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Major risks to aquatic biotic condition in a Neotropical Savanna River basin

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Abstract

Conditions in freshwater ecosystems are responsible for maintaining biodiversity and other ecosystem services. Identifying and understanding how anthropogenic disturbances affect biotic conditions are important steps in rehabilitating and protecting environmental quality. The relative risk, relative extent, and attributable risk approaches are used to determine ecosystem conditions in ecological monitoring programs conducted across large spatial extents. Our study was conducted in the Pandeiros River basin, which is a protected area in Minas Gerais, Brazil, that contains 233 km of mapped streams that were perennial and accessible. Field sampling was conducted in the dry period (April and June 2016) at 40 randomly selected sites. Ten multimetric indices (MMIs), previously determined to be sensitive in this river basin, were calculated. All the physical habitat disturbance metrics were significantly correlated with the MMIs. The risk of finding poor MMI scores was 1.6–1.7 times higher at sites with a high integrated disturbance index (IDI) or local disturbance index (LDI) score. Pasture was the most extensive disturbance, affecting 40.8% of the stream length, followed by 40.1% for low bed stability, 29% for fine substrates (<16 mm), 24.4% for high IDI scores, and 21.7% for high LDI scores. This is useful to know for five reasons: (1) standardized MMIs can assess environmental quality. (2) MMIs clarify that both catchment and local disturbances may represent serious risks to aquatic assemblages. (3) MMIs indicate which disturbances represent the most risk by comparing MMI scores against disturbance scores. (4) MMI risk assessments facilitate choosing the most appropriate mitigation actions. (5) Our results suggest environmental conservation actions for similar river basins.

KEYWORDS
bioassessment, biotic indices, Cerrado, conservation unit, macroinvertebrates, streams

1 | INTRODUCTION

The increased human demands for water affect the quality and availability of this resource and threaten aquatic biodiversity (Reid et al., 2018). Identifying the most threatening anthropogenic disturbances of these ecosystems and understanding how they affect biotic conditions are important steps in improving environmental quality and proposing recovery options (Feio et al., 2021; Sánchez-Bayo & Wyckhuys, 2019).

The relative risk (RR), relative extent (RE), and attributable risk (AR) approaches are used by the United States Environmental Protection Agency (USEPA) to report regional and national aquatic ecosystem conditions in its national-extent biomonitoring and bioassessment program (USEPA, 2016a, 2016b, 2016c). They have also been implemented by some USA state biomonitoring programs (e.g., Merrick, 2015; Mulvey, Leferink, & Borisenko, 2009; Rowe, Pierce, & Wilton, 2009) and in Brazilian research (Jiménez-Valencia, Kaufmann, Sattamini, Mugnai, &

Baptista, 2014; Silva, Herlihy, Hughes, Macedo, & Callisto, 2018). This approach is based on its ability to provide quantifiable associations between major anthropogenic disturbance metrics and biological responses (Paulsen et al., 2008). The RE provides the magnitude in which high disturbance levels occur in a region or basin. The RR describes the probability of good versus poor biological condition, given the presence/absence of low versus high disturbance levels. The AR is the percentage reduction in the regional extent (RE) of a poor biological condition if the stressor is eliminated (Van Sickle & Paulsen, 2008). By implementing a probabilistic survey design for site selection, results can be statistically inferred from a relatively small set of sampled sites to an entire channel network across a basin, region, or nation (Van Sickle & Paulsen, 2008). This is important because it ensures representation across the entire studied area, allowing the physical, chemical, and biological characteristics of the sampled sites to reflect the ecological conditions of the region or basin as a whole (Herlihy et al., 2008; Herlihy, Sifneos, Hughes, Peck, & Mitchell, 2020; Mulvey et al., 2009; Silva et al., 2018). Also, probability survey designs have three other advantages: (1) They are economical because they allow accurate and reliable inferences to the ecological condition of large areas based on a minimum number of probabilistic sampled sites (Paulsen et al., 2008). (2) They allow rigorous statistical estimation of the channel length of the entire river basin with known confidence limits (Herlihy, Larsen, Paulsen, Urquhart, & Rosenbaum, 2000). (3) Because they are random approaches, sites are not selected for convenience, thereby avoiding biased conclusions in ecological assessment studies, including in studies across difficult and unroaded subtropical and subarctic terrains (Hughes, Boxall, Herlihy, Adams, & Young, 2020; Jiménez-Valencia et al., 2014).

RR and RE approaches have included metrics that quantify instream and riparian physical characteristics (Kaufmann, Levine, Robison, Seeliger, & Peck, 1999). In general, physical habitat includes all structural attributes that influence or enable the maintenance of organisms in an aquatic ecosystem (Peck et al., 2006). The decrease in physical habitat diversity can lead to the simplification of biological communities, therefore, its assessment is of fundamental importance for assessing ecological conditions (Barbour, Gerritsen, Snyder, & Stribling, 1999).

