



Functional responses of aquatic invertebrates to anthropogenic stressors in riparian zones of Neotropical savanna streams

Kele R. Firmiano^{a,b,c}, Diego M.P. Castro^{a,*}, Marden S. Linares^a, Marcos Callisto^a

^a Universidade Federal de Minas Gerais, Instituto de Ciências Biológicas, Departamento de Genética, Ecologia e Evolução, Laboratório de Ecologia de Bentos, Av. Antônio Carlos 6627, CP 486, CEP 31270-901 Belo Horizonte, Minas Gerais, Brazil

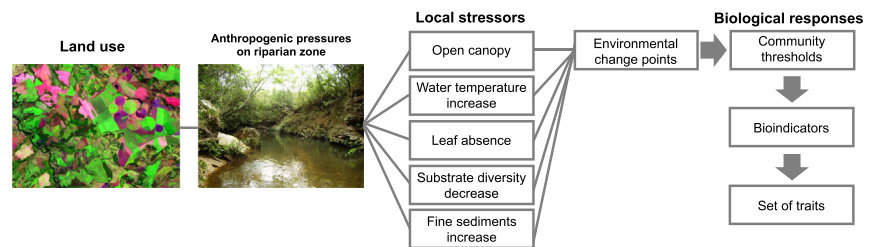
^b Programa de Capacitação Institucional (PCI), Instituto Nacional Mata Atlântica, Av. José Ruschi, N° 4, Santa Teresa – ES – Cep: 29.650-000

^c Instituto de Pesquisa Jardim Botânico, Rio de Janeiro, RJ, Brazil

HIGHLIGHTS

- Five local stressors due to human pressures on riparian zones were assessed.
- Fifty-one freshwater invertebrate taxa (60%) were detected as robust bioindicators.
- Local stressors act as environmental filters for aquatic invertebrates.
- Traits related to percentage of fines, substrate heterogeneity and water temperature were identified.
- A fuzzy coded trait profile of 51 neotropical stream invertebrate taxa is made available.

GRAPHICAL ABSTRACT



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ABSTRACT

Riparian zones ensure freshwater ecosystem processes such as microclimate regulation, organic matter inputs, and fine substrate retention. These processes illustrate the importance of riparian zones for freshwater ecosystem functioning, maintaining biodiversity, and mitigating the effects of anthropogenic pressures on aquatic ecosystems. We aimed to determine the freshwater invertebrate biological traits that are most affected by anthropogenic stressors in the riparian zones of 210 Neotropical savanna headwater streams. We assessed % canopy cover over the streambed, % fine bottom substrate, % leaf pack, substrate heterogeneity, and water temperature. Firstly, we identified bioindicator taxa in response to each local metric gradient. We assessed the functional response, based on biological traits of bioindicators previously selected. We identified 324,015 specimens belonging to 84 freshwater invertebrate taxa. Fifty-one taxa (60%) were bioindicators of anthropogenic stressors. We found three main sets of traits. (1) a set of traits linked to increased disturbance (higher percentage of fine sediments), consisting of organisms with aquatic adult stages, spherical body shape, and long adult life stages. (2) A set of traits linked to lower disturbance (higher substrate heterogeneity), including taxa with short or very short lifespans that live attached to substrates. (3) A set of traits linked to higher water temperature, including organisms with short adult lifespans and lower body flexibility. These patterns suggest that the stressors act as environmental filters and do not act independently on single traits, but rather, selecting sets of biological traits that facilitate taxa surviving and persisting in local environmental conditions. Our results support the development of powerful evaluation tools for environmental managers and decision makers. Because degraded freshwater communities respond in similar ways across large biogeographic areas, these sets of traits can be used for ecological monitoring efforts along other tropical savanna headwaters worldwide.

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* Corresponding author.

E-mail addresses: diegobioufla@gmail.com (D.M.P. Castro), callisto@ufmg.br (M. Callisto).

1. Introduction

Freshwater ecosystems host high levels of biodiversity, housing 9.5% of globally recognized animal species, despite comprising only 0.01% of the water on Earth (Balian et al., 2008). This is even more impressive in the tropics, which hold the vast majority of the world's freshwater biota (Barlow et al., 2018). However, these ecosystems are constantly threatened by anthropogenic pressures, such as land use intensification, pollution, habitat degradation, and riparian zone deforestation (Reid et al., 2019; Sundar et al., 2020). These anthropogenic pressures produce multiple stressors on freshwater ecosystems (Hughes et al., 2019) that alter physical habitats (e.g., substrate, water flow) and water quality (e.g., nutrients, temperature, turbidity) compromising the persistence and abundance of many taxa living in these ecosystems (Li et al., 2019). Thus, anthropogenic stressors act as environmental filters, regulating community structure and composition (Castro et al., 2018; Statzner et al., 2004).

Various environmental factors act as filters by selecting species with a set of traits that allow them to persist under the local biotic (e.g. competition) and abiotic (e.g. physical environment) conditions (Poff, 1997; Statzner et al., 2004). In addition, local community composition is constrained by historical and stochastic environmental factors (HilleRisLambers et al., 2012). According to the Habitat Templet Theory (Townsend and Hildrew, 1994), specific combinations of traits determine the ability of individuals to coexist in a local community under specific environmental conditions. Supported by this idea, the habitat-filtering hypothesis (Poff, 1997) postulates that the least suitable sets of biological traits are eliminated in a given environment and that only taxa possessing traits that pass through the habitat filter will be present in the community. In this context, anthropogenic stressors are considered additional environmental filters that change the expected trait composition of assemblages compared to those in natural conditions (Floury et al., 2017). Identifying assemblage traits also facilitates mechanistic understanding of cause-effect relationships between local stressors and biodiversity thereby indicating the stressors most likely responsible for biological impairment (Berger et al., 2018; Mondy et al., 2016; Verberk et al., 2013).

