Research paper

Land cover disturbance homogenizes aquatic insect functional structure in neotropical savanna streams

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ARTICLE INFO

ABSTRACT

By modifying local habitat conditions, the alteration of land cover can result in severe faunal impairment, subsequently affecting ecosystem functioning. We analyzed changes in the composition of Ephemeroptera, Plecoptera and Trichoptera (EPT) assemblages along a gradient of human disturbance intensity in 66 neotropical savanna headwater stream sites. Whereas EPT genus richness did not differ among the levels of disturbance, functional richness and diversity significantly decreased from least- to highly disturbed sites. Using a combination of RLQ and fourth-corner analyses, we found that 12 of 28 trait categories were significantly associated with changes in catchment land use and cover intensity, local physical habitat, and water quality. EPT assemblages in least-disturbed sites were characterized by higher proportions of large body size and low body flexibility with < 1 reproductive cycle per year, case building, and climbing functional traits. In addition, disturbed sites demonstrated high assemblage functional homogenization compared with least disturbed sites. To our knowledge, this study is the first that examines the response of invertebrate functional structure along a gradient of human disturbance at a large spatial scale in the Neotropics. In doing so, we demonstrate the applicability of multiple trait-based approaches and the higher reliability of functional structure in comparison to taxonomic composition for assessing the effects of human disturbances in neotropical streams.

1. Introduction

Human pressures related to agriculture and land cover modifications are the main drivers of ecological degradation in stream and river ecosystems, primarily resulting from increases in nutrients and fine sediments and hydromorphological alterations (Carpenter et al., 2011; Dudgeon et al., 2006; Vörösmarty et al., 2010). These changes have often resulted in the simplification of habitats and reduced the diversity of aquatic communities, imperiling the ecological integrity and the sustainability of ecological processes and associated ecosystem services (Cardinale et al., 2012; Hooper et al., 2005). Furthermore, multiple human pressures yield taxonomic and functional losses in assemblages through the reduction of the number of specialized species (i.e., species with narrower niche breadth), leading to biotic homogenization (Devictor et al., 2010; Mondy et al., 2014; Leitão et al., 2016). Olden et al. (2004) identified three components of biotic homogenization: genetic, taxonomic, and functional. Taxonomic homogenization (the most common component) represents the increase in species similarity in space over time whereas functional homogenization stands for the decrease in functional diversity among species in the community

(Olden and Rooney, 2006). As a result, functional homogenization at the community level limits the functions provided by the community and its ability to respond to human pressures (Clavel et al., 2011; Gámez-Virués et al., 2015).

Trait-based approaches have been successfully used to assess mechanisms structuring communities and the effects of human pressures on aquatic invertebrate assemblages (e.g., Dolédec et al., 1999; Culp et al., 2011; Gutiérrez-Cánovas et al., 2015; Piló et al., 2016; Kuzmanovic et al., 2017) because of several advantages: (1) Biological trait responses are spatially stable in both natural and human least-disturbed situations (e.g., Statzner et al., 2004; Bonada et al., 2007), which potentially offer a more reliable assessment of ecological integrity compared with the use of taxonomic composition that naturally varies across large spatial scales. (2) Biological traits allow a more mechanistic understanding to identify the causes of changes in assemblages (e.g., Dolédec et al., 2011; Verberk et al., 2013). (3) Biological traits respond to a broad range of stressors in a predictable manner (e.g., Dolédec et al., 1999; Usseglio-Polatera and Beisel 2002; Culp et al., 2011; Mondy et al., 2014; Mouillot et al., 2013).

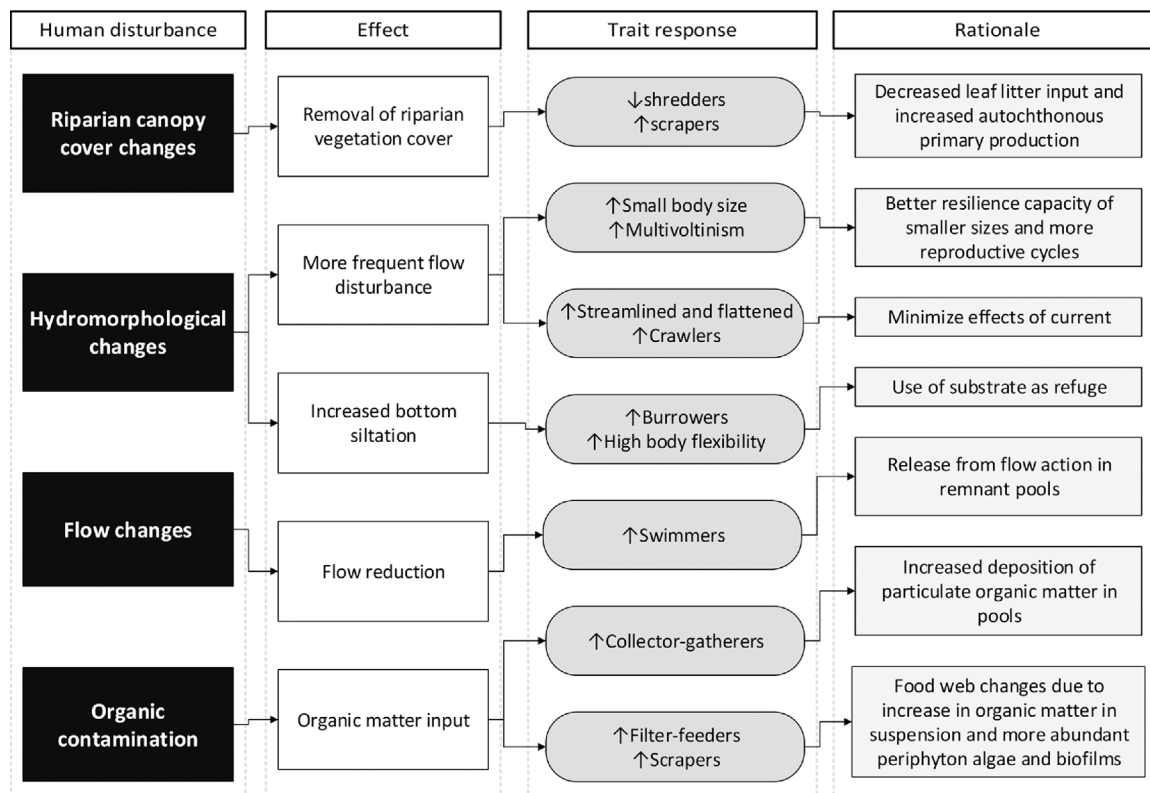In the Neotropics, the main human disturbances include habitat