In addition to physical habitat assessment, it is essential to evaluate the biological condition of entire aquatic ecosystems. The index of biotic integrity approach proposed by Karr (1981), has been widely used for water quality assessment (Ruaro, Gubiani, Hughes, & Mormul, 2020). Multimetric indices (MMIs), which are variants of Karr's index, are composed of a combination of various biological attributes or metrics that reflect anthropogenic disturbances along a gradient of environmental disturbance (Karr, Fausch, Angermeier, Yant, & Schlosser, 1986). Macroinvertebrate assemblages are commonly and effectively used in environmental monitoring programs globally (Buss et al., 2015; Feio et al., 2021) because they respond to environmental conditions and integrate physical, chemical, and biological aspects of ecosystems (Bonada, Prat, Resh, & Statzner, 2006).

Because of their usefulness in assessing environmental quality, over 400 MMIs have been developed globally (Ruaro et al., 2020). However, such a diversity of indices hinders making regional, let alone global, comparisons and condition assessments across the various studies (Buss et al., 2015). Therefore, Martins, Macedo, Hughes, and Callisto (2020) assessed the efficacy of MMIs developed in different regions and continents in the Pandeiros River basin and showed that 10 MMIs passed all validation stages and were extremely effective for assessing anthropogenic impacts. However, a knowledge gap remained regarding their applicability, combined with a probabilistic survey, in environmental diagnostic studies. Therefore, we sought to assess the RR of various types of anthropogenic disturbances on those 10 MMI scores in a river and its tributaries in a priority area for biodiversity conservation. The results of such studies can be used to inform managers of conservation units and agencies responsible for conserving aquatic ecosystems.

2 | MATERIALS AND METHODS

2.1 | Study area

The study area included the entire 3,960 km^2 Pandeiros River basin. The basin is located in Minas Gerais state, Brazil, in the Cerrado (Neotropical Savanna) biome (Figure 1). The basin is considered an area of special biological importance because it is a unique environment (Azevedo, Nunes, Veloso, Neves, & Fernandes, 2009), having flooded regions (wetland and marginal lagoon complexes) and palm swamps. Both are among the international priority areas for biome conservation. Most of the basin area (85.7%) is part of the Rio Pandeiros State Environmental Protection Area (APAERP) (IEF, 2019). The region's climate is tropical, with an April–September dry season (Aw climate; Alvares, Stape, Sentelhas, De Moraes Gonçalves, & Sparovek, 2013), so the perennial flow regime of most Pandeiros tributaries is of great importance to guarantee water supply for local human populations. However, most first- and second-order streams mapped at 1:100,000 scale in the basin were dry or inaccessible (Figure 1).

2.2 | Survey design and sampling

Stream and river sites were selected through the use of spatially balanced procedures employing a random and systematic survey design, following the method used by the USEPA in its national rivers and streams survey (Olsen & Peck, 2008) adapted for Cerrado aquatic ecosystems (Callisto, Hughes, Lopes, & Castro, 2014). To ensure a gradient of ecological conditions, some presumably degraded sites were handpicked (Whittier, Stoddard, Larsen, & Herlihy, 2007). A random set of 40 potential sampling sites and an additional set of substitute sites were selected to ensure that we had a final set of 40 because we assumed that some sites initially selected would be dry, inaccessible, or have access denied (Macedo, Hughes, et al., 2014; Macedo, Pompeu, et al., 2014). Each site was at least 1 km from any other to minimize spatial autocorrelation and it received a weight, proportional to the inverse of its selection probability. That probability is the length