Among the various anthropogenic pressures on freshwater ecosystems, land use change within the riparian zone of streams is recognized as having one of the most severe effects on aquatic biodiversity (Dala-Corte et al., 2020; Feld et al., 2018; Tanaka et al., 2016). Riparian zones ensure ecosystem processes such as microclimate regulation, organic matter inputs, and fine substrate retention (Gregory et al., 1991; Riis et al., 2020). Canopy cover by riparian vegetation provides shading, which limits direct solar radiation on stream beds and consequently decreases water temperatures (Gregory et al., 1991) and limits instream primary production (Neres-Lima et al., 2017). Moreover, organic matter inputs by leaf fall on stream beds constitute important feeding and shelter resources for freshwater biota (Ligeiro et al., 2020; Tiegs et al., 2019). Riparian zones are also responsible for fine substrate retention from shore and floodplain erosion, which reduces substrate homogenization and consequent species losses (Feld et al., 2018). These processes illustrate the importance of riparian zones for freshwater ecosystem functioning, maintaining biodiversity, and mitigating the effects of anthropogenic pressures (Dala-Corte et al., 2020; Feld et al., 2018; Luke et al., 2019).

Recent studies in temperate freshwater ecosystems have assessed which set of traits in aquatic invertebrates are related to various anthropogenic pressures, such as water quality (Berger et al., 2018), human land use (Krynak and Yates, 2018), and agrochemicals (Collins and Fahrig, 2020). For neotropical freshwater ecosystems, however, this knowledge is still lacking, and because of differences in environmental conditions between temperate and neotropical ecosystems, we should not directly extrapolate the results of studies from one to the other. As neotropical freshwater ecosystems are increasingly threatened by agrobusiness expansion (Strassburg et al., 2017), it is critical to

understand the effects of that anthropogenic pressure on riparian zone stressors and aquatic biodiversity (Dala-Corte et al., 2020).

Therefore, our aim is to understand how riparian zone stressors relate with trait assemblages of aquatic invertebrates and shape the functional structure of these communities in neotropical streams. We assumed that land use intensification generates pressures on riparian zones, resulting in local environmental stressors. We hypothesized that a set of local riparian stressors (reduced % canopy cover, increased % fine substrate, reduced % leaf packs, reduced substrate heterogeneity, and increased water temperature) selects certain sets of biological traits, following the rationale presented in Table 1. We expected to find trait combinations of aquatic invertebrates that are selected by local stressors acting as environmental filters.

2. Methods

2.1. Study area

The study area is located in the Brazilian Neotropical savanna (Cerrado biome). The local climate is characterized by temperatures ranging from 22° to 27 °C and an average annual rainfall of 1500 mm (Klink and Machado, 2005). It has two well-defined seasons, a dry season from April to September, and a rainy season from October to March. The native vegetation is composed of forest patches, shrubs, and seasonally wet grasslands, all adapted to high acidity and aluminum and low nutrient concentrations (Fernandes et al., 2018; Wantzen et al., 2006). The soil can be chemically amended, which enables large-scale agricultural commodity production, such as soybean, sugarcane, and cattle (Strassburg et al., 2017). Because of increased human activities threatening many endemic species, the Brazilian Neotropical savanna is one of 25 global biodiversity hotspots (Myers et al., 2000).

2.2. Sampling design

We sampled 210 first- to third- order stream sites (1:100,000 scale; Strahler, 1957). Sites were defined through the use of a spatially dispersed random survey design (Stevens and Olsen, 2004). We sampled 166 randomly selected sites and 44 hand-picked sites to ensure that the sites were regionally representative and that both minimally disturbed and highly disturbed sites were represented. The sites were located in five different hydrological units (drainage areas within 35 km upstream of each of five major hydropower reservoirs): Três Marias, Volta Grande, São Simão, Nova Ponte, and Pandeiros (Fig. 1), comprising a total geographic area of 49,100 km². One sampling campaign per site in each hydrologic unit was conducted in September from 2011 to 2016, ensuring that samples were all taken during the dry season (Silva et al., 2017; Stevens and Olsen, 2004). The sites covered a wide range of land use (natural, pasture, agriculture, urbanized) as demonstrated in previous studies (Callisto et al., 2019; Castro et al., 2017; Macedo et al., 2018).

2.3. Local stressors

The length of each stream site sampled was 40 times its mean wetted width, with a minimum length of 150 m. Each stream site was divided into 11 equally spaced transects. We calculated five metrics as surrogates of local stressors generated by anthropogenic pressures on riparian zones: percentage of canopy cover over the streambed (PCT_Canopy), percentage of fine substrate (PCT_Fines), percentage of leaf pack cover (PCT_Litter), substrate heterogeneity index (DIV_Substrate), and water temperature (°C) (Wat_Temperature). Those five metrics were selected based on previous studies showing their influence on the diversity of aquatic communities (e.g., Berger et al., 2018; Burdon et al., 2013; Feld et al., 2018).

PCT_Canopy was measured with a hemispherical densiometer in the middle of each transect in four directions (East, West, North, and South)

Table 1

Predictions about trait responses according to site stressor gradient increases. + indicates an increase in the frequency of the trait category; - indicates a decrease in the frequency of the trait category with the site stressor increase.

