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Setting thresholds of ecosystem structure and function to protect streams of the Brazilian savanna

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Freshwater environments are among the most threatened by human activities, consequently, their ecosystem structures and functions are targets of significant transformations. It makes monitoring an essential tool in the management of these environments. Ecological metrics have been proven to be effective in monitoring programs aimed at assessing freshwater ecosystem integrity. Structural and functional aspects of the ecosystem may allow for a comprehensive view of the multiple human impacts that occur at different scales. However, a gap in the effective use of such ecological tools lies in the identification of the relative importance of different mechanisms that cause impacts and the interactions between them. Using Boosted Regression Tree (BRT) models, we evaluated the relative importance of natural and human impact factors, from local to catchment scales, on metrics related to diatom and macroinvertebrate assemblages and ecosystem processes. The study was carried out in 52 stream reaches of the Brazilian savanna in central Brazil. Conductivity was the most relevant factor to explain the variation of ecological metrics. In general, macroinvertebrate metrics and algal biomass production responded to both water quality and land use factors, while metrics of diatoms and microbial biomass responded more strongly to water quality variables. The nonlinear responses allowed the detection of gradual or abrupt-changes curves, indicating potential thresholds of important drivers, like conductivity (100–200 $\mu S\ cm^{-1}$), phosphate (0.5 $mg\ L^{-1}$) and catchment-scale urbanization (10–20%). Considering the best performance models and the ability to respond rather to stress than to natural factors, the potential bioindicators identified in the study area were the macroinvertebrates abundance, the percentage of group Ephemeroptera/Plecoptera/Trichoptera abundance, the percentage of group Oligochaeta/Hirudinea abundance, the percentage of genus *Eunotia* abundance, the Trophic Diatom Index and the algal biomass production. The results reinforced the importance of consider in the national monitoring guidelines validated ecological thresholds. Thus, maintaining the balance of aquatic ecosystems may finally be on the way to being achieved.

KEYWORDS

ecosystem integrity, boosted regression tree, ecological metrics, freshwater management, monitoring programs

1 Introduction

Freshwater ecosystems are among the most threatened by human activities (Gatti 2016). The knowledge of the various components of these ecosystems is of paramount importance to the elaboration of public policies on conservation or recovery (Bunn et al., 2010). Biomonitoring data has been increasingly used in determining the ecological conditions of aquatic environments, in addition to the traditional physical and chemical indicators of water quality (Leese et al., 2018; Pardo et al., 2018; Gieswein et al., 2019). A comprehensive ecosystem integrity assessment should consider both structural and functional characteristics (Bunn & Davies 2000). While the structure of an ecosystem comprises physical and chemical attributes related to water quality, composition of biological assemblages and habitat conditions, its functioning is related to the processes regulating energy and matter fluxes (Tilman et al., 2014).

The most commonly used metrics to assess freshwater ecosystem integrity are those related to biological assemblages, such as species richness and diversity, abundance, the proportion of tolerant and sensitive taxa, organismal traits (e.g., feeding habits, body size, mobility), and indices of sensitivity to pollution (Hering et al., 2006). Macroinvertebrates, diatoms, macrophytes and fish are often used for that purpose (Son et al., 2018; Waite et al., 2019) as they are robust to the identification of several human disturbances and present particular features that facilitate such application (e.g., life cycle, habitat, size; Merritt & Cummins 1996; Kelly et al., 2008). Much less explored in the context of biomonitoring are aquatic fungi and bacteria, which are key decomposers of organic matter in streams. The responses of some ecosystem processes to stressors are fundamental to understanding the effects of human disturbances on ecosystem services that produce direct benefits to people. But despite this, there is still a lot of reluctance among managers and little use of functional indicators (e.g., litter decomposition) in monitoring programs (Schiller et al., 2017).

Although many studies have pointed out to the applicability of several ecological metrics for assessing freshwater ecosystem integrity, the main gap lies in the relative importance of different mechanisms that cause impacts and the interactions between them (Wenger et al., 2009). According to Sutherland et al. (2013), one solution is the use of modelling as a tool for measuring and monitoring systems. In the context of environmental management, most models used in monitoring programs consider biological assemblages, especially benthic invertebrates (AUSRIVAS, Smith et al., 1999; RIVPACS, Wright et al., 1984; USEPA, 2016), as indicators. Some studies suggest the use of ecosystem processes for this purpose (Gessner & Chauvet 2002; Feio et al., 2010; Woodward et al., 2012), and rare are those that present a multi-metric approach using structural and functional aspects (but see Castela et al., 2008; Clapcott et al., 2014).

In Brazil, as in many other tropical countries, monitoring programs focus on physical and chemical variables of water, with a substantial gap in knowledge about the structure and functioning of aquatic ecosystems. The Brazilian savanna (Cerrado) is a global biodiversity hotspot (Myers et al., 2000), and its headwaters are responsible for 70% of all water supply to other Brazilian regions (Lima & Silva 2007). However, the devastation of Cerrado has been taking place at levels proportional to its ecological and social relevance (Strassburg et al., 2017). It is urgent to know the behaviour of these threatened ecosystems through two valuable management tools: 1) the identification of variables that respond strongly to anthropogenic impacts than to natural variations, and 2) the identification of ecological thresholds that are adopted as standards in monitoring programs. We define the term 'ecological threshold' as a point along a stressor gradient where the relationship between the stressor and an ecological indicator shows an abrupt change in the response curve that can be ecologically explained and significantly relevant for management (Wagenhoff et al., 2017).