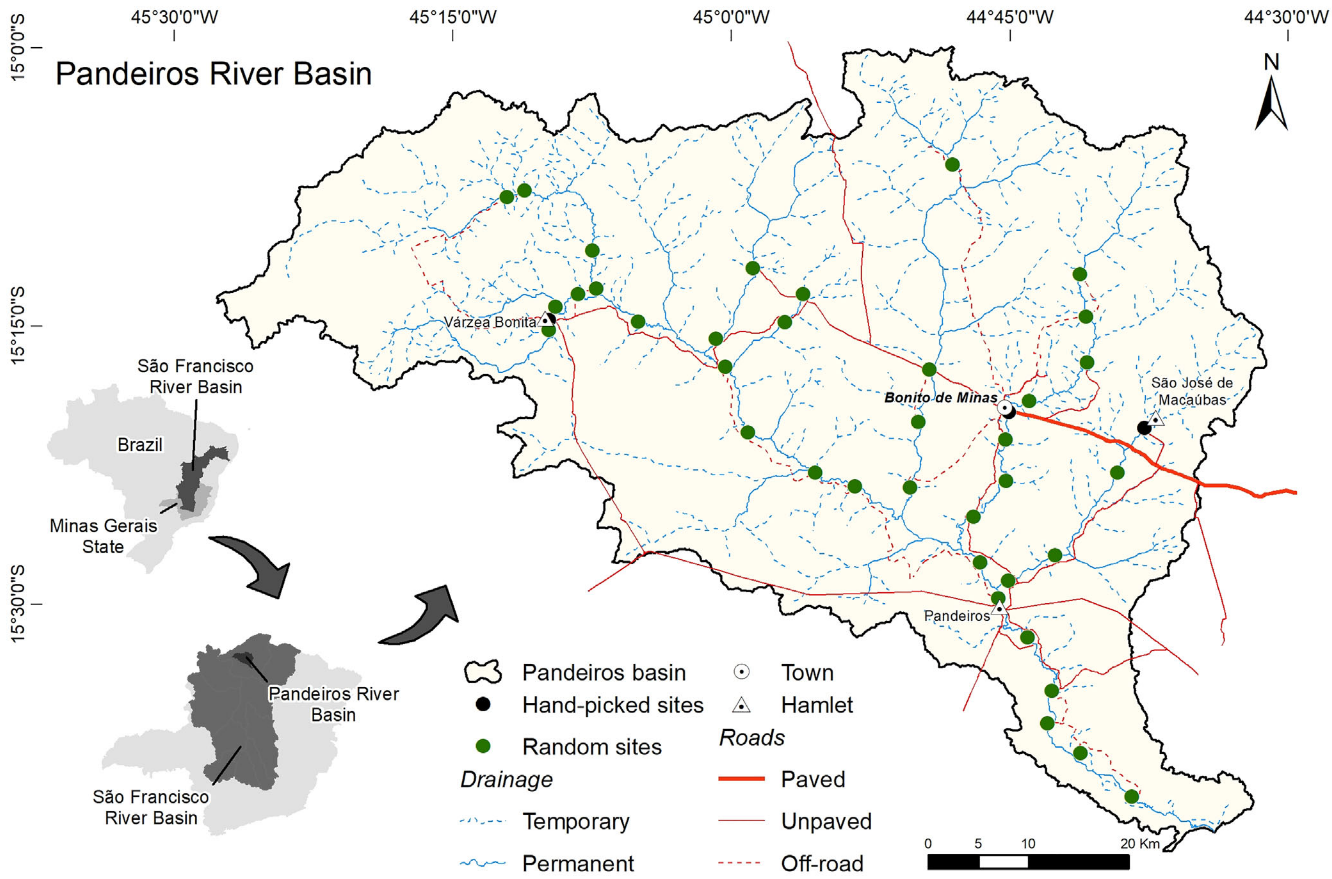

FIGURE 1 Location of sample sites in the Pandeiros River basin, Minas Gerais [Color figure can be viewed at wileyonlinelibrary.com]

of the entire channel network that represents the entire target population, that is, the entire stream length in that stream order. We used those weights to balance the number of sites across third-, fourth-, and fifth-order streams and rivers to ensure that most sites were not in the more abundant lower-order streams. The weights were also used to estimate the extent of the stream environmental and biological conditions and their RRs to the biota (Van Sickle, Stoddard, Paulsen, & Olsen, 2006). The sites chosen manually were not used to make extent estimates because they had zero weights. But both probabilistic and handpicked sites were considered for establishing thresholds for metrics and MMIs (Van Sickle et al., 2006). To confirm the set of sampling sites (target length), field reconnaissance was required. In this phase, sites were checked for access and flows; those that were not sampled for any reason (dry, non-wadeable, access denied, etc.) were substituted for by sites having the same weights (Silva et al., 2018).

During April and June 2016, we sampled 15 third-order sites, 13 fourth-order sites, and 12 fifth-order sites for a distance equal to 40 times their mean wetted width, with a minimum length of 150 m (Hughes & Peck, 2008). In each site, 11 transverse transects (perpendicular to the stream flow) were established defining 10 sections, where physical habitat structure and biota were sampled (Peck et al., 2006; USEPA, 2016b). For details on sampling, identification, calculation of indices, and results see Martins et al., 2020.

2.3 | Anthropogenic disturbances

The quantification of types of land use and the cover was carried out using supervised classification of digital images, whereby classes were assigned to pixels of satellite images, creating homogeneous patterns to which different classes of land use and cover are associated (Santos, Martins, Callisto, & Macedo, 2017). We used 2016 imagery from the Landsat-8 satellite, sensor OLI (30-m spatial resolution), orbit scene 219/71 and 219/70 made available by INPE (Instituto Nacional de Pesquisas Espaciais, 2016). The anthropogenic land-use classes included human settlements, row crop agriculture, and pasture that were calculated as the percent of each class in the total catchment, as described in Callisto et al. (2014).

We selected disturbance variables based on the results from other studies in Brazil (Jiménez-Valencia et al., 2014; Macedo et al., 2016; Martins et al., 2020; Silva et al., 2018). We used the concept of least-disturbed or minimally disturbed (Martins, Ligeiro, Hughes, Macedo, & Callisto, 2018; Stoddard, Larsen, Hawkins, Johnson, & Norris, 2006) because there were no pristine sites in the basin. To identify these sites, we used integrated disturbance index (IDI) scores, which were calculated from local anthropogenic disturbances (local disturbance index [LDI]) and total catchment disturbances (catchment disturbance index [CDI]) for each site (Ligeiro et al., 2013).