Stressor	Trait category	Trait prediction	Rationale	Reference
Open canopy	Aquatic stages	- Larval + Adult	Less food available in riparian habitats and more available energy to the lotic ecosystem	Berger et al. (2018)
Water temperature increase	Respiration	- Gill and tegument + Plastron	Higher temperature decreases dissolved oxygen concentration in the water	Berger et al. (2018); Castro et al. (2018); Krynak and Yates (2018)
	Voltinism	- Univoltine + Multivoltine	Higher temperatures favor species with rapid development and more reproductive cycles	Berger et al. (2018); Castro et al. (2018); Krynak and Yates (2018)
Leaf absence	Adult lifespan	- Long + Short	Higher temperatures favor species with short adult lifespan	Krynak and Yates (2018)
	Locomotion	- Crawler + Burrower	Litter banks are more unstable microhabitats and favor species with crawling locomotion	Di Sabatino et al. (2014)
Substrate heterogeneity decrease	Body shape	- Flattened and spherical + Cylindrical	Loss of hard substrate and its associated body shapes	Castro et al. (2018); Krynak and Yates (2018)
Fine sediments increase	Body flexibility	- Low and medium + High	Habitat selects species adapted for interstitial lifestyle	Castro et al. (2018)
	Locomotion and substrate relation	- Crawler - Attached to substrate + Burrower	Loss of attachable substrates	Castro et al. (2018); Krynak and Yates (2018)

(Peck et al., 2006). The final metric is the mean of all 11 channel canopy-cover means at the stream site (Kaufmann et al., 1999).

We recorded the proportion of the different types and substrate size classes (inorganic: % of bedrock, boulder, cobble, gravel, sand, mud, and clay; organic: % of wood, living roots, algae, macrophyte, fine and coarse leaf) in each stream site, thereby assessing 105 individual points distributed across the 11 cross-sections of the wetted channel. This procedure was adopted to ensure stable and precise substrate estimates (Kaufmann et al., 1999). PCT_Litter was the percentage of coarse leaf cover over the channel bottom at those 11 transects. PCT_Fines refers to the total percentage of all inorganic substrates <16 mm size. DIV_Substrate was calculated from the Simpson index considering all types and class sizes of substrate.

We assessed only one water quality variable, which was most closely associated with riparian conditions (Feld et al., 2018). Wat_Temperature was measured once per site at the end of the visit via a model YSI 6600 multiprobe meter. We observed weak correlations among the five metrics (Pearson correlation coefficient < 0.7) allowing us to conduct further analyses (Appendix A).

2.4. Freshwater invertebrate sampling and identification

We collected freshwater invertebrates at all 210 sites using a D-frame kick net (30 cm opening, 0.5 mm mesh sieve). Following a systematic zig-zag pattern along the site, eleven sub-samples (0.09 m² quadrat) were taken per site, generating a 0.99 m² multi-habitat composite sample for each site. We fixed the samples in the field with 10% formalin, and took them to the laboratory, where invertebrates were sorted and identified to family (except non-insect taxa: Bivalvia, Decapoda, Nematoda, and Oligochaeta) using taxonomic keys (Costa et al., 2006; Fernández and Domínguez, 2001; Merritt and Cummins, 1996; Mugnai et al., 2010). This procedure was adopted because the current knowledge of Neotropical freshwater invertebrates does not allow identifying all taxa to lower taxonomic levels (Heino et al., 2018). Lower taxonomic level patterns (e.g. genus or family) are good proxies of those produced at higher taxonomic resolution (i.e., species) (Whittier and Van Sickle, 2010). Therefore, they are commonly applied in biomonitoring programs (Heino, 2014). Furthermore, the trait structure of freshwater invertebrate assemblages is generally conserved when lower taxonomic levels are used (Dolédéc et al., 2000; Gayraud et al., 2003). In addition, we identified high congruence

between EPT genera and families (PROTEST: $r = 0.77$, $p = 0.00001$; Appendix A), which allowed us to conduct statistical analyses at the family level. All specimens were deposited in the reference collection of benthic macroinvertebrates of the Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais.

2.5. Traits compilation

Community functional structure was indirectly assessed based on multiple biological traits representing biological characteristics that are connected to ecosystem functions (Dolédéc and Statzner, 2010). In the Neotropics, the use of multiple traits approaches is increasing (e.g., Castro et al., 2018; Céréghino et al., 2018; Saito et al., 2016; Tomanova and Usseglio-Polatera, 2007), but knowledge about freshwater invertebrate biological and ecological traits remains limited (Brito et al., 2018; Heino, 2014). Therefore, we selected seven biological traits (as a proxy to functional traits) expected to respond to stressors in the studied region. We compiled trait information available in the literature (Appendix B) following a priority criteria: i) trait databases from Neotropics (Tomanova and Usseglio-Polatera, 2007; Reynaga and Santos, 2012; Saito et al., 2015; Castro et al., 2018); ii) from North America (Poff et al., 2006); iii) and from Europe (Tachet et al., 2002). The affinity of each taxon for each category within a trait was described using a fuzzy coding approach, ranging from 0 to 3, with 0 indicating no affinity of the taxon with the category, 1 indicating weak affinity, 2 indicating moderate affinity and 3 indicating strong affinity (Chevenet et al., 1994). This methodology allowed us to compensate for different types and levels of information available. Affinity scores were standardized so that their sums for a given taxon and a given trait equaled 1. We produced a trait database comprising 7 biological traits in 25 trait categories expected to respond to local metrics in the studied sites (Table 2). Those 7 selected traits were chosen because they are traits that we could compile sufficiently, were reliably documented in the literature and with which we could make meaningful predictions of their responses to local stressors (Table 1).