In this context, Boosted Regression Tree (BRT) models have been used as a robust tool to identify the influence of environmental variables, natural or those related to human activities, on ecological metrics, making it possible to evaluate the shape of the responses and to make forecasts by using new data (Clapcott et al., 2012; Waite et al., 2019). This approach allows the identification of gradients, from which is possible to detect non-linear responses, interactions among predictors and potential threshold zones. However, identification of potential thresholds alone is not helpful for management if it does not accompany by an analysis of their ecological consequences relevant to management decisions. Furthermore, it is important to consider a group of non-redundant variables so that the impacts of different stressors on the structure and function of ecosystems are detected. This approach would lead to more robust in-stream objectives and provide options for adopting goals that protect the aspects of ecosystems that people value most (Wagenhoff et al., 2017). Several studies have used BRT models to identify thresholds in a diversity of ecological areas, highlighting the potential of this tool to environmental management (Davis et al., 2019; Giri et al., 2019; Wherry et al., 2021).

This study evaluated how ecological metrics respond to natural environmental gradients and human-related stressors (local and catchment) at different spatial scales. We also identified the most suitable metrics to be used as indicators of stream integrity and assessed the response and potential thresholds of ecological metrics along environmental gradients to inform the ecological management of Cerrado freshwaters.

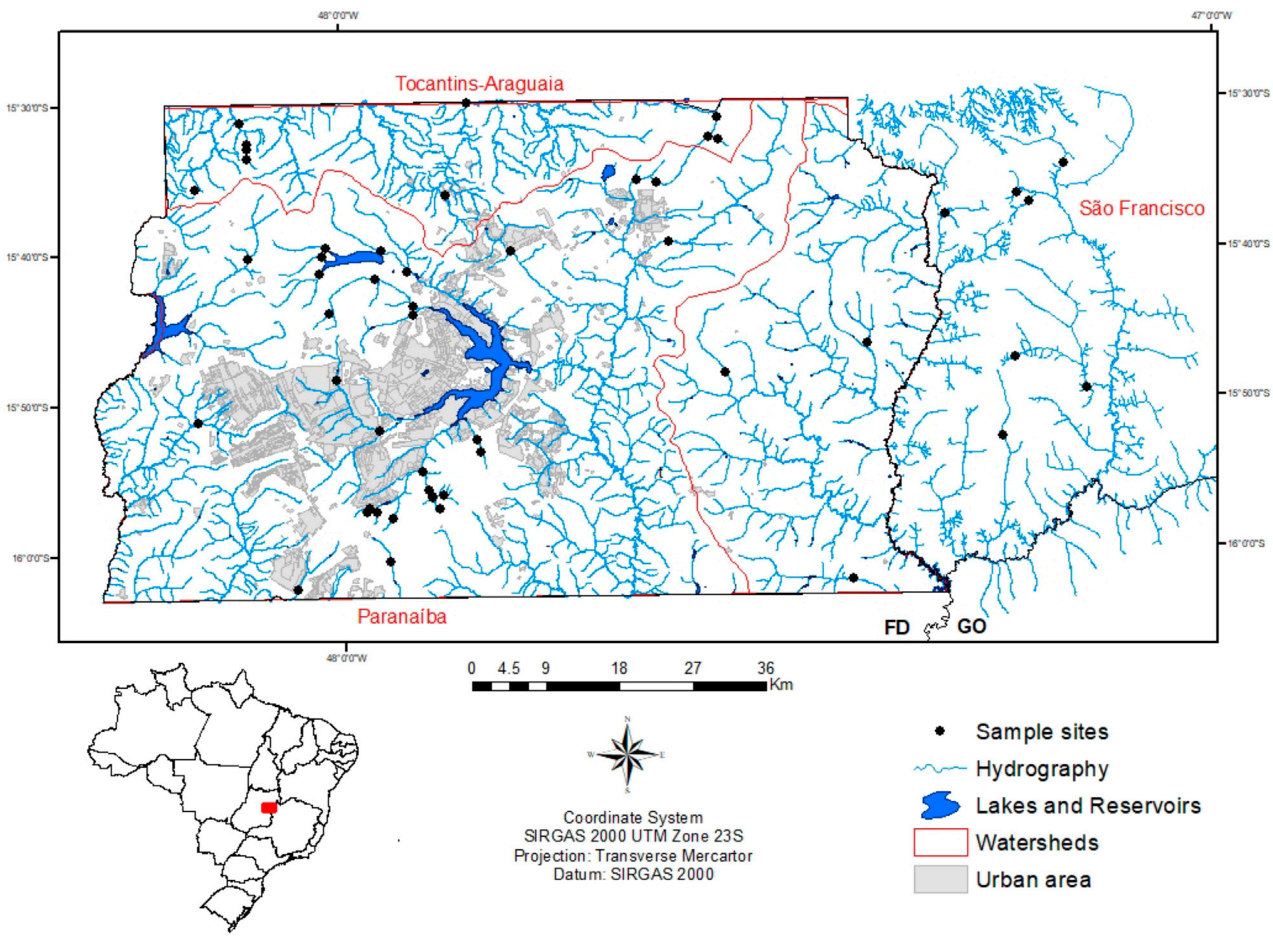

FIGURE 1
Spatial distribution of the 52 stream reaches in the regional river network. The red limits represent the portion of the national watersheds (Tocantins-Araguaia, São Francisco and Paranaíba) located in the study area (Federal District and Goiás) in central Brazil.

2 Materials and methods

2.1 Study area

The study was conducted in the central Brazilian plateau (*ca.* 1,000 m a.s.l.) in an area of approximately 6,700 km^2 dominated by Cerrado (Brazilian savanna) vegetation. Fifty-two stream reaches were selected to represent a broad range of natural environmental conditions (Figure 1). Briefly, sample sites were chosen to represent regions with different land uses and watersheds with different natural characteristics; accessibility for sampling was also taken into account for site selection. When more than one reach was sampled in the same stream, they were at least 500 m apart from each other (to reduce their spatial dependence) and comprised different natural characteristics. All streams are wadeable and perennial of up to 5th order (Strahler 1957).