We measured three additional disturbance indicators: (1) Percent fine substrates (<16 mm) included fine gravel, sand, silt, and clay. (2) Bed stability estimated the relationship between the average geometric diameter of the bed substrate and the critical theoretical diameter that the flow and channel might support, indicating % excess fine sediment. (3) Percent pasture in the total catchment, the dominant land use in the Pandeiros basin, was determined from satellite images (Macedo, Hughes, et al., 2014; Macedo, Pompeu, et al., 2014).

2.4 | Selection of MMIs

Ten different MMIs (Table 1) had been tested and validated to be effective in assessing biological quality in the Pandeiros River basin (Martins et al., 2020). Each MMI was built following the original procedures described in its publication; their metrics are described in Appendix 1. To standardize and classify the indices used in this study, we defined thresholds by anthropogenic disturbances based on the distribution of each of the 10 MMI scores in the least-disturbed sites. Seven least-disturbed sites were classified according to their IDI values (see below), as described in Martins et al. (2020) and were the same sites for all MMIs. Each MMI had a different range of values, so we scored each one as MMI scores <5th percentile of the IDI distribution of reference sites equaled poor and MMI scores >25th percentile equaled good (Table 1). Sites classified as fair were combined with poor sites to create a not-good class for subsequent risk analyses (Silva et al., 2017).

2.5 | Anthropogenic disturbance thresholds

Disturbance thresholds are generally based on regional distributions of values observed in least-disturbed sites (Herlihy et al., 2020; Kaufmann et al., In Review). Using an approach similar to that used for the biological conditions, we defined sites with fine substrates, % total catchment pasture, and IDI having ≥75th percentile of the distribution in least-disturbed sites as being in not-good condition. Sites with percentages <75th percentile were considered as being in good condition (Van Sickle & Paulsen, 2008). The LDI sites with values <1 were classified as good and those >1 were classified as not good (Silva et al., 2018). The substrate stability thresholds were based on their score distributions, with values less than −1.5 being classified as not-good, and higher values considered as good (Table 2) (Kaufmann, Larsen, & Faustini, 2009).

2.6 | RE, RR, and AR analyses

RE measures extents across a study area, as represented by the stream length and proportion with high disturbance scores of each predictor variable used. Proportions are obtained as a sum of the sample weights of the sites found with high disturbance scores divided by the sum of all the weights of the sites (expressed in % channel length) (Van Sickle & Paulsen, 2008).

RR was used to assess the severity of the disturbances previously selected to affect MMI scores (for each of the 10 MMIs used) and the RE of those disturbances (Van Sickle & Paulsen, 2008). RR was based on conditional probability obtained from a 2 × 2 contingency table, in which all possible situations of having a good or not-good MMI condition were obtained, given a site's high or low disturbance value. The analysis uses the concept of conditional probability to measure RR and is calculated as:

$$\mathrm{RR} = \frac{\Pr(\mathrm{MMIp}|\mathrm{ng})}{\Pr(\mathrm{MMIp}|\mathrm{g})}, \tag{1}$$

the numerator is the probability of finding poor biological condition (MMIp) at a site where the disturbance indicates a not-good environmental condition (ng). The denominator is the probability of finding poor biological conditions at a site, where the disturbance indicates good environmental condition (g). A RR equal to or <1 indicates the absence of an association between the biological indicator and the disturbance. For an RR > 1, we interpreted the value as how many times more likely a not-good MMI condition would occur, given the

TABLE 1 Multimeric indices (MMIs) used, references, development locations, and threshold values for biological condition classification in the Pandeiros River basin

MMI	Reference	Location	Poor	Good
MMI_Baptista	Baptista et al. (2007)	Brazil—Atlantic forest	≤20.1	≥27
MMI_Ferreira	Ferreira, Paiva, and Callisto (2011)	Brazil—Cerrado	≤22.7	≥25.5
MMI_Macedo	Macedo et al. (2016)	Brazil—Cerrado	≤50	≥59
MMI_Silva	Silva, Herlihy, Hughes, and Callisto (2017)	Brazil—Cerrado	≤56	≥70
MMI_Helson	Helson and Williams (2013)	Panamá—Rainforest	≤5.04	≥7.08
MMI_Fierro	Fierro, Arismendi, Hughes, Valdovinos, and Jara-Flores (2018)	Chile—Mediterranean shrub	≤3.33	≥3.98
MMI_Ode	Ode, Rehn, and May (2005)	USA—Mediterranean shrub	≤66.78	≥68.64
MMI_Li	Li, Cai, and Ye (2010)	China—Rainforest	≤5.25	≥6.31
MMI_Nguyen	Nguyen, Everaert, Gabriels, Hoang, and Goethals (2014)	Vietnam—Rainforest	≤0.55	≥0.58
MMI_Jun	Jun, Won, Lee, Kong, and Hwang (2012)	South Korea—Temperate broadleaf Forest	≤29.5	≥36

TABLE 2 Disturbance metric thresholds

	Good	Not-good
% Fines (substrates <16 mm)	≤93	>93
Local disturbance index (LDI)	≤1	>1
Bed stability	≥−1.5	<−1.5
% Pasture (in the total watershed)	≤40%	>40%
Integrated disturbance index (IDI)	≤0.23	>0.23

high disturbance compared to the low disturbance level. We calculated 95% confidence intervals for RR estimates; for the RR to be significant, the lower bound of the 95% confidence interval of RR must also have been >1.

