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Mayfly bioindicator thresholds for several anthropogenic disturbances in neotropical savanna streams

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ABSTRACT

Anthropogenic disturbances are widely recognized as major threats to terrestrial and aquatic biodiversity worldwide, including areas located in non-forest ecosystems. Headwater streams in the neotropical savanna are severely threatened by large-scale landscape changes that degrade local habitat characteristics and lead to biodiversity loss. The objective of our study was to evaluate Ephemeroptera assemblages as bioindicators of catchment land use and cover, local streambed and riparian vegetation conditions, and instream water quality. To do so, we sampled mayfly nymphs in 184 stream sites across a broad disturbance gradient in four hydrologic units of the Brazilian neotropical savanna. We selected seven metrics without significant co-variation with natural variability: % catchment urban, riparian vegetation condition index (RCOND), human disturbances of the stream channel and riparian zone (W1_HALL), substrate mean embeddedness (XEMBED), dissolved oxygen (mg L^{-1}), pH, and total phosphorus (mg L^{-1}). We ran threshold indicator taxa analysis (TITAN) for each disturbance metric to detect change points in mayfly genera responses (whether sensitive or tolerant) and assemblage turnover pattern. TITAN showed that 20 of the 39 genera found were robust bioindicators (based on purity and reliability values >0.95), sixteen of them being sensitive to increased disturbance. The most sensitive genera were *Tricorythopsis* (Leptohyphidae) and *Camelobaetidius* (Baetidae), showing decreased abundance to most disturbance metrics. We found a turnover pattern of mayfly genera in response to W1_HALL in a narrow variation range. For total phosphorus, the benchmark value defined in Brazilian Federal Legislation is higher than the turnover threshold of several mayfly genera. This indicates that we will lose many sensitive genera even within the limits imposed by national environmental legislation. The indicator taxa approach, based on multiple taxa rather than univariate metrics or single indicator species, demonstrates the value of quantitative ecological information for conserving and managing freshwater ecosystems globally.

1. Introduction

Freshwater ecosystems in good ecological status are indispensable for providing high-quality water supplies for humans and for biodiversity maintenance and conservation (Vörösmarty et al., 2010). Rivers and streams achieve those conditions only if their channels, upstream reaches, riparian vegetation and catchments are in good ecological status (Dudgeon et al., 2006). Increased human developments have largely modified the natural condition of freshwater ecosystems, leading to reduced ecological function and biodiversity (Steffen et al., 2015). Such human-induced ecosystem changes are observed at multiple spatial scales (global, regional, local), constituting a complex and interconnected feedback system (Rockström et al., 2009). For instance, land uses affect geomorphological processes, causing many impacts to stream channels such as channel incision (Beschta et al., 2013), bottom siltation, decreased substrate and flow diversities (Allan, 2004), diminished litter input from riparian vegetation (Boyero et al.,

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2016), and degraded water quality (Taylor et al., 2014; Woodward et al., 2012).

Headwater streams are the smaller riverine sections (Strahler, 1957), comprising around 80% of the cumulative stream length in watersheds (Benda et al., 2005). Considering their contribution to river basins and their high degree of exposure to anthropogenic disturbances, it is important to protect them (Dudgeon et al., 2006). Varying levels of anthropogenic disturbances generate disturbance gradients, yielding streams ranging from nearly pristine to severely disturbed (Davies and Jackson, 2006). The local stream biota are a result of natural environmental drivers (Feld et al., 2016), but a wide range of anthropogenic disturbances also influence their structure (Stoddard et al., 2006). To develop reliable bioindicators, it is necessary to separate the effects of natural variability from anthropogenic disturbance on assemblage structure (Chen et al., 2014; Hughes and Peck, 2008).

Assemblage turnover is observed in response to disturbance gradients, wherein the abundance and/or frequency of taxa increase or decrease abruptly at some threshold point of the gradient (King and Baker, 2014). Baker and King (2010) proposed the threshold indicator taxa analysis (TITAN), which detects points of change in environmental gradients where assemblage response becomes most evident. This allows direct inference from the data and concrete actions to minimize impacts or propose rehabilitation strategies (King et al., 2011). Different studies have demonstrated threshold responses of stream assemblages to various anthropogenic disturbances, considering them as direct cause-and-effect relationships. For instance, assemblage composition change has been related to sedimentation (Burdon et al., 2013), urbanization (King et al., 2011), and natural vegetation suppression (Rodrigues et al., 2016). However, it is necessary to demonstrate how different anthropogenic disturbances alter stream biota by selecting disturbance metrics that are weakly related to natural variability (Shimano and Juen, 2016; Stoddard et al., 2008).

Much attention has been given to conserving and restoring tropical forests, but not much attention has been given to neotropical savannas (Overbeck et al., 2015; Veldman et al., 2015). The Brazilian neotropical savanna is the source of several important large rivers in South America (Wantzen et al., 2006), and their headwaters encompass high levels of biodiversity (Agostinho et al., 2005). However, agriculture, livestock grazing and urbanization are major threats to the biological integrity of this biome (Carvalho et al., 2009; Macedo et al., 2014; Silva et al., 2006). Likewise, hydropower dams and water supply reservoirs create major barriers to dispersal of native species (Agostinho et al., 2005).