2.2 Sampling, analysis and metrics

Two sampling campaigns were conducted in 2018, one at the end of the wet season (April/May)—which is from November to March—and a second at the end of dry season (August/September)—which is from May to September.

2.2.1 Predictor variables

A large number of variables related to natural conditions and human stressors were previously measured at each stream reach (Campos et al., 2021). From this dataset, we retained only uncorrelated variables (absolute Pearson's $r < 0.6$) which include natural characteristics (drainage area, elevation, riparian shading, and percentage of organic matter and coarse sediments in the riverbed), water quality variables commonly used in monitoring programs and considered as indirect indicators of human disturbances (dissolved oxygen, conductivity, turbidity, nitrate,

TABLE 1 Description, average and range (minimum and maximum) of natural and human disturbances variables. (*) Data collected four times, but for analysis, we consider the average between April/May and August/September. (**) For categorical variables, we indicated the number of samples in each category.

Variables	Description	Average (min-max)	Category—number of samples**
drai_area	Drainage area upstream of the sample site (Km^2)	40.52 (2.21–215.42)	
elevation	Altitude of the sample site relative to the sea level (m)	1,015 (744–1,220)	
shading	% of riparian shading (0 = 0%; 1 = < 30%; 2 = between 30 and 60%; 2 = > 60%)		0–3; 1–9; 2–7; 3–33
OM	% of organic matter in the riverbed sediment	6.15 (0.61–26.66)	
coa_sed	% of coarse sediments (>2000–710 mm) in the riverbed sediment	60.49 (4.19–97.28)	
DO*	Dissolved Oxygen (mg L^{-1})	7.11 (1.88–8.85)	
cond*	Electrical conductivity (μS cm^{-1})	56 (1–584)	
turb*	Turbidity (NTU)	8 (0.04–197)	
NO_3^-*	Nitrate (mg L^{-1})	0.35 (0–10.29)	
PO_4^{-3}*	Phosphate (mg L^{-1})	0.23 (0–6.26)	
RIP_urb	% of urban area in the riparian corridor	1 (0–33)	
RIP_agr	% of agricultural and livestock areas in the riparian corridor	8 (0–56)	
CAT_urb	% of urban area in upstream catchment	7 (0–70)	
CAT_agr	% of agricultural and livestock areas in upstream catchment	21 (0–86)	
CAT_mod	% of modified area in upstream catchment (allotment, exposed soil, eucalyptus)	3 (0–39)	
SR**	Presence (1)/absence (0) of point-source treated sewage release upstream		0–49; 1–3
Dam**	Presence (1)/absence (0) of dams upstream		0–39; 1–13

and phosphate), and primary sources of human disturbances (urbanization and agriculture in the catchment area and in the riparian corridor, other uses in the catchment, presence of upstream point-source sewage release and dam) (Table 1). All of them will be considered hereinafter as predictors. The season (wet and dry) was also considered as a predictor since it may affect some of our biological response metrics.

2.2.2 Response metrics

A large number of ecological metrics were considered in this study (Table 2). The structural metrics are related to the diatom and macroinvertebrate assemblages' composition. The functional metrics include relevant ecosystem processes such as leaf litter decomposition (microbial and total), sediment respiration and algal and microbial biomass production.

2.2.3 Biological assemblages sampling

Macroinvertebrates were sampled using a *surber* (0.09 m^2 area and 0.25 mm mesh size) to collect five sub-samples per site covering the proportional diversity of habitats. The sub-samples were then integrated and preserved in 96% alcohol to be sorted and identified under a stereomicroscope to the lower taxonomic level possible (until family). Diatoms were sampled from five 10 × 10 cm pieces of artificial substrates (slate stones) that were incubated in the riverbed for approximately 30 days. Nearly 250 cm^2 were scraped and the shaved material was preserved in vials containing 0.33% Lugol solution. The identification and quantification of the organisms were carried out under an inverted microscope (Utermöhl 1931). Identification of macroinvertebrates and diatoms was carried out mostly to family and species level, respectively, with the assistance of taxonomic specialists (see Acknowledgments).

2.2.4 Biological assemblage metrics

We considered in this study metrics related to the structure and sensitivity to pollution of diatom and macroinvertebrate assemblages. The structure was composed of richness, abundance, diversity (Shannon-Wiener, Simpson), and evenness (Pielou) indices. The percentage abundance of pollution-sensitive taxa was calculated for the diatom genus *Eunotia*, for diatoms, and for the macroinvertebrate orders Ephemeroptera/Plecoptera/Trichoptera (EPT) and the Plecoptera order alone. The percentage abundance of pollution-tolerant taxa was calculated for the diatom species *Nitzschia palea*, and for the macroinvertebrate classes Oligochaeta and Hirudinea.

Some pollution sensitivity indices were adapted for diatoms and macroinvertebrates. The TDI (Trophic Diatom Index) was adapted from Kelly (1998). Although this index has been developed in Europe, it has the most complete species list. Only 8 of the 74 species identified were not described in the TDI list, hence we attributed the lowest value 1) to them, not to have too much influence on the result. The Biological Monitoring Working Party (BMWP) was adapted from four BMWP indices developed in different regions. The main reference was Monteiro et al. (2008), followed by Junqueira & Campos (1998), Uherek & Gouveia (2014), and Alba-Tercedor & Sánchez-Ortega (1988).

TABLE 2 Description, average and range (minimum and maximum) of ecological response metrics.