The AR is expressed as a combination of a RE and its RR. If AR $\neq$ 0, any increase in a stressor RE or in its RR will also increase its AR. Conversely, decreases in RE or RR will decrease AR. We report 100 $\times$ AR as the % reduction that could be achieved by eliminating a stressor (Van Sickle & Paulsen, 2008). The RR and RE confidence intervals were obtained using R statistical software version 2.2.1 (R Development Core Team, 2005) and the R spsurvey package (version 2.9).

3 | RESULTS

The total mapped perennial stream length in the Pandeiros River basin is 431 km, from this, 233 km (CI = 3.04 km) (or 54% of the total length) constituted the target length, defined as perennial, accessible, third- to fifth-order and with flowing water. A total of 84 sites were visited, 40 were sampled and 44 were not. The main reasons sites were not sampled were lack of access (34%), including the absence of owner, locked gates, GPS error, or inaccessible roads for our vehicles or by walking. Dry third-order sites mapped as permanent represented 12% of the total. The number of sites classified by IDI as good, fair, and poor was 7, 26, and 7, respectively.

Regarding RE, percent pasture is the most widespread disturbance in the basin, present in 40.8% of the target stream length, followed by low streambed stability, present in 40.1% of the target stream length. Percent fine substrates (<16 mm) were found in 29%, high IDI scores in 24.4%, and high LDI in 21.7% of the target stream length in the Pandeiros River basin (Figure 2).

The disturbance metrics evaluated (LDI, IDI, % fine substrate, and % pasture) were associated (RR > 1 and lower bound of CI >1) with two or more of the MMIs (Table 3). For example, high LDI and IDI scores were the greatest risks for low MMI_Macedo and MMI_Silva scores (Table 3). In other words, the risk of finding a poor MMI score with a high IDI or LDI score was 1.6–1.7 times higher than for sites where the IDI or LDI did not exceed thresholds. The risk of finding low MMI_Silva and MMI_Ode scores in the presence of high % fines was 1.2–1.6 times higher. In the presence of pasture, the risk was 1.2–1.6 higher for finding low scores from the indexes of MMI_Jun, MMI_Baptista, and MMI_Silva (Figure 3).

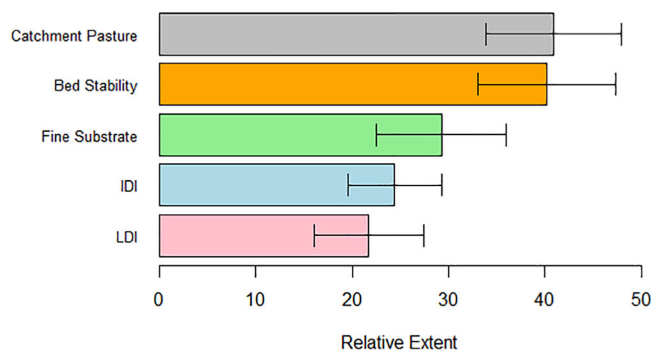

FIGURE 2 Relative extent of disturbances (with 95% confidence intervals) in the Pandeiros River basin [Color figure can be viewed at wileyonlinelibrary.com]

AR assessments offer insights into possible cost-effective management options (Table 3, Figure 3). Elimination of excessive levels of the fine substrate could decrease the risk of low MMI scores by 14% (MMI_Ode, MMI_Silva). Elimination of the pasture pressure would result in 19%, 12%, or 8% decrease in the risk of finding low MMI_Silva, MMI_Baptista, and MMI_Jun scores, respectively. Eliminating local riparian disturbances (LDI) could allow decreases of 4.5%–12% in the risk of finding low scores for the significant indices. Similarly, eliminating catchment plus local disturbances (IDI) could allow decreases of 5 to 15% in the risk of finding low scores. Only IDI and LDI (0.91) and % fines and bed stability (−0.52) were highly or moderately correlated (Appendix 2).

4 | DISCUSSION

The 10 MMIs, combined with a probabilistic survey allowed us to assess the ecological condition in an environmental protection area and to estimate the risks of each disturbance contributing to poor MMI scores. The disturbances (% catchment pasture, IDI, LDI, streambed stability, and % fine substrate) that we evaluated occurred in 20%–40% of the target stream length, with the presence of anthropogenic disturbances (IDI, LDI, and pasture) being the most important threats to poor biological condition. Nonetheless, our results were limited by the 40 sample sites, which can create statistically unstable RR estimates (Van Sickle & Paulsen, 2008).

In the presence of large amounts of the fine substrate, the risk for poor MMI scores was 1.59 (MMI_Ode) to 1.60 (MMI_Silva) times greater. And, this disturbance was present in 29% of the target stream length. In aquatic ecosystems, the presence of fine substrates in stream beds is one of the most important threats to their ecological condition (Burdon, McIntosh, & Harding, 2013). This is because fine sediments reduce habitat availability for macroinvertebrate assemblages, directly compromising their structure, composition, and function (Beermann et al., 2018).