2.6. Statistical analyses

We conducted two major analytical steps, described in detail below. We first identified bioindicator taxa in response to each local stressor gradient. We then assessed their functional responses based on the

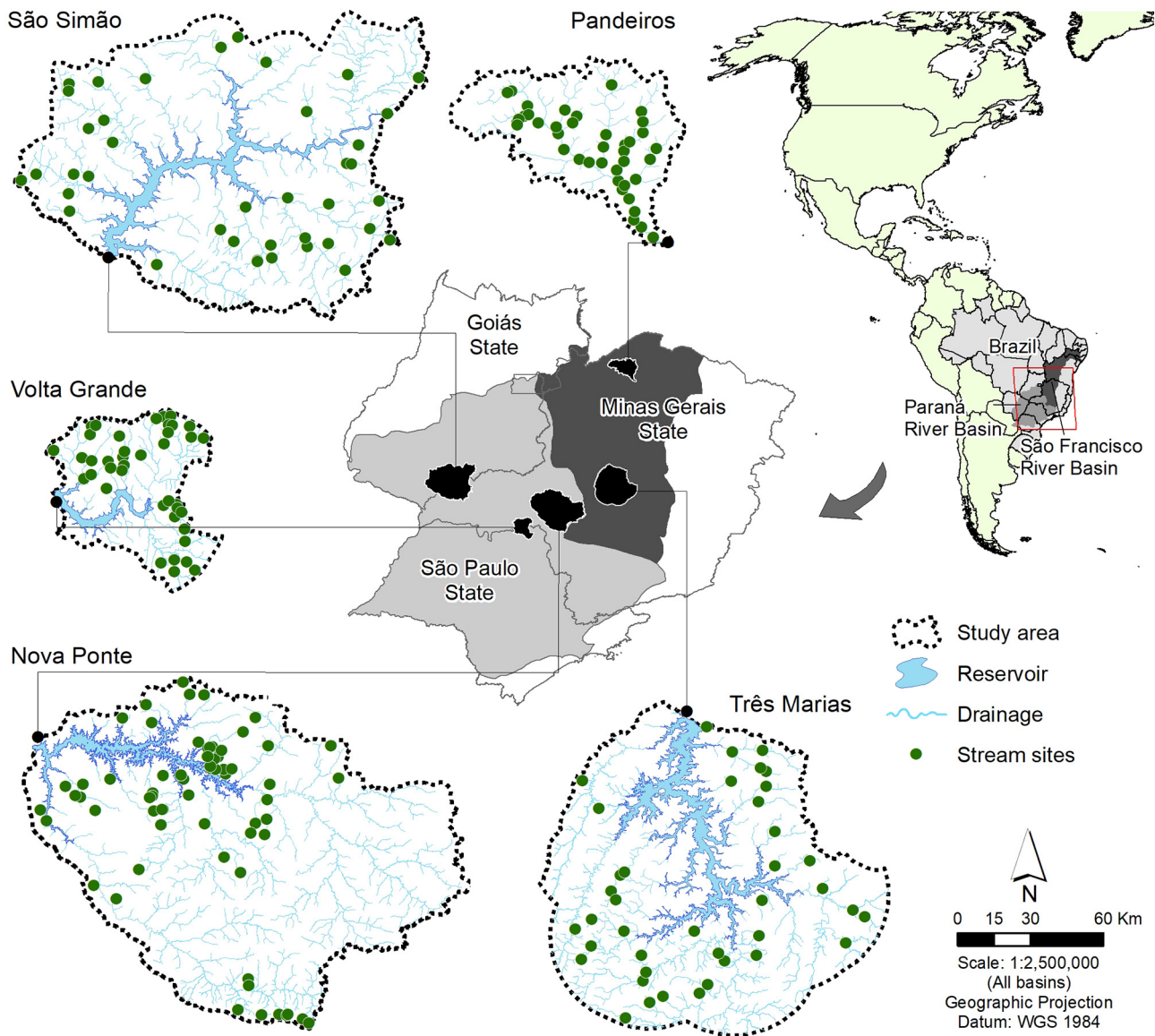


Fig. 1. Location of hydrologic units and stream sites sampled in the Brazilian Neotropical savanna.

biological traits of the previously selected taxa. All statistical analyses were conducted in R version 3.5.1 (R Development Core Team, 2018), using *vegan* (P.R. et al., 2015), *TITAN2* (Baker and King, 2010), and *ade4* (Dray and Dufour, 2007).

2.7. Threshold indicator taxa analysis

We performed Threshold Indicator Taxa Analysis (TITAN) to detect change points in the freshwater invertebrate taxa responses to each site stressor. Our goal was to select statistically robust bioindicator taxa, and then, conduct subsequent analytical steps with the most suitable taxa. Through this initial screening we evaluate which taxa were most responsive to the site stressors. This procedure may help to develop more stressor-specific trait-based biomonitoring tools, by combining taxonomic and functional indicators. A similar approach was applied in temperate regions to assess the effects of land use at different spatial extents (Krynak and Yates, 2018), and multiple local stressors (Berger et al., 2018).