Ecological group	Response metrics	Description	Average
Diatoms	Diat_Rich	Diatom species richness	7.52 (1–17)
	Diat_Abund	Diatom species abundance	2,857.61 (6.12–9 × 10^4)
	Diat_Shannon	Shannon-Wiener index	1.40 (0–2.52)
	Diat_Simpson	Simpson index	0.64 (0–0.9)
	Diat_Pielou	Pielou index	0.74 (0–1)
	%Eunotia	% abundance of *Eunotia*	56.81 (0–100)
	%Nitz_palea	% abundance of *Nitzschia palea*	2.28 (0–76.76)
	TDI	Trophic Diatom Index (Kelly (1998), adapted)	15.44 (0–92.01)
Macroinvertebrates	Inv_Rich	Macroinvertebrate taxa richness	14.66 (3–28)
	Inv_Abund	Macroinvertebrate taxa abundance	513.94 (6–6.4 × 10^3)
	Inv_Shannon	Shannon-Wiener index	1.49 (0.43–2.23)
	Inv_Simpson	Simpson index	0.62 (0.19–0.86)
	Inv_Pielou	Pielou index	0.58 (0.19–0.96)
	%EPT	% abundance of Ephemeroptera, Plecoptera and Trichoptera	17.72 (0–76.47)
	%Plecoptera	% abundance of Plecoptera	2.53 (0–31.82)
	%OLI_HIR	% abundance of Oligochaeta and Hirudinea	3.74 (0–70.29)
	BMWP	Biological Monitoring Work Party (Monteiro et al. (2008); Junqueira & Campos (1998); Uherek and Gouveia (2014); & Alba-Tercedor & Sanches-Ortega (1988), adapted)	87.00 (15–170)
	ASPT	Average Score per Taxon Armitage et al., (1983)	5.90 (4.83–6.93)
Ecosystem Processes	Mic_dec	% of decomposed leaf litter in fine mesh litter bags	65.2 (32.79–119.70)
	Tot_dec	% of decomposed leaf litter in coarse mesh litter bags (microbial + invertebrates)	61.69 (15.28–118.05)
	Resp	Sediment respiration rate (mg O_2 h^{-1})	0.13 (0–1.31)
	Chl	Algal biomass (Chlorophyll *a* concentration ug m^{-2})	0.69 (0–11.63)
	Erg	Fungal biomass (Ergosterol concentration mg Erg/g AFDM)	0.05 (0–0.27)
	ATP	Microbial biomass (ATP concentration nmol ATP/g AFDM)	0.01 (0–0.07)

Taxa without published sensitivity grades were attributed with the lowest score (1). The Average Score per Taxon (ASPT) index Armitage et al. (1983) was calculated by dividing the score of each taxon by the total number of scoring taxa.

2.2.5 Ecosystem processes

The respiration rates on river sediments were measured following Feio et al. (2010), with some adaptations, as an indication of river metabolism. Three PVC chambers (30 cm long, ø 4.4 cm) were half-filled with riverbed sediment (<1 cm diameter; collected up to 15 cm depth) and then filled in with stream water and sealed with rubber stoppers. To control, one PVC chamber was filled in only with river water. Respiration rates were measured as the depletion of dissolved oxygen in the chambers after approximately 30 min. The volume of water in each chamber was measured using a beaker.

The respiration rate for each site was given by the expression (1):

$$\mathrm{Rr} = \sum \mathbf{s}\left[\mathbf{Vx}\left(\mathbf{Of}-\mathbf{Oi}\right)\mathbf{xt}\right]-\mathbf{c}\left[\mathbf{Vx}\left(\mathbf{Of}-\mathbf{Oi}\right)\mathbf{x}\,\mathbf{t}\right] \quad (1)$$

where Rr (mg O_2 L^{-1} h^{-1}) is the respiration rate, “s” is each chamber, V is the volume (L) of water in each chamber, Of is the final O_2 concentration (mg L^{-1}), measured with a YSI probe), Oi is the initial O_2 concentration (mg L^{-1}), “t” is the incubation period (hours) and “c” is the control chamber. Respiration was measured only in September (dry season).

The microbial (fine mesh bag-FMB) and total (coarse mesh bag-CMB) leaf litter decomposition rates were calculated by the decrease in leaves weight after 30 days of incubation on riverbeds. Portions with approximately 3 ± 0.5 g of dry air leaves (*Hyeronimia alchorneoides*) were placed in fine- (0.25 mm mesh; 13 cm × 20 cm size) and coarse-mesh litter bags (10 mm mesh; 18 cm × 23 cm size). The use of FMB (only microbial effects) and CMB (microbial and invertebrates assemblages’ effects) allows distinguishing the contribution of microorganisms and macroinvertebrates to the loss of leaf litter mass. Moreover, CMB may also add the physical water abrasion effect (Tonin et al., 2018).

In the laboratory, six leaf discs (10 mm diameter) were cut from each sample. A set of a three-leaves disc was used to determine ergosterol content (as an indirect measure of fungal biomass on decomposing leaves; Gessner 2005) and another similar set was used to determine the total ATP content (as an indirect measure of the total microbial biomass; Abelho 2005). The results were expressed in % of decomposed biomass standardized for 30 days.

A similar piece of artificial substrate area scraped for diatoms (approx. 250 cm^2) was scraped off for Chlorophyll *a* determination, an indirect measure of periphytic algal biomass. The material was filtered (glass fibre 0.45 mm filters) and frozen until analysis. Chlorophyll *a* concentration ($\mu g\ m^{-2}$) was determined spectrophotometrically after acetone extraction (Wetzel & Likens 1991).