Biological assessments are recommended for developing effective stream and catchment conservation and management (Hughes et al., 1986; Stoddard et al., 2008). Benthic macroinvertebrates have been widely recognized for their ability to detect impacts on freshwater ecosystems because of their sensitivity to multiple anthropogenic disturbances (Bonada et al., 2006). When natural environments are altered, sensitive taxa are lost and those that are tolerant prevail, producing assemblage turnover (Davies and Jackson, 2006; King and Baker, 2014). Mayfly nymphs are considered good bioindicators because they are highly diverse, abundant in streams in good ecological condition (Bauernfeind and Moog, 2000; Dedieu et al., 2015), represent multiple trophic levels (Brittain, 1982), and are relatively easy to identify to genus (Domínguez et al., 2006).

Our objective in this study was to evaluate the effects of anthropogenic disturbances on neotropical savanna streams based on thresholds of mayfly assemblage responses. Specifically, we sought to find disturbance metrics that most altered mayfly assemblages. The stream sites represented multiple land use and cover types, streambed and riparian vegetation condition levels, and instream water quality covering a wide disturbance gradient. We classified the mayfly genera by their sensitivity or tolerance based on their threshold responses for each disturbance metric as well as the overall assemblage turnover. Such information can contribute to the conservation of stream integrity and watershed management by identifying critical disturbance thresholds based on reliable bioindicators, as well as for determining priorities for biodiversity conservation and ecosystem restoration.

2. Material and methods

2.1. Study area

We conducted our study in 184 wadeable stream sites (1st-3rd order sensu Strahler, 1957; defined at a 1:100,000 scale), averaging 3.4 m (±1.9) wide, and 35.5 cm (±17.1) deep in the states of Minas Gerais, Goiás, and São Paulo, southeastern Brazil. The sites were located in four hydrologic units (Seaber et al., 1987) of the upper São Francisco and Paraná River Basins (Fig. 1), comprising a total geographic area of 45,180 km^2. Nova Ponte, Três Marias, Volta Grande, and São Simão hydrologic units were defined as the contributing drainage areas within 35 km upstream of each of four major hydropower reservoirs. The Nova Ponte hydrologic unit also included a set of 25 handpicked reference sites. The sites were far enough upstream of the reservoirs to be unaffected by variable water levels in the reservoirs.

We sampled in September from 2010 to 2013, one year for each aforementioned hydrologic unit, ensuring that samples were standardized by the low flow season. Dry season sampling facilitates data collection and reduces the effect of freshets, thereby clarifying the effects of the disturbance gradient on mayfly assemblages (Hughes and Peck, 2008). We believe that rainfall differences between the sampling years did not prevent the comparability of data because the average annual precipitation in the four hydrological units were comparable (2010: 958 mm, 2011: 968 mm, 2012: 1155 mm, 2013: 1171 mm) and within the normal climatological average for the neotropical savanna (ANA, 2016). In each hydrologic unit, small and medium-size cities (up to 80,000 inhabitants) occurred and the main land uses were irrigated agriculture (soy, coffee, corn, and sugarcane) and livestock grazing (hereafter pasture) (Ligeiro et al., 2013; Macedo et al., 2014).

2.2. Survey design

Selection of sites employed a spatially balanced probabilistic survey (Stevens and Olsen, 2004), used by the U.S. Environmental Protection Agency (US-EPA) in its regional and national biomonitoring programs (Olsen and Peck, 2008). To ensure that the disturbance gradient would be well represented, in each hydrological unit we also handpicked least- and most- disturbed sites to sample. Other studies have demonstrated the effectiveness of spatially balanced methods together with targeted sampling for strengthening disturbance gradients (Bryce et al., 2010; Ligeiro et al., 2013; Smucker et al., 2013). Least-disturbed sites included 25 sites located in Serra da Canastra National Park and Serra do Salitre region in the Paraná River Basin (Nova Ponte hydrologic unit), considered *a priori* as least-disturbed sites (Hughes et al., 1986; Stoddard et al., 2006). We assumed their good ecological condition based on the effectiveness of national parks in protecting ecosystems, Google Earth® images of the area showing landscape conditions, and field reconnaissance. Most-disturbed sites included a set of 23 urban sites ranging from 0.2% to 85% of catchment urban land use.