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Fig. 1. Graphical framework showing predictions for trait category responses to human impacts. Impacts may decrease (↓) or increase (↑) the relative abundance of a single trait category (after Statzner and Bêche 2010 and Feio and Dolédec 2012).

fragmentation and land cover conversion for agricultural purposes (Overbeck et al., 2015; Hunke et al., 2015). Those disturbances have several consequences for stream environments, such as reduced water quality, excess bottom siltation, altered flow regimes, and changes in stream channel structure (Allan et al., 1997; Leal et al., 2016). In other regions, authors have demonstrated that human disturbances not only affect taxonomic composition and richness but also the functional structure of benthic invertebrate assemblages (e.g., Archaimbault et al., 2010; Mondy et al., 2016; Ding et al., 2017). The neotropical savanna (Cerrado biome), the second largest biome in Brazil and a biodiversity hotspot (Myers et al., 2000), encompasses three of the largest basins in South America (i.e., Paraná, São Francisco, and Tocantins-Araguaia Rivers). Despite its enormous importance for freshwater biodiversity and the provision of ecosystem services (Strassburg et al., 2017), the Cerrado biome has been largely overlooked in terms of human disturbance effects on the functional structure of aquatic invertebrate assemblages. Therefore, assessing changes in functional structure may facilitate disentangling the effects of disturbance on species assemblages (Leitão et al., 2017) and understanding how human disturbances affect aquatic ecosystems in this region.

We designed our study using Ephemeroptera, Plecoptera and Trichoptera (EPT) assemblages to answer two questions: (1) Are potential differences in reliability between taxonomic and functional structure of aquatic insect assemblages to depict human disturbance in the Neotropics similar to those observed elsewhere (e.g., Dolédec et al., 1999)? (2) Do human disturbances also alter assemblage specialization? We had two hypotheses: (1) Because taxa possess traits that confer different sensitivities to land cover alteration intensity and associated hydromorphological degradation, co-adapted traits within benthic assemblages would be selected [life-history strategies include different combinations of traits that represent the solution to a given ecological problem or disturbance (Verberk et al., 2013)]. (2) The more diversified habitat conditions in least-disturbed streams will result in higher

taxonomic richness, functional diversity, and assemblage specialization compared with streams affected by anthropogenic stressors. Specifically, we made *a priori* predictions on the potential responses of the functional structure of benthic invertebrate assemblages to land cover alteration (e.g., deforestation) and the associated impairment of the hydromorphology (e.g., sedimentation and flow regime) and stream water quality (e.g., increase in nutrient inputs). Direct effects of riparian vegetation cover removal include the reduction of litter input, which should subsequently limit the proportion of shredders. Indirect effects of riparian vegetation cover removal yield more frequent flow disturbances. Those disturbances should select organisms with traits conferring resilience, such as small size, several reproduction cycles per year (multivoltinism), and resistance capabilities (streamlined/flattened shape and crawling locomotion) (Townsend and Hildrew, 1994). In addition, changing land cover for agricultural purposes may directly involve an increase in fine sediment deposition, which should favor burrowers and potentially lead to flow reduction (because of water abstraction), which should favor swimmers (e.g., Statzner et al., 2010). Finally, changes in water quality associated with organic contamination should favor filter feeders and collector gatherers, and the associated expansion of biofilms should favor scrapers (Fig. 1). Although we made individual predictions, we are aware that traits correlate in organisms (Verberk et al., 2013).

2. Methods

2.1. Study area

We conducted our study in wadeable streams located in the neotropical savanna (Cerrado biome) of southeastern Brazil. A total of 160 sites (1st- to 3rd-order streams sensu Strahler (1957), defined at a 1:100,000 scale) were randomly selected and sampled in four hydrological units (defined as the contributing drainage areas within 35 km
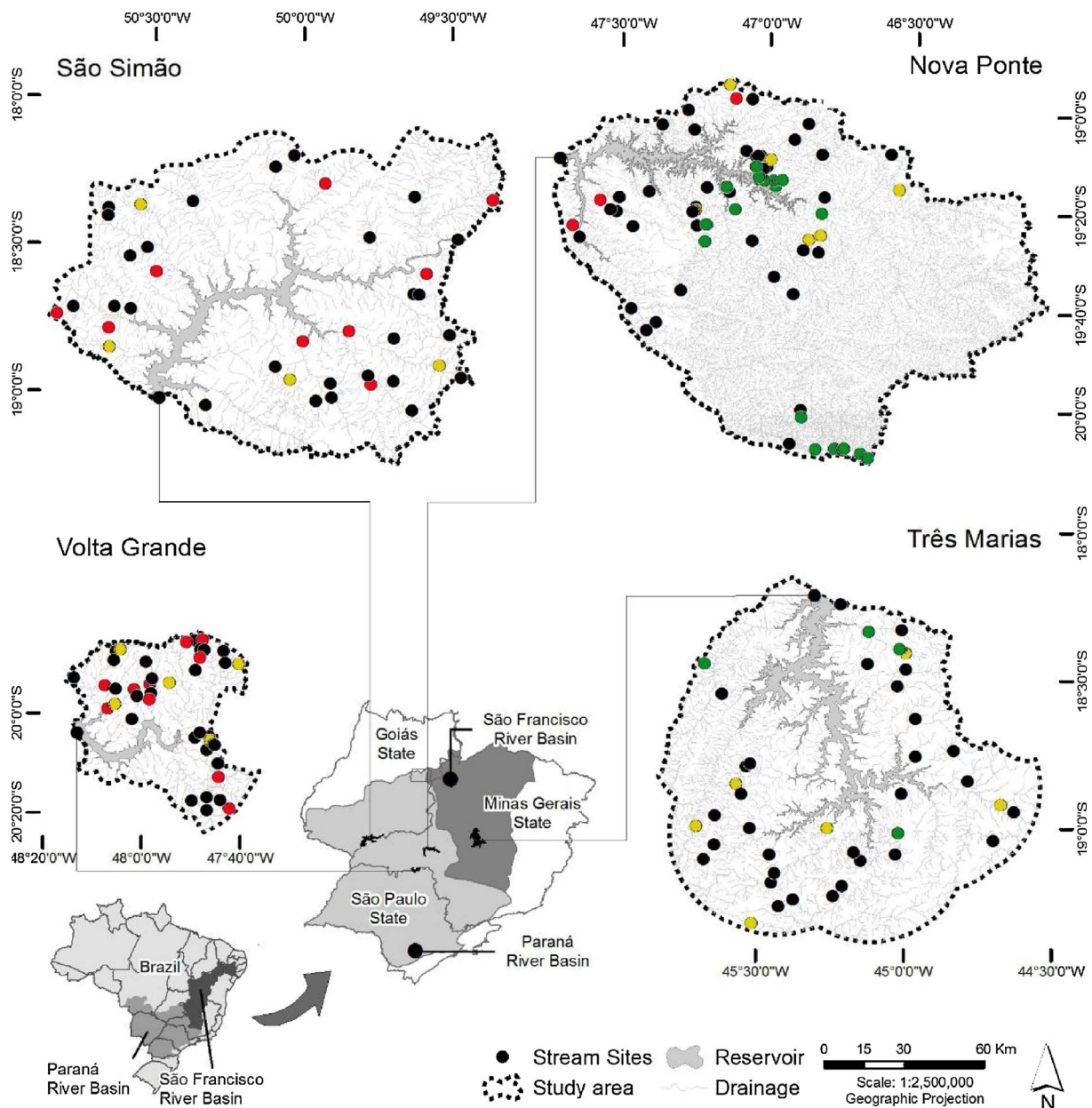
Fig. 2. Locations of wadeable stream sites (n = 186) in four hydrological units in the Neotropical savanna, Minas Gerais state, southeastern Brazil. Green circles represent least disturbed sites, yellow circles represent intermediately disturbed sites, and red circles represent disturbed sites. Black dots represent removed sites after categorization based on the Integrated Disturbance Index. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

