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Diatom and Macroinvertebrate assemblages to inform management of Brazilian savanna's watersheds

Camila Aida Campos [a,b,*], Mark J. Kennard [c], José Francisco Gonçalves Júnior [a]

[a] *Laboratório de Limnologia, Departamento de Ecologia, Universidade de Brasília, Campus Universitário Darcy Ribeiro, Bloco E s/n 1° andar - Asa Norte, CEP 70910-900, Brasília, DF, Brazil*

[b] *Agência Reguladora de Águas, Energia e Saneamento Básico do Distrito Federal (ADASA), SAIN Estação Rodoferroviária de Brasília, s/n, Ala Norte, CEP 70631-900, Brasília, DF, Brazil*

[c] *Australian Rivers Institute, Griffith University, 170 Kessels Road, Nathan, 4111 QLD, Australia*

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ABSTRACT

Human activities are increasingly affecting freshwater ecosystems and biodiversity, especially in neotropical regions like the Brazilian savanna. Limited research and data availability have inhibited the development and implementation of systematic bioassessment programs and management guidelines. Identifying drivers of biological assemblages' composition and ecological thresholds along human disturbance gradients is an important step to protect and recover freshwater ecosystems while avoiding threats to biodiversity, goods and services of value to humans. The objectives of this study were to: 1) assess changes in the composition and density of periphytic diatom and macroinvertebrate assemblages relative to natural and human disturbance gradients in Brazilian savanna's streams, and 2) identify ecological thresholds for direct and indirect human disturbances and potential indicator taxa that could inform the development of biomonitoring programs in Brazil and other neotropical countries. Samplings were carried out in 52 stretches of streams located in central Brazil during two campaigns throughout 2018. Ordination analyses (NMDS) were applied to identify the main drivers of biological assemblages' composition and analyses of taxa distribution in disturbance gradients (TITAN) were carried out to detect possible thresholds and potential bioindicator taxa. Our results pointed out that the scale of land use in the catchment, treated sewage input, and water quality variables (nitrate, phosphate, and conductivity) were the main drivers of diatom and macroinvertebrate assemblages. Taxa such as *Eunotia* (diatoms) and some families of the orders Ephemeroptera, Plecoptera, and Trichoptera (EPT, macroinvertebrates) were associated with natural conditions, while *Nitzschia palea* and *Gomphonema* (diatoms) and Oligochaeta and Hirudinea (macroinvertebrates) were related to environments with a higher degree of disturbance. Although ecological thresholds along disturbance gradients varied among taxa and biotic groups, our results revealed that relatively minor increases in land use in the riparian zone (>0%) and in the upstream catchment (0–33%) were sufficient to trigger significant changes in macroinvertebrate and diatom assemblages. The limits of tolerance for conductivity, nitrate and phosphate were also low, varying between 7-163 $\mu S.cm^{-1}$, 0.3–1.0 $mg.L^{-1}$ and 0.03–1.5 $mg.L^{-1}$, respectively. In general, the values we found were more restrictive than those provided by Brazilian government guidelines, suggesting that the latter would be insufficient to maintain the integrity of biological assemblages in Brazilian savanna's watersheds. We provided valuable knowledge about the sensitivities and tolerances of diatom and macroinvertebrate's taxa/assemblages that can be especially useful for the proper freshwater and watersheds management.

1. Introduction

Human activities are increasingly affecting freshwater ecosystems and biodiversity (Reid et al., 2019), which are sensitive to the cumulative impacts of multiple interacting stressors (Jackson et al., 2016). In addition to persistent threats such as habitat fragmentation, degradation

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[*] Corresponding author at: Laboratório de Limnologia, Departamento de Ecologia, Universidade de Brasília, Campus Universitário Darcy Ribeiro, Bloco B s/n térreo - Asa Norte, CEP 70910-900, Brasília, DF, Brazil.

E-mail address: camila.campos@adasa.df.gov.br (C. Aida Campos).

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and loss, species invasions and pollution, a range of emerging threats associated with climate change, new contaminants, and other human stressors are disproportionately impacting freshwater ecosystems in many parts of the world (Vörösmarty et al., 2010; Reid et al., 2019). In Brazil as in many other developing economies in the neotropics, human threats to the integrity of freshwater ecosystems are rapidly increasing due to high urban population densities, poor sewage and wastewater treatment, water resource development, mining, and native vegetation clearing for large scale pastures and cropping (Ríos-Touma & Ramírez 2018).

To understand the consequences of human activities on aquatic ecosystems and to prioritize remedial management actions, a range of monitoring indicators has been applied for assessment, diagnosis, and prognosis of ecosystem health and sustainability (Norris & Thoms, 1999; Gergel et al., 2002; Rossberg et al., 2017). These include indicators of water quality (physical and chemical), ecosystem process (e.g. leaf litter decomposition, primary productivity) and biological assemblages' composition since they can be sensitive to a variety of anthropogenic disorders (Bunn et al., 2010). Although physical and chemical variables have been the most widely used in water quality monitoring programs around the world, indicators that incorporate biological assemblages are increasingly being used to evaluate river health as they can integrate processes over a range of spatial and temporal scales, providing a holistic view of the aquatic ecosystem (Feio et al., 2010, Buss et al., 2015, Bo et al., 2017).

Both large (e.g. watershed, regional) and local scale factors are important to determining variations in the composition and abundance of biological assemblages since they generate conditions that are appropriate or not for their survival and maintenance (Li et al., 2018, He et al., 2020). However, quantifying taxa and assemblages' responses to natural and anthropogenic changes remains an ongoing challenge for river health assessment and watershed management (Firmiano et al., 2017). Examining changes in the composition and abundance of taxa along environmental gradients allows not only to understand how individual taxa respond to different conditions but also to identify critical change points or thresholds in assemblages' responses (Baker & King, 2010; Snyder & Young, 2020). Although there are controversies regarding the applicability of thresholds to environmental impact assessment (Murray et al., 2018), the ability to identify such change points might be useful since it informs the limits of disturbance tolerated by biological assemblages and constituent taxa (Baker & King 2010), often indicating the existence of a gap between the limits acceptable by the assemblages and those applied by government guidelines. This scenario is well exemplified by the study developed by Rodrigues et al. (2016), which, by analysing damselfly assemblage, were able to verify the inefficiency of Brazilian legislation in preserving riparian vegetation and consequently the associated aquatic biodiversity in the Brazilian savanna. On the other side of the globe, Tibby et al. (2020) demonstrated that thresholds for diatoms in gradients of electrical conductivity and of phosphorous were considerably lower than the trigger values used in South Australia guidelines.

Many countries, such as Australia (Bunn et al., 2010), the European Union countries (European Union, 2000), New Zealand (Schallenberg et al., 2011), and the United States of America (USEPA, 2016), have incorporated biological assemblages as part of their river health assessment programs. In rapidly developing neotropical countries like Brazil, an increasing number of studies have demonstrated the potential of the analyses of biological assemblages in assessing and monitoring the health of aquatic environments (Buss et al., 2016; Macedo et al., 2016; Pereira et al., 2016). A recent, complete and comprehensive study in the Brazilian savanna region, developed a robust multimetric index (MMI) based on different aspects of macroinvertebrate assemblage characteristics to assess and monitor the ecological condition in neotropical savanna's streams (Silva et al., 2017). However, the development of systematic bioassessment programs and guidelines in national resolutions on environmental and water resources is in its infancy in this region, despite increasing concerns about the degradation of freshwater ecosystems (Sundar et al., 2020).

Macroinvertebrate assemblages are commonly used as bioindicators around the world (Buss et al., 2015; Sumudumali & Jayawardana, 2021), not only because they are an essential part of the aquatic environment - since they transfer the energy to other trophic levels in the aquatic food web, but also because of their restricted mobility, relatively long life cycle if compared to other aquatic organisms, high taxa diversity with different levels of stress tolerance, pollution sensitivity, suitability of taxonomic keys and existence of well-defined sampling protocols (Sumudumali & Jayawardana 2021). Diatoms (Bacillariophyceae) are also widely used in monitoring programs as bioindicators (Pandey et al., 2018) due to their fundamental ecological role at the base of the food web and their sensitivity and rapid response to environmental fluctuations in comparison with organisms in higher trophic positions (Kelly et al., 2008). Changes in macroinvertebrate and diatom assemblages' composition and abundance are expected in the face of all pressures that aquatic ecosystems have been suffering (Silva et al., 2017; Gonzáles-Paz et al., 2020).

Most studies aimed at identifying ecological thresholds for aquatic biota typically focus only on a single biotic group (e.g. Smucker et al., 2013; Zhang et al., 2016; Brito et al., 2020) and very few consider two or more groups (e.g. Schröder et al., 2015). In Brazil, especially in neotropical savanna, fundamental knowledge about environmental controls of macroinvertebrates and diatoms biodiversity and responses to anthropogenic disturbances is critically scarce (Overbeck et al., 2015), posing a challenge for the implementation of biomonitoring to inform freshwater management.

Having these considerations in mind, the objectives of this study were (i) to assess changes in the composition and density of periphytic diatom and macroinvertebrate assemblages in relation to natural and human disturbance gradients in Brazilian savanna's streams, and (ii) to identify ecological thresholds and potential indicator taxa that could further inform the development of biomonitoring programs in Brazil and other neotropical countries.

2. Material and methods

2.1. Study area

The study was carried out mostly in the Distrito Federal (DF; 5,802 km^2), located in the central Brazilian plateau (Fig. 1). It is characterized by regions with distinct land uses: agriculture in the east; preservation and mining areas in the north; and urban densification in the central and south-western portion (GDF, 2012). Eastern tributaries draining from the adjacent state of Goiás (GO), that present similar physical characteristics to those of the DF, were also included in the study area.