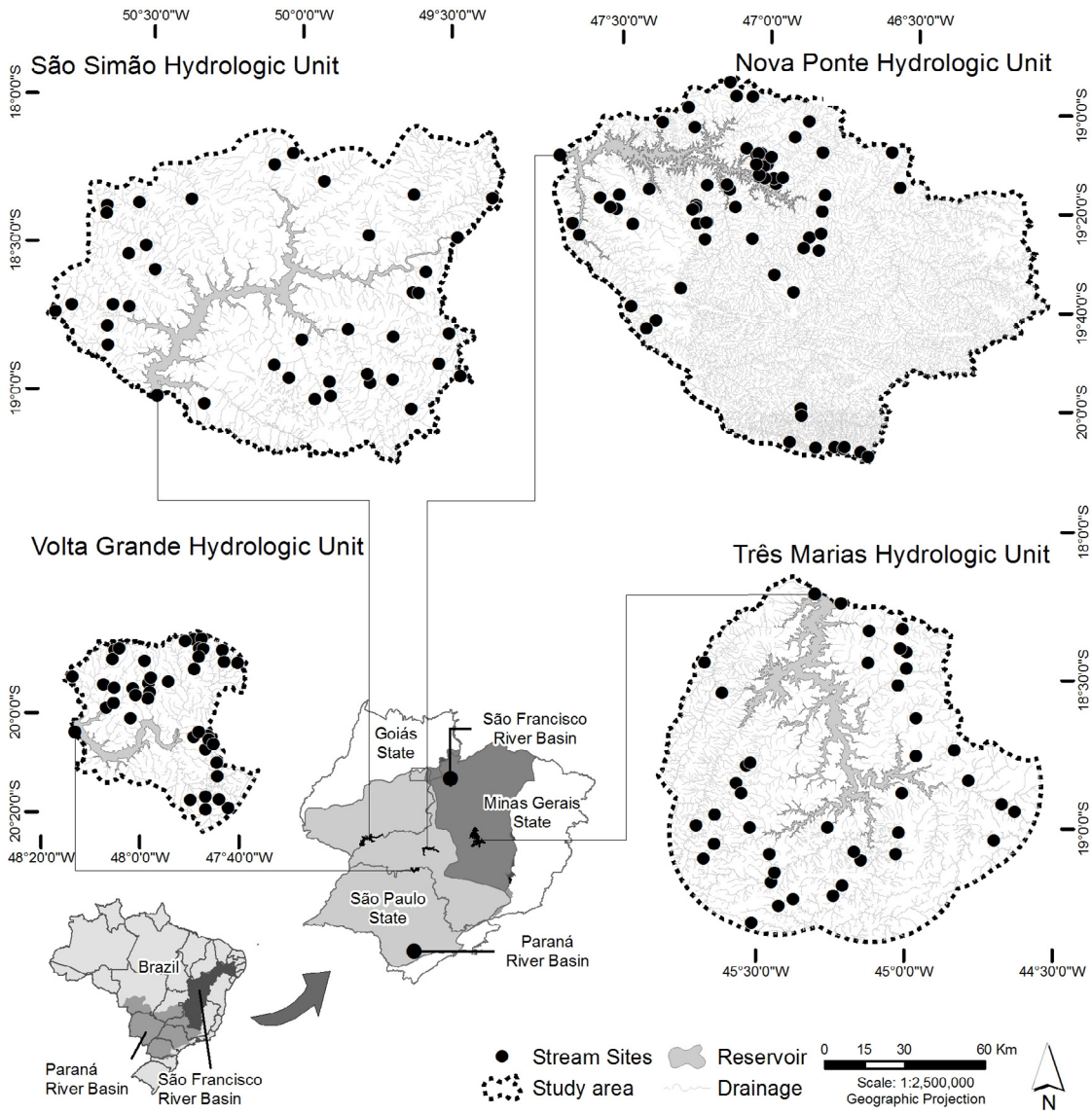

Fig. 1. Locations of hydrologic units and stream sites sampled in the Brazilian neotropical savanna.

2.3. Natural environmental variables

We extracted the average annual rainfall and temperature (~50 years climate baseline) from the worldclim dataset (Hijmans et al., 2005). Catchment area and catchment elevation and slope (range and average) were measured by DEM of Shuttle Radar Topographic Mission (1 arc-sec; USGS, 2005).

2.4. Catchment scale anthropogenic disturbance metrics

We assessed the land use and cover of the catchments of each stream site by interpreting satellite images. This method consisted of combined interpretation of September Landsat TM sensor multispectral imagery (R4G3B2 false color band combination) and fine resolution images from Google Earth® (0.6–5 m spatial resolution; Google, 2014). This method allows one to distinguish leaf structure showed in the multispectral Landsat response from the shape and texture of targets in fine resolution images (Macedo et al., 2014). We identified four natural vegetation cover phytophysiognomies (woodland savanna, parkland savanna, grassy-wood savanna and wetland palm swamp) and four anthropogenic land uses (pasture, agriculture, urban and *Eucalyptus* afforestation), and calculated the percentage of each cover type in each catchment. To further characterize anthropogenic influences we calculated household and population densities using 2010 Brazilian Census data (IBGE, 2016). Road density was calculated by Open Street Map data (OSM Foundation, 2016).

2.5. Local scale anthropogenic disturbance and water quality metrics

To determine the size of the site to be sampled, we multiplied the average wetted width of each site by 40, with a minimum longitudinal site length of 150 m. In each site we placed 11 equidistant cross sectional transects. In the field, we visually assessed anthropogenic pressures in the stream channel and riparian zone following a protocol developed by the US-EPA (Peck et al., 2006). We used: (1) mean substrate embeddedness (XEMBED) (Kaufmann et al., 1999), and (2) log-transformed relative bed stability (LRBS) as indicators of sediment input to the stream bed (Kaufmann et al., 2008); (3) W1_HALL (Kaufmann et al., 1999), which is a metric calculated from the sum of eleven types of disturbance in the stream margin (revetments, buildings, pavement, roads, pipes, trash, lawn, row crop, pasture, mines, and logging), distance

weighted from the channel; (4) riparian woody cover (XCMGW); and (5) a composite riparian condition index (RCOND), which summarizes anthropogenic effects on riparian vegetation cover and structure (Kaufmann et al., 2008). The RCOND score decreases with increased W1_HALL, and increases with increased riparian vegetation complexity. We used an Integrated Disturbance Index (IDI) that combines local (W1_HALL) and catchment (percentage of different land use) disturbance (Ligeiro et al., 2013). Increased XEMBED, LRBS, W1_HALL and IDI scores suggest an increase in the intensity of anthropogenic disturbance in the sites, whereas increased XCMGW and RCOND suggest a decrease in anthropogenic disturbance.