2.3 Data analysis

To quantify the relationships between selected predictors and response metrics we used Boosted Regression Tree (BRT) analysis. BRTs provide a means to fit nonlinear relationships between predictors to response metrics, including interaction effects, by using a boosting strategy to combine results from a large number (often thousands) of simple regression tree models (Friedman 2001). Three elements are fundamental in the execution of the BRT models: 1) tree complexity (*tc*), which controls whether the interactions are fitted; 2) the learning rate (*lr*), which determines the contribution of each tree to the growing model; and 3) the number of trees (*nt*) necessary for the optimization of the model, which is determined based on the two previous parameters (Elith et al., 2008). We adopted the tree complexity (*tc*) equal to 5, and the learning rate varying between 0.01 and 0.0001, guaranteeing that at least 1,000 trees were generated for each metric (see all settings in Supplementary Material). The bag fraction (*bf*) represents the proportion of training data to be selected, without replacement, at each interaction, thus controlling the stochasticity of randomization. We applied *bf* equal to 0.75. Within the BRT, the cross-validation (CV) technique provides a means for testing the model using part of the training data, while still using all data at some stage to fit the model. It is useful especially in cases of relatively low sample sizes (Elith et al., 2008), as is the case of this study.

BRT outputs included the performance of training data (% variation explained) and test data (CV correlation), the relative influence (contribution) of each predictor to explain the training data (sum adds up to 100%). Lastly, partial dependence plots indicated the shapes of relationships between predictors and the response variable (e.g., linear, curvilinear, and sigmoidal) taking into account the average effect of all other predictors (Elith et al., 2008). We also used the shapes for visual identification of thresholds (Wagenhoff et al., 2017).

In a second step, the models were reduced with the exclusion of predictor variables that contributed less than 2% to explain each response variable, since the reduction of variables is desirable considering that BRT models tend to overfit models (Elith et al., 2008; Brown et al., 2012). The results presented refer to the reduced final models. Sewage release (SR) was excluded from the reduced models in all response metrics (less than 2% of relative contribution). All statistical analyses were performed using the gbm package (Greenwel et al., 2018) from R v.4.0.3 (R Core Team 2020) and specific code for BRT provided by Elith et al. (2008).

3 Results

3.1 Performance of boosted regression tree models

For macroinvertebrate metrics, the highest percentages of variance explained were observed for % Oligochaeta/Hirudinea (91%), Macroinvertebrate abundance (84%), % Plecoptera (82%) and % EPT (70%). For diatom metrics, BRT models explained the highest percentage of variation for: %Eunotia (87%), Trophic Diatom Index (TDI, 84%) and Diatom richness (77%). Metrics of ecosystem processes were best predicted for algal biomass production (Chl, 96%), microbial decomposition (Mic_dec, 82%) and total decomposition (Tot_dec, 73%) (Figure 2). The medians of the structural and functional metrics were very similar, around 60% (Figure 2).

3.2 Relative contributions of predictor variables

Predictors related to the river size (drainage area and elevation) were important to explain some metrics, but especially %Plecoptera, for which the two predictors combined explained 29% of its variation. Habitat variables explained large portions of variation in a few metrics, most noteworthy among them was the percentage of organic matter in river sediment for macroinvertebrate metrics, ergosterol, and ATP (Figure 3).

Water quality variables were relevant in explaining almost all metrics. Conductivity highly contributed for most metrics (macroinvertebrates, diatoms and ecosystem processes). Turbidity, dissolved oxygen, nitrate, and phosphate were also relevant for some response metrics (Figure 3).

Among the land use predictors, agricultural and urban cover in the upstream catchment (CAT_agr and CAT_urb) explained the

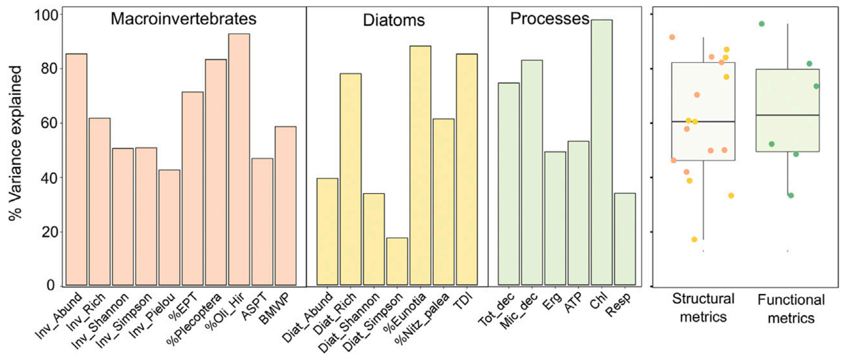

FIGURE 2
Percentage of variance explained for Macroinvertebrates, Diatoms, and Ecosystem Processes metrics models (see the metrics description in Table 2). Diat_Pielou is not shown because it was not possible to run the model. Boxplot of structural (Diatoms and Macroinvertebrates) and functional (ecosystem processes) metrics results. See all model settings and statistics in the Supplementary Material.