Fifty-two randomly selected sample sites were widely distributed within the study area to represent a broad range of natural conditions - relating to stream size, elevation, slope, geology, and other factors -, different land uses and anthropogenic disturbances in the drainage area (Table1). Sample sites were separated by at least 500 m when located in the same watercourse, and as long as different natural characteristics were observed. Site selection was constrained by road accessibility and all of them were located in wadeable streams of up to 5th order (Strahler 1957). The influence of the river size variation was considered in the study by some natural variables such as the drainage area, distance from the source and elevation.

2.2. Natural variables and human disturbances

Natural variables (Table 1) minimally affected by human activities, were defined by Geographic Information System (GIS) processing. A Digital Elevation Model (DEM) (30 m resolution) was used to outline the upstream watershed borders, using the hydrology tools in ArcMap 10.4 and topographic data from Brazil's Geomorphometric Database

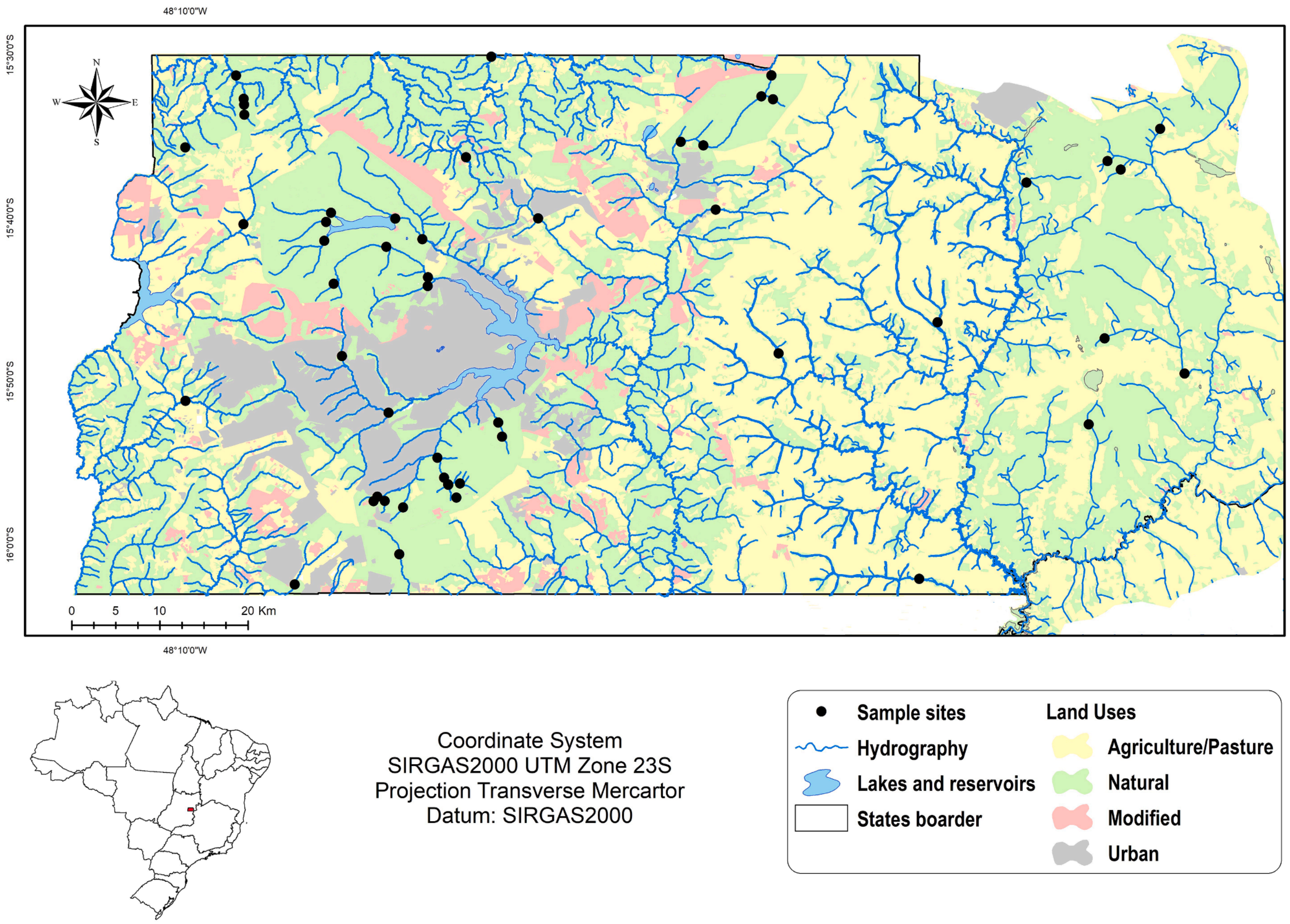

Fig. 1. Study area, sample sites and land uses. Land uses were classified in agricultural (any kind of agriculture and pasture), natural (native vegetation), modified (new settlements, exposed soil and eucalyptus), or urban.

(TOPODATA; INPE, 2018) on a scale of 1:50,000. Each sample site was treated as an outlet point. From this model, we extracted the upstream drainage area, the average slope of the river, elevation, and the distance from the source for each sample site.

Soil type and geological group data for Distrito Federal and its surroundings were sourced from the Federal District's Water, Energy and Basic Sanitation Agency (ADASA). Both were determined on a local scale, i.e. at each sample site, using the ArcMap 10.4. The three geological groups present in the study area were the Bambui, Paranoa, and Canastra; and the three kinds of soils were red oxisol, plinthosol, and cambisol.

Natural variables that could potentially be affected by human activities (Table 1) included: local habitat (riverbed sediment granulometry and canopy cover shading), hydromorphological characteristics, and water quality (see description below).

The main anthropogenic disturbances in the study area are the removal of natural land cover for agriculture, urban development, mining, point-source sewage release (treated or not); and the presence of dams (GDF, 2012). To evaluate the land use in the upstream catchment (CAT) we adapted the Land Use Index (LUI) proposed by Rawer-Jost et al. (2004). This index considers that the impact of urban areas is greater than agriculture and other kinds of land uses. Our adapted index considers agricultural and pasture areas with the same weight and includes modified areas (allotment, exposed soil, mining, and eucalyptus). The proposed Land Use Index (LUI) is calculated as (1):

$$LUI = 4x\%\,urban\,areas\,(CAT\_urb) + 2x\%\,agricultural\,and\,pasture\,areas\,(CAT\_agr) + \%modified\,areas\,(CAT\_mod) \tag{1}$$

To evaluate the influence of alterations on the riparian corridor (RIP) in the biological assemblages we considered an upstream 30 m-buffer along each bank. The Brazilian Forest Code (Federal Law 12.651/2012) determines that the extent of riparian vegetation depends on the river width. Rivers up to 10 m in width should have, at least, 30 m of riparian vegetation on each bank. Rivers with 10 to 50 m width should have 50 m of riparian vegetation on each bank. Since most rivers in the study area have width below 10 m, the land use analyses in the riparian zone covered 30 m from each bank. The riparian corridor was also generated from the DEM model, considering the land uses within the 30 m along each bank. Similar to the catchments, land uses in the riparian corridor were also grouped into 3 categories: urban, agricultural and modified (Table 1). The percentage of occupation by native vegetation was not considered since it represents exactly the total area having subtracted the other uses. We calculated the proportion of each kind of land use in the upstream catchment and in the riparian corridor of each sample site using a land uses shapefile for Distrito Federal (Reis & Lima 2015) and

Table 1
Description, average, standard deviation (SD), range (min = minimum and max = maximum) and number of data (N) of natural and human disturbances variables. (*) Data collected four times, but for analyses, we consider the average between April/May and August/September as representing dry and wet season, respectively. In one sample site, data were obtained only in the wet period. (**) For categorical variables, we indicated the number of samples in each category. (***) used only in TITAN analyses. Variables highlighted in bold were used in ordination analyses (the others were excluded by Spearman correlation analysis).