We took one water sample per site for measurements of electrical conductivity ($\mu S\,cm^{-1}$), pH, and total dissolved solids ($mg\,L^{-1}$) with a multi-probe. Dissolved oxygen ($mg\,L^{-1}$), turbidity (NTU), and total phosphorus ($mg\,L^{-1}$) were measured in the laboratory following Standard Methods (APHA, 2005).

2.6. Mayfly assemblage sampling and taxonomic identification

We sampled mayfly nymphs in all 184 stream sites with a D-net (30 cm wide mouth, 500 μm mesh, and 0.09 m^2 area), taking one subsample per transect (Peck et al., 2006). The sampling was performed in six equidistant cross sectional transects, following a systematic zigzag pattern along transects to represent the predominant habitats at each site. We grouped all six subsamples into a single pooled sample for each site, fixing the samples in the field with 10% formalin. In the laboratory, samples were washed in a 500 μm sieve and then stored in 70% alcohol.

We identified mayfly nymphs under a stereomicroscope (80×) to genus using taxonomic keys and consulting taxonomists when necessary (Domínguez et al., 2006; Salles and Lima, 2014). All specimens were deposited in the reference collection of benthic macroinvertebrates of the Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais.

2.7. Data analyses

2.7.1. Screening metrics

We conducted an ordinary least square regression on all metrics related to natural variability and all anthropogenic disturbance metrics. Our objective was to select disturbance metrics without significant co-variation with natural variability, excluding all disturbance metrics with $r^2 > 0.10$ (Chen et al., 2014). The metrics retained were submitted to a correlation matrix and metrics at $r_{Pearson} > |0.50|$ were excluded to avoid multicollinearity (Zar, 1999). Distribution frequencies of all metrics are shown in the Supplementary material (SM1).

2.7.2. Identification of threshold responses

We performed TITAN to detect change points in the genera responses to each final disturbance metric. TITAN combines change point (King and Richardson, 2003) and indicator value (Dufrêne and Legendre, 1997) analyses to detect abrupt change in the abundance and frequency of taxa along environmental gradients (Baker and King, 2010). TITAN also measures purity and reliability properties based on the bootstrap technique (500 resamples with replacement) to confirm the thresholds for each taxa and assemblage. Purity corresponds to the proportion of change points (if z− or z+) along the resampling that agree with the observed value. Reliability corresponds to the proportion of the resampling that reports an indicator value with significant p-values (Baker and King, 2010). After indicator taxa have been identified, TITAN supplies an assemblage-level threshold, reflecting the magnitude of assemblage changes as an indicator of coincident change point in the entire assemblage structure [sum(Z)]. Following the recommendations of Baker and King (2010), we excluded taxa occurring at fewer than three sites and with fewer than five individuals, resulting in 176 stream sites analyzed. We performed TITAN analysis in R version 3.3.1. (R Development Core Team, 2016), using the TITAN2 package (Baker and King, 2010).

3. Results

We collected 26,167 nymphs belonging to 39 genera and seven families. The most abundant genera were *Americabaetis* (18%) (Baetidae), *Traverhyphes* (14%) (Leptohyphidae), *Thraulodes* (10%), *Farrodes* (9%) (Leptophlebiidae), and *Caenis* (5%) (Caenidae). Those five represented 56% of all individuals collected, and occurred in 72%, 65%, 35%, 65% and 47% of the sites respectively. Sixteen genera were considered rare, and together accounted for less than 1% of all individuals collected.

3.1. Correlation between anthropogenic disturbance and natural variability metrics

Several disturbance variables were correlated with natural variability. Agriculture, *Eucalyptus*, natural native vegetation, and pasture were correlated with at least two natural variability metrics ($r^2 = 0.11$-0.35; $p < 0.001$) (Table 1). Population density was correlated with a natural variability metric ($r^2 = 0.12$; $p < 0.001$). LRBS was correlated with several natural variability metrics ($r^2 = 0.12$–0.51; $p < 0.001$) as was IDI ($r^2 = 0.16$–0.19; $p < 0.001$). Conductivity ($r^2 = 0.11$; $p < 0.001$) and total dissolved solids ($r^2 = 0.14$; $p < 0.001$) were correlated with natural variability metrics also. Nine disturbance metrics were not correlated with natural variability metrics: % catchment urban, household density, road density, RCOND, XEMBED, W1_HALL, dissolved oxygen, pH, and total phosphorus (Table 1). We selected% catchment urban, RCOND, XEMBED, W1_HALL, dissolved oxygen, pH, and total phosphorus as disturbance metrics to be used in the TITAN (Table 2). We selected% catchment urban versus household density or road density because it is more comprehensive.