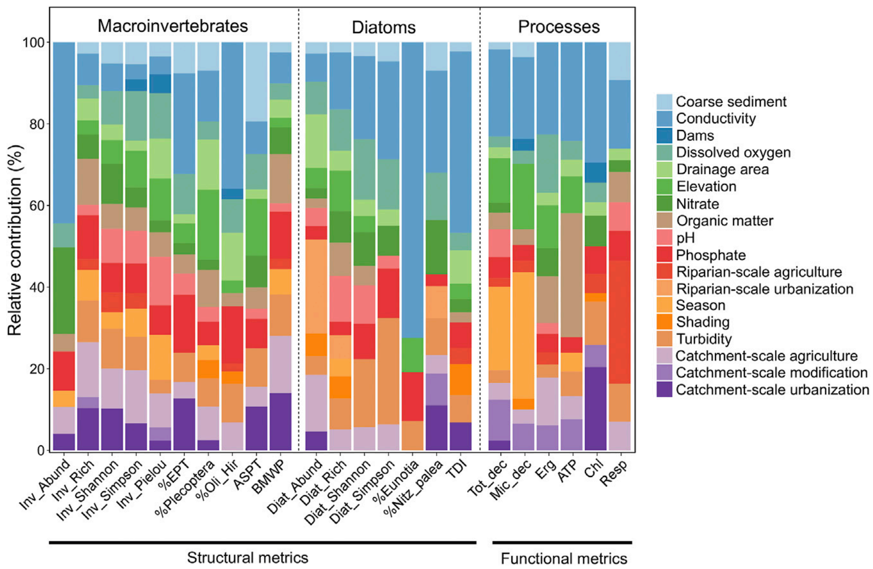

FIGURE 3
Relative contribution (0–100%) of predictor variables on the variance explained of each response ecological metrics (structural and functional; see the metrics description in Table 2). Sewage release (SR) was excluded because its contribution was 0% in all models.

largest fraction of variation of the response metrics (Figure 3). Macroinvertebrates metrics were the most influenced by them, but also the abundance of diatoms and sediment respiration. For macroinvertebrates, some metrics were rather explained by urban cover in the upstream catchment (e.g., %EPT, 13%), others by agricultural (e.g., Inv_Simpson, 13%) and others by both, like the macroinvertebrates richness (CAT_agr 14%, CAT_urb 10%) and the BMWP (CAT_agr 14%, CAT_urb 13%). Generally, catchment-scale metrics explained more variation in ecological variables than riparian-scale metrics, except for the abundance of diatoms and respiration rate, which were mostly influenced by urbanization (RIP_urb) and agricultural activities in the riparian corridor (RIP_agr), respectively. The influence of the presence of dams was minimal in all models.

3.3 Ecological response relationships with environmental gradients

The relationships between predictors and response metrics presented some features in common: 1- most

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response shapes were non-linear; 2- some of the response metrics presented an early increase or decrease followed by the continuity of the curve in the opposite direction; 3- for some of them, it is possible to identify common values from which the curves abruptly changed, which points out to the existence of potential thresholds. For example, change points of most conductivity curves were around 100 µS cm^{-1}. For phosphorus, change points were around 0.5 mg L^{-1}, and CAT_urb between 10 and 20% (Figures 4–6).

Conductivity, phosphate, nitrate and land use in the catchment had a positive influence on Macroinvertebrates abundance, %Oligochaeta/Hirudinea, TDI, Diatom richness and algal biomass; and positive on %EPT and %Eunotia. The increase in the drainage area and the reduction in elevation were negatively related to the %Plecoptera, %Eunotia and Diatom richness, and positively related to the increase in % Oligochaeta/Hirudinea, TDI, total and microbial decomposition (Figures 4–6).

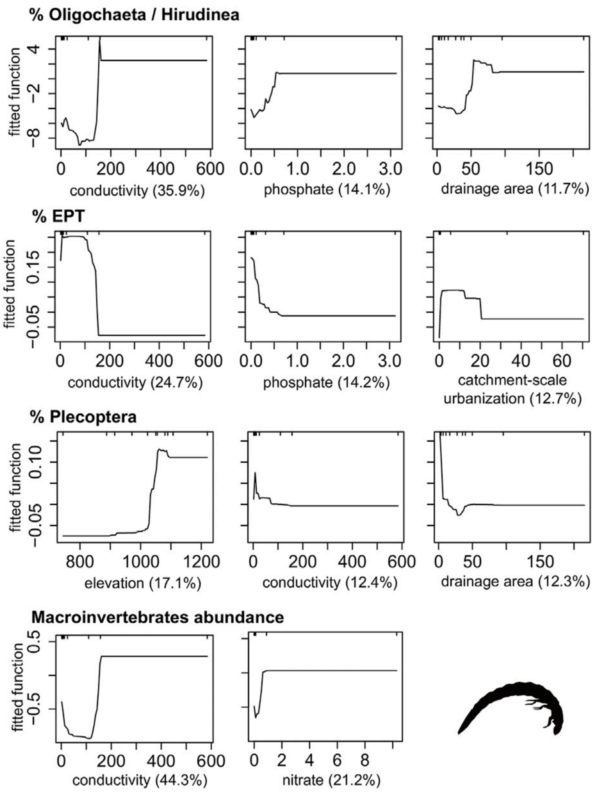

FIGURE 4
Boosted regression tree (BRT) fitted functions for the best performance models of Macroinvertebrate metrics. Plots are only shown for those predictors that explained more than 10% deviance in the metric. Rug plots show the distribution of data, in deciles, of the variable on the X-axis.

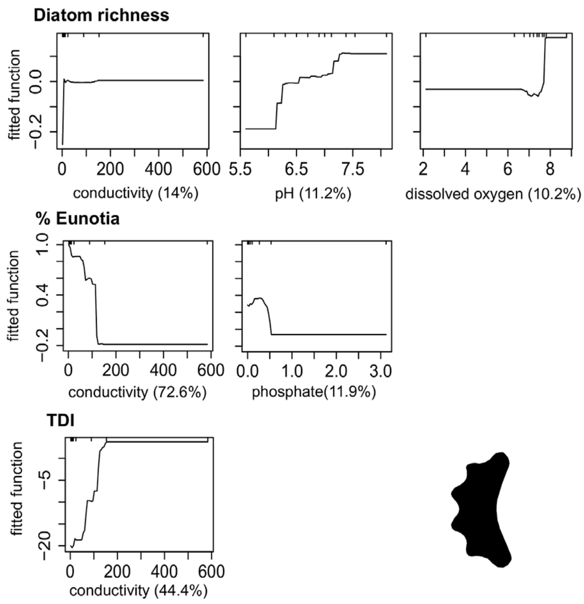

FIGURE 5
Boosted regression tree (BRT) fitted functions for the best performance models of Diatom metrics. Plots are only shown for those predictors that explained more than 10% deviance in the metric. Rug plots show the distribution of data, in deciles, of the variable on the X-axis. (TDI) Trophic Diatom Index.