upstream of each of four major hydropower reservoirs) of Nova Ponte, Três Marias, Volta Grande, and São Simão (Fig. 2), comprising a total geographic area of 45,180 km². Sampling was conducted in September from 2010 to 2013, one year for each of the aforementioned hydrological units, ensuring that samples were all taken in the low flow season. Site details are available in Ferreira et al. (2017) and Firmiano et al. (2017). A set of 26 handpicked sites representing least-disturbed conditions was additionally sampled in the Nova Ponte hydrological unit (Martins et al., 2017). As recommended in previous studies (Hughes et al., 1986; Stoddard et al., 2006), we defined sites in least disturbed conditions based on the minimal human disturbance in the catchment (by means of GIS analysis), the absence of direct local influence of human alterations, and the presence of native riparian vegetation at sampling sites. We tested the homogeneity for both taxon and trait composition in each hydrological unit, which would allow using all 186 sites together. We compared the observed variance across the sites in each hydrological unit (further details are described in

Castro et al., 2017). Therefore, there is no spatial dependency on selected sites that may cause potential bias in our analysis.

2.2. Site selection

We ranked sites from least- to most-disturbed based on the Integrated Disturbance Index (IDI) developed by Ligeiro et al. (2013). At each site, we measured human activities at local (LDI – Local Disturbance Index) and catchment (CDI – Catchment Disturbance Index) scales and analyzed both scales together through the IDI. Briefly, CDI measures land uses in the catchment using a weighted average procedure. Urban areas receive a higher weight than agricultural areas, which receive a higher weight than pasture. LDI is based on the presence of local human pressures (garbage, sewage, buildings, domestic animals, row crops, pasture, erosion and dams) at each site (see Kaufmann et al., 1999). IDI scores were ranked (Table A1 in Supplementary material), and we calculated the mean and standard deviation

Table 1

Traits and their categories and codes used for 73 EPT genera collected in neotropical savanna streams.

Trait	Code	Category
Body size (mm)	less_1.5	< 1.5
	bet_1.5_2.5	> 1.5–2.5
	bet_2.5_3.5	> 2.5–3.5
	bet_3.5_5	> 3.5–5.0
	bet_5_10	> 5.0–10.0
	more_10	> 10
Potential number of reproductive cycles per year	less_1y	≤1
	more_1y	> 1
Feeding habits	collector_gat	Collector-Gatherer
	shredder	Shredder
	scraper	Scraper
	filter_feeder	Collector-Filterer
	predator	Predator
Locomotion	burrow	Burrower
	climber	Climber
	sprawler	Sprawler
	clinger	Clinger
	swimmer	Swimmer
Body flexibility (°)	flex_less_10	< 10
	flex_10_45	> 10–45
	flex_more_45	> 45
Body form	streamlined	Streamlined
	flattened	Flattened
	cylindrical	Cylindrical
	spherical	Spherical
Relation to substrate	free_living	Free living
	silk_net_builder	Silk net builders
	case_builder	Case builder

(SD) of the IDI distribution to define the limits of least- and most-disturbed sites. Least- and most-disturbed sites were assessed as those deviating from the mean by −1SD and +1SD, respectively. The intermediately disturbed sites were those that occurred between these values (Fig. A1 in Supplementary material).

After eliminating five sites with a genus richness < 4 (most composed of a single individual in a taxon, outliers in the analyses), we retained 37 sites classified as least-disturbed (IDI < 0.21), 22 as disturbed (IDI > 0.63) and 122 classified as intermediately disturbed (> 0.21 and < 0.63). To respect an equal number of sites across human disturbance levels, we randomly selected 22 sites among the least disturbed sites and 22 among the intermediately disturbed sites (Fig. A1 in Supplementary material). This is a prerequisite of the subsequent data analyses because an unequal sample size creates an additional risk that different site weights would markedly bias the difference between classes. Following this random selection, we eliminated those sites with IDI scores close to boundaries (i.e. sites with IDI scores of 0.21 ± 0.05 and between 0.60–0.63). Therefore, we analyzed a total of 66 sites with 22 sites in each level of human disturbance (least, intermediate, and most).