3.2. TITAN versus anthropogenic disturbance metrics

TITAN detected 20 (52%) of 39 genera as robust bioindicators (purity and reliability ≥ 0.95) (Fig. 2). Sixteen genera were considered sensitive to at least one disturbance metric. The most sensitive genera were *Tricorythopsis* (Leptohyphidae) and *Camelobaetidius* (Baetidae), showing decreased abundance and frequency to several disturbance metrics. *Aturbina*, *Callibaetis*, *Waltzoyphius*, *Zelusia* (Baetidae), and *Caenis* (Caenidae) were considered tolerant to at least one disturbance metric. TITAN results for all genera are shown in Supplementary material (SM2).

Regarding catchment and physical habitat disturbance, none of the genera were tolerant to increased urbanization. Only *Caenis* was sensitive (z−) to increased% of urban area, starting to disappear from sites with >0% catchment urban (Fig. 2a). Two genera showed positive associations with increased riparian zone integrity, being observed in increased abundance and frequency in sites with RCOND values ranging from 8 (*Zelusia*) to 13 (*Askola*) (Fig. 2b). Six genera were sensitive, whereas three genera were tolerant, to riparian disturbance (W1_HALL) (Fig. 2c). We detected a clear turnover pattern, wherein two genera decreased while three other genera increased in abundances along an interval ranging from (Z−) = 0.5 and (Z+) = 2.4 W1_HALL values. Eleven genera were sensitive to increased fine sediments across a wide range of XEMBED values (Fig. 2d). The most sensitive genus to XEMBED started to decrease in abundance at an XEMBED of 6%, and the least sensitive started to decrease in abundance at an XEMBED of 87%.

Among water quality variables, we detected a turnover pattern wherein five genera showed positive associations with increased

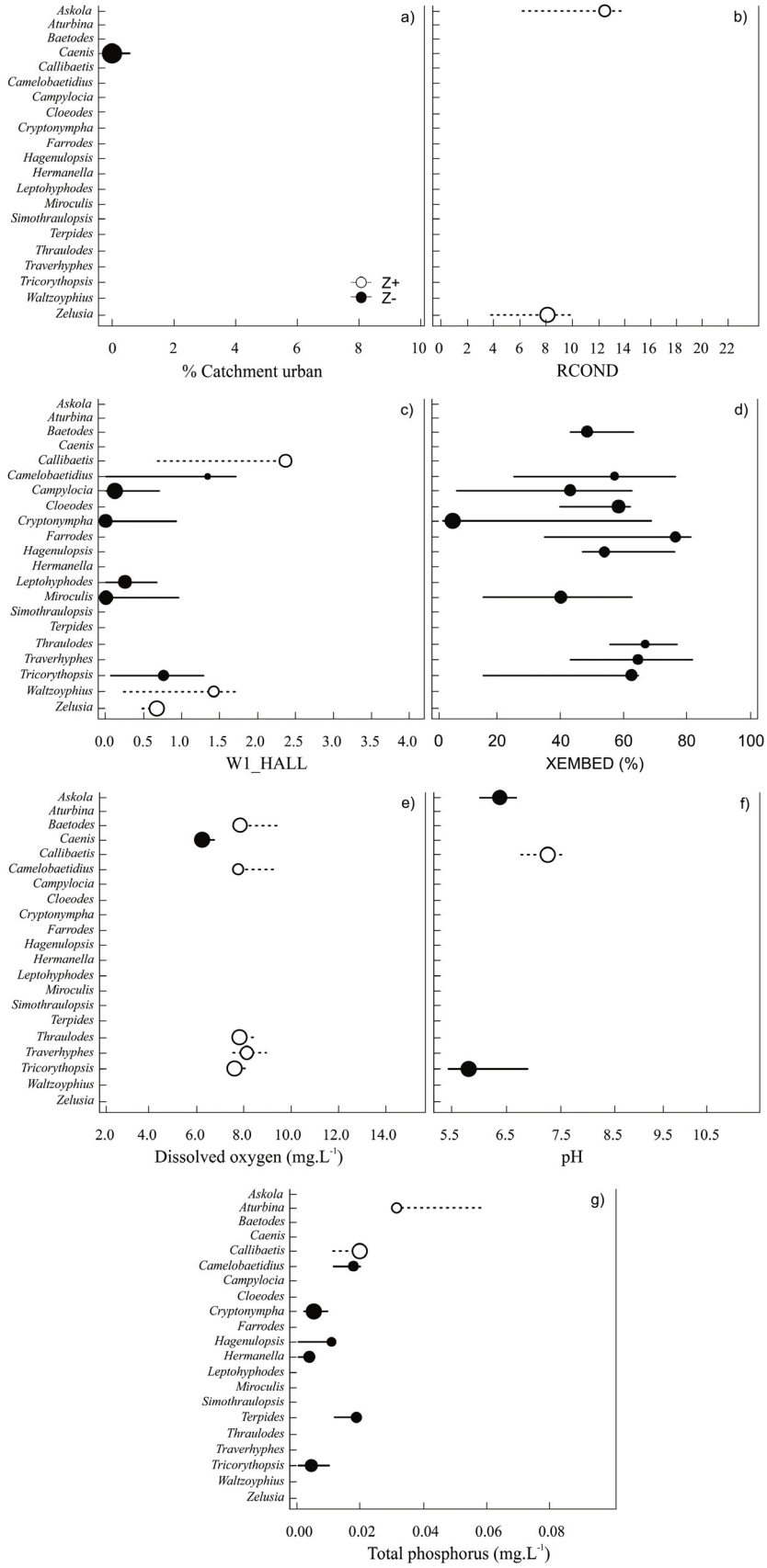

Fig. 2. Robust indicator taxa identified by TITAN in response to anthropogenic disturbance gradients, shown as declining (z −), or increasing genera (z +). Lines (solid or dashed) represent 95% confidence intervals of observed change points (open or black circles).

