labeled in gray

eating habits depending on the availability of food items (Butakka et al. 2016; Jovem-Azevêdo et al. 2019). Their body shape, longer pseudopods, retractable antennae and fused eyes also make them more mobile and hydrodynamically efficient (Trivinho-Strixino 2014; Saulino and Trivinho-Strixino 2018a).

Elevated percentages of sand and substrate embeddedness often indicate anthropogenic changes in aquatic ecosystems. In another studies conducted in the same river basin (Martins et al. 2020, 2021a, b), we observed that increased levels of fine substrate were associated with greater relative risk for poor biological condition. In aquatic ecosystems, the presence of fine substrates in stream beds is one of the most important threats to their ecological condition (Bryce et al. 2010; Burdon et al. 2013). This is because fine sediments reduce the availability of habitat for macroinvertebrate assemblages, directly compromising their structure, composition and function (Wood and Armitage 1997; Angradi 1999; Matthaei et al. 2010; Buendia et al. 2013; Beermann et al. 2018). Fine sediments also have been found to be important stressors of macroinvertebrate condition in regional and national assessments in the USA (Van Sickle et al. 2006; Paulsen et al. 2008; Herlihy et al. 2020; USEPA 2020), Cerrado (Silva et al. 2018a) and Amazonia (Leitão et al. 2018). Excess riverbed fine substrates are associated with human activities that increase erosion, such as agriculture, pasture, roads and deforestation (Kaufmann et al. 2009; Burdon et al. 2013; Strassburg et al. 2017; Brito et al. 2020; Dala-Corte et al. 2020).

The RLQ and fourth-corner analyses showed positive relationships between riparian canopy cover and herbivorous organisms such as *Endotribelos*, *Beardius* (shredders) and *Stenochironomus* (miner). In addition, these characteristics were related to larger-sized chironomids. Riparian vegetation is essential for important ecological processes in aquatic ecosystems, such as providing allocthonous nutrients, temperature balance and habitat heterogeneity. The absence or minimization of riparian vegetation reduces and homogenizes biological diversity (Castro et al. 2018; Firmiano et al. 2021) and eliminates sensitive species (Martins et al 2021a, b; Oliveira-Junior et al. 2015; Brito et al. 2020; Dala-Corte et al. 2020). In addition, riparian vegetation is an important component in herbivory processes in aquatic ecosystems. Herbivores play an important ecological role in determining the energy flow from primary producers to higher consumers (Wood et al. 2017). Freshwater herbivorous macroinvertebrates are composed mostly of scrapers (algae grazers) and shredders (leaf, wood and debris fragments) (Saulino et al. 2020). Shredders have fundamental importance in fragmenting coarse particulate organic matter (CPOM) present on the streambed into fine particulate organic matter (FPOM) (Graça 2001; Boyero et al. 2015). Low canopy coverage can cause changes in the functional composition of chironomid assemblages (Cañedo-Argüelles et al. 2016). Riparian canopy cover was the main factor responsible for the functional structure of least disturbed sites (Castro et al. 2018) and was positively correlated with more sensitive chironomid genera (Sensolo et al. 2012). On the other hand, the absence of riparian vegetation had a negative effect on abundance and richness of specialist organisms in wood processing, such as *Endotribelos*, *Beardius* and *Stenochironomus* (Valente-Neto et al. 2015). These results reinforce the importance of riparian vegetation for the functional structure of Chironomidae assemblages and other aquatic assemblages (Dala-Corte et al. 2020).

The largest Chironomidae larvae were significantly enhanced by the presence of riparian vegetation. Relative to the sizes of chironomids reported in the literature (Serra et al. 2016), we found intermediate sizes. In general, smaller larvae are benefited by high temperatures, low rainfall and anthropogenic disturbances (Feio et al. 2015; Jovem-Azevêdo et al. 2019). However, some traits, such as body size, are still not well described for Chironomidae, even though this trait is of great importance for other macroinvertebrates in discriminating various types of anthropogenic impact (Dolédec and Statzner 2008). Body size is linked with key ecological functions of macroinvertebrates (production/biomass, production/respiration) (Robson et al. 2005). Greater effort is needed to describe some traits for Chironomidae (body size, voltinism and forms of resistance) (Serra et al. 2017) in the neotropics.

Percent catchment agriculture, % fines and W1_hall were all related to the presence of *Polypedilum*. This cosmopolitan genus is generally associated with sandy substrate, silt and aquatic macrophytes (Cenzano and Würdig 2006) and has a general detritivore food habit (Higuti and Takeda 2002; Amorim et al. 2004). The genus includes species tolerant to a wide range of environmental conditions (Silva et al. 2018b), such as eutrophication (Saito and Fonseca-Gessner 2014), moderate concentrations of dissolved inorganic nitrogen and low levels of dissolved oxygen (Cranston et al. 1997; Roque et al. 2010; Cortelezzi et al. 2020). This is because *Polypedilum* contains large amounts of hemoglobin and can store oxygen (Trivinho-Strixino 2011). These characteristics help explain its relationship with anthropogenic disturbance metrics.

Riparian zone disturbance, as indicated by W1_hall, can alter habitats and biota (Death and Joy 2004; Kaufmann and Hughes 2006; Bryce et al. 2010). The riparian zone strongly influences the organization, diversity and dynamics of aquatic communities (Gregory et al. 1991; Allan 2004). Changes in soil conditions, vegetation and other factors directly reflect terrestrial aquatic interactions (Naiman et al. 2000) and services provided by the riparian meta-ecosystem (Callisto et al. 2019b). Thus, it is essential to consider the important role of the riparian zone in the organization, diversity and dynamics of aquatic communities.

Chironomid genera proved to be effective for assessing ecological status in the Pandeiros River basin and some environmental characteristics were fundamental for structuring chironomid assemblages. As predicted, chironomid traits and functional indices were affected by the anthropogenic pressures identified in the catchment and the resultant stressors measured at the sites. This approach allowed us to identify cause–effect relationships, such as the reduced herbivorous feeding strategy associated with reduced riparian and channel canopy cover. Despite the relatively low levels of basin disturbance, we observed that riparian vegetation and substrate size were particularly important for structuring chironomid assemblages. The Chironomidae is a very diverse family, and its functional relationships are still little explored in the neotropics. This shows the importance of genus-level identifications, because marked differences in tolerance levels occur within the family, as observed in this study (Saulino and Trivinho-Strixino 2018a). However, its predominance in sandy environments where EPT (Ephemeroptera, Plecoptera, Trichoptera) are uncommon, such as the Pandeiros River basin, make it an important tool for ecological assessment of places with these characteristics (Li et al. 2014). Thus, this study adds an important contribution to that knowledge and consolidated those aspects that are most important for maintaining aquatic ecosystem condition in the Pandeiros River basin.

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Authors' contributions I.M., D.M.P.C, D.R.M., R.M.H. and M.C. elaborated the research design and wrote the paper. I.M. collected and processed the data. I.M. and D.M.P.C conducted the statistical analyses.

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Data availability The data are available by requesting the corresponding author's email.

Declarations

Conflicts of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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