2.3. Environmental variables

Environmental variables at each site were described in terms of catchment land use and cover, local riparian canopy cover, physical habitat (habitat hydromorphology, substrate size, flow regime, instream habitat cover), and water quality (Table A2 in Supplementary material). Sites initially were described by 250 environmental metrics. After screening, those metrics having low variability (more than 90% with no variance) and being highly correlated with each other (r > 0.8), we retained a set of 61 environmental metrics (see Castro et al., 2017, for further details).

2.4. Sampling and biological traits

Benthic macroinvertebrates were sampled with a D-frame kick net (30 cm aperture, 500 μm mesh). Eleven sub-sample units were taken at each site following a systematic zig-zag trajectory along the site, generating one composite sample for each site (see Firmiano et al., 2017 for further details). Identification keys at genus or species levels remain limited for neotropical macroinvertebrates (Moya et al., 2011), and the use of family level identification can lead to the loss of potentially important ecological information (Tomanova et al., 2008). Therefore, we restricted our study to Ephemeroptera, Plecoptera and Trichoptera (EPT) assemblages, given that they are the most studied groups of aquatic invertebrates in the Neotropics. In the laboratory, EPT individuals were identified at the genus level using various taxonomic keys (e.g., Dominguez et al., 2006; Mugnai et al., 2010; Pes et al., 2005). EPT abundances were log-transformed [ln(x + 1)] to reduce the effect of dominant species and approximate a normal distribution.

In the Neotropics, the use of multiple trait-based (MTB) approaches is still in its infancy. One important limitation of the use of MTB approaches involves the lack of biological trait information for most neotropical aquatic invertebrate species. We used a trait database recently developed for neotropical macroinvertebrates fully described in Castro et al. (2017). This database contains biological traits and their respective trait categories describing EPT genus profiles in terms of morphology, life cycle, resilience or resistance ability to natural disturbance or human disturbance, and feeding behavior (Table 1).

2.5. Taxonomic richness, functional diversity and assemblage specialization

We assessed traditional community metrics, including taxonomic richness (rarefied) and Shannon index. In addition, using the relative abundance of taxa in each trait category, we calculated functional diversity in two different facets. First, we computed functional richness (FRic), which measures the overall spread of traits of all taxa in the community (Villéger et al., 2008). Second, we computed the functional dispersion index (FDis), which represents the dispersion of species in trait space from the centroid of all species weighted by their relative abundances (Laliberté and Legendre, 2010).

Finally, we assessed functional homogenization using the indices proposed by Mondy and Usseglio-Polatera (2014). For each taxon and each trait, we calculated a taxon specialization index (TSI). In this case, a truly specialist taxon (i.e., using only one category in a given trait) is characterized by a maximal specialization score (i.e., 1.00) whereas a generalist (i.e., evenly using all the trait categories in a given trait) exhibits a low TSI score. We measured the 7 community specialization indices (CSI), which estimate functional homogenization associated with each trait and each site by averaging the scaled TSIs of taxa weighted by the respective log-transformed abundances (see Mondy and Usseglio-Polatera, 2014 for further details). We further computed a global specialization index by averaging each trait CSI across all sites.

After testing for normality of residuals and homoscedasticity, we used ANOVA to evaluate whether the above biological metrics were affected by disturbance. We used the *post hoc* Tukey HSD test to identify differences among the categories when tests were significant ($P < 0.05$).

2.6. Trait response of EPT assemblages to human disturbance

To assess the significance of associations between a combination of trait categories and environmental variables, we combined RLQ (described below) and fourth-corner analyses as recommended by Dray et al. (2014). This method involves performing correspondence analysis (COA) on the log-transformed abundance of EPT genera (66 sites and 73 EPT genera) to derive scores between sites and genera that have the maximal covariance (Dolédec et al., 1996). To reduce the number of environmental variables (61 initial variables) involved in RLQ,

principal components analysis (PCA) was conducted on each group of variables (i.e., catchment land use and cover, habitat morphology, substrate, flow, riparian canopy cover, in-stream cover, and water quality), and we extracted the first four axes of each PCA. Axes significantly related (using ANOVA) to the IDI index (i.e., human disturbance) were then used as new environmental variables in the RLQ analysis. RLQ is a weighted averaging technique. A given RLQ axis provides site scores (according to environmental variable loadings). Each EPT genus score equals the weighted average distribution of sites where the genus is present, i.e. each site being averaged is multiplied by its weight based on its relative importance (site weight yielded by COA). Furthermore, a trait category score is equal to the average distribution of the genera that have this trait category, i.e. each genus being averaged is multiplied by its weight based on its abundance.

We tested the global significance of the relationship between EPT biological traits and these environmental variables using a Monte Carlo test (9999 permutations). As suggested by Dray et al. (2014), we considered

model 6, which combines two permutation tests to determine the overall significance of the relationship between trait categories and environmental variables. The first model tests the null hypothesis that "species assemblages are randomly attributed to sites, irrespective of the site characteristics" (rows of the abundance table; model 2). The second model tests the null hypothesis that "species are distributed irrespective of their traits" (columns of the abundance table; model 4). Finally, for a more detailed and specific interpretation of trait-environment associations, fourth-corner analysis helped us quantify and test all bivariate correlations between each trait category or their combination and each environmental variable. In all tests, we applied a false discovery rate adjustment of probabilities to correct for multiple comparisons. Fourth-corner tests were then directly applied onto the RLQ axes.