Anthropogenic disturbances in the riparian zone (LDI) represented a risk for poor MMI scores that varied from 1.22 (MMI_Jun) to 1.62 (MMI_Silva) and represented 21.7% of the target stream length. This

TABLE 3 Values of relative risk (RR), 95% lower confidence intervals (LCI) for each calculated multimetric index (MMI)

	% Fine substrate		LDI		Bed stability		% Pasture		IDI	
	RR	LCI	RR	LCI	RR	LCI	RR	LCI	RR	LCI
MMI_Macedo	0.88	0.5	**1.61**	**1.08**	0.72	0.19	0.99	0.57	**1.72**	**1.12**
MMI_Fierro	0	n.a.	0	n.a.	1.12	0.19	1.91	0.34	0	n.a.
MMI_Helson	1.10	0.75	**1.57**	**1.26**	0.79	0.55	1.08	0.79	**1.60**	**1.25**
MMI_Li	1.11	0.76	**1.43**	**1.09**	0.73	0.48	1.27	0.90	**1.47**	**1.10**
MMI_Jun	1.04	0.81	**1.22**	**1.05**	0.91	0.71	**1.22**	**1.00**	**1.23**	**1.05**
MMI_Baptista	1.24	0.93	**1.44**	**1.17**	0.81	0.57	**1.35**	**1.02**	**1.46**	**1.17**
MMI_Ode	**1.59**	**1.00**	1.44	0.92	1.09	0.68	0.84	0.52	**1.55**	**1.00**
MMI_Silva	**1.60**	**1.13**	**1.62**	**1.18**	1.04	0.70	**1.60**	**1.08**	**1.68**	**1.21**
MMI_Nguyen	1.27	0.94	**1.47**	**1.19**	0.83	0.59	1.21	0.88	**1.49**	**1.18**
MMI_Ferreira	1.24	0.93	**1.30**	**1.03**	1.02	0.75	1.08	0.82	**1.33**	**1.05**

Note: RR values >1 and 95% LCIs >1 represent a negative influence of the disturbance on an MMI score (bold).

type of disturbance, although local, can alter habitats and biota (Kaufmann & Hughes, 2006; Kaufmann et al., In Review). Changes in soil conditions, vegetation, and other factors directly reflect the aquatic-terrestrial interactions (Naiman, Bilby, & Bisson, 2000) and meta-ecosystem services (Callisto, Macedo, Linares, & Hughes, 2019). In other studies, macroinvertebrate abundance was predominantly affected by local land use (Allan, 2004) and only 1.4%–6.5% reduction in riparian vegetation coverage was associated with the loss of sensitive macroinvertebrate species (Brito et al., 2020; Dala-Corte et al., 2020).

Low bed stability represented a risk of producing poor MMI scores that varied from 0.72 (MMI_Macedo) to 1.12 (MMI_Fierro) and represented 40.1% of the target stream length. However, the lower confidence intervals indicated a statistically insignificant effect on MMI scores. Lower streambed stability values suggest that there are ongoing landscape or channel erosion processes (Kaufmann et al., 2009). Other studies have found that this process is intensified by reduced vegetation cover resulting from agricultural activities (de Castro, Dolédec, & Callisto, 2017; Leal et al., 2018; Leitão et al., 2018).

The most extensive land use impact was % total catchment pasture, representing 40.8% of the target stream length and risk for poor MMI scores varying from 1.60 (MMI_Silva), 1.35 (MMI_Baptista), and 1.22 (MMI_Jun). Diffuse disturbances, such as pasture, contribute to excess fine sediments, nutrients, and pollutants in freshwater ecosystems (Allan, 2004; Hughes, Infante, Wang, Chen, & Terra, 2019). As the extent of cattle grazing increases in river basins, there is an increase in pollutants and sediments, as well as channel degradation, which affects the habitat available to organisms (Beschta et al., 2013), particularly when that grazing occurs in riparian zones (Kauffman, Beschta, Otting, & Lytjen, 1997).

In the presence of high levels of anthropogenic disturbances measured by the IDI, the risk for poor MMI scores varied between 1.23 (MMI_Jun) and 1.72 (MMI_Macedo). Considering that poor IDI condition is present in 24.4% of the target stream length, it is a considerable concern for the management and conservation of biological conditions. High IDI values were also associated with an increased risk of biological changes related to human activities in other Cerrado streams (Ligeiro et al., 2013). Bed instability and excess fine sediments are associated with disturbances in the catchment and riparian zone, thereby reducing MMI scores and sensitive taxa (Brito et al., 2020; Dala-Corte et al., 2020).

In all field studies based on correlative relationships, one can ask whether the observed relationships are truly causal, only correlated by chance, or driven by some unmeasured variable. This is particularly a concern when the RR values are only slightly greater than one when relatively small proportions of observed biological variability are explained by the study variables, and if the sample size is relatively small (40 sites in our case). In these cases, it is useful to use a weight-of-evidence approach based on six factors for supporting conclusions (Kaufmann, Herlihy, & Baker, 1992; Kaufmann & Hughes, 2006).