Natural Variables	Description	Average (SD)	Min-Max	N
GIS obtained variables				
drai_area	Total drainage area upstream of the sample site (Km^2)	40.52 (48,91)	2.21–215.42	52
Source_dist	Distance from the sample site to the river source (Km)	7.96 (7.37)	0.54–33.63	52
elevation	Altitude of the sample point relative to sea level (m)	1015.48 (96.63)	744–1220	52
slope	Average slope between two points along the river channel (m)	3.02 (1.72)	0.63–7.32	52
Soil**	Dominant soil type: red oxisol (S1) plinthosol (S2) cambisol (S3)	S1 − 46; S2 − 5; S3 − 1		52
Geol_group**	Geological group Paranoá (G1) Canastra (G2) Bambuí (G3)	G1 − 42; G2 − 1; G3 − 9		52
Habitat variables				
shading	% of riparian shading (0 = 0%; 1 = < 30%; 2 = between 30 and 60%; 2 = > 60%)	2.36 (0.96)	0–3	52
OM	% of organic matter in riverbed sediment	6.15 (5.27)	0.61–26.66	52
coa_sed	% of coarse sediments (2000–710 mm) in the riverbed sediment	60.49 (25.86)	4.18–97.28	52
med_sed	% of medium sediments (500–250 mm) in the riverbed sediment	21.99 (13.97)	1.06–52.16	52
fin_sed	% of fine sediments (180–125 mm) in the riverbed sediment	10.94 (9.42)	0.42–45.39	52
v_fin_sed	% of very fine sediments (90–63 mm) in the riverbed sediment	3.67 (3.75)	0.18–15.43	52
silt	%of silt and clay sediments (<63 mm) in the riverbed sediment	2.91 (4.51)	0.08–20.48	52
*Hydromorphological variables**				
av_depth	Average depht (m)	0.31 (0.18)	0.04–1	103
av_veloc	Average velocity (m)	0.27 (0.20)	0.01–1.5	103
av_width	Average width ($m\ s^{-1}$)	3.17 (2.72)	0.3–16.3	103
disch	Discharge ($m^3\ s^{-1}$)	0.35 (0.59)	0–4.77	103
*Water Quality (WQ) variables**				
temp	Temperature (°C)	20.52 (1.88)	13.8–26.6	103
DO	Dissolved Oxygen ($mg\ L^{-1}$)	7.11 (0.92)	1.88–8.85	103
pH	Potential of Hydrogen	6.60 (0.45)	5–8.1	103
Cond	Electrical conductivity ($uS\ cm^{-1}$)	56.07 (93.98)	1.3–584	103
turb	Turbidity (NTU)	7.92 (15.37)	0.04–197	103
K^+	Potassium ($mg\ L^{-1}$)	1.27 (2.87)	0–46.55	103
Fe^{+2}	Iron ($mg\ L^{-1}$)	1.47 (0.08)	1.3–1.85	103
F^-	Fluorine ($mg\ L^{-1}$)	0.10 (0.64)	0–0.57	103
Cl^-	Chlorine ($mg\ L^{-1}$)	0.92 (2.95)	0–35.4	103
NO_2^-	Nitrite ($mg\ L^{-1}$)	0.04 (0.10)	0–1.23	103
Br^-	Bromide ($mg\ L^{-1}$)	0.01 (0.01)	0–0.09	103
NO_3^-	Nitrate ($mg\ L^{-1}$)	0.35 (1.11)	0–10.29	103
PO_4^{-3}	Phosphate ($mg\ L^{-1}$)	0.23 (0.60)	0–6.26	103
SO_4^{-2}	Sulphate ($mg\ L^{-1}$)	0.18 (0.51)	0–2.66	103
Human disturbances variables				
RIP_urb	% of urban area in riparian area	1.22 (5.08)	0–33.44	52
RIP_agr	% of agricultural and livestock areas in riparian area	7.82 (15.48)	0–55.93	52
RIP_mod	% of modified area in riparian area (allotment, exposed soil, eucalyptus)	0.70 (3.41)	0–23.91	52
CAT_urb***	% of urban area in upstream catchment	6.92 (16.23)	0–70.39	52
CAT_agr***	% of agricultural and livestock areas in upstream catchment	20.61 (25.03)	0–86.48	52
CAT_mod***	% of modified area in upstream catchment (allotment, exposed soil, eucalyptus)	3.28 (6.55)	2.21–215.42	52
LUI	Land Use Index for upstream catchment (see equation (1))	72.20 (75.25)	0–296.03	52
SR**	Presence/absence of point-source treated sewage release upstream	0–49; 1–3	0–1	52
Dam**	Presence/absence of upstream dams	0–39 ;11–13	0–1	52
N_dams	Number of upstream dams	0.47 (0.98)	0–4	52
Dist_dam	Distance between the sample point and the nearest dam	5.62 (3.33)	0.74–17.87	52
C_roads	Number of road crossings upstream	0.72 (1.65)	8–0	52

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by photointerpretation of RAPIDEYE satellite image with 5 m resolution for Goiás state (Table 1).

The number of point-source treated sewage releases and dams present in the upstream catchment of each sample site were obtained from the Federal District's Water, Energy and Basic Sanitation Agency (ADASA). The number of upstream road crossings was calculated by Open Street Map data (OSM Foundation, 2017). All geoprocessing analyses were carried out in ArcMap 10.4.

2.3. Field sampling and laboratory analyses

Two sampling campaigns were conducted in 2018, one at the end of the wet season (April/May) and another at the end of the dry season (September/October). Although rainfall data were not available for this study, since the regional hydrometeorological stations were out of operation throughout 2018, the historical rainfall in Distrito Federal is characterized by two well-marked periods, the wet season (October to March) and the dry season (April to September; INMET, 2019). As it is an exploratory work, we consider it necessary to collect data that involves two sampling periods, thus guaranteeing a greater coverage on the conditions of our systems.

2.3.1. Water quality

A large number of water quality variables were measured at each sample site on each sampling occasion. Dissolved oxygen and water temperature were measured in situ using a YSI probe. A water sample (approximately 300 mL) was collected 15 cm under the surface – or between the surface and riverbed in case of the depth was <15 cm – and taken to the lab under refrigeration. A small portion of this sample was used immediately after samples arrived at the lab to measure turbidity, conductivity and pH (Jenway, Quimis e Metrohm probes). The remainder was filtered (glass fibre 0.22 mm filter) and stored in 15 mL Falcon tubes at −20 °C for analyses of ionic composition. Cation, anions, and metals were analysed on a Metrohm chromatograph using specific columns for cation (potassium), anions (fluoride, chloride, nitrite, bromide, nitrate, phosphate and sulphate) and metals (iron). Cation and metal determinations were performed on the MetroSep C4 250/4.0 mm column using 1.7 mM nitric acid / 0.7 nM dipicolinic acid as eluent with unsuppressed conductivity detection. Anion samples determinations were performed on the MetroSep A Supp 5 250/4.0 mm column using 3.2 $mmol.L^{-1}$ sodium carbonate and 1 $mmol.L^{-1}$ sodium bicarbonate as eluent, with suppressed conductivity detection.

2.3.2. Hydromorphology

Hydromorphological parameters were calculated in field, in a representative section of the stream stretch, and followed the middle section methodology (Santos et al., 2001). Measurements were taken on each sampling occasion, except when high water velocities prevented this. In these cases, gauged discharge values were obtained from the Federal District's Water, Energy and Basic Sanitation Agency (ADASA). Riparian shading was estimated visually and allocated on an ordinal scale (ranging from 0 to 3) to one of the following classes: 0% − 0; 0–30% − 1; 30–60% − 2; >60% − 3.

The granulometry of the riverbed sediments was analysed only once (April) by the sieving method (Suguio, 1973). Around 1 kg of sediments was sampled and air-dried for at least 72 h. After drying, the samples were homogenised and a sub-sample (approximately 100 g) was incinerated in a muffle furnace at 550 °C for 4 h. The difference in weight between pre- and post-drying was used to determine the percentage of organic matter in each sub-sample. Each dried sub-sample was then sifted for 15 min passing through sieves of different mesh diameters (2 mm, 1 mm, 0.710 mm, 0.500 mm, 0.355 mm, 0.250 mm, 0.180 mm, 0.125 mm, 0.090 mm e 0.063 mm) with the help of an automatic shaker. Each size sample was weighed, and the results were expressed as a percentage of the total sub-sample weight. Prior to further analyses sediments samples were grouped into 5 categories: coarse sediments (>2–0.710 mm), medium sediments (0.500–0.250 mm), fine sediments (0.180–0.125 mm), very fine sediments (0.090–0.063 mm) and silt/clay (<0.063 mm).

2.3.3. Macroinvertebrates

Macroinvertebrates were collected in May and September 2018 with a *surber* sampler (0.09 m^2 area and 0.250 mm mesh size). Five sub-samples distributed proportionally across the area covered by the most representative habitats were collected (e.g. macrophytes, stones, sand, and leaves), covering a length of 5 to 50 m, depending on access conditions along the riverbed. The sampling duration was 1 min in each sub-sample site. The five sub-samples were then combined to generate a single composite sample and it was preserved in 96% alcohol. Later, the macroinvertebrates were sorted and identified under a stereomicroscope. Identification was carried out mostly to family level following Hamada et al. (2018) and Hamada et al. (2019) and with the help of taxonomic specialists (see Acknowledgments). Few groups were classified only up to class or order. The results were expressed as the number of individuals/m^2 for each taxon.

2.3.4. Periphytic diatoms

Periphytic diatoms were sampled in May and September 2018 following the Water Framework Directive (WFD) protocol for Portuguese rivers (INAG I.P., 2008). Five 10 × 10 cm (total 500 cm^2 of surface area) pieces of slate stone tile were deployed in the riverbed and after approximately 30 days of incubation, they were retrieved. On average, a total surface area of about 250 cm^2 of tile at each site was scraped with a toothbrush to obtain the periphytic diatom sample. The scraped material was preserved in vials containing 0.33% Lugol solution and the identification and quantification of the periphytic diatoms were carried out under an inverted microscope using the Utermöhl method (Utermöhl, 1931). We did not proceed the oxidation of organic matter previously to the identification as suggested by INAG I.P. (2008), because we were interested only in the active cells (which means cells with cellular content) at the time of sampling. Identification was carried out mostly to species level following specific taxonomic literature, with the help of taxonomic specialists (see Acknowledgments). The results were expressed as cells/cm^2/month for each taxon.

2.4. Data analyses

Since data distribution was not normal for many variables, Spearman correlation analysis was used to identify higher inter-correlated environmental and human disturbances variables. For pairs of variables with absolute correlation coefficients ≥ 0.6, we retained those correlated with the greatest number of other variables and also the most straightforward to obtain in the field or lab. These analyses resulted in the exclusion of 21 of the 40 initial variables considered (Table 1; Appendix B). All hydromorphological variables were excluded since they were highly correlated with the drainage area. Regarding habitat variables, the shading, the percentage of organic matter in the sediment (OM) and the percentage of coarse sediment (coa_sed) were retained. Among water quality variables, Cl, NO_2^- and SO_4^{-2} were excluded. The percentage of modified area in the riparian corridor (RIP_mod) was also excluded since it was correlated with the percentage of urban area (RIP_urb). The only correlated variables we retained for subsequent analyses were LUI and RIP_agr since they refer to different scales of land use and occupation. Importantly, the retained human disturbance variables were minimally correlated with the natural environmental gradients (absolute Spearman's rho < 0.6).

