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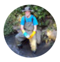

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Thresholds of freshwater biodiversity in response to riparian vegetation loss in the Neotropical region

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Abstract

1. Protecting riparian vegetation around streams is vital in reducing the detrimental effects of environmental change on freshwater ecosystems and in maintaining aquatic biodiversity. Thus, identifying ecological thresholds is useful for defining regulatory limits and for guiding the management of riparian zones towards the conservation of freshwater biota.
2. Using nationwide data on fish and invertebrates occurring in small Brazilian streams, we estimated thresholds of native vegetation loss in which there are abrupt changes in the occurrence and abundance of freshwater bioindicators and tested whether there are congruent responses among different biomes, biological groups and riparian buffer sizes.
3. Mean thresholds of native vegetation cover loss varied widely among biomes, buffer sizes and biological groups: ranging from 0.5% to 77.4% for fish, from 2.9% to 37.0% for aquatic invertebrates and from 3.8% to 43.2% for a subset of aquatic invertebrates. Confidence intervals for thresholds were wide, but the minimum values of these intervals were lower for the smaller riparian buffers (50 and 100 m) than larger ones (200 and 500 m), indicating that land use should be kept away from the streams. Also, thresholds occurred at a lower percentage of riparian vegetation loss in the smaller buffers, and were critically lower for invertebrates: reducing only 6.5% of native vegetation cover within a 50-m riparian buffer is enough to cross thresholds for invertebrates.
4. *Synthesis and applications*. The high variability in biodiversity responses to loss of native riparian vegetation suggests caution in the use of a single riparian width for conservation actions or policy definitions nationwide. The most sensitive bioindicators can be used as early warning signals of abrupt changes in freshwater biodiversity. In practice, maintaining at least 50-m wide riparian reserves on each side of streams would be more effective to protect freshwater biodiversity in Brazil. However, incentives and conservation strategies to protect even wider riparian reserves (~100 m) and also taking into consideration the regional context will promote a greater benefit. This information should be used to set conservation goals and to create complementary mechanisms and policies to protect wider riparian reserves than those currently required by the federal law.

KEYWORDS
forest code, freshwater, land use, native vegetation, private property, riparian reserves, stream fauna, tipping point

1 | INTRODUCTION

Identifying thresholds (also termed tipping points or breakpoints) of land use above which ecosystems shift abruptly is paramount to set conservation goals and to support policies aiming to maintain biodiversity and ecological services within safe boundaries (Rockström et al., 2009). However, we know little about the existence of general threshold ranges of habitat change at which substantial biodiversity loss occurs in freshwaters draining human-dominated landscapes (Dodds, Clements, Gido, Hilderbrand, & King, 2010; Leal et al., 2018). The paucity of such information for guiding management strategies and environmental legislation is worrying given the projected expansion of agricultural lands in the next decades, which will drive species extinctions and compromise fundamental ecological services (e.g. water quality and sediment retention). These concerns are particularly acute in the hyperdiverse tropics that hold the vast majority of the world's freshwater biota (Barlow et al., 2018).

Land use change within the riparian zone of streams is recognized as having one of the most severe effects on aquatic biodiversity (Dala-Corte et al., 2016; Gregory, Swanson, McKee, & Cummins, 1991; Jones, Helfman, Harper, & Bolstad, 1999). However, little consensus exists on whether the reduction of riparian vegetation below a certain level leads to non-linear changes in ecosystem dynamics, the so-called threshold responses (Swift & Hannon, 2010). Estimating how species respond to vegetation loss can improve our understanding of the processes that cause species extinctions and can also support the definition of conservation and restoration strategies (Suding & Hobbs, 2009), such as the minimum riparian size required to maximize protection of aquatic life.

In general, there are few scientific-based recommendations of riparian widths needed to protect aquatic life in tropical freshwaters (Luke et al., 2019). Although there has been a growing number of studies that identified thresholds in tropical aquatic systems (e.g. Brejão, Hoeinghaus, Pérez-Mayorga, Ferraz, & Casatti, 2018; Brito et al., 2019), it is not even clear how different aquatic communities respond to native vegetation loss, and whether specifying different minimum widths for different regions would be more effective to avoid biodiversity declines. This understanding is critical: if many species show synchronous responses to habitat loss, then freshwater ecosystems can undergo abrupt changes (Dodds et al., 2010) and communities can enter an alternative state where ecosystem functioning and services shift unpredictably (Beisner, Haydon, & Cuddington, 2003; Folke et al., 2004). Under this scenario, restoration and recovery to a previous state may be difficult or even impossible (van Nes et al., 2016), specially under a shifting baseline syndrome, where we would be unable to know the previous state of a system due to rapid biodiversity losses (Soga & Gaston, 2018).

We investigated the congruence in thresholds for different biological groups, riparian buffer sizes and Brazilian biomes, assessing the values of riparian vegetation cover loss at which abrupt decline of freshwater biodiversity could occur. We focused on stream fish and invertebrates because they are abundant, widespread and species-rich groups. Also, these groups include several reliable bioindicators of environmental change, and play a key role in various ecosystem processes and services (e.g. nutrient cycling and transport; Karr, 1981; López-López & Sedeño-Díaz, 2015; Wallace & Webster, 1996). We identified the extent of native riparian vegetation cover at which there are synchronous and abrupt population losses of most bioindicators for independent datasets. The existence of congruent thresholds among riparian buffer sizes, biological groups and biomes would support the implementation of a single, accurate and science-based value to regulate land use in riparian areas across the country. Alternatively, the lack of a clear and unique threshold would suggest the need for defining land use or region-specific regulations.