TITAN combines change point and indicator species analyses to detect abrupt change in the abundance and frequency of taxa along each

site stressor (Baker and King, 2010). The robust indicator taxa are established by purity (i.e., proportion of change points along the resampling that agree with the observed value) and reliability properties (i.e., proportion of the resampling that reports indicator p value < 0.05) by the bootstrap technique to confirm the thresholds for each taxa (500 resamples with replacement) (King and Baker, 2014). Taxa considered robust bioindicators are those with purity > 0.95 , reliability > 0.95 , and achieving $p < 0.05$ to bootstrapping replicates (Baker and King, 2010; King and Baker, 2014).

We identified 324,015 specimens belonging to 84 freshwater invertebrate taxa. Fifty-one taxa (60%) were detected as robust bioindicators by TITAN (Table 3) (see Appendix C for detailed taxa results) and were used in the following analyses.

2.8. RLQ and Fourth-corner analyses

After selecting the freshwater invertebrates that were robust bioindicator taxa, we assessed the associations between their trait categories and site stressors through use of RLQ analysis (Dolédec et al., 1996). RLQ is an extension of the co-inertia analysis (Dolédec and

Table 2

Traits, categories and codes used for freshwater invertebrates considered in the present study.

Trait	Code	Category
Voltinism	Volt_uni	≤1 reproductive cycle per year
	Volt_mult	>1 reproductive cycle per year
Locomotion	Loc_burr	Burrower
	Loc_craw	Crawler
	Loc_attac	Attached
	Loc_swim	Swimmer
	Loc_flier	Flyer
Body flexibility	Flex_10	<10°
	Flex_10_45	>10–45°
	Flex_45	>45°
Aquatic stages	Stag_egg	Egg
	Stag_larv	Larvae
	Stag_nymp	Nymph
	Stag_adult	Adult
Adult lifespan	Adult_v.short	Very short
	Adult_short	Short
	Adult_long	Long
Body shape	Shape_lined	Streamlined
	Shape_flatt	Flattened
	Shape_cylin	Cylindrical
	Shape_spheri	Spherical
Respiration	Resp_teg	Tegument
	Resp_gil	Gill
	Resp_pla	Plastron
	Resp_spi	Spiracle

Chessel, 1994) that allows relating three tables: a site stressor table (R), a taxa abundance table (L), and a trait table (Q). RLQ aims to identify the main co-structures between traits and site stressor variations mediated by taxa abundances. Prior to RLQ analysis, the three tables were analyzed separately. Correspondence analysis (CA) was performed on the abundance table and principal component analysis (PCA) on the trait and site stressor tables, respectively. Local metrics were standardized (mean = 0 and standard deviation = 1) before running all analyses. RLQ summarizes the multivariate structures by searching for linear combinations of traits and site stressors on which sites and taxa are projected, providing new site and taxa scores that are the most covariant. The overall significance was further 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). Fourth-corner analysis was used to test bivariate relationships between traits and local metrics. Furthermore, 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 the 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).

3. Results

The relationships between the trait composition of robust bioindicators selected by TITAN and site stressors were globally significant (Model 2 simulated $p < 0.001$; Model 4 simulated $p < 0.035$). The relationships between traits and site stressors can be summarized by the first two RLQ axes (68.9% and 19.1% of the cross-variance between the traits and environment for axis 1 and 2, respectively). The first two axes accounted for 85% of the variability of the site stressors table and 70% of the variance of the trait table. In addition, the new set of site and taxa scores had a correlation of 0.14 along the first RLQ axis, which was 33% of the best possible correlation (i.e., obtained from the separate CA of the invertebrate abundance table) (Table 4).

The left (negative) part of the first RLQ axis identified taxa (*Bivalvia*, *Hydracarina*, *Planorbiidae*, *Corixidae*, *Gerridae*, *Veliidae*; Fig. 2a) with longer adult lifespans, spherical body shapes, and flier or burrower locomotion (Fig. 2b). Those taxa were mostly found in sites with higher PCT_Fines, PCT_Canopy, PCT_Litter, and Wat_Temperature (Fig. 2c). The right (positive) part of the axis highlighted organisms (*Hydropsychidae*, *Philopotamidae*, *Psephenidae*, *Glossosomatidae*, *Simuliidae*; Fig. 2a) that live attached to substrate, have short and very short lifespans, and have streamlined body shapes (Fig. 2b) in sites with higher DIV_Substrate (Fig. 2c). The second RLQ axis indicated sites with warmer Wat_Temperature and DIV_Substrate. Those habitats were characterized by organisms with short adult lifespans, lower body flexibility and crawling locomotion (e.g. *Glossosomatidae*, *Helicopsychidae*, *Psephenidae*).

We did not find significant bivariate associations between traits and local stressors after applying the *P*-value adjustment. This result suggests that a combination of traits, rather than a single trait, is affected by local stressors. Therefore, we further 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 local stressors) was significantly negatively correlated to organisms with adult aquatic stages and longer adult lives, whereas the second environmental axis (AxcR2) was positively associated with organisms with crawling locomotion (Fig. 3a). The first RLQ trait axis (AxQ1, combination of traits) was negatively related to PCT_Fines and PCT_Canopy and positively related to DIV_Substrate. Wat_Temperature was the only metric positively associated with the second RLQ trait axis (AxQ2) (Fig. 3b).

4. Discussion

We found three main sets of traits: (1) a set of traits linked to higher percentages of fine sediments, consisting of organisms with aquatic adult stages, spherical body shapes, and long adult lives; (2) a set of traits linked to higher substrate heterogeneity, that included taxa that live attached to substrates and have short or very short lifespans; (3) a set of traits associated with higher water temperatures, represented by organisms with short adult lifespan, lower body flexibility and crawling locomotion. These findings imply that the site stressors resulting from anthropogenic pressures on riparian zones act as environmental filters and do not act independently on single traits, but rather, selecting sets of biological traits that aid taxa in surviving to local environmental conditions in headwater streams.