Table 1
Linear regression coefficient (r^2) among anthropogenic disturbance and natural variability metrics.

Type of descriptor	Metric code	Altitude	Annual rainfall average	Annual temperature average	Catchment area	Catchment elevation average	Catchment elevation range	Catchment slope average	Catchment slope range
Land use and cover	Agriculture (%)	0.12***	0.12***	0.10***	0.01	0.11***	0.11***	0.23***	0.03**
	Eucalyptus (%)	0.01	0.36***	0.01	0.17***	0.02	0.15***	0.04**	0.00
	Natural (%)	0.32***	0.00	0.25***	0.02	0.32***	0.00	0.35***	0.00
	Pasture (%)	0.01	0.17***	0.01	0.05***	0.01	0.17***	0.02	0.05***
	Urban (%)	0.01	0.00	0.01	0.00	0.02	0.01	0.01	0.00
Urbanization	Household density	0.01	0.00	0.01	0.00	0.01	0.00	0.00	0.00
	Population density	0.02	0.00	0.00	0.12***	0.01	0.01	0.03*	0.00
	Road density	0.01	0.00	0.01	0.00	0.01	0.00	0.02	0.01
Local	LRBS	0.49***	0.12***	0.43***	0.03*	0.51***	0.03*	0.16***	0.00
	RCOND	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00
	XCMGW	0.11***	0.10***	0.10***	0.03*	0.10***	0.02*	0.02	0.00
	XEMBED (%)	0.03**	0.00	0.03**	0.00	0.05**	0.00	0.02	0.00
	W1_HALL	0.06***	0.02*	0.06***	0.00	0.06***	0.00	0.02	0.00
Integrated	IDI	0.19***	0.00	0.16***	0.00	0.19***	0.00	0.19***	0.02
Water quality	Conductivity	0.10***	0.04**	0.11***	0.02	0.11***	0.01	0.03**	0.00
	Dissolved oxygen	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01
	pH	0.00	0.00	0.00	0.00	0.01	0.00	0.01	0.00
	Total dissolved solids	0.14***	0.09***	0.14***	0.08***	0.14***	0.01	0.02*	0.02
	Total phosphorus	0.01	0.00	0.00	0.00	0.01	0.00	0.01	0.00
	Turbidity	0.03**	0.00	0.03**	0.00	0.03*	0.00	0.02	0.00

* $P < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

Table 2
Correlations among selected anthropogenic disturbance metrics.

Metric code	% catchment urban	Household density	Road density	RCOND	XEMBED (%)	W1_HALL	Dissolved oxygen	pH	Total phosphorus	Turbidity
% catchment urban	–	0.90*	0.98*	−0.01	0.12	0.46*	0	−0.01	−0.01	−0.01
Household density	0.90*	–	0.86*	0.02	0.11	0.48*	0	−0.01	−0.01	0
Road density	0.98*	0.86*	–	0	0.12	0.46*	0.01	−0.02	0	0.01
RCOND	−0.01	0.02	0	–	0.11	0.14	−0.01	0.09	0.04	−0.06
XEMBED (%)	0.12	0.11	0.12	0.11	–	0.1	−0.15*	−0.01	0.06	0.20*
W1_HALL	0.46*	0.48*	0.46*	0.14	0.1	–	−0.06	−0.03	−0.05	0.02
Dissolved oxygen	0	0	0.01	−0.01	−0.15*	−0.06	–	−0.01	−0.23*	−0.18*
pH	−0.01	−0.01	−0.02	0.09	−0.01	−0.03	−0.01	–	−0.01	−0.01
Total phosphorus	−0.01	−0.01	0	0.04	0.06	−0.05	−0.23*	−-0.01	–	0.69*
Turbidity	−0.01	0	0.01	−0.06	0.20*	0.02	−0.18*	−0.01	0.69*	–

* $P < 0.05$.

dissolved oxygen ranging from 5 to 10.65 mg L^{-1}, whereas one genus started declining in abundance in sites with dissolved oxygen > 6.4 mg L^{-1} (Fig. 2e). We detected a turnover pattern for two genera that were sensitive to pH values of 5.8 and 6, whereas one genus persisted in pH up to 7.3, being considered tolerant (Fig. 2f). We observed a turnover pattern comprising a range of 0.001 to 0.02 mg L^{-1} of total phosphorus (Fig. 2g). Six genera were sensitive to increased total phosphorus concentrations ranging from 0.001 to 0.02 mg L^{-1}, whereas two genera were considered tolerant, increasing in abundance in sites ranging from 0.02 and 0.06 mg L^{-1} of total phosphorus. Finally, we observed a wide range of change points when we considered disturbance variables and the assemblage as a whole, based on loss of sensitive genera [Sum(Z−)], and increase in tolerant genera [Sum(Z+)] (Table 3).