We used ordination analyses (Nonmetric Multidimensional Scaling - NMDS) based on a Bray-Curtis dissimilarity matrix of the sample-by-taxon density data to summarise diatom and macroinvertebrate assemblages and evaluate relationships with natural and disturbance variables. Three dimensions ($k = 3$) was considered because of the reduced stress value compared with two dimensions. We used two-way Permutation Analyses of Variance (PERMANOVA) to test for differences in assemblages' composition between the dry/wet seasons. The p-values for pseudo-F were obtained from 999 data permutation. Different colours were used to distinguish seasonal samples in the ordination biplots. The R function "envfits" was used to relate the natural and human disturbances variables with sample position in ordination space. The projections of points onto vectors have a maximum correlation with corresponding gradients.

We used Threshold Indicator Taxa Analyses (TITAN; Baker & King 2010) to estimate thresholds for land uses and water quality gradients that were strongly related to variation in diatom and macroinvertebrate assemblages (as identified by ordination and envfit correlation analyses). Human disturbance variables included the Land Use Index (LUI) and its components – land use in the catchment scale (CAT_urb, CAT_agr, and CAT_mod), land use in the riparian scale (RIP_agr and RIP_urb), conductivity, phosphate and nitrate concentrations. TITAN is based on change point (King & Richardson 2003) and indicator value (z-value, Dufrêne and Legendre, 1997) analyses to detect significant changes in the frequency of occurrence and relative abundance of taxa along environmental gradients (Baker & King 2010). TITAN differentiates taxa with positive (Z+) and negative (Z−) responses to all gradients, with Z+ (tolerant) taxa increasing in frequency and abundance from the change point and Z− (sensitive) taxa decreasing. The response quality of each indicator taxon is measured by purity and reliability; both indices are obtained by resampling using the bootstrap method (500 resamples with replacement) to confirm the thresholds for each taxon and assemblage. Purity corresponds to the proportion of change points (if Z− or Z+) along with resampling that concurs with the observed value. Reliability corresponds to the proportion of the resampling that reports an indicator value with significant p-values (Baker & King 2010). We consider robust taxa those with purity and reliability above 90%. After taxon-specific change points have been identified, TITAN supplies an assemblage-level threshold, reflecting the magnitude of assemblage changes as an indicator of coincident change point in the assemblage structure [sum (Z)], considering only the robust taxa (filtered results). Following recommendations (Baker & King 2010), we excluded taxa occurring at fewer than three sites. TITAN analyses were carried out with both seasons data together since we obtained much more consistent results (greater number of robust taxa) than considering wet and dry season separately, and also because it allowed us to have a broader view of the studied streams' system.

Prior to TITAN analyses, taxon density data were log (x + 1) transformed because of the wide ranges observed, although it is certainly acceptable to use untransformed data in this nonparametric analysis (King & Baker 2014). All statistical analyses were performed using the R program (R Development Core Team, 2018) with specific packages for NMDS (Vegan; Oksanen et al. 2017), PERMANOVA (Adonis2, Anderson 2001; *in* Vegan), TITAN (TITAN2; Baker and King 2010) and graphs (Ggplot2, Wickham 2016).

3. Results

3.1. Biological assemblages

We sampled 18 families, 26 genera, and 74 species of diatoms across the 52 study sites and two sampling seasons (wet and dry). The most frequent genus in both seasons was *Eunotia*, with *Eunotia* sp.1 presents in 78% of all samples. In the wet season, the most dense species were *Eunotia* sp.1 (16%), *Gomphonema* cf. *parvulum* (10%) and *Nitzschia* sp.1 (9%). In the dry season, the most dense species were *Nitzschia* cf. *palea* (31%), *Eunotia* sp.2 (12%) and *Gomphonema* cf. *lagenula* (11%; Appendix A).

We collected 50,880 macroinvertebrates belonging to 4 phyla, 10 classes, 11 orders and 54 families across all sites and sampling periods. The most common and widespread taxon was Chironomidae, occurring in all samples and forming 55% and 54% of the total density in the wet and dry seasons, respectively. After Chironomidae, the next most frequently occurring taxa, were Elmidae (90%), Ceratopogonidae (74%) and Perlidae (59%) in the wet season, and Elmidae (88%), Ceratopogonidae (82%) and Leptophlebiidae (82%) in the dry season. The most dense taxa were Simulidae (36%), Oligochaeta (14%) and Elmidae (12%) in the wet season and Elmidae (17%), Simuliidae (17%) and Oligochaeta (14%) in the dry season (Appendix A).

3.2. Relationships of biological assemblages with environmental and human disturbances

The ordination of diatom and macroinvertebrate assemblages allowed the identification of a major gradient, predominantly characterized by direct and indirect human impacts (Figs. 2 and 3). The land use in the upstream catchment (LUI), the presence of point-source sewage release (SR), and water quality variables commonly related to pollution like phosphate, nitrate and conductivity, were the most relevant to taxa distribution. Among the natural variables, shading and elevation stand out as relevant factors for diatoms, while the percentage of organic matter in the sediment (OM) was more relevant for macroinvertebrates (Figs. 2 and 3, see "envfits" values in Appendix C).

Sites with a high density of genus *Eunotia* (diatom) and taxa Perlidae, Megapodagrionidae, Noteridae, Leptoceridae and Decapoda (macroinvertebrates) were characterised by low levels of human disturbance, more shading, higher elevation and higher percentage organic matter (OM). Sites with higher levels of human disturbance and greater drainage area, were characterised by several diatom taxa including *Nitzschia* cf. *palea*, *Gomphonema* cf. *lagenula*, *Diploneis* sp. and *Pinnularia* sp.1. Macroinvertebrates taxa displayed distinctive associations with different types of human disturbance. Simuliidae, Oligochaeta, Chironomidae and Hirudinea were related to sites with the presence of point-source sewage release and PO_4^{-2}, while Physidae, Hydropsychidae and Dicteriadidae were more related to the land uses and higher conductivity values.

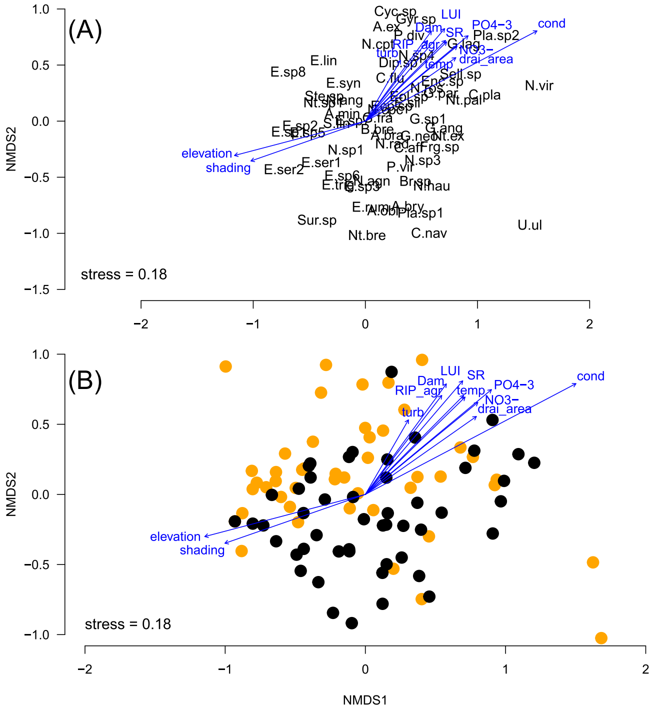

Fig. 2. Distribution of diatom species (A) and sites (B), and the fitted vectors for significant ($p < 0.01$) environmental and human disturbance variables (blue arrows). Stronger relationships are represented by longer vectors. In B, sites are separated by season - wet (orange) and dry (black). Abbreviations: see Table 1. The complete list with taxa codes is in Appendix A. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Diatom and macroinvertebrate assemblages' composition differed significantly between seasons (PERMANOVA; pseudo-F = 2.77, p = 0.003 for diatoms, and pseudo-F = 4.60, p = 0.001 for macroinvertebrates). But the contribution of the predictor variables (envfit) did not vary significantly when we performed the ordination (NMDS) analysis separated by season or with all data together (ANOVA F = 2.343, p = 0.107 for diatoms, and F = 0.347, p = 0.708 for macroinvertebrates). So here we only represent the results with both seasons data together.

3.3. Environmental thresholds identified by TITAN analyses

TITAN analyses revealed substantial changes in diatom and macroinvertebrate assemblages along environmental disturbance gradients and different thresholds between the biotic groups. The assemblage threshold of the catchment land use index (LUI) was lower for sensitive (Z-) taxa of diatoms compared with macroinvertebrates (42 *versus* 173, Table 2). In contrast, the assemblage threshold of tolerant (Z+) taxa was higher for diatoms (159) than macroinvertebrates (42.5; Table 2). This suggests that sensitive diatoms were more responsive to minor changes in LUI than the sensitive macroinvertebrates, but the tolerant diatoms required greater changes in land uses than macroinvertebrates before they increased in density.