1. Is there a clear scientific mechanism for the relationship? We found that landscape pressures (as measured by increased IDI and LDI scores) were associated with increased % fine substrate at the sites as well as the condition of the macroinvertebrate assemblages (measured by several MMIs) at those sites. The four most sensitive MMIs to our disturbance measures all include a taxa richness or diversity metric as well as an EPT (Ephemeroptera, Trichoptera, Plecoptera) metric (Martins et al., 2020). Those metrics are also the most commonly used macroinvertebrate MMI metrics globally (Ruaro et al., 2020). In other words, what humans do to the land produces stressors (fine sediments) that negatively affect the benthic macroinvertebrates living on stream bottoms.
2. What is the statistical rigor of the study design? We used a probability survey to ensure our sites were statistically representative of an entire river basin. This is a much more rigorous study design than ad hoc site selection, which tends to be biased by convenience or ease of sampling, or a disturbance gradient design, which is biased along a single presumed disturbance (Stevens & Olsen, 2004).
3. What is the statistical strength of the observed RR associations? Those associations and their confidence intervals were only slightly above one in our study (Table 3). However, this is not

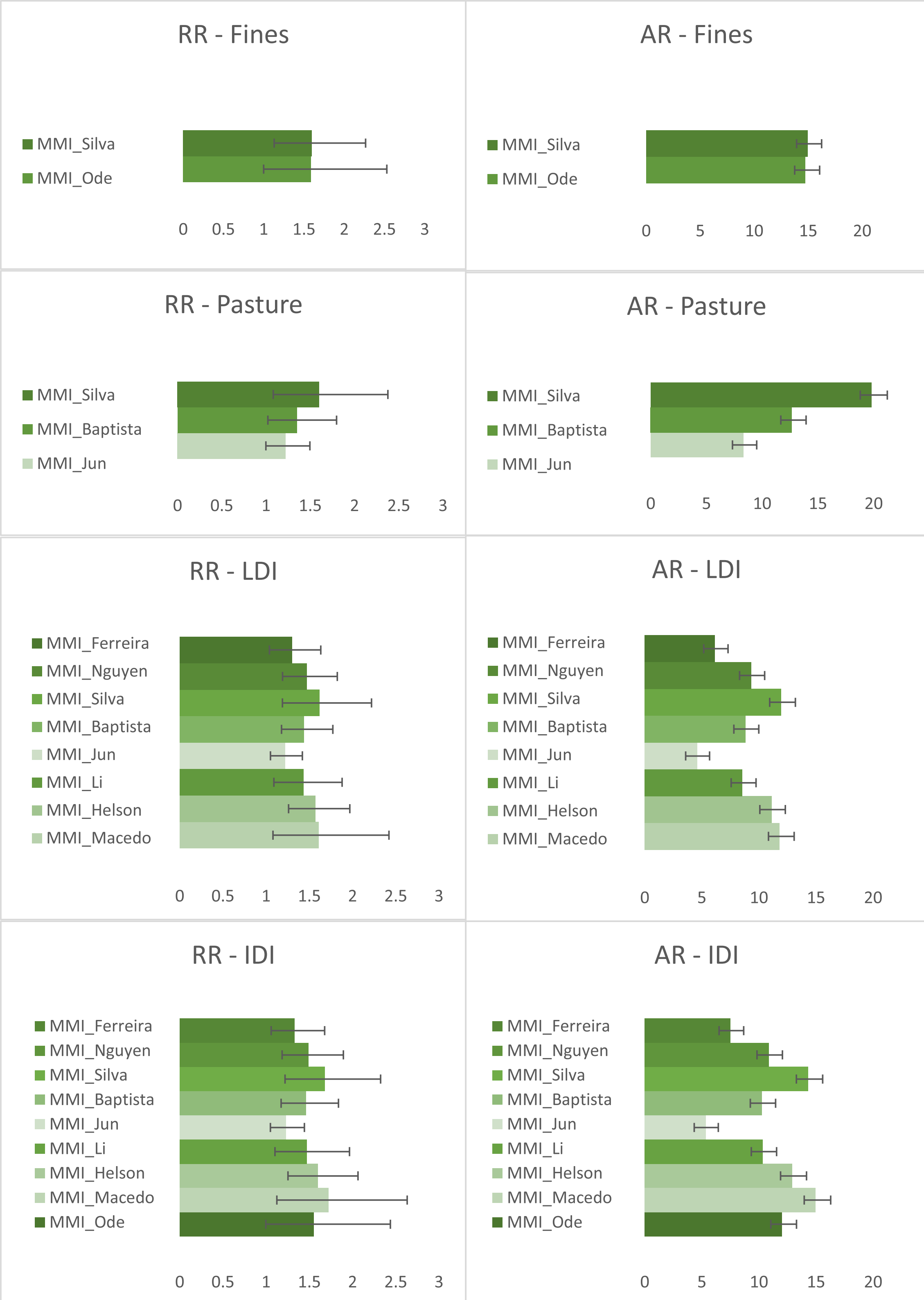

FIGURE 3 Relative risk (RR), 95% lower confidence intervals (LCI) of the indices (MMI) that are significantly related to the disturbances assessed in the Pandeiros River basin and their respective attributable risks and confidence intervals [Color figure can be viewed at wileyonlinelibrary.com]