Table 3
TITAN assemblage-level thresholds estimated from mayfly genera responses to anthropogenic disturbance metrics. Sum(z) associated with decrease (−) or increase (+) along the gradient; CP is the assemblage change point; 5%, 10%, 50%, 90% and 95% are bootstrap quantile intervals capturing true thresholds.

Metric code	Sum(z)	CP	5%	10%	50%	90%	95%
Urban	–	0.00	0.00	0.00	0.00	0.00	0.08
	+	7.18	0.00	0.00	3.53	7.57	8.57
RCOND	–	0.00	0.00	0.00	3.28	9.26	9.32
	+	1.75	1.51	1.75	8.65	12.46	12.77
W1_HALL	–	0.00	0.00	0.00	0.13	1.43	1.66
	+	1.34	0.39	0.43	1.08	2.53	2.61
XEMBED (%)	–	63.00	27.83	48.86	58.05	67.14	77.23
	+	11.38	11.38	11.38	70.76	92.58	92.91
Dissolved oxygen	–	6.40	5.60	5.90	6.35	6.50	7.25
	+	8.25	7.10	7.20	8.20	10.25	10.70
pH	–	6.34	5.43	5.80	6.37	6.86	7.50
	+	7.30	7.10	7.11	7.28	8.30	8.45
Total phosphorus	–	0.010	0.001	0.003	0.010	0.017	0.020
	+	0.020	0.002	0.003	0.015	0.058	0.059

4. Discussion

Tropical freshwater ecosystems harbor high biodiversity and their protection should be a priority considering the growing pressure of anthropogenic alterations (Dudgeon et al., 2006; Sala et al., 2000). This is particularly true of aquatic ecosystems in neotropical savannas (Rodrigues et al., 2016; Silva et al., 2006; Wantzen et al., 2006). Their high biodiversity can be properly evaluated only if ecological assessments use continuous disturbance gradients to represent the extent and severity of anthropogenic disturbances, rather than setting discrete disturbance categories (Davies and Jackson, 2006; Ligeiro et al., 2013). In our study, we confirmed the value of using a combination of streams selected *a priori* as minimally disturbed reference sites and highly disturbed urban sites,

together with sites selected through a probabilistic survey design (Bryce et al., 2010; Smucker et al., 2013). Likewise, we confirmed the importance of using sensitive or relatively rare taxa when making those assessments, as did Pond et al. (2008) for temperate forest streams and Leitão et al. (2016) for Amazonian streams.

The scientific literature reports a broad range of land use effects on freshwater ecosystems as a result of human activities near watercourses (Sala et al., 2000). We could demonstrate the effects of land use pressures on stream biota directly only for urbanization, because the other types were significantly correlated with several natural environmental variables. This finding suggests a deterministic relationship, where some areas exhibit favorable natural conditions (like favorable climate and flat terrain) for food production and other commodities whereas sites in better ecological condition are more likely in rugged terrain. Such relationships have been demonstrated in other studies in the neotropical savanna (Carvalho et al., 2009; Silva et al., 2006) as well as in the western USA (Whittier et al., 2006), New Zealand (Burdon et al., 2013), and eastern Australia (Kath et al., 2014). Such correlations between natural environmental gradients, anthropogenic disturbances, and biological responses are why others have used regression analysis residuals to calibrate biological metrics against natural environmental gradients when developing multimetric indices in Bolivia (Moya et al., 2011), Brazil (Macedo et al., 2016; Pereira et al., 2016), China (Chen et al., 2014), and the USA (Mazor et al., 2016; Stoddard et al., 2008).

We selected disturbance metrics not correlated with natural variability to better separate disturbance effects from natural effects on freshwater ecosystems. Our results suggest that different anthropogenic disturbances may act independently in impairing stream sites, as demonstrated by other studies conducted in Mexico (Ávila-Gómez et al., 2015) and Bolivia (Moya et al., 2011). For instance, Macedo et al. (2014) showed a decrease in the macroinvertebrates richness due to alterations in the local conditions affected by influences of land use and cover composition at catchment scales. Besides catchment-scale anthropogenic disturbances, tropical ecosystems are also under more subtle effects, such as modifications in riparian zone structure (as indicated by RCOND and W1_HALL) (Sloan and Sayer, 2015). Similar patterns have been reported for riparian vegetation condition in the western USA (Paulsen et al., 2008). Alterations in water quality parameters can also reflect anthropogenic disturbances that represent serious threats to human water supply and biodiversity maintenance globally (Sala et al., 2000; Steffen et al., 2015; Woodward et al., 2012). For this reason water quality variables are used as normative parameters in Brazil (Brasil, 2005), and other nations, such as the USA (Clean Water Act), European Union (Water Framework Directive), and Australia (Sustainable Rivers Audit).