4 Discussion

The study made it possible to identify the main predictors driving each ecological metric and how metrics responded to natural and human-related predictors, allowing the detection of potential indicators of stream integrity. While the percentage of EPT group abundance and the algal biomass would be good indicators of urbanization in the upstream catchment, the percentage of *Eunotia* abundance would indicate changes in water quality. In contrast, other metrics were poorly explained by the predictors or mainly influenced by natural predictors, making them inappropriate indicators of environmental disturbances for management purposes (Norris & Hawkins 2000). Like the Simpson Index for Diatoms showed a low variance explained (17%), the percentage of Plecoptera, which was influenced mainly by natural characteristics (elevation and drainage area), and decomposition primarily influenced by seasonality. Most of our models presented a unidirectional response for direct (land use) and indirect (water quality) human disturbances. Overall, increasing human disturbance (e.g., conductivity and changes in land use) led to a decrease in pollution-sensitive taxa (e.g., percentage of EPT group and *Eunotia*) and an increase in pollution-tolerant taxa (e.g., percentage of Oligochaeta and Hirudinea, the Trophic Diatom Index and the algal biomass production).

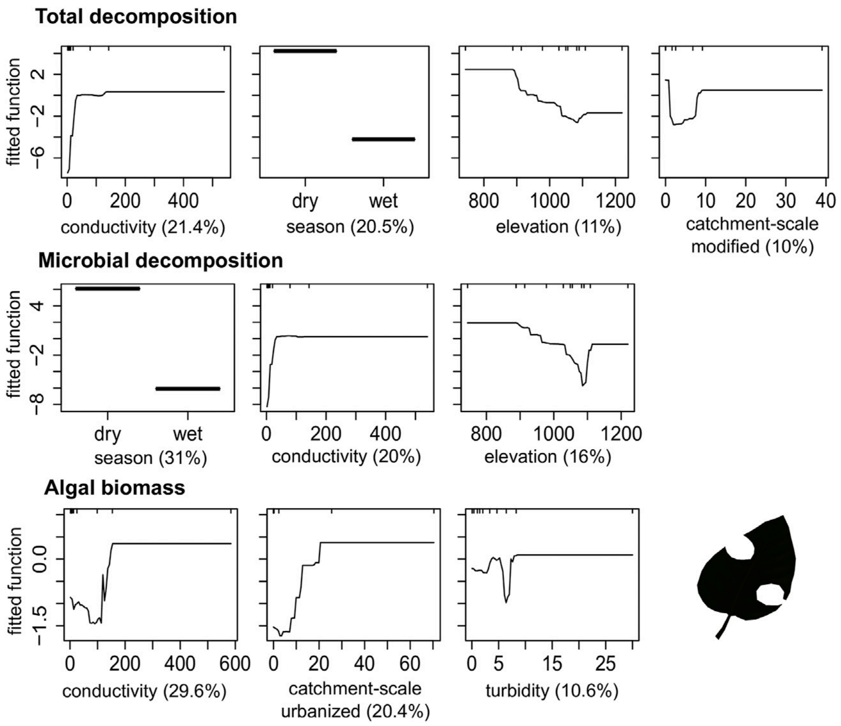

FIGURE 6
Boosted regression tree (BRT) fitted functions for the best performance models of functional metrics. Plots are only shown for those predictors that explained more than 10% deviance in the metric. Rug plots show the distribution of data, in deciles, of the variable on the X-axis.

The nonlinear responses promoted insights into the subsidy-stress theory (too much of a good thing syndrome; Odum 1983), which predicts that the increase of limited resources (e.g., nutrients, light) in an environment may have an initial positive effect on biological communities and ecosystem functions. However, this effect rises to a certain threshold; after then, it can lead to adverse effects. In this context, considering that Brazilian savanna streams are poor in nutrients (Markewitz et al., 2006), nutrient inputs possibly promote the maintenance of more species/individuals. But at the other extreme of the gradient, intense disturbances are expected to reduce the number of species that can colonize or tolerate high impact levels (Odum 1983). The shape of the EPT curve (initial low value followed by a sharp rise, lately a decrease) indicates their sensitivity to disturbed environments face to the increase in conductivity, and catchment urbanization was an example of this (Ligeiro et al., 2013; Siegloch et al., 2017). The evaluation of the response curves from BRT models was also a good starting point for discussing thresholds for the considered predictors. Notable change points could be observed, such as conductivity, phosphate, and the percentage of urbanization in the upstream catchment.

Our study showed the most important predictors to explain the ecological metrics were physical and chemical variables often used to indicate human disturbances (Heathwaite 2010; Uriarte et al., 2011; Alvarez-Cabria et al., 2016), such as phosphorus and nitrate concentrations, but especially conductivity. For instance, we reported significant changes in ecological metrics when conductivity stood between 100 and 200 μS cm^{-1}, suggesting a potential threshold. Values above this threshold indicate loss of water quality, except when high conductivity is due to the natural background (Fravet & Cruz 2007; Fundação Nacional de Saúde, 2014; CETESB 2020). Conductivity was the main predictor for the studied metrics in terms of relative importance. Comparing water bodies of preserved and anthropogenic (especially those without vegetation protection) areas, the diffuse sources of pollution resulted in higher electrical conductivity (Gardiner et al., 2009; Rezende et al., 2014). Like in anthropogenic areas with inadequately treated effluents flowing to water bodies, increasing the nutrient concentrations of the water (Myrka et al., 2008).