2 | MATERIALS AND METHODS

2.1 | Datasets

We assembled data on fish and aquatic invertebrates distributed across streams in four of the six Brazilian biomes: Amazon, Cerrado (Neotropical savanna), Atlantic Forest and Pampa (Subtropical grassland). The other two Brazilian biomes (Caatinga and Pantanal) were not represented in our datasets. We used the Brazilian official classification of biomes because it is the one used as reference by the government to implement environmental regulations (see Figure 1 for a world biome correspondence of Brazilian nomenclature). Datasets included both well-preserved areas and landscapes dominated by agriculture, with few urban areas. Each dataset comprised a site by taxon matrix with their respective geographic coordinates. Datasets satisfied three a priori inclusion criteria: (a) covering a nearly complete gradient of native vegetation loss (minimum range was 0%–80% of native vegetation cover; see below), (b) including at

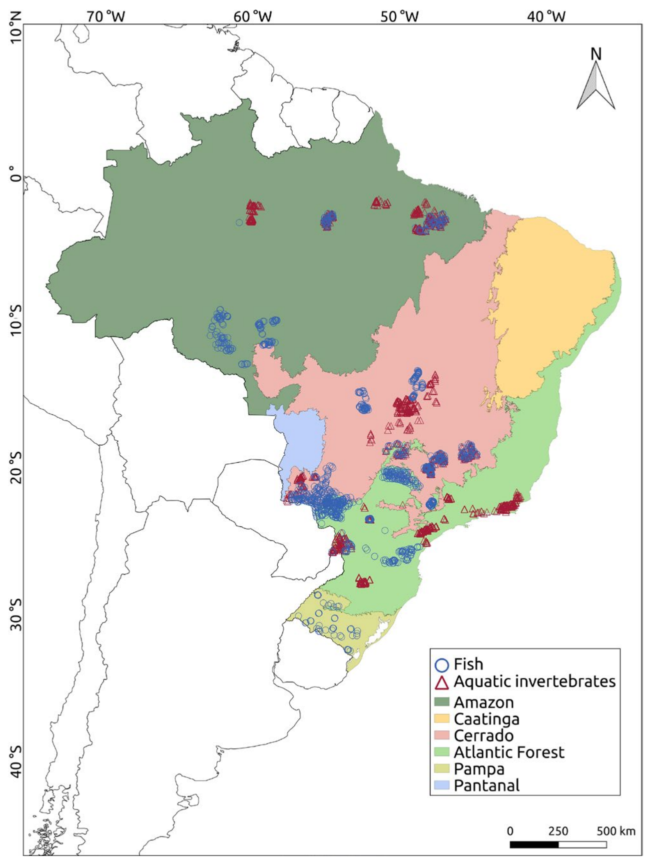

FIGURE 1 Distribution of stream sites for fish (1,149 sites) and aquatic invertebrate (1,449 sites) taxa across the Brazilian biomes (correspondence with world biomes, sensu Olson et al., 2001: Amazon (Tropical Moist Forest), Caatinga (Xeric shrublands), Cerrado (Tropical Savannas), Pantanal (Flooded Grasslands), Atlantic Forest (Tropical and Subtropical Moist Forest), Pampa (Subtropical Grasslands). A subset of aquatic invertebrates taxa, including aquatic insects of the orders Ephemeroptera, Plecoptera, Trichoptera, Odonata and Diptera (EPTOD) sampled in 955 sites was also investigated

least 20 stream sites and (c) including streams no wider than 10 m (because riparian protection may be based on watercourse width, as in the Native Vegetation Protection Law of Brazil, Law number 12,651/2012, which has a specific regulation of 30-m width riparian reserves for up to 10-m width streams).

After filtering out the datasets considering the above criteria, analyses were based on 1,149 stream sites sampled for fish and 1,449 stream sites for aquatic invertebrates (subdivided into 18 datasets for fishes and 18 datasets for aquatic invertebrates). The number of datasets for fish, per biome, was the following: Amazon = 3; Pampa = 1; Cerrado = 8; Atlantic Forest = 6; for invertebrates: Amazon = 7; Cerrado = 5; Atlantic Forest = 6. All fish data were identified to species level. Aquatic invertebrates included multiple taxa commonly sampled in the streams (e.g. crustaceans, mollusks, annelids and insects) identified to various taxonomic levels (e.g. order, family, genera). In addition, we used subsets of aquatic insect immature stages of the orders Ephemeroptera, Plecoptera, Trichoptera, Odonata and Diptera (EPTOD), regarded as bioindicators (Bonada, Prat, Resh, & Statzner, 2006), spanning 955 stream sites from 13 datasets (these data are available in Dala-Corte et al., 2020).

We identified the biome in which each sampling site was located by overlaying the site geographic coordinates with a polygon layer of the Brazilian biomes. If a dataset encompassed sites distributed in more than one biome, we separated it into two or more datasets according to the corresponding biomes and conducted the subsequent analysis separately. Fish data were located mainly in the Cerrado (494 sites), Atlantic Forest (384 sites) and Amazon (225 sites), with a few sites from the Pampa (46). Invertebrate data were primarily from the Amazon (546 sites), Cerrado (452 sites) and Atlantic Forest (451 sites; Figure 1). The methods used for sampling each stream assemblage varied according to the dataset, so we ran the analyses separately by dataset to control for differences in the sampling methods.