Assemblage turnover responses are usually related strictly to the gradient being evaluated as demonstrated by many studies, including the effect of deforestation in bat assemblages in Mexico (Ávila-Gómez et al., 2015), and the effect of groundwater in the decline of trees in New Zealand (Kath et al., 2014). In our study, we demonstrated the negative effects of anthropogenic disturbances on freshwater ecosystems based on the evaluation of several metrics, indicating the importance of measuring multiple disturbances, as confirmed by Shimano and Juen (2016) for Amazonian streams and Paulsen et al. (2008) for USA streams. We suggest that future studies also investigate response thresholds and turnover in response to different anthropogenic disturbance metrics. This is particularly important because, based on the range effects of each type of stressor, decision makers can target conservation actions and rehabilitation programs more efficiently (Steffen et al., 2015).

Our results corroborate the negative effects of urbanization and local anthropogenic disturbances (W1_HALL, RCOND, XEMBED) on assemblage structure, as observed with aquatic insects in New Zealand (Burdon et al., 2013); neotropical savanna (Rodrigues et al., 2016), and Amazonia (Dedieu et al., 2015; Shimano and Juen, 2016). We observed a wide variation in the response of each genus, corroborating the negative effects resulting from an increase of very low levels of urbanization in Mexico (Ávila-Gómez et al., 2015) and the USA (Hughes and Dunham, 2014; King et al., 2011). On the other hand, we observed a strong positive effect of increasing riparian vegetation integrity (RCOND) on the stream biota, as did Rodrigues et al. (2016) for adult damselflies in neotropical savanna, and Kaufmann et al. (2014) for fish and birds in northeastern USA lakes. Local anthropogenic disturbances can decrease habitat availability for many aquatic taxa (Allan, 2004). We demonstrated this from the turnover pattern in mayfly assemblages in response to a narrow range of change in the W1_HALL gradient. Maintaining and restoring the riparian zone is crucial to ensure stream habitat integrity. For this reason, riparian zones are regulated by the Brazilian Forest Code (Brasil, 2012), which limits riparian vegetation removal to protect stream biota (Sloan and Sayer, 2015). For that same reason, anthropogenic disturbances are restricted for 50–100 m in the riparian zones of USA Pacific Northwest forests, depending on whether streams support fish or not (Thomas and Raphael, 1993).

Intensive land use tends to increase streambed sedimentation, leading to loss of appropriate habitat for several aquatic organisms and causing many local extinctions (Bryce et al., 2010; Burdon et al., 2013). Our study corroborates the effectiveness of sedimentation metrics (e.g., mean embeddedness) as surrogates for intensity of land use. Researchers studying Ephemeroptera, Plecoptera, and Trichoptera assemblages in temperate regions found very low sedimentation thresholds (10% in Bryce et al., 2010; 20% in Burdon et al., 2013), whereas we found a Sum(z−) threshold of 63% XEMBED for the whole mayfly assemblage. Despite the wide range in response thresholds of individual taxa, we observed that mayflies as a whole assemblage do not have a positive association with increased sedimentation, which supports the use of individual target taxa to evaluate sedimentation thresholds. In our study, we observed that sensitive genera showed considerable diversity in gill morphology (e.g., filamentous: *Askola*; operculate: *Tricorythopisis*), mobility (e.g., swimmers: *Campylocia*; crawlers: *Camelobaetidius*), and feeding group (e.g., collector-gatherer: *Cloeodes*; scrapers: *Miroculis*). Specific morphological features associated with improved absorption of oxygen, such as presence of filamentous gills (Shimano and Juen, 2016), or small body-size genera are abundant in minimally disturbed sites (Dedieu et al., 2015). However, associations between traits and stressors need to be better investigated in neotropical savanna streams.

The threshold values for pH and dissolved oxygen demonstrated by mayfly genera agreed with the water quality thresholds established by Brazilian legislation. However, the total phosphorus criterion is 0.05 mg/L (Brasil, 2005). That value is higher than the threshold response of the whole mayfly assemblage [sumz(−) = 0.010 mg L^{-1}], and much higher than the individual response thresholds of the most sensitive genera (e.g., *Hagenulopsis, Hermanella*, and *Tricorythopsis*). The scientific literature shows biodiversity loss as a result of freshwater eutrophication (e.g., Baker and King, 2010; Taylor et al., 2014), calling attention to global eutrophication tendencies (Steffen et al., 2015; Stoddard et al., 2016; Woodward et al., 2012). Our results reveal that small streams can be particularly sensitive to nutrient enrichment, and the changing urban thresholds of the assemblages can be much lower than the value considered in legislation or regulations. Thus, we suggest that environmental legislation and regulations set benchmarks based on actual biological information such as we provided here.

We demonstrated how specific anthropogenic disturbances reduce ecological quality in headwater streams by using disturbance metrics not correlated with natural variability. Thus, we

observed biodiversity loss and assemblage turnover responses to independent disturbance gradients. We highlight the applications of our results, including: (i) the use of biological thresholds when developing regulations for water quality and biological integrity; (ii) the use of the indicator taxa approach based on multiple taxa rather than using only univariate metrics such as taxonomic richness and abundance; and (iii) the necessity of dialog, based on high quality ecological data and scientific information, among decision makers, industrial leaders, and the scientific community to find common goals for managing and conserving ecosystems.

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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.2016.11.033.

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