We found that an aquatic adult stage was a common trait affected by site stressors. This might be explained by the tendency of freshwater invertebrates in streams affected by land use intensification to have lower capacities for aerial dispersal, avoiding adverse conditions in the surrounding terrestrial ecosystems and becoming confined to the river channel (Carlson et al., 2016). Thus, a riparian zone affected by site anthropogenic stressors would act as an environmental filter, barring those species that rely on terrestrial life stages for their distribution. Similar results were found in previous studies in temperate streams (Krynak and Yates, 2018).

Similarly, another set of traits was linked with higher water temperatures, which is associated with increased availability of autochthonous food sources (Linares et al., 2018; Md Rawi et al., 2013; Santos et al., 2019). These food sources are more readily consumable by freshwater invertebrates than more lignified allochthonous organic matter (Death and Collier, 2010; Thorp and Delong, 1994). Sites with these conditions had higher prevalence of *Bivalvia*, *Gastropoda* and some *Coleoptera*, and thus again a higher prevalence of fully aquatic taxa (Cummins et al., 2005; Ding et al., 2017).

We found that burrower locomotion and spherical body shape were also traits closely related to site stressors. This result can be explained as a function of a common anthropogenic stressor in the riparian zone

Table 3
Robust indicator taxa identified by TITAN in response to each site stressor assessed. The change point values, and taxa association with gradient for each taxon (negative “-”, or positive “+”) are shown. Below each site stressor are the range values, as well the mean and standard deviation values between parenthesis.

Taxa		PCT_canopy 0–100 (74 ± 26)		PCT_litter 0–89 (9 ± 11)		Wat_temperature 14–26 (20 ± 2)		DIV_substrate 0–0.9 (0.7 ± 0.2)		PCT_fines 0–100 (50 ± 26)	
		-	+	-	+	-	+	-	+	-	+
Acari	Hydracarina					24					
Coleoptera	Dryopidae										54
	Dytiscidae				26						
	Elmidae					22					
	Gyrinidae								0.8		
	Lutrochidae		100		20						
	Psephenidae	51									
	Ptilodactylidae				31						
Crustacea	Decapoda		99				25				
Diptera	Chaoboridae					17					
	Culicidae				26	15		0.1			
	Empididae						16		0.9		
	Muscidae				37						
	Psychodidae	21						0.2			
	Simuliidae					22					
	Tabanidae		22								
Ephemeroptera	Baetidae	13				23			0.3		96
	Caenidae					16					
	Euthyplociidae										27
	Leptohyphidae								0.2		
	Leptophlebiidae				33	25		0.1			96
	Polymitarcyidae					15		0.3			
Hemiptera	Corixidae					16					0
	Gerridae	89									
	Naucoridae								0.1		0
	Notonectidae					16					
	Pleidae	9									12
	Veliidae		22				25				
Lepdoptera	Pyralidae	9		0							0
Megaloptera	Corydalidae								0.8		
	Sialidae				28	18					
Mollusca	Bivalvia							0.1			1
	Hydrobiidae		98				24				
	Physidae				34						
	Planorbidae		73					0.4			
Odonata	Coenagrionidae				0	25			0.1		94
	Gomphidae		9		34						
	Libellulidae				20	16					
	Megapodagrionidae					22					
	Perilestidae					15					
Plecoptera	Gripopterygidae		100						0.9		
	Perlidae					22			0.8		94
Trichoptera	Calamoceratidae		35		19				0.8		
	Glossosomatidae								0.7		94
	Helicopsychidae						24				
	Hydrobiosidae				20		23				
	Hydropsychidae								0.1		
	Hydroptilidae	9			24						0
	Philopotamidae								0.8		48
	Polycentropodidae				34	19					94
	Sericostomatidae						22				

leading to increased fine sediment deposition in the stream bed, reducing sediment heterogeneity (Bryce et al., 2010; Burdon et al., 2013). Therefore, taxa with these traits are better adapted to this modified

Table 4
RLQ analysis summary outputs: eigenvalues and percentage of total co-inertia, correlation with the L matrix (taxa), ratio of inertia and co-inertia for R (local metrics) and Q (trait table) for Axis 1 and Axis 2.

RLQ analysis outputs	Axis 1	Axis 2
% of total co-inertia	68.9	19.1
Eigenvalues decomposition	0.004	0.001
Correlation (L)	0.14	0.08
Ratio of inertia and co-inertia (R)	0.84	0.85
Ratio of inertia and co-inertia (Q)	0.60	0.70

habitat. These results can be explained by reduced sediment retention by riparian vegetation (Sánchez-Bayo and Wyckhuys, 2019).

Short or very short lifespans, attached locomotion, and streamlined body shape were a set of traits closely related with sites with reduced anthropogenic stressors. These traits reflect adaptations to least-disturbed sites in the Neotropical savanna, small forested streams with high substrate heterogeneity and available hard substrates (Martins et al., 2018). Substrate heterogeneity provides a more diverse set of microhabitats and presumably greater taxonomic and trait diversity (Milesi et al., 2016; Townsend and Hildrew, 1994).