2.2 | Native vegetation loss

Using ArcGIS to process the land cover/use classification available in MapBiomas (http://mapbiomas.org), which is based on 30-m resolution Landsat imagery from year 2017, we obtained the percentage of native riparian vegetation cover within four buffer sizes (50-, 100-, 200- and 500-m buffer radius area) around the sites. Native vegetation along the riparian zone was mainly composed by shrubs, trees or any other native woody vegetation even in non-forest biomes (i.e. Cerrado and Pampa), which were easily detected and discriminated using satellite images. Therefore, we assumed that the proportion of native woody vegetation cover is a good proxy for native riparian vegetation remnant in all biomes. To transform the percentage of vegetation cover remnant into vegetation loss, we obtained complement values by subtracting the cover percentage from 100. We chose different buffer sizes to test whether vegetation loss that occurs close to streams lead to threshold values that differ from those calculated when vegetation loss occurs far from streams. We did not control for potential effects of other human disturbances (e.g. point source pollution) because we were specifically interested in investigating whether we could give clear recommendations for regulating land use of riparian zones considering the assessment of riparian vegetation cover only.

2.3 | Data analysis

We investigated thresholds of bioindicators because aggregating community data into univariate metrics has not always been efficient in demonstrating community changes following disturbance (Baker & King, 2010). For example, species richness and abundance can either stay constant or increase after disturbances, while important species and functions are lost (Leitao et al., 2016). Thresholds were estimated using the Threshold Indicator Taxa Analysis (TITAN; Baker & King, 2010). The analysis was performed separately for each dataset of fish, aquatic invertebrates and EPTOD families. TITAN identifies the level at which a stressor causes simultaneous changes in the abundance and frequency of occurrence of many taxa of a given community. For this, TITAN calculates the indicator value (IndVal) for each taxon using the analysis proposed by Dufrêne and Legendre (1997) and considers several splits in the variable used to define the environmental gradient (native vegetation loss in our study). For each split, TITAN calculates IndVal scores for groups on each side of the split, one at a time. The higher the IndVal score, the stronger is the association with one side of the split (negative or positive response). The maximum IndVal obtained after multiple comparisons for one of the two groups (negative or positive response) is used as an indicator of change in a specific value of the environmental gradient. Afterward, standardized IndVal scores (z scores) are obtained to allow cross-taxa comparison and to calculate community-level thresholds (Baker & King, 2010). Thus, for each taxon, the maximum z score identified along the environmental gradient represents the most abrupt change in frequency and abundance. Negative ($z-$) and positive ($z+$) responses are used to calculate the overall cumulative responses of declining [sum($z-$)] and increasing [sum($z+$)] taxa in the community (Baker, King, & Kahle, 2015).

We were interested in the cumulative response of declining taxa [sum($z-$)] in relation to native vegetation loss around streams. Thus, thresholds for each dataset correspond to the value of native vegetation loss around which the aggregated sum($z-$) scores were maximum, indicating that many taxa declined in frequency and abundance. As recommended by Baker et al. (2015), we used 1,000 bootstraps to estimate threshold values for each dataset and uncertainty around these values (5% and 95% confidence intervals—CIs). We log(x + 1) transformed abundances before running TITAN analysis. We removed taxa with less than five occurrences from the analyses because they do not present enough information along the environmental gradient for allowing threshold identification (Baker et al., 2015). Thresholds based on reliable indicator taxa only, which consisted of the taxa that responded strongly and significantly ($p < 0.05$) to native vegetation loss, were also calculated, corresponding to the filtered z-scores [fsum($z-$)]. We performed these analyses using the R package TITAN2 (Baker et al., 2015).

Subsequently, using thresholds based on fsum($z-$) scores only, we tested for differences in threshold values between riparian buffer sizes of 50, 100, 200 and 500 m using a blocked analysis of variance (ANOVA), where datasets were included as block factors. Afterward, because we had multivariate data with thresholds of four different buffer sizes, we tested differences in threshold values between biomes with a multivariate analysis of variance (MANOVA), using the Pillai–Bartlett statistic in the R environment (R Core Team, 2018). We applied Tukey's HSD post-hoc test to evaluate pairwise differences when ANOVAs or MANOVAs indicated significant differences ($p < 0.05$). All the data and R scripts used in our analyses are available in Dala-Corte et al. (2020).

3 | RESULTS

Fish, aquatic invertebrates and EPTOD showed wide variation in threshold values at which native riparian vegetation loss was related to the abrupt decline of bioindicators (Table 1; Figure 2). Mean thresholds of riparian vegetation loss, across the different biomes, ranged from 0.5% to 77.4% for fishes, 2.9% to 37.0% for aquatic invertebrates and 3.8% to 43.2% for EPTOD (Tables 1 and 2). Despite this variation, threshold values clearly decreased in smaller buffer sizes for both aquatic invertebrates and EPTOD, with the lowest thresholds observed in 50-m buffers. Also, confidence intervals increased with buffer size, mainly for aquatic invertebrate and EPTOD, indicating that modifications near to streams consistently lead to loss of bioindicators (Tables 1 and 2; Figure 3). In general, fish showed higher thresholds than aquatic invertebrates and EPTOD, indicating that aquatic invertebrates include more bioindicators that are highly sensitive to loss of riparian vegetation (Table 1; Figure 3).