The Land Use Index in the upstream catchment (LUI) were the land use gradient that presented the largest number of robust taxa identified by TITAN analyses for both biological assemblages (Table 2, Figs. 4 and 5). We obtained different results in each LUI component. For diatoms, no sensitive (Z-) taxa were identified in CAT_urb. The thresholds observed

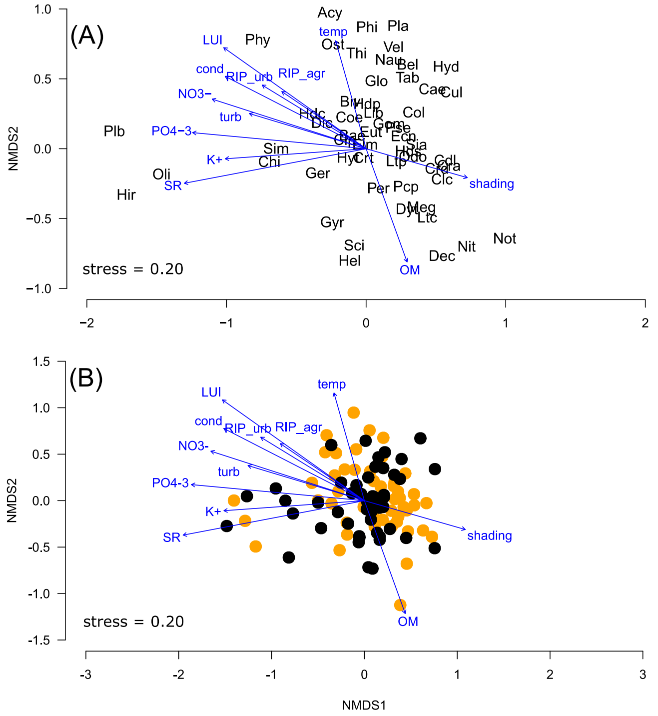

Fig. 3. Distribution of macroinvertebrates taxa (A) and sites (B), and the fitted vectors for significant ($p < 0.01$) environmental and human disturbance variables (blue arrows). Stronger relationships are represented by longer vectors. In B, sites are separated by season - wet (orange) and dry (black). Abbreviations: see Table 1. The complete list with taxa codes is in Appendix A. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

in CAT_urb were higher for diatoms (Z+, 33.2%) than for macroinvertebrates (Z- 19.9%, Z+ 0.75%). The Z- thresholds in CAT_agr were lower for macroinvertebrates (0.18%) than diatoms (1.63%), but vice-versa for Z+ (macroinvertebrates 20.8%, diatoms 9.8%). The CAT_mod presented similar thresholds for both assemblages, near 0% for sensitive taxa and up to 5% for tolerant taxa. The thresholds were lower in the riparian land uses (RIP) than in the upstream catchment land use (LUI) components for both assemblages, with values around zero and no sensitive species detected for diatoms in RIP_urb gradient.

Among the physical and chemical parameters, conductivity gradient presented the largest number of robust sensitive and tolerant taxa for both assemblages (Table 2, Figs. 4 and 5). The assemblage thresholds for both sensitive (Z-) and tolerant (Z+) taxa were higher for diatoms (Z- 22.6 μS.cm^{-1}, Z+ 124.7 μS.cm^{-1}) than for macroinvertebrates (Z- 7.24 μS.cm^{-1}, Z + 163 μS.cm^{-1}). The change point in nitrate gradient was lower for both sensitive and tolerant diatoms (0.347 mg.L^{-1}, 0.486 mg.L^{-1}) than macroinvertebrates (0.976 mg.L^{-1}). The change point in phosphate gradient was around 0.04 mg.L^{-1} (Z-) and 0.90 mg.L^{-1} (Z+) for diatoms and 0.028 mg.L^{-1} (Z-) and 1.47 mg.L^{-1} (Z+) for macroinvertebrates (Table 2).

A wide range of change points were identified along disturbance gradients for taxa in each biotic group (Figs. 4 and 5). All sensitive (Z-) diatom species had LUI change points <50 and all the tolerant (Z+) species between 50 and 200 (Fig. 4). For macroinvertebrates, most of the sensitive taxa had LUI change points >100 and most tolerant (Z+) taxa had change points <100 (Fig. 5). For conductivity, most of the diatom and macroinvertebrate sensitive taxa (Z-) presented change points lower than 50 μS.cm^{-1} , but tolerant taxa (Z+) indicated a group increasing in low values and another group in higher than 100 μS.cm^{-1} values (Figs. 4 and 5). In some land use gradients, taxa started to increase or decrease just after 0%, for example, the Megapodagrionidae (Z-) in CAT_agr gradient and all tolerant (Z+) diatom species in RIP_urb gradient.

The most common sensitive taxon among diatoms was *Eunotia* and the most common tolerant taxa were *Gomphonema, Nitzschia and Navicula* (Appendix E). The most sensitive macroinvertebrate taxa were Perlidae, Dytiscidae, Leptoceridae and Leptophlebiidae while the most common tolerant taxa were Hirudinea, Oligochaeta, Physidae and Planorbidae. Taxa belonging to EPT (Ephemeroptera/Plecoptera/Trichoptera) group represented 43.5% of the total sensitive (Z-) macroinvertebrate taxa (Appendix E).

4. Discussion

4.1. *Influence of natural and human disturbance gradients on biological assemblages*

Combined natural and human factors lead to changes in freshwater environment, that can ultimately impact biological assemblages. Considering both factors and their covariations allows a better understanding of the freshwater ecosystem (Tang et al., 2019). In this study, we observed that a large part of the natural factors considered did not have great relevance on the composition of both assemblages, with few exceptions. This may have occurred due to the relative homogeneity of the region since natural factors tend to be more relevant in explaining the variation in ecoregions and on national scales (Tang et al., 2019).

The natural variables that presented some correlation with the

Table 2
TITAN responses for diatom and macroinvertebrate assemblages among all gradients. For all gradients abbreviations, see Table 1. NI = not identified. For each group (diatoms and macroinvertebrates): number of robust sensitive (Z-) and tolerant (Z+) taxa, the assemblage threshold for filtered sensitive (Fsum Z-) and tolerant (Fsum Z+) taxa followed by 5th and 95th percentiles among bootstrap replicates (n = 500). SumZ plots of all gradients for both assemblages can be found in Appendix D.

	LUI	LUI components			RIP		Water Quality		
		CAT_urb	CAT_agr	CAT_mod	RIP_urb	RIP_agr	Cond	NO3	PO4
Diatoms									
No. robust taxa (Z−)	4	0	3	3	0	2	8	3	5
No. robust taxa (Z+)	9	6	1	4	7	8	13	5	3
Fsum Z- (5th-95th percentiles)	42.48 (3.52–48.66)	NI	1.63 (0.58–9.83)	0 (0–2.610	NI	0 (0–0.12)	22.57 (2.72–50.27)	0.347 (0.001–0.545)	0.037 (0.005–0.310)
Fsum Z+ (5th-95th percentiles)	159.21 (105.16–206.85)	33.20 (2.10–53.77)	9.83 (8.37–79.60)	5.75 (0.63–11.38)	0 (0–0.06)	0.88 (0–11.32)	124.75 (13.58–139.47)	0.486 (0.082–2.122)	0.8975 (0.248–1.927)
Macroinvertebrates									
No. robust taxa (Z−)	9	8	2	1	6	5	14	9	10
No. robust taxa (Z+)	9	5	11	7	4	8	13	5	1
Fsum Z- (5th-95th percentiles)	172.95 (151.55–175.73)	19.94 (14.32–33.15)	0.18 (0.12–7.73)	0 (0–0.51)	3.20 (0–6.43)	0 (0–6.55)	7.24 (2.84–9.28)	0.976 (0.015–1.253)	0.028 (0.022–0.160)
Fsum Z+ (5th-95th percentiles)	42.49 (42.49–239.65)	0.75 (0.59–56.13)	20.83 (9.83–38.21)	7.91 (4.66–8.24)	0 (0–3.90)	0.12 (0–2.64)	163.00 (14.82–184.82)	0.976 (0.044–1.904)	1.470 (0.160–1.499)