All analyses were performed in R (R Core Development Team, 2016) with the *vegan* (Oksanen et al. 2017), *FD* (Laliberté et al., 2014), and *ade4* (Chessel et al., 2004) packages and following predetermined methodological steps (Fig. 3).
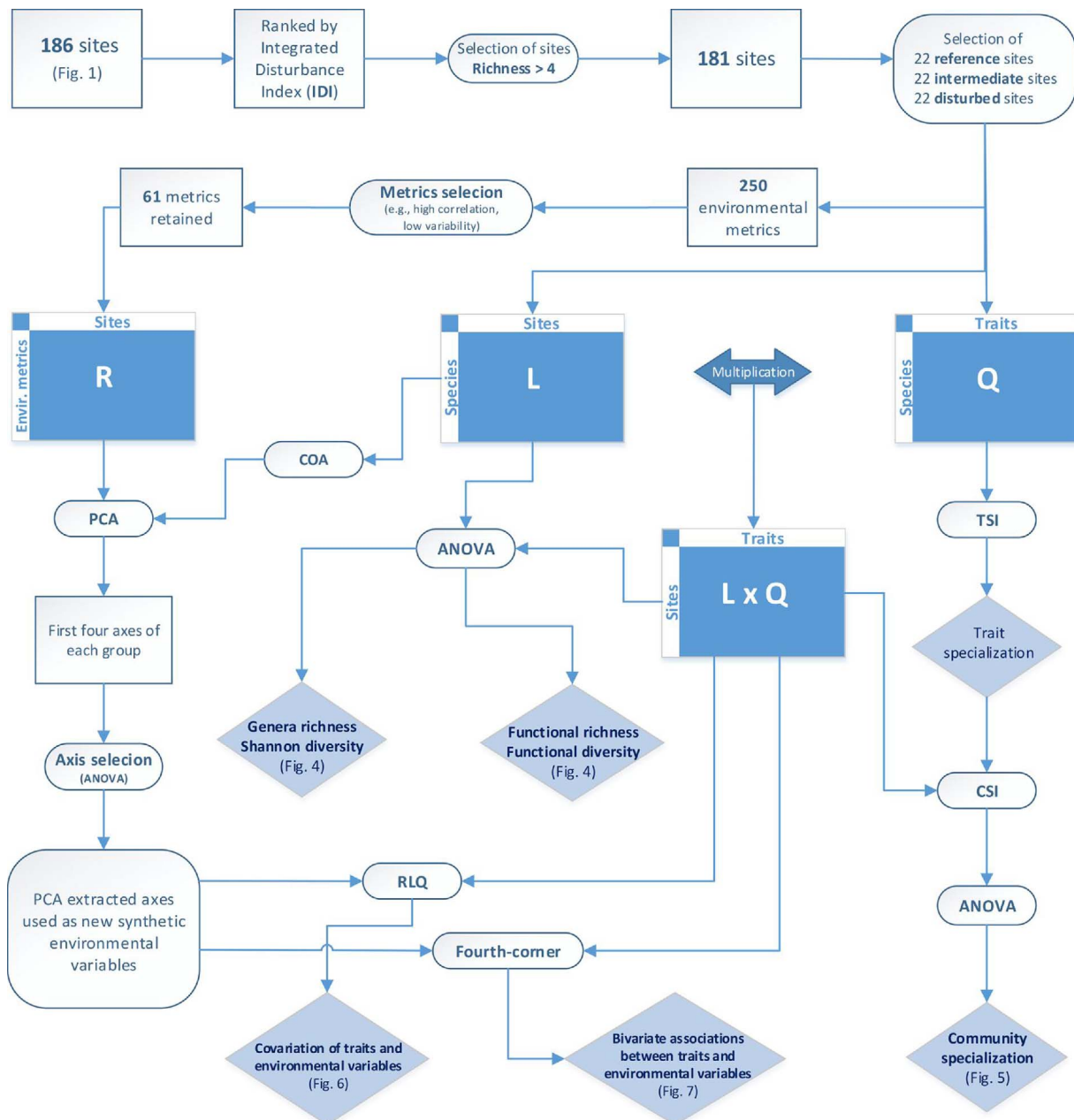

Fig. 3. Schematic diagram presenting the methodological design of site selection and statistical analyses used in this study. RLQ and fourth corner allow relating three tables: environmental metrics at sites (R), species abundance at sites (L) and species traits (Q).
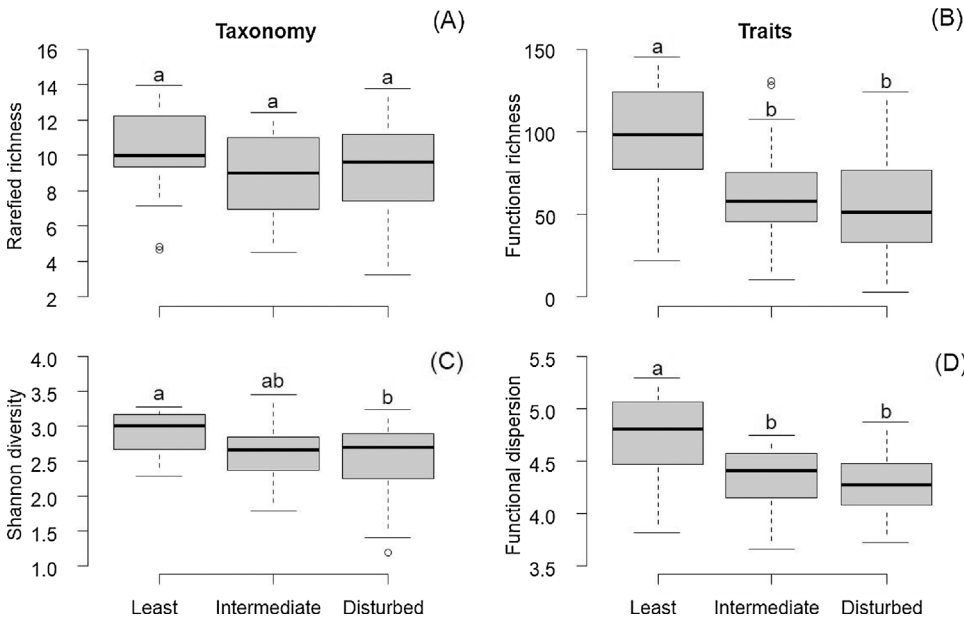
Fig. 4. Difference in (A) genus rarefied richness, (B) functional richness, (C) Shannon diversity, and (D) functional dispersion among the three categories of human pressure. Lines in boxes are medians, and box end are quartiles. Whiskers indicate maximum and minimum values (excluding outliers), and circles represent outliers (values with more than 1.5 times of upper or lower quartile). Tukey HSD (Honest Significant Difference) *post hoc* tests were used for pairwise comparisons. Different letters indicate significant differences among the categories of disturbance.

3. Results

3.1. Taxonomic richness, functional diversity and assemblage specialization

EPT genus rarefied richness did not significantly differ among the levels of human disturbance ($F_{2,63}$ = 1.39, P = 0.255, Fig. 4a). In contrast, functional richness was increased in the least-disturbed sites ($F_{2,63}$ = 9.13, P < 0.001, Fig. 4b), which also supported EPT assemblages with higher Shannon ($F_{2,63}$ = 4.04, P = 0.023, Fig. 4c) and functional dispersion indices ($F_{2,63}$ = 10.6, P < 0.001, Fig. 4d). In addition, EPT assemblages were overall more specialized in least-disturbed compared with most-disturbed sites ($F_{2,63}$ = 7.52, P = 0.001, Fig. 5).