The mean proportion of bioindicator taxa (i.e. taxa that declined with native riparian vegetation loss in relation to the total number of taxa in the assemblage) ranged from 5.4% to 7.6% for fishes, 12.1% to 18.4% for aquatic invertebrates and 15.4% to 25.5% for EPTOD. Interestingly, the proportion of bioindicator taxa tended to increase in larger riparian buffer sizes, suggesting that some taxa responded to native vegetation loss in larger buffers only, mainly in 100 and 200-m buffers (Table 1).

No differences were observed between biomes in terms of thresholds for aquatic invertebrates and EPTOD (Table 3; Figure 3). For fish, however, thresholds were, in general, higher for the Atlantic Forest than for the Amazon or Cerrado; there was no difference between the Amazon and the Cerrado (Table 3; Figure 3).

4 | DISCUSSION

4.1 | There is no magic number

We detected several cases of abrupt changes in freshwater biodiversity along gradients of riparian vegetation loss in Brazil. Although threshold values varied widely among biomes and biological groups, they were on average below 50% for fish and below 40% for invertebrates and EPTOD. Also, there was no clear difference in thresholds among biomes, except for fish, with the highest thresholds for the Atlantic Forest biome. The wide variation in thresholds indicates that a single threshold value (or a one-size-fits-all criterion) does not exist across biomes or biological groups for aquatic biodiversity. This result can be partially attributed to the contingency effects of anthropogenic impacts on biodiversity (Brejão et al., 2018). For example, the Atlantic Forest is by far the most degraded biome in Brazil, with a long history of deforestation since early European colonization (Rezende et al., 2018). Hence, the highest thresholds observed for fish decline in the Atlantic Forest may reflect a legacy effect (Harding, Benfield, Bolstad, Helfman, & Jones, 1998), where past land use changes have persistent effects on currently observed thresholds (Roque et al., 2018). In this case, streams in Atlantic Forest landscapes, under a long history of land use effects (e.g. agriculture and cattle ranching), may lack several indicator species, even if the streams have high riparian vegetation coverage currently, suggesting that fish diversity is already largely reduced in this biome. Thus, our findings indicate that protecting only a specific width of riparian vegetation, although better than nothing, is still not enough if we want to maximize the conservation of freshwater biodiversity while considering the land use needs across the Brazilian territory.

Other factors not evaluated herein can also explain the highly variable thresholds that we observed. Landscape features such as slope, soil characteristics, geomorphology and phytophysiognomies of each watershed can mediate the effects of riparian vegetation on stream biodiversity (Gregory et al., 1991; Lowrance et al., 1997). Also, land use upstream the sampled sites, in the whole watershed, can have profound impacts on aquatic biodiversity due to increases in turbidity, siltation, and loads of nutrients and other pollutants (Dala-Corte et al., 2016; Dodds & Oakes, 2006; Leal et al., 2018). In addition, considering that biomes have large areas in Brazil (e.g. Cerrado has around 2 million km^2), thresholds within each biome may be influenced by the different species pool of the different freshwater ecoregions within the biomes, especially for fish, which are constrained to disperse by the watersheds boundaries (Abell et al., 2008). Therefore, although our results support that maintaining largely intact riparian reserves should be the major strategy for protecting aquatic life in the neotropics, the high variability in the thresholds indicates that considering the regional context and land use practices beyond riparian zones can contribute to define regional-specific riparian reserve widths and to elaborate complementary strategies of land use at the catchment scale (Azevedo-Santos et al., 2019; Wahl, Neils, & Hooper, 2013).

Even considering all sources of variation described above, thresholds of native vegetation loss were in general lower for smaller buffer sizes, with the minimum values observed in the 50-m wide buffers, suggesting that vegetation loss near streams are more harmful to biodiversity, and that land conversion should be kept away from watercourses (Dala-Corte et al., 2016; King et al., 2005). This reinforces the idea that strict protection of large riparian reserves should be a priority to minimize the impacts of land use on freshwater ecosystems, and that protecting only part of the riparian zone

TABLE 1 Thresholds (mean values) of indicator taxa loss in response to percentage of native riparian vegetation loss estimated at 50-, 100-, 200- and 500-m buffers (across 1,000 bootstrap replicates), per biome, for fish, aquatic invertebrates and EPTOD (insect orders Ephemeroptera, Plecoptera, Trichoptera, Odonata and Diptera) and their respective confidence intervals (CI). Thresholds correspond to the value of native vegetation loss at which many taxa exhibit strong declines in their frequency and abundance based on *z*-scores. Thresholds were based on reliable taxa only, which consists of the taxa that responded strongly and significantly (negatively) to native vegetation loss [fsum(*z*−) scores]. NTaxa = mean number of bioindicator taxa identified that decline in response to native vegetation loss; %Taxa = percentage of bioindicators in relation to the total. Number of datasets for estimating thresholds using the Threshold Indicator Taxa Analysis (TITAN) was 18 for fish, 18 for aquatic invertebrates and 13 for EPTOD. NA = no significant species indicator identified. Boldface indicates overall mean values per taxa for each buffer size