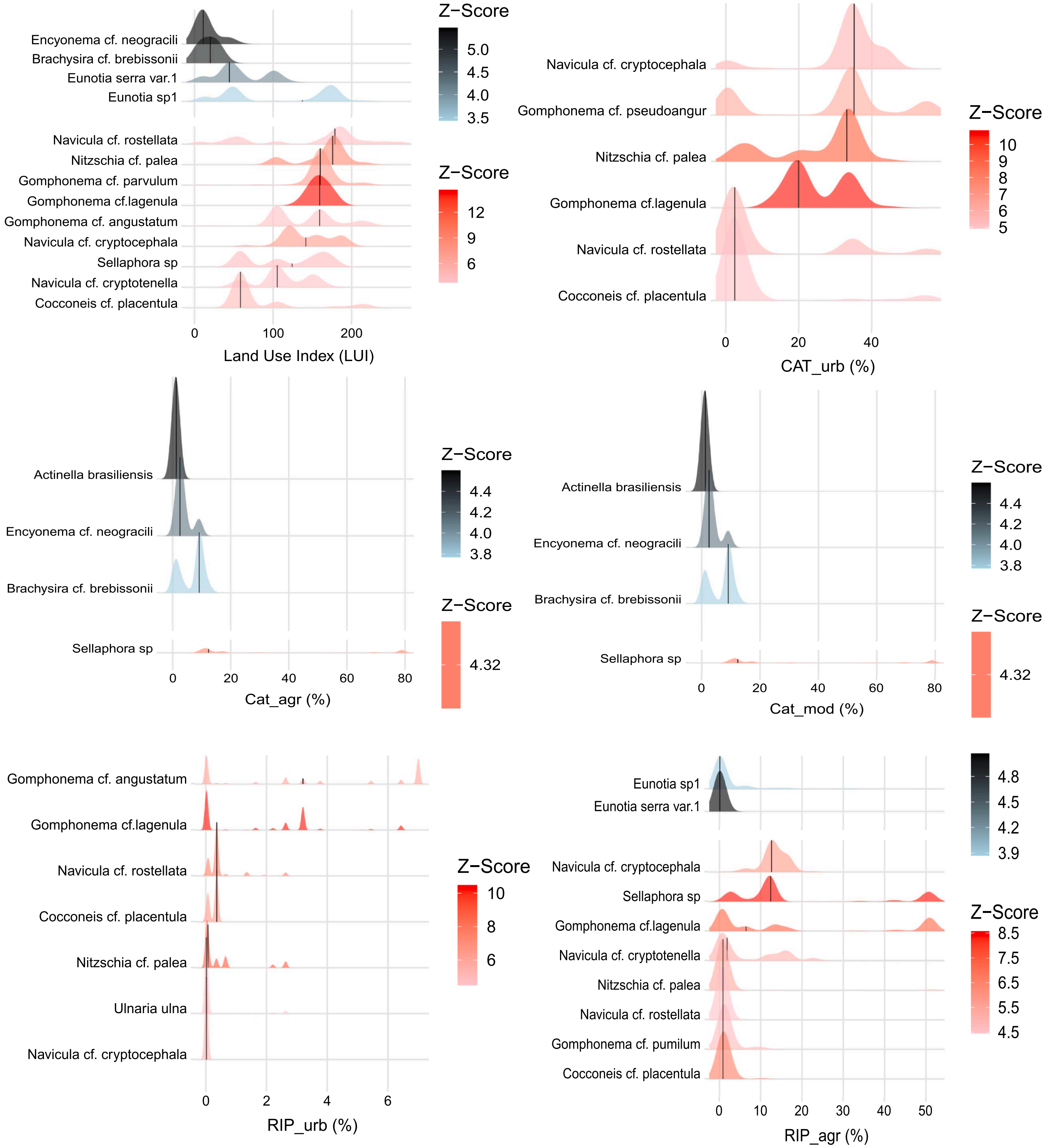

Fig. 4. Results of TITAN showing taxon-specific maximum change points along all gradients for Diatoms. Robust sensitive taxa (Z-) and tolerant taxa (Z+) are represented in blue and red scales respectively. The blue/red colour intensity is proportional to the magnitude of the response. Black vertical bars = change point of each taxon. The area below the curve represent 5th and 95th percentiles among 500 bootstrap replicates. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

assemblages were elevation, shading and the percentage of organic matter (OM) in the river sediments. Jointly with local aspects, the elevation is reported as a determinant factor of the diatoms and macroinvertebrates' diversity (He et al., 2020). The last two factors - shading and OM - may be related to natural phenomena such as river enlargement from the source towards the mouth (Vannote et al., 1980; Hughes et al., 2011) but also to human disturbances, such as the removal of natural vegetation. Light availability has been reported as the most important factor controlling diatom assemblages in oligotrophic forested headwaters (Tornś & Sabater 2010). Besides this, the allochthonous OM from the riparian vegetation is the main source of energy in headwater streams and its input is fundamental for organisms

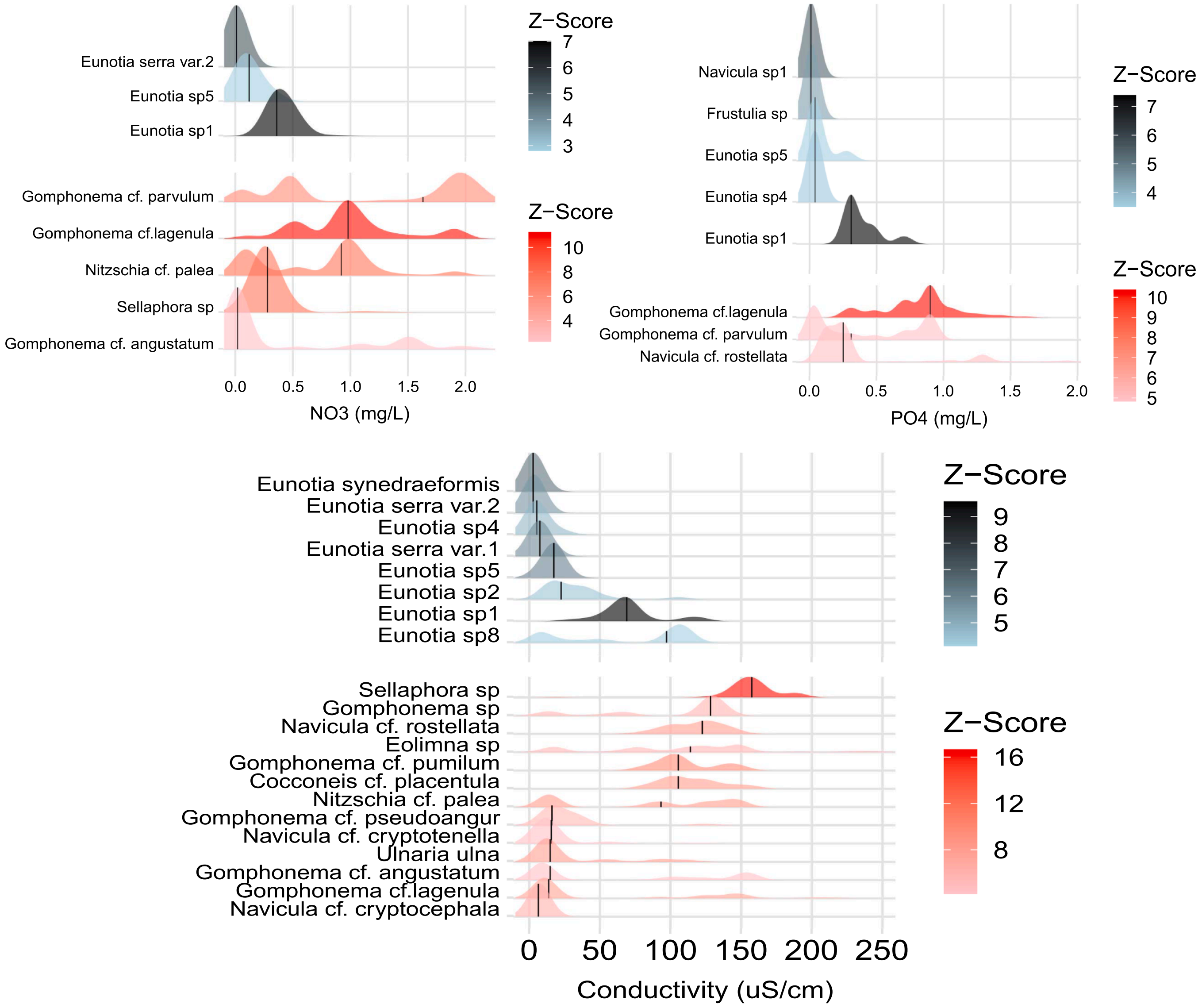

Fig. 4. (*continued*).

that depend on this resource, like some macroinvertebrates (Gonçalves et al., 2006; Sánchez-Argüello et al., 2010).

Land uses on both scales (catchment and riparian) were important drivers in the composition of diatom and macroinvertebrate assemblages. It is known that human activities on different scales can lead to in-stream modifications, often through successive and complex processes that ultimately manifest in changes in the structure of biological assemblages (Allan 2004). Riparian vegetation plays a critical role in maintaining the structure and function of freshwater ecosystems, especially in headwaters or small rivers (Bunn & Davies, 2000; Perona et al., 2009) as is the case of the streams in the study area. However, intact riparian zones can be insufficient to buffer the effects of altering catchment land uses, as demonstrated by Hlúbiková et al. (2014) and Giling et al. (2016). In this study, small changes in riparian vegetation were sufficient to trigger significant changes in biological assemblages. Therefore, this natural buffer is already broken under a small percentage of deforestation, leading to a greater effect of the catchment scale factors under the freshwater ecosystem.

We also detected strong relationships between biological assemblages' structures and the presence of point-source sewage release in the upstream catchment, particularly for macroinvertebrates. Organic pollution is a worldwide threat and the number of people affected by this is bound to increase from 1.1 billion in 2000 to 2.5 billion in 2050, with developing countries being disproportionately affected (Wen et al., 2017). The deficit in sewage collection and treatment in Brazilian cities has resulted in a significant input of pollutants reaching the streams and rivers, causing negative implications to the multiple uses of water resources (Agência Nacional de Águas (ANA) (2017)). Despite the self-depuration capacity of water bodies, the pollutant input causes changes in physical and chemical water characteristics, especially increasing turbidity, electrical conductivity, nutrient concentration and decreasing dissolved oxygen(Copetti et al., 2018). Nutrient pollution can have major and widespread impacts on biotic structure and function (Woodward et al., 2012) especially in headwater streams and small rivers that have relatively low discharge with minimal flushing or capacity for pollutant dilution, as is the case of the majority of watercourses in the study area (GDF, 2012).