3.2. Trait response of EPT assemblages to human disturbance

The global RLQ test revealed a significant relationship between genus abundance and environmental variables (model 2, P = 0.001) as well as genus abundance and biological traits (model 4, P = 0.045). We focus on the first RLQ axis, given that it explained most of the variability (i.e., 79.3% vs. 8.9% for the second axis; Fig. 6; Table 2) and strongly differentiated least- from intermediately disturbed and most-disturbed sites (Fig. 6b). Environmental variables associated with least-disturbed sites included increased mean herbaceous and woody ground-layer and mid-layer cover, reduced mean exposed soil (Cano_1 in Fig. 6a), higher percentages of grassy-wood and woodland, lower percentage of agriculture (LandUse_2), more flow types, and greater mean velocity (Flow_4; see Supplementary material Fig A1). Intermediate and disturbed sites on the positive side of the first RLQ axis exhibited increased conductivity, total dissolved solids, total nitrogen, and total phosphorus (Quality_1 in Fig. 6a), increased percentage of boulders and cobbles, greater mean substrate diameter, greater percentage of agriculture, reduced relative bed stability (Subs_3), lower house density, lower percentages of natural and parkland cover, and closer highway distance (LandUse_1; see Supplementary material Fig A2).

Trichoptera genera such as *Barypenthus, Marilia, Triplectides, Oxyetira* and *Nectopsyche* were associated with least-disturbed sites, whereas Ephemeroptera genera mainly occurred in intermediate and most-disturbed sites (e.g., *Zelusia, Callibaetis, Waltzoyphius, Farrodes, Miroculis*) (Fig. 6c). Least-disturbed sites had higher proportions of individuals with large body size (> 10 mm), low flexibility (< 10°), < 1 reproductive cycle per year, case builders, and climbers (Fig. 6d).

Intermediate and disturbed sites had increased proportions of individuals that are free-living, highly flexible (> 45°), with small body size (< 3.5 mm), streamlined bodies, reproducing more than once a year, and swimmers (Fig. 6d).

Fourth-corner analysis revealed significant correlations between several trait categories and environmental variables (Fig. 7). Higher riparian vegetation cover was positively associated with individuals with < 1 reproductive cycle per year, low body flexibility (< 10°), and case builders, and negatively associated with swimmers, high body flexibility (> 45°), and smaller body size (< 3.5 mm). Lower water quality and bed stability and higher mean substrate diameter were positively associated with organisms with small body size (< 3.5 mm), swimmers, and high body flexibility (Fig. 7). The first trait axis (AxcQ1) was significantly negatively correlated with canopy cover (Cano_1) and positively correlated with land use and cover (LandUse_1), substrate (Subst_3), in-stream cover (Instream_1), and water quality (Quality_1) (Table 3).
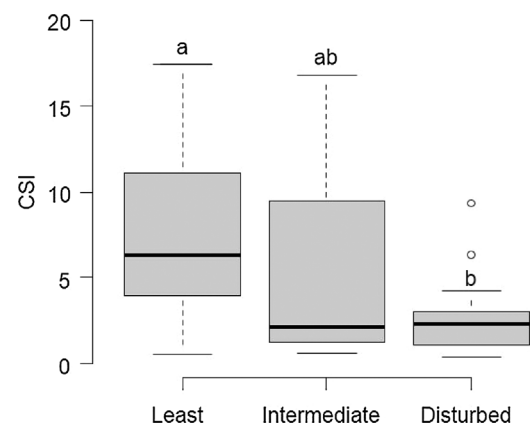

Fig. 5. Overall community specialization index (CSI) for EPT assemblages in each disturbance category. Lines in boxes are medians, and box end are quartiles. Whiskers indicate maximum and minimum values (excluding outliers), and circles represent outliers (values with more than 1.5 times of upper or lower quartile). Tukey HSD (Honest Significant Difference) *post hoc* tests were used for pairwise comparisons. Different letters indicate significant differences among the categories of human pressure.
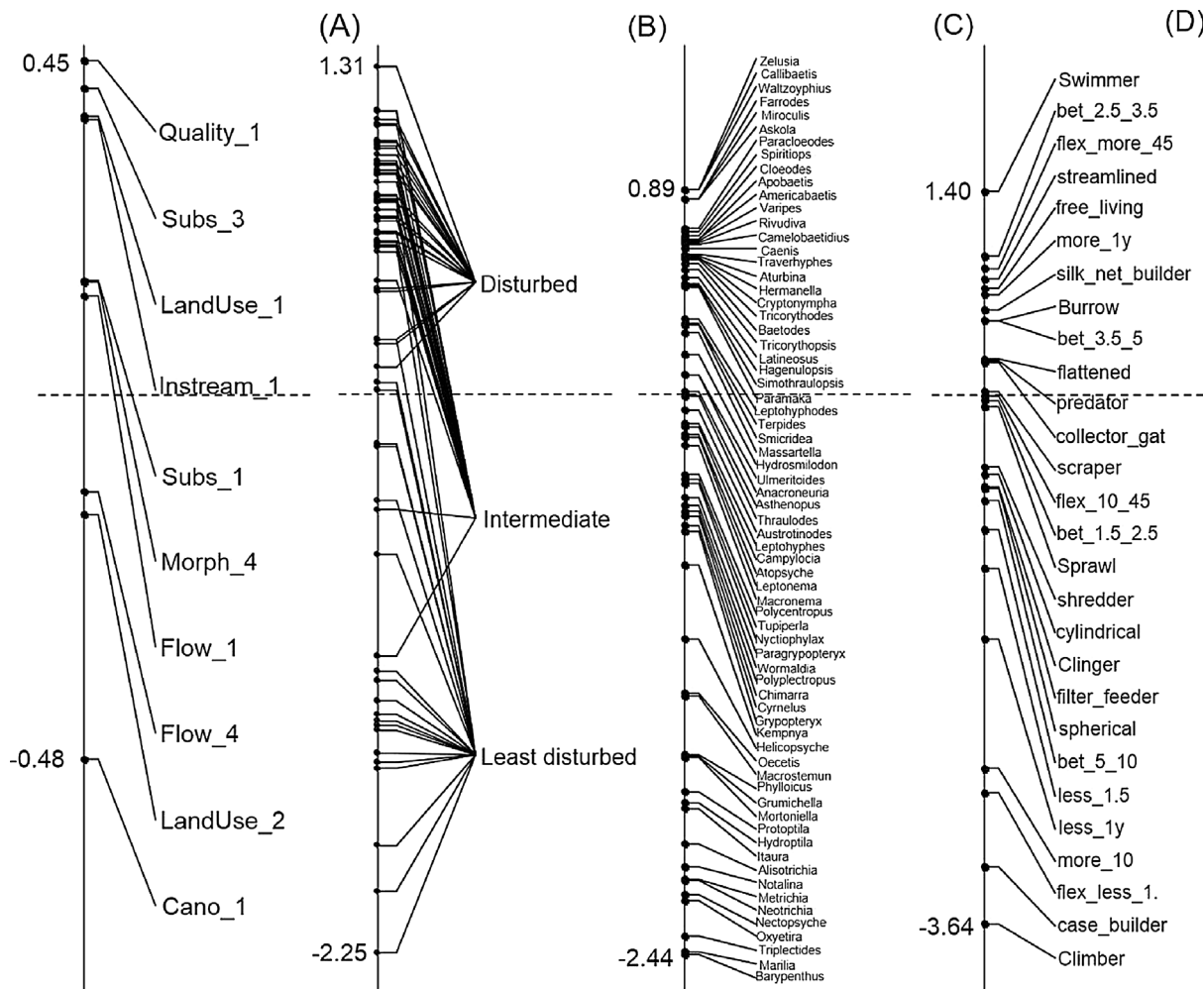
Fig. 6. RLQ analysis first axis scores demonstrating (A) environmental variable loadings (acronyms stand for the type of variable and the axis of the separate PCA), (B) distribution of site scores grouped by disturbance category, (C) EPT genus scores at the weighted average of sites where they are present, and (D) trait scores at the weighted average of their species. Dotted line is the axis center. Acronyms for traits and environmental variables are provided in Tables 1 and A.2, respectively.