Taxa	Riparian buffer	Overall/biome	Threshold (%)	5% CI	95% CI	NTaxa	%Taxa
Fish	50 m	Overall	25.9	6.2	52.8	3.4	5.4
		Amazon	24.8	0.0	62.0	6.7	8.9
		Atlantic Forest	68.5	33.5	73.0	2.0	3.2
		Cerrado	21.3	5.5	49.0	1.3	3.8
		Pampa	0.5	0.0	16.5	1.0	1.7
	100 m	Overall	33.8	8.9	46.2	3.4	6.3
		Amazon	49.3	0.3	54.5	7.3	9.7
		Atlantic Forest	45.5	24.3	74.8	2.0	4.7
		Cerrado	23.4	8.9	33.8	1.8	5.4
		Pampa	16.0	4.0	26.5	2.0	3.5
	200 m	Overall	46.2	28.1	61.1	3.8	7.4
		Amazon	30.3	9.7	56.3	8.0	10.7
		Atlantic Forest	74.6	50.1	80.4	2.0	5.2
		Cerrado	32.8	21.6	50.8	2.8	7.2
		Pampa	47.0	28.0	49.5	4.0	6.9
	500 m	Overall	48.5	39.3	65.6	4.0	7.6
		Amazon	18.2	16.8	49.3	10.0	13.4
		Atlantic Forest	77.4	67.5	90.8	1.8	4.4
		Cerrado	44.4	31.8	56.9	2.5	6.8
		Pampa	NA	NA	NA	NA	NA
Aquatic invertebrates	50 m	Overall	6.5	2.1	38.0	7.7	12.1
		Amazon	2.9	0.2	42.2	11.8	18.6
		Atlantic Forest	9.1	6.0	44.2	7.0	11.1
		Cerrado	8.5	0.0	24.0	2.5	3.8
	100 m	Overall	11.2	4.5	31.4	10.7	16.4
		Amazon	6.5	4.4	26.0	15.3	23.5
		Atlantic Forest	12.0	8.2	34.8	10.2	16.6
		Cerrado	17.3	0.1	35.3	4.5	5.5
	200 m	Overall	20.9	11.1	34.6	12.0	17.7
		Amazon	13.1	6.1	27.2	18.8	28.2
		Atlantic Forest	25.9	14.5	36.9	10.4	16.9
		Cerrado	25.2	13.8	41.1	5.4	5.8
	500 m	Overall	29.6	16.8	46.8	12.3	18.4
		Amazon	20.5	8.5	37.4	19.5	29.2
		Atlantic Forest	37.0	26.4	54.3	11.0	17.8
		Cerrado	31.5	15.2	49.1	5.2	6.3
EPTOD	50 m	Overall	8.7	2.9	31.6	4.9	15.4
		Amazon	10.3	0.3	44.4	5.5	18.4
		Atlantic Forest	9.5	6.9	23.8	5.5	16.8

(Continues)

TABLE 1 (Continued)

Taxa	Riparian buffer	Overall/biome	Threshold (%)	5% CI	95% CI	NTaxa	%Taxa
		Cerrado	3.8	0.0	21.5	2.5	6.4
	100 m	Overall	16.5	7.4	33.7	6.7	21.4
		Amazon	10.5	5.8	35.0	9.5	32.1
		Atlantic Forest	20.4	8.2	26.1	6.4	20.3
		Cerrado	18.2	8.2	44.5	3.3	8.9
	200 m	Overall	27.3	12.9	40.3	7.7	25.5
		Amazon	29.9	4.5	43.5	11.0	38.0
		Atlantic Forest	21.1	14.5	33.9	7.4	24.3
		Cerrado	34.3	21.3	46.5	3.7	10.7
	500 m	Overall	35.2	20.4	55.7	6.8	22.6
		Amazon	31.3	14.8	56.9	10.3	34.6
		Atlantic Forest	33.9	26.0	53.8	6.7	22.2
		Cerrado	43.2	16.8	58.0	2.7	7.6