Short adult lifespans and lower body flexibility were a set of traits linked to the second axis of our analysis. They were closely related to warmer water temperatures but not to the other site stressors, implying that these traits are linked to less-disturbed open-canopy streams. As opposed to closed-canopy streams, these ecosystems are more

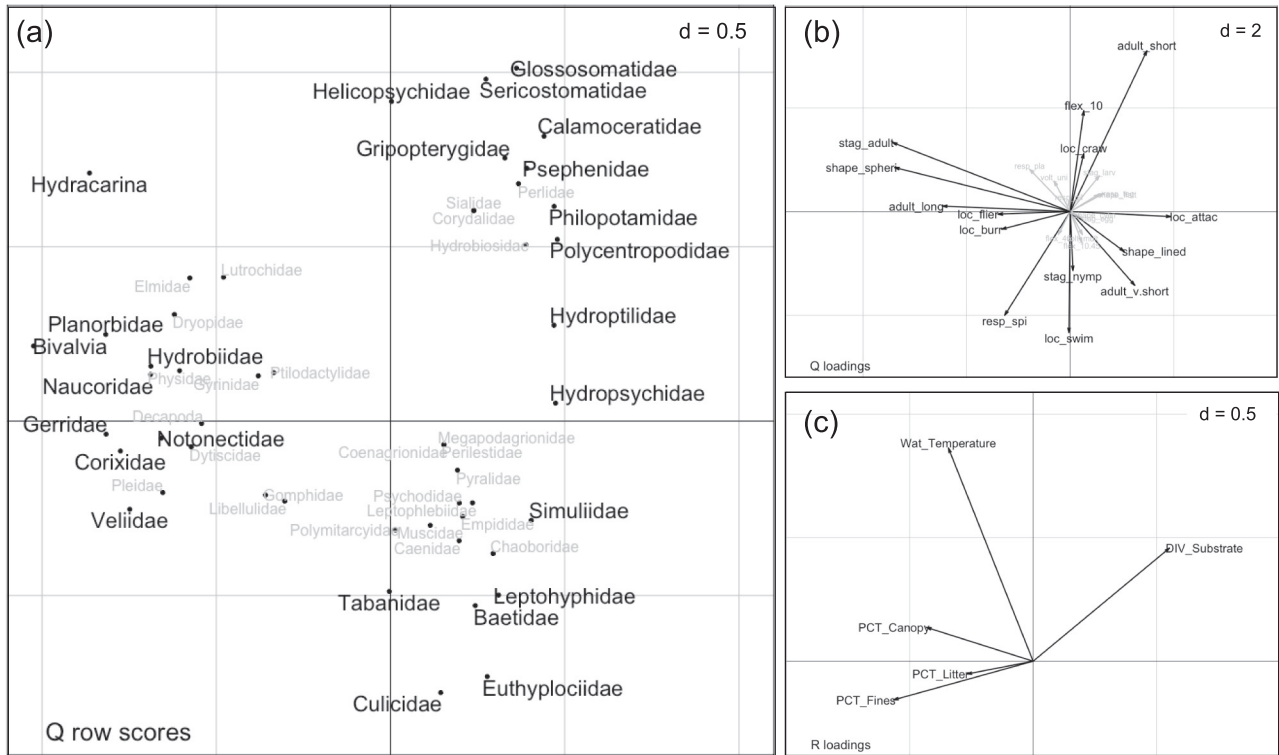


Fig. 2. Axis 1 and axis 2 of the RLQ analysis from 210 stream sites in the Neotropical savanna. (a) taxon scores; (b) trait scores; (c) site stressor scores. The values for 'd' indicate grid sizes for scale comparison across the four figures.