Phosphorus increase is responsible for triggering the eutrophication of freshwaters (Figueredo et al., 2016; Zhang et al., 2017) coming from agricultural fields and urban effluents (Ockenden et al., 2016). Our results showed potential thresholds for phosphate around 0.5 mg L-1, and its contribution was especially relevant for metrics sensitive to pollution, such as %EPT, %Oli_Hir and %Eunotia, as shown elsewhere (Kelly.

1998; Salomoni et al., 2006; Ferreira et al., 2014; Pardo et al., 2020). Both conductivity and phosphorus were positively related to effluent discharge and deforestation. Anthropogenic areas (remarkably urbanized areas) strongly influence biological assemblages, and their effects are disproportionate to the size of the area used (Rezende et al., 2014; Campos et al., 2021).

Urban and agricultural cover in the upstream catchment was the most important land-use factor to explain the response metrics. The adverse effects of replacing native vegetation with urban or agricultural areas in the upstream catchment have been reported for the stream via complex pathways (Allan 2004) like changes in temperature, habitat diversity, hydromorphology, sunlight, and nutrient availability (Einheuser et al., 2013). These changes have translated into alterations in the structure and functioning of the stream ecosystem (Clapcott et al., 2012). We observed that values between 10 and 20% of urban cover in the upstream catchments led to a decrease in the abundance of the EPT group and an increase in algal biomass. Brito et al. (2020) reported abrupt changes in the composition of macroinvertebrates with the removal of 57–79% of native vegetation in the Amazon Forest, while Dala-Corte et al. (2020) reported threshold values between 3 and 40% of native vegetation removal across biomes in Brazil. Therefore, our results in the study region indicated more restrictive values suggesting that parts of the Brazilian savanna are more susceptible to the conversion of native areas. Additionally, the increase of algal biomass related to the urbanization process confirms a recent study that shows a 32% greater effect on stream functioning than in its structure in the tropics (Wiederkehr et al., 2020).

Changes in biological assemblages and ecosystem processes are commonly associated with alterations in the riparian plants (Encalada et al., 2010; Fierro et al., 2017), especially in headwaters that are light-limited systems and rely on plant litter inputs from surrounding vegetation (Bunn & Davies, 2000; Perona et al., 2009). However, we did not observe a robust relationship with macroinvertebrates. On the other hand, we found a consistent negative relationship among diatoms, urbanization and agriculture in the riparian zone, indicating a higher local than catchment-scale effect. This finding suggests reliable benefits of forested riparian buffers for stream biological diversity in urban environments, supported by previous studies (e.g., Mutinova et al., 2020).

The different responses to the set of predictors, including structural and functional ecosystem metrics, can lead to a comprehensive interpretation of river conditions (Feio et al., 2010). The prediction of ecological conditions is relevant from the management's point of view since these are more complex data to be acquired but of extreme relevance for understanding the health of water bodies (Karr 2006). Knowledge about the importance of each predictor for the response metrics allows, for example, to predict some ecological conditions in places with limited availability of biological data.

Finally, the potential thresholds identified in the present study are important signs of significant changes in ecological responses. They should be employed in eventual review processes of guidelines to public policies for river health preservation and recovery (Huggett 2005). Brazilian national environmental guidelines do not consider, for example, conductivity (CONAMA n° 357, Brasil 2005), notwithstanding the importance of this variable as an ecosystem driver, as demonstrated in our study. In addition, further attention should be paid to the context of land use, especially to the urbanization processes in the upstream catchment. Currently, Brazil has increased awareness of riparian vegetation (Federal Law n° 12.651, Brasil 2012). However, for purposes of biodiversity conservation and maintenance of ecosystem processes, we also have shown it necessary to consider the entire context of the catchment in which the stream is located.

5 Conclusion

Our results demonstrated the importance of considering a set of ecological response metrics (structural and functional) and environmental factors (natural and disturbances), allowing a complete view of the freshwater ecosystem condition. The relative importance of predictors on ecological metrics pointed to metrics most affected by factors on a local scale (e.g., percentage of *Eunotia* abundance) and catchment scale (e.g., algal biomass). Also, the nonlinear responses permitted the detection of gradual or abrupt change curves, pointing out the existence of potential thresholds of important drivers, like the conductivity (100–200 µS cm-1), phosphate (0.5 mg L-1), and catchment-scale urbanization (10–20%). The potential bioindicators (considering the best performance models and the ability to respond more strongly to the human disturbances) were macroinvertebrates abundance, EPT abundance percentage, Oligochaeta and Hirudinea abundance percentage, percentage of *Eunotia* abundance, Trophic Diatom Index, and algal biomass. Although we have worked with many biotic and abiotic variables and the BRT model considered the interaction between them, models are simplified representations of a complex system, therefore presenting limitations. Nevertheless, the consistency and reasonableness of influential metrics within a given set of ecological metrics provide a weight of evidence in support of the models' results. The BRT models approach proved to be powerful tools that can be effectively employed to enhance and give better direction to freshwater management, not only to the streams of the Brazilian savanna but also to water bodies in other regions.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

Campos, CC: Term, Conceptualization, Methodology, Formal analysis, Investigation, Writing–Original Draft, Writing–Review and Editing, Visualization. Tonin, AT: Writing–Review and Editing, Visualization. Kennard, MK: Conceptualization, Writing–Review and Editing, Supervision. JG: Conceptualization, Resources, Funding acquisition, Writing–Review and Editing, Supervision.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fenvs.2022.867905/full#supplementary-material

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