The water quality parameters considered in this study were not correlated with the direct human disturbances listed above, but in the ordination plots, some of them presented similar vectors (size and direction) to the main impacts, which may indicate that these parameters reflect unmeasured disturbances, such as the input of wastewater and diffuse pollutant into waterbodies. Electrical conductivity, nitrate, phosphate, turbidity and dissolved oxygen are among the water quality

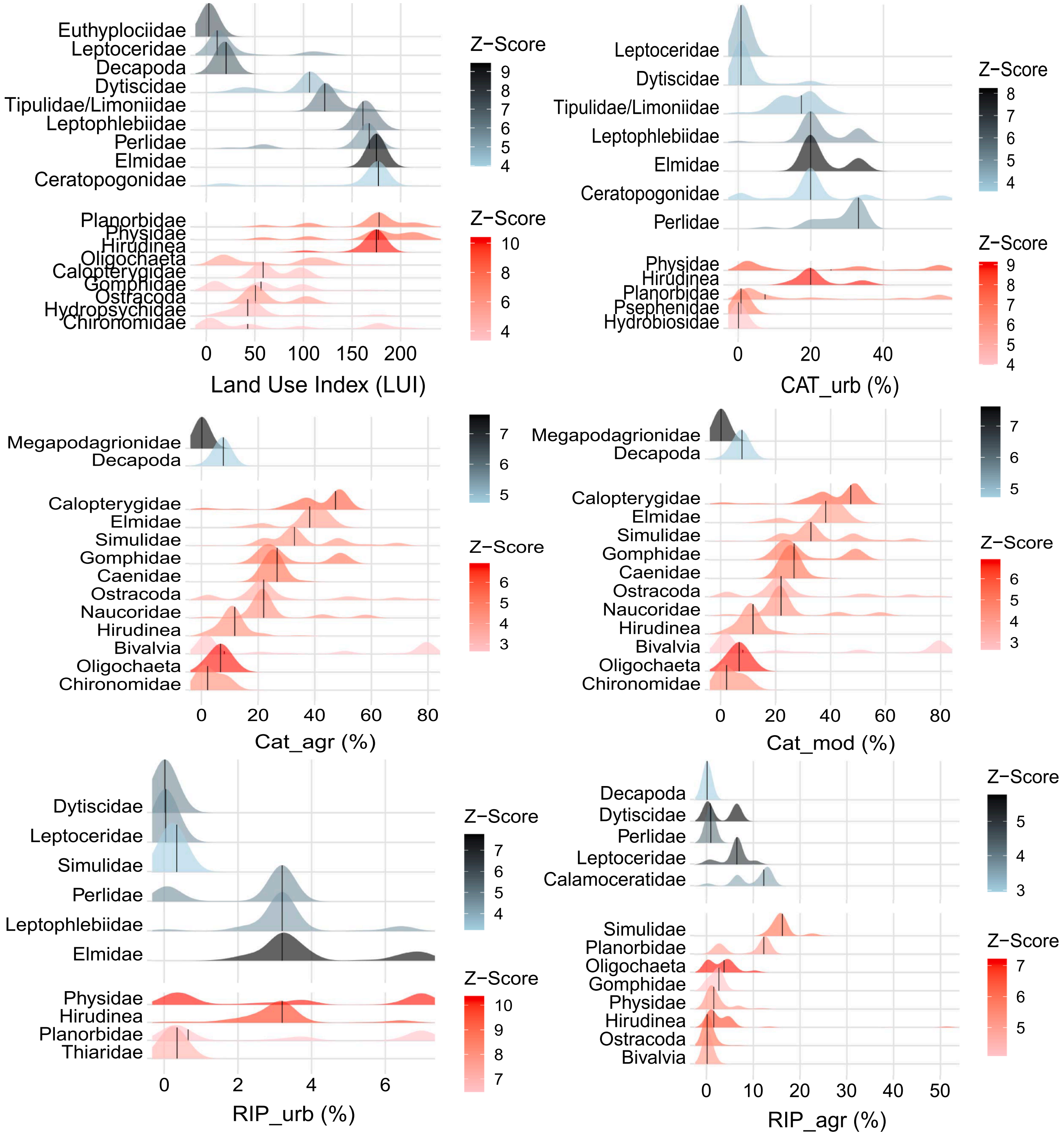

Fig. 5. Results of TITAN showing taxon-specific maximum change points along all gradients for Macroinvertebrates. Robust sensitive taxa (Z-) and tolerant taxa (Z+) are represented in blue and red scales respectively. The blue/red colour intensity is proportional to the magnitude of the response. Black vertical bars = change point of each taxon. The area below the curve represent 5th and 95th percentiles among 500 bootstrap replicates. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

variables most frequently altered by anthropogenic activities such as the release of domestic and industrial effluents, land use, and the use of fertilizers along with other agricultural inputs (Mello et al., 2020). Studies have already demonstrated the relationship of some of these parameters with the structure of biological assemblages, such as conductivity and phosphorus related to diatoms (Potapova & Charles, 2003; Waite et al., 2019), and dissolved oxygen and turbidity related to macroinvertebrates (Corijmans et al., 2021; Okano et al., 2017).

4.2. *Land use and water quality thresholds for diatom and macroinvertebrate assemblages*

Catchment and riparian land uses often result in changes in biological assemblages that are characterised by a decrease in the density and richness of taxa sensitive to environmental changes and an increase in the density of tolerant taxa (Feio et al., 2013; Martins et al., 2017). To reiterate: an ecological threshold refers to the point at which a small or

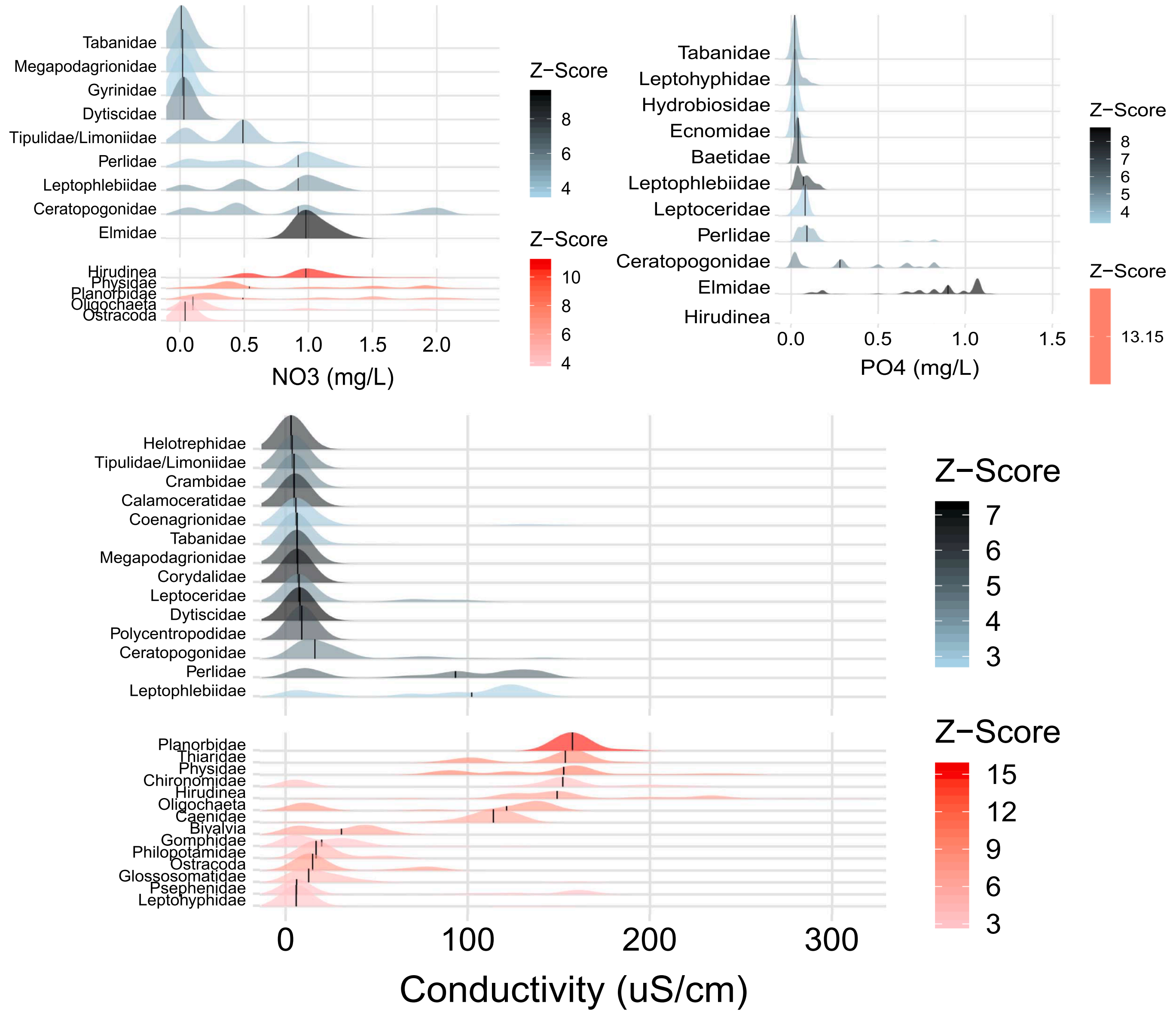

Fig. 5. (*continued*).

abrupt change in a driver gradient may produce large responses in the ecosystem or particular components (e.g., species; Baker and King 2010). The ability to identify such thresholds is seen as an important aspect of managing ecological systems (Huggett, 2005; Dodds et al., 2010) not only because they can influence ecosystem goods and services that people value (Martin et al., 2009) but also because they prevent the loss of biodiversity and ecosystem function (King & Richardson 2003).