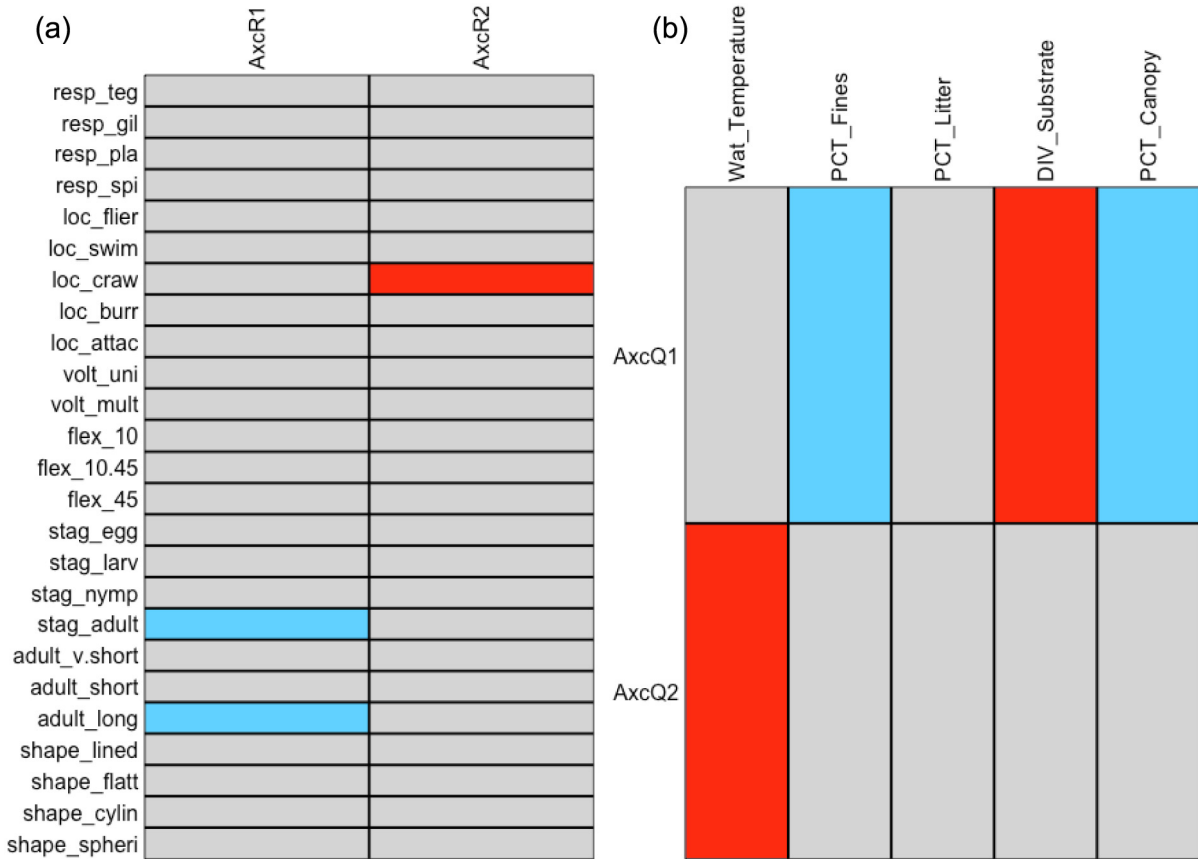


Fig. 3. Significant relationships (P-adjusted <0.05) between (a) the RLQ environmental axes and individual traits and (b) between the RLQ trait axes and individual local stressors. Red indicates positive and blue indicates negative correlations between factors. Non-significant relationships are labeled in grey. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

dependent on autochthonous primary production (Ceneviva-Bastos and Casatti, 2014; Detry et al., 2016), which explains their association with taxa that feed preferentially on algae (e.g. Glossosomatidae, Helicopsychidae, Psephenidae). It also shows that open-canopy streams are important for supporting high biological diversity and complexity of freshwater invertebrate assemblages in the Neotropical savanna (Linares et al., 2018; Santos et al., 2019).

Selection pressures do not act independently on single traits, but rather, on whole organisms carrying many interacting traits. Consequently, species performance will be a function of trait combinations that together present an adaptive response to the environmental conditions (Verberk et al., 2013). This justifies the fact that we did not find significant bivariate associations between one trait and one stressor, but, instead, trait combinations as shown in other studies (Berger et al., 2018; De Castro-Català et al., 2020). Therefore, our study provides evidence that trait combinations of aquatic invertebrates define stressor-specific tolerance to local riparian stressors. Nevertheless, it is important to highlight that a different combination of traits can provide a similar overall solution to specific ecological problems. This type of trait interrelationship provides a complex adaptive solution (strategy) to the multitude of environmental filters faced by an organism in its environment, but still unexplored. Therefore, the trait combinations in response to different stressors that we reported may encourage further attempts to identify life-history strategies and to develop stressor-specific indices, an approach still little explored (Mondy et al., 2016).

The riparian zone mediates important ecological processes for stream ecosystems, such as the quality and quantity of energy inputs, temperature regulation and habitat heterogeneity (Gregory et al., 1991). When these processes are disrupted local stressors then act as highly selective environmental filters, drastically reducing biological diversity and homogenizing it across large geographical areas (Castro et al., 2018; Rahel, 2002). This disruption is especially worrying for the Neotropical Savanna, because of its high endemism, which can result in the extinction of many species and a possible collapse in the biodiversity of the biome (Strassburg et al., 2017).

By using the alteration of the trait structure of benthic macroinvertebrate communities as a proxy to changes in ecosystem processes, our results also suggest that the anthropogenic stressors can cause significant shifts in the ecosystem functioning of neotropical streams. As a general trend, we found that sites affected by the stressors favored taxa associated with a fully aquatic lifestyle and autotrophic food sources. This may result in more isolated communities, due to taxa with aquatic adult stages not being as good dispersers as those with terrestrial (flying) adult stages (Sarremejane et al., 2017). Also, benthic macroinvertebrate communities that are more dependent on autotrophic food sources are less stable and resilient than those more dependent on allochthonous food sources (Death and Collier, 2010). Therefore, these communities would be more vulnerable to stochastic disturbances and more prone to local extinctions, and therefore should be prioritized for restoration efforts.

5. Conclusion

Our results highlight the importance of riparian zones for the taxonomic and functional structure of aquatic assemblages and anthropogenic stressors as environmental filters in neotropical stream ecosystems. They show that streams affected by local anthropogenic stressors select taxa with sets of traits distinct from those in sites least affected by those stressors, which can compromise ecosystem functioning. Our results can also support the development of evaluation tools for environmental managers and decision makers. For example, they can be used to indicate that the effects of a stressor are significantly affecting the functioning of a stream ecosystem. Also, assessing specific groups of site stressors and responses of specific traits simplifies environmental impact studies and provides mechanistic understanding of different anthropogenic stressors. Moreover, these results encourage the

application of bioindicators to assess anthropogenic pressures in riparian zones, providing an effective approach for singling out the most vulnerable sites in the scenario of global changes and dramatic changes in land use, helping conservation and restoration efforts. Because freshwater assemblage traits will respond in similar ways across large biogeographic areas, this set of traits can be used with relative ease for ecological monitoring in other tropical savanna headwater streams worldwide.

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CRedit authorship contribution statement

K.R.F., D.M.P.C, M.S.L., and M.C. elaborated the research design, collected and processed the data. K.R.F. and D.M.P.C conducted the statistical analyses. All authors wrote the paper.

Declaration of competing 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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