4. Discussion

Our aim was to examine the reliability of the taxonomic and trait responses of EPT assemblages to overall human disturbance and to assess the effects of human disturbances on assemblage specialization.

Table 2
Statistics obtained from multivariate analysis with (A) separate analyses demonstrating eigenvalues and percentages of variance that represented the first axis of each analysis, and (B) RLQ analysis demonstrating eigenvalues and percentage of total variance that accounted for the first RLQ axis, covariance and correlation, projected variance of the environment, abundance, trait tables onto the first RLQ axis, and associated percentage of variance compared with separate analyses along the same axis.

	Axis 1	
	eigenvalue	% of variance
(A) Separate analysis		
Environment (PCA)	4.07	40.7
Abundance (COA)	0.27	10.1
Trait (FCA)	0.35	23.9
(B) RLQ analysis		
Eigenvalue	0.03	78.9
Covariance	0.18	
Correlation	0.16	
R/RLQ	3.92	96.2
L/RLQ	0.16	30.9
Q/RLQ	0.30	88.1

Functional structure was more reliable than taxonomic composition in the assessment of the effects of human disturbances on the stream environment. In addition, the consequences of human disturbance included a reduction of the functional diversity in EPT assemblages and an increase in their functional homogenization.

4.1. Taxonomic richness, functional diversity and assemblage specialization

In aquatic systems, land cover modification is a major driving force of biodiversity loss (Allan, 2004). The reduced functional diversity observed in our study in the disturbed streams corroborates the habitat-filter hypothesis (Poff, 1997). This hypothesis postulates that only those taxa possessing a specific set of traits can pass through the habitat filter and establish local populations. Environmental filtering occurs when environmental pressures exceed the physiological tolerance limits of individuals in local populations (Somero, 2010). In this context, human disturbances are additional environmental filters that can change the expected functional structure of assemblages observed in natural conditions (Floury et al., 2017). An increased functional richness implies that more niches are filled by taxa, whereas an increased functional dispersion indicates a more uniform distribution of a dissimilar trait. In our study, both functional richness and functional dispersion were increased in the least-disturbed sites, suggesting that most of the functional trait space was occupied and that the species were well distributed in the functional space in least-disturbed streams despite a lack of significant differences in taxonomic richness among the three
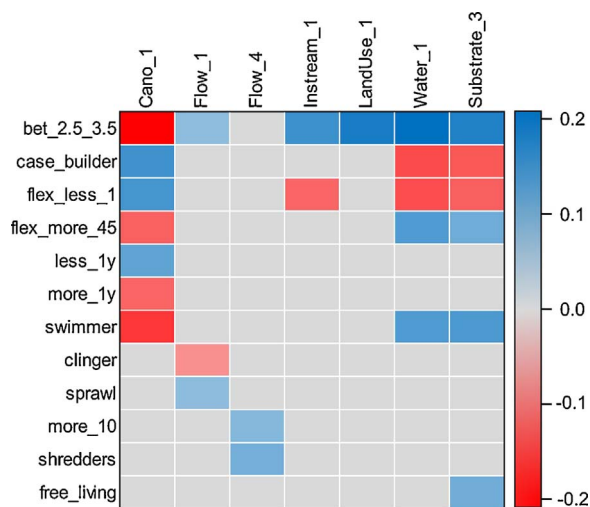
Fig. 7. Trait environment relationship resulting from fourth-corner analysis. Significant positive associations ($P < 0.05$) between the environmental variables and EPT trait categories are represented by blue cells, whereas significant negative associations are represented by red cells. The relative color tone indicates the strength of association. Non-significant associations are gray. Environmental variables and traits categories without any association are not presented for clarity. Codes for traits and environmental variables are provided in Tables 1 and A.2, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

categories of human disturbance.