Our results revealed that relatively minor increases in land uses in the riparian zone and in the upstream catchment were sufficient to trigger significant changes in macroinvertebrates and diatom assemblages. This suggests that the biotic assemblages in the small headwater streams we analysed are especially vulnerable due to the high connectivity of these ecosystems to adjacent landscapes (Taniwaki et al., 2018). In a study comprising several Brazilian biomes, Dala-Corte et al. (2020) demonstrated that the narrower the riparian-buffer the lower the threshold for removal of native vegetation. It was especially critical for macroinvertebrates considering that the reduction of only 6.5% of native vegetation cover within a 50-m riparian buffer was enough to cross thresholds for that biological assemblage. Similar low values were detected in Amazonian streams, where the thresholds for macroinvertebrates varied between 1 and 15%(~9%) and 0–35% (~1.4%) of forest-loss at the catchment and riparian levels, respectively (Brito et al., 2020). Even though the Brazilian Forest Code (Federal Law 12.651/2012) determines the maintenance of a riparian corridor between 30 and 100 m, depending on the river width, the preservation of the natural vegetation in private zones is limited to 35% in agricultural areas and unlimited in urban areas, which means that a much higher percentage of logging is permitted than it is tolerable according to the assemblages studied.

The limits of tolerance for conductivity were very low (7 – 163 μS.cm^{-1}) when compared to other studies. Sultana et al. (2019) demonstrated that for two catchments in Australia the conductivity threshold for macroinvertebrates ranged between 407 and 931 μS.cm^{-1}, furthermore Nguyen et al. (2017) found in western Ecuador change points between 930 and 1430 μS.cm^{-1}. Tibby et al. (2020) detected in South Australia significant declines in the relative abundance of sensitive species of diatoms at ~ 280 μS cm^{-1}. A possible reason for this discrepancy may be the limited amount of samples in the highest extremity of the gradient, which led to a low average conductivity (around 50 μS.cm^{-1}) and a few high values reaching a maximum of 500 μS.cm^{-1}. Nevertheless, our results indicate that some diatom and macroinvertebrate taxa responded robustly to even subtle changes in conductivity, indicating that it has an important correlation with biological assemblage's composition.

Nitrogen and phosphorus are fundamental nutrients in triggering the eutrophication process of rivers and lakes (Figueredo et al., 2016; Zhang et al., 2017), and the export of phosphorus from agricultural land to water bodies is predicted to increase (Ockenden et al., 2016). Our results showed nitrate and phosphate thresholds values around 0.3–1.0 $mg.L^{-1}$ and 0.03–1.5 $mg.L^{-1}$, respectively, while the Brazilian legislation provides reference values of 10 and 0.1–0.15 $mg.L^{-1}$ for nitrate and total phosphorous, respectively. The threshold values of nitrate and phosphate were similar to those found in other regions of the world, both for diatoms (e.g. Tibby et al., 2020; Hausmann et al., 2016) and macroinvertebrates (e.g. Nguyen et al., 2017; Kail et al., 2012). The scientific literature indicates freshwater eutrophication as a major stressor (Brönmark & Hansson 2002) and cause of biodiversity loss in freshwater ecosystems (Taylor et al., 2014), but here we demonstrated that the loss of sensitive taxa occurs even before the risk of eutrophication.

That is very clear in the taxa-specific graphs where it is noticed that the change points of the sensitive taxa (Z-) are in general smaller and sharper than those of the tolerant taxa (Z+). The decline in sensitive taxa occurs due to increasing pressures such as physiological limitations, inaccessibility of resources, interruption of interspecific interactions and the strengthening of competition and predation pressure. In contrast, tolerant taxa are not directly affected by increasing environmental pressure, but abundance arises with increasing pressure as they fill ecological niches that become vague with the disappearance of competitors or when they are exposed to reductions in predation and subsidy of experimental resources. This makes the response of the tolerant taxa more gradual and less abrupt than that of the sensitive ones (King & Baker 2011; Sundermann et al., 2015).

Threshold analyses allowed for the identification of robust sensitive (ex: *Eunotia*, Coridalidae, Perlidae, Helotrephidae, Leptoceridae, Leptophlebiidae and others) and tolerant taxa (ex: *Gomphonema*, Hirudinea, Oligochaeta, Planorbidae, and others) and their individual responses to different disturbances. Factors such as taxa life history attributes, habitat diversity, and the influence of stochastic events appear to be implicated in producing these differential threshold responses (Huggett 2005), but also an important factor is the taxonomic resolution. In this study, we use the levels of genus/species for diatoms and family/order/class for macroinvertebrates. More specific identifications can lead to new taxa-specific change points and must provide more informative description than higher taxonomic levels, however, studies have already shown that, in general, a coarse identification is sufficient for biomonitoring proposals (Bailey et al., 2001; Rimet & Bouchez, 2012; Vilmi et al., 2016).

Eunotia was related to good water quality conditions in studies carried out in Brazil (Salomoni et al., 2006) and England (Kelly, 1998), but Oeding & Taffs (2017) demonstrated that species within this genus exhibited a range of sensitivity values in Australian rivers. There is wide scientific consensus that *Nitzschia palea* is a specie tolerant to pollution (Kelly, 1998; Lobo et al., 2015; Oeding & Taffs, 2017). For macroinvertebrates, the EPT (Ephemeroptera, Plecoptera, and Trichoptera) group represented 43.5% of the sensitive taxa identified by TITAN, and Oligochaeta, Hirudinea, and Physidae were the most common tolerant taxa, confirming the findings of other studies (Wagenhoff et al., 2012; Ferreira et al., 2014; Pardo et al., 2020). The robust response to a range of human disturbance gradients demonstrates the potential utility of these taxa as bioindicators for the study area. Ecological thresholds may support the development of numerical indicators of ecosystem health and also the identification of reference conditions to characterize assemblages' composition in the absence of disturbance (King & Richardson, 2003; Baker & King, 2010).

4.3. Implications for freshwater bioassessment

Despite its biological importance, the Brazilian savanna has been threatened by agribusiness and is one of the world's richest biodiversity hotspots with the lowest percentage of areas under full protection (MMA, 2020). In addition to the low percentage of protected areas, the acceptable percentage of native vegetation removal and the water quality mandated by Brazilian legislation and management guidelines may be insufficient to maintain the integrity of biological assemblages (Martins et al., 2017). Our study has important implications and offers valuable information for the development of efficient management and policy tools on freshwater and watershed in the Brazilian savanna. Corroborating previous research in Brazil (Rodrigues et al., 2016; Dala-Corte et al. 2020; Brito et al., 2020) and elsewhere (e.g. Sultana et al., 2019; Tibby et al., 2020) we indicated that there is a gap between the needs of biological assemblages and current management guidelines.

Additionally, the study also demonstrated the importance of management considering more than one biological assemblage (Vilmi et al., 2016) and multiple stressors (Waite et al., 2019) since the responses are complementary. Changes in the biological composition attributed to a single variable do not necessarily represent a simple relationship to the environmental variable in focus but could include responses to other pressure variables linked with the corresponding variable (Sundermann et al., 2015). The study brings a simplification of a complex system, and although it considered multiple direct and indirect stressors, the change points cannot be used as a basis to derive causalities and the reduction of any stress factor below the change point of a sensitive taxon will be no guarantee for a recovery. The mapping of taxon-specific and assemblages change points can be used, in addition to the conservation of ecosystems and prevention of loss of biodiversity, in the indication of potentially polluted places and also in the prediction of future scenarios. (Sundermann et al., 2015).

The extent of temporal variability in biotic assemblages has important implications for bioassessment programs that aim to evaluate the health or integrity of aquatic ecosystems based on biological assemblages' structure and to assess the magnitude and likely sources of human disturbances (Tonkin et al., 2017). As it is an exploratory work, we consider it necessary to proceed sampling in two periods, thus guaranteeing greater coverage on the conditions of our systems. Although natural and human impact gradients were very similar, significant differences were detected in the composition of both biological assemblages between dry and wet seasons, as it has been shown in previous studies in the region (Bispo & Oliveira 2007) and elsewhere (e.g. Karaouzas et al., 2019; Snell et al., 2019). In this study, joint analysis of data from both sesons allows future monitoring programs to have reference taxa and thresholds, regardless of the period of sampling. In addition, with a single sampling in each season, we would not be able to affirm seasonality effects with certainty since this would require sampling in more than one year. Further research is required to understand the extent and drivers of temporal variation in biotic assemblages in Brazilian savanna's streams.

5. Conclusions

Working with different scales, stressors and responses is challenging but necessary to understand at least part of the environment's complexity (Allan, 2004; Wagenhoff et al., 2012). Here we demonstrate that, despite being influenced by some natural characteristics, the structures of the diatom and macroinvertebrate assemblages were strongly affected by the gradients of anthropogenic disturbances, from local to the catchment scale. Our results may provide new perspectives on the management of freshwater in the study area and in other neotropical regions aided by the identification of specific taxa of diatoms and macroinvertebrates as potential bioindicators, and thresholds of important anthropogenic disorders (land use and water quality). In general, the values found were more restrictive than those provided by Brazilian government guidelines, which means they would be insufficient to maintain the integrity of biological assemblages in Brazilian savanna's watersheds. We offered valuable knowledge about the sensitivities and tolerances of diatom and macroinvertebrate's taxa/assemblages that can be especially useful for the proper freshwater and watersheds management.

CRediT authorship contribution statement

Camila Aida Campos: Conceptualization, Methodology, Formal analysis, Investigation, Writing - original draft, Writing - review & editing. **Mark J. Kennard:** Conceptualization, Supervision, Writing - review & editing. **José Francisco Gonçalves Júnior:** Conceptualization, Resources, Funding acquisition, Supervision, Writing - review & editing.

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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Appendix A–E. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2021.107834.

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Further reading

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