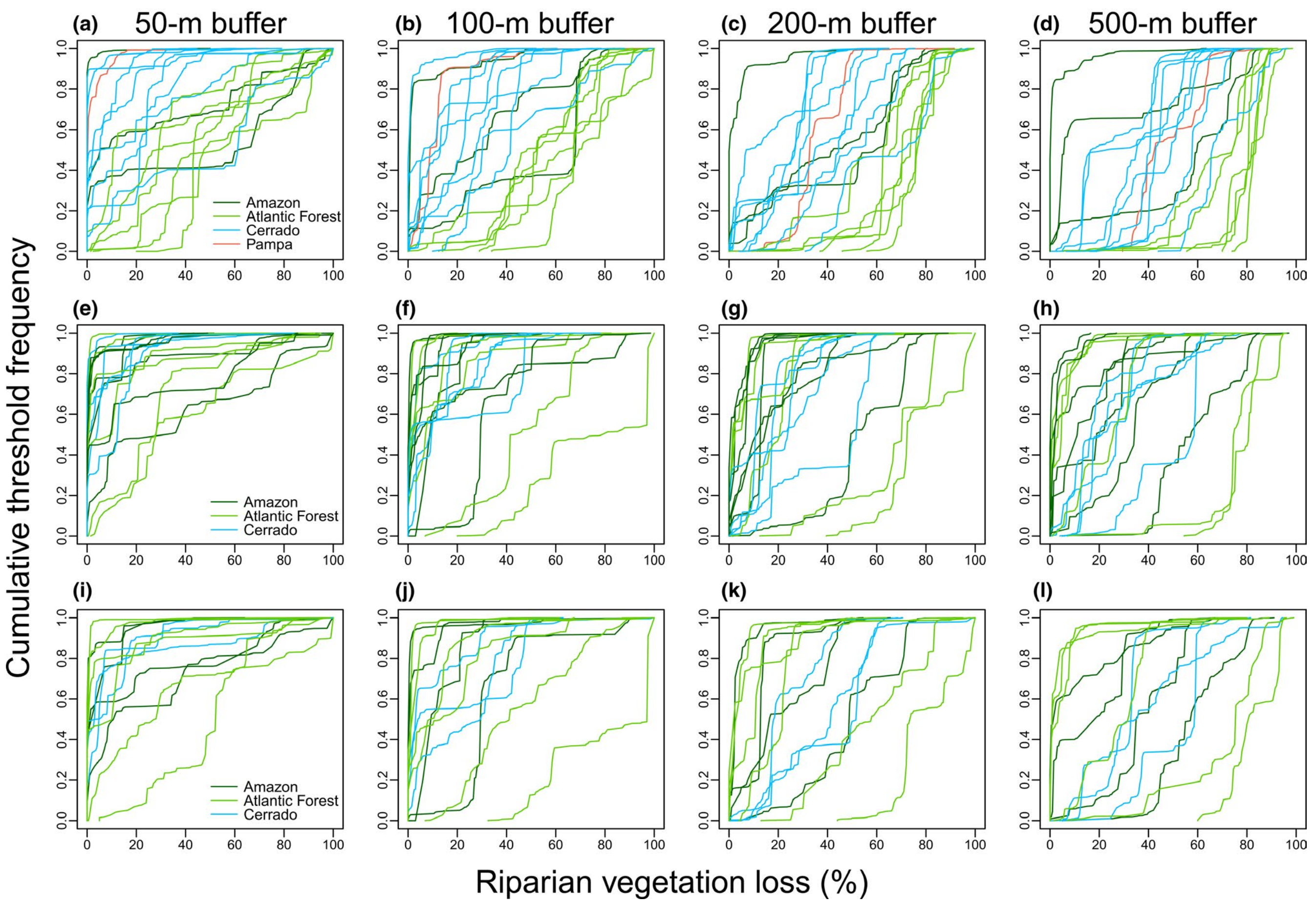

FIGURE 2 Threshold indicator taxa analysis (TITAN) for fish (a–d), aquatic invertebrates (e–h) and Ephemeroptera, Plecoptera, Trichoptera, Odonata and Diptera (EPTOD) (i–l) in response to percentage of native vegetation loss around streams (buffers of 50, 100, 200 and 500 m). Lines are cumulative frequency distributions of negative *z* scores of all taxa [sum(*z*−)], including non-significant values, that decline in response to native vegetation loss (across 1,000 bootstrap replicates). Maximum values (1.0) show declines of all indicator taxa. Each line represents a distinct dataset. Sharp and vertical lines show abrupt declines and low uncertainty around change-point, whereas diagonal lines suggest more even declines and a large uncertainty around change-point. Numbers of datasets were 18 for fish, 18 for aquatic invertebrates and 13 for EPTOD

TABLE 2 Blocked ANOVA comparing thresholds of native riparian vegetation loss between four buffer sizes (50, 100, 200 and 500 m) for different biological groups. Datasets entered as blocks. Models were fitted separately for fish species, aquatic invertebrate taxa and EPTOD (Ephemeroptera, Plecoptera, Trichoptera, Odonata and Diptera) families. Threshold values for each dataset were obtained running the threshold indicator taxa analysis (TITAN). Contrasts comparing mean threshold value difference (m.diff) were tested with TukeyHSD only for significant ANOVAs (p < 0.05)

Taxa	Contrasts	m.diff	*df*	*F*	*p*
Fish			16, 28	2.83	0.056
	50 m versus 100 m	7.88			
	50 m versus 200 m	20.25			
	50 m versus 500 m	22.56			
	100 m versus 200 m	12.37			
	100 m versus 500 m	14.68			
	200 m versus 500 m	2.30			
Aquatic invertebrates			19, 43	12.71	<0.001
	50 m versus 100 m	4.73			0.677
	50 m versus 200 m	14.14			0.007
	50 m versus 500 m	22.58			<0.001
	100 m versus 200 m	9.41			0.121
	100 m versus 500 m	17.85			<0.001
	200 m versus 500 m	8.44			0.169
EPTOD			15, 31	4.03	<0.001
	50 m versus 100 m	7.89			0.225
	50 m versus 200 m	18.68			<0.001
	50 m versus 500 m	26.58			<0.001
	100 m versus 200 m	10.79			0.040
	100 m versus 500 m	18.68			<0.001
	200 m versus 500 m	7.90			0.176