In freshwater ecosystems, the replacement of habitat specialists by habitat generalists occurs as a response of assemblages to habitat degradation and land cover alteration (Petsch, 2016). In our study, EPT assemblages of disturbed sites were more functionally homogenous compared with least-disturbed sites, suggesting a non-random effect of stress on homogenization (e.g., Mondy and Usseglio-Polatera, 2014; Smart et al., 2006). Specifically, we observed an increase of traits providing resilience (e.g., body size, number of reproductive cycles per year) and resistance (e.g., relation to substrate, body form) capabilities of individuals in most-disturbed sites. Given that homogenization causes a reduction in ecosystem resilience and resistance to environmental changes (Olden et al., 2004), we infer that most-disturbed streams have lost the ability to resist future changes in a global human change scenario.

4.2. Trait response of EPT assemblages to human disturbance

Predictions of response to human disturbance made for 28 trait categories were fulfilled in 12 cases. Organisms characterized by large body size, low body flexibility, < 1 reproductive cycle per year, case builders, and climbers characterized EPT assemblages of least-disturbed sites, whereas disturbed sites had increased proportions of organisms characterized by small body size, streamlined, highly flexible, free-

Table 3
Correlations between environmental variables (PCA loadings) and trait axis (AxcQ1). *P*-values in bold are significant relationships.

Test	Std.Obs	*P* value
LandUse_1/AxcQ1	0.1200	**0.0188**
LandUse_2/AxcQ1	−0.0510	0.1329
Morph_4/AxcQ1	0.0488	0.1512
Subs_1/AxcQ1	0.0495	0.2697
Subs_3/AxcQ1	0.1319	**0.0005**
Flow_1/AxcQ1	0.0428	0.1511
Flow_4/AxcQ1	−0.0411	0.1794
Cano_1/AxcQ1	−0.1557	**0.0003**
Instram_1/AxcQ1	0.1188	**0.0096**
Quality_1/AxcQ1	0.1436	**0.0035**

living, swimmers, and reproducing more than once a year.

The first RLQ ordination axis accounted for a large fraction of the total variance explained, indicating the existence of a strong environmental gradient structuring the trait composition of EPT assemblages, which partially matched our *a priori* classification based on IDI scores (Fig. 1). Least-disturbed sites were clearly separated from most-disturbed sites, whereas intermediately disturbed sites wer e generally superimposed with most-disturbed sites (Fig. 6). Riparian canopy cover and water quality were the main environmental drivers of the functional structure of EPT assemblages. In their natural state, wooded riparian zones are effective in preserving the ecological integrity and trophic dynamics of aquatic ecosystems (Ferreira et al., 2017). The replacement, reduction, or removal of riparian vegetation cover generally changes physical habitat and hydromorphological and water quality characteristics (Ferreira et al., 2012; Pusey and Arthington, 2003), leading to macroinvertebrate assemblages with more generalist feeding behaviors and greater overlap of trophic niches (Castro et al., 2016). Leitão et al. (2017) reported similar changes in Amazonian fish functional traits as local deforestation altered instream physical habitat structure and water quality.

The observed patterns of body size and number of reproductive cycles per year were in agreement with our predictions, with the proportion of large body size individuals having < 1 reproductive cycle per year occurring in the least-disturbed sites, and small body size organisms with > 1 reproductive cycles per year occurring in the disturbed sites. These latter trait categories confer resilience and/or resistance to disturbance, allowing for faster recolonization of disturbed environments as observed by other authors (Díaz et al., 2008; Dolédec et al., 2006; but see Ding et al., 2017 who recently observed the opposite effects in Chinese headwater streams).

Organisms with small body size and high body flexibility increased in sites with reduced bed stability, heterogeneity and higher proportion of fine sediments. Heterogeneous substrates allow for more diverse benthic communities and provide refuge, whereas homogeneous substrates imply more exposure to physical disturbances, such as current velocity and shear stress (Beisel et al., 2000; Milesi et al., 2016). In addition, high flexibility provides advantages to taxa in sites with a high percentage of fine sediments, enabling the use of small interstices within the bed sediments (Gayraud and Philippe, 2001; Lamouroux et al., 2004; Descloux et al., 2014).

Small body size, high body flexibility, and swimmers were also positively associated with low water quality related to wastewater (Stalter et al., 2013) and agricultural activities (Johnson et al., 1997). Conductivity, total dissolved solids, and other water parameters may also affect invertebrate composition, acting as one of the environmental filters determining assemblage composition (Moya et al., 2011; Olson and Hawkins, 2017). For example, salinity and total dissolved solids can induce physiological stress in freshwater organisms adapted to low concentrations and could affect their fitness and growth (Berger et al., 2017; Cañedo-Argüelles et al., 2013; Olson and Hawkins, 2017), eliminating some species and indirectly changing the trait composition of assemblages. However, in our study, water quality correlated with land use intensity, especially with an increased percentage of agriculture and pasture areas and house density. We believe that the association of these traits is related to the fact that water quality is directly influenced by land use intensification, which has been shown to affect the trait composition of assemblages (Moya et al., 2011; Castro et al., 2017). As a result, the above-mentioned correlation may result from an indirect effect of land use intensification mediated through water quality.

Functional feeding groups exhibited weak relationships with environmental variables. We did not identify the expected relationship between shredders and riparian cover or filter feeders and water quality. These results can be associated with the great trophic plasticity and opportunistic feeding habits of neotropical aquatic insects (Castro et al., 2016; Ceneviva-Bastos et al., 2017; Ferreira et al., 2015).

Identifying how human pressures modify the functional structure of

assemblages can improve our predictive capability about patterns and processes in freshwater ecosystems and help us develop tools that complement traditional assessment for management actions and conservation initiatives (Jonsson et al., 2016; Pallottini et al., 2016). Our MTB approach has proven to improve our ability to evaluate the functional response of assemblages to human disturbance gradients. The RLQ and fourth-corner integrated analysis significantly discriminated streams with different levels of human disturbance. However, the lack of information about neotropical invertebrate traits is one of the main limitations in using the trait approach. Nevertheless, few studies have established the foundation for building a database for neotropical aquatic invertebrate species (Dedieu et al., 2015; Milesi et al., 2016; Reynaga and Santos, 2013; Tomanova and Usseglio-Polatera, 2007; Castro et al., 2017). Gathering the maximum information possible and including more biological traits known to respond to specific human pressures is crucial to improve our understanding of the functioning of lotic ecosystems, providing tools for biomonitoring and conservation of neotropical streams.

Acknowledgements

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ecolind.2017.09.030.

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