unusual for similar field studies conducted in the USA and Brazil. USEPA (2020) reported RR scores of 1.4–1.8 for four measures of physical habitat structure, yet that study was based on data from 1,853 sites. Studies employing probability designs with far fewer sites (20–190) in Brazil reported RR scores of 1.9–2.5 for watershed and riparian disturbance (Jiménez-Valencia et al., 2014; Silva et al., 2018). Those Brazilian scores reflected much stronger landscape disturbance gradients than were observed in our study and therefore had much stronger RR scores.

4. What alternative or unmeasured explanations might exist for explaining the study relationships? Frequently, unmeasured disturbances or substantial and unmeasured natural gradients, such as channel slope, lithology, or climate confound observed disturbance-biology patterns (Macedo, Hughes, et al., 2014; Macedo, Pompeu, et al., 2014; Silva et al., 2017; Stoddard et al., 2008). We are aware of no other anthropogenic disturbances in the minimally disturbed Pandeiros basin nor are there any large natural background gradients (Azevedo et al., 2009).
5. To what degree does one study agree with or contradict similar studies? Our results conform with a large body of evidence indicating that land uses that remove the natural vegetation of catchments and riparian zones lead to increased stream sedimentation and degradation of benthic macroinvertebrate assemblages (Allan, 2004; Beschta et al., 2013; Callisto et al., 2019; Herlihy et al., 2020; Hughes, 2019; Kaufmann et al., 2009; Wood & Armitage, 1997).
6. Lastly, is there any evidence that mitigating a major disturbance can reduce its impact on ecosystems? In this case, improved management of livestock grazing or pasturing does reduce stream sedimentation and improve the condition of benthic macroinvertebrates (Agouridis, Workman, Warner, & Jennings, 2005; Quinn, Croker, Smith, & Bellingham, 2009; Weigel, Lyons, Paine, Dodson, & Undersander, 2000). The AR results in our study indicate that decreasing the disturbances that we measured could reduce the risks of finding low MMI scores by up to 19%.

The identification of disturbances that represent the greatest risk to biological conditions is essential for assessment and management purposes, especially in protected areas worldwide. However, our study basin has low levels of anthropogenic disturbances (Callisto et al., 2019) compared with those in other Cerrado hydrologic units that have been studied (e.g., Ligeiro et al., 2013; Macedo et al., 2016; Silva et al., 2017). Protected areas, such as the Rio Pandeiros State Environmental Protection Area, are of fundamental importance to limit anthropogenic disturbances (Barlow et al., 2018; Leal et al., 2020), thereby maintaining basin environmental quality.

Composite measures, such as MMIs, are useful for detecting the overall degradation of aquatic ecosystems. MMIs combined with probabilistic analyses of RR and RE are important tools for decision making (Nõges, van de Bund, Cardoso, Solimini, & Heiskanen, 2009) and for implementing more cost-effective measures for protecting high-quality systems and rehabilitating degraded ecosystems (Statzner & Bêche, 2010). Probabilistic studies like ours can help policymakers and managers identify important local and regional disturbances and estimate the possible benefits of their remediation (Van Sickle & Paulsen, 2008). In protected areas, still influenced by human activities, local impacts can still affect overall basin environmental quality and consequently its biological condition (Barlow et al., 2018). Creating new protected areas and improving existing ones should be a priority for any strategy for conserving tropical aquatic ecosystems (Sundar et al., 2020).

The results of our study also support the use of standard sampling methods and MMIs in neotropical environmental quality assessments because all 10 MMIs had similar responses to a set of five common disturbance metrics. They also indicated which disturbances were associated with the most risk to poor MMI scores, which disturbances, when eliminated, would most decrease risk, and thereby those that should be primarily monitored and mitigated. Our results indicate that managers in the Pandeiros basin (and other Cerrado basins) should focus on reducing erosion and sedimentation through better livestock management across the river basin, but particularly in riparian zones.

5 | CONCLUSIONS

We found that it was possible to identify the main disturbances associated with the poor biological conditions present and to assess the extent, RRs and ARs of those disturbances. The Pandeiros River basin is an important tributary in the São Francisco River basin. Therefore, it is necessary to focus river rehabilitation efforts on reducing key landscape disturbances that generate risks to losing good biological conditions. Our scientific information has been presented to the pertinent state and national environmental agencies, electrical company, riverine citizens, and members of the river basin committee to support them in rehabilitation efforts. Improved pasture management, avoiding erosion, and reduced siltation of river courses are key priorities for the better freshwater ecological condition in the entire river basin. This joint management effort offers an example for other tropical river basins globally.

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CONFLICT OF INTEREST

The authors declare no direct or potential conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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