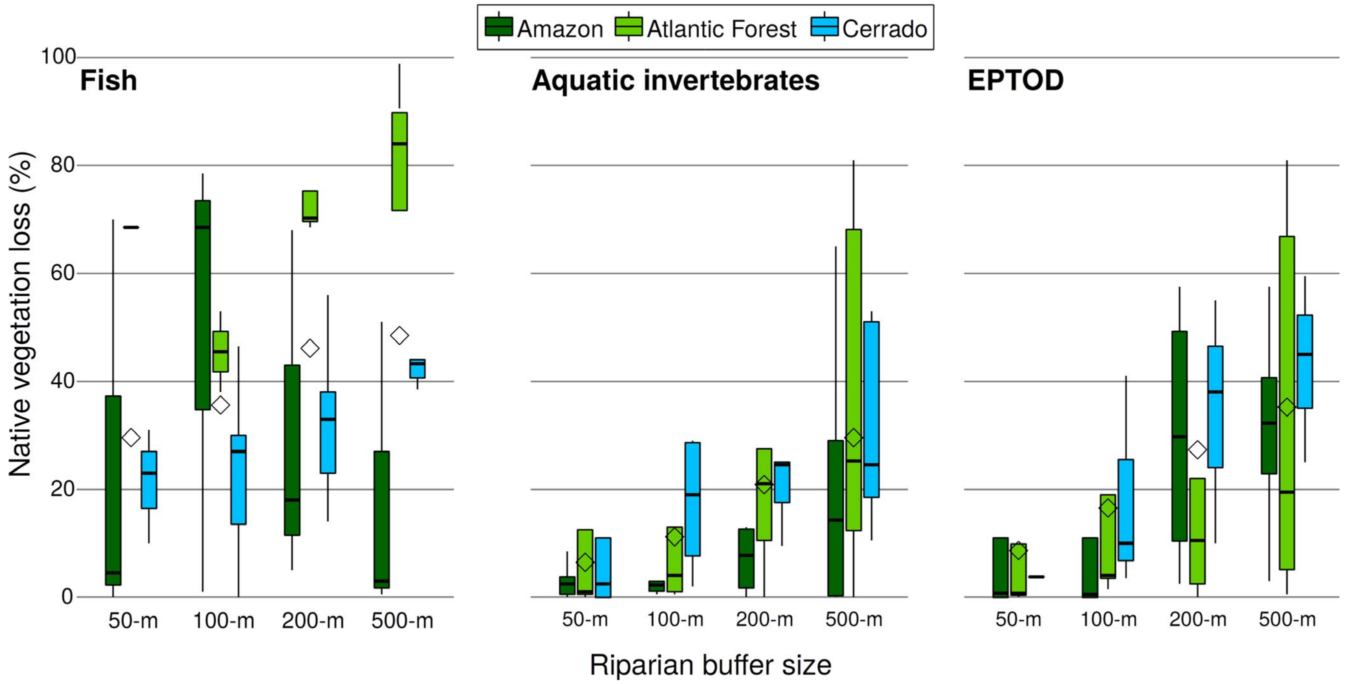

FIGURE 3 Variation in percentage of native vegetation loss in 50-, 100-, 200- and 500-m riparian buffer sizes that drives abrupt decline of fish, aquatic invertebrates and EPTOD insects (groups Ephemeroptera, Plecoptera, Trichoptera, Odonata and Diptera) for three biomes of Brazil. Diamonds show overall mean values per buffer size for each biological group. The lower, central and upper hinges correspond to the 25th (Q1), median and 75th (Q3) percentiles. Lower and upper whiskers represent the range within 1.5 × IQR, where IQR is the Inter-Quartile Range (distance between Q1 and Q3). Number of datasets was 18 for fish, 18 for aquatic invertebrates and 13 for EPTOD

TABLE 3 Multivariate analysis of variance (MANOVA) for testing threshold differences for decline in stream biodiversity between biomes. Response matrices in each MANOVA included thresholds calculated for 50-, 100-, 200- and 500-m riparian buffers. Models were fitted separately for fish species (Fish), aquatic invertebrate taxa and EPTOD (Ephemeroptera, Plecoptera, Trichoptera, Odonata and Diptera) families. Threshold values for each dataset were obtained with the threshold indicator taxa analysis (TITAN). MANOVA was performed with the Pillai–Bartlett statistic. For significant MANOVA models (p < 0.05) we tested mean threshold differences (m.diff) of contrasts with Tukey's HSD test

Taxa	Contrasts	m.diff	*df*	*F*	*p*
Fish			2, 4	6.69	0.042
	Amazon versus Atlantic Forest	39.11			<0.001
	Amazon versus Cerrado	1.52			0.996
	Atlantic Forest versus Cerrado	37.59			<0.001
Aquatic invertebrates			2, 11	0.41	0.903
	Amazon versus Atlantic Forest	11.01			
	Amazon versus Cerrado	10.72			
	Atlantic Forest versus Cerrado	0.29			
EPTOD			2, 6	0.83	0.602
	Amazon versus Atlantic Forest	1.98			
	Amazon versus Cerrado	6.30			
	Atlantic Forest versus Cerrado	4.32			

(as established by the current Brazilian Native Vegetation Protection Law) will probably not be enough to maintain high freshwater diversity across the country (see below).

4.2 | Incorporating uncertainty and the precautionary principle into the law

Policies regulating land use are essential to protect riparian zones and to avoid losing the fundamental ecosystem services provided by freshwater and its biodiversity, but scientific-based orientation is scarce for tropical regions (Luke et al., 2019). In Brazil, the Native Vegetation Protection Law (Federal Law Number 12,651/2012) states that landowners in all biomes must protect a minimum width of riparian reserves. The extent of these riparian reserves varies according to watercourse width (e.g. from 30 m on each side for watercourses up to 10-m wide to 500-m for watercourses larger than 600-m width). In addition, for riparian reserves cleared before 2008, the law allows agricultural activities within them and states that restoration depends on property size (Brancalion et al., 2016). As a consequence, riparian reserves are even smaller in private properties where deforestation occurred before 2008, and watercourse width is not taken into consideration in these cases. Despite Brazilian Native Vegetation Protection Law provides a legal guideline across the country, it is weakly supported by scientific evidence (Brancalion et al., 2016; Metzger, 2010).

Because of the high variability observed in the ecological thresholds, we suggest using the most-sensitive freshwater groups (bioindicators) as reference to avoid biodiversity loss owing to the decrease of native riparian vegetation. This recommendation incorporates the precautionary principle because groups with the lowest thresholds can be used as early warning signals of incoming tipping points in ecosystems (Roque et al., 2018). For example, aquatic invertebrate bioindicators had the lowest, less variable (more congruent) and sharp thresholds to native vegetation loss in the 50-m buffer. This may be so because aquatic invertebrate bioindicators include more species that are highly responsive to stream substrate quality and directly dependent on the riparian zones for feeding, refuge and dispersal (Ruaro, Gubiani, Cunico, Moretto, & Piana, 2016). In this sense, using thresholds for aquatic invertebrates as a reference for regulating the minimum width of riparian reserves would include most of the thresholds observed for fish.

Our study was not designed to answer precise questions about the minimum width and shape of riparian reserves that should be incorporated in the Brazilian legislation. Such a study would need to test spatially explicit hypotheses by directly measuring the size and shape of the riparian zones based on the values stated in the law (instead of buffers as we did), and to measure the amount of native vegetation at a finer scale (the MapBiomas data used in our study is based on 30-m resolution satellite images). Despite these limitations, our results indicate the need for full protection of the smaller buffers, instead of a threshold level of habitat change for orientation of conservation actions or policy definitions. The abrupt decline of aquatic invertebrates after losing a very low amount of riparian vegetation in the smallest buffer size of 50-m radius (mean = 6.5%) and the uncertainty observed around this value (e.g. only 2.9% of vegetation loss for the Amazon biome) suggests that all the vegetation within the 50-m buffers should be maintained. Therefore, maintaining 50 m of riparian reserves on each side of the stream channel (resulting in a 100-m wide strip in total) would most effectively avoid crossing thresholds of aquatic biodiversity loss in Brazil. However, because the number of bioindicator taxa that declined was higher when we evaluated larger buffer sizes (mainly 100- and 200-m buffers), and considering the small values of the coefficient intervals, a great benefit to freshwater biodiversity would be achieved by encouraging the protection of even larger riparian reserves around small watercourses (up to 10-m wide).

4.3 | Strategies to protect Brazilian freshwater biodiversity

Our findings indicate the need to create incentives and strategies to protect large riparian zones around small streams (>50 m wide) in order to maximize the protection of freshwater biodiversity across Brazilian biomes. In this sense, management strategies already proposed for terrestrial ecosystems could also be beneficial for freshwater biodiversity. For instance, increasing pasture productivity and incentives to direct expansion of croplands over already converted lands, mainly pasturelands, could offset the loss of native vegetation in Brazil (Strassburg et al., 2017). In addition, land use should be intensified far away from riparian zones, as we showed that loss of vegetation near to streams is more harmful to freshwater biodiversity.

There is also an opportunity for legislators to complement the Brazilian Native Vegetation Protection Law by enforcing more stringent protection of the riparian zones at state and municipal levels. For example, the city of Bonito (Mato Grosso do Sul State), which relies on ecotourism, has a specific regulation that mandates the protection of 50-m wide riparian reserves around watercourses of rural areas (Bonito, 2004). Aparecida de Goiânia (Goiás State) has also a specific municipal regulation of 50-m wide riparian reserves for small watercourses, and 100 m for other larger rivers (Aparecida de Goiânia, 2018). Considering the context-dependency, such fine-tuned legislation can be more efficient if based on scientific data obtained in smaller scales that consider regional differences (e.g. topography, type of land use and species pool of each watershed).

Creating and expanding economic incentives for landowners that protect large riparian reserves can be more effective than traditional command-and-control approaches. Economic incentives may include payment for ecosystem services, access to lower interest rate loans and reduced rural territorial taxes. For instance, the city of Extrema (Minas Gerais State), in Brazil, has an initiative to pay to rural landowners for adopting management actions that improve and protect water resources, including the increase of vegetation cover in the catchment basin (Jardim & Bursztyn, 2015). Similarly, the 'Manancial Vivo' program promotes payment for ecosystem services to rural landowners in the city of Campo Grande (Mato Grosso do Sul), with positive outcomes to water provision (Sone et al., 2019). In this sense, Brazilian Native Protection Law has a whole chapter (Law 12,651/2012, Chapter X) encouraging the executive branch of the Federal Government to increase the provision of economic incentives to protect native vegetation, which can be used as a basis for implementing legal incentives to protect large riparian stripes.

Brazilian streams harbour one of the highest freshwater biodiversity and levels of endemism in the world (Abell et al., 2008). About 62% of Brazil's territory is privately owned and most of the existing public areas are concentrated in the Amazon (Freitas et al., 2018), meaning that no sound conservation across the country will be successful without reaching private properties and without considering regional characteristics. Therefore, agriculture, ranching and forestry expansion over the native vegetation around watercourses represent a challenge for implementing conservation policies in the country, calling for rigorous control of compliance with the Brazilian Native Vegetation Protection Law. Nonetheless, our results indicate that additional strategies are needed to protect wider riparian reserves than required by the current federal law if we want to maximize the efficiency of both agricultural activities across the country and the conservation of freshwater biodiversity. We hope these findings encourage renewed dialogue among stakeholders, and a national and international effort to safeguard the freshwater life of this hyperdiverse country.

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AUTHORS' CONTRIBUTIONS

F.d.O.R. conceived the idea, R.B.D.-C. organized the data and carried out the analyses and R.B.D.-C., F.d.O.R., T.S., A.S.M. and L.M.B. led the writing of the manuscript. All the authors provided biological data for the analyses, contributed with the draft writing and gave their final approval for publication.

DATA AVAILABILITY STATEMENT

Data available via Zenodo: https://doi.org/10.5281/zenodo.3765802 (Dala-Corte et al